CHEMOTHERAPY AND SURGERY IN COLON CANCER: FOR BETTER TREATMENT OUTCOMES

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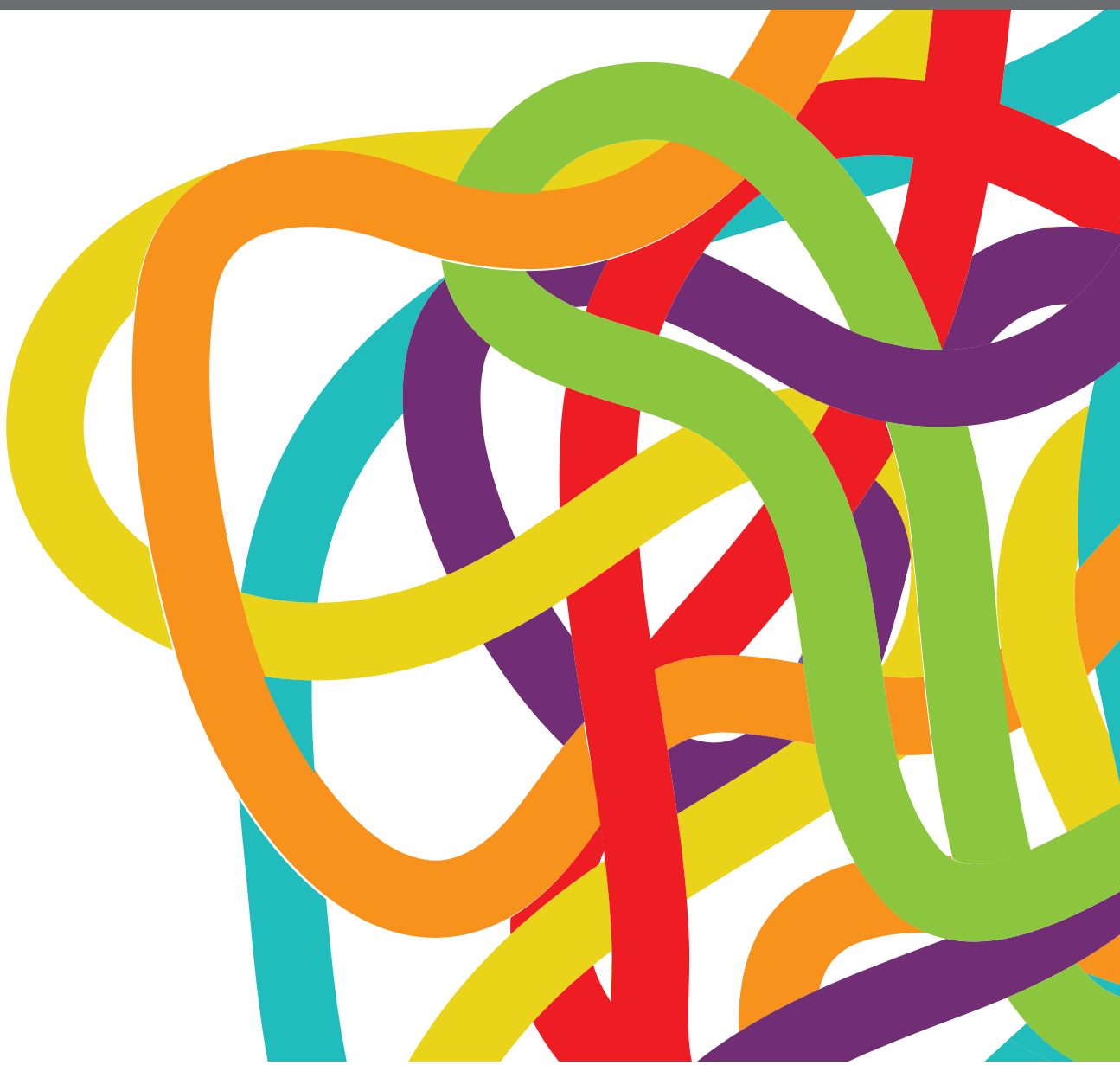

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CHEMOTHERAPY AND SURGERY IN COLON CANCER: FOR BETTER TREATMENT OUTCOMES

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Adjuvant Chemotherapy and Tumor Sidedness in Stage II Colon Cancer: Analysis of the National Cancer Data Base

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Background: Current guidelines recommend discussion of adjuvant chemotherapy (AC) for stage II colon cancer (CC) with high-risk features despite lacking conclusive randomized trial data. We examined AC administration in this population and its effect on overall survival (OS) for available patient, tumor, and treatment characteristics

Methods: Using National Cancer Data Base, a cohort of 42,971 stage II CC patients diagnosed from 2004 to 2009, who underwent surgery with curative intent, was identified. Chi-square test and multivariate logistic regression were used to analyze baseline characteristics and to calculate odds of chemotherapy administration, respectively. Survival analysis was conducted using Kaplan Meier survival analysis with log-rank test and Cox proportional hazards regression modeling.

Results: AC was administered to 26% patients. The use decreased with advancing age and elderly patients received more single-agent than multi-agent chemotherapy (3 vs. 2.4%, $p < 0.0001$). Major predictors of AC use included pT4 status, evaluation of <12 lymph nodes, high grade tumors, positive resection margins, age < 65 years, left sided tumors, and low comorbidity score. AC was associated with improved OS regardless of high-risk features (pT4, undifferentiated histology, <12 lymph node evaluation, or positive resection margins), tumor location, age, gender, comorbidity index, chemotherapy regimen or type of colectomy (adjusted HR: single-agent 0.55, multi-agent 0.6; $p < 0.0001$). In subgroup analysis, AC use compensated for the survival differences otherwise seen between left and right sided tumors in the non-chemotherapy population.

Conclusion: AC in stage II CC was associated with improved OS regardless of age, chemotherapy type or high-risk features. It improved 5-years OS irrespective of tumor location and seemed to compensate for the survival difference seen between right and left sided tumors noted in the non-chemotherapy group.

Keywords: colorectal cancer, stage 2, adjuvant chemotherapy, tumor sidedness, national cancer data base

INTRODUCTION

Colorectal cancer is the third leading cause of cancer diagnosis in the United States (U.S.), both among men and women (1, 2). It is also the second most common cause of cancer death when men and women are combined. As of January 2019, it was estimated that there were in excess of 1.5 million patients in the U.S. with a diagnosis of colorectal cancer (3). In 2020, it is estimated that an additional 147,950 new cases and 53,200 deaths will occur in the U.S (2). Surgical resection remains the mainstay of treatment for non-metastatic colon cancer, with adjuvant chemotherapy having demonstrated improved overall survival (OS) for stage III colon cancer patients (4–7). In patients with stage II colon cancer, the role of adjuvant chemotherapy remains a point of debate (8–16). Since there are few definitive prospective clinical trials which have evaluated the use of adjuvant chemotherapy in stage II colon cancer, current clinical practice guidelines by the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) recommend discussing chemotherapy in patients with tumors possessing high-risk features or microsatellite stability (MSS) and all T4 tumors (17–20). Stage II microsatellite instability high (MSI-H) patients may have a good prognosis and do not benefit from 5-fluorouracil (5-FU) adjuvant chemotherapy (21). In contrast, population-based studies have failed to demonstrate substantial OS benefit with the use of adjuvant chemotherapy for all patients with poor-prognostic or high-risk features (22, 23).

Recently, a study looking at stage II colon cancer patients diagnosed from 1998 to 2006 using the National Cancer Data Base (NCDB) was able to demonstrate an OS benefit associated with adjuvant chemotherapy (24). Though the sample size in this study was large ($N = 153{,}110$), it included patients with other malignancies, thereby resulting in a competing mortality bias.

The primary objective of our study was to utilize data from the NCDB and assess for OS benefit of adjuvant chemotherapy in stage II colon cancer patients, with no other malignancies, diagnosed from 2004 to 2009. Since the US Food and Drug Administration (FDA) approved combination of 5-FU, leucovorin and oxaliplatin (FOLFOX) in 2004 (25), all patients receiving multi-agent chemotherapy in our study population were hypothesized to have received the FOLFOX regimen. The secondary objectives of our study were to assess the impact of tumor sidedness on OS with or without adjuvant chemotherapy, as well as to evaluate the association between adjuvant chemotherapy and the two major high-risk features of stage II colon cancer i.e., T4 tumors and inadequate lymph node evaluation (<12 lymph nodes). In addition, a multivariate analysis of determinant factors in utilization of chemotherapy from 2004 to 2009 was also performed.

Abbreviations: OS, Overall survival; ASCO, American Society of Clinical Oncology; NCCN, National Comprehensive Cancer Network; MSS, microsatellite stability; NCDB, National Cancer Data Base; FDA, Food and Drug Administration; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; PUF, Participant User File; AJCC, American Joint Committee on Cancer; CCS, Collaborative Stage Data Collection System; OR, odds ratio; HR, hazards ratio; CI, confidence interval; MSI, micro-satellite instability; SEER, Surveillance, Epidemiology and End Results.

PATIENTS AND METHODS

Data Source

The NCDB is a joint quality improvement initiative of the American College of Surgeons' Commission on Cancer and the American Cancer Society. The NCDB contains 34 million patient records, and represents ∼70% of all newly diagnosed cases of cancer in the United States (26, 27). Data access was approved by the NCDB after a thorough review of the study proposal. Participant User File (PUF), which included patients diagnosed with colon cancer from 2004 to 2014, was utilized to extract the study cohort.

Study Population

The American Joint Committee on Cancer (AJCC) sixth edition was used for staging purposes and site-specific information was defined according to the AJCC's Collaborative Stage Data Collection System (CCS). Using CCS we excluded patients with appendiceal adenocarcinoma along with exclusion of those who underwent surgical procedures spanning less than a partial colectomy. Only patients with a pathologically confirmed diagnosis were included for analysis. Patients lacking documentation about the variables of interest were also excluded. A final cohort of 42,971 patients diagnosed with stage II colon cancer from 2004 to 2009 was identified using an age-mandated eligibility criteria (18 years and above), along with above mentioned inclusion and exclusion criteria (**Figure 1**). The year 2009 was chosen as a cut-off to enable a minimum follow-up of 5 years for all patients while maintaining a uniform cancer staging system (AJCC, 6th edition).

Measured Outcomes and Variables

The primary endpoint of this study was the 5-years OS. In patients who were alive at the last follow-up, OS was censored at 60 months. Age was analyzed as an ordinal variable after being grouped into 18–64 years, 65–74 years and above. Patients were categorized into four ethnic groups; Caucasians, African-Americans, Hispanics, and others. Patient performance status was analyzed using the Charlson/Deyo comorbidity index and the primary site of colon cancer was recoded into left, right, or transverse part of the colon. Other variables analyzed included gender, institution (academic vs. non-academic), insurance status, average neighborhood income level, year of diagnosis, geographic location of treating institution, histologic grade, involvement of margins, adequacy of lymph nodes evaluated during surgery, pathologic primary tumor characteristics (pT), type of colectomy, and adjuvant chemotherapy.

Statistical Analysis

Descriptive analysis of patient's demographic and clinical information according to receipt of adjuvant chemotherapy was performed using the Pearson chi-square test. Multivariate logistic regression model was used to calculate the odds ratio (OR) of chemotherapy administration based on several determinant factors including age, race, baseline comorbidity index, institution, geographic location, year of diagnosis, tumor laterality, grade, adequate lymph node evaluation, pT, surgery, and margins.

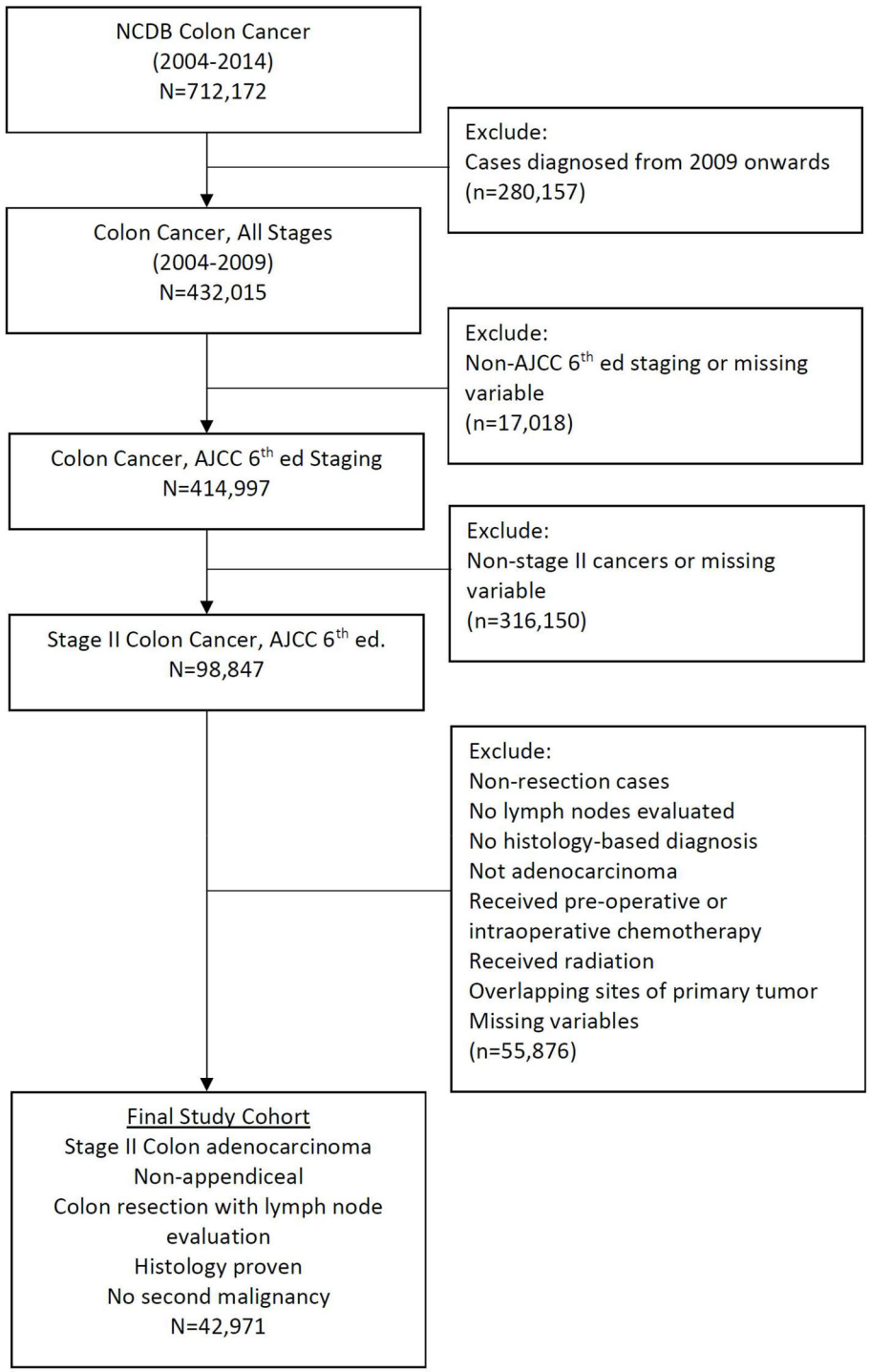

FIGURE 1 | CONSORT diagram.

Kaplan Meier survival curves and Cox proportional hazards model were utilized to perform survival analysis. Kaplan Meier survival curves were adjusted and tested with the log-rank test. A Cox proportional hazards model was constructed using age, average neighborhood income level and Charlson/Deyo comorbidity index as ordinal variables. Other variables including gender, race, institution, insurance status, year of diagnosis, location of primary tumor, histologic grade, adequacy of lymph node evaluation, pT, margins, type of surgery and adjuvant chemotherapy were analyzed as categorical variables in this

TABLE 1 | Baseline patient characteristics, stratified according to receipt of adjuvant chemotherapy.

	No chemotherapy (n = 33,986)	Chemotherapy (n = 8,985)	p
Age, (%)			<0.0001
18–64 years	59	41	
65–74 years	77.1	22.9	
≥75 years	93.8	6.2	
Gender, (%)			<0.0001
Male	77.5	22.5	
Female	80.4	19.6	
Race, (%)			<0.0001
White	80	20	
Black	75.6	24.4	
Hispanic	71.9	28.1	
Others	76.6	23.4	
Charlson/Deyo score, (%)			<0.0001
0	76.2	23.8	
1	82.9	17.1	
2	90	10	
Institution, (%)			0.7356
Academic	79	21	
Non-academic	79.1	20.9	
Location, (%)			<0.0001
East North Central	78.6	21.4	
East South central	77.2	22.8	
Middle Atlantic	77	23	
Mountain	79.9	20.1	
New England	82.6	17.4	
Pacific	81.5	18.5	
South Atlantic	79.5	20.5	
West North Central	78.8	21.2	
West South Central	77.7	22.3	
Insurance, (%)			<0.0001
Insured	79.6	20.4	
Uninsured	62.4	37.6	
Income, (%)			0.1188
<$30,000	78.7	21.3	
$30,000–$34,999	78.3	21.7	
$35,000–$45,999	79.7	20.3	
≥$46,000	79	21	
Year of diagnosis, (%)			<0.0001
2004	79.5	20.5	
2005	78.9	21.1	
2006	77.6	22.4	
2007	78	22	
2008	80.1	19.9	
2009	80.6	19.4	
Primary site, (%)			<0.0001
Left	74.4	25.6	
Right	82.1	17.9	
Transverse	79.6	20.4	
Grade, (%)			<0.0001
Well-differentiated	81.1	18.9	
Moderately diff	79.7	20.3	
Poor/Undifferentiated	75.2	24.8	
Nodes evaluated, (%)			0.6588
Adequate (≥12)	79.1	20.9	
Inadequate (<12)	78.9	21.1	
AJCC pT, (%)			<0.0001
3	81.5	18.5	
4	56.5	43.5	
Colectomy, (%)			<0.0001
Partial	77.5	22.5	
Subtotal	80.1	19.9	
Total	75.4	24.6	
Margins, (%)			<0.0001
Negative	79.6	20.4	
Positive	62.8	37.2	

TABLE 2 | Modality of chemotherapy administered by age (chi-square test, $p < 0.0001$).

Modality of chemotherapy	18–64 years, *n* (%)	65–74 years, *n* (%)	≥75 years, *n* (%)	Total (by chemotherapy, *N*)
None	7,786 (59)	8033 (77.1)	18,167 (93.8)	33,986
Single agent	1,491 (11.3)	852 (8.2)	576 (3)	2,919
Multi agent	3,338 (25.3)	1,236 (11.9)	465 (2.4)	5,039
Type not documented	581 (4.4)	296 (2.8)	150 (0.8)	1,027
Total (by age group, *N*)	13,196	10,417	19,358	**42,971**

Bold value means total sample size.

model. Hazards ratios (HR) and 95% confidence intervals (CI) were generated with HR <1.0 indicating survival benefit.

The $p < 0.05$ was considered statistically significant and Statistical Analysis Software (SAS version 9.4; SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Patient Characteristics

We identified 42,971 patients from NCDB who were diagnosed with stage II colon cancer between 2004 and 2009. Patient and tumor characteristics are shown in **Table 1**, stratified according to receipt of adjuvant chemotherapy. The overall frequency of adjuvant chemotherapy administration was 26% and did not differ significantly by the academic level of treating institution, median family income level or adequacy of lymph node evaluation.

Very elderly patients (age ≥ 75 years) received significantly less chemotherapy as compared to the elderly (65–74 years)

TABLE 3 | Adjusted odds ratios of adjuvant chemotherapy administration based on multivariate logistic regression.

	Odds ratio	95% CI	*p*
Age			
≥75 years	Ref = 1		
18–64 years	11.69	10.84–12.6	<0.0001
65–74 years	4.89	4.52–5.29	<0.0001
Gender			
Male	Ref = 1		
Female	1.06	1.0–1.12	0.0368
Race			
Black	Ref = 1		
White	1.11	1.02–1.21	0.0139
Hispanic	1.3	1.12–1.49	0.0003
Others	1.13	0.96–1.34	0.1337
Charlson/deyo score			
2	Ref = 1		
0	2.11	1.88–2.38	<0.0001
1	1.68	1.48–1.91	<0.0001
Institution			
Non-academic	Ref = 1		
Academic	1.19	1.12–1.27	<0.0001
Location			
Pacific	Ref = 1		
East North Central	1.4	1.26–1.55	<0.0001
East South Central	1.26	1.11–1.44	0.0005
Middle Atlantic	1.62	1.45–1.8	<0.0001
Mountain	1.05	0.9–1.23	0.5254
New England	1.16	1.01–1.34	0.0316
South Atlantic	1.15	1.04–1.27	0.006
West North Central	1.38	1.22–1.57	<0.0001
West South Central	1.2	1.06–1.36	0.0043
Insurance			
Uninsured	Ref = 1		
Insured	1.1	0.96–1.26	0.1694
Income			
<$30,000	Ref=1		
$30,000–$34,999	1.06	0.97–1.17	0.2071
$35,000–$45,999	0.98	0.9–1.08	0.7154
≥$46,000	1.01	0.93–1.11	0.7599
Year of diagnosis			
2009	Ref = 1		
2004	1.22	1.11–1.34	<0.0001
2005	1.24	1.13–1.36	<0.0001
2006	1.37	1.25–1.5	<0.0001
2007	1.29	1.18–1.41	<0.0001
2008	1.1	0.99–1.2	0.0526
Primary site			
Right	Ref = 1		
Left	1.27	1.19–1.35	<0.0001
Transverse	1.11	1.02–1.22	0.0194
Grade			
Well–differentiated	Ref = 1		
Moderately differentiated	1.14	1.04–1.25	0.0061
Poor/Undifferentiated	1.76	1.58–1.97	<0.0001
Nodes evaluated			
Adequate (≥ 12)	Ref = 1		
Inadequate (<12)	1.15	1.08–1.22	<0.0001
AJCC, pT			
3	Ref = 1		
4	3.54	3.27–3.34	<0.0001
Colectomy			
Total	Ref = 1		
Partial	1.08	0.93–1.26	0.3234
Subtotal	1.14	0.98–1.33	0.0797
Margins			
Negative	Ref = 1		
Positive	1.63	1.42–1.87	<0.0001

Ref, Reference.

and young (18–64 years) patient population (6.2 vs. 22.9% vs. 41%, $p < 0.0001$). Women were less likely to receive adjuvant chemotherapy as compared to men (19.6 vs. 22.5%, $p < 0.0001$). Among Caucasians, only 20% received chemotherapy as compared to Hispanics (28.1%), African Americans (24.4%), and other ethnicities (23.4%), which was a significant difference ($p < 0.0001$). Patients with higher comorbidity index i.e., the Charlson/Deyo score, were less likely to receive adjuvant chemotherapy (10 vs. 17.1 vs. 23.8%, $p < 0.0001$). Among the nine broadly divided geographic regions in the U.S., patients in New England were least likely to receive chemotherapy (17.4%, $p < 0.0001$). Adjuvant chemotherapy administration was more common in those without insurance than with (37.6 vs. 20.4, $p < 0.0001$). Frequency of chemotherapy administration steadily increased from 2004 to 2006 (20.5–22.4%, $p < 0.0001$), but then gradually decreased to 19.4% as of 2009.

Patients with tumors located on the left side of colon more often received adjuvant chemotherapy (25.6%) compared to those with tumors of the right (17.9%) or transverse colon (20.4%), which was significantly different as depicted by $p < 0.0001$. Furthermore, patients with high-risk features including pT4 (43.5%), positive margins (37.2%), and high grade tumors (24.8%) more often received adjuvant chemotherapy as compared to other respective risk groups ($p < 0.0001$). Majority of the patients underwent subtotal colectomy (data not shown) and were least likely to receive adjuvant chemotherapy (19.9%) as compared to those who underwent partial (22.5%) or total (24.6%) colectomy.

Comparison of Type of Adjuvant Chemotherapy by Age

Table 2 demonstrates differences of chemotherapy administration among various age groups stratified according to the type of chemotherapy. Young patients more often received

TABLE 4 | 5-years overall survival analysis using Cox proportional hazards regression model.

	5-years survival, %	Hazards ratio	95% CI	*p*
Age				
≥75 years	45.3	Ref = 1		
18–64 years	71.9	0.45	0.43–0.47	<0.0001
65–74 years	65.4	0.56	0.54–0.58	<0.0001
Gender				
Male	57.6	Ref = 1		
Female	59	0.86	0.83–0.88	<0.0001
Race				
Black	58.4	Ref = 1		
White	58.2	0.9	0.86–0.95	0.0001
Hispanic	58.1	1.03	0.94–1.12	0.5622
Others	63.4	0.95	0.86–1.06	0.3364
Charlson/deyo score				
2	39.2	Ref = 1		
0	62.6	0.58	0.55–0.61	<0.0001
1	53.7	0.7	0.68–0.74	<0.0001
Institution				
Non–academic	58.1	Ref = 1		
Academic	59	0.99	0.95–1.03	0.5834
Location				
Pacific	63.8	Ref = 1		
East North Central	58.9	1.16	1.1–1.24	<0.0001
East South Central	57.9	1.2	1.11–1.3	<0.0001
Middle Atlantic	54.5	1.32	1.24–1.4	<0.0001
Mountain	56.7	1.27	1.16–1.4	<0.0001
New England	56.4	1.15	1.07–1.24	0.0002
South Atlantic	58.7	1.16	1.1–1.23	<0.0001
West North Central	60.1	1.12	1.04–1.2	0.0037
West South Central	55.6	1.31	1.22–1.41	<0.0001
Insurance				
Uninsured	58.8	Ref = 1		
Insured	58.3	0.71	0.64–0.78	<0.0001
Income				
<$30,000	54.7	Ref=1		
$30,000–$34,999	56.6	0.95	0.9–0.99	0.0470
$35,000–$45,999	59.4	0.87	0.82–0.91	<0.0001
≥$46,000	61	0.85	0.81–0.89	<0.0001
Year of diagnosis				
2009	52.4	Ref = 1		
2004	59	0.79	0.75–0.83	<0.0001
2005	60.7	0.75	0.71–0.79	<0.0001
2006	59.7	0.78	0.74–0.82	<0.0001
2007	60.2	0.79	0.75–0.83	<0.0001
2008	57.9	0.86	0.81–0.9	<0.0001
Primary site				
Left	59	Ref = 1		
Right	58	0.93	0.9–0.97	0.0003
Transverse	58	0.95	0.9–1.00	0.0574
Grade				
Poor/Undifferentiated	55.2	Ref = 1		
Well-differentiated	60.1	0.88	0.82–0.93	<0.0001
Moderately diff	58.8	0.91	0.87–0.95	<0.0001
Nodes evaluated				
<12, inadequate	51.4	Ref = 1		
≥12, adequate	60.6	0.74	0.71–0.76	<0.0001
AJCC pT				
4	46.4	Ref=1		
3	59.6	0.59	0.57–0.62	<0.0001
Chemotherapy				
None	54.3	Ref = 1		
Single–agent	73.9	0.55	0.51–0.59	<0.0001
Multi–agent	73.6	0.6	0.56–0.64	<0.0001
Type not known	72.2	0.65	0.57–0.73	<0.0001
Colectomy				
Total	54	Ref = 1		
Partial	58.8	0.84	0.76–0.91	<0.0001
Subtotal	58.3	0.86	0.78–0.94	0.0005
Margins				
Positive	41.3	Ref = 1		
Negative	58.9	0.64	0.59–0.68	<0.0001

Ref, Reference.

chemotherapy, either single- or multi-agent, as compared to the elderly and the very elderly (11.3/25.3% vs. 8.2/11.9% vs. 3/2.4%). It is interesting to note that among the very elderly population, more patients received single-agent rather than multi-agent chemotherapy (3 vs. 2.4%).

Determinant Factors for Adjuvant Chemotherapy Administration

In multivariate logistic regression analysis, patients with high-risk features were more likely to receive adjuvant chemotherapy (**Table 3**). Patients with pT4 lesions had higher likelihood of receiving chemotherapy compared to pT3 lesions (adjusted OR 3.54, 95% CI 3.27–3.34), as did patients with inadequate lymph node evaluation compared to those with adequate lymph node assessments (adjusted OR 1.15, 95% CI 1.08–1.22). High grade tumors were more likely to receive chemotherapy compared to well-differentiated tumors (adjusted OR 1.76, 95% CI 1.58–1.97) and patients with positive tumor margins had higher likelihood of receiving chemotherapy (adjusted OR 1.63, 95% CI 1.42–1.87). Young patients had higher odds of receiving adjuvant chemotherapy compared to very elderly (adjusted OR 11.69, 95% CI 10.84–12.6) and so did left sided tumors compared to right sided lesions (adjusted OR 1.27, 95% CI 1.19–1.35). Other significant factors associated with receipt of adjuvant chemotherapy included gender, race, comorbidity score,

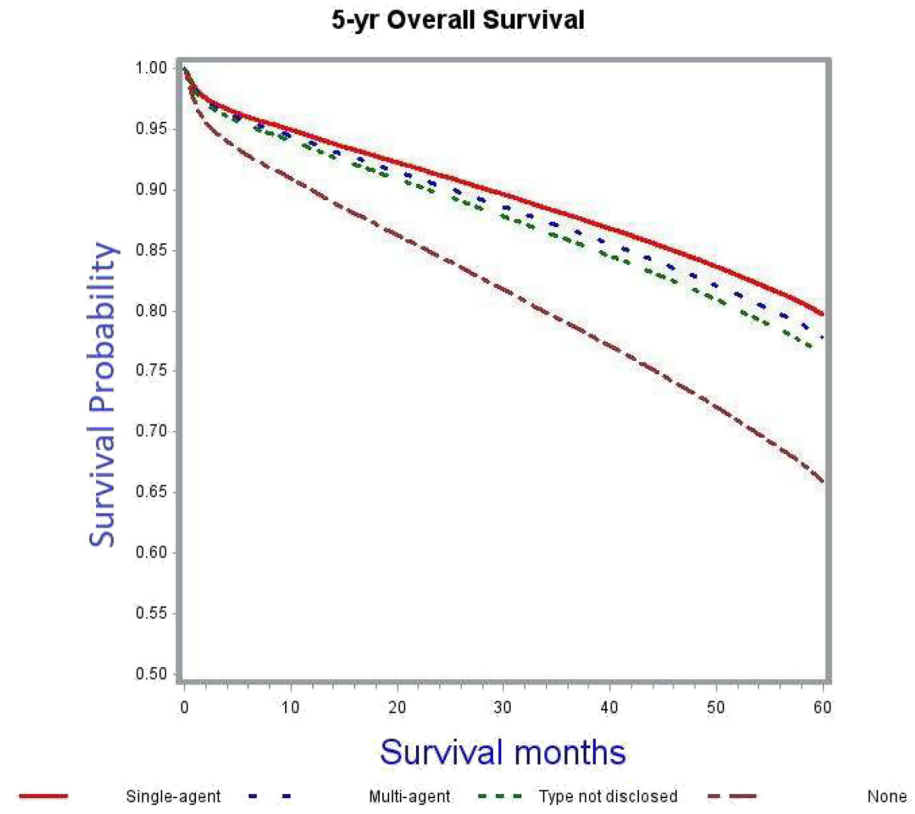

FIGURE 2 | Adjusted survival curves stratified by adjuvant chemotherapy ($p < 0.0001$).

academic level of treating institution, geographic location and year of diagnosis.

Independent Predictors of Overall Survival

The crude 5-years OS rate for all patients receiving any kind of adjuvant chemotherapy was 73.5% as compared to 54.3% among those not receiving chemotherapy (**Table 4**). After adjusting for patient, tumor and treatment characteristics, the probability of death was significantly lower in patients receiving chemotherapy, irrespective of type and modality, as compared to patients not receiving adjuvant chemotherapy (single-agent: adjusted HR 0.55, 95% CI 0.51–0.59; multi-agent: adjusted HR 0.6, 95% CI 0.56–0.64; unknown type of chemotherapy: adjusted HR 0.65, 95% CI 0.57–0.73). **Figure 2** demonstrates the association between adjuvant chemotherapy and improved OS. This further reflects the similar survivals associated with single- and multi-agent chemotherapy regimens in adjuvant setting.

Patients with right sided tumors had better OS compared to left sided tumors (adjusted HR 0.93, 95% CI 0.9–0.97). **Figure 3** demonstrates the association between tumor sidedness and improved OS. Among the four high-risk features evaluated in the study, including pT, grade, adequacy of lymph node evaluation and margins, improved OS was associated with pT3 lesions (adjusted HR 0.59, 95% CI 0.57–0.62, compared to pT4), low grade tumors (adjusted HR 0.88, 95% CI 0.82–0.93, compared to high grade tumors), adequate lymph node evaluation (adjusted HR 0.74, 95% CI 0.71–0.76, compared to inadequate lymph node assessment) and negative margins at surgery (adjusted HR 0.64, 95% CI 0.59–0.68, as compared to positive margins).

After adjusting for all other covariates, age, gender, ethnicity, Charlson/Deyo comorbidity index, geographic location, insurance status, median family income, year of diagnosis, and colectomy remained independent predictors of OS in stage II colon cancer (**Table 4**).

Overall Survival Advantage of Adjuvant Chemotherapy by Tumor Sidedness

After adjusting for covariates, tumor sidedness was noted to demonstrate a significant survival benefit in thenon-chemotherapy subgroup (**Table 5**). In this subgroup right sided tumors had improved survival compared to left sided tumors (adjusted HR 0.92, 95% CI 0.88–0.96). This survival difference based on tumor location was however compensated with administration of chemotherapy, as demonstrated by the absence of significant OS benefit in the adjuvant chemotherapy subgroup analysis.

Furthermore, the OS benefit of adjuvant chemotherapy was confirmed in the subgroup analyses based on tumor sidedness. Irrespective of tumor location, adjuvant chemotherapy was associated with improved 5-years OS outcomes.

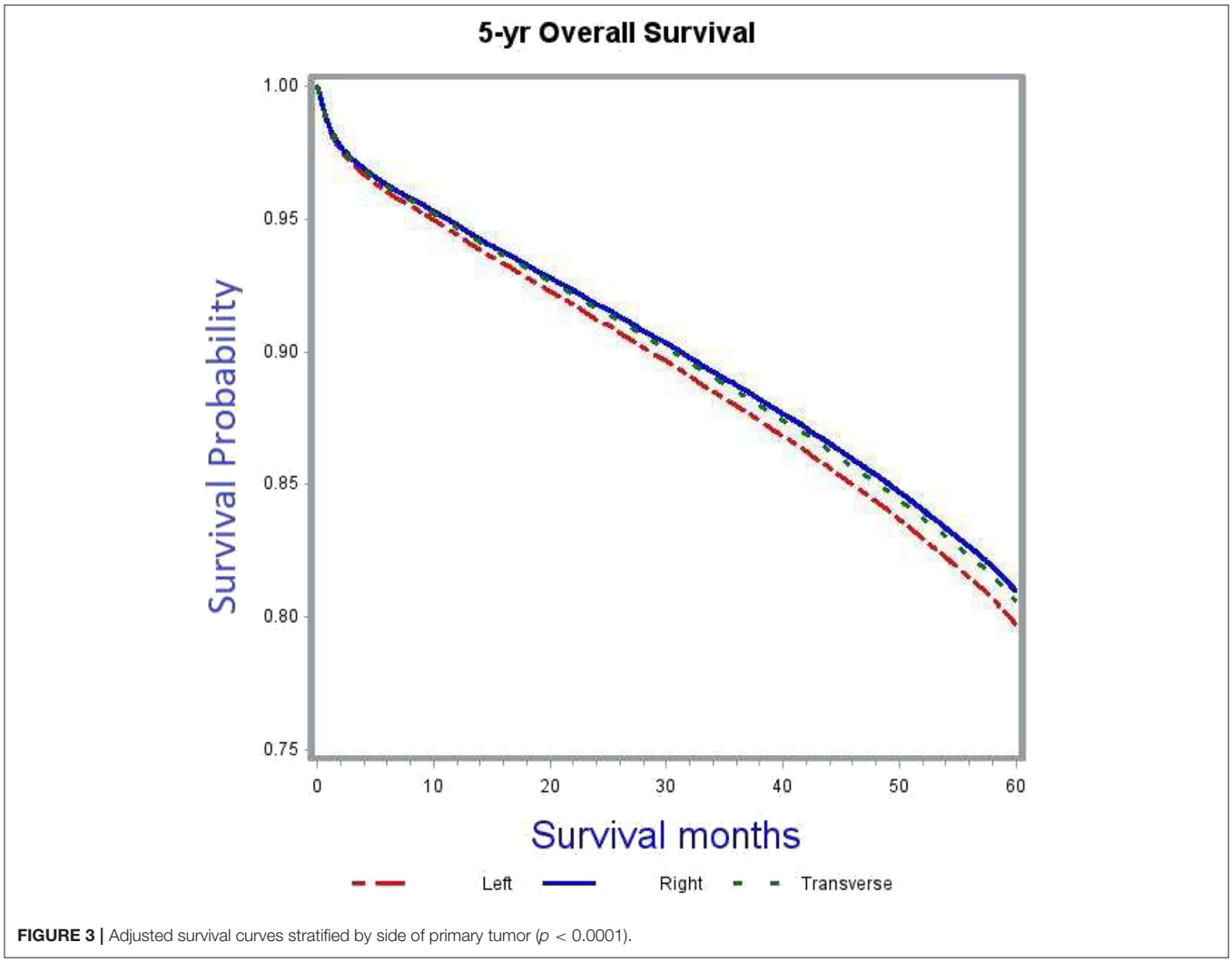

FIGURE 3 | Adjusted survival curves stratified by side of primary tumor ($p < 0.0001$).

DISCUSSION

In stage II colon cancer, surgical resection is the mainstay of treatment with a wide 5-years OS range which highlights the heterogeneity that exists among stage II colon cancers in term of recurrence. The survival benefit of adjuvant chemotherapy in stage III patients is well-established but a definitive benefit in stage II patients remains unclear (8–16, 22–24, 28–30). Some of these studies have demonstrated disease specific and/or overall survival benefit of adjuvant chemotherapy in stage II colon cancers with high-risk features. Current clinical practice guidelines and the consensus statement from the recent Cochrane review recommend discussion of adjuvant chemotherapy with stage II colon cancer patients having high risk features (19, 20, 31). As a result, there is a wide variation regarding the decision to administer adjuvant chemotherapy among individual physicians, institutions, and countries.

The current study was undertaken to provide a better assessment of the OS benefit of adjuvant chemotherapy in stage II colon cancer with regard to various high-risk features including pT4, inadequate lymph node evaluation, high grade tumors and those with positive surgical margins, along with the other important prognostic factor of tumor sidedness (which was considered as a surrogate for MSI status) (32, 33). We found that after controlling for various patient, tumor, and treatment characteristics, adjuvant chemotherapy had a significant OS benefit in stage II colon cancer. This is consistent with the findings of earlier studies including the QUASAR trial (12) and a retrospective analysis by Casadaban et al. (24). Single-agent chemotherapy fared as well as multi-agent chemotherapy when compared to no adjuvant chemotherapy (**Figure 2**). Though the overall use of adjuvant chemotherapy decreased with advancing age; the elderly and very elderly patients were more likely to receive single-agent chemotherapy (**Table 2**) compared to young patients. Despite this difference, the outcomes favored adjuvant chemotherapy, which is in line with findings from the ACCENT database and the MOSAIC trial (9, 34).

In the multivariate Cox proportional hazards model analysis, patients with right sided tumors had better OS compared to left sided tumors. A previous retrospective analysis by Weiss et al. (30) using the Surveillance, Epidemiology and End Results (SEER)-Medicare data showed no OS benefit of adjuvant

TABLE 5 | Multivariate subgroup analysis of 5-years OS, according to adjuvant chemotherapy and tumor sidedness.

	5-years survival, %	HR	95% CI	*P*
Adjuvant chemotherapy (*n* = 8,985)				
Left sided	73.4	Ref = 1		
Right sided	73.5	1.04	0.94–1.15	0.4623
Transverse	74.1	0.96	0.83–1.13	0.6022
No adjuvant chemotherapy (*n* = 33,986)				
Left sided	54	Ref=1		
Right sided	54.6	0.92	0.88–0.96	<0.0001
Transverse	53.8	0.95	0.9–1.00	0.0677
Left sided (*n* = 15,107)				
No chemotherapy	54	Ref = 1		
Chemotherapy	73.4	0.58	0.53–0.62	<0.0001
Right sided (*n* = 23,151)				
No chemotherapy	54.6	Ref = 1		
Chemotherapy	73.5	0.6	0.56–0.65	<0.0001
Transverse (*n*= 4,713)				
No chemotherapy	53.8	Ref = 1		
Chemotherapy	74.1	0.55	0.48–0.64	<0.0001

chemotherapy for either right- or left-sided tumors. However, this study included only Medicare beneficiaries aged 66 years and older. To further delineate into the survival interactions between tumor sidedness and adjuvant chemotherapy in our study cohort, a set of subgroup analyses was carried out. In the subgroup not receiving any adjuvant chemotherapy right sided tumors had better OS outcomes compared to left sided tumors (adjusted HR 0.92, 95% CI 0.88–0.96). This difference was compensated for in the subgroup receiving adjuvant chemotherapy (**Table 5**). Right-sided tumors clinically correlate for MSI-H status and based on available evidence of MSI-H tumors not responding to 5-FU based adjuvant chemotherapy (21), it could be argued that administration of chemotherapy in these tumors could have resulted in worsening of survival outcomes thereby nullifying the survival difference between right- and left-sided tumors. This was further put to test through additional subgroup analyses of left-sided, right-sided, and transverse colon only cancers. In these multivariate Cox proportional regression analyses, administration of chemotherapy resulted in significant OS benefit for all tumor locations (adjusted HR and 95% CI: left-sided 0.58, 0.53–0.62; right-sided 0.6, 0.56–0.65; transverse 0.55. 0.48–0.64). These results support the benefit of adjuvant chemotherapy irrespective of tumor location.

High-risk features in stage II colon cancer traditionally included pT4, tumor perforation or bowel obstruction, high grade or poorly differentiated tumors, lympho-vascular invasion and <12 lymph nodes examined. According to the current guidelines, it is recommended to discuss adjuvant chemotherapy with patients having any one or combination of these risk factors (19, 20). Using the current study cohort, we evaluated the odds of adjuvant chemotherapy administration, from 2004 to 2009, based on some of these high-risk features that were available through NDCB. These included pT4, high-grade tumors and examination of <12 lymph nodes. As depicted in **Table 3**, patients with pT4 lesions were more likely to receive chemotherapy than those with pT3 lesions. Verhoeff et al. (23) analyzed data from the Netherlands Cancer Registry and found improved survival outcomes with adjuvant chemotherapy in patients with pT4 stage II colon cancer. Tumors with undifferentiated histology or higher-grade were more likely to receive adjuvant chemotherapy. This is in contrary to the idea of poorly differentiated tumors being clinically correlated to MSI-H status, which portends a poor response to 5-FU based chemotherapy (21, 35). Inadequate lymph node evaluation was shown to carry a poor prognostic effect on OS in a recent study by Reha et al. (36) using the NCDB. On the same note, patients with inadequate lymph node evaluation in our study cohort were more likely to receive adjuvant chemotherapy to help improve the odds of OS attributable to disease recurrence.

There are several limitations to this study. First, this is a non-randomized, retrospective analysis that allows for a potential selection bias. Second, information on the type of chemotherapy regimen, adherence and completion rates were not available in the NCDB. This creates a heterogeneous population in which patients could receive substandard duration of therapy. Lack of information on the type of chemotherapy regimen was partially compensated by the availability of data on single vs. multi-agent chemotherapy. Analysis of certain data variables was restricted by availability in the NCDB file, including MSI status, disease specific mortality, colonic obstruction or perforation and missing information from the lympho-vascular invasion data collection.

Despite these limitations, the current study is the largest population-based analysis of stage II colon cancer only patients in the U.S. Though the previous study by Casadaban et al. (24) included nearly 4-times the number of patients in our study, their cohort included stage II colon cancer patients with other malignancies, who were excluded from our study cohort. Our study population included adult patients belonging to all age groups as compared to the SEER-Medicare study by Weiss et al. (30) that included patients aged 66 years and older.

CONCLUSION

The results of our study suggest the use of adjuvant chemotherapy in stage II colon cancer. There was a statistically significant 5-years OS benefit seen after adjusting for available patient, tumor and treatment characteristics including high-risk features as well as tumor location. Subgroup analysis further confirmed the survival benefit associated with adjuvant chemotherapy irrespective of tumor sidedness. However, owing to the observational nature of this study, interpretation and clinical application should be undertaken with caution. Future validation with prospective trials including MSI status is warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

DISCLOSURE

This study was presented in poster format at the ASCO Gastrointestinal Cancers Symposium; January, 2017; San Francisco, California.

AUTHOR CONTRIBUTIONS

RR and SM had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, acquisition, analysis, or interpretation of data, and administrative, technical, or material support. SM and DH drafting of the manuscript. PS and RR critical revision of the manuscript for important intellectual content. SM statistical analysis. RR study supervision. All authors study concept and design.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.568417/full#supplementary-material

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The Role of Tumor Deposits in Predicting the Efficacy of Chemotherapy in Stage III Colon Cancer

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Purpose: To evaluate the role of tumor deposits (TDs) in predicting the efficacy of chemotherapy in stage III colon cancer.

Methods: Using the SEER*Stat software Version 8.3.6, we started with a national cohort of colon cancer cases diagnosed between 2004 and 2016. We used the χ^2 (Chi-square) test to compare differences between different categorical variables according to the number of TDs. The Cox proportional hazards regression model was used to determine the independent association of different clinical and pathological variables with CSS, which were adjusted for other significant prognostic factors.

Results: We have identified 29,017 patients diagnosed with stage III colon cancer from the SEER database. The results of multivariate analyses showed that patients with the receipt of chemotherapy had 54.7% decreased risk of cancer-specific mortality compared with those not (HR = 0.453, 95% CI = 0.425–0.483, $P < 0.0001$) in the no-TD group; In the 1–2-TD group, patients with the receipt of chemotherapy had 56.8% decreased risk of cancer-specific mortality compared with those not (HR = 0.432, 95% CI = 0.364–0.512, $P < 0.0001$); In the ≥3-TD group, patients with the receipt of chemotherapy had 51.8% decreased risk of cancer-specific mortality compared with those not (HR = 0.482, 95% CI = 0.389–0.597, $P < 0.0001$).

Conclusions: Our study demonstrated that the presence of TDs was associated with a dismal prognosis and high number of TDs would also contribute to the worse survival of colon cancer. High number of TDs did not affect the survival benefit of chemotherapy in stage III colon cancer.

Keywords: tumor deposits, stage III, colon cancer, chemotherapy, survival

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INTRODUCTION

Colon cancer is one of the most malignant tumors and occupies the fifth leading cause of cancer deaths worldwide (1). It is reported that more than one-third of colon cancer patients would present with lymph node metastases, that is, stage III colon cancer. Stage III colon cancer is considered to be an aggressive disease and has a clinically significant risk of distant metastasis after resection (2). 5-Fluorouracil (5-FU)-based chemotherapy regimens are commonly used in stage III colon cancer followed by surgical resection of the primary tumor, and it is known that 50% of the stage III colon

cancer patients are cured by surgery alone, 20% with addition of adjuvant chemotherapy; however, 30% of those patients would experience recurrence, which is generally fatal within 2–3 years (3–6). Therefore, it is necessary to predict the efficacy of stage III colon cancer chemotherapy.

In 1935, tumor deposit (TD) was firstly reported in some node-negative colorectal cancer patients after meticulous pathological dissection of colorectal cancer specimens, and the researchers believed that these non-lymphatic metastases were the result of vascular spread (7). Over the years, the understanding of TDs was constantly changing, and the definition of TDs (also known as N1c) in colorectal cancer had been revised in the 8^{th} edition of the American Joint Committee on Cancer (AJCC) staging system. Tumor deposit was a discrete nodule of cancer in pericolic/perirectal fat or adjacent mesentery, without histological evidence of residual lymph node or identifiable vascular or neural structures (7, 8).

Tumor deposits had been considered as an indicator of poor prognosis in colorectal cancer (9–11). What is more, it was reported that patients with both TDs and lymph node metastasis would have a worse prognosis than patients with either alone (12, 13). However, no previous studies had evaluated the role of TDs in predicting the efficacy of chemotherapy in stage III colon cancer.

MATERIALS AND METHODS

Data Source and Study Population

Sponsored by the National Cancer Institute (NCI), the Surveillance, Epidemiology and End Results (SEER) database is representative of the United States population and consists of population-based cancer registries that cover approximately 28% of the United States population with patient-level data abstracted from 18 geographically diverse populations that represent rural, urban, and regional populations and is freely available for cancer-based epidemiology investigation and survival analysis (14). Using the SEER*Stat software Version 8.3.6, we started with a national cohort of 298637 colon cancer cases diagnosed between 2004 and 2016 (**Figure 1**). Of these, we excluded patients without the exact number of TDs because we want to evaluate the clinical role of TDs in colon cancer. In addition, we also excluded some colon cancer patients with the following exclusion criteria: (1) unknown TNM stage, (2) non-adenocarcinoma histological type, (3) node-negative, (4) number of nodes examined was unknown, (5) without surgical treatment, and (6) unknown race and with distant metastases. Finally, only qualified patients diagnosed with stage III colon cancer were included in this study, and all the patients were divided into three groups: no TDs (N = 24740) vs. 1–2 TDs (N = 3103) vs. ≥3 TDs (N = 1174). We then ascertained variables of interest from the SEER database, including T stage (T1 stage, T2 stage, T3 stage, or T4 stage), N stage (N1 stage or N2 stage), age at diagnosis (years), race (white race, black race, or other race), gender (male or female), tumor grade (well/moderate tumor grade, poor/anaplastic tumor grade or unknown tumor grade), histological type (adenocarcinoma or mucinous/signet-ring cell carcinoma), the receipt of chemotherapy (no/unknown or yes), and the number of TDs (no TDs, 1–2 TDs, or ≥3 TDs).

Statistical Analysis

We used the χ^2 (Chi-square) test to compare differences between different categorical variables according to the number of TDs. Cancer-specific survival (CSS) was used as the outcome of interest, which was calculated from the date of diagnosis to the date of colon cancer death. The Cox proportional hazards regression model was used to determine the independent association of different clinical and pathological variables with CSS, which were adjusted for other significant prognostic factors. Moreover, only variables considered significant ($P < 0.20$) in univariable analyses would be incorporated into multivariate Cox models. An accumulated risk curve was also constructed by the Kaplan–Meier method to compared CSS of colon cancer patients according to the number of TDs. Significant differences in survival were tested with the log-rank tests. The 95% confidence intervals (CI) for hazard ratios (HRs) were generated and reported. All calculations were performed with SPSS 22.0 (Chicago, IL, United States), and all P values were two-sided and would be considered of statistical significance when P values were less than 0.05.

RESULTS

Baseline Patient Characteristics

We have identified 29,017 patients diagnosed with stage III colon cancer with the known number of TDs from the SEER database, and all the patients were divided into three groups, including no TDs (N = 24740), 1–2 TDs (N = 3103), and ≥3 TDs (N = 1174). There were 12,581 (43.4%) patients with ≤65 years and 16,436 (56.6) with >65 years. The mean age of the study population was 67.41 years, and the median age was 68 years. Among the study population, 14,132 (48.7%) patients were males and 14,874 (51.3%) patients were females; 1412 (4.9%) patients were T1 stage, 2679 (4.9%) patients were T2 stage, 18,725 (9.2%) patients were T3 stage, and 6201 (64.5%) patients were T4 (21.4) stage; and 20,120 (69.3%) patients were N1 stage, 8897 (30.7%) patients were N2 stage.

The detailed clinicopathological characteristics of patients based on the number of TDs were summarized in **Table 1**. It was found that a higher number of TDs preferred to be associated with higher T stage ($P < 0.001$), higher N stage ($P < 0.001$), higher tumor grade ($P < 0.001$), and mucinous/signet-ring cell carcinoma ($P = 0.001$), showing that the presence of TDs preferred to be associated with aggressive features of pathology. However, age at diagnosis ($P = 0.054$), race ($P = 0.249$), gender ($P = 0.197$), and the receipt of chemotherapy ($P = 0.106$) between different TD groups did not achieve statistical significance.

The Prognosis of TDs in Stage III Colon Cancer

Kaplan–Meier curves of CSS according to the number of TDs are shown in **Figure 2**. It was found that the presence of TDs

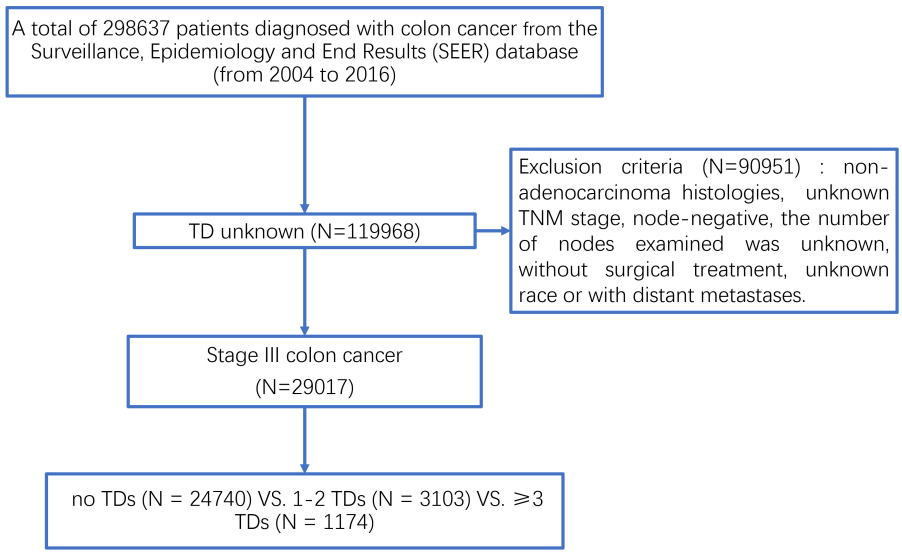

FIGURE 1 | The selection process of patients diagnosed with stage III colon cancer from the SEER database.

TABLE 1 | Demographic and clinicopathological characteristics of stage III colon cancer patients.

Groups	Number of Patients (%)			*P*
	No TDs	1–2 TDs	≥3 TDs	
T stage				<0.001
T1	1340 (5.4)	63 (2.0)	9 (0.8)	
T2	2465 (10.0)	181 (5.8)	33 (2.8)	
T3	16054 (64.9)	1998 (64.4)	673 (57.3)	
T4	4881 (19.7)	861 (27.7)	459 (39.1)	
N stage				<0.001
N1	17268 (69.8)	2234 (72.0)	618 (52.6)	
N2	7472 (30.2)	869 (28.0)	556 (47.4)	
Age at diagnosis (years)				0.054
≤65	10747 (43.4)	1297 (41.8)	537 (45.7)	
>65	13993 (56.6)	1806 (58.2)	637 (54.3)	
Race				
White	19139 (77.4)	2398 (77.3)	912 (77.7)	0.249
Black	3218 (13.0)	410 (13.2)	132 (11.2)	
Other	2383 (9.6)	295 (9.5)	130 (11.1)	
Gender				0.197
Male	12008 (48.5)	1539 (49.6)	596 (50.8)	
Female	12723 (51.5)	1564 (50.4)	578 (49.2)	
Grade				<0.001
Well/moderate	18039 (72.9)	2299 (74.1)	753 (64.1)	
Poor/anaplastic	6292 (25.4)	757 (24.4)	408 (34.8)	
Unknown	409 (1.7)	47 (1.5)	13 (1.1)	
Histology				0.001
Adenocarcinoma	22162 (89.6)	2818 (90.8)	1020 (86.9)	
Mucinous/signet-ring cell carcinoma	2578 (10.4)	285 (9.2)	154 (13.1)	
Chemotherapy				0.106
No/unknown	9682 (39.1)	1241 (40.0)	428 (36.5)	
Yes	15058 (60.9)	1862 (60.0)	746 (63.5)	

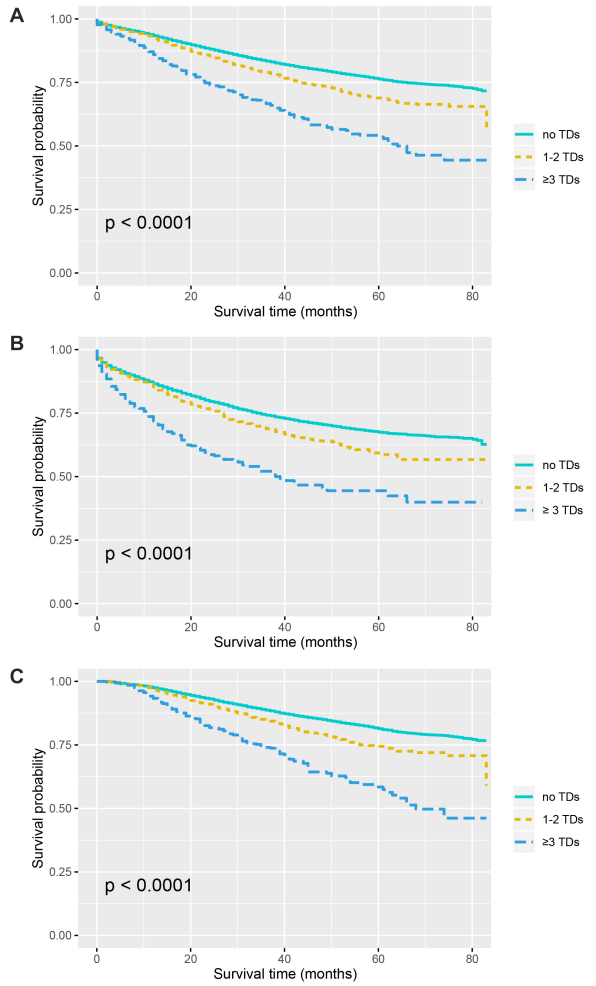

FIGURE 2 | Survival curves for patients stratified by the number of TDs in panel **(A)** all the patients. **(B)** Patients without chemotherapy. **(C)** Patients treated with chemotherapy.

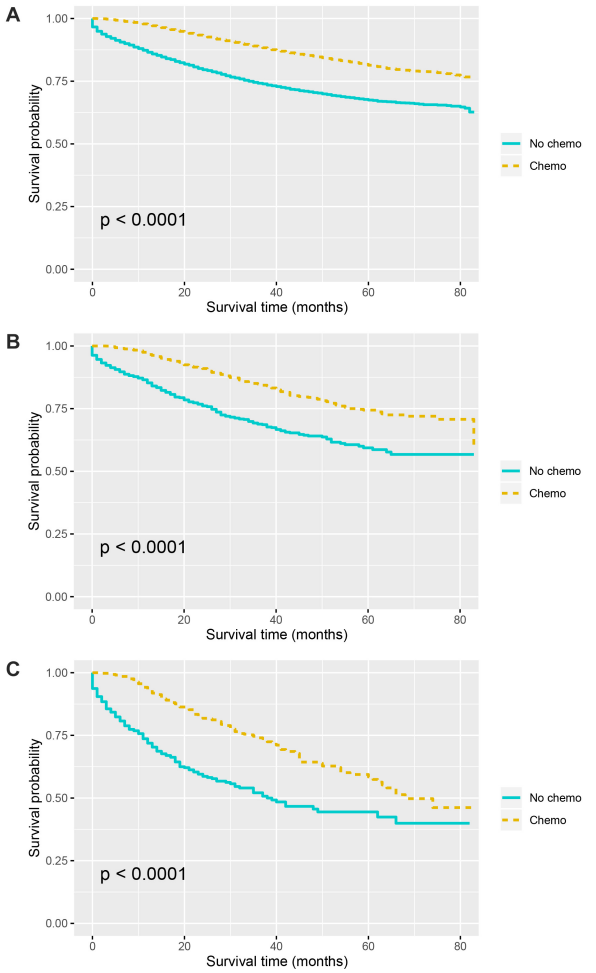

FIGURE 3 | Survival curves for patients stratified by the receipt of chemotherapy or not in colon cancer patients with **(A)** No TDs. **(B)** 1–2 TDs. **(C)** ≥3 TDs.

would reduce CSS of colon cancer patients: in all the stage III colon cancer patients, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 76.3, 68.9, and 53.6%, respectively ($P < 0.0001$, **Figure 2A**); in the stage III colon cancer patients without the receipt of chemotherapy, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 67.5, 59.4, and 44.4%, respectively ($P < 0.0001$, **Figure 2B**); in the stage III colon cancer patients with the receipt of chemotherapy, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 81.4, 74.4, and 58.4%, respectively ($P < 0.0001$, **Figure 2C**).

The Role of Tumor Deposits in Predicting the Efficacy of Chemotherapy in Stage III Colon Cancer

Kaplan–Meier curves of CSS according to the number of TDs are shown in **Figure 3**. It was found that the receipt of chemotherapy would significantly improve CSS of stage III colon cancer patients: in the no-TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 67.5 and 81.4%, respectively ($P < 0.0001$, **Figure 3A**); in the 1–2-TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 59.4 and 74.4%, respectively ($P < 0.0001$, **Figure 3B**); in the ≥3-TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 44.4 and 58.4%, respectively ($P < 0.0001$, **Figure 3C**). In addition, the Cox proportional hazards regression model was also used to verify the above findings. In the no-TD group, the univariate Cox analysis produced eight variables, which were then incorporated into multivariate Cox models, including T stage, N stage, age at diagnosis, race, gender, tumor grade, histological type, and the receipt of chemotherapy. What is more, the results of multivariate analyses showed that patients with the receipt of chemotherapy had 54.7% decreased risk of cancer-specific mortality compared with those not (HR = 0.453, 95% CI = 0.425–0.483, $P < 0.0001$; **Table 2**) in the no-TD group. In the 1–2-TD group, univariate Cox analysis produced seven variables, which were then incorporated into multivariate Cox

TABLE 2 | HRs of different demographic and clinicopathological characteristics in stage III colon cancer with no tumor deposits.

Groups	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T1			1	
T2			1.654 (1.222–2.238)	0.001
T3			3.380 (2.585–2.238)	<0.001
T4			7.306 (5.572–9.579)	<0.001
N stage		<0.001		<0.001
N1			1	
N2			1.807 (1.698–1.924)	
Age at diagnosis (years)		<0.001		<0.001
≤65			1	
>65			1.439 (1.345–1.540)	
Race		0.027		<0.001
White			1	
Black			1.257 (1.150–1.374)	<0.001
Other			0.933 (0.839–1.037)	0.199
Gender		0.018		0.974
Male			1	
Female			0.999 (0.940–1.062)	
Grade		<0.001		<0.001
Well/Moderate			1	
Poor/Anaplastic			1.369 (1.282–1.461)	<0.001
Unknown			1.328 (1.058–1.665)	0.014
Histology		<0.001		0.055
Adenocarcinoma			1	
Mucinous/signet-ring cell carcinoma			1.093 (0.998–1.198)	
Chemotherapy		<0.001		<0.001
No/unknown			1	
Yes			0.453 (0.425–0.483)	

models, including T stage, N stage, age at diagnosis, race, gender, tumor grade, and receipt of chemotherapy. What is more, the results of multivariate analyses showed that patients with receipt of chemotherapy had 56.8% decreased risk of cancer-specific mortality compared with those not (HR = 0.432, 95% CI = 0.364–0.512, $P < 0.0001$; **Table 3**) in the 1–2-TD group. In the ≥3-TD group, the univariate Cox analysis produced six variables, which were then incorporated into multivariate Cox models, including T stage, N stage, age at diagnosis, tumor grade, histology and the receipt of chemotherapy. What is more, the results of multivariate analyses showed that patients with the receipt of chemotherapy had 51.8% decreased risk of cancer-specific mortality compared with those not (HR = 0.482, 95% CI = 0.389–0.597, $P < 0.0001$; **Table 4**) in the ≥3-TD group.

DISCUSSION

In 1935, TD was firstly reported in some node-negative colorectal cancer patients after meticulous pathological dissection of colorectal cancer specimens, and in 1997, TD was first introduced into the AJCC TNM staging system (7). In our study, a total of 4277 (14.7%) stage III colon cancer patients were diagnosed with TDs, and the proportion was a little lower than in the previous study (15). It was reported by some researches that, some variables, such as T4 and N2, which were definitively associated with worse survival expectations, were found to occur more frequently in stage III colon cancer with TDs (9–11). In our analyses, it was found that a higher number of TDs preferred to be associated with higher T stage, higher N stage, higher tumor grade, and mucinous/signet-ring cell carcinoma, indicating that the presence of TDs preferred to be associated with aggressive features of pathology, which we believed will provide further understanding of the nature and origin of TDs.

The most novel finding of a study from Canada had shown that a high number of TDs (≥3) were indicative of worse prognosis than 1 to 2 TDs; therefore, all patients diagnosed with TDs in the present study were divided into two subgroups, including the 1–2-TD and ≥3-TD groups. In addition, the present study also investigated the prognostic value of TDs in different TD subgroups. Recently, a retrospective analysis had found that the survival difference between N1b and N1c did not achieve statistical difference; what is more, N1c was associated with worse prognosis compared to N1a (16). Moreover, the results of survival analyses in this study showed that the presence of TDs would reduce CSS of colon cancer patients: in all the

TABLE 3 | HRs of different demographic and clinicopathological characteristics in stage III colon cancer with 1–2 tumor deposits.

Groups	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T1			1	
T2			0.863 (0.364–2.045)	0.738
T3			1.361 (0.642–2.883)	0.421
T4			2.859 (1.343–6.083)	0.006
N stage		<0.001		<0.001
N1			1	
N2			1.798 (1.520–2.127)	
Age at diagnosis (years)		<0.001		<0.001
≤65			1	
>65			1.517 (1.266–1.819)	
Race		0.105		0.002
White			1	
Black			1.468 (1.171–1.841)	0.001
Other			0.911 (0.680–1.221)	0.534
Gender		0.079		0.435
Male			1	
Female			1.066 (0.907–1.253)	
Grade		<0.001		<0.001
Well/moderate			1	
Poor/anaplastic			1.429 (1.200–1.701)	<0.001
Unknown			0.462 (0.191–1.118)	0.087
Histology		0.589		
Adenocarcinoma				
Mucinous/signet-ring cell carcinoma				
Chemotherapy		<0.001		<0.001
No/unknown			1	
Yes			0.432 (0.364–0.512)	

stage III colon cancer patients, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 76.3, 68.9, and 53.6%, respectively ($P < 0.0001$); in the stage III colon cancer patients without the receipt of chemotherapy, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 67.5, 59.4, and 44.4%, respectively ($P < 0.0001$); in the stage III colon cancer patients with the receipt of chemotherapy, the 5-year CSS rates of no TDs, 1–2 TDs, and ≥3 TDs were 81.4, 74.4, and 58.4%, respectively ($P < 0.0001$). The subgroup analyses in our study indicated that the presence of TDs was associated with a dismal prognosis and a high number of TDs would also contribute to worse survival, which was in agreement with previous findings that some previous studies had demonstrated that TD was an adverse prognostic factor in colon cancer (15, 17, 18). Moreover, in 2018, it was reported by a recent study that the presence of TDs had 220% increased risk of developing disease recurrence (2).

It should also be noted that the origins of TDs were diverse. By serial sectioning in a series of 30 irregular TDs from 418 stage III colon adenocarcinomas patients, it was found that almost 40% of all the TDs showed a combined perineural, perivascular, and intravascular origin. In addition, a perineural origin was present in 77% of cases and an intravascular origin in 83% of cases (19). In 2010, Wünsch and his colleagues conducted a study with 69 TDs and it also showed the similar diversity to the above finding (20). The worse prognosis of patients diagnosed with TDs was partly explained by the presence of vessels and nerves in TDs, because it would add more anatomic highways for the metastases and spread of tumor cells (21).

In the Kaplan–Meier CSS analyses according to the number of TDs in the current study, it was found that the receipt of chemotherapy would significantly improve CSS of stage III colon cancer patients: in the no-TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 67.5 and 81.4%, respectively ($P < 0.0001$); in the 1–2-TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 59.4 and 74.4%, respectively ($P < 0.0001$); in the ≥3 TD group, the 5-year CSS rates of patients without and with the receipt of chemotherapy were 44.4 and 58.4%, respectively ($P < 0.0001$). The efficacy of adjuvant chemotherapy in stage III colon cancer had been widely recognized in previous studies, and it was also confirmed in different TD subgroups of our study (22, 23).

What is more, the main significance of this study was to evaluate the survival benefit difference in stage III colon cancer

TABLE 4 | HRs of different demographic and clinicopathological characteristics in stage III colon cancer with ≥3 tumor deposits.

Groups	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T1			1	
T2			0.367 (0.023–5.903)	<0.001
T3			2.801 (0.390–20.123)	0.479
T4			4.341 (0.603–31.238)	0.306
N stage		<0.001		0.145
N1			1	
N2			1.848 (1.484–2.301)	
Age at diagnosis (years)		<0.001		<0.001
≤65			1	
>65			1.662 (1.332–2.074)	
Race		0.453		
White				
Black				
Other				
Gender		0.325		
Male				
Female				
Grade		<0.001		0.002
Well/moderate			1	
Poor/anaplastic			1.474 (1.179–1.843)	0.001
Unknown			1.981 (0.906–4.331)	0.087
Histology		0.018		0.511
Adenocarcinoma			1	
Mucinous/signet-ring cell carcinoma			1.105 (0.820–1.488)	
Chemotherapy		<0.001		<0.001
No/Unknown			1	
Yes			0.482 (0.389–0.597)	

with high number of TDs. Moreover, we have found that, in the no TD group, the Cox proportional hazards regression analyses showed that patients with the receipt of chemotherapy had 54.7% decreased risk of cancer-specific mortality compared with those not. In the 1–2-TD group, patients with the receipt of chemotherapy had 56.8% decreased risk of cancer-specific mortality compared with those not. In the ≥3-TD group, similarly, the results of multivariate analyses showed that patients with the receipt of chemotherapy had 51.8% decreased risk of cancer-specific mortality compared with those not.

Tumor deposits have become a hotspot in colon cancer study during recent years, and it was demonstrated that a high number of TDs was indicative of worse prognosis (21, 24). In 2017, Nagtegaal et al. (21) proposed that TDs and their actual number were equal to the number of lymph node metastases in making treatment decisions and the number of TDs should be fully included in the TNM staging. To our knowledge, however, few studies investigated the efficacy of survival benefit in stage III colon cancer with high number of TDs. In the present study, we demonstrated that, for the first time, the efficacy of chemotherapy was similar in different TD subgroups and a high number of TDs did not affect the survival benefit of chemotherapy in stage III colon cancer.

We also need to address the shortcomings in the current study. The first was the inherent weakness SEER data set, such as the lack of some detailed clinicopathological characteristics, which might introduce some selection biases in our study. The other limitation in our work was that our study was a retrospective one. In addition, patients without the information of TDs of some patients are excluded from our analyses, which might cause the selection bias. More researches, especially large prospective ones, were needed in the future to generalize the results.

In summary, it was found that the presence of TDs was associated with a dismal prognosis and a high number of TDs would also contribute to the worse survival of colon cancer. Moreover, we demonstrated that, for the first time, the efficacy of chemotherapy was similar in different TD subgroups and high number of TDs did not affect the survival benefit of chemotherapy in stage III colon cancer.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/.

AUTHOR CONTRIBUTIONS

YR was responsible for the conception and design of this study. MS, JW, CZ, and XZ performed the study selection, data extraction, and statistical analyses. MS, HZ, and GY performed the literature search and wrote the first draft of the manuscript. MS, HZ, and YR revised and edited the final version of manuscript. All authors reviewed and approved the submitted version of the manuscript.

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Is Radical Surgery Alone Enough in T1-3N1a Colon Cancer?

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Background: Low lymphatic tumor burden is associated with a better prognosis. However, it is uncertain whether those patients diagnosed as cN0 found to be pN+ could be a favorable subgroup in stage III disease. Radical surgery alone might avoid overtreatment in those patients.

Methods: Eligible patients diagnosed with colon cancer without metastasis were recruited from the Surveillance, Epidemiology, and End Results (SEER) database from 2004 to 2016 using SEER*Stat 8.3.5 software (Surveillance Research Program, National Cancer Institute) and divided into two groups: surgery group (n = 3,081) and surgery followed by adjuvant chemotherapy group (n = 4,591). Overall survival (OS) and cause-specific survival (CSS) differences were assessed by Kaplan–Meier analysis, and survival differences were estimated with log-rank tests. Univariate and multivariate Cox proportional hazard regressions were used to assess hazard ratios (HRs) and 95% confidence intervals (CIs) for colon cancer patients.

Results: A total of 7,672 pT1-3N1a colon cancer patients were recruited from 208,751 colon cancer patients. The 5-year CSS rates of patients without and with adjuvant chemotherapy were 80.0 and 90.7%, respectively. The receipt of adjuvant chemotherapy after the radical resection of the primary tumor was independently associated with 57.3% decreased risk of colon cancer-specific mortality compared with surgery alone (HR = 0.427, 95% CI = 0.370–0.492, $P < 0.001$, using surgery alone as the reference).

Conclusions: Adjuvant chemotherapy was significantly associated with improved prognosis and radical surgery alone did not provide enough treatment for colon cancer with very low lymphatic tumor burden.

Keywords: radical surgery, adjuvant chemotherapy, colon cancer, lymph node, burden

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BACKGROUND

Colorectal cancer (CRC) is one of the most common cancers and among the leading causes of cancer-related mortality worldwide (1–3). Lymph node status is the most important prognostic factor in non-metastatic colon cancer (4–6). It has been estimated that the sensitivity of nodal involvement in colon cancer with preoperative CT was 71% (95% CI, 59–81%), which indicates that ∼30% of lymph node involvement is missed due to the normal size (caused by very low lymphatic tumor burden) and because it is only revealed in the pathological report after surgery (7). Previous research has suggested that T4N0 colon cancer

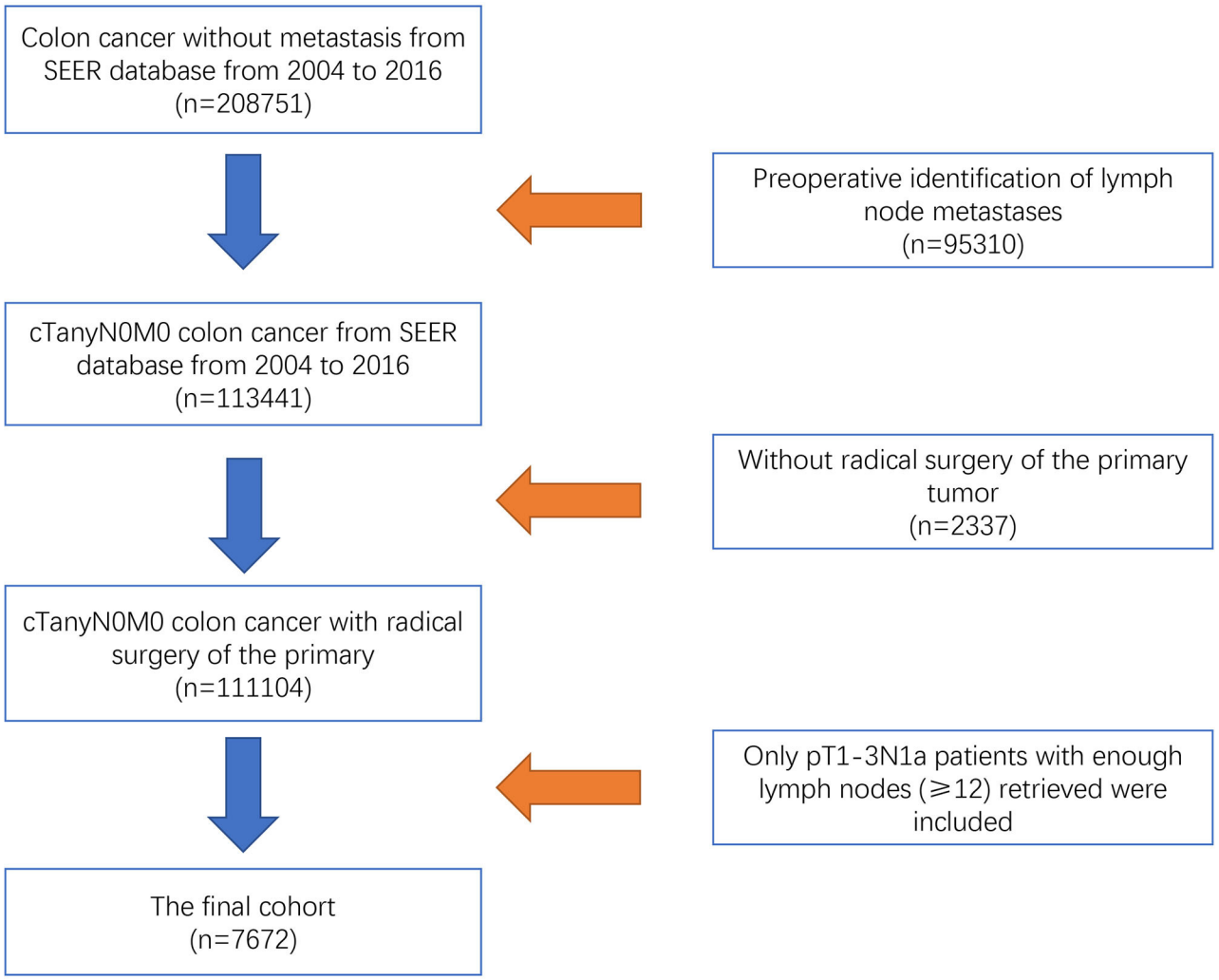

FIGURE 1 | Flow chart of the selection process for pT1-3N1a colon cancer patients from the SEER database.

patients have inferior 5-year overall survival (5-OS) compared with T1-2N1 patients (8–10). A low lymphatic tumor burden could be associated with a better prognosis compared to the higher T stage without lymph node metastasis. However, it remains uncertain whether those with clinically node-negative colon cancer and pathologically diagnosed node involvement could be a favorable subgroup in stage III disease. This study explores this, examining whether this subgroup of colon cancer patients could be treated with radical surgery alone to avoid overtreatment.

MATERIALS AND METHODS

Database and Study Population

As a public database with free access, the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI) covers ∼27.8% of cancer cases in the United States. Using SEER*Stat 8.3.5 software (Surveillance Research Program, National Cancer Institute), we collected data from patients who were diagnosed with colon cancer without metastasis from the SEER database from 2004 to 2016. Patients without complete information on the TNM stage or active follow-up were excluded from the study. We also excluded patients with preoperative identification of lymph node metastases (n = 95,310) or who did not receive radical surgery of the primary tumor (n = 2,337). The efficacy of adjuvant chemotherapy in T4 disease has been confirmed in recent studies (11–13). For example, Kumar et al. (12) found that the survival benefits of adjuvant chemotherapy were mainly observed in patients with T4 disease compared with other high-risk factors. This study aimed to investigate whether radical surgery alone was enough in colon cancer with a very low lymphatic tumor burden, the study subjects were focused on colon cancer with only one lymph node metastasis by postoperative pathologic results (pN1a), which was not confirmed by preoperative examination. Therefore, only pT1-3N1a colon cancer patients from whom enough lymph nodes (≥12) were retrieved are included in our analyses (n = 7,672, **Figure 1**). For the final cohort, we divided patients into

TABLE 1 | Clinical characteristics of two groups of patients included in the final study cohort.

Variables	Number (%)		*P*
	Surgery (*N* = 3081)	**Surgery and adjuvant chemotherapy (*N* = 4591)**	
T stage			0.001
T1	238 (7.7)	463 (10.1)	
T2	467 (15.2)	739 (16.1)	
T3	2,376 (77.1)	3,389 (73.8)	
Age (years)			<0.001
≤65	670 (21.7)	2,522 (54.9)	
>65	2,411 (78.3)	2,069 (45.1)	
Race			0.070
White	2,444 (79.3)	3,545 (77.2)	
Black	391 (12.7)	623 (13.6)	
Other	246 (8.0)	423 (9.2)	
Gender			0.024
Male	1,443 (46.8)	2,271 (49.5)	
Female	1,638 (53.2)	2,320 (50.5)	
Grade			0.007
Grade I/II	2,407 (78.1)	3,718 (81.0)	
Grade III/IV	626 (20.3)	801 (17.4)	
Unknown	48 (1.6)	72 (1.6)	
Histological type			0.149
Adenocarcinoma	2,806 (91.1)	4,224 (92.0)	
Mucinous adenocarcinoma/signet ring cell carcinoma	275 (8.9)	367 (8.0)	

two groups: surgery group (n = 3,081) and surgery followed by adjuvant chemotherapy group (n = 4,591). The continuous variables were transformed into categorical variables based on recognized cut-off values. The relevant variable definitions and information including T stage (including T1, T2, and T3), age (years), race/ethnicity (including white, black, and other), gender (including male and female), grade (including grade I/II, grade III/IV, and unknown), and histological type (including adenocarcinoma, and mucinous adenocarcinoma/signet ring cell carcinoma) were extracted from the SEER database.

Statistical Analysis

In our analyses, the Chi-square test was performed to compare categorical variables between patients in the surgery group and surgery and adjuvant chemotherapy group. The outcomes of interest included overall survival (OS) and cause-specific survival (CSS). OS and CSS differences were assessed by Kaplan–Meier analysis, and survival differences were estimated with log-rank tests. Univariate and multivariate Cox proportional hazard regressions were used to assess hazard ratios (HRs) and 95% confidence intervals (CIs) of patient characteristics for pT1-3N1a colon cancer patients. Only clinicopathologic characteristics that showed prognostic significance (log rank, $P < 0.20$) in univariate Cox analyses were entered in multivariate Cox analyses. For CSS, these prognostic factors included the receipt of chemotherapy, T stage, age, race/ethnicity, grade, and histological type; for OS, these prognostic factors included the receipt of chemotherapy, T stage, age, race/ethnicity, gender,

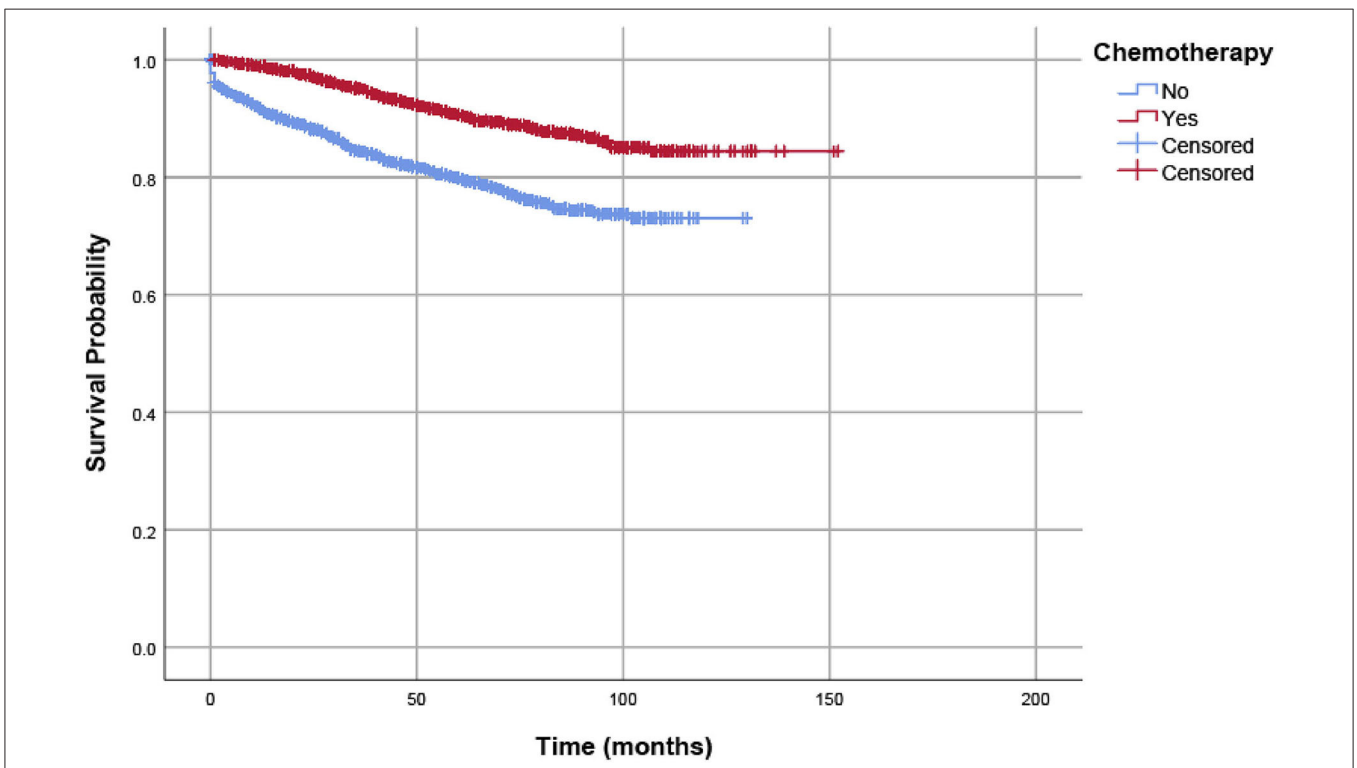

FIGURE 2 | CSSs of eligible patients were assessed according to the receipt of chemotherapy by Kaplan–Meier analysis, and survival differences were estimated with log-rank tests.

grade, and histological type. All tests were two sided, and two sided $P < 0.05$ was considered statistically significant in our analyses. All analyses were conducted using SPSS version 23 statistical software (IBM Corporation).

RESULTS

Patient Clinicopathological Characteristics

A total of 7,672 pT1-3N1a colon cancer patients who met with the strict inclusion criteria of our analyses, were recruited from 208,751 colon cancer patients included on the SEER database between 2004 and 2016, including 3,714 male (48.4%) and 3,958 female (51.5%) patients. Among them, 238 (7.7%) patients were T1 stage, 467 (15.2%) patients were T2 stage, and 2,376 (77.1%) patients were T3 stage. The mean age was 69 years. Clinicopathologic characteristics of the whole cohort regarding the receipt of chemotherapy, T stage, age, race/ethnicity, gender, grade, and histological type are listed in **Table 1**. The median follow-up durations were 42 months. The 3 and 5-year CSS rates in the SEER cohort were 91.0 and 86.6%, respectively. The 3 and 5-year OS rates in the SEER cohort were 79.2 and 69.9%, respectively.

As shown in **Table 1**, the T3 stage was less likely to receive adjuvant chemotherapy ($P = 0.001$); older patients were less likely to receive adjuvant chemotherapy ($P < 0.001$); male patients were more likely to receive adjuvant chemotherapy ($P = 0.024$).

The Survival Benefits of Adjuvant Chemotherapy in pT1-3N1a Colon Cancer Patients

In our analyses, OS and CSS were assessed by Kaplan–Meier analysis, and survival differences were estimated with log-rank tests. As shown in **Figure 2**, in pT1-3N1a colon cancer patients without preoperative identification of lymph node metastases, adjuvant chemotherapy was significantly associated with improved CSS. The 5-year CSS rates of patients without adjuvant chemotherapy and patients with adjuvant chemotherapy were 80.0 and 90.7%, respectively.

We used Cox proportional hazard regression analyses to evaluate potential risk factors and the efficacy of adjuvant chemotherapy. **Table 2** shows the results of univariate and multivariate Cox regression analyses for CSS in the whole cohort. Clinicopathologic characteristics that showed prognostic significance (log rank, $P < 0.20$) in univariate Cox analyses were entered in multivariate Cox analyses, including the receipt of adjuvant chemotherapy, T stage, age (years), race/ethnicity, grade, and histological type. In multivariate Cox analyses, higher T stage was associated with increased risk of colon cancer-specific mortality (HR = 1.570, 95% CI = 1.019–2.419, P =0.041 for T2 stage; HR = 3.320, 95% CI = 2.272–4.852, $P < 0.001$ for T3 stage; using T1 stage as the reference). Older patients were also associated with increased risk of colon cancer-specific mortality (HR = 1.381, 95% CI = 1.186–1.608, $P < 0.001$, using ≤65 years as the reference), and people of black ethnicity are also associated with an increased risk of colon cancer-specific mortality (HR = 1.451, 95% CI = 1.210–1.740, $P < 0.001$, using white ethnicity as the reference). More importantly, the receipt of adjuvant chemotherapy after the radical resection of the primary tumor was independently associated with 57.3% decreased risk of colon cancer-specific mortality compared with surgery alone (HR = 0.427, 95% CI = 0.370–0.492, $P < 0.001$, using surgery alone as the reference).

The study also used OS as the endpoint to evaluate the prognostic value of clinicopathologic features in the whole cohort. This also found that adjuvant chemotherapy was significantly associated with improved OS. The 5-year OS rates of patients without and with adjuvant chemotherapy were

TABLE 2 | Univariate and multivariate Cox regression analyses for CSS in the whole cohort.

Variable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Group		< 0.001		< 0.001
Surgery	1		1	
Surgery and adjuvant chemotherapy	0.386 (0.337–0.442)		0.427 (0.370–0.492)	
T stage		<0.001		< 0.001
T1	1		1	
T2	1.703 (1.107–2.621)		1.570 (1.019–2.419)	0.041
T3	3.620 (2.483–5.279)		3.320 (2.272–4.852)	<0.001
Age (years)		< 0.001		<0.001
≤65	1		1	
>65	1.845 (1.598–2.132)		1.381 (1.186–1.608)	
Race		<0.001		<0.001
White	1		1	
Black	1.343 (1.121–1.608)		1.451 (1.210–1.740)	<0.001
Other	0.767 (0.586–1.006)		0.817 (0.623–1.071)	0.144
Gender		0.729		
Male	1			
Female	0.977 (0.855–1.116)			
Grade		0.039		0.416
Grade I/II	1		1	
Grade III/IV	1.233 (1.048–1.451)		1.111 (0.943–1.309)	0.209
Unknown	0.942 (0.532–1.667)		1.159 (0.652–2.059)	0.615
Histological type		0.128		0.622
Adenocarcinoma	1		1	
Mucinous adenocarcinoma/ signet ring cell carcinoma	1.194 (0.950–1.501)		1.060 (0.841–1.335)	

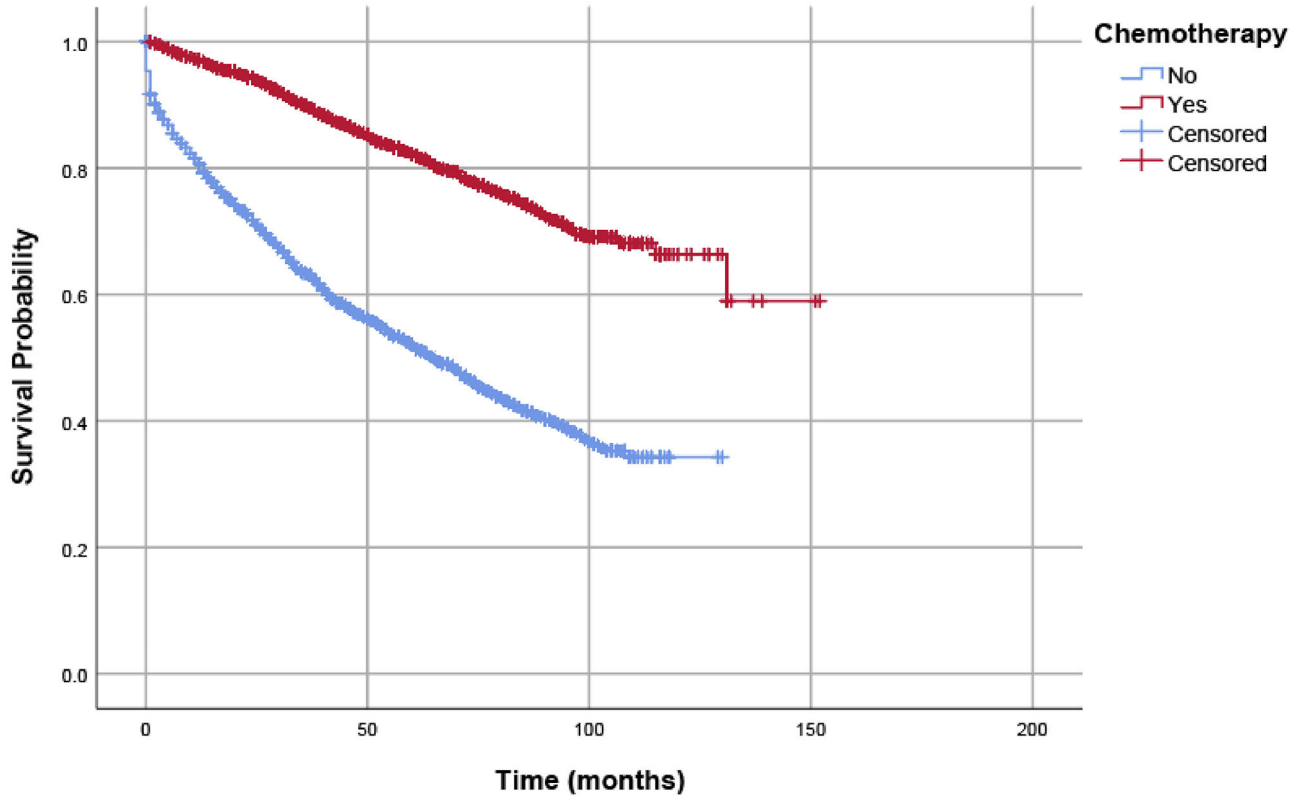

FIGURE 3 | OSs of eligible patients were assessed according to the receipt of chemotherapy by Kaplan–Meier analysis, and survival differences were estimated with log-rank tests.

52.1 and 82.1%, respectively (**Figure 3**). **Table 3** shows the results of univariate and multivariate Cox regression analyses for OS in the whole cohort. Clinicopathologic characteristics that showed prognostic significance (log rank, $P < 0.20$) in univariate Cox analyses were entered in multivariate Cox analyses, including the receipt of adjuvant chemotherapy, T stage, age (years), race/ethnicity, gender, grade, and histological type. In multivariate Cox analyses, higher T stage was associated with increased risk of overall mortality (HR = 1.219, 95% CI = 0.985–1.508, $P = 0.068$ for T2 stage; HR = 1.688, 95% CI = 1.403–2.032, $P < 0.001$ for T3 stage; using T1 stage as the reference), older patients were associated with increased risk of overall mortality (HR = 2.262, 95% CI = 2.034–2.517, $P < 0.001$, using ≤65 years as the reference), and black ethnicity was associated with increased risk of overall mortality (HR = 1.187, 95% CI = 1.049–1.344, $P = 0.007$, using white ethnicity as the reference), it was also found that gender was significant, and female patients were associated with a decreased risk of overall mortality (HR = 0.848, 95% CI = 0.779–0.922, $P < 0.001$, using male gender as the reference), and mucinous adenocarcinoma/signet ring cell carcinoma was associated with increased risk of overall mortality (HR = 1.212, 95% CI = 1.056–1.391, $P = 0.006$, using adenocarcinoma as the reference). The receipt of adjuvant chemotherapy after the radical resection of the primary tumor was also shown to be independently associated with a 64.0% decreased risk of overall mortality compared with surgery alone (HR = 0.360, 95% CI = 0.329–0.394, $P < 0.001$, using surgery alone as the reference).

DISCUSSION

Similar to cases with rectal cancer, the preoperative diagnosis of lymph node positivity with CT (computerized tomography) in colon cancer was a problem for radiologists, and false-negative results were usually caused by microscopic metastatic lymph nodes with a normal size (7). However, node-negative colon cancers that were not clinically visible on preoperative imaging also indicated very low lymphatic tumor burdens. As we already know, the overuse of chemotherapy increases economic burdens because of the high cost of chemotherapy and has adverse effects on the personal lives and work of patients (14, 15). With this in mind, this study aimed to explore whether those with clinically node-negative colon cancer, pathologically diagnosed with node involvement, could be a favorable subgroup in a study of stage III disease and whether they could be treated with radical surgery alone to avoid overtreatment. The investigation of this question is of clinical significance, and to the best of our knowledge, has not been specifically discussed in previous studies.

In the current study, to investigate whether those with clinically node-negative colon cancer found to be pathologically

TABLE 3 | Univariate and multivariate Cox regression analyses for OS in the whole cohort.

Variable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Group		<0.001		<0.001
Surgery	1		1	
Surgery and adjuvant chemotherapy	0.289 (0.265–0.316)		0.360 (0.329–0.394)	
T stage		<0.001		<0.001
T1	1		1	
T2	1.417 (1.147–1.752)	0.001	1.219 (0.985–1.508)	0.068
T3	1.990 (1.656–2.390)	<0.001	1.688 (1.403–2.032)	<0.001
Age (years)		<0.001		<0.001
≤65	1		1	
>65	3.126 (2.823–3.462)		2.262 (2.034–2.517)	
Race		<0.001		<0.001
White	1		1	
Black	1.029 (0.910–1.164)	0.649	1.187 (1.049–1.344)	0.007
Other	0.687 (0.577–0.817)	<0.001	0.749 (0.630–0.892)	0.001
Gender		0.051		<0.001
Male	1		1	
Female	0.920 (0.847–1.000)		0.848 (0.779–0.922)	
Grade		0.001		0.245
Grade I/II	1		1	
Grade III/IV	1.207 (1.090–1.338)	<0.001	1.058 (0.954–1.173)	0.286
Unknown	1.193 (0.869–1.639)	0.275	1.250 (0.907–1.721)	0.172
Histological type		<0.001		0.006
Adenocarcinoma	1		1	
Mucinous adenocarcinoma/ signet ring cell carcinoma	1.352 (1.180–1.549)		1.212 (1.056–1.391)	

diagnosed with node involvement could be treated with radical surgery alone, only pT1-3N1a colon cancer patients without preoperative identification of lymph node metastases were included into analyses. As this was to our knowledge, the first research to explore this problem, we believed that a large-scale database was the most suitable option as it enabled us to access more data.

According to the patient data included in the study, for those with very low lymphatic tumor burden, the 5-year CSS rates of patients without and with adjuvant chemotherapy were 80.0 and 90.7%, respectively. The results of multivariate Cox regression analyses also showed that the receipt of adjuvant chemotherapy after the radical resection of the primary tumor was independently associated with a 57.3% decreased risk of colon cancer-specific mortality compared with surgery alone. In addition, the 5-year OS rates of patients without adjuvant chemotherapy (52.1%) was lower than patients with adjuvant chemotherapy (82.1%), and the receipt of adjuvant chemotherapy after the radical resection of the primary tumor was also shown to be independently associated with a 64.0% decreased risk of overall mortality compared with surgery alone. All of the above findings demonstrated that adjuvant chemotherapy was significantly associated with improved prognosis and radical surgery alone was not enough in colon cancer with very low lymphatic tumor burden.

In stage III colon cancer, patients were commonly treated with chemotherapy and it was a standard treatment of node-positive disease after the radical resection of the primary tumor, which was reported to result in 8–10% improvement in overall survival (16, 17). However, both patients and medical oncologists should be aware of the toxicity caused by chemotherapy, and it has been reported for example, that patients treated with oxaliplatin might develop late-onset neuropathy with adverse impact on the personal lives and work of patients (18, 19). There is, therefore, a need to stratify stage III colon cancer patients into different prognostic subgroups to generate better therapeutic options for low-risk patients who may not require high dose chemotherapy.

Recently, the IDEA (International Duration Evaluation of Adjuvant therapy) demonstrated that CAPOX (capecitabine and oxaliplatin) treatment for 3 months is as effective as 6 months in ensuring disease-free survival among low-risk (T1-3N1) but not high-risk (T4 or N2) patients. These results have led many medical oncologists to consider 3 months of adjuvant treatment as the new standard of care for low-risk stage III disease, indicating that the standard long-course chemotherapy was not necessary for stage III colon cancer with low lymphatic tumor burden (20, 21).

In 2014, Tashiro et al. (22) examined the efficacy of adjuvant chemotherapy in stage III colon cancer. Although adjuvant therapy raised their chances of survival by three-fold compared with curative surgery alone, this study found that chemotherapy did not affect the 3-year OS and the 3-year RFS of stage IIIA patients. This led them to believe that chemotherapy might be omitted for certain cases of stage IIIA colon cancer with low risk of recurrence, such as node positive T1/T2 patients.

In 2016, Akeel et al. conducted a retrospective analysis with 218 clinically node negative rectal cancer patients undergoing radical surgery using total meso-rectal excision (TME) techniques for rectal cancer with curative intent from 2000 to 2012. These cases were later confirmed to be stage III disease on final pathology from a prospectively maintained database, and they found that TME surgery alone was not sufficient for those patients, which is consistent with our study (23).

In research by Tashiro et al. it was noted that stage IIIA diseases were mixed with non-N1a colon cancer. Moreover, the sample size of stage IIIA colon cancer was too small (22 patients in the adjuvant chemotherapy group and 17 patients in surgery alone group) and the survival differences between the two groups did not reach statistical significance. Therefore, as mentioned

above, we strongly believed that adjuvant chemotherapy was significantly associated with improved prognosis, and that radical surgery alone was not enough in colon cancer with very low lymphatic tumor burden.

The main strengths are first, that it was reasonable to ask whether those with clinically node-negative colon cancer found to be pathologically diagnosed with node involvement, could be a favorable subgroup in stage III disease and could they be treated with radical surgery alone to avoid overtreatment, based on previous findings. Second, to the best of our knowledge, this is the first study to examine the necessity of adjuvant chemotherapy in colon cancer patients with a very low lymphatic tumor burden. Third, our study was based on a large population-based using the SEER database, which increased the credibility of the results.

However, the present study also had two weaknesses. First, the chemotherapy regimens and the information of comorbidities were not available in the SEER database. Many previous studies (24–27) have reported that the presence of comorbidities was associated with a decrease in cancer treatment, which would cause a selection bias in the present study. Second, due to its retrospective nature, selection bias may exist in the current study, and results subsequently need to be interpreted cautiously.

CONCLUSIONS

In conclusion, our study retrospectively analyzed the efficacy of adjuvant chemotherapy in patients with clinically node-negative colon cancer found to be pathologically diagnosed with node involvement and demonstrated that adjuvant chemotherapy was significantly associated with improved prognosis and radical surgery alone was not enough in colon cancer with very low lymphatic tumor burden.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov/).

AUTHOR CONTRIBUTIONS

ZZ and CS participated in the design of the study. GX, YJ, and CF participated in collecting data. GX, YJ, and JY conducted the statistical analysis and wrote the first draft of the manuscript. ZZ and CS reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Bevacizumab Plus FOLFOX-4 Combined With Deep Electro-Hyperthermia as First-line Therapy in Metastatic Colon Cancer: A Pilot Study

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Bevacizumab plus FOLFOX-4 regimen represents the first-line therapy in patients affected by metastatic colorectal cancer (mCRC). Hyperthermia has been considered an effective ancillary treatment for cancer therapy through several anti-tumor mechanisms, sharing with Bevacizumab the inhibition of angiogenesis. Up to now, scientific literature offers very few clinical data on the combination of bevacizumab plus oxaliplatin-based chemotherapy with deep electro-hyperthermia (DEHY) for metastatic colon cancer (mCC) patients. Therefore, we aimed at evaluating the efficacy of this combination based on the possible interaction between the DEHY and bevacizumab anti-tumor mechanisms. We conducted a retrospective analysis on 40 patients affected by mCC treated with the combination of bevacizumab plus FOLFOX-4 (fluorouracil/folinic acid plus oxaliplatin) and DEHY (EHY2000), between January 2017 and May 2020. DEHY treatment was performed weekly, with capacitive electrodes at 80–110 W for 50 min, during and between subsequent bevacizumab administrations, on abdomen for liver or abdominal lymph nodes metastases and thorax for lung metastases. Treatment response assessment was performed according to the Response Evaluation Criteria for Solid Tumors (RECIST). The primary endpoints were disease control rate (DCR) and progression-free survival (PFS). The secondary endpoint was overall survival (OS). DCR, counted as the percentage of patients who had the best response rating [complete response (CR), partial response (PR), or stable disease (SD)], was assessed at 90 days (timepoint-1) and at 180 days (timepoint-2). DCR was 95% and 89.5% at timepoint-1 and timepoint-2, respectively. The median PFS was 12.1 months, whereas the median OS was 21.4 months. No major toxicity related to DEHY was registered; overall, this combination regimen was safe. Our results suggest that the combined treatment of

DEHY with bevacizumab plus FOLFOX-4 as first-line therapy in mCC is feasible and effective with a favorable disease control, prolonging PFS of 2.7 months with respect to standard treatment without DEHY for mCC patients. Further studies will be required to prove its merit and explore its potentiality, especially if compared to conventional treatment.

Keywords: tumor angiogenesis, bevacizumab, hyperthermia, chemotherapy, metastatic colon cancer

INTRODUCTION

Colorectal cancer (CRC) is the fourth most frequently diagnosed cancer and the second leading cause of cancer-related death in the United States (1). In 2019, approximately 49,000 new cases of CRC were diagnosed in Italy, while about 20,000 people died of CRC during 2016 (2). CRC is the second leading cause of cancer in both sexes in Italy, with a 5-year survival rate of 65% (2). Despite these high numbers, the incidence and mortality of CRCs decreased during the last decades, thanks to cancer prevention, earlier diagnosis through preventing screening and better treatment approaches (1, 2).

Approximately 50 to 60% of patients affected by CRC develop metastases (3–5), and the liver represents the most frequent metastatic site (6), leading to death in most patients (7).

Both oral and intravenous fluoride pyrimidines, irinotecan, oxaliplatin, anti-EGFR, and antiangiogenic monoclonal antibodies, regorafenib, trifluridine/tipiracil (TAS-102) and, in very dated studies, mitomycin C proved to be effective in the treatment of advanced disease (8–17).

The patients' health conditions drive the choice of the appropriate recommended first-line basic schedules which include intensive therapies, such as FOLFOX, XELOX, FOLFIRI, and FOLFOXIRI (8–14). In addition, biologic agents such as bevacizumab, cetuximab or panitumumab, can be combined to chemotherapy depending on the K-RAS biomarker status of the tumor. Further systemic therapies for patients with progressive disease always depend on the chosen first-line therapy.

Bevacizumab has been the first recombinant humanized murine IgG1 monoclonal antibody blocking the biomolecular activity of all the isoforms of the circulating Vascular Endothelial Growth Factor A (VEGF-A), a natural ligand that plays a pivotal role in tumor angiogenesis, being up-regulated in several human tumors (18–22). In particular, bevacizumab inhibits the VEGF/VEGF receptor signaling pathway, blocking tumor angiogenesis (20, 23) decreasing microvessel density but inducing HIF-1 gene expression (24), which is a fleeting molecular balance that stimulates VEGF activity. Some recent studies have demonstrated that non-responder colorectal cancer patients had high pre-treatment HIF-1 levels (25).

Since 2004 FDA has recognized its revolutionary mechanism of action, approving its clinical use in several tumor diseases, and the first was mCRC.

The activity of bevacizumab as first-line therapy in mCRC was evaluated in several randomized phase II and III studies with significant improvements in clinical outcomes (8–11). In more detail, NO16966 trial is a phase III study comparing XELOX plus bevacizumab or placebo *versus* FOLFOX plus bevacizumab or placebo as first therapy in patients with mCRC (12). Clinical results confirm that bevacizumab plus oxaliplatin-based chemotherapy regimens increase progression-free survival (PFS) of 1.4 months with respect to the same regimens without bevacizumab, while no statistical significance difference in overall survival (OS) was reached (12).

Bevacizumab toxicity is far from the common cytotoxic chemotherapy-associated side effects, such as myelosuppression, alopecia, diarrhea, nausea, and vomiting (26). It is rather associated with proteinuria, hypertension, arterial thromboembolic events, wound healing complications, bleedings, and gastrointestinal perforation (11).

In the cases of liver or lung metastases from CRC, loco-regional therapies often play an additional key role along the cure pathway: surgery is the gold standard treatment for resectable metastases, while tumor ablation is indicated for non-surgery eligible patients or for small metastases that can be treated with adequate margins (27–32). Ablative techniques include radiofrequency ablation, microwave ablation, cryoablation, and irreversible electroporation (2, 32–36).

In contrast, liver-only or liver-dominant metastatic disease not eligible for surgery or ablation can be a candidate for locally arterial directed treatments, such as hepatic arterial infusion chemotherapy, yttrium-90 microsphere radioembolization, and transcatheter arterial chemoembolization (2, 37–47).

Among other physical treatments, hyperthermia (HT) proved to be an effective anti-tumor approach in combination with standard therapies. HT increases the temperature of tumor tissue up to 40–45°C and is applied as an enhancer of the effects both of radiotherapy and, to a lesser degree, of chemotherapy, in the treatment of different tumors, such as breast cancer, cervix carcinoma, head and neck cancer, glioblastoma, melanoma, peritoneal carcinomatosis, hepatocellular carcinoma, and soft tissue sarcoma, with significant improvements in clinical outcomes (48–56). Multiple direct and indirect mechanisms are responsible for the synergistic anti-cancer effect performed by HT. First of all, it has an evident cytotoxic action on cancer cells living in hypoxic, nutrient-deprived, and acid microenvironments (57, 58). Secondly, HT enhances the activity of anti-cancer drugs, by influencing plasmatic membrane protein distribution and transmembrane efflux pumps (59), so that the increased membrane permeability facilitates the uptake of antineoplastic agents within cancer cells (59). Thirdly, the application of a higher temperature in a specific area of the body is responsible for the denaturation of intracellular proteins, the inhibition of repair enzymes implying alteration of DNA repair processes and the

expression of heat-shock proteins (HSPs) (60, 61). The activation of HSP-mediated pathways determinates the induction of apoptosis and other cell-death mechanisms (62). Fourthly, HT hinders DNA homologous recombination, preventing the reparation of DNA breaks due to chemotherapy (63). Therefore, the combination of chemotherapy and hyperthermia boosts up DNA damage in the tumoral cells, selectively. There are several *in vitro* studies that have demonstrated the increased cytotoxicity of several chemotherapeutic agents, thanks to thermal exposure, such as platinum, melphalan, fluorouracil, and doxorubicin (64–66). Fifthly, HT induces a local vasodilatation which brings a greater drug dose into the tumor area. Finally, HT inhibits tumor angiogenesis through two different mechanisms. On the one hand, it directly damages endothelial cells because of the absorption of the electric field related thermal energy in the extracellular liquid, with a subsequent temperature gradient between the extra- and intracellular compartments, which threatens and/or destroys cancer cell membranes (67, 68). On the other hand, it is well known that hypoxic tumor microenvironment stimulates the expression of hypoxia-inducible factor-1 (HIF-1), which is the main VEGF inducer, the most powerful angiogenic factor. Moreover, HIF-1 also induces the expression of genes involved in an exceeding metabolism, shifting cells towards glycolysis and reducing oxygen consumption rate (69–71). In contrast, HT favors reoxygenation and down-regulates the expression of HIF-1, both through vasodilatation that enhances tumor perfusion and by decreasing oxygen consumption (70, 72, 73).

Deep electro-hyperthermia (DEHY), also known as oncothermia, is a method of locoregional HT. DEHY works by generating a modulated electric field with a carrier radiofrequency of 13.56 MHz through two active electrodes. Since malignant tissue has higher conductivity than healthy human tissue, the electric field tends to flow predominantly through the malignant tumor tissue. Thanks to the interaction between the electric field and the heat, selection at the cellular level takes place, and the system self-focuses on the tumor. In fact, the electric field tends to move through the pathways with the lowest impedance, *i.e.* through the malignant tissue (71, 74–77).

To the best of our knowledge, scientific literature offers very few data on the efficacy of anti-angiogenetic agents plus chemotherapy combined with HT (78).

Based on promising clinical results, its multiple anti-cancer mechanisms and possible unexplored advantages of its combination with an anti-angiogenic agent, we evaluated the synergic efficacy of DEHY in combination with bevacizumab plus FOLFOX-4 (fluorouracil/folinic acid plus oxaliplatin) as first-line therapy in 40 patients affected by metastatic colon cancer.

PATIENTS AND METHODS

Patient Population

Forty patients with untreated mCC were referred to the "Interventional and Medical Oncology Unit" of the National Cancer Research Centre, Istituto Tumori "Giovanni Paolo II" in Bari (Italy) between January 2017 and May 2020. Patients who met the following criteria were included in this study: (1) patients age ≥18 years with histologically confirmed colon cancer with clinical–instrumental and/or histological evidence of distant metastases; (2) life expectancy ≥3 months; (3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2; (4) measurable disease consistent with the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, not suitable for curative resection based on surgical criteria (**Figure 1**); (5) no prior systemic therapy for mCC; no previous treatment with oxaliplatin in the last year, bevacizumab or DEHY; (6) adequate organ function, including liver, kidney, and bone marrow; (7) provided signed informed consent.

The key exclusion criteria were: (1) history of malignancy other than CRC; (2) the presence of clinically significant cardiovascular disease; (3) uncontrolled hypertension; (4) proteinuria ≥500 mg/24 h; (5) bleeding diathesis or coagulopathy; (6) central nervous system metastasis; (7) use of full-dose anticoagulants or thrombolytics; (8) pregnancy or lactation; (9) non-healing wounds, ulcer, or bone fracture; (10) contraindications for hyperthermia treatment.

Patients with no completed clinic-pathological and survival data were also excluded.

Figure 1 is a flow diagram of study inclusion/execution criteria.

Bevacizumab Plus FOLFOX-4 Regimen

All patients received bevacizumab plus FOLFOX-4 regimen as first-line therapy for metastatic disease. FOLFOX-4 schedule includes on day 1, Oxaliplatin intravenous infusion at a dose of 85 mg/m^2 dissolved in glucose 5% was administered over 120 min contemporary to Leucovorin 200 mg/m^2 dissolved in glucose 5%. Leucovorin was given also on day 2 before fluoropirimidine. 5-Fluorouracil, as intravenous bolus at the dosage of 400 mg/m^2, was administered before its intravenous continuous infusion over 22 h at the dosage of 600 mg/m^2/die on days 1 and 2. As for bevacizumab, intravenous administration at a dose of 5 mg/kg in 100 ml sodium chloride 0.9% was administered before Oxaliplatin over 90 (for the first time) and 60 (for the sequent infusions) min on day 1 and repeated every 14 days.

Anti-emetic prophylaxis was conducted with a serotonin-5HT3-antagonist.

Treatment was continued until disease progression or unacceptable drug-related toxicities (by oxaliplatin above all), considering the shift to bevacizumab alone or plus 5-FU/LV as maintenance therapy.

Deep Electro-Hyperthermia

DEHY was performed by using the Oncotherm EHY-2000 medical device (Oncotherm GmbH, Traisdorf, Germany). Oncotherm EHY-2000 is made up of three components: a therapy bed with built-in waterbed mattress, a generator unit, and a web box system. The system's electronics is housed in the generator unit. A mobile computer unit allows viewing and saving the treatment data.

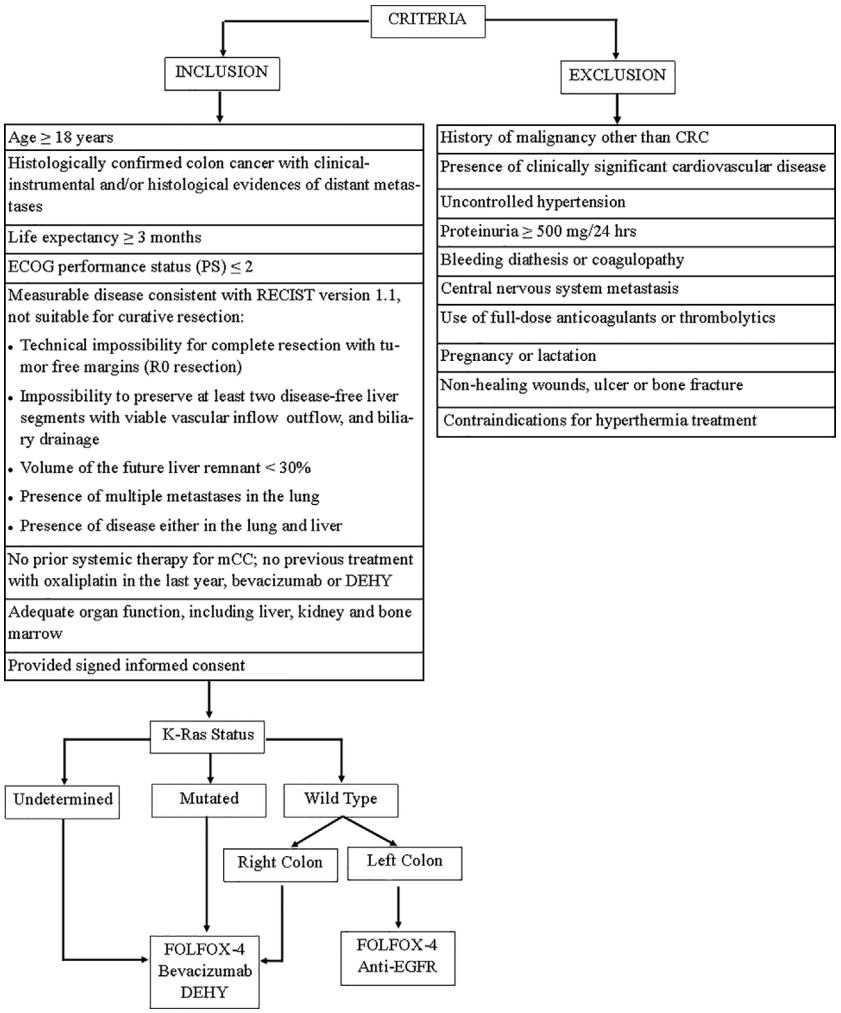

FIGURE 1 | Flow diagram of study inclusion/execution criteria. FOLFOX-4, fluorouracil/folinic acid plus oxaliplatin; DEHY, Deep electro-hyperthermia.

A modulated electric field with a carrier radiofrequency of 13.56 MHz is generated by two active electrodes: the large bolus electrode (30 cm in diameter) positioned at the site where the patient is to be treated and the counter electrode positioned under the mattress of the waterbed. During treatment, the patient lies on the waterbed and becomes part of the electric field *via* the bolus electrode.

DEHY was performed at an output power of 80–110 W generated by the generator unit, obtaining a calculated temperature of 41.5–42°C for 50 min as the whole hyperthermia time, including the 2–3 min of preheating until the therapeutic temperature is reached.

A water bag was used to protect the skin from overheating.

All patients received DEHY treatment weekly, during and between subsequent bevacizumab administrations. The target area of DEHY was the abdomen (n = 36) for liver or abdominal lymph nodes as sites of metastasis and thorax (n = 10) for lung metastasis on the basis of CT imaging guidance. If more than one target area was present, a maximum of two target sites were used alternately, each in one of the two DEHY treatments within a cycle (n = 9). Patients were carefully instructed to report any discomfort during treatment. Moreover, late DEHY-associated adverse events (AEs) were recorded for each patient. DEHY treatment was stopped if an adverse event occurred or by patients will.

Assessments

A clinical–instrumental evaluation based on general condition, clinical signs, laboratory tests, chest–abdomen–pelvis contrast enhancement computed tomography (ceCT) scan were required before starting the treatment after 90 days (timepoint-1), 180 days (timepoint-2), and then every 3 months to assess tumor response, monitor safety, compliance and determine AEs. Moreover, ^{18}F-FDG PET/CT at baseline and at timepoint-2 was performed to all patients. Two different radiologists and nuclear physicians evaluated and checked all ceCT and ^{18}F-FDG PET/CT exams independently.

Dimensional tumor measurements were performed on ceCT according to Response Evaluation Criteria for Solid Tumors (RECIST-Version 1.1) (79), and treatment response was indicated as Complete Response (CR), Partial Response (PR), Stable Disease (SD), and Progressive Disease (PD).

AEs were estimated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and reported in the clinical folder at each cycle of treatment. A decrease of white blood cell counts below 2×10^3 µl, of granulocytes below 0.5×10^3 µl, and of platelets below 100×10^3 µl implied a treatment delay of 1 week or more. Chemotherapy doses were reduced in the following cycle to 75% if nadir of granulocytes was $<1.5 \times 10^3$ µl, platelets $<100 \times 10^3$ µl, or any non-hematological toxicity grade 3 occurred.

Statistical Analysis

The primary endpoints were disease control rate (DCR) and PFS. The secondary endpoint was OS. DCR was considered as the percentage of patients who had the best response rating [complete response (CR), partial response (PR), or stable disease (SD)] and was assessed at 90 days (timepoint-1) and at 180 days (timepoint-2).

PFS was defined as the time from the start of treatment until the date of the first radiological evidence of PD or the date of death derived from any cause, whichever occurred first. OS was specified as the time from the start of treatment until the date of death.

Fisher's exact test was used to assess the correlation between DCR, PFS, OS, and tumor location (left-sided CRC/right-sided CRC), K-RAS status (wild type/mutation), number of metastatic sites (1–2, ≥3), liver involvement (yes/no), and/or lung involvement (yes/no). R *barplot()* function was used to create barplots. For survival analyses, the Kaplan–Meier method was used to estimate the correlation between PFS, OS rates, and clinic-pathological variables at 95% CI. The log-rank test was used to compare survival curves. The "survival" R package has been used to perform survival analyses. Cox proportional-hazards regression test using the 'coxph' function of the R 'survival' package has been elaborated. Survival curves have been graphically depicted by "ggplot 2" R package. All statistical analyses were performed using R version 3.6.

RESULTS

Patient Characteristics

Forty patients affected by mCC (21 female, 19 male; median age 64.4 years old) treated with bevacizumab plus FOLFOX-4 combined with DEHY between January 2017 and May 2020 in the "Interventional and Medical Oncology" of the National Cancer Research Centre, Istituto Tumori "Giovanni Paolo II" in Bari (Italy) were collected and retrospectively analyzed.

Patients presented an ECOG PS of 0 (n = 25), 1 (n = 11), 2 (n = 4) at the first therapy administration. Among 40 patients, five patients had primitive tumor in site (12.5%), while 35 patients had previously undergone resection of primitive tumor (87.5%); 26 patients (65%) harbored left-sided CC, 27 (67.5%) were KRAS mutated, 32 (80%) had ≤2 metastatic sites with liver as the most common metastatic organ (36 patients, 90%). All patients were not eligible for surgery because of unresectable disease according to abovementioned criteria. Patients' characteristics are shown in **Table 1**.

Efficacy

Clinical–instrumental evaluation at timepoint-1 was assessed in all patients of our study: PR was detected in 12/40 (30%) patients, SD in 26/40 (65%) patients, and PD in 2/40 (5%) patients, with a DCR of 95% (**Table 2**).

38 patients (95%) completed the clinical–instrumental evaluation at timepoint-2: CR was achieved in 2/38 (5.3%) patients, PR in 10/38 (26.3%) patients, SD in 22/40 (55%) patients, and PD in 4/38 (10%) patients, with a DCR of 89.5% (**Table 2**).

DCR decreased of 5.55% from timepoint-1 to timepoint-2 treatment response evaluations.

Figure 2 represents the best response rate according to colon site and K-Ras status.

TABLE 1 | Baseline patient Characteristics.

Characteristics	Enrolled Patients (n = 40)	%
Gender		
Male	19	47.5%
Female	21	52.5%
Median age, years	64,4	Range 45–80
ECOG Performance Status		
0	25	62.5%
1	11	27.5%
2	4	10%
Primitive tumor in site		
Yes	5	12.5%
No	35	87.5%
Primitive tumour side		
Right Colon	13	32.5%
Left Colon	27	67.5%
Biomarker status (K-Ras)		
Wild Type	13	32.5%
Mutated	27	67.5%
No. of metastatic sites		
1–2	32	80%
≥3	8	20%
Major involvement site		
Liver	36	90%
Lung	4	10%

TABLE 2 | Clinical response assessment according to RECIST at timepoint-1 and timepoint-2.

Clinical Response	Timepoint 1 (*n* = 40)	%	Timepoint 2 (*n* = 38)	%
CR	/	/	2	5.3
PR	12	30	10	26.3
SD	26	65	22	55
PD	2	5	4	10.5
DCR	38	95	34	89.5

Figures 3 and **4** represent two exemplar cases of patients judged to be in PR and CR.

Median PFS, the other primary endpoint, was 12.1 months (range 2.9–32.6 months) (**Figure 5**).

Concerning the secondary endpoint, median OS was 21.4 months (range 3.5–52 months) (**Figure 6**).

Three patients (7.5%) underwent an attempt at curative metastasectomy obtaining complete R0 resection of the whole disease.

31 of 40 patients (77.5%) crossed over to receive a second-line therapy after disease progression. The most common regimens used were: FOLFIRI plus aflibercept (71%) or panitumumab/cetuximab (29%) based on biomarker status of the tumor. A small group also received a third-line therapy.

Neither K-Ras status ($p = 0.68$; $p = 0.48$) (**Figures 7A**, **8A**) nor primitive site ($p = 0.092$; $p = 0.68$) (**Figures 7B**, **8B**), as well as any clinic-pathological variables resulted in influencing PFS and OS significantly. A Cox-hazard regression analysis has been performed aggregating K-Ras mutational status and tumor sidedness. Considering the small sample size, surprisingly, we found that patients with K-Ras wild type left-sided tumors had a double risk to have a shorter PFS [HR: 2.58 (95%CI: 0.96 ÷ 6.92)] (**Table 3; Figure 9**).

Toxicity

All AEs reported in this study are shown in **Table 4**.

Bevacizumab-FOLFOX4 regimen was substantially well-tolerated: only one patient interrupted this therapeutic scheme for sensory neuropathy, continuing treatment with bevacizumab and fluorouracil/folinic acid. In spite of this premature suspension of oxaliplatin infusions, he achieved a good response to treatment.

The main non-hematological AEs were nausea and vomiting, which occurred in nine patients. Other AEs were fatigue (four cases), peripheral sensory neuropathy (three cases), high blood pressure (two cases), epistaxis (two cases) and gastrointestinal discomfort (two cases). As for hematological AEs, leucopenia in six cases (three patients required granulocyte-colony stimulating factor), anemia (5 cases), and thrombocytopenia (three cases) were observed. However, none of the listed AEs led to a break-up of the treatment.

The addition of DEHY did not result in additional AEs on chemotherapy-related toxicity. The main DEHY-related AE was mild positional pain during treatment sessions which occurred in four patients. Erythema in the target area of DEHY was observed in three patients; power-related pain occurred in two cases during the first session and solved by power adjustment.

No patient required a significant treatment interruption because of treatment complications nor resistance phenomena to DEHY

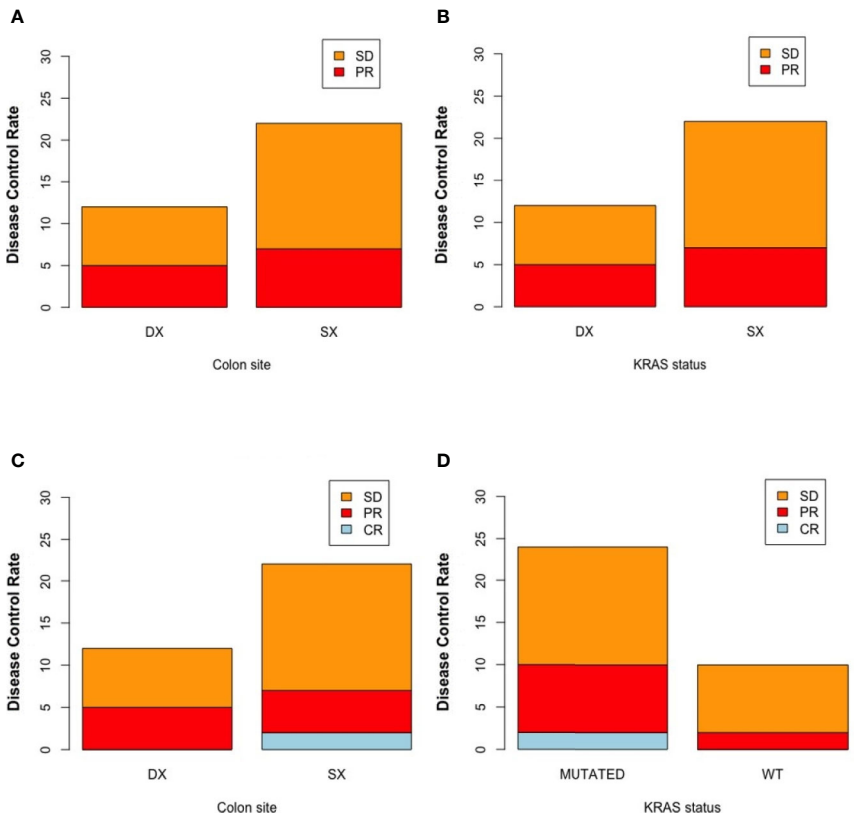

FIGURE 2 | Disease control rate according to colon site and K-Ras status at timepoint-1 (respectively **A, B**) and at timepoint 2 (respectively **C, D**). SX, left colon; DX, right colon.

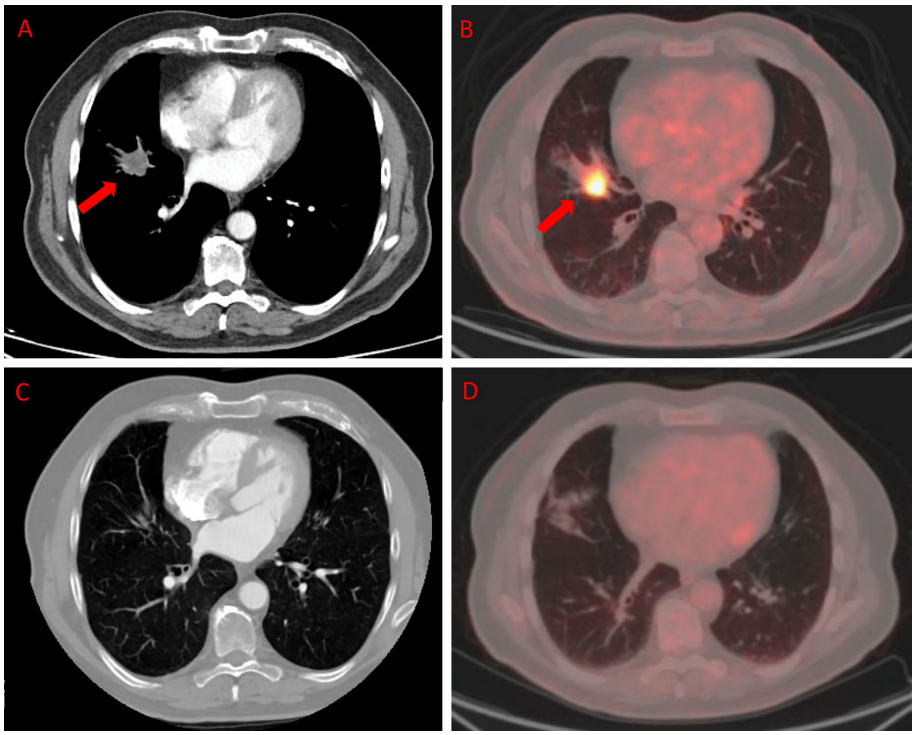

FIGURE 3 | ceCT and ^{18}F-FDG PET/CT in a patient affected by colon cancer with lung metastasis. A 79-year-old male affected by colorectal cancer with lung metastasis, subjected to 12 cycles of Bevacizumab-based chemotherapy and 24 DEHY sessions on the thorax as first-line therapy. Baseline ceCT **(A)** showed metastasis in the middle lobe (red arrows). Baseline whole body ^{18}F-FDG PET/CT **(B)** confirmed lung involvement by the increased ^{18}F-FDG uptake (red arrows) detectable axial fused PET/CT images in the same site. Timepoint-2 ceCT **(C)** and ^{18}F-FDG PET/CT **(D)** evaluation demonstrated CR of lung metastasis.

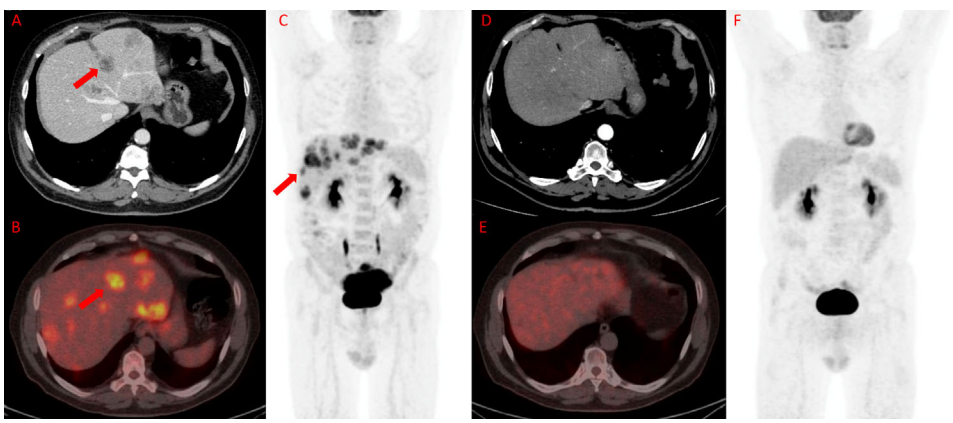

FIGURE 4 | ^{18}F-FDG PET/TC in a patient affected by colon cancer with liver metastasis. A 55-year-old male affected by colorectal cancer with multiple liver metastases, subjected to 12 cycles of Bevacizumab-based chemotherapy and 24 hyperthermia sessions on the abdomen as first-line. Baseline ceCT showed massive liver involvement (A and red arrows) and whole-body ^{18}F-FDG PET/TC showed increased ^{18}F-FDG uptake in the liver lesions (red arrows) detectable also on axial fused PET/CT and MIP images **(B** and **C)** in the same site. Timepoint-2 ceCT **(D)** evaluation demonstrated significant size decrease of liver metastasis with no evidence of ^{18}F-FDG uptake on whole-body PET/CT **(E** and **F)**. According to RECIST, the patient was classified as CR.

were ever observed due to the mild weekly administration, whose specific aim was to catalyze vasodilatation and a major uptake of drug in the target site. In light of our results, we can affirm that no major toxicity has been observed and reported by patients.

DISCUSSION

In literature, several reports demonstrate that the addition of bevacizumab to chemotherapy improves OS and/or PFS for

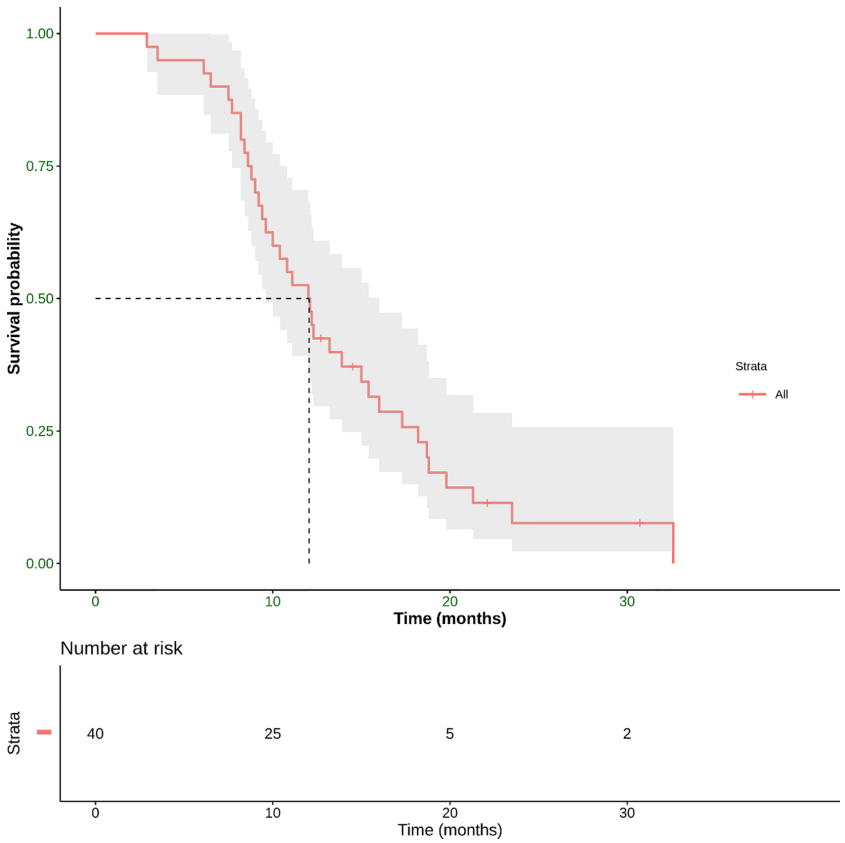

FIGURE 5 | Progression-free Survival (PFS) defined as the time from the start of treatment until the date of the first radiological evidence of PD or the date of death derived from any cause, whichever occurred first. This panel shows Kaplan–Meier estimate of PFS in our patient population. The mean PFS was 12.1 months (range 3.5–32.6 months).

patients affected by untreated mCRC. As reported by Kabbinavar et al., in randomized phase II studies, mCRC treated with bevacizumab and 5-FU/LV obtained an OS improvement with respect to the same regimen without bevacizumab (8, 10, 11). Hurwitz H. et al. conducted a phase III trial comparing irinotecan, bolus fluorouracil, and leucovorin (IFL) with and without bevacizumab demonstrating an increased mOS in the bevacizumab group (9). Moreover, the combination of bevacizumab with FOLFOX-4 or XELOX resulted in a statistically significant improvement in mPFS compared with those patients treated with chemotherapy alone (hazard ratio [HR], 0.83; 97.5% CI, 0.72 to 0.95; p = .0023), while no statistically significant difference in mOS was reached (HR, 0.89; 97.5% CI, 0.76 to 1.03; p = .077) (NO16966 trial) (12). The last reported phase III trial is one of the registered clinical studies of bevacizumab in its first commercial formulation Avastin®, which contributed to the final approval of the drug for therapy. To our aims, the critical end-point results of this trial have represented the right and immediate comparison group, lacking in our study an internal control patient group randomized to the same chemotherapy treatment without DEHY.

Bevacizumab binds VEGF, the key promoter of vasculogenesis and angiogenesis, preventing its bond to the specific receptors, Flt-1 (VEGFR-1) and KDR (VEGFR-2), on the surface of endothelial cells. Therefore, bevacizumab blocks the biological activity of VEGF, reverses the vascularization of tumors, normalizes the residual tumor vascularization and inhibits the formation of new vascularization, thus preventing tumor growth (80).

Its complex tridimensional structure has a molecular weight of 149 kDa; its bioavailability is 100% only by intravenous administration, and its half-life of about 20 days (range: 11–50 days) is compatible with the frequency of standard chemotherapy in mCRC, as well as for other chemotherapeutic schedules in different tumors. This favorable half-life is due to its peculiar metabolic and elimination profiles which are comparable to native IgGs, unable to link VEGF. It is initially attacked by proteolytic enzymes everywhere in the body, including endothelial cells and is not principally eliminated through liver or kidney because IgG link with FcRn receptor protects them from elimination, conferring a long terminal half-life. Bevacizumab's clearance value of 0.231 l/die completes its pharmacokinetic profile, summarizing that initial half-life of 1.4 days and a terminal half-life of 20 days are comparable to native IgG terminal half-life, swinging from 18 to 23 days.

In this contest, the combination therapy bevacizumab plus DEHY finds a fertile field of application in clinics.

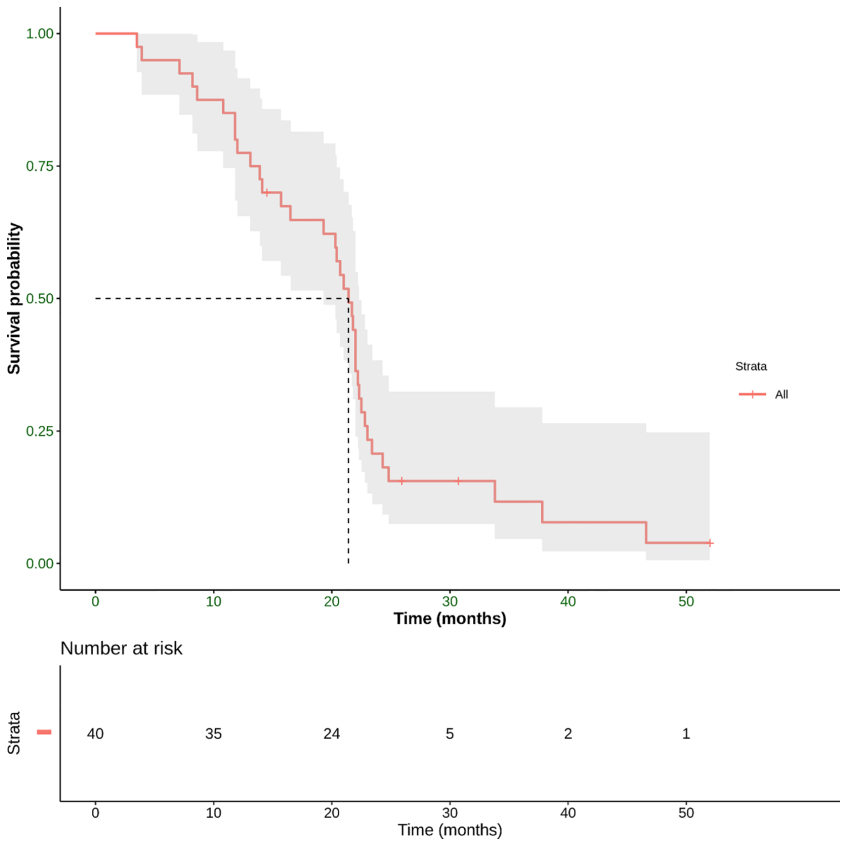

FIGURE 6 | Overall survival (OS) defined as the time from the start of treatment until the date of death. This panel shows Kaplan–Meier estimate of OS in our patient population. The mean OS was 21.4 months (range 3.5–52 months).

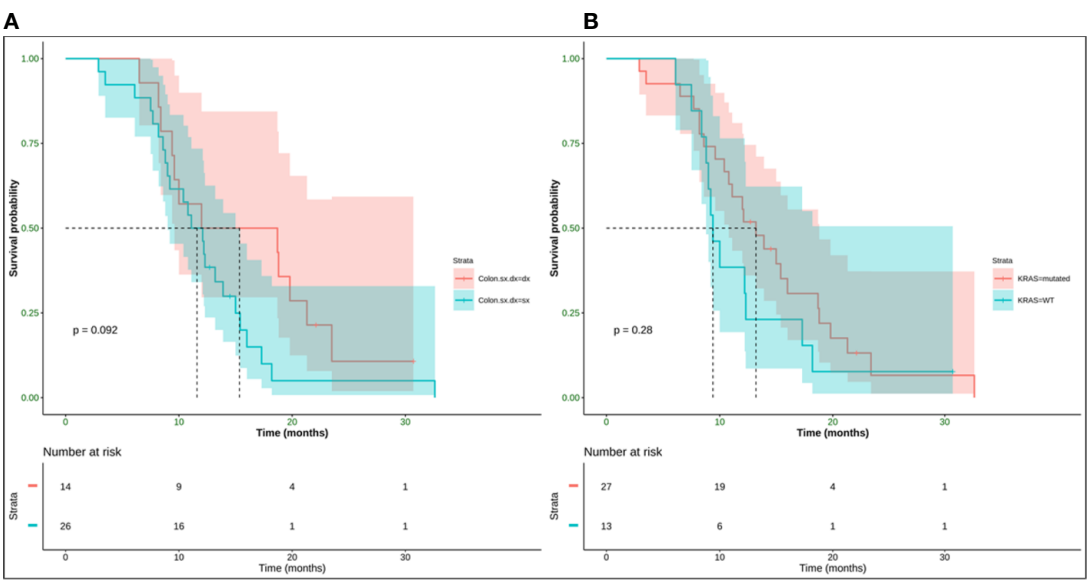

FIGURE 7 | No statistically significant difference in terms of mPFS was observed (*p*-value = 0.092) between right (mPFS 15.3 months) and left (mPFS 11.6 months) colon cancer **(A)**, and (*p*-value = 0.28) between K-Ras wild type (mPFS 13.2 months) and K-Ras mutated (mPFS 9.4 months) patients **(B)**. SX, left colon; DX, right colon.

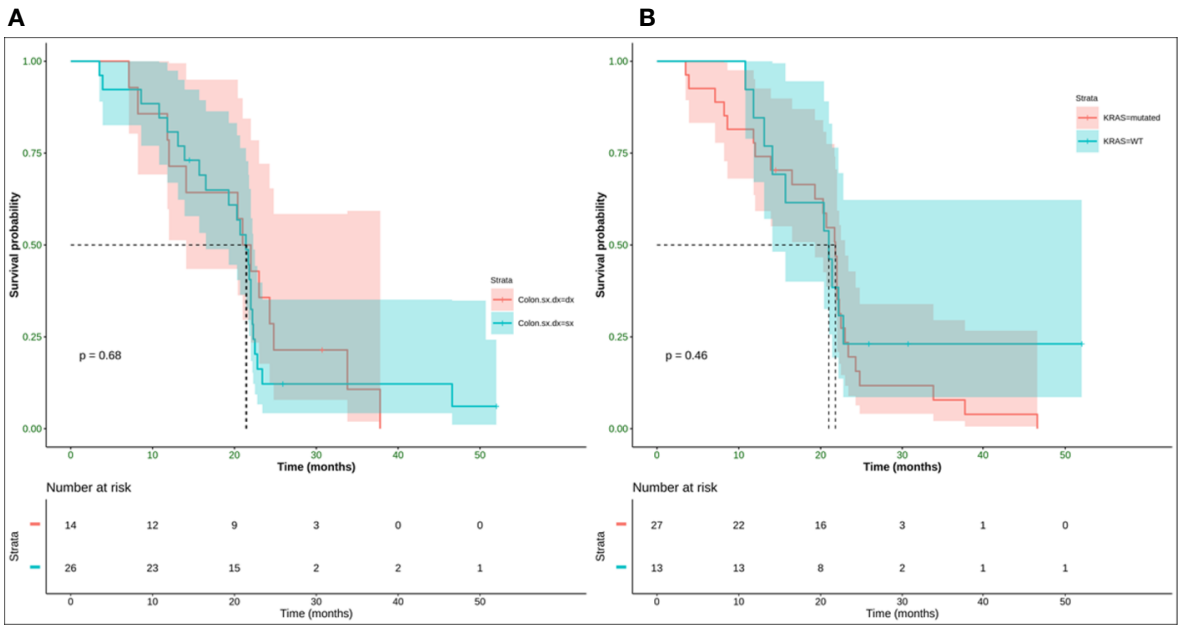

FIGURE 8 | No statistically significant difference in terms of mOS was observed (*p*-value = 0.68) between right (mOS 21.5 months) and left (mOS 21.4 months) colon cancer **(A)** and (*p*-value = 0.46) between K-Ras wild type (mOS 21 months) and K-Ras mutated (mOS 21.8 months) patients **(B)**. SX, left colon; DX, right colon.

TABLE 3 | Cox-hazard regression analysis results.

		OS	
Variable		**HR (95%CI)**	**p-value**
KRAS mutational status and sidedness	Right colon/KRAS mutated		Ref
	Right colon/KRAS WT	0.98 (0.26 ÷ 3.62)	0.98
	Left colon/KRAS mutated	1.35 (0.59 ÷ 3.06)	0.46
	Left colon/KRAS WT	0.87 (0.32 ÷ 2.31)	0.78
	PFS		
	Right colon/KRAS mutated		Ref
	Right colon/KRAS WT	1.23 (0.33 ÷ 4.65)	0.75
	Left colon/KRAS mutated	1.75 (0.57 ÷ 0.72)	0.21
	Left colon/KRAS WT	2.58 (0.96 ÷ 6.92)	**0.05**

Bold values denote statistical significance at the p < 0.05 level.

More recent anti-tumoral combination strategies have proven to be helpful to bevacizumab increasing its activity and clinical efficacy. In particular, HIF-1 inhibitors having limited activity as single agents, proved to enhance bevacizumab efficacy by contrasting the intratumor-hypoxia it induces by increasing HIF-1 dependent gene in the target tissue. Experimental models testing bevacizumab in combination with the HIF-1 inhibitor topotecan proved that this association generates a profound inhibition of HIF-1 transcriptional activity, a more significant inhibition of proliferation than bevacizumab alone, and the induction of apoptosis that bevacizumab alone is unable to induce. Waiting for further results about this pharmacologic combination strategy, the inhibition of HIF-1 expression can be physically reached, thanks to hyperthermia.

HT has an anti-cancer effect by means of multiple direct and indirect mechanisms including the inhibition of angiogenesis. HT can explicate this action through the direct damage of endothelial cells, the vasodilatation that enhances tumor reoxygenation, and by decreasing oxygen consumption (70, 72, 73). These mechanisms reduce tumor hypoxia and, subsequently, the expression of HIF-1 which plays a central role in the regulation of angiogenesis and cell metabolism. Specifically, HIF-1 is a transcription factor that triggers VEGF expression, the most powerful angiogenic factor, and shifts cells towards glycolysis decreasing oxygen consumption rate. By combining the two agents, bevacizumab and HT, the synergism of action, the goal they share and the possibility to organize subsequent administrations with an additional intermediate cycle of HT can probably further lengthen bevacizumab's terminal half-life so that a major and safe accumulation of drug due to HT vasodilatation can occur in the target district. Moreover, HT is able to contrast HIF-1 gene expression induced by bevacizumab, leading to a stable negative balance of this tumor marker.

These are perhaps hypothesis to explain the longer mPFS than historical control (NO16966 trial) in our population and could also predict even better data of mOS than standard treatment still to prove.

To the best of our knowledge, no authors investigated the possible clinical advantages deriving from the combination of bevacizumab plus FOLFOX-4 with DEHY in untreated mCC. In a previous pilot study, we evaluated bevacizumab–chemotherapy combined with DEHY in multi-treated patients affected by colorectal, breast, and ovarian cancers.

Our data demonstrated that DEHY may enhance the bevacizumab-based treatment, in particular improving tumor response (78).

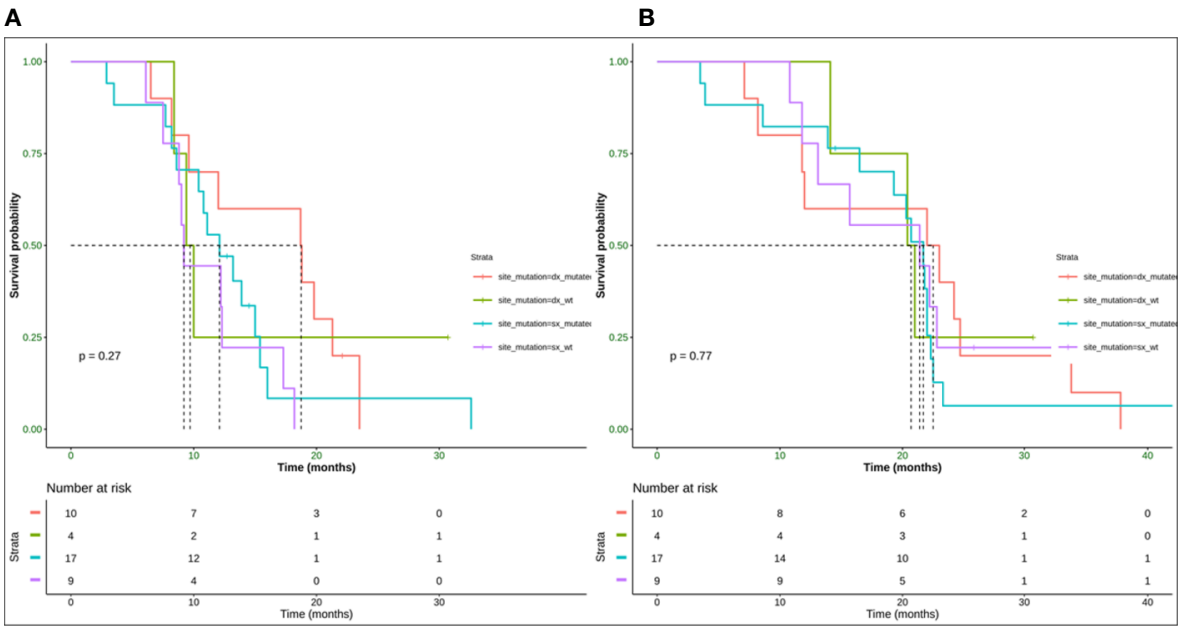

FIGURE 9 | Median PFS for mutated right colon was 18.8 months, for wild type right colon was 9.7 months, for mutated left colon was 12.1 months, wild type left colon was 9.8 months **(A)**. Median OS for mutated right colon was 22.5 months, for wild type right colon was 20.7 months, for mutated left colon was 21.7 months, for wild type left colon was 21.4 month **(B)**. SX, left colon; DX, right colon.

TABLE 4 | Adverse events.

Events	No. (%)
Hematologic	
Leucopenia	6 (15%)
Anemia	5 (12.5%)
Thrombocytopenia	3 (7.5%)
Non-hematologic	
Nausea and vomiting	9 (22.5%)
Positional pain	4 (10%)
Fatigue	4 (10%)
Erythema	3 (7.5%)
Peripheralsensoryneuropathy	2 (5%)
High blood pressure	2 (5%)
Epistaxis	2 (5%)
Gastrointestinal discomfort	2 (5%)
Power-related pain	2 (5%)

Based on the possible biological and clinical interaction between bevacizumab and DEHY effects, we conducted a pilot study to analyze the efficacy of bevacizumab plus FOLFOX-4 combined with DEHY in untreated mCC patients.

Our results showed that this combination determined a high disease control. In more detail, we obtained a DCR of 95 and 89.5% at timepoint-1 and timepoint-2, respectively. The median value of PFS was 12.1 months, while median OS was 21.4 months. The other important result was the absence of major toxicity related to DEHY.

Another result of our statistical Cox-analysis was the discovery that patients with KRAS wild type left-sided tumors have a double risk to have a shorter PFS.

Therefore, our study suggests that the addition of DEHY to bevacizumab-FOLFOX-4 regimen enhances the efficacy of the gold standard treatment, as studied in NO16966 clinical trial, through a high disease control and a longer mPFS (our report: 12.1 months *vs* historical control: 9.4 months) without additional adverse events or chemotherapy-related toxicity. The second clinical parameter we evaluated, DCR, cannot be directly compared to the response rate (RR) in NO16966, where there was no specific reference to times within the course of clinical evaluations. We chose to study DCR as a natural continuation of our previous pilot study, where we observed through DCR very promising stabilized diseases and objective responses.

Although NO16966 represents a historical comparison, our results support the validity of this study since the baseline patient characteristics of enrolled population, the inclusion and exclusion criteria, and the administered chemotherapy regimen are comparable to the historical control.

Moreover, the main limitations of our study, the small sample size, and the absence of comparison with an internal control group, did not allow us to adequately analyze mOS that, in relation to mPFS data, could prove to be longer than the standard treatment.

In spite of these aspects, we succeeded in focusing on a homogeneous patient population, and our results represent the first clinical data on the potential benefit of DEHY in addition to bevacizumab-based chemotherapy. We also demonstrated the safety of this type of combination treatment due to a different specific use of DEHY as catalyzing agent of vasodilatation and drug uptake.

CONCLUSION

Bevacizumab plus FOLFOX-4 combined with DEHY as first-line therapy in colon cancer patients demonstrated both tolerability and efficacy. Bevacizumab's pharmacokinetic data will interweave with DEHY's ability to retain drugs in the selected treatment areas so that drug elimination undergoes a delay compatible with our results of the principal clinical endpoints. Moreover, their opposite effects on HIF-1 expression play a key role in controlling disease progression and represent a new field of research in oncology. This regimen, as adopted in our study, has also demonstrated that DEHY in combination with chemotherapy schedules has no relevant toxicity, is safe and receives acceptable compliance by patients. A randomized trial will be necessary in the near future to further confirm these data.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Hyperthermia is recognized and reimbursed by Italian Health System therapeutic strategy in association with chemotherapy or radiotherapy in the treatment of tumors, being identified by the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) with the code 9985. Consequently, this treatment does not need a clinical trial, but only the signed informed consent. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, GRa, CL, and CG. Methodology, SDS. Software, SDS, MP. Validation, FM. Formal analysis, MA. Investigation, PM. Resources, GL, CG. Writing—original draft preparation, CL, ML. Writing—review and editing, ML. Visualization, AP, GRu, CF. Supervision, GRa, CG. Project administration, GRa, CL. All authors contributed to the article and approved the submitted version.

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Previous Use of Anti-Vascular Endothelial Growth Factor Receptor Agents Decreases Efficacy of Fruquintinib in Metastatic Colorectal Cancer Refractory to Standard Therapies

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Purpose: Fruquintinib is an anti-vascular endothelial growth factor receptor (VEGFR) agent. The FRESCO trial demonstrated that patients with metastatic colorectal cancer (mCRC) refractory to standard therapies could benefit from fruquintinib with tolerable adverse events (AEs). However, the efficacy and safety of fruquintinib in clinical practice has scarcely been reported, especially in patients with previous use of anti-VEGFR agents.

Methods: This retrospective study investigated the efficacy and safety of fruquintinib in patients with mCRC between January 2019 and December 2019. Progression-free survival (PFS) and overall survival (OS) were assessed by a Kaplan-Meier analysis and log-rank test. A Cox regression model was performed to identify independent prognostic factors.

Results: A total of 46 patients were included. The median PFS and OS were 3.1 months (95% confidence interval [CI], 1.9–4.3 months) and 9.0 months (95% CI, 7.2–10.8 months), respectively. Patients previously treated with anti-VEGFR agents had shorter median PFS compared with those without previous use of anti-VEGFR agents (1.9 *vs.* 3.7 months, P = 0.006), while the median OS was similar between the two groups (8.5 *vs.* 9.0 months, P = 0.992). Multivariate analysis revealed that the neutrophil-lymphocyte ratio (NLR) was an independent prognostic factor in PFS (hazard ratio [HR], 2.230; 95% CI, 1.191–4.517, P = 0.014) and OS (HR, 4.221; 95% CI, 1.683–10.586; P = 0.002). The most common non-hematological and hematological AEs were hand-foot syndrome (37.0%) and anemia (39.1%), respectively.

Conclusion: Fruquintinib was an effective third-line therapy in mCRC with tolerable AEs. Efficacy of fruquintinib was decreased in patients with previous use of anti-VEGFR agents. NLR was an independent prognostic factor in PFS and OS in patients treated with fruquintinib.

Keywords: metastatic colorectal cancer, third-line therapy, fruquintinib, neutrophil-lymphocyte ratio, survival outcome

INTRODUCTION

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths globally (1). Metastasis occurs in approximately 20% of newly diagnosed patients, and approximately 50% of early stage CRC develop metastasis (2). Although cytotoxic drugs combined with targeted agents are standard first- and second-line therapies for metastatic CRC (mCRC) in National Comprehensive Cancer Network (NCCN) guidelines (3), patients still experience disease progression after standard treatment. The third-line therapy in mCRC has not been well-established, although regorafenib and trifluridine/tipiracil (TAS-102) are available. Regorafenib is an inhibitor targeting vascular endothelial growth factor receptor (VEGFR), which has an anti-angiogenic function (4). Both the CORRECT (5) and CONCUR (6) trials observed improved median progression-free survival (PFS) and overall survival (OS) in patients treated with regorafenib compared with those treated with placebo. TAS-102 is an orally administered combined chemotherapy agent. The RECOURSE (7) and TERRA (8) trials revealed a survival benefit of TAS-102 compared with placebo in mCRC refractory to standard therapies.

However, disease control of mCRC refractory to standard therapies is still limited. As a result, the anti-VEGFR tyrosine kinase inhibitor (TKI), fruquintinib, has been investigated (9). A phase Ib study and a randomized double-blind phase II study showed a significant improvement of PFS in the fruquintinib group compared with the placebo group ($P < 0.001$) in patients with treatment-refractory mCRC (10). In the FRESCO trial, 278 patients were randomized to the fruquintinib group and 138 patients to the placebo group. The median PFS and OS were significantly improved in the fruquintinib group compared with the placebo group (3.7 *vs.* 1.8 months, $P < 0.001$; and 9.3 *vs.* 6.6 months, $P < 0.001$; respectively), and treatment-related adverse events (AEs) in the fruquintinib group were tolerable (11). Therefore, fruquintinib was approved in China in September 2018 and in the United States in June 2020 (12).

The association between inflammatory and immune status, including neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR), and the efficacy of anti-VEGFR therapy has also been investigated. Santoni and colleagues (13) evaluated the prognostic role of NLR in patients treated with anti-VEGFR therapy in metastatic renal cell carcinoma and revealed that NLR was an independent prognostic factor for both OS ($P < 0.001$) and PFS ($P = 0.03$). Moreover, Hu et al. (14) conducted a prospective study assessing the efficacy of regorafenib in metastatic gastrointestinal stromal tumors in a Taiwanese population, and the results suggested that high NLR and PLR predicted unfavorable OS ($P = 0.033$ and $P = 0.019$, respectively). However, the prognostic role of NLR and PLR in treatment-refractory mCRC treated with fruquintinib has rarely been explored.

Clinical practice is complex in mCRC refractory to standard therapies. Although clinical trials revealed notable efficacy and tolerance of fruquintinib, patients with previous anti-VEGFR agents were excluded. Therefore, the efficacy and safety of fruquintinib require further assessment. It remains unclear whether NLR and PLR can predict fruquintinib efficacy. Thus, this retrospective study was conducted to provide insight for clinical practice.

Abbreviations: CRC, colorectal cancer; mCRC, metastatic colorectal cancer; NCCN, National Comprehensive Cancer Network; TAS-102, trifluridine/tipiracil; VEGFR, vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; TKI, tyrosine kinase inhibitor; AE, adverse event; PFS, progression-free survival; OS, overall survival; ECOG PS, Eastern Cooperative Oncology Group performance status; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response; DCR, disease control rate; CI, confidence interval; HR, hazard ratio; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; CEA, carcinoembryonic antigen; HFS, hand-foot syndrome.

METHODS AND MATERIALS

Patient Selection

In this retrospective study, patients with mCRC between January 2019 and December 2019 at Sun Yat-sen University Cancer Center were reviewed. The inclusion criteria were as follows: (1) CRC with metastasis; (2) refractory to at least two lines of standard systemic therapies; and (3) received at least one dose of fruquintinib. The exclusion criteria were as follows: (1) combination therapy of fruquintinib with other anti-tumor drugs; (2) lack of treatment data; and (3) lost follow-up. All clinical records, image information, and blood profiles were reviewed. The study was approved by the Medical Ethics Committee of Sun Yat-sen University Cancer Center (B2020-256). Key data of this study has been uploaded onto the Research Data Deposit public platform (http://www.researchdata.org.cn), with approval number of RDDA2020001709.

Statistical Analysis

NLR was categorized as ≤3 and >3 (15). PLR was categorized as <150, 150–300, and >300 (16). Tumor response was defined by the Response Evaluation Criteria in Solid Tumors version 1.1 (17). The objective response rate (ORR) referred to the rate of complete response (CR) and partial response (PR), and the disease control rate (DCR) referred to the rate of CR, PR, and stable disease (SD). PFS was defined as the beginning of fruquintinib treatment to disease progression or death, and OS was defined as the beginning of fruquintinib treatment to death from any cause. AEs during treatment were assessed based on the Common Terminology Criteria for Adverse Events version 3.0 (18).

Continuous and categorical variables were compared by chi-squared and Mann-Whitney U tests, respectively. Survival outcomes were evaluated by the Kaplan-Meier method and log-rank test. A Cox regression model was performed to identify independent prognostic factors. All tests were two-sided and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 21.0 software.

RESULTS

Patient Characteristics

A total of 46 mCRC patients treated with fruquintinib monotherapy were identified (**Table 1**). The median age was 59 years (range, 21–85 years), and 60.9% of patients were male.

TABLE 1 | Baseline characteristics of 46 patients treated with fruquintinib.

Characteristics	Number
Age (years)	
<60	25 (54.3)
≥60	21 (45.7)
Gender	
Male	28 (60.9)
Female	18 (39.1)
ECOG PS	
0–1	42 (91.3)
2	4 (8.7)
Primary site	
Colon	33 (71.7)
Rectum	8 (17.4)
Unknown	5 (10.9)
Metastatic organs	
1–2	19 (41.3)
≥3	27 (58.7)
RAS mutant	
Yes	25 (54.3)
No	12 (26.1)
Unknown	9 (19.6)
Lines of previous therapy	
2	33 (71.7)
≥3	13 (28.3)
Previous anti-tumor agents	
Fluoropyromidine	45 (97.8)
Irinotecan	42 (91.3)
Oxaliplatin	43 (93.5)
Bevacizumab	38 (82.6)
Cetuximab	13 (28.3)
Anti-VEGFR	14 (30.4)

VEGFR, vascular endothelial growth factor receptor; ECOG PS, Eastern Cooperative Oncology Group performance status.

Four of the 46 (8.7%) patients had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 2. The most common primary site was the colon (71.7%), followed by the rectum (17.4%). More than half of the patients had metastatic lesions in more than two organs (58.7%) and the incidence of the RAS mutation was 54.3%. Moreover, 38/46 (82.6%) patients were previously treated with bevacizumab, and 14/46 (30.4%) patients had previously been treated with anti-VEGFR agents (8 of regorafenib alone; 3 of apatinib alone; and 3 of both regorafenib and apatinib). Baseline characteristics between the 14 patients previously treated with anti-VEGFR agents and 32 patients not treated with anti-VEGFR agents were well balanced except for previous lines of therapy (**Supplementary Table**).

Treatment

A total of 43 patients were initially treated with 5 mg per day with a 28-day treatment cycle (3 weeks on/1 week off). Three patients had an initially reduced dose of 4 mg (two patients) and 3 mg (one patient) according to the judgment of clinicians. Six (13.0%) patients experienced dose reduction, 12 (26.1%) patients had treatment interruption, and 6 (13.0%) patients discontinued the therapy. Therapies after fruquintinib were as follows: rechallenge of chemotherapy with or without bevacizumab in 16 (34.8%) patients and local therapy in 4 (8.7%) patients.

Efficacy

With a median follow-up time of 9.7 months (range, 1.2–16.4 months), the median PFS and OS were 3.1 months (95% confidence interval [CI], 1.9–4.3 months) and 9.0 months (95% CI, 7.2–10.8 months), respectively (**Figure 1**). The ORR and DCR rates were 4.9 and 51.2%, respectively. Patients previously treated with anti-VEGFR agents had shorter median PFS than patients not previously treated with anti-VEGFR agents (1.9 months [95% CI, 1.1–2.6 months] *vs.* 3.7 months [95% CI, 3.4–4.1 months], P = 0.006). However, no significant difference was observed in median OS between the two groups (9.0 months [95% CI, 6.8–11.2 months] *vs.* 8.5 months [95% CI, 6.4–10.7 months], P = 0.992) (**Figure 2**).

Univariate and Multivariate Analyses

Univariate and multivariate analyses were performed to identify independent prognostic factors (**Tables 2** and **3**). The univariate analysis revealed that previous anti-VEGFR agents (hazard ratio [HR], 2.423; 95% CI, 1.245–4.715; P = 0.009), NLR (HR, 1.976; 95% CI, 1.061–3.682; P = 0.032), and hand-foot syndrome (HFS) (HR, 2.153; 95% CI, 1.077–4.304; P = 0.030) were significantly associated with PFS. NLR (HR, 2.332; 95% CI, 1.085–5.011;

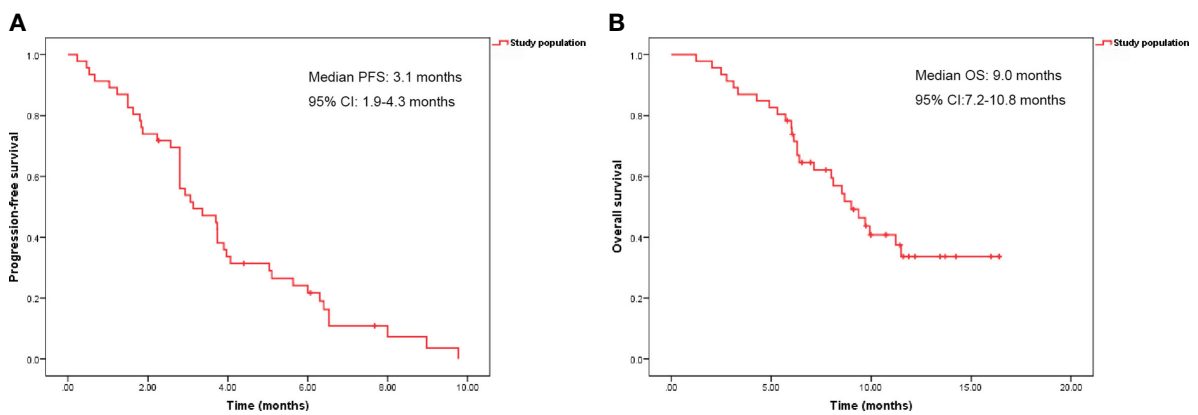

FIGURE 1 | Kaplan-Meier curves of progression-free survival **(A)** and overall survival **(B)** in 46 patients.

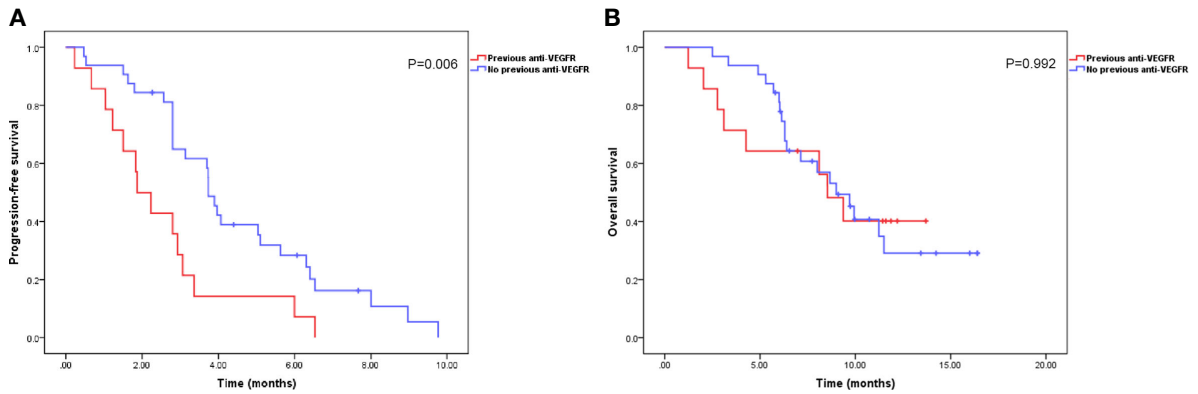

FIGURE 2 | Kaplan-Meier curves of progression-free survival **(A)** and overall survival **(B)** in patients with or without previous anti-vascular endothelial growth factor receptor (VEGFR) agent treatment.

P = 0.030) was significantly associated with OS. Multivariate analysis demonstrated that previous anti-VEGFR therapy (HR, 2.021; 95% CI, 1.009–4.074; P = 0.047) and elevated NLR (HR, 2.230; 95% CI, 1.191–4.517; P = 0.014) were independent prognostic factors in PFS. Moreover, elevated NLR (HR, 4.221; 95% CI, 1.683–10.586; P = 0.002) was an independent prognostic factor in OS.

Subgroup Analysis

Since patients with elevated NLR were likely to have poor survival outcomes, we investigated whether NLR was able to predict survival outcomes in patients with or without previous treatment with anti-VEGFR agents (**Table 4**). In the previous anti-VEGFR group, patients with NLR >3 had shorter median PFS compared with patients with NLR ≤3 (1.8 *vs.* 3.4 months; P = 0.026). In the no previous anti-VEGFR group, patients with NLR >3 had shorter median OS compared with patients with NLR ≤3 (6.0 *vs.* 11.5 months; P = 0.003).

Safety

AEs during treatment are listed in **Table 5**. The most common non-hematological AEs were HFS (37.0%), hepatotoxicity (32.6%), and hypertension (28.3%), while the most common ≥ Grade 3 AEs were HFS (13.0%), hypertension (6.5%), and hepatotoxicity (4.3%). Diarrhea and proteinuria occurred in 13.0 and 6.5% patients, respectively, with no patients suffering from these two AEs above Grade 2. Fatigue affected 26.1% of patients with 1 patient experiencing ≥ Grade 3 fatigue. Two patients experienced Grade 1 bleeding. The most common hematological AEs was anemia (39.1%), while the most common ≥ Grade 3 hematological AEs was thrombocytopenia (8.7%). No treatment-related death occurred. The most common AEs related to dose reduction was HFS (3/6, 50.0%), while the leading three causes of treatment interruption were thrombocytopenia (4/12, 33.3%), HFS (3/12, 25.0%), and proteinuria (2/12, 16.7%). In addition, fruquintinib discontinuation was observed in six patients due to HFS (3/6, 50%), proteinuria (1/6, 16.7%), and patients' own reasons (2/6, 33.3%).

DISCUSSION

Angiogenesis plays a crucial role in tumor growth, because the tumor-associated neovasculature supplies oxygen and nutrients to support tumor cell survival (19). Since Kim et al. (20) found that anti-vascular endothelial growth factor (VEGF) antibodies impaired neovascularization and tumor growth in mice, VEGF/VEGFR inhibitors have been widely explored in various advanced cancers (21). As treatment after first- and second-line therapies in mCRC is limited, the use of VEGFR inhibitors has been investigated. In the CORRECT trial (5), the median PFS and OS were improved in the regorafenib group compared with the placebo group (1.9 *vs.* 1.7 months, P < 0.0001; and 6.4 *vs.* 5.0 months, P = 0.0052; respectively) in mCRC refractory to at least two line standard therapies. These results were confirmed in the CONCUR trial (median PFS: 3.2 *vs.* 1.7 months, P < 0.0001; median OS: 8.8 *vs.* 6.3 months, P = 0.00016; respectively) (6). A recent prospective study of apatinib also showed efficacy in chemotherapy-refractory mCRC with median PFS and OS of 4.8 months (95% CI, 3.653–5.887 months) and 9.1 months (95% CI, 5.155–13.045 months), respectively (22). In the present study, we retrospectively explored the efficacy of the newly approved VEGFR inhibitor, fruquintinib, and observed shorter median PFS (3.1 *vs.* 3.7 months) and OS (8.6 *vs.* 9.3 months) compared with the FRESCO trial. The DCR rate was also lower in the present study compared with that in the FRESCO trial (51.2 *vs.* 62.2%).

The results of this retrospective study could be interpreted in several aspects. First, bevacizumab combined with chemotherapy is recommended as standard therapy for mCRC (3), but the impact of prior bevacizumab on later treatment is still unclear. Retrospective and prospective studies (22, 23) regarding the efficacy of apatinib in mCRC revealed no significant differences between patients with or without prior treatment with bevacizumab in PFS and OS. Besides, clinical trials (10, 11) evaluating the efficacy of fruquintinib did not exclude patients with previous use of bevacizumab. In the present study, 82.6% of patients had received bevacizumab previously, and multivariate analysis showed that previous use of bevacizumab had a non-statistically significant impact on OS (P = 0.078) in treatment-refractory mCRC treated with fruquintinib. This was

TABLE 2 | Univariate analysis of PFS and OS in 46 patients.

Variables	PFS		OS	
	Univariate analysis		Univariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Age (years)				
<60	Ref.		Ref.	
≥60	1.362 (0.734–2.527)	0.328	1.036 (0.486–2.210)	0.926
Gender				
Male	Ref.		Ref.	
Female	1.048 (0.556–1.974)	0.886	1.273 (0.589–2.752)	0.540
ECOG PS				
0–1	Ref.		Ref.	
2	1.240 (0.377–4.081)	0.723	1.061 (0.251–4.491)	0.936
Primary site				
Colon	Ref.		Ref.	
Rectum	0.509 (0.168–1.541)	0.232	0.875 (0.333–2.305)	0.788
Unknown	0.615 (0.163–2.325)	0.474	0.668 (0.207–2.157)	0.500
Metastatic organs				
1–2	Ref.		Ref.	
≥3	1.703 (0.886–3.275)	0.110	1.993 (0.134–7.376)	0.398
RAS mutant				
Yes	Ref.		Ref.	
No	1.225 (0.518–2.896)	0.644	1.494 (0.494–4.519)	0.477
Unknown	1.805 (0.690–4.723)	0.229	1.867 (0.559–6.232)	0.310
Lines of previous therapy				
2	Ref.		Ref.	
≥3	1.187 (0.589–2.392)	0.631	1.830 (0.691–4.844)	0.188
Previous Bevacizumab				
Yes	Ref.		Ref.	
No	1.017 (0.446–2.319)	0.968	2.659 (0.997–7.091)	0.051
Previous anti-VEGFR				
Yes	Ref.		Ref.	
No	2.423 (1.245–4.715)	0.009	1.004 (0.438–2.303)	0.992
Sufficient Treatment*				
Yes	Ref.		Ref.	
No	1.093 (0.584–2.045)	0.782	1.236 (0.577–2.650)	0.586
NLR				
≤3	Ref.		Ref.	
>3	1.976 (1.061–3.682)	0.032	2.332 (1.085–5.011)	0.030
PLR				
>300	Ref.		Ref.	
150–300	0.659 (0.283–1.534)	0.333	1.122 (0.425–2.962)	0.816
<150	0.570 (0.234–1.385)	0.214	0.396 (0.137–1.147)	0.088
CEA				
≤5	Ref.		Ref.	
>5	1.308 (0.506–3.382)	0.579	1.072 (0.321–3.577)	0.910
Hand-foot syndrome				
Yes	Ref.		Ref.	
No	2.153 (1.077–4.304)	0.030	1.262 (0.565–2.814)	0.570
Hypertension				
Yes	Ref.		Ref.	
No	1.796 (0.869–1.796)	0.114	1.291 (0.545–3.060)	0.562
Fatigue				
Yes	Ref.		Ref.	
No	1.128 (0.544–2.340)	0.746	1.987 (0.750–5.264)	0.167

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; VEGFR, vascular endothelial growth factor receptor; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio, CEA, carcinoembryonic antigen.
**Fruquintinib was administered without dose reduction, treatment interruption, or therapy discontinuation.*

consistent with previous results, indicating that different mechanisms might exist between VEGF and VEGFR inhibitors in suppressing tumor growth, resulting in scarce cross-resistance. However, the underlying mechanism is still unclear, and further studies are expected. Second, we observed poor median PFS in patients previously treated with anti-VEGFR agents (regorafenib and apatinib). Regorafenib is an anti-VEGFR TKI that inhibits angiogenesis (VEGFR-1, -2, -3, and TIE2) and oncogenic receptor tyrosine kinases (4). Apatinib is an anti-VEGFR TKI that targets VEGFR-2, as well as c-KIT, RET, and c-SRC (24). Fruquintinib is an

TABLE 3 | Multivariate analysis of PFS and OS in 46 patients.

Variables	PFS		OS	
	Multivariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Metastatic organs				
1–2	Ref.			
≥3	1.701 (0.814–3.552)	0.143		
Lines of previous therapy				
2			Ref.	
≥3			1.745 (0.649–4.695)	0.270
Previous Bevacizumab				
Yes			Ref.	
No			2.458 (0.906–6.673)	0.078
Previous anti-VEGFR				
Yes	Ref.			
No	2.021 (1.009–4.074)	**0.047**		
NLR				
≤3	Ref.		Ref.	
>3	2.320 (1.191–4.517)	**0.014**	4.221 (1.683–10.586)	**0.002**
PLR				
>300			Ref.	
150–300			2.295 (0.920–9.296)	0.771
<150			0.836 (0.252–2.780)	0.069
Hand-foot syndrome				
Yes	Ref.			
No	1.807 (0.703–4.462)	0.219		
Hypertension				
Yes	Ref.			
No	1.005 (0.664–1.521)	0.920		
Fatigue				
Yes			Ref.	
No			2.153 (0.759–6.108)	0.149

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; VEGFR, vascular endothelial growth factor receptor; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; CEA, carcinoembryonic antigen.
Bold values represent the P-values that are statistically significant.

TABLE 4 | Survival outcomes in patients with or without previous anti-VEGFR agents stratified by NLR.

Variables	Median PFS (months, 95% CI)			Median OS (months, 95% CI)		
	NLR ≤3	NLR >3	P	NLR ≤3	NLR >3	P
Previous anti-VEGFR	3.4 (2.4–4.3)	1.8 (0.9–2.8)	0.026	9.4 (6.7–12.1)	8.5 (6.4–10.7)	0.751
No previous anti-VEGFR	4.0 (3.5–4.4)	3.7 (3.4–4.1)	0.365	11.5 (8.7–14.3)	6.0 (5.0–7.1)	0.003

PFS, progression-free survival; OS, overall survival; CI, confidence interval; VEGFR, vascular endothelial growth factor receptor; NLR, neutrophil-lymphocyte ratio.

anti-VEGFR TKI that inhibits VEGFR-1, -2, and -3 (25). Thus, we postulated that prior regorafenib and apatinib might decrease the efficacy of fruquintinib because of their overlapping functions, leading to shorter median PFS. These results suggested that fruquintinib might not be a good choice following treatment with anti-VEGFR agents. The current NCCN guidelines recommend ramucirumab combined with FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) for the treatment of mCRC with disease progression after previous oxaliplatin based therapy without irinotecan (3). Moreover, the REVERCE study (26) reported longer median OS with regorafenib followed by cetuximab ± irinotecan rather than cetuximab ± irinotecan followed by regorafenib (17.4 *vs.* 11.6 months; P = 0.0293) in KRAS exon 2 wild-type mCRC after failure of fluoropyrimidine, oxaliplatin, and irinotecan. With further investigations of anti-VEGFR therapy in mCRC as first- or second-line therapy, the potential use of fruquintinib requires further study. We are thus anticipating the results of two clinical trials, a global phase III trial (NCT04322539) investigating efficacy and safety of fruquintinib in patients with refractory mCRC, and a phase II trial (NCT04296019) exploring efficacy and safety of fruquintinib as a maintenance therapy following first-line treatment for mCRC. On the other hand, mCRC is molecularly heterogeneous with various biomarkers predicting response to treatment, such as RAS and BRAF mutations. This leads to significant challenges in planning an optimal treatment strategy for the refractory population (27). Clinical trials have explored the possibility of rechallenge of chemotherapy with or without targeted drugs such as cetuximab or bevacizumab (28–30), combination

TABLE 5 | Adverse events of 46 patients treated with fruquintinib.

Adverse events	Any grade	Grade ≥3
Non-hematologic		
Hypertension	13 (28.3)	3 (6.5)
Hand-foot syndrome	17 (37.0)	6 (13.0)
Proteinuria	3 (6.5)	0 (0.0)
Hepatotoxicity	15 (32.6)	2 (4.3)
Fatigue	12 (26.1)	1 (2.2)
Bleeding	2 (4.3)	0 (0.0)
Diarrhea	6 (13.0)	0 (0.0)
Hematologic		
Leukopenia	6 (13.0)	2 (4.3)
Neutropenia	5 (10.9)	1 (2.2)
Thrombocytopenia	11 (23.9)	4 (8.7)
Anemia	18 (39.1)	2 (4.3)

therapy including anti-BRAF and anti-HER2 agents according to BRAF mutation and HER2 amplification status (31, 32), and interventional techniques for local disease (33) in treatment-refractory mCRC. These explorations reported promising efficacy and safety, although high-quality evidence is still limited. Therefore, treatment strategies based on biomarker examination and disease evaluation might be the future direction for the refractory population. Third, we observed statistical significance of HFS in PFS in univariate analysis (HR, 2.153; 95% CI, 1.077–4.304; P = 0.030). A previous meta-analysis reported that VEGFR TKIs increased the risk of HFS (P < 0.00001) (34). Studies have indicated that HFS might result from inhibition of targeted receptors in healthy tissue by anti-angiogenic agents, revealing potential effectiveness of VEGF/VEGFR blockade (35, 36). Thus, we proposed that the presence of HFS might be an indicator of effectiveness of fruquintinib, and further studies with large sample size are expected. Fourth, the predictive potential of systemic inflammation in CRC has been widely investigated (37). Neutrophils are a major component of peripheral blood and are considered inflammatory cells. They are engaged in supporting adaptive immunity yielding signaling molecules that include the angiogenetic growth factor VEGF (38). Lymphocytes are considered an important part of anti-tumor immunity leading to cytotoxic cell death. High neutrophils or low lymphocytes in the peripheral circulation may result in a reduced immunological response, which can weaken treatment efficacy and lead to poor survival outcomes. In the present study, we identified NLR as an independent prognostic factor in PFS and OS among patients treated with fruquintinib, consistent with previous results (13, 14). Fifth, patients in our cohort showed shorter OS compared with the FRESCO trial. We prefer to attribute these results to older patients with poor performance status as well as the presence of metastatic lesions in multiple organs in the patients enrolled in our study.

The AEs profile in our study had some differences compared with that in the FRESCO trial (11). Hypertension (55.4%) was the most common side effect in the FRESCO trial, while HFS (37.0%) was the most common AE in our cohort. We prefer to attribute this difference to outpatient treatment, resulting in unsatisfactory observation of hypertension. In addition, the incidence of hematologic AEs was frequent in the present study. Anemia, which was not reported in the FRESCO trial, had a high rate of 39.1% in our cohort, and 8.7% patients experienced ≥ Grade 3 thrombocytopenia. This might result from heavy previous treatment (lines of previous therapy ≥3, 58.7%) and poor performance status (ECOG PS = 2, 8.7%) prior to treatment with fruquintinib.

The present study also had limitations. This was a retrospective study from a single institution with a small sample size. Although bias was unavoidable, we collected detailed data to reveal real-world treatment of fruquintinib monotherapy in mCRC refractory to standard therapies.

CONCLUSION

Our study demonstrated that fruquintinib was effective as a third-line therapy for mCRC refractory to standard therapies with tolerable AEs. The benefit of fruquintinib in patients previously treated with anti-VEGFR agents was decreased. In addition, NLR was an independent prognostic factor in the efficacy of fruquintinib. Because this is a retrospective study with a small sample size from a single institution, further prospective investigations are warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Sun Yat-sen University Cancer Center. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LX: concept and design. LW, HC: data collection and statistical analysis. LW, HC, CJ, WH: results interpretation and manuscript writing. YY, KP, YJ: manuscript revision. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.587692/full#supplementary-material

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SYSTEMATIC REVIEW
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Clinical Efficacy and Safety of Hyperthermic Intraperitoneal Chemotherapy in Colorectal Cancer Patients at High Risk of Peritoneal Carcinomatosis: A Systematic Review and Meta-Analysis

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Background: Hyperthermic intraperitoneal chemotherapy (HIPEC) is an effective measure for improving the prognosis of colorectal cancer (CRC) patients with peritoneal carcinomatosis (PC). However, the role of HIPEC in CRC patients at high risk of PC remains controversial. The current systematic review and meta-analysis aimed to evaluate the clinical efficacy and safety of HIPEC in CRC patients at high risk of PC.

Methods: We performed a systematic search of PubMed, Embase, Cochrane Library, and other online databases up to July 30, 2020. The clinical data, including overall survival, disease free survival, peritoneal metastasis rate, and postoperative adverse reaction were screened and analyzed after data extraction. Risk ratios (RRs) were applied to analyze these dichotomous outcomes with a random effects model.

Results: A total of 6 available clinical studies involving 603 patients were finally included. CRC patients at high risk of PC who proactively underwent HIPEC treatment showed a significantly reduced peritoneal metastasis rate (RR: 0.41, 95% CI: 0.21–0.83, $P = 0.01$; $I^2 = 58\%$) compared to the similarly high-risk in CRC patients who did not receive HIPEC treatment. However, in terms of overall survival (RR: 1.13, 95% CI: 0.97–1.33, $P = 0.12$; $I^2 = 77\%$), disease-free survival (RR: 1.10, 95% CI: 0.75–1.59, $P = 0.63$; $I^2 = 53\%$), progression free survival (RR: 1.85, 95% CI: 0.48–7.14, $P = 0.37$; $I^2 = 93\%$), and postoperative adverse reactions (RR: 0.1.07, 95% CI: 0.36–3.15, $P = 0.90$; $I^2 = 78\%$), there was no significant difference between the HIPEC treatment and control groups.

Conclusions: Proactive HIPEC treatment did not show the expected clinical efficacy in prolonging the overall survival time, disease-free survival time, and progression-free

survival time of CRC patients at high risk of PC. However, the preemptive administration of HIPEC was associated with a reduced peritoneal metastasis rate and did not cause adverse additional postoperative effects.

Keywords: colorectal cancer, HIPEC, peritoneal carcinomatosis, survival, meta- analysis

INTRODUCTION

Colorectal cancer (CRC) is the most common malignancy of the digestive system, and the latest statistics show that the mortality of CRC ranks second for men and women combined in the United States (1, 2). The peritoneum is the second most likely metastasis site of CRC (3), and the prognosis of CRC patients with peritoneal metastasis (PM) is extremely poor, with a median survival time of 16.8 months (4). Methods of improving the survival rate of CRC patients with PM is the main focus and challenge of CRC research.

In the past few decades, the most common clinical treatment for CRC patients with PM has been systemic intravenous chemotherapy or palliative tumor reduction surgery (5, 6). The emergence of hyperthermic intraperitoneal chemotherapy (HIPEC) treatment greatly alleviated the previous dilemma. HIPEC refers to a novel treatment technique that can prevent and treat primary or secondary peritoneal cancer (PC) by heating the perfusate containing chemotherapeutics to the treatment temperature, and then infusing it into the patients' abdominal cavity for a certain period of time (7, 8). Baratti's study showed that HIPEC was effective in treating CRC patients with PM, and treatment by cytoreductive surgery (CRS) combined with HIPEC extended the median survival time of these patients up to 32 months (9). In addition, multiple clinical studies have shown that patients with peritoneal spread of CRC who undergo CRS plus HIPEC have a 5-year survival rate of 33–58% (10–12). CRS plus HIPEC has gradually become the mainstream treatment for CRC patients with PM on account of its relatively stable safety and efficacy. However, some CRC patients have high risk factors for PM, but their imaging and pathology reports do not confirm peritoneal metastases, such as ovarian metastases from CRC or perforation of the tumor (13). Unfortunately, the detection of PM at an early stage remains elusive due to the lack of typical clinical symptoms and the poor accuracy of imaging (14). In the case of such patients, prophylactic treatment by HIPEC may increase potential clinical benefits. However, some experts have raised different opinions since they believe that the preemptive administration of HIPEC has no clinical basis and may increase adverse reactions in patients (15, 16).

Many systematic reviews and meta-analyses explore the efficacy of HIPEC in CRC patients with PM, but whether HIPEC is effective in CRC patients at high risk of PM is rarely mentioned (17–19). This systematic review and meta-analysis, therefore, aimed to evaluate all published available clinical studies on the efficacy and safety of HIPEC in patients with CRC who are at a high risk of peritoneal carcinomatosis.

Abbreviations: CRC, Colorectal cancer; PC, peritoneal carcinomatosis; PM, peritoneal metastasis; HIPEC, hyperthermic intraperitoneal chemotherapy; CRS, cytoreductive surgery; RRs, Risk Ratios; 95%CI, 95% confidence intervals; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; RCTs, randomized controlled trials.

METHODS

Search Strategy

A comprehensive literature search, irrespective of language, was conducted in multiple online databases including PubMed, Embase, and Cochrane Library, up to July 30, 2020. Our strategy that included a combination of exploded medical subject heading (MeSH) terms and the entry terms: "Colorectal Neoplasm," "Neoplasm, Colorectal," "Colorectal Carcinoma," "Carcinoma, Colorectal," "Colorectal cancer," "Cancer, Colorectal," "Colorectal Tumor," "Tumor, Colorectal," "Hyperthermic Intraperitoneal Chemotherapy," "Chemotherapy, Hyperthermic Intraperitoneal," "Hyperthermic Intraperitoneal Chemotherapies," "Intraperitoneal Chemotherapy, Hyperthermic," and "HIPEC." Studies were also identified by screening the reference lists of systematic reviews on similar subjects. The current study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statements (20).

Study Selection

The current meta-analysis included clinical studies comparing the efficacy and safety of HIPEC administration with control groups that did not undergo HIPEC treatment among adult CRC patients at high risk of PC (minimal PC that was completely resected at the same time as the primary tumor; synchronous or metachronous ovarian metastasis; perforated primary tumor inside the peritoneal cavity for some pathologies or iatrogenic reasons), regardless of study type (RCTs or non-RCTs). Considering the limited evidence on gray data, we did not include conference abstract-type research.

The exclusion criteria were as follows: (1) studies with CRC patients who had developed peritoneal metastases or liver metastases; (2) studies with no control groups or with CRC patients in the control group who also underwent HIPEC treatment; (3) studies involving PM that might have originated from areas other than a colorectal origin; (4) ongoing clinical trials; and (5) studies with a lack of sufficient information or without follow-up.

All studies were independently identified by two reviewers. In both the inclusion and exclusion processes, titles and abstracts were initially screened, and any conflicts between two reviewers were resolved by consensus. After screening the titles or abstracts, the full-text was subsequently assessed to determine the eligibility of a particular study.

Data Extraction and Quality Evaluation

Two reviewers independently extracted data from all eligible studies with a standardized and predesigned form. Characteristics including the first author, year of publication, type of study, the total number of enrolled patients, intervention of treatment and comparison groups, HIPEC methodologies, endpoints, and follow-up times were recorded. Similarly, we resolved inconsistencies in the extracted data by discussion until a consensus was reached.

The risk of bias of each included study was assessed according to its study type. The quality of randomized controlled trials (RCTs) was judged using the Jadad scoring system composed of randomization, double-blinding, withdrawals, and dropouts (21). A score of 0 to 5 was assigned to each trial. If a study scored higher than 3, it was deemed a high-quality study with a low risk of bias. Likewise, the Newcastle Ottawa Scale (NOS) was used to assess the quality of the observational study (cohort study and case-control study) (22). According to this scale, each study was judged through 3 categories (selection, comparability, exposure, or outcomes) and assigned a score of 0 to 9 stars. A study with a score of higher than 5 stars indicated high quality and a low risk of bias.

Outcome Measurements

We chose 3- or 5-year overall survival (OS) as the primary endpoint due to its generalizability in determining the prognosis of tumor patients. In addition, OS was preferentially reported by the majority of the included studies.

The secondary endpoints included 3- or 5-year disease-free survival (DFS), progression-free survival (PFS) and the incidence of peritoneal metastases or local recurrence. In addition, the rate of postoperative adverse reactions was also analyzed as it reflected the safety of treatment.

Statistical Analysis

Meta-analysis was conducted using ReviewManager (RevMan 5.3, Copenhagen: the Nordic Cochrane Center, the Cochrane Collaboration, 2014). We applied risk ratios (RRs) for dichotomous outcomes, and pooled proportions were calculated with a 95% confidence interval (95% CI). The I^2 statistic was calculated to evaluate the heterogeneity of each outcome. If $I^2 > 50\%$ was considered significant heterogeneity, a random effects model was applied; otherwise, a fixed-effects model was used accordingly. A funnel plot was constructed and visually inspected to assess publication bias. We also conducted Begg's and Egger's tests. A two-sided P-value of < 0.05 was deemed statistically significant. Considering the great heterogeneity in risk factors for PC and methodology of HIPEC, we performed subgroup analysis combined with sensitivity analysis to seek potential influencing factors, as well as to validate the consistency and robustness of our findings. All included clinical studies were stratified by type of study (RCT or non-RCT), outcome report, patients subgroup, and HIPEC drug (oxaliplatin alone or not).

RESULTS

Literature Search and Characteristics of Included Studies

A total of 1,895 potentially relevant records were identified through the database search (681 from PubMed, 1,086 from Embase, and 107 from Cochrane Library) and other sources (6 from conference abstracts and 15 from reference lists). After screening titles and abstracts, 1,869 studies were excluded on account of duplication, already occurring peritoneal metastasis, studies that included other sources of cancer, texts that were not original publications, and those that performed the wrong intervention or comparison. The 26 remaining studies were further evaluated for eligibility via a full-text review, and 20 of them were excluded due to no comparisons, incorrect interventions, and irrelevant outcomes, etc. Finally, 6 clinical studies met the criteria and were included in the meta-analysis. The detailed screening process is shown in **Figure 1**.

The characteristics of all included studies are presented in **Table 1**. Six clinical studies (4 observational studies and 2 RCTs) involving 603 patients were enrolled in this systematic review and meta-analysis (23–30). Among all included studies, 2 studies administered HIPEC treatment only in the experimental group, while the remaining 4 studies conducted treatment by HIPEC combined with curative surgery or adjuvant systemic chemotherapy in the intervention group. The majority of studies also chose OS as the primary outcome, while Goéré, Charlotte, and Elias selected 3-year DFS, 18-month PFS, and 3-year DFS, respectively, as their first outcome.

Quality Assessment

All the observational studies (3 cohort studies and 1 case-control study) scored higher than 5 stars according to NOS and were thus considered to be of high quality and low risk of bias (**Supplementary Tables 1**, **2**). Only 2 RCTs were included in our study, and these had a score of 3 in the Jadad scoring system (**Supplementary Table 3**). Both RCTs met the randomization requirements but the blinding method was not effectively implemented, which indicated a high risk of performance bias.

Primary Outcome: Overall Survival

All six studies included in the systematic review and meta-analysis consistently reported OS as one of the clinical outcomes, while the follow-up time of each study remained irregular (3 years, 5 years, or other timespans). We found that CRC patients at high risk of PC who were undergoing HIPEC treatment had no survival time benefit compared to those undergoing standard treatment without HIPEC (RR: 1.13; 95% CI: 0.97–1.33; $P = 0.12$; $I^2 = 77\%$) (**Figure 2**). The heterogeneity test showed that the conclusion was proven to be stable by excluding every study, each at a given time.

Secondary Outcomes

The detailed characteristics of each secondary outcome are summarized in **Table 2**. As shown in this table, 3 clinical studies reported DFS, and 2 clinical articles mentioned PFS. In addition, 6 and 5 clinical studies selected the incidence

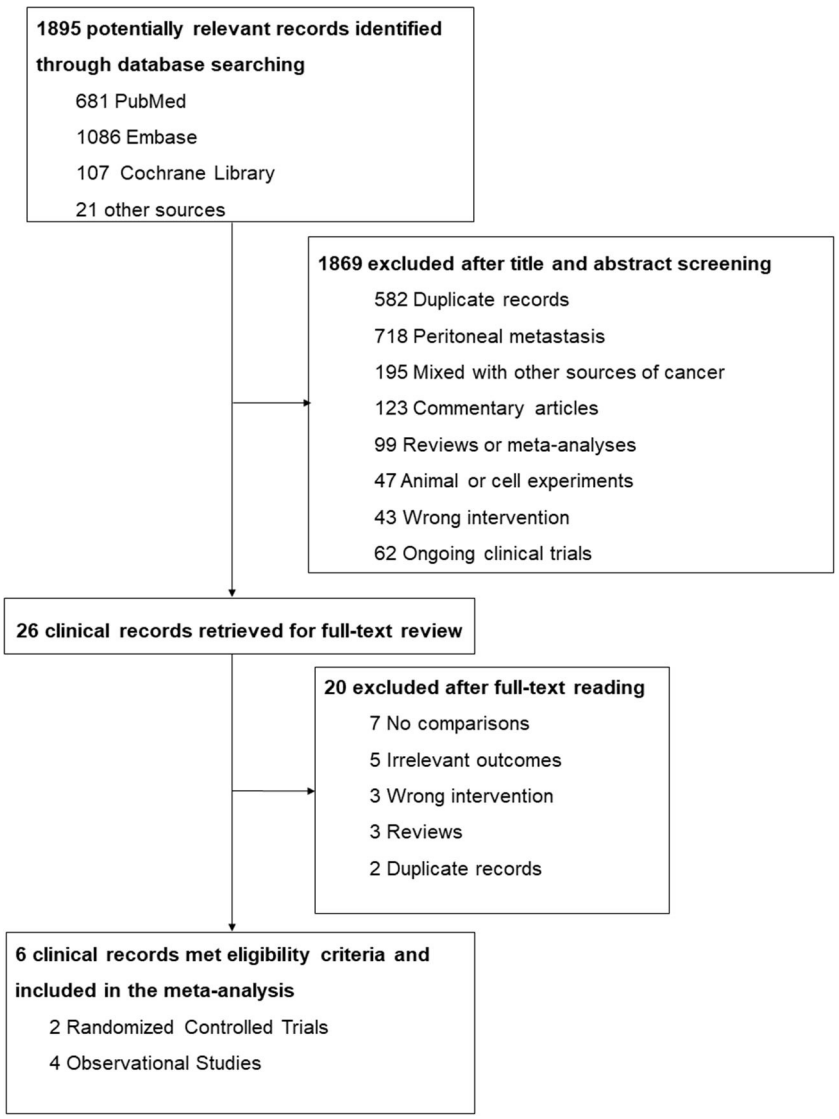

FIGURE 1 | Flowchart for the selection process.

of PM and postoperative adverse reactions, respectively, as outcome indicator.

Disease-Free Survival

A total of 3 studies involving 238 patients reported DFS, and the results showed that HIPEC treatment did not extend the DFS of CRC patients at high risk of PC (RR: 1.10; 95% CI: 0.75–1.59; $P = 0.63$; $I^2 = 53\%$) (**Figure 3**). Through performing a sensitivity analysis that excluded every study, each at a given time, we found that the conclusion was proven to be stable.

Progression Free Survival

Similar to the case of DFS, there were 2 studies with a total of 268 participants choosing PFS as secondary outcomes. Unfortunately, in terms of PFS, the HIPEC group did not show the expected efficacy (RR: 1.85; 95% CI: 0.48–7.14; $P = 0.37$; $I^2 = 93\%$) (**Figure 4**). However, this outcome displayed fairly high heterogeneity. Through sensitivity analysis, we found that Charlotte's study was the main source of heterogeneity and that if we excluded this study, the robustness of our conclusion would also be affected (RR: 3.75; 95% CI: 1.88–7.47; $P < 0.01$).

Incidence of Peritoneal Metastasis

The incidence of peritoneal metastasis after treatment was documented in all eligible studies. The results showed that prophylactic HIPEC treatment significantly reduced the incidence of peritoneal metastases in CRC patients at high risk of PC compared to the control group (RR: 0.41; 95% CI: 0.21–0.83; $P = 0.01$; $I^2 = 58\%$) (**Figure 5**). Although the heterogeneity was relatively high, the conclusion was quite stable after conducting a sensitivity analysis by excluding each study at a time from all qualified clinical studies.

TABLE 1 | Characteristic of included clinical studies.

Study	Types of studies	No. of patients	Intervention		Patients subgroup			HIPEC methodologies	Endpoints	Followup time
			Treatment group	Comparison group	Synchronous PC	Ovarian mtastases	Perforated tumor			
Elias et al. 2008 (23)	Cohort study	13	HIPEC	Complete exploration of the peritoneal cavity	6	1	6	43°C 30 min Oxaliplatin(460 mg/m^2)	DFS; OS; relapsed in the peritoneum; isolated visceral metastases; postoperative complication	3 years
Tentes et al. 2011 (24)	Cohort study	97	HIPEC	Intraperitoneal chemotherapy	Locally advanced colorectal carcinomas			42.5–43°C 90 or 60 min Mitomycin-C (15 mg/m^2) or Oxaliplatin(130 mg/m^2)	OS; peritoneal metastases; The incidence of recurrence; postoperative complication	3 years
Sammartino et al. 2014 (25)	Case-control study	75	HIPEC + proactive surgical	Standard surgical resection	Advanced colonic cancer			43°C 30 min Oxaliplatin(460 mg/m^2)	OS; DFS; peritoneal metastases local recurrence; postoperative complication	5 years
Baratti et al. 2016 (26)	Cohort study	66	HIPEC + curative surgery+ adjuvant systemic chemotherapy	Standard treatments	18	6	42	42.5°C 60 min Mitomycin-C (3.3 mg/m^2) and Cisplatin (25 mg/m^2)	OS; PFS; cumulative PM incidence; postoperative complication	5 years
Charlotte et al. 2019 (27)	Randomized controlled trials	202	HIPEC+ Chemotherapy	Chemotherapy	Resectable primary clinical or pathological T4N0–2M0 stage or perforated colon cancer			42°C 30 min Oxaliplatin(460 mg/m^2)	PFS; OS; peritoneal metastases quality of life; costs	1.5 years
Goéré et al. 2020 (28)	Randomized controlled trials	150	HIPEC + systematic second-look surgery	Surveillance	69	20	61	43°C 30 min Oxaliplatin(460 mg/m^2)	DFS; OS; peritoneal relapse postoperative complications	3 years

HIPEC, hyperthermic intraperitoneal chemotherapy; DFS, disease-free survival; OS, overall survival; PFS, progression-free survival.

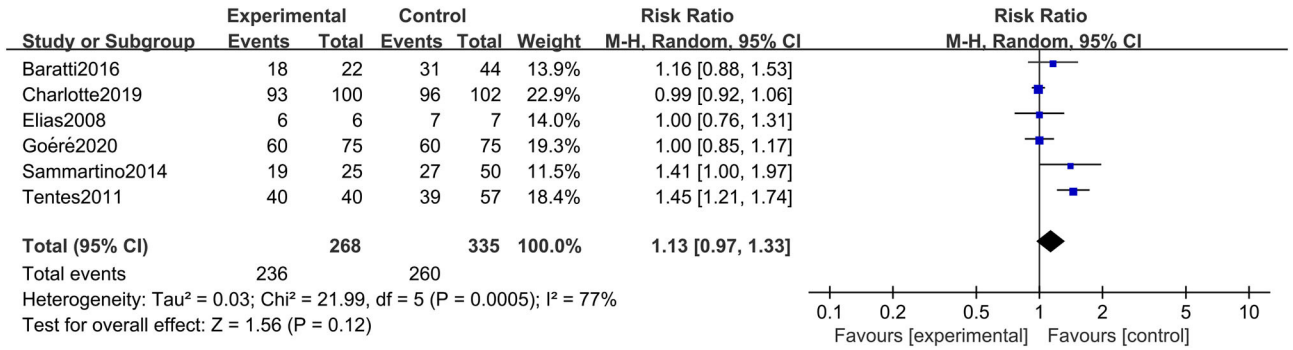

FIGURE 2 | Forest plot of OS comparing the experimental group to control group among CRC patients at high risk of PC.

TABLE 2 | Summary of primary and secondary outcomes.

Outcome	No. of studies	RR	95%CI	I^2	*P*-value
Primary endpoint					
OS	6	1.13	0.97–1.33	77%	0.12
Secondary endpoint					
DFS	3	1.10	0.75–1.59	53%	0.63
PFS	2	1.85	0.48–7.14	93%	0.37
PM	6	0.41	0.21–0.83	58%	**0.01**
Postoperative adverse reaction	5	1.07	0.36–3.15	78%	0.90

OS, overall survival; DFS, disease-free survival; PFS, progression free survival; PM, peritoneal metastasis; RR, risk ratio; CI, confidence interval.

Rate of Postoperative Adverse Reactions

The rate of postoperative adverse reactions was reported in 5 studies. As presented in **Figure 6**, we observed that there was no significant difference in the rate of postoperative adverse reactions between the two groups (RR: 1.07; 95% CI: 0.36–3.15; $P = 0.90$; $I^2 = 78\%$). Similarly, this conclusion was quite stable after conducting the sensitivity analysis.

Subgroup Analysis

The rigorous design and standardized implementation of RCTs mean that they have a high level of evidence. Considering that most of the included studies were observational studies, we conducted a subgroup analysis according to the study type. Two RCTs containing 352 patients were enrolled. As presented in **Figure 7**, although the heterogeneity was significantly reduced, it did not change the conclusion of our research, that is, there was no significant relationship between overall survival and the preventive implementation of HIPEC in the target patients (RR: 0.99; 95% CI: 0.93–1.06; $P = 0.77$; $I^2 = 0\%$). A total of 3 enrolled studies with 238 patients chose OS as the primary endpoint, while the remaining 3 studies chose DFS or PFS as their primary outcome. We observed statistically significant differences in OS between the experimental group and the control group in studies that chose OS as the primary endpoint (RR: 1.37; 95% CI: 1.19–1.57; $P < 0.01$; $I^2 = 0\%$) (**Figure 8**), which showed that preventive HIPEC treatment could extend the OS of CRC patients at high risk of PC. The reason might be that we unconsciously tended to report positive results when we took OS as the primary outcome.

Given the differences in the baseline statistics of the included patients, we also conducted a subgroup analysis according to the patient subgroup. Three studies including 229 patients carefully described and listed the number of colorectal cancer patients with peritoneal metastasis among different high-risk factors. As shown in **Figure 9**, there were still no significant differences in OS between the two arms when performing a subgroup analysis of studies that carefully described the patients' baseline data (RR: 1.03; 95% CI: 0.91–1.17; $P = 0.63$; $I^2 = 0\%$). Moreover, we conducted a subgroup analysis in the included studies that chose oxaliplatin alone as the chemotherapy drug during HIPEC treatment, and 4 studies involving 440 patients were accordingly enrolled. Surprisingly, choosing oxaliplatin alone as a HIPEC drug did not improve the overall survival time of CRC patients, while when such treatment was combined with other chemotherapy drugs, such as cisplatin or mitomycin-C, CRC patients at high risk of PM had significant survival benefits (RR: 1.33; 95% CI: 1.07–1.65; $P = 0.009$; $I^2 = 44\%$) (**Figure 10**). The subgroup analysis and sensitivity analyses on primary outcomes are presented in **Table 3**.

Publication Bias

A funnel plot was constructed to assess the possible publication bias of the primary outcome (**Figure 11**). There appeared to be no publication bias by

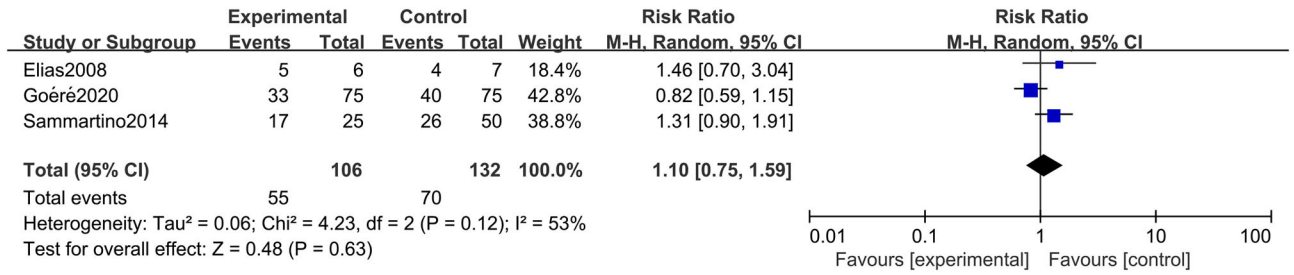

FIGURE 3 | Forest plot of DFS comparing the experimental group to control group among CRC patients at high risk of PC.

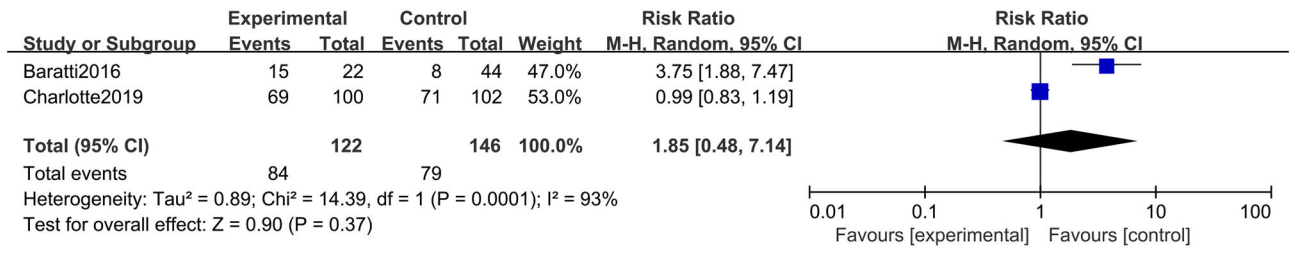

FIGURE 4 | Forest plot of PFS comparing the experimental group to control group among CRC patients at high risk of PC.

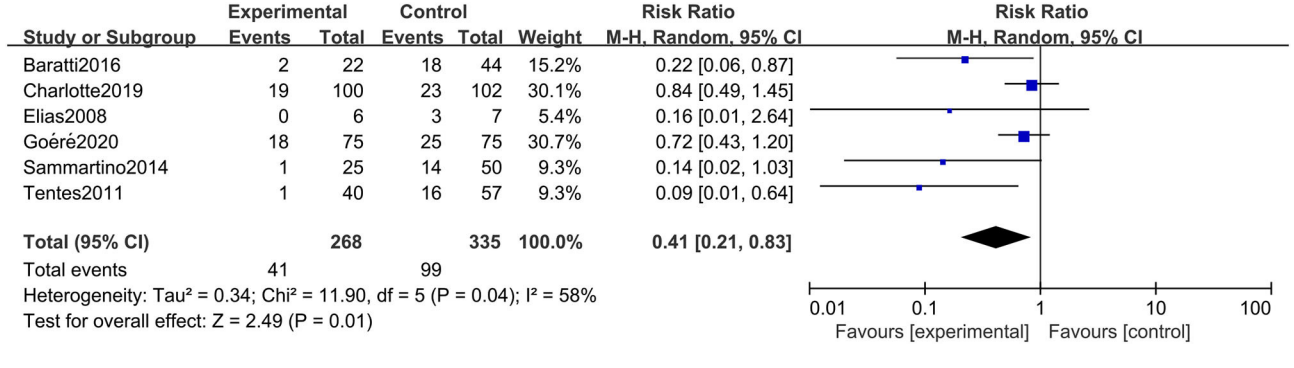

FIGURE 5 | Forest plot of incidence of PM comparing the experimental group to control group among CRC patients at high risk of PC.

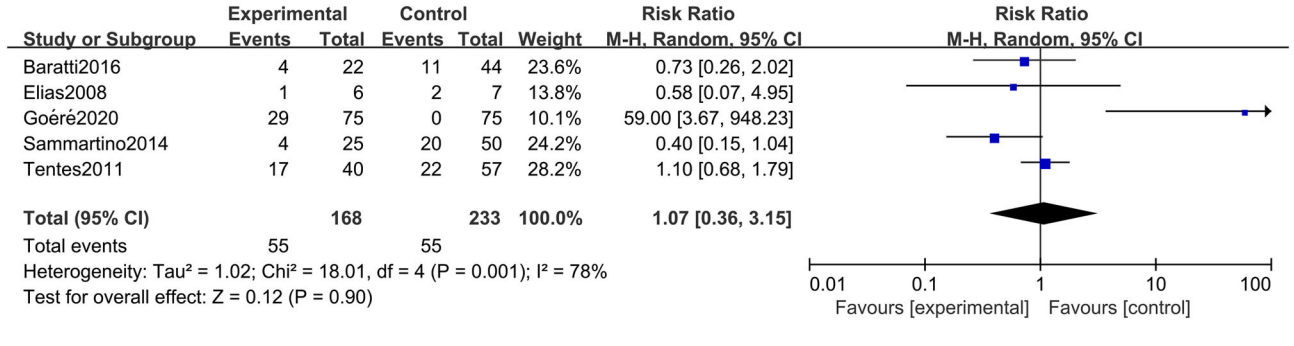

FIGURE 6 | Forest plot of rate of postoperative adverse reactions comparing the experimental group to control group among CRC patients at high risk of PC.

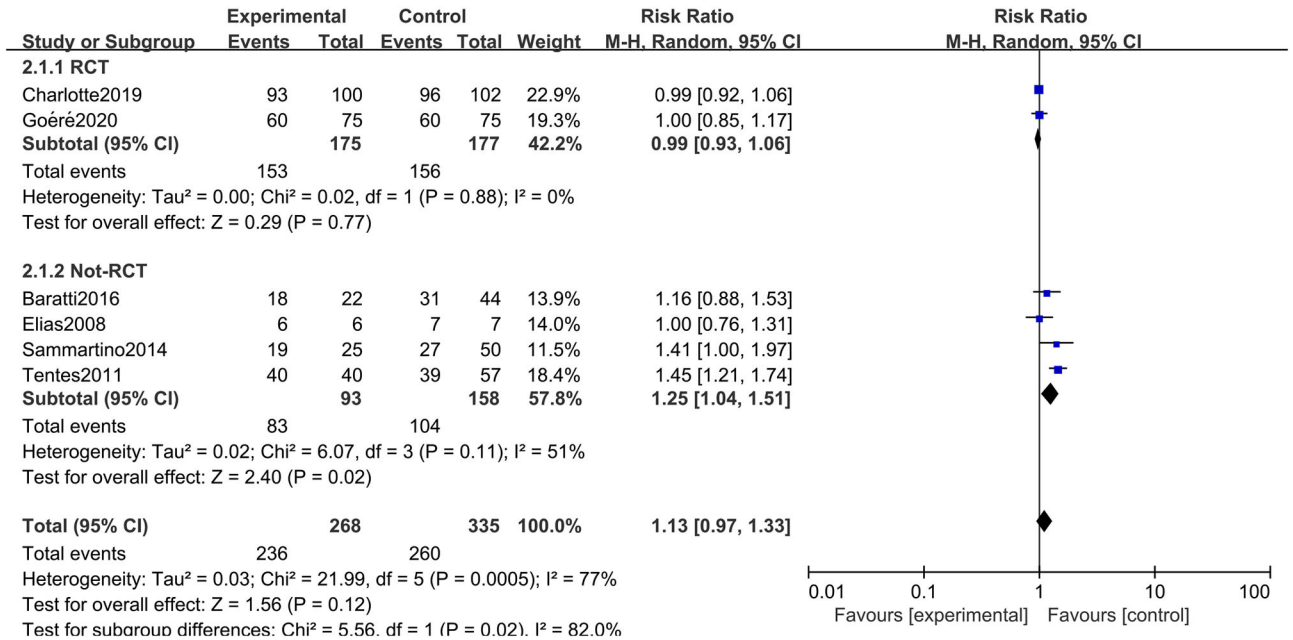

FIGURE 7 | Forest plot of subgroup analysis of RCTs.

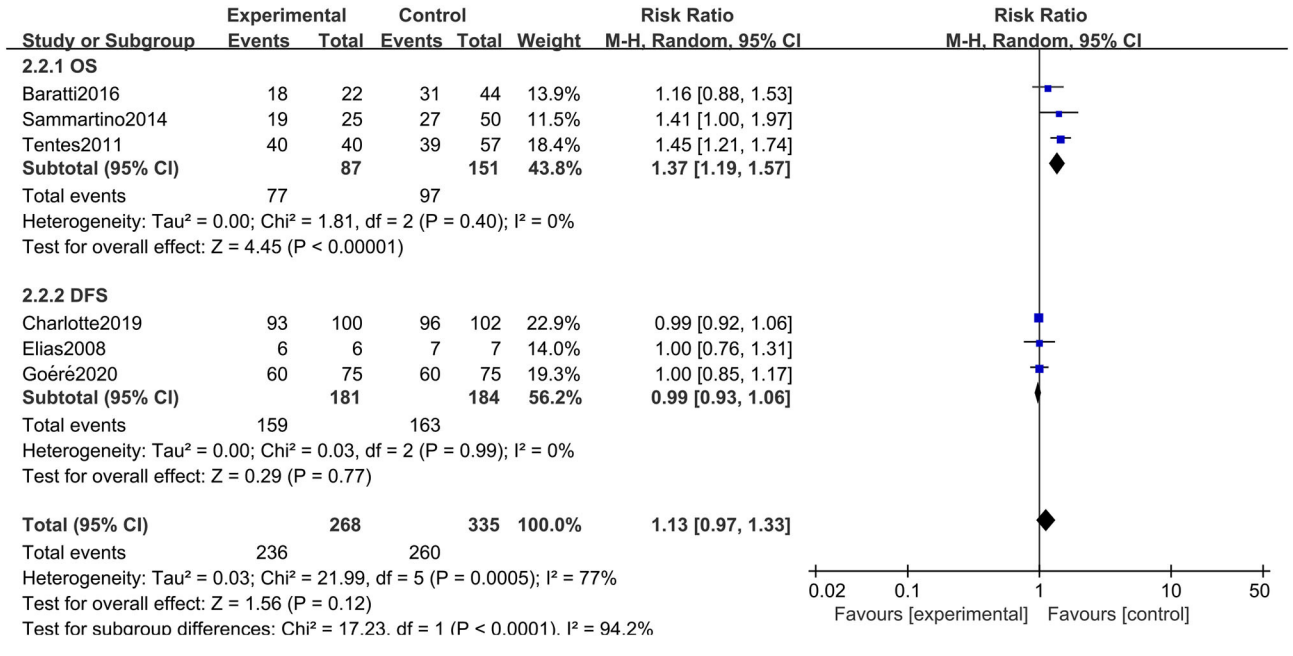

FIGURE 8 | Forest plot of subgroup analysis of studies whose primary outcome was OS.

visually inspecting the funnel plot. For further verification, we conducted Begg's test and Egger's test to evaluate the funnel plot of OS. The results showed that there was no statistically significant evidence of publication bias (Begg's test: $P = 0.12$; Egger's test: $P = 0.36$).

DISCUSSION

In this systematic review and meta-analysis, we evaluated the clinical efficacy and safety of the preventative administration of HIPEC among CRC patients at high risk of PC. Unfortunately, we found that preemptive HIPEC did not improve the OS of selected

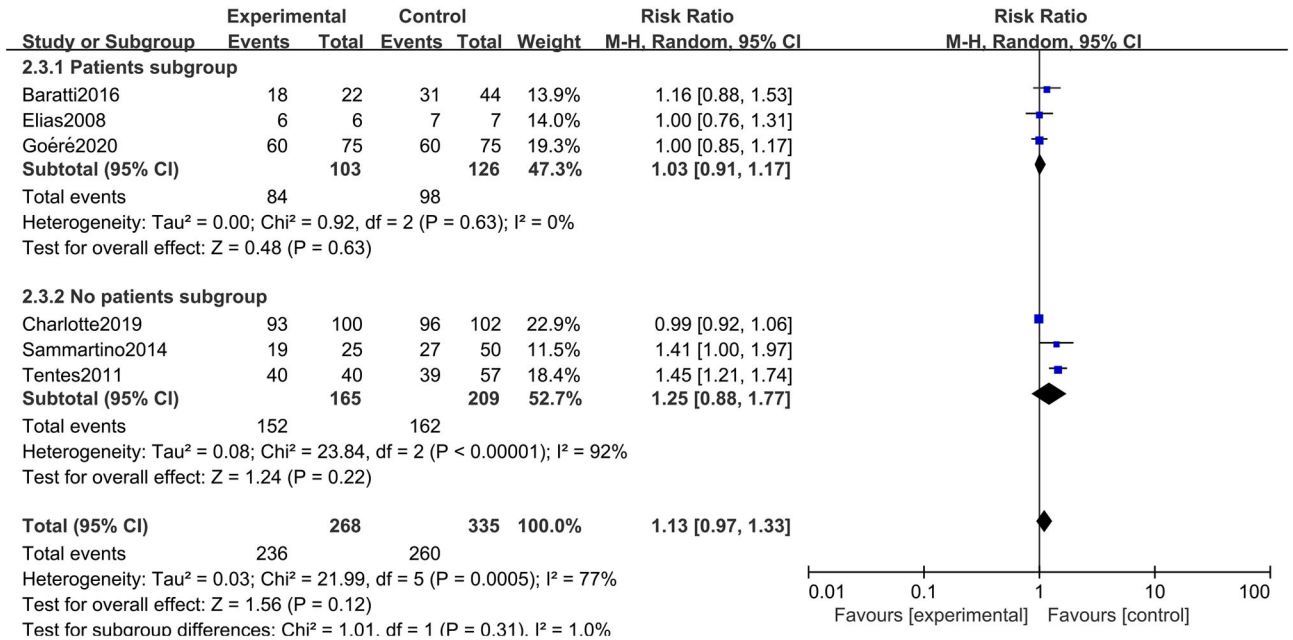

FIGURE 9 | Forest plot of subgroup analysis of patient subgroups.

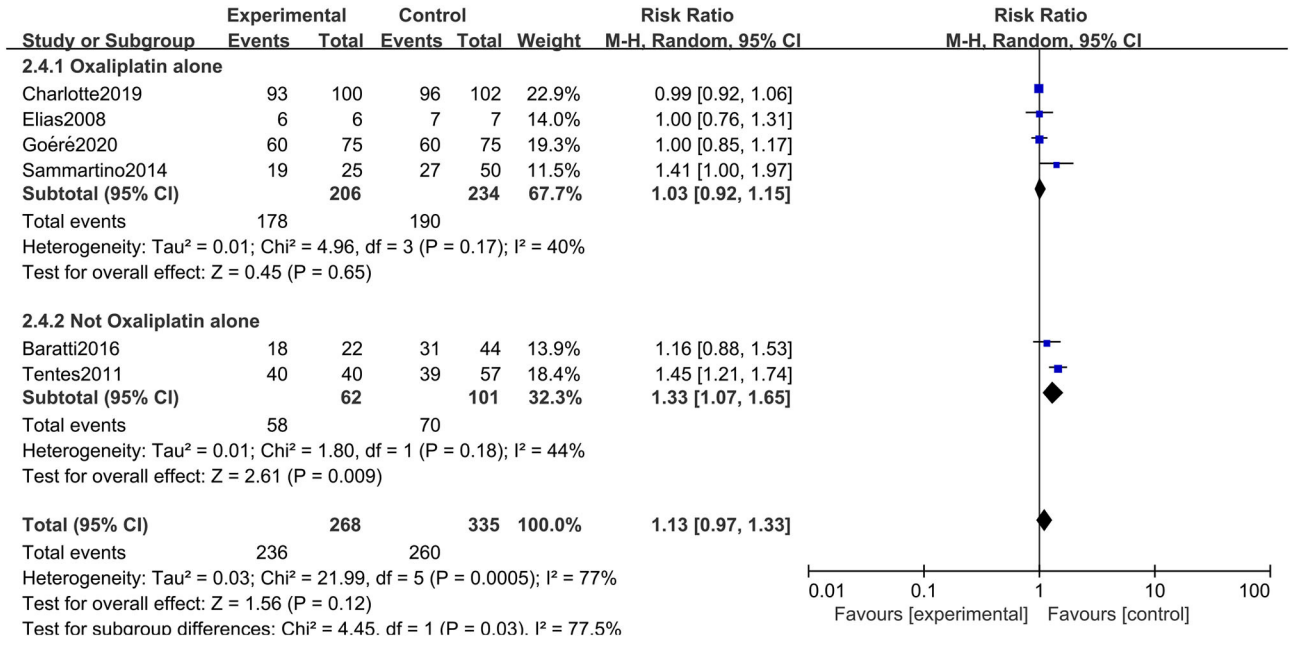

FIGURE 10 | Forest plot of subgroup analysis of HIPEC drugs.

patients with advanced CRC. Additionally, the preemptive HIPEC treatment also showed no benefit in extending DFS or PFS. This may be because HIPEC has clear indications and contraindications. At present, HIPEC is mainly applied to primary or secondary peritoneal tumors in clinical practice. The use of HIPEC therapy in CRC patients at high risk of PM may be a relatively extreme approach, since the existing published RCT research conclusions are similar to ours, and preventive HIPEC does not benefit the long-term survival of the target populations. This conclusion may provide some reference value for other ongoing RCT studies (27, 28). Intriguingly, the incidence of PM was significantly reduced by HIPEC treatment compared to

TABLE 3 | Subgroup analysis and sensitivity analyses on primary outcomes.

Subgroup	No. of studies	No. of patients	RR	95%CI	I^2	*P*-value
Type of studies						
RCT	2	352	0.99	0.93–1.06	0%	0.77
Not-RCT	4	251	1.25	1.04–1.51	51%	**0.02**
Outcome measurement						
OS	3	238	1.37	1.19–1.57	0%	**<0.001**
DFS or PFS	3	365	0.99	0.93–1.06	0%	0.77
Patients subgroup						
Yes	3	229	1.03	0.91–1.17	0%	0.63
No	3	374	1.25	0.88–1.77	92%	0.22
HIPEC drugs						
Oxaliplatin alone	4	440	1.03	0.92–1.15	40%	0.65
Not oxaliplatin alone	2	163	1.33	1.07–1.65	44%	**0.009**

RCT, randomized controlled trials; OS, overall survival; DFS, disease-free survival; HIPEC, hyperthermic intraperitoneal chemotherapy; RR, risk ratio; CI, confidence interval.

control treatments. In addition, we did not observe an increased rate of postoperative adverse reactions in the experimental group, which verified the safety of preventive HIPEC treatment to some extent. Considering the high heterogeneity, we performed several subgroup analyses according to the primary outcome. The results showed that when we chose all RCTs, in all studies reporting patient subgroups, or those that chose oxaliplatin alone as a HIPEC drug to perform the subgroup analysis, the heterogeneity was markedly reduced while the conclusion was still unchanged. In summary, upfront HIPEC treatment was safe and reduced the incidence of PM, however, there was no significant long-term survival benefit in CRC patients at a high risk of PC, conferred by preventatively administering HIPEC treatment. Therefore, the clinical efficacy of HIPEC in CRC patients at high risk of PC still needs more clinical studies and evidence-based medicine for confirmation.

In the past few decades, there have been several prospective cohort studies and retrospective case-control studies conducted to explore the effectiveness of HIPEC treatment in protecting CRC patients from peritoneal metastasis (15, 31–33). Some related RCTs have also published preliminary results in recent years (27, 29), while to our knowledge, this is the first meta-analysis to summarize previous relevant studies and to evaluate the clinical efficacy and safety of HIPEC in CRC patients at high risk of PC. In 2018, Stamou et al. (14) conducted a similar systematic review that included 12 studies and found that prophylactic HIPEC administered during primary surgery might improve the oncological results (peritoneal recurrence rate, 3-year, and 5-year DFS, and 3-year and 5-year OS) in patients at high risk of developing PC. However, they did not draw firm conclusions due to insufficient evidence. What is exciting about these results, is the fact that the Dutch randomized COLOPEC trial published these clinical results last year. Charlotte et al. (27) finally included 202 patients with clinical or pathological T4N0-2M0-stage tumors or perforated colon cancer and randomly assigned them to a HIPEC treatment group or a control group. Unfortunately, after 18 months of regular follow-up, they found that there was no difference in peritoneal-free survival at 18 months between the two groups (80.9% for the experimental group vs. 76.2% for the control group) and concluded that the administration of adjuvant HIPEC was not advocated on the basis of their trial. Similarly, the randomized phase 3 PROPHYLOCHIP trial also did not show the benefits of a second-look surgery plus HIPEC in patients at high risk of developing colorectal peritoneal metastases (29). Although these two RCTs were conducted based on different protocols, their results questioned the efficacy of preventative HIPEC treatment in CRC patients at high risk of PC. Intriguingly, several prospective cohort studies and retrospective case-control studies reported promising results of preventative HIPEC. Baratti et al. (26, 30), Sammartino et al. (25), Tentes et al. (24), and Elias et al. (23) found that adjuvant HIPEC appeared to improve survival and decrease the incidence of recurrence in advanced colorectal cancer patients who were considered at high risk for peritoneal spread. These were all observational studies with small sample sizes, whose level of evidence was limited to some extent. The diametrically inverse conclusion of RCTs vs. observational studies may be attributed to publication bias, as researchers and publishers tended to report positive results. To reduce the influence of differences in study type as much as possible, we performed a corresponding subgroup analysis, whose results were similar to those of previous research. The subgroup analysis of RCTs did not confirm the benefits of preventive HIPEC in improving the long-term survival of CRC patients at high risk of PC, while the subgroup analysis of observational studies found that prophylactic administration of HIPEC significantly extended the OS, DFS, and PFS of eligible patients. The consistency between the conclusions of our meta-analysis and the results of the RCTs might be due to the larger number of patients included in the RCTs, which meant more weight in the results.

Given the high mortality in CRC patients with peritoneal metastases, early diagnosis and treatment may be the most effective measures to improve their prognosis. Unfortunately, identifying these high-risk patients at an early stage is beyond the sensitivity of current clinical, biological, and imaging techniques. The emergence of HIPEC therapy provides insights

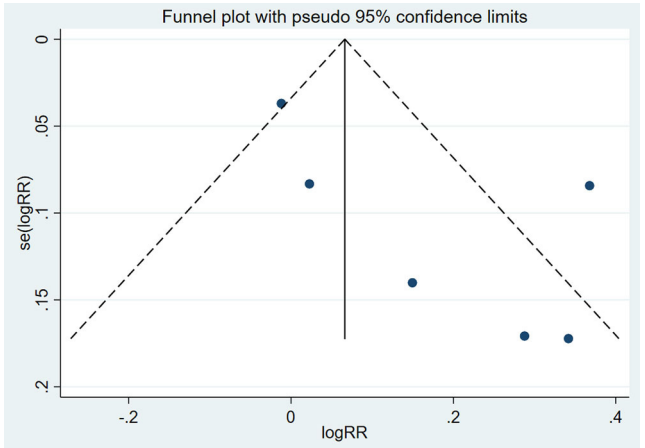

FIGURE 11 | Funnel plot of all included clinical studies.

into these high-risk patients and the goal of the treatment of CRC with PM has changed from being purely palliative or supportive to being considered curative in selected high risk patients. Unlike traditional surgical treatments and systemic chemotherapy, HIPEC can be used to treat small lesions that are beyond the scope of visual observation. We found that the preemptive administration of HIPEC significantly reduced the incidence of PM. This finding was understandable since preventive HIPEC was a local treatment that could cover all potential peritoneal metastases; consequently, preventive HIPEC can achieve better locoregional control thus reducing local recurrence and peritoneal spread. Moreover, we found that performing HIPEC did not cause additional postoperative adverse effects, the reason might be that HIPEC did not involve an additional surgical intervention and was able to concentrate chemotherapeutic drugs in the abdominal cavity, which was not limited by the contraindications of neoadjuvant chemotherapy to some extent. Unexpectedly, preventative HIPEC treatment did not show the expected superiority in terms of improving OS, DFS, and PFS in CRC patients at high risk of PC. The reasons might be as follows: first, given the limited means of examination, a substantial proportion of the included patients might have developed peritoneal metastasis, for which there was no window of time to administer a preventive intervention. Second, some patients received neoadjuvant systematic chemotherapy before receiving HIPEC treatment. If the drugs used by HIPEC were the same as those in the intravenous chemotherapy, the efficacy of HIPEC might be affected because this neoadjuvant treatment potentially induced a certain degree of resistance to certain drugs in the tumor cells; third, the adjuvant HIPEC procedure commonly used in the literature involved adding chemotherapy drugs to the infusion solution for 30 min at a minimum infusion temperature of 42°C, and a single 30-min exposure of malignant cells to chemotherapeutic drugs might also be too short to obtain a clinically relevant antitumor effect; additionally, the optimal timing of early surgery and HIPEC treatment remained unclear and requires further evaluation. If the treatment time was not appropriate, the clinical efficacy would also be affected. Numerous related to ongoing clinical trials have not yet reported their results, including the PROMENADE (NCT02974556) trial, HIPECT4 (NCT02614534) trial, CHECK (NCT03914820) study, and some other similar trials worldwide (34–39). The outcomes of these clinical trials might contribute to drawing a more definitive conclusion on the efficacy and safety of preventative HIPEC treatment in CRC patients at high risk of PC.

Several limitations should be taken into account in this systematic review and meta-analysis. First, we enrolled only 6 clinical studies including 2 RCTs and 4 observational studies with small sample sizes, so it was difficult to confirm the conclusion. Moreover, RCTs and observational studies are fundamentally different. Mixing them for a meta-analysis may lead to unconvincing results. Considering this limitation, we conducted strict quality assessments on all included studies. The evaluation results showed that all included RCTs and observational studies were of high quality and low risk of bias. We ruled out the existence of publication bias through Begg's test and Egger's test. Second, the heterogeneity of some outcomes was relatively high, indicating a large variability in results among studies. We further performed a subgroup analysis combined with sensitivity analysis to find potential influencing factors. Third, the methodologies of HIPEC, such as the timing, techniques, duration, and agents, were disparate across the different enrolled studies. Moreover, the treatments in the control groups including surveillance, systematic chemotherapy, and standard surgical resection, were also uneven. All of the above factors might have affected the robustness of our conclusions. Finally, we could not evaluate the quality of evidence of outcomes in line with the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) criteria due to the inconsistent types of eligible studies.

CONCLUSION

The current systematic review and meta-analysis did not show the expected superiority of preventative HIPEC treatment in improving OS, DFS, and PFS in CRC patients at high risk of PC. However, the preemptive administration of HIPEC was found to significantly reduce the incidence of PM and, at the same time, did not cause additional postoperative adverse effects.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

P-yZ, S-dH, and Y-xL conceived the study and co-wrote the paper. P-yZ and S-dH extracted all data. Y-xL, R-qY, CR, C-zH, S-yL, and Y-mY undertook and refined the search. P-yZ, R-qY, and CR undertook statistical analyses. Y-fW, Y-mY, X-hH, and X-hD helped to revise the

intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2020.590452/full#supplementary-material

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Re-Evaluation of the Survival Paradox Between Stage IIB/IIC and Stage IIIA Colon Cancer

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Objective: We conducted this large population-based study to re-evaluate the survival paradox between stage IIB/C and stage IIIA colon cancer based on the newest staging criteria.

Methods: Colon cancer patients were recruited from the Surveillance, Epidemiology, and End Results (SEER) database using SEER*Stat software (version 8.3.4) with strict inclusion criteria. We used Chi-square test to compare categorical variables between patients diagnosed with stage IIB/IIC and stage IIIA colon cancer. Survival probabilities were then assessed using the Kaplan–Meier method. Cox proportional hazards models were used to analyze hazard ratios (HRs) and 95% confidence intervals (CIs) of clinicopathologic characteristics in stage IIB/IIC and stage IIIA colon cancer patients.

Results: In the current study, a total of 9,227 eligible colon cancer patients were collected from the SEER database between 2010 and 2015. It was found that stage IIIA had 66.4% decreased risk of colon cancer-specific mortality compared with stage IIB (HR = 0.336, 95%CI = 0.286–0.394 for stage IIIA, $P < 0.001$, using stage IIB as the reference) after the adjustment for other known prognostic factors. And T1N2a colon cancer had significantly lower 5-year overall survival (OS) rate compared with T2N1 disease (74.7% vs. 57.1%, $P = 0.018$).

Conclusions: Our study confirmed the existence of survival paradox between stage IIB/IIC and stage IIIA colon cancer based on the newest staging criteria. What is more, the subgroup analyses revealed that T1N2a had the least influence on the survival paradox. N2a colon cancer seemed to be associated with worse prognosis than T2 disease, which would give us a better understanding of tumor biology of colon cancer and be conducive to the refinement of individualized treatment regimens in stage III disease.

Keywords: survival paradox, stage IIB/IIC, stage IIIA, colon cancer, T1N2a

INTRODUCTION

Colon cancer was one of the most common malignant tumors worldwide (1). And the American Joint Committee on Cancer (AJCC) TNM staging system was the most commonly used reference index for the guidance of treatment and the judgment of prognosis in many solid cancers. The AJCC staging system could accurately predict the prognosis of cancer patients, with lower stage cancers having better prognosis than higher stage cancers in most solid cancers (2). For colon cancer, however, a survival paradox could be observed between stage IIB/C (T4N0) and stage IIIA (T1-2N1, T1N2a) tumors in previous studies (3–7).

From the 6th to 7th editions of the AJCC staging system, T4 had been subdivided into T4a and T4b, and N1 had been subdivided into N1a, N1b, and N1c. However, no large population-based studies had been reported to evaluate the prognosis of subgroups in stage IIB/C and stage IIIA colon cancer behind the survival paradox according to the newest AJCC TNM staging criteria.

Several reasons had been reported to contribute to the inferior survival in stage IIB/IIC compared with that of stage IIIA, such as the lower use of systemic chemotherapy in stage IIB/IIC colon cancer patients and the stage migration due to inadequate retrieval of lymph nodes. We then conducted this large population-based study to evaluate the prognosis of different subgroups based on the newest staging criteria, together with inclusion of the retrieval of lymph nodes and the receipt of adjuvant chemotherapy, which we believed would contribute to a better understanding of the survival paradox between stage IIB/C and stage IIIA colon cancer.

MATERIALS AND METHODS

Data Source

The Surveillance, Epidemiology, and End Results (SEER) program covered approximately 28% of the US population and was considered representative of the US in terms of cancer-related data. It collected de-identified data including cancer incidence, clinicopathological characteristics, treatment modalities, and survival from 18 participating population-based cancer registries annually (8). We then used SEER*Stat software (version 8.3.4, Surveillance Research Program, National Cancer Institute) to identify cases meeting the requirements of our study.

Study Population

Shown as **Figure 1**, at first, 298,637 colon cancer patients were recruited from the SEER database between 2004 and 2015. The present study aimed to conduct a detailed evaluation of survival paradox between stage IIB/IIC and stage IIIA colon cancer according to newest staging classification. Therefore, patients diagnosed before 2010 were excluded from the present study, only patients with complete information regarding the American Joint Committee on Cancer (AJCC) 7th TNM staging system and diagnosed with stage IIB/IIC or stage IIIA were retained. In addition, patients with unknown race, without positive histological confirmation, without active follow-up, or without surgical resection were excluded from our analyses. The final cohort included patients diagnosed with stage IIB, stage IIC, and stage IIIA, and we collected the relevant patient information including age (≤65 and >65 years), race (including white, black, and other), gender (including male and female), grade (including

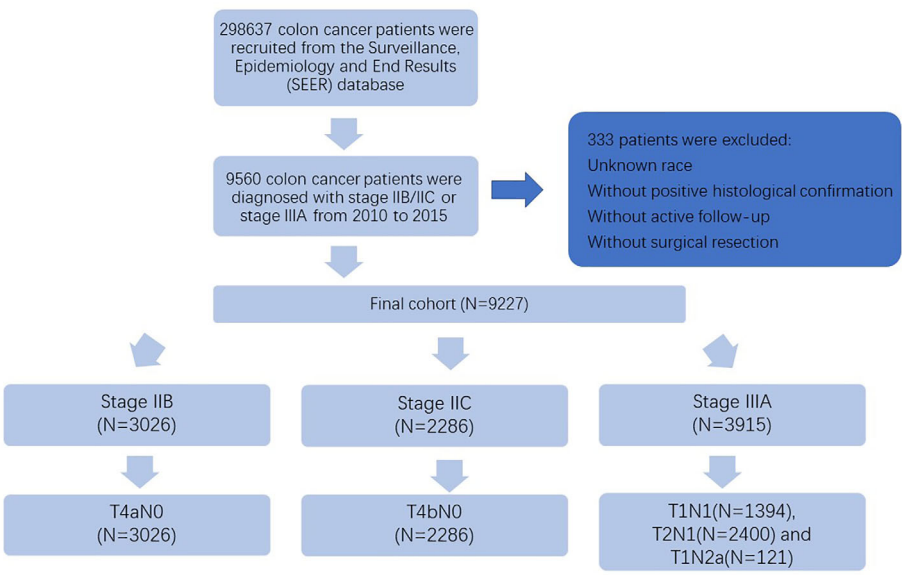

FIGURE 1 | Flow diagram showed how eligible cases were selected from the SEER database.

grade I/II, grade III/IV, and unknown), histology (including adenocarcinoma, and mucinous adenocarcinoma/signet ring cell carcinoma), No. of examined lymph nodes (<12 and ≥12), chemotherapy (no/unknown and yes), and TNM stage (stage IIB, stage IIC, and stage IIIA). Because we wanted to re-evaluated the survival paradox between stage IIB/IIC and stage IIIA colon cancer in detail, furtherly, all the cases were divided into five subgroups, including T4aN0, T4bN0, T1N1, T2N1, and T1N2a.

Statistical Analysis

In our analyses, the outcomes variables of interest were colon cancer-specific survival (CCSS, from the time of diagnosis to the time of colon cancer-related death) and overall survival (OS, from the time of diagnosis to the time of death from any cause). First of all, in the present study, we used Chi-square test to compare categorical variables between patients diagnosed with stage IIB/IIC and stage IIIA colon cancer. Survival probabilities were then assessed using the Kaplan–Meier method, and the log-rank tests were used to evaluate any significant differences in CCSS and OS. Univariate and multivariate cox proportional hazards models were used to analyze hazard ratios (HRs) and 95% confidence intervals (CIs) of clinicopathologic characteristics for stage IIB/IIC and stage IIIA colon cancer patients. Only factors with a statistical significance (log rank, $P < 0.20$) in the univariate Cox analysis would be included in the multivariate Cox analyses. In our univariate analyses, clinicopathologic characteristics including age, race, gender, grade, histology, No. of examined lymph nodes, chemotherapy, and TNM stage were included in the multivariate Cox analyses. A two-sided p value less than 0.05 was considered statistically significant. Analyses were performed using SPSS version 23 statistical software (IBM Corporation).

RESULTS

Patient Baseline Characteristics

In the current study, a total of 9,227 eligible colon cancer patients were collected from the SEER database between 2010 and 2015. The median follow-up duration was 33 months. Patient characteristics were listed in **Table 1**. Of the 9,227 patients diagnosed with stage IIB/IIC and stage IIIA colon cancer, 4,459 (48.3%) patients were female and 4,768 (51.7%) patients were male. A total of 3,897 (42.2%) patients were ≤65 years, and 5,330 (57.8%) patients were >65 years. Among these patients, 7,778 (84.3%) patients had enough lymph node retrieved, while 1,449 (15.7%) patients did not. The 3-year and 5-year CCSS rates of all the patients were 86.8% and 81.6%, respectively; The 3-year and 5-year OS rates in the SEER cohort were 73.7% and 63.2%, respectively. Based on the chi-squared test between stage IIB/IIC and stage IIIA colon cancer, stage IIIA was found to be associated with younger age ($P < 0.001$), black race ($P < 0.001$), male ($P = 0.016$), grade I/II ($P < 0.001$), adenocarcinoma ($P < 0.001$), low number of lymph nodes retrieved ($P < 0.001$), and the receipt of chemotherapy ($P < 0.001$), indicating that stage IIIA patients were more likely to be associated with some favorable clinicopathological characteristics (**Table 1**).

Survival Paradox Between Stage IIB/IIC and Stage IIIA Colon Cancer

Stratified by AJCC TNM stage (stage IIB, stage IIC, and stage IIIA), Kaplan–Meier CCSS curves were shown in **Figure 2A**, and survival differences were estimated with log-rank tests. The CCSS rate of stage IIIA colon cancer patients was significantly higher than stage IIB, stage IIC colon cancer patients (3-year CCSS rates

TABLE 1 | Clinical features of stage IIB/IIC and stage IIIA colon cancer.

Characteristics	Number of patients (%)		*P*
	Stage IIB/IIC(N = 5,312)	Stage IIIA(N = 3,915)	
Age (years)			<0.001
≤65	2,002 (37.7)	1,895 (48.4)	
>65	3,310 (62.3)	2,020 (51.6)	
Race			<0.001
White	4,350 (81.9)	2,941 (75.1)	
Black	552 (10.4)	566 (14.5)	
Other	410 (7.7)	408 (10.4)	
Gender			0.016
Male	2,510 (47.3)	1,949 (49.8)	
Female	2,802 (52.7)	1,966 (50.2)	
Grade			<0.001
Grade I/II	3,928 (73.9)	3,139 (80.2)	
Grade III/IV	1,259 (23.7)	631 (16.1)	
Unknown	125 (2.4)	145 (3.7)	
Histology			<0.001
Adenocarcinoma	4,554 (85.7)	3,700 (94.5)	
Mucinous adenocarcinoma/signet ring cell carcinoma	758 (14.3)	215 (5.5)	
No. of examined lymph nodes			<0.001
<12	774 (14.6)	675 (17.2)	
≥12	4,538 (85.4)	3,240 (82.8)	
Chemotherapy			<0.001
No/unknown	3,450 (64.9)	1,457 (37.2)	
Yes	1,862 (35.1)	2,458 (62.8)	

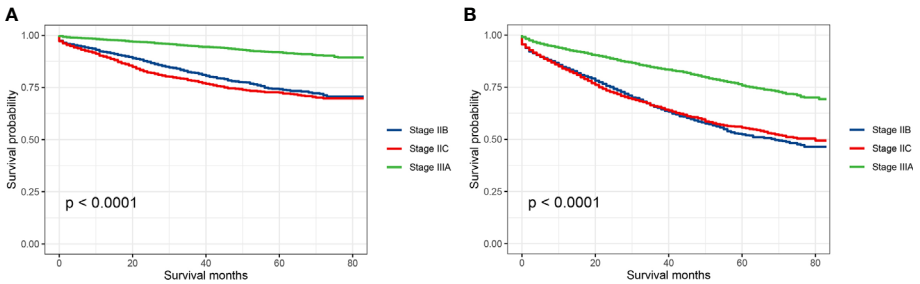

FIGURE 2 | **(A)** Colon cancer-specific survival and **(B)** overall survival for stage IIB, stage IIC, and stage IIA colon cancer patients.

for stage IIB *vs.* stage IIC *vs.* stage IIIA, 82.2% *vs.* 78.2% *vs.* 94.9%, P < 0.0001; 5-year CCSS rates for stage IIB *vs.* stage IIC *vs.* stage IIIA, 74.2% *vs.* 72.5% *vs.* 91.9%, P < 0.0001; **Figure 2A**). Consistent with CCSS, the result of Kaplan–Meier OS analysis also showed that the OS rate of stage IIIA colon cancer patients was significantly higher than stage IIB, stage IIC colon cancer patients (3-year OS rates for stage IIB *vs.* stage IIC *vs.* stage IIIA, 65.6% *vs.* 65.8% *vs.* 84.4%, P < 0.0001; 5-year OS rates for stage IIB vs. stage IIC *vs.* stage IIIA, 52.4% *vs.* 55.6% *vs.* 75.8%, P < 0.0001; **Figure 2B**).

We also carried out univariate and multivariate Cox proportional hazards analyses to evaluate potential risk factors associated with the CCSS and the CCSS difference between stage IIB/IIC and stage IIIA colon cancer. All the clinicopathologic characteristics with prognostic significance were included in multivariate Cox proportional hazards analyses and the result of multivariate analyses was shown in **Table 2**: age [hazard ratio (HR) = 1.524, 95% confidence interval (CI) = 1.338–1.736 for >65 years, P < 0.001, using ≤65 years as the reference], race (HR = 1.429, 95%CI = 1.214–1.681 for black race; HR = 0.956, 95%CI = 0,773–1.183 for other, P < 0.001, using white race as the reference), grade (HR = 1.349, 95%CI = 1.187–1.533 for grade III/IV; HR = 1.250, 95%CI = 0.889–1.758 for unknown, P < 0.001, using grade I/II as the reference), No. of examined lymph nodes (HR = 0.530, 95%CI = 0.464–0.606 for ≥12 resected lymph nodes, P < 0.001, using <12 lymph nodes as the reference), and chemotherapy (HR = 0.595, 95%CI = 0.522–0.678 for the receipt of chemotherapy, P < 0.001, using no chemotherapy as the reference) were independently associated with the risk of colon cancer-specific mortality. What is more, it was found that stage IIIA had 66.4% decreased risk of colon cancer-specific mortality compared with stage IIB, and stage IIC had 32.4% increased risk of colon cancer-specific mortality compared with stage IIB (HR = 1.324, 95%CI = 1.169–1.500 for stage IIC; HR = 0.336, 95%CI = 0.286–0.394 for stage IIIA, P < 0.001, using stage IIB as the reference) after the adjustment for other relevant covariables.

Further Analyses of Survival Paradox Between Stage IIB/IIC and Stage IIIA Colon Cancer

Then, we further investigated which subgroup would contribute to survival paradox between stage IIB/IIC and stage IIIA colon cancer. Stratified by detailed stage (T4aN0, T4bN0, T1N1, T2N1, and T1Na), Kaplan–Meier CCSS curves were shown in **Figure 3A**, and survival differences between different subgroups were estimated with log-rank tests: 5-year CCSS rates for T4aN0 *vs.* T4bN0 *vs.* T1N1 *vs.* T2N1 *vs.* T1N2a, 74.2% *vs.* 72.5% *vs.* 94.2% *vs.* 91.0% *vs.* 81.2% (P < 0.0001), indicating that T1N2a colon cancer had inferior CCSS compared with T2N1 colon cancer though the survival difference did not achieve statistical significance (P = 0.406); Kaplan–Meier OS curves were shown in **Figure 3B**, and survival differences between different subgroups were estimated with log-rank tests: 5-year OS rates for T4aN0 *vs.* T4bN0 *vs.* T1N1 *vs.* T2N1 *vs.* T1N2a, 52.4% *vs.* 55.6% *vs.* 79.4% *vs.* 74.7% *vs.* 57.1% (P < 0.0001), and T1N2a colon cancer had significantly inferior OS compared with T2N1 colon cancer (P = 0.018).

Univariate and multivariate Cox proportional hazards analyses were carried out to evaluate potential risk factors associated with the CCSS and the CCSS differences between different subgroups (T4aN0, T4bN0, T1N1, T2N1, and T1Na). It was found that T2N1 had a 25.7% decreased risk of colon cancer-specific mortality compared with T1N2a (HR = 0.743, 95%CI = 0.392–1.408 for T2N1, using T1N2a as the reference) after the adjustment for other relevant covariables, though the survival difference did not achieve statistical significance (P = 0.362 **Table 3**).

DISCUSSION

The AJCC staging system contained information about the tumor status at diagnosis which could assist clinicians to predict survival, impart prognostic information, and give the guidance to select the most effective treatments. As early as 2000, the colorectal working group proposed to subdivide T4 into T4a (tumor penetrated the surface of the visceral peritoneum) and T4b (tumor directly invaded or was histologically adherent to other organs or structures) according to the absence or presence of tumor involving the surface of the specimen based on the evidence that previous study had found that peritoneal involvement had an adverse outcome (9, 10). Then, the 7th edition AJCC TNM staging system published in 2010 subdivided T4 into T4a and T4b and further divided N1 into

TABLE 2 | Cox regression analyses of factors associated with CSS.

Variable	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Stage		<0.001		<0.001
Stage IIB	1		1	
Stage IIC	1.199 (1.059–1.356)	0.004	1.324 (1.169–1.500)	<0.001
Stage IIIA	0.284 (0.243–0.331)	<0.001	0.336 (0.286–0.394)	<0.001
Age (years)		<0.001		<0.001
≤65	1		1	
>65	1.954 (1.730–2.206)		1.524 (1.338–1.736)	
Race		0.035		<0.001
White	1		1	
Black	1.161 (0.988–1.364)	0.069	1.429 (1.214–1.681)	<0.001
Other	0.840 (0.680–1.038)	0.107	0.956 (0,773–1.183)	0.680
Gender		0.001		0.136
Male	1		1	
Female	1.210 (1.081–1.353)		1.090 (0.973–1.221)	
Grade		<0.001		<0.001
Grade I/II	1		1	
Grade III/IV	1.528 (1.346–1.734)	<0.001	1.349 (1.187–1.533)	<0.001
Unknown	1.079 (0.770–1.513)	0.659	1.250 (0.889–1.758)	0.199
Histology		0.017		0.488
Adenocarcinoma	1		1	
Mucinous adenocarcinoma/signet ring cell carcinoma	1.231 (1.038–1.459)		0.941 (0.792–1.118)	
No. of examined lymph nodes		<0.001		<0.001
<12	1		1	
≥12	0.598 (0.523–0.682)		0.530 (0.464–0.606)	
Chemotherapy		<0.001		<0.001
No/unknown	1		1	
Yes	0.407 (0.360–0.459)		0.595 (0.522–0.678)	

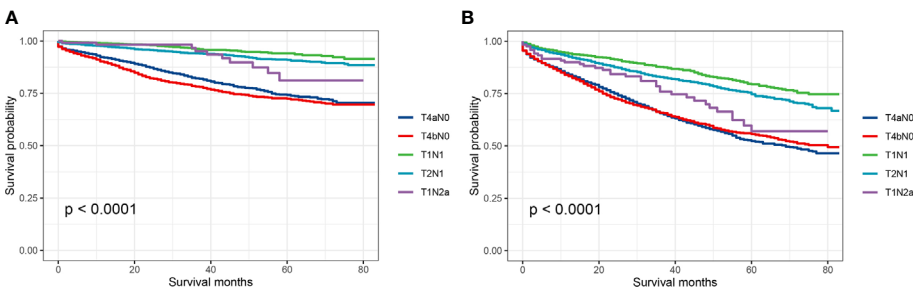

FIGURE 3 | **(A)** Colon cancer-specific survival and **(B)** overall survival for stage T4aN0, stage T4bN0, stage T1N1, stage T2N1, and stage T1Na colon cancer patients.

N1a (metastasis in 1 node), N1b (metastasis in 2–3 nodes) and N1c (the presence of tumor deposit, there was no regional lymph node metastasis), and N2 into N2a (metastasis in 4–6 nodes) and N2b (metastasis in ≥7 nodes). Therefore, stage II colon cancer was subdivided into IIA (T3N0), IIB (T4aN0), or IIC (T4bN0) and stage III colon cancer became IIIA (T1-2 N1, T1N2a), IIIB (T3-4 N1, T2-3N2a, T1-2N2b), and IIIC (T4aN2a, T3-T4aN2b, T4bN1-2) (2, 11, 12). And the eighth AJCC TNM staging system was the same as seventh staging system in regards to stage II and stage III colon cancer. Although the survival paradox between stage IIB/IIC and stage IIIA colon cancer had long been known, few population-based studies reported this phenomenon based on the newest AJCC TNM staging system or evaluated prognosis of subgroups in stage IIB/IIC and stage IIIA to further reveal the survival paradox in colon cancer (2–7).

In our analyses, it was found that the CCSS rate of stage IIIA colon cancer patients was significantly higher than stage IIB, stage IIC colon cancer patients (5-year CCSS rates for stage IIB *vs.* stage IIC *vs.* stage IIIA, 74.2% *vs.* 72.5% *vs.* 91.9%). Similarly, 5-year OS rates of stage IIB, stage IIC and stage IIIA were 52.4%, 55.6%, and 75.8%, respectively, which was consistent with previous report by Edge et at. (2) that the 5-year OS rate for patients with stage IIIA was approximately 70% *vs.* 46–61% for stage IIB/C. More importantly, we also conduct multivariate analyses to exclude the possibility of the influence of other prognostic factors including age, race, gender, grade, histology,

TABLE 3 | Cox regression analyses of factors associated with CSS (including T4aN0, T4bN0, T1N1, T2N1, and T1Na).

Variable	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Stage		<0.001		<0.001
T4aN0	1		1.956 (1.044–3.665)	0.036
T4bN0	1.199 (1.059–1.356)	0.004	2.588 (1.382–4.848)	0.003
T1N1	0.204 (0.156–0.267)	<0.001	0.478 (0.244–0.934)	0.031
T2N1	0.325 (0.272–0.388)	<0.001	0.743 (0.392–1.408)	0.362
T1N2a	0.421 (0.225–0.786)	0.007	1	
Age (years)		<0.001		<0.001
≤65	1		1	
>65	1.954 (1.730–2.206)		1.511 (1.326–1.721)	
Race		0.035		<0.001
White	1		1	
Black	1.161 (0.988–1.364)	0.069	1.422 (1.208–1.673)	<0.001
Other	0.840 (0.680–1.038)	0.107	0.953 (0.770–1.180)	0.660
Gender		0.001		0.129
Male	1		1	
Female	1.210 (1.081–1.353)		1.092 (0.975–1.223)	
Grade		<0.001		<0.001
Grade I/II	1		1	
Grade III/IV	1.528 (1.346–1.734)	<0.001	1.342 (1.181–1.524)	<0.001
Unknown	1.079 (0.770–1.513)	0.659	1.298 (0.922–1.826)	0.135
Histology		0.017		0.464
Adenocarcinoma	1		1	
Mucinous adenocarcinoma/signet ring cell carcinoma	1.231 (1.038–1.459)		0.938 (0.790–1.114)	
No. of examined lymph nodes		<0.001		<0.001
<12	1		1	
≥12	0.598 (0.523–0.682)		0.526 (0.460–0.601)	
Chemotherapy		<0.001		<0.001
No/unknown	1		1	
Yes	0.407 (0.360–0.459)		0.595 (0.522–0.678)	

No. of examined lymph nodes, and the receipt of chemotherapy. It was found that stage IIIA had 66.4% decreased risk of colon cancer-specific mortality compared with stage IIB. In other words, stage IIB/C (T4N0) colon cancer had worse prognosis compared with stage IIIA (T1-2 N1, T1N2a) even after adjusting for the number of lymph nodes retrieved and the receipt of adjuvant chemotherapy, which was in agreement with previous study and once again demonstrated the existence of survival paradox between stage IIB/IIC and stage IIIA colon cancer (3).

Previous studies had suggested that the poor survivals of T4N0 might attribute to the following factors: preferential administration of chemotherapy for stage IIIA compared with T4N0 disease (while the present study had shown that 35.1% of T4N0 colon cancer patients would receive adjuvant chemotherapy compared with 62.8% of stage IIIA colon cancer patients); T4N1 colon cancer was understaged as T4N0 due to inadequate retrieval of lymph nodes (while 85.4% of T4N0 colon cancer patients had enough retrieval of lymph nodes compared with 82.8% of stage IIIA colon cancer patients in our analyses) and biologically more aggressive tumors in T4N0 (13, 14). In 2016, Quyen and his colleagues (3) carried out a retrospective analysis and found that the survival paradox between stage IIB/IIC and stage IIIA colon cancer cannot be entirely explained by inadequate lymph nodes retrieved and lack of receipt of adjuvant chemotherapy, which was consistent with the current study. A previous study showed that T4N0 colon cancers were associated with higher proportion of MSI-H and poor histological grade, indicating that T4N0 carcinomas might have different entity of tumor biology from T1-2N1 disease (4, 15).

In 2016, Quyen et al. (6) reported that stage IIB/C were associated with a greater proportion of positive margins (19%) than did stage IIIA (1%; P <.0001), from this they believed that positive surgical margins might contribute to the survival paradox between stage IIB/C and stage IIIA colon cancer patients. Our study also showed that stage IIB/IIC colon cancer was more likely to be associated with grade III/IV compared with stage IIIA disease (23.7% *vs.* 16.1%, P < 0.001), which could add new evidence supporting the above hypothesis.

Although the fact that the use of adjuvant chemotherapy had been widely accepted as the routine treatment for patients with stage III colon cancer and stage IIB/IIC colon cancer had significant poor survivals compared with stage IIIA disease, some researchers have suggested that the efficacy of adjuvant chemotherapy in T4 disease was not significant (16–21). Therefore, future studies were still needed to investigate the necessity of intensive chemotherapy in T4 colon cancer. In further exploration of the present study, Kaplan–Meier survival curves showed the 5-year CCSS rates (T4aN0 *vs.* T4bN0 *vs.* T1N1 *vs.* T2N1 *vs.* T1N2a, 74.2% *vs.* 72.5% *vs.* 94.2% *vs.* 91.0% *vs.* 81.2%) and 5-year OS rates (5-year OS for T4aN0 *vs.* T4bN0 *vs.* T1N1 *vs.* T2N1 *vs.* T1Na, 52.4% *vs.* 55.6% *vs.* 79.4% *vs.* 74.7% *vs.* 57.1%) of different subgroups in stage IIB/IIC and stage IIIA colon cancer, indicating that T1N2a colon cancer had inferior CCSS (P = 0.406) and OS (P = 0.018) compared with T2N1 colon

cancer though the CCSS difference between T1N2a and T2N1 colon cancer did not achieve statistical significance.

The results of multivariate analyses in the present study also showed the similar result that T2N1 colon cancer had a 25.7% decreased risk of colon cancer-specific mortality compared with T1N2a (HR = 0.743, 95%CI = 0.392–1.408 for T2N1, using T1N2a as the reference) after the adjustment for other relevant covariables, though the survival difference did not achieve statistical significance (P = 0.362). The above findings also indicated the inconsistency of subgroups in stage IIIA colon cancer, especially between stage T2N1 and stage T1N2a though they were both classified as stage IIIA. And N2a seemed to be a stronger factor for poor prognosis than T2 stage, the increase of one positive lymph node seemed to be a worse indicator of survival compared with the penetration of tumor from submucosa to muscular layer. That the CCSS difference between T1N2a and T2N1 colon cancer did not achieve statistical significance might be because of the small sample size of T1N2a (N = 121).

The main strengths of the present study were that, as far as we know, this was the first population-based analysis to evaluate prognosis of detailed subgroups in stage IIB/IIC and stage IIIA to further reveal the survival paradox in colon cancer based on the newest staging criteria and a large population. The finding that N2a colon cancer seemed to be a worse prognostic factor than T2 disease revealed the inconsistence in stage IIIA colon cancer and T1N2a had the least influence on the survival paradox between stage IIB/IIC and stage IIIA, which could give us a better understanding of tumor biology of colon cancer and be conducive to the refinement of individualized treatment regimens in stage III disease.

However, this study had two limitations. On the one hand, information on the surgical margin status, molecular and genetic markers that were confirmed as prognostic factors of colon cancer were lacking because of the limitation of the SEER database (22–24). On the other hand, our research was a retrospective type of study with inherent deficiencies that could lead to confusion or observer bias, and future research could overcome this problem by the use of a prospective diary. In addition, external validation is missing because of insufficient eligible patients in our center.

In conclusion, our study confirmed the presence of survival paradox between stage IIB/IIC and stage IIIA colon cancer based on the newest staging criteria. What is more, the subgroup analyses revealed the inconsistence in stage IIIA colon cancer and T1N2a had the least influence on the survival paradox. N2a colon cancer seemed to be a worse prognostic factor than T2 disease, which would give us a better understanding of tumor biology of colon cancer and be conducive to the refinement of individualized treatment regimens in stage III disease.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov/).

AUTHOR CONTRIBUTIONS

HL, GF, WW, and WZ conceived and designed the study. YH, ZW, and TL collected and analyzed the data. HL, TL, ST, and HC performed the statistical analysis. HL and GF wrote the first draft of the manuscript. HC and WZ revised the final data and the manuscript. All the authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.595107/full#supplementary-material

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Duration of FOLFOX Adjuvant Chemotherapy in High-Risk Stage II and Stage III Colon Cancer With Deficient Mismatch Repair

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Background: We evaluated the impact of 3 months of mFOLFOX6 adjuvant chemotherapy or surgery alone in comparison with 6 months of mFOLFOX6 on disease-free survival (DFS) in deficient mismatch repair (dMMR) colon cancer (CC) patients.

Methods: This retrospective study identified a cohort of patients with high-risk stage II and III dMMR CC who underwent curative surgery between May 2011 and July 2019. DFS was compared using the Kaplan-Meier survival methods and Cox proportional hazards models. Propensity-score matching was performed to reduce imbalance in baseline characteristics.

Results: A total of 242 dMMR CC patients were identified; 66 patients received 6 months of mFOLFOX6, 87 patients received 3 months of mFOLFOX6, and 89 patients were treated with surgery alone. The 3-year DFS rate was 72.8% in 3-month therapy group and 86.1% in 6-month therapy group, with a hazard ratio (HR) of 2.78 (95CI%, 1.18 to 6.47; P= 0.019). The difference in DFS between surgery alone group and 6-month therapy group was also observed but was nonsignificant (HR= 2.30, 95%CI, 0.99 to 5.38; P=0.054). The benefit of 6-month therapy in DFS compared with 3-month therapy group was pronounced for patients with stage III (HR=2.81, 95%CI, 1.03 to 7.67; P=0.044) but not for high-risk stage II patients. Propensity score matched analysis confirmed a DFS benefit in the 6-month therapy group.

Conclusion: This study suggested that a 6-month duration of mFOLFOX6 adjuvant chemotherapy in dMMR CC patients may be associated with improved DFS compared with 3-month therapy, particularly in patients with stage III. The observational nature of the study implies caution should be taken in the interpretation of these results.

Keywords: colon cancer, adjuvant chemotherapy, deficient mismatch repair, microsatellite instability, duration of therapy

INTRODUCTION

As the fourth most commonly seen cancer worldwide, colon cancer (CC) leads to 550,000 deaths each year (1). On the basis of positive findings from three phase-3 trials, 6 months of FOLFOX (fluorouracil, leucovorin, and oxaliplatin) or CAPOX (capecitabine and oxaliplatin) became the standard adjuvant therapy for patients with stage III CC (2–6). Given the neurotoxicity of oxaliplatin accumulated amid therapy that might affect patients' daily-life activities, shorter duration of adjuvant therapy was afforded to reduce adverse effects (7). However, the non-inferiority of 3 months of adjuvant therapy with either FOLFOX or CAPOX versus 6 months was not confirmed for overall CC patients in an IDEA collaboration study. Furthermore, among the patients who received FOLFOX, results of those who received 6 months of adjuvant therapy were superior to those receiving 3 months (8, 9).

Colorectal cancer is a biologically heterogeneous disease that develops *via* 2 well described pathways of colorectal carcinogenesis, including chromosomal instability and, less commonly, microsatellite instability (MSI). MSI is a consequence of a deficient mismatch repair (dMMR) system that results in error accumulation within microsatellite region, and it occurs in approximately 15% of all colorectal cancers (10). Several studies have shown that dMMR non-metastatic CC patients were associated with a more favorable stage-adjusted prognosis compared to patients with proficient mismatch repair (pMMR) (11–14). Whether a shorter duration of adjuvant therapy for patients with dMMR would lead to any decrease in efficacy is still unclear. Thus, we evaluated the impact of 3 months of mFOLFOX6 adjuvant chemotherapy or surgery alone in comparison with 6 months of mFOLFOX6 on disease-free survival (DFS) in high-risk stage II and stage III patients with dMMR.

METHOD

Study Population

This retrospective study included all consecutive patients with histologically confirmed high-risk stage II or III CC and determined dMMR tumors who received radical surgical resection from May 2011 to July 2019 at The Sixth Affiliated Hospital of Sun Yat-Sen University. Patients with rectal cancer, incomplete curative resection (R1 or R2 resection), stage I and stage II without any high-risk factors, or adjuvant chemotherapy with fluoropyrimidine alone, CAPOX regimen (capecitabine and oxaliplatin), or duration less than 3 months were excluded. High-risk stage II CC was defined by pathologic stage T4, vascular invasion, lymphatic infiltration, perineural invasion, initial bowel obstruction, tumor perforation, or fewer than 12 excised lymph nodes (15). This study was approved by the Institutional Review Boards of The Sixth Affiliated Hospital of Sun Yat-Sen University.

IHC Analysis of MMR Protein Expression

Formalin-fixed, paraffin-embedded tumors were stained for MLH1, MSH2, MSH6, and PMS2 proteins. Mismatch repair protein loss is defined as the absence of nuclear staining in neoplastic cells but positive nuclear staining in lymphocytes and normal adjacent colonic epithelium (16). Primary monoclonal antibodies against MLH1 (clone M1, Ventana, prediluted), MSH2 (clone G219-1129, Ventana, prediluted), MSH6 (clone 44, Ventana, prediluted), and PMS2 (clone EPR3947, Ventana, prediluted) were applied.

MSI Testing

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissues. Comparative analysis of normal colon and tumor DNA using the five consensus monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, NR-27) obtained through polymerase chain reaction (PCR)-based assay was adopted to assess the microsatellite instability (MSI). Specimens with at least 2 unstable markers were scored as highly unstable, while those with fewer than 2 unstable markers were stable (17).

MMR Status Determination

MMR status was tested though the analysis of MMR protein expression by immunohistochemistry (IHC), and to be further confirmed by PCR-based MSI testing when the IHC result was undetermined. Deficient MMR phenotype tumors were defined as exhibiting the loss of expression of 1 or more MMR protein by IHC or high-level tumor DNA MSI by PCR. Tumors with discordant results between MMR protein expression and DNA MSI testing were not included in the study.

Gene Mutation Detection

Under adequate quality-control procedures, mutation analysis was performed at the Molecular Diagnostic Laboratory of the Sixth Affiliated Hospital of Sun Yat-sen University. Genomic DNA was extracted from FFPE samples of surgery with an EZgene Tissue gDNA miniprep kit (Cat no: GD2211, Biomiga, China). KRAS (exons 2, 3, 4), NRAS (exons 2, 3, 4), BRAF (exon 15, V600E mutations), and PIK3CA (exon 9 and 20) were evaluated by bidirectional sequencing using ABI Prism 3 500 DX genetic Analyzer (Applied Biosystems, Foster City, CA).

Treatment and follow-Up

All patients in this study underwent curative surgical resection, followed by either adjuvant chemotherapy with mFOLFOX6 regimen (oxaliplatin 85 mg/m^2, leucovorin 400 mg/m^2, fluorouracil 400 mg/m^2, and fluorouracil 2400 mg/m^2 by 48 hours continuous intravenous infusion) for 3 to 6 months or observation only. Follow-up routines consisted of physical examination, serum carcinoembryonic antigen (CEA) assay, and computed tomography scan (chest/abdominal/pelvic) every 3 to 6 months in the first 3 years and every 6 months in the following 2 years. The data were updated in December 2019.

Propensity Score Matching

We performed propensity score matching to reduce imbalances in baseline characteristics between patients who received 3 months of mFOLFOX6 or surgery alone and those who received 6 months of mFOLFOX6. A multivariable logistic regression model was constructed to generate propensity scores. We selected covariates for inclusion in the propensity model based on factors presumed to be associated with the patient's survival outcomes. The following baseline data were included in the model: age at diagnosis,

pathologic T stage, pathologic N stage, initial bowel obstruction, vascular invasion and/or lymphatic infiltration, and perineural invasion. Patients who received 3 months of mFOLFOX6 and surgery alone were matched to those who received 6 months of mFOLFOX6 in a 1:1 ratio respectively, according to a greedy nearest-neighbor matching algorithm with no replacement. A caliper width equal to 0.2 of the standard deviation was utilized as the logit of the propensity score. We compared baseline characteristics between the propensity score-matched group using standardized differences. A standardized difference of less than 0.1 can be regarded as negligible imbalance between groups (18).

Statistical Analysis

Categorical variables were compared by dint of the Chi-square test or Fisher's exact test. DFS was defined as the time from surgery to the first event of local or metastatic recurrence, second primary cancer, or death from any cause. DFS curves were estimated *via* the Kaplan-Meier method and were compared by means of COX proportional hazards regression model with hazard ratios (HR), 95% confidence intervals (CI), and P values for candidate prognostic factors. Variables with P values of 0.05 or less in univariate analysis or clinically relevant were eligible for the multivariate analyses. Two-sided P values of less than 0.05 were designated as statistically significant. Apart from propensity score matching, which was implemented in R, version 3.3.2 (R Foundation), using the package Matching, all statistical analyses were performed with the 22 version of SPSS software (SPSS Inc. Chicago, IL, USA).

RESULTS

Patient Characteristics

A total of 242 patients with high-risk stage II and III dMMR CC were identified. A complete consolidated standards of reporting trials (CONSORT) diagram depicting the selection process is outlined on **Figure 1**. The median age at diagnosis was 55 (range, 22 to 88), with 64.0% of the patients being men. All patients were tested for MMR status by IHC, and 38 cases were also confirmed by PCR-MSI testing. Among 242 patients, 139 (57.4%) had lost MLH1 and PMS2 protein expression, 60 (24.8%) with MSH2 and MSH6 expression, 14 (5.8%) with MSH6 expression, and 29 (12.0%) with PMS2 expression. Additionally, 176 patients had complete data for KRAS, NRAS, BRAF, and PI3KCA status.

Overall, 153 patients received adjuvant chemotherapy with mFOLOFX6 after surgery, which consisted of 6-month therapy (27.3%, n = 66; median cycles [range] = 12 [9-12]) or 3-month therapy (36.0%, n = 87; median cycles [range] = 6 [4-7]), and 89 patients (36.7%) were treated with surgery alone. Baseline patients and tumor characteristics between treatment durations were presented in **Table 1**. Patients in the 6-month therapy group were more likely to be younger (< 65 years: 95.5%, 85.1%, 55.1%), with lower proportions of initial bowel obstruction (27.3%, 33.3%, 47.2%), but more of them proceeded to stage III (74.2%, 57.5%, 42.7%) with more positive lymph nodes examined (N1: 53.0%, 42.5%, 36.0%; N2: 21.2%, 14.9%, 6.7%) than those in the 3-month therapy and surgery alone group.

Association of Adjuvant Chemotherapy Duration and Disease-Free Survival

For the overall cohort at the time of data cutoff, the median follow-up was 21.9 months. There were 17 DFS events in patients with high-risk stage II and 29 in stage III disease that led to a 3-year DFS rate of 79.5% (95%CI, 70.1% to 88.9%) and 73.4% (95% CI, 64.2% to 82.6%) respectively.

The 3-year DFS rate was 72.8% (95%CI, 61.0% to 84.6%) for patients who received 3 months of mFOLFOX6 therapy and 86.1% (95%CI, 77.1% to 95.1%) for patients who received 6 months of therapy, along with an estimated multivariate HR of 2.78 (95CI%, 1.18 to 6.47; P= 0.019). For the patients treated with surgery alone, the 3-year DFS rate was 72.4% (95%CI, 60.8% to 84.0%). The multivariate HR for DFS compared with 6-month therapy was 2.30 (95%CI, 0.99 to 5.38; P=0.054) (**Figure 2**, **Table 2**, and **Supplementary Table S1**).

Subgroup analysis demonstrated 3-year DFS rate for patients with stage III was 70.8% in the 3-month therapy group and 86.2% in the 6-month therapy group (HR=2.81, 95%CI, 1.03 to 7.67; P=0.044). In high-risk stage II subgroup analysis, no significant effect of adjuvant chemotherapy duration on DFS was observed in the 3-month therapy group compared with the 6-month therapy group (**Figure 3** and **Supplementary Table S1**).

Disease-Free Survival After Propensity Score Matching

At 1:1 propensity score matching, 51 patients who received 3-month therapy and 35 patients treated with surgery alone were matched to the patients who received 6-month therapy respectively. As shown in **Table 3**, after propensity score matching, standardized differences for all included covariates among patients who received 6-month, 3-month therapy, and surgery alone were all less than 0.1, indicating a well-balanced covariate distribution after matching.

After matching, a significant difference in DFS in favor of the 6-month therapy group compared to the 3-month therapy group was observed. The 3-year DFS rate was 88.7% (95%CI, 79.3% to 98.1%) and 68.7% (95%CI, 52.2% to 85.2%) respectively, plus an estimated multivariate HR of 4.35 (95CI%, 1.46 to 13.00; P= 0.008). In subgroup analysis, we identified a benefit on DFS of the 6-month adjuvant chemotherapy compared with the 3-month therapy for stage III patients (3-year DFS rate: 90.3% vs. 64.5%; HR=5.88, 95% CI, 1.44 to 24.04; P=0.014) (**Figure 4**). In contrast, there was still no significant difference in DFS between the two groups for high-risk stage II patients (**Supplementary Table S2**). Marginally significant difference in DFS between the 6-month therapy group and the surgery alone group was observed in the multivariable analysis (HR=3.02, 95%CI, 1.00 to 9.07; P=0.049). The 3-year DFS rate was 84.6% (95%CI, 72.1% to 97.1%) and 65.4% (95%CI, 46.6% to 84.2%) respectively (**Figure 4** and **Supplementary Table S3**).

DISCUSSION

To our knowledge, this is the first study to explore the effect of the duration of mFOLFOX6 adjuvant chemotherapy on DFS in high-risk stage II and III dMMR CC patients, and it showed a statistically significant benefit in DFS in 6-month therapy group

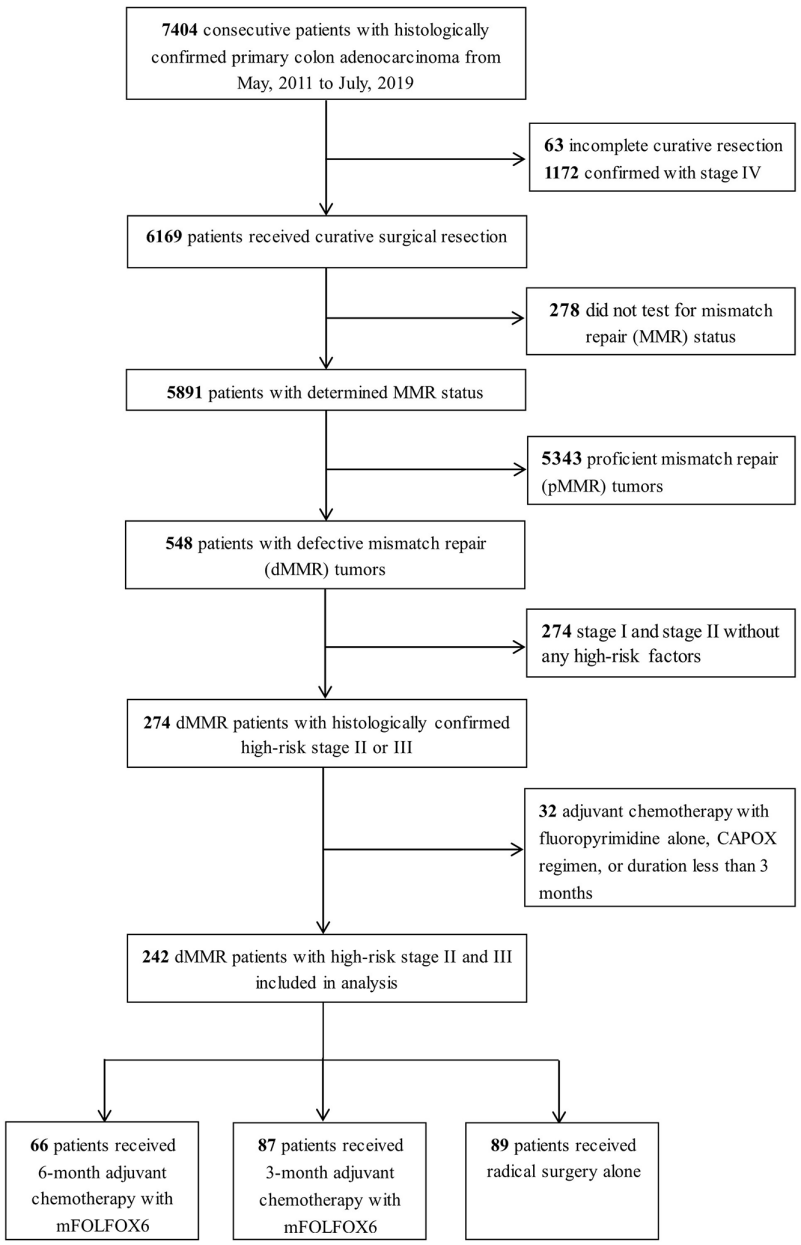

FIGURE 1 | Flow diagrams of the study population.

compared with the 3-month therapy group, particularly in patients with pathologic stage III.

Current guidelines recommend that all dMMR stage II CC patients, regardless of high-risk factors, should not receive 5-FU-based adjuvant therapy (11, 19–25). For dMMR stage III CC patients, adjuvant therapy with CAPOX or FOLFOX regimen is recommended, but the role of the MMR status as a predictive biomarker is still not completely clear (23, 24, 26–28). IDEA collaboration and IDEA France study have failed to demonstrate the non-inferiority of 3-month FOLFOX to 6-month FOLFOX in stage III CC patients; however, these analyses did not perform a subgroup analysis of patients' dMMR status (8, 9). In keeping with the IDEA study, we observed that the 6-month duration of mFOLFOX6 adjuvant therapy may provide an additional DFS benefit compared with 3-month duration for dMMR stage III CC patients. In this subgroup of high-risk stage II CC patients, there were no significant differences in DFS among three groups, which implied that dMMR high-risk stage II CC patients did not significantly benefit from the FOLFOX adjuvant therapy however long the therapy duration was. The good prognosis of dMMR stage II CC patients, compared with stage III patients, might comparatively benefit less from the adjuvant therapy.

TABLE 1 | Patients and tumor characteristics.

Characteristics	Missing values	All the population, n=242 No. (%)	6-month therapy group, n=66 No. (%)	3-month therapy group, n=87 No. (%)	Surgery alone group, n=89 No. (%)	*P*
Adjuvant therapy duration						
Median (range), weeks		–	24 (20–27)	12 (12–16)	0	
Completion no. of cycles						
Median (range)		–	12 (9–12)	6 (4–7)	0	
Age, years						<0.001
< 65		186 (76.9%)	63 (95.5%)	74 (85.1%)	49 (55.1%)	
≧65		56 (23.1%)	3 (5.5%)	13 (14.9%)	40 (44.9%)	
Gender						0.331
Female		87 (36.0%)	19 (28.8%)	35 (40.2%)	33 (37.1%)	
Male		155 (64.0%)	47 (71.2%)	52 (59.8%)	56 (62.9%)	
Grade of differentiation						0.214
Well or moderately		120 (49.6%)	27 (40.9%)	44 (50.6%)	49 (55.1%)	
Poorly		122 (50.4%)	39 (59.1%)	43 (49.4%)	40 (44.9%)	
Primary tumor site						0.259
Left (splenic flexure, descending colon, and sigmoid colon)		94 (38.8%)	21(31.8%)	39 (44.8%)	34 (38.2%)	
Right (cecum, ascending colon, hepatic flexure, and transverse colon)		148 (61.2%)	45 (68.2%)	48 (55.2%)	55 (61.8%)	
Initial bowel obstruction						0.028
No		153 (63.2%)	48 (72.7%)	58 (66.7%)	47 (52.8%)	
Yes		89 (36.8%)	18 (27.3%)	29 (33.3%)	42 (47.2%)	
Vascular invasion and/or lymphatic infiltration						0.282
No		186 (76.9%)	55 (83.3%)	63 (72.4%)	68 (76.4%)	
Yes		56 (23.1%)	11(16.7%)	24 (27.6%)	21 (23.6%)	
Perineural invasion						0.621
No		220 (90.9%)	61(92.4%)	77 (88.5%)	82 (92.1%)	
Yes		22 (9.1%)	5 (7.6%)	10 (11.5%)	7 (7.9%)	
No. of lymph nodes excised						0.503
< 12		19 (7.9%)	3 (4.5%)	8 (9.2%)	8 (9.0%)	
≧12		223 (92.1%)	63 (95.5%)	79 (90.8%)	81(91.0%)	
Pathologic T stage						0.411
T1–T3		193 (79.8%)	51 (77.3%)	67 (77.0%)	75 (84.3%)	
T4		49 (20.2%)	15 (22.7%)	20 (23.0%)	14 (15.7%)	
Pathologic N stage						0.002
N0		105 (43.4%)	17 (25.8%)	37 (42.5%)	51 (57.3%)	
N1		104 (43.0%)	35 (53.0%)	37 (42.5%)	32 (36.0%)	
N2		33 (13.6%)	14 (21.2%)	13 (14.9%)	6 (6.7%)	
Pathologic TNM stage						<0.001
High-risk stage II		105 (43.4%)	17 (25.8%)	37 (42.5%)	51 (57.3%)	
Stage III		137 (56.6%)	49 (74.2%)	50 (57.5%)	38 (42.7%)	
KRAS mutation						0.602
No		109 (61.2%)	30 (57.7%)	43 (59.7%)	36 (66.7%)	
Yes		69 (38.8%)	22 (42.3%)	29 (40.3%)	18 (33.3%)	
Missing values	64					
NRAS mutation						
No		174 (98.9%)	51 (100%)	71 (100%)	52 (96.3%)	0.102
Yes		2 (1.1%)	0	0	2 (3.7%)	
Missing values	64					
BRAF mutation						0.073
No		161 (90.4%)	50 (96.2%)	66 (91.7%)	45 (83.3%)	
Yes		17 (9.6%)	2 (3.8%)	6 (8.3%)	9 (16.7%)	
Missing values	66					
PIK3CA mutation						0.765
No		148 (84.1%)	44 (86.3%)	58 (81.7%)	46 (85.2%)	
Yes		28 (15.9%)	7 (13.7%)	13 (18.3%)	8 (14.8%)	
Missing values	66					

Some previous data suggested that the effect of 5-FU chemotherapy was dependent on the MMR status, and that dMMR CC patients might not benefit from 5-FU monotherapy compared to patients with pMMR CC (11, 19, 23, 29). A possible biological explanation is that in the absence of a functional MMR system, repair may only occur through the "base excision repair" system, a process that is less affected by the dNTP disequilibrium induced by 5-FU (30). However, prolonged treatment with 5-FU

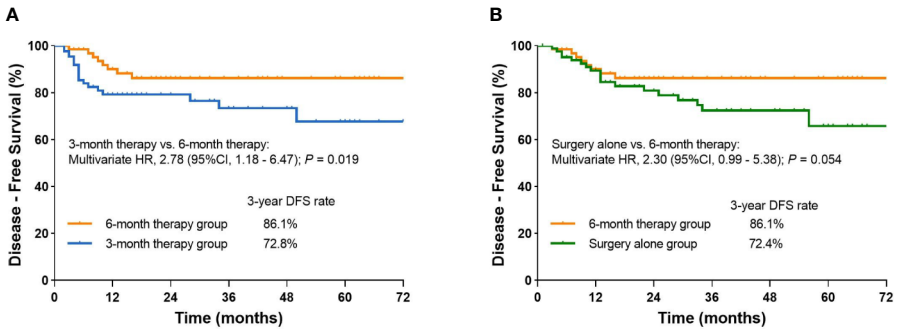

FIGURE 2 | **(A)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and 3-month therapy group; **(B)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and surgery alone group.

leads to the accumulation of DNA lesions that are targeted by another repair pathway (31), and oxaliplatin forms DNA adducts that result in the distortion of secondary DNA structure that is poorly recognized by MMR complexes (32, 33). These might explain why 6-month FOLFOX adjuvant therapy was superior to 3-month therapy in this present study.

Perineural invasion, less than 12 lymph nodes excised, and T4 are independently associated with the decreased DFS in the present analyses. There was no significant difference in DFS among different therapy duration groups in these subgroups of patients, which might be due to the limited number of the cases (data are not shown). These assessments of high-risk factors in daily practice should be discussed because they provide potentially important prognostic information for dMMR CC patients and will help to tailor adjuvant therapy.

Molecular testing (RAS, BRAF) is currently a routine part of clinical practice in colorectal cancer. However, for stage II and III CC patients, the prognostic role of these markers is controversial,

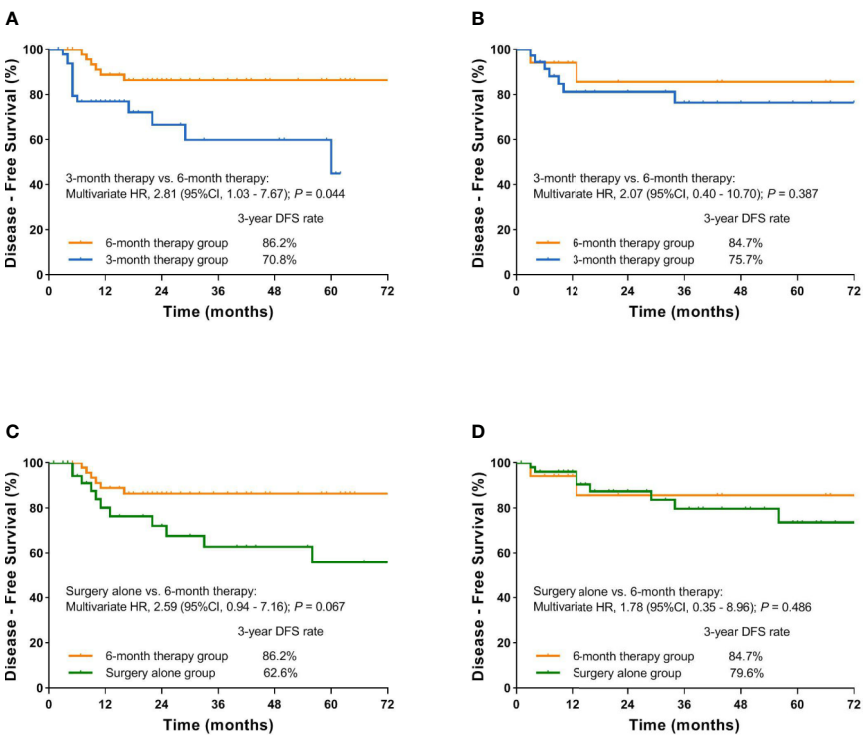

FIGURE 3 | **(A)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and 3-month therapy group for patients with pathologic stage III; **(B)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and 3-month therapy group for patients with high-risk stage II; **(C)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and surgery alone group for patients with pathologic stage III; **(D)** Kaplan-Meier curve of disease-free survival comparing 6-month therapy group and surgery alone group for patients with high-risk stage II.

TABLE 2 | Univariate and multivariate Cox proportional hazards regression model for disease-free survival.

Variable	No. patients	No. events	Univariate analysis		Multivariate analysis	
			HR (95% CI)	*P*	HR (95% CI)	*P*
Total	242	46				
Age						
<65 years	186	33	1	0.360		
≧65 years	56	13	1.35 (0.71–2.56)			
Gender						
Female	87	16	1	0.900		
Male	155	30	0.96 (0.52–1.76)			
Grade of differentiation						
Well or moderately	120	23	1	0.970		
Poorly	122	23	1.01 (0.57–1.80)			
Primary tumor site						
Left	94	23	1	0.170		
Right	148	23	0.67 (0.37–1.19)			
Initial bowel obstruction						
No	153	29	1	0.580		
Yes	89	17	0.84 (0.46–1.54)			
Vascular invasion and/or lymphatic infiltration						
No	186	31	1	0.014	1	0.059
Yes	56	15	2.16 (1.17–4.01)		1.89 (0.98–3.68)	
Perineural invasion						
No	220	37	1	0.001	1	0.004
Yes	22	9	3.37 (1.62–6.99)		3.26 (1.47–7.24)	
No. of lymph nodes excised						
≧12	223	38	1	0.002	1	<0.001
< 12	19	8	3.33 (1.55–7.14)		5.09 (2.23–11.62)	
Pathologic T stage						
T1-T3	193	29	1	<0.001	1	<0.001
T4	49	17	3.07 (1.68–5.61)		3.39 (1.83–6.30)	
Pathologic N stage						
N0	105	17	1	0.220	1	0.081
N1-2	137	29	1.45 (0.80–2.64)		1.80 (0.93–3.47)	
KRAS mutation						
No	109	15	1	0.278		
Yes	69	12	1.53 (0.71–3.27)			
BRAF mutation						
No	161	25	1	0.971		
Yes	17	2	0.97 (0.23–4.12)			
PIK3CA mutation						
No	148	22	1	0.315		
Yes	28	5	1.65 (0.62–4.38)			
Adjuvant chemotherapy duration						
6-month therapy group	66	8	1		1	
3-month therapy group	87	19	2.43 (1.06–5.55)	0.036	2.78 (1.18–6.47)	0.019
Surgery alone group	89	19	1.95 (0.85–4.46)	0.113	2.30 (0.99–5.38)	0.054

CI, confidence interval; HR, hazard ratio.

particularly among dMMR patients. In a pooled analysis of PETACC-8 and N0147, and a post hoc analysis of the PETACC-8, both included resected stage III colon cancer patients receiving adjuvant FOLFOX, BRAF or KRAS mutations are independently associated with the decreased DFS in patients with pMMR, but not dMMR tumors (34, 35). These findings could explain why there was no difference of DFS in patients with KRAS, BRAF, or PIK3CA mutation tumors as compared with wild-type patients in our study.

There were some limitations of our study. First, this was a single-center retrospective study that caused the imbalances in baseline characteristics among the three groups. Fewer patients in the 3-month therapy group and the surgery alone group were in stage III than those in the 6-month therapy group. Propensity score matching was conducted to mitigate the potential bias caused by confounding covariates. The differences in DFS between the 6-month therapy group and the 3-month therapy group were consistent and robust before and after matching. Nevertheless, high-quality randomized controlled clinical trials or subgroup analyses based on a large sample size from IDEA collaboration study are demanded to confirm the optimal duration of chemotherapy for patients with stage III dMMR CC. Second, the duration of adjuvant therapy was left to investigators' discretion in this observation, which was mainly based on not just the disease characteristics but also patient's age and preference. Before matching, the median age of patients in the surgery alone group was significantly older than that of patients in the 3-month therapy group and the 6-month therapy group, that is, 63, 50, and 49 years

TABLE 3 | Selected baseline characteristics before and after propensity score matching.

Characteristics	No. (%)				No. (%)			
	Before matching				After matching			
	6-month therapy group, n=66	3-month therapy group, n=87	*P*	Standardized difference	6-month therapy group, n=51	3-month therapy group, n=51	*P*	Standardized difference
Age, years								
Median (range)	49 (22–78)	50 (23–77)	0.337	0.190	50 (22–78)	48 (26–76)	0.269	0.030
< 65	63 (95.5%)	74 (85.1%)			49 (96.1%)	45 (88.2%)		
≧65	3 (5.5%)	13 (14.9%)			2 (3.9%)	6 (11.8%)		
Pathologic T stage			1.000	0.006			1.000	<0.001
T1-3	51 (77.3%)	67 (77.0%)			38 (74.5%)	38 (74.5%)		
T4	15 (22.7%)	20 (23.0%)			13 (25.5%)	13 (25.5%)		
Pathologic N stage			0.040	0.357			1.000	0.042
N0	17(25.8%)	37(42.5%)			16 (31.4%)	15 (29.4%)		
N1-2	49 (74.2%)	50 (57.5%)			35 (68.6%)	36 (70.6%)		
Initial bowel obstruction			0.481	0.131			1.000	0.042
No	48 (72.7%)	58 (66.7%)			36 (70.6%)	35 (68.6%)		
Yes	18 (27.3%)	29 (33.3%)			15 (29.4%)	16 (31.4%)		
Vascular invasion and/or lymphatic infiltration			0.124	0.264			1.000	0.046
No	55 (83.3%)	63 (72.4%)			40 (78.4%)	39 (76.5%)		
Yes	11 (16.7%)	24 (27.6%)			11 (21.6%)	12 (23.5%)		
Perineural invasion			0.585	0.133			1.000	0.068
No	61 (92.4%)	77 (88.5%)			47 (92.2%)	46 (90.2%)		
Yes	5 (7.6%)	10 (11.5%)			4 (7.8%)	5 (9.8%)		

Characteristics	No. (%)				No. (%)			
	Before matching				After matching			
	6-month therapy group, n=66	Surgery alone group, n=89	*P*	Standardized difference	6-month therapy group, n=35	Surgery alone group, n=35	*P*	Standardized difference
Age, years								
Median (range)	49 (22–78)	63 (22–88)	<0.001	1.053	49 (22–78)	56 (23–76)	1.000	<0.001
< 65	63 (95.5%)	49 (55.1%)			32 (91.4%)	32 (91.4%)		
≧65	3 (5.5%)	40 (44.9%)			3 (8.6%)	3 (8.6%)		
Pathologic T stage			0.302	0.177			1.000	<0.001
T1-3	51 (77.3%)	75 (84.3%)			27 (77.1%)	27 (77.1%)		
T4	15 (22.7%)	14 (15.7%)			8 (22.9%)	8 (22.9%)		
Pathologic N stage			<0.001	0.671			1.000	<0.001
N0	17 (25.8%)	51 (57.3%)			14 (40.0%)	14 (40.0%)		
N1-2	49 (74.2%)	38 (42.7%)			21 (60.0%)	21 (60.0%)		
Initial bowel obstruction			0.013	0.418			1.000	0.063
No	48 (72.7%)	47 (52.8%)			25 (71.4%)	26 (74.3%)		
Yes	18 (27.3%)	42 (47.2%)			10 (28.6%)	9 (25.7%)		
Vascular invasion and/or lymphatic infiltration			0.322	0.172			1.000	0.094
No	55 (83.3%)	68 (76.4%)			32 (91.4%)	31 (88.6%)		
Yes	11 (16.7%)	21 (23.6%)			3 (8.6%)	4 (11.4%)		
Perineural invasion			1.000	0.011			1.000	<0.001
No	61 (92.4%)	82 (92.1%)			32 (91.4%)	32 (91.4%)		
Yes	5 (7.6%)	7 (7.9%)			3 (8.6%)	3 (8.6%)		

old respectively. MOSAIC and NSABP C-07 study revealed no statistically significant survival benefit for the addition of oxaliplatin to fluorouracil with leucovorin as adjuvant treatment for patients older than 70 (36, 37). In addition, patients with dMMR tumors may not benefit from adjuvant chemotherapy with 5-FU alone. For stage III patients in the surgery alone group, 26.3% of them were older than 70. These may be a potential reason why 38 stage III patients did not receive any adjuvant chemotherapy. Third, dMMR in colon cancer is most commonly caused by epigenetic inactivation of MLH1 by promoter hypermethylation in a setting of CpG island methylator phenotype (CIMP) in sporadic tumors (approximately 75%) (38, 39), and the remainder of dMMR tumors are associated with germline mutations (40). A study based on a large database from randomized trials in colon cancer stage III patients suggested that the dMMR tumors with suspected germline mutations were associated with improved DFS after 5-FU-based adjuvant treatment compared with sporadic tumors where no benefit was observed (24). The absence of the family history information and methylation status of MLH1 of our cohort made it difficult to analyze the mechanism of MMR deficiency.

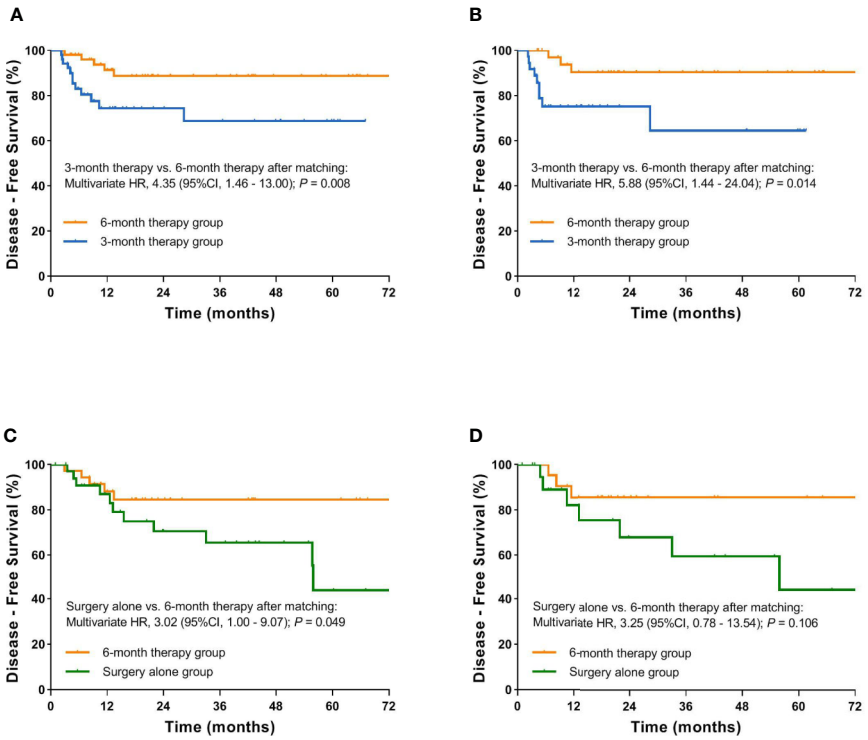

FIGURE 4 | **(A)** Kaplan-Meier curve of disease-free survival comparing 3-month therapy group and 6-month therapy group after propensity score matching; **(B)** Kaplan-Meier curve of disease-free survival comparing 3-month therapy group and 6-month therapy group for patients with stage III after propensity score matching; **(C)** Kaplan-Meier curve of disease-free survival comparing surgery alone group and 6-month therapy group after propensity score matching; **(D)** Kaplan-Meier curve of disease-free survival comparing surgery alone and 6-month therapy group for patients with stage III after propensity score matching.

In conclusion, this study suggests that 6-month duration of mFOLFOX6 adjuvant chemotherapy in dMMR CC patients may be associated with improved DFS compared with 3-month therapy, particularly in stage III patients. However, these results are limited by the presence of potential unmeasured confounding in this retrospective study and require further investigations for confirmation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This study was approved by the Institutional Review Boards of The Sixth Affiliated Hospital of Sun Yat-Sen University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HH, ZW, and YH carried out the studies, participated in collecting data, and drafted the manuscript. CW, JZ, YC, JXL, XYX, CS, WL, and JYL performed the statistical analysis and participated in its design. XHX and YD participated in acquisition, analysis, or interpretation of data and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.579478/full#supplementary-material

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HMGB1, the Next Predictor of Transcatheter Arterial Chemoembolization for Liver Metastasis of Colorectal Cancer?

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HMGB1 is an important mediator of inflammation during ischemia–reperfusion injury on organs. The serum expression of HMGB1 was increased significantly on the 1st day after TACE and decreased significantly which was lower on the 30th day after TACE. Tumor markers of post-DEB-TACE decreased significantly. The correlational analysis showed that patients with low HMGB1 expression had lower risks of fever and liver injury compared those with the higher expression, while the ORR is relatively worse. Patients with lower expression of HMGB1 had longer PFS, better efficacy, and higher quality of life. With the high post-expression, the low expression had lower incidence of fever and liver injury too. There was no statistical difference in the one-year survival among the different groups. The quality of life of all patients was improved significantly. The over-expression of HMGB1 in LMCRC is an adverse prognostic feature and a positive predictor of response to TACE.

Keywords: liver metastasis of colorectal cancer, drug-eluting beads transarterial chemoembolization, transarterial chemoembolization, high mobility group box 1, HMGB1

HIGHLIGHTS

The findings of this study show that patients with low expression of HMGB1 before TACE have a lower incidence of severe liver damage. Post-TACE liver damage is proportional to the pre-TACE expression level of HMGB1, and respondents who reported post-TACE lower levels of HMGB1 also reported significantly lower liver damage. The findings from these studies suggest that higher HMGB1 expression levels before TACE may be a prognosis of liver damage and efficacy. Taken together, these results exhibit that patients with severe HMGB1 changes after TACE had more severe liver damage and were less sensitive, but ORR and PFS of them were relatively better. These results confirm that the changes of HMGB1 maybe the predictor of liver damage and efficacy after TACE.

INTRODUCTION

Colorectal cancer is one of the most common malignant tumors in the world, including colorectal cancer and rectal cancer (1). About 10–25% of colorectal cancer patients find simultaneous liver metastasis at the time of diagnosis (2, 3). When patients have distant metastasis outside the primary site, it is difficult to obtain satisfactory results only by surgical resection (4–6). For unresectable metastatic liver cancer, cryoablation, local thermal ablation of liver, transcatheter arterial infusion (TAI), proton therapy, liver radioactive particle implantation, and transcatheter arterial chemoembolization (TACE) are some good non-surgical treatment methods (7–10).

TACE is considered one of the most effective and safe treatment for advanced liver cancer (11). It is generally assumed to play a considerable role in liver solid tumors, but it has also been reported to have the potential to cause significant damage to liver function (12). There are three reasons that liver injury after TACE are common: the history of concomitant cirrhosis, chemotherapeutic drugs, and the process of ischemia–reperfusion in the liver (13). After TACE, the block of blood supply to local liver tissue at the embolic site leads to local ischemia and hypoxia. After a period of time (usually 7–30 days), local blood supply is restored under the dual action of flowing blood and the establishment of collateral circulation. Therefore, we can assume that the liver undergoes a complete ischemia–reperfusion process after TACE. Conventional TACE (c-TACE) and drug-eluting bead transcatheter aterial chemoembolization (DEB-TACE) are widely used at present. Lipiodol suspended with an anticancer drugs and gelatin sponge particles served as embolic-agents are widely used in c-TACE. Chemotherapeutic drugs were delivered to the tumor by super-selective catheterization, and then the nutrient vessels were sealed with embolic materials. At present, several novel spherical embolic drugs-carrying/drug-eluting beads (DEBs) have been developed to release the drug slowly and long-term, reduce liver damage and improve the local concentration of anticancer drugs. The biggest difference between the two is that DEB-TACE combines drugs and embolic materials in drug-loaded microspheres, but their effects on local blood disruption in the liver are similar. Regardless of the difference of treatment modalities, some chemotherapeutic drugs (such as irinotecan, doxorubicin, oxaliplatin, *etc.*) inevitably have a killing effect on peritumoral tissue (14). Lead to the powerful killing effect of chemotherapeutics, normal liver tissues appeared damaged, necrotic, and apoptotic (14). On the other hand, some recent findings show that inflammatory mediators after TACE play a role in the reestablishment of collateral circulation. Therefore, after TACE, timely prediction and clinical treatment of patients' liver damage can effectively reduce the possibility of tragic outcomes. However, there are certain drawbacks of the current liver function test, like insufficient sensitivity and higher latency. When the results of the liver function test after TACE showed obvious abnormalities, patients often have reached the level of severe liver damage. It is necessary to find a critical demand for prognostic and predictive biomarkers in liver damage (15).

Previous studies in patients with primary liver cancer have shown that expression of high mobility group box-1 (HMGB1) in local liver tissues can rise dramatically in a few hours after TACE (16). Significant changes in serum expression of HMGB1 could be detected at 12 to 24 h after TACE, and it could reach the highest level at 28 to 36 h. Finally, HMGB1 gradually returned to the normal level within the following month (17). In view of the repeated traumatic examination of the liver that will bring certain risks of complication to patients, the concentration level of HMGB1 in the blood is the predictor to analyze the liver damage and avoid the bad impact of repeated liver biopsy. In this study, we studied the level of HMGB1 in the blood after TACE and verified the predictive ability of HMGB1 on liver damage and the efficacy of TACE. To this end, we generated a comprehensive review after TACE at the liver damage, safety and progression-free survival time (PFS) by blood samples, clinical information, and the results of follow-up.

METHOD

Study Design

A prospective, randomized study recruited 106 LMCC patients from December 2017 to July 2019 in Shandong Tumor Hospital as previously described. All procedures were performed with a protocol approved by the ethics committee. Patients were required to be 18 years of age or older and have a diagnosis of liver metastases from colorectal cancer. Patients with a prior anticancer treatment within 2 months were not eligible for enrollment. Prior to the collection of biological samples and TACE, all patients were required to give full informed consent. All patients had radiologic imaging either by computed tomography scanning (CT) and/or magnetic resonance imaging (MRI) before TACE to document the presence of any other metastases. Serum tumor markers (CA19-9, CEA), serum HMGB1 level, liver function and blood cell cluster differentiation antigen were examined 4 to 6 h before TACE and 1, 3, 5, 7, 30 days after treatment. Laboratory analysis of serum preparation was performed at the Shandong Province Cancer Hospital Central Laboratory. Approximately 10 ml of peripheral blood was drawn by the peripheral vein puncture in two standard serum tubes and centrifuged (10 min, 2,000g, room temperature) within 24 h following the collection time to remove clots. The researcher collected and dispensed the serum into multiple 2 ml cryotubes and stored it at −80℃. Any contaminated samples were excluded from the analysis. The concentration of HMGB1 in serum was measured by the ELISA kit (Novus Biologicals, LLC, US) and immune cells were determined by the flow cytometry assay (Thermo Fisher Scientific Inc. US). All assays were run according to the manufacturer's instructions, and all controls were within the ranges provided by the manufacturer. In this study, all patients underwent TACE for the liver metastases and symptomatic treatment for the possible adverse reaction. Enhanced imaging examination obtained from all cases was centrally reviewed by two radiologists to verify the diagnoses made by the researcher. The patients had a regular review with their physician every month during the first six months and then every two months until the end of follow-up.

The treatments were performed by two designated interventional radiology physicians (20- and 11-years' experience). The medical

imaging results were viewed by a radiologist (minimum 10 years of experience) and reviewed by another radiologist. The follow-up information and results were compiled and maintained by a designated researcher. Data analysis was conducted independently by two researchers.

Group

Due to the differences in drug release rate and local concentration between the two TACE modalities, we initially divided patients into DEB-TACE (CalliSpheres®, Jiangsu Hengrui Medicine Co. Ltd., Jiangsu, P.R. China) group and c-TACE groups. Then, we classified that patients with pre-HMGB1 level in serum above 17.5 pg/ml as the preoperative high expression group and others as the preoperative low expression group. Whether the change of HMGB1 concentration in the sample on the first day after TACE is more than 50% is defined as the grouping standard. According to the high change group of HMGB1 before TACE increased by more than 50%, and the patients with variation less than 50% were low change group.

Follow-Up

PFS, the most important efficacy indicator in this study, is the time between the date the patient enters the group for treatment and any documented tumor progression or death from any cause (not limited to death from cancer). The treatment outcomes of TACE can be classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) according to mRECIST1.1. The objective remission rate (ORR) in this study is the proportion of patients whose target tumor shrinks to the SD level and remains there for a period.

Statistical Analysis

The statistical data in this study were analyzed using SPSS version 22.0 (SPSS Inc., Chicago, US) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA 92108). Sample size in this study was calculated by PASS 15.0 (NCSS, LLC. Kaysville, Utah, USA). The sample size was determined by power analysis using preliminary data obtained in our laboratory with the following assumptions: α of 0.05 (two-tailed), power of 90%, difference in patients between before and after TACE, and a standard deviation of 17.5 pg/L. Fisher's exact test (two-tailed) was used to compare categorical variables and Mann–Whitney U test (two-tailed) for continuous variables. PFS was analyzed using the Kaplan–Meier method and compared *via* the log-rank test. Comparisons were made using the log-rank test (for univariate analysis). Between-group comparisons were examined using either the t-test or the chi-square test. The correlation analysis was performed using Poisson's test, and p-values less than 0.05 were considered statistically significant. All tables are drawn by Microsoft Office Word 2019 (Microsoft, Redmond, WA, USA).

RESULTS

A total of 126 patients enrolled in the study, and 106 of them (82 males and 24 females) were evaluable. Patients were divided into two groups: 56 of them received DEB-TACE and the rest received c-TACE. The mean age of the evaluable study cohort was 61 years old (range: 30 to 88), with a mean age for the c-TACE group of 60 years old (range: 30 to 79) and DEB-TACE 62 years old (range: 38 to 88). There were 51 patients with rectal cancer (23 c-TACE and 28 DEB-TACE) and 55 patients with colon cancer (27c-TACE and 28 DEB-TACE) diagnosed in the study group. Patient demographics and characteristics are illustrated in **Table 1**. Data analysis was conducted independently by two researchers.

Level of HMGB1 of Post-TACE

The patients' average HMGB1 of pre-TACE was 19.68 pg/ml and at 1st after TACE was 32.25 pg/ml, $p<0.05$. At 30 days after treatment, the level was 17.19 PG/ml, which was statistically significant. The changes of HMGB1 expression in patients are shown in **Table 1**.

Pre-TACE Level of HMGB1 and Prognosis

The patients were grouped by the level of HMGB1 in the serum before TACE. The basic information about the four groups of patients is shown in **Table 2**.

A comparison of the changes in liver function during treatment in each group (**Figure 1**) revealed that most of the index markers failed to show sufficient statistical significance (**Table 3**), but the liver damage seems more severe in the high pre-TACE HMGB1 expression group, regardless of whether they received DEB-TACE or c-TACE.

TABLE 1 | Patients' characteristics before TACE.

Characteristics	DEB-TACE Patients	c-TACE Patients
Gender		
Male	41 (73.21%)	41 (82.00%)
Female	15 (26.79%)	9 (18.00%)
Age		
<60	22 (39.29%)	22 (44.00%)
≥60	34 (60.71%)	28 (56.00%)
ECOG Score [a]		
0	2 (3.57%)	6 (12.00%)
1	28 (50.00%)	27 (54.00%)
2	20 (35.71%)	12 (24.00%)
3	6 (10.72%)	5 (10.00%)
BCLC [b]		
A	12 (21.43%)	9 (18.00%)
B	44 (78.57%)	41 (82.00%)
Tumor differentiation		
No reported	22 (39.29%)	20 (40.00%)
Low	10 (17.86%)	6 (12.00%)
Moderate	12 (21.43%)	10 (20.00%)
High	12 (21.43%)	14 (28.00%)
The expression of HMGB1 (pg/ml)		
Pre-TACE	19.14 ± 3.91	19.98 ± 3.98
1st after TACE	31.55 ± 7.15	32.86 ± 7.62
3rd after TACE	31.31 ± 7.10	32.55 ± 7.65
5th after TACE	30.75 ± 7.21	32.08 ± 7.52
7th after TACE	28.78 ± 6.69	30.16 ± 6.81
30th after TACE	17.39 ± 2.86	17.01 ± 2.44

[a]*ECOG Score : Eastern Cooperative Oncology Group Score Standard.*
[b]*BCLC: Barcelona Clinic Liver Cancer.*

TABLE 2 | Characteristics of patients of HMGB1 expression subgroup.

	DEB-TACE			c-TACE			DEB-TACE			c-TACE		
	High-expression (n = 34)	Low-expression (n = 22)	*P*-value	High-expression (n = 28)	Low-expression (n = 22)	*P*-value	High-change (n = 33)	Low-change (n = 23)	*P*-value	High-change (n = 30)	Low- change (n = 20)	*P*-value
Age	60.47 ± 11.60	64.32 ± 10.15	0.21	58.86 ± 10.59	61.59 ± 12.48	0.41	60.45 ± 10.73	64.17 ± 11.51	0.22	56.90 ± 12.41	64.80 ± 7.87	0.02
Sex(male)	26	15	0.50	22	19	0.49	28	13	0.03	26	15	0.33
Tumor differentiation			0.05			0.66			0.75			0.43
High	10	2		5	9		7	5		8	6	
moderate	8	4		8	2		7	5		8	2	
low	5	4		5	1		5	5		3	3	
HMGB1 expression(pg/ml)	22.36 ± 2.83	15.79 ± 1.02	<0.01	21.78 ± 2.69	15.19 ± 1.09	<0.01	88.84%	33.64%	<0. 01	87.93%	31.77%	<0.01
BCLC [a]			0.40			0.45			0.19			0.77
A	6	6		4	5		9	3		5	4	
B	28	16		24	17		24	20		25	16	

[a]*BCLC, Barcelona Clinic Liver Cancer.*

Figure 2 depicts the changes of tumor markers and immune function of patients during the treatment, which have more detailed comparisons in **Table 3**. There was a significant decrease of tumor marker in all patients after treatment. The patients in the four groups had transient immune disorders after TACE, but the degree of inhibition in the low expression group was slight than the others. Immune function of all could be recovered to the level of pre-treatment for one month.

The results of statistics of some common adverse reactions rate after TACE are shown in **Table 4**. Patients had similar risks of vomiting, abdominal pain, and nausea, but those who exhibited high expression of HMGB1 before TACE had a higher risk of fever.

The analysis of the treatment outcomes of the four groups of patients is shown in **Table 4**. Combined with the significant decrease of tumor markers, it can be found that most patients have a good treatment effect even if they receive different TACE. The table also shows that the quality of life of all patients was significantly improved after TACE.

As shown in **Figure 3A**, a correlation was found between the pre-expression of HMGB1 and PFS. The differences between the level of HMGB1 and 1-year survival are highlighted in **Figure 3B**.

The Change of Post-TACE Level of HMGB1 and Prognosis

The basic information about the four groups of patients is shown in **Table 3**.

From the information of **Figures 4** and **Table 5**, we can find that there is a certain relation between the changes of liver function and the changes of HMGB1. The rise of HMGB1 is accompanied by subsequent liver function damage, which means that the rise of HMGB1 probably indicates the severity of the liver injury. It can be seen from the change trend chart that as the change curve of HMGB1 showed a significant rise, the markers of liver damage also showed a significant upward trend in the following days.

Table 5 shows the changes of tumor markers and immune function after TACE. The trend chart (**Figure 5**) shows that the degree of immunosuppression after DEB-TACE was slightly severer than that after c-TACE; however, the difference between them revealed no statistically significant differences.

The adverse reactions after the TACE of the four groups were analyzed, and the results are shown in **Table 6**. Fever is the most closely related adverse reaction to the change of HMGB1.

The treatment results of the four groups are shown in **Table 6**. The patients with a small increase in HMGB1 after TACE have a relatively good treatment effect. The PFS and one-year survival are shown in **Figures 3C, D**.

As can be seen from the data in **Table 6**, the quality of life of all patients benefits from treatment.

DISCUSSION

In this study, the level of HMGB1 was found significantly elevated in the blood after TACE. It expands the knowledge on the association between HMGB1 and treatment outcome in LMCRC by showing its magnitude rather than just showing that there is a statistically

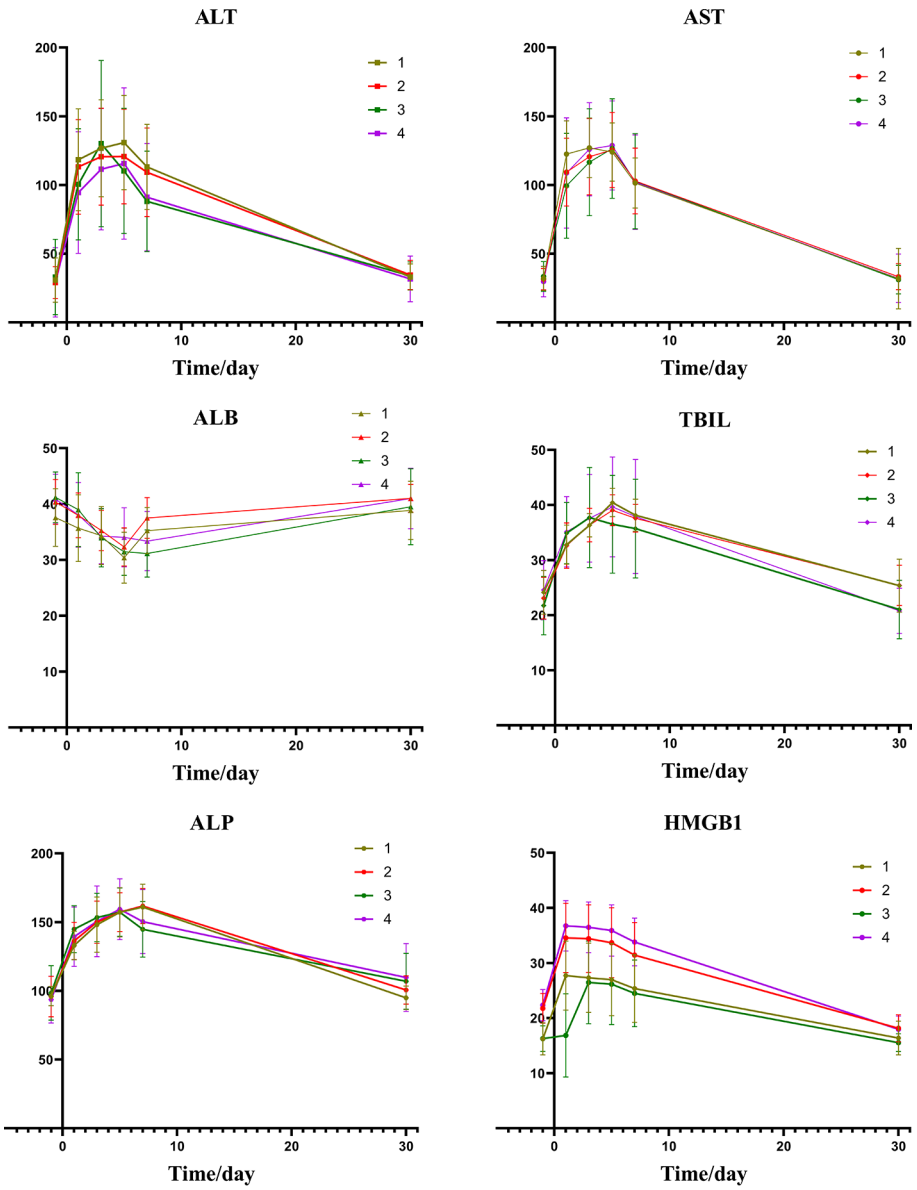

FIGURE 1 | Group1: low pre-expression of HMGB1 with c-TACE; Group2: high pre-expression of HMGB1 with c-TACE; Group3: low pre-expression of HMGB1 with DEB-TACE; Group4: high pre-expression of HMGB1 with DEB-TACE.

significant relationship. HMGB1 was more enriched in the serum of patients with severe liver impairment compared to the preoperative low-expression group. We identified a highly significant relation among HMGB1expression, liver damage, and PFS.

In addition, the analysis of the changes of HMGB1 after TACE can improve the sensitivity of it in the diagnosis of liver function damage. HMGB1 may be a possible prognostic factor for adverse reactions in patients with LMCRC.

Due to their stability and specificity in most bodily fluids, HMGB1 provides a high potential to serve as a liquid biopsy tool for some cancers and sterile inflammation (18). Some researchers performed proteomic analyses to the clinical significance of HMGB1 in serum-purified exosomes from malignant mesothelioma cancer patients and identified it as potential biomarkers in diagnosis and prognosis (19, 20). Dr. Venereau believes that high levels of serum hyper-acetylated HMGB1 are sensitive disease biomarkers (21). He also found that injection of HMGB1 accelerates tissue repair by acting on muscle stem cells, hepatocytes, and infiltrating cells (22). Dr. Liu concludes that HMGB1 protein is a valuable marker for the progression of CRC patients. High HMGB1 expression is associated with poor overall survival in patients with CRC (23).

In the case of LMCRC, we have further confirmed strong correlations between elevated expression of HMGB1 and liver damage or PFS and discovered strong correlations between

TABLE 3 | Changes of HMGB1, liver function, tumor markers and immune cells after TACE.

	DEB-TACE				c-TACE			
	High HMGB1 expression	Low HMGB1 expression	*p*-value	95%CI	High HMGB1 expression	Low HMGB1 expression	*p*-value	95%CI
Pre-ALT	29.14 ± 25.26	33.12 ± 27.48	0.58	3.97(–10.37–18.31)	29.07 ± 11.55	30.82 ± 16.12	0.66	1.75(–6.11–9.62)
1st post-ALT	94.48 ± 44.22	100.57 ± 40.44	0.61	6.09(–17.39–29.56)	113.21 ± 34.44	118.42 ± 37.04	0.61	5.21(–15.19–25.60)
3rd post-ALT	111.59 ± 44.25	130.13 ± 60.53	0.19	18.53(–9.55–46.62)	120.67 ± 35.33	126.74 ± 35.32	0.55	6.07(–14.17–26.30)
5th post-ALT	115.69 ± 55.04	110.23 ± 45.58	0.70	–5.47(–33.75–22.82)	120.77 ± 34.39	130.92 ± 34.28	0.30	10.15(–9.52–29.83)
7th post-ALT	91.17 ± 39.10	88.15 ± 36.50	0.77	–3.02(–23.93–17.88)	109.31 ± 32.20	113.28 ± 30.96	0.66	3.97(–14.17–22.11)
30th post-ALT	31.74 ± 16.61	33.94 ± 10.18	0.58	2.20(–5.73–10.13)	34.57 ± 10.60	33.07 ± 9.56	0.61	–1.49(–7.31–4.32)
Pre-AST	29.80 ± 11.08	33.49 ± 10.87	0.23	3.70(–2.34–9.73)	31.54 ± 7.73	32.08 ± 8.88	0.82	0.53(–4.20–5.26)
1st post-AST	108.78 ± 40.11	99.49 ± 38.18	0.39	–9.30(–30.89–12.30)	109.40 ± 24.73	122.55 ± 24.09	0.07	13.14(–0.86–27.15)
3rd post-AST	126.04 ± 33.92	116.64 ± 38.83	0.34	–9.40(–29.10–10.30)	120.69 ± 27.76	127.07 ± 21.70	0.38	6.37(–8.12–20.86)
5th post-AST	128.75 ± 32.42	126.58 ± 36.26	0.82	–2.17(–20.80–16.64)	125.54 ± 27.37	124.07 ± 21.24	0.84	–1.47(–15.71–12.78)
7th post-AST	102.14 ± 34.33	102.74 ± 34.61	0.95	0.60(–18.29–19.49)	103.01 ± 23.99	101.51 ± 18.19	0.81	–1.50(–13.50–10.90)
30th post-AST	32.10 ± 17.73	31.23 ± 10.43	0.82	–0.87(–8.42–6.69)	33.33 ± 9.46	31.94 ± 22.00	0.63	–1.39(–7.21–4.44)
Pre-ALB	40.89 ± 4.43	41.22 ± 4.54	0.78	0.34(–2.12–2.79)	40.37 ± 4.01	37.60 ± 5.17	0.04	–2.77(–5.37–4.57)
1st post-ALB	38.07 ± 5.78	39.00 ± 6.61	0.58	0.93 (–2.42–4.28)	37.97 ± 4.01	35.70 ± 5.97	0.11	–2.27(–5.12––0.57)
3rd post-ALB	34.26 ± 4.95	33.98 ± 5.22	0.84	–0.28(–3.05–2.50)	35.28 ± 3.62	34.36 ± 5.19	0.46	–0.93(–3.43–1.58)
5th post-ALB	34.05 ± 5.29	31.46 ± 4.22	0.06	–2.59(–5.28–0.10)	32.33 ± 3.40	30.41 ± 4.57	0.10	–1.92(–4.18–0.35)
7th post-ALB	33.36 ± 5.31	31.17 ± 4.23	0.11	–2.19(–4.89–0.51)	37.50 ± 3.65	35.24 ± 4.11	0.04	–2.26(–4.47––0.05)
30th post-ALB	41.00 ± 5.41	39.53 ± 6.82	0.37	–1.46(–4.75–1.83)	41.04 ± 2.49	38.87 ± 5.22	0.08	–2.17(–4.64–0.31)
Pre-TBIL	24.57 ± 5.34	21.76 ± 5.33	0.06	–2.82(–5.75–0.11)	23.10 ± 3.77	24.15 ± 3.97	0.35	1.04(–1.17–3.25)
1st post-TBIL	35.14 ± 6.39	34.91 ± 5.56	0.89	–0.23(–3.57–3.10)	32.64 ± 4.12	32.83 ± 3.54	0.86	1.10(–2.02–2.41)
3rd post-TBIL	37.61 ± 7.97	37.73 ± 9.09	0.96	0.12(–4.50–4.74)	36.37 ± 3.06	36.32 ± 2.12	0.95	–0.05(–1.59–1.49)
5th post-TBIL	39.66 ± 9.07	36.52 ± 8.89	0.21	–3.14(–8.08–1.79)	39.05 ± 2.79	40.46 ± 2.57	0.07	1.40(–.0.14–2.95)
7th post-TBIL	37.92 ± 10.35	35.72 ± 8.96	0.42	–2.20(–7.60–3.19)	37.61 ± 2.51	38.16 ± 2.91	0.48	0.55(–0.99–2.09)
30th post-TBIL	20.81 ± 4.10	21.05 ± 5.32	0.85	0.24(–2.30–2.77)	25.42 ± 3.66	25.36 ± 4.81	0.96	–0.06(–2.47–2.35)
Pre-ALP	93.63 ± 17.09	98.52 ± 19.79	0.30	4.89(–4.43–14.20)	95.84 ± 14.74	96.20 ± 6.99	0.91	0.36(–6.50–7.23)
1st post-ALP	139.34 ± 21.65	144.91 ± 17.02	0.31	5.58(–5.38–16.54)	136.35 ± 13.60	133.06 ± 10.40	0.35	–3.29(–10.34–3.76)
3rd post-ALP	150.61 ± 25.76	153.41 ± 17.67	0.63	2.79(–8.85–14.44)	149.94 ± 15.35	148.18 ± 20.17	0.73	–1.76(–11.85–8.34)
5th post-ALP	159.43 ± 22.13	157.26 ± 17.56	0.70	–2.18(–13.41–9.05)	157.31 ± 14.06	157.13 ± 17.90	0.97	–0.18(–12.25–8.74)
7th post-ALP	150.40 ± 23.28	144.74 ± 20.31	0.36	–5.66(–17.82–6.51)	161.76 ± 12.85	161.03 ± 16.70	0.86	–0.73(–9.13–7.67)
30th post-ALP	109.69 ± 24.76	106.94 ± 20.36	0.67	–2.75(–15.44–9.95)	100.65 ± 10.29	94.91 ± 8.61	0.04	–5.74(–11.23––0.24)
Pre-CEA	547.90 ± 1,506.18	369.10 ± 646.92	0.60	–178.80(–861.57–503.97)	433.67 ± 444.94	542.61 ± 1139.32	0.65	108.95(–363.17–581.06)
30th post-CEA	416.60 ± 1,120.93	247.60 ± 409.36	0.86	20.63(–204.03–245.29)	204.43 ± 206.56	214.52 ± 318.59	0.89	10.09(–139.74–159.91)
Pre-CA19-9	547.39 ± 1,777.83	416.60 ± 1120.93	0.76	–130.78(–984.19–722.62)	565.02 ± 890.67	592.34 ± 1437.19	0.94	27.32(–638.22–692.86)
30th post- CA19-9	137.11 ± 234.16	286.40 ± 712.42	0.35	149.28(–174.99–473.56)	330.32 ± 567.47	161.34 ± 231.36	0.20	–168.99(–428.07–70.09)
Pre-CD3+	64.45 ± 7.46	66.94 ± 9.76	0.29	2.49(–2.13–7.11)	64.65 ± 13.57	65.42 ± 8.63	0.82	0.78(–5.91–7.46)
7th post-CD3+	56.42 ± 5.21	57.09 ± 5.24	0.64	0.66(–2.20–3.53)	58.43 ± 5.59	58.87 ± 5.09	0.78	0.43(–2.65–3.51)
30th post-CD3+	66.02 ± 7.10	67.68 ± 8.93	0.45	1.66(–2.66–5.97)	67.58 ± 8.78	66.40 ± 7.30	0.62	–1.17(–5.85–3.50)
Pre-CD19+	11.55 ± 3.51	11.48 ± 3.90	0.94	–0.07(–2.09–1.84)	11.44 ± 4.05	9.88 ± 2.41	0.09	–1.56(–3.42–0.29)
7th post–CD19+	10.19 ± 3.15	10.13 ± 3.41	0.94	–0.06(–1.85–1.72)	10.05 ± 3.34	8.31 ± 2.25	0.03	–1.75(–3.34––0.15)
30th post–CD19+	11.94 ± 3.36	11.94 ± 3.83	0.99	–0.01(–1.95–1.95)	11.75 ± 3.89	10.15 ± 2.44	0.08	–1.59(–3.40–0.22)
Pre-NK cell	24.89 ± 8.79	27.03 ± 8.04	0.36	2.14(–2.53–6.81)	25.41 ± 9.34	25.38 ± 8.20	0.70	0.97(–4.11–6.04)
7th post-NK cell	20.18 ± 7.69	22.54 ± 7.92	0.27	2.37(–1.90–6.63)	19.43 ± 7.68	20.21 ± 7.14	0.71	0.79(–3.48–5.05)
30th post-NK cell	24.93 ± 8.76	27.50 ± 7.98	0.27	2.57(–2.07–7.21)	24.65 ± 9.16	25.55 ± 7.98	0.72	0.90(–4.026–5.86)
Pre-CD3+CD4+	39.67 ± 6.70	37.24 ± 5.87	0.17	–2.43(–5.94–1.07)	36.47 ± 6.77	39.07 ± 8.56	0.24	2.60(–1.76–6.96)
7th post-CD3+CD4+	43.10 ± 6.31	40.55 ± 7.22	0.17	–2.55(-6.21–1.12)	41.94 ± 6.54	44.24 ± 6.50	0.22	2.30(–1.43–6.04)
30th post-CD3+CD4+	39.95 ± 7.37	38.11 ± 7.78	0.38	–1.85(–5.98–2.29)	36.16 ± 6.78	39.04 ± 8.99	0.20	2.89(–1.59–7.37)

(Continued)

TABLE 3 | Continued

	DEB-TACE				c-TACE			
	High HMGB1 expression	Low HMGB1 expression	*p*-value	95%CI	High HMGB1 expression	Low HMGB1 expression	*p*-value	95%CI
Pre-CD3+CD8+	28.23 ± 4.38	29.73 ± 4.22	0.21	1.50(−0.87–3.87)	26.75 ± 6.66	28.17 ± 5.38	0.42	1.41(−2.09–4.93)
7th post-CD3+CD8+	26.62 ± 4.37	27.88 ± 3.39	0.26	1.26(−0.94–3.47)	24.91 ± 6.30	26.30 ± 4.95	0.39	1.40(−1.90–4.69)
30th post-CD3+CD8+	28.59 ± 4.03	29.46 ± 4.40	0.45	0.87(−1.42–3.16)	26.54 ± 6.43	28.18 ± 5.16	0.34	1.64(−1.74–5.02)
Pre-CD4/CD8	1.43 ± 0.30	1.27 ± 0.25	0.04	−0.16(−0.36–−0.01)	1.41 ± 0.30	1.42 ± 0.37	0.89	0.013(−0.18–0.20)
7th post-CD4/CD8	1.65 ± 0.33	1.47 ± 0.32	0.04	−0.18(−0.36–−0.01)	1.76 ± 0.39	1.73 ± 0.38	0.79	−.03(−0.25–0.19)
30th post-CD4/CD8	1.41 ± 0.28	1.33 ± 0.38	0.35	−0.08(−0.26–0.11)	1.41 ± 0.30	1.41 ± 0.34	0.97	0.00(−0.18–0.19)

elevated the HMGB1 and the effect of palliative treatment including the c-TACE and DEB-TACE.

This study focused on the expression and changes of HMGB1 in the serum of colorectal cancer patients with liver metastasis before and after TACE, and we explored the possibility of predicting liver injury, adverse reaction, and PFS after TACE by monitoring the changes of HMGB1. In this study, we detected and compared the expression of HMGB1 before TACE and on the first, third, fifth, and seventh days after TACE. The results of data analysis and change trend chart showed that the expression of HMGB1 changed significantly and reached the peak on the 1st day after TACE, and the expression was stable on the 3rd, 5th, and 7th days. The expression of HMGB1 returned to pre-TACE until the 30th day. For those with low expression of HMGB1 before TACE, the liver injury was slighter, while for those with the severe rise of HMGB1 after treatment, the liver injury was more serious. The expression level of HMGB1 was positively correlated with the degree of liver injury. This result suggests that the level of HMGB1 on the 1st day after treatment compared with pre-TACE expression may predict liver injury.

Embolization and reperfusion after TACE are a standard process of liver ischemia–reperfusion injury in patients (13). After complete embolization of the target vessel by the doctor, the tumor focus of the liver and the surrounding normal liver tissue will form a temporary ischemic area (13). The interruption of the blood supply has left the area in a state of ischemia, hypoxia, and nutrient deprivation, with a large number of tumor cells and hepatocyte death (24). However, the thrombolytic effect of TACE gradually declined with the subsequent constant flushing of blood from the ischemic area. At the same time, collateral circulation was established in the embolized area, which made the embolized area get blood perfusion again. In the whole process, the embolization of drugs and blood vessels not only killed tumor cell, but also produced liver damage. Treatment stress and the changes of tumor microenvironment can aggravate the acute liver failure (25).

HMGB1 plays an important role in the process of ischemia–reperfusion after TACE, and its acetylation and release are mainly regulated by four main modes (18). Firstly, large amounts of hypoxanthine accumulated converted to xanthine during anaerobic respiration after the interruption of blood supply, and the resulting ROS prompt cells to release acetylated HMGB1 (26, 27). Secondly, Kupffer cells can be activated to release IL-1β, IL-6, and TNF-α, which can promote the acetylation of HMGB1 (28, 29). Thirdly, during the late stage of ischemia/reperfusion (the phase of injury caused by blood reperfusion), activated neutrophils and macrophages begin to converge and accumulate towards the ischemic area and stimulate HMGB1 release through the release of inflammatory factors. Lastly, ischemia–reperfusion can lead to Ca^{+} overload, which caused abnormal mitochondrial membrane permeability transporter pore and abnormal electron transport in the respiratory chain to produce ROS, then stimulates the local massive release of HMGB1 (30, 31). Under the combined action of various factors, a large amount of acetylated HMGB1 is released into the extracellular space, and its expression in the ischemic area and body blood rises rapidly. After TACE, the blood vessels of the tumor and surrounding tissue are embolized, which causes

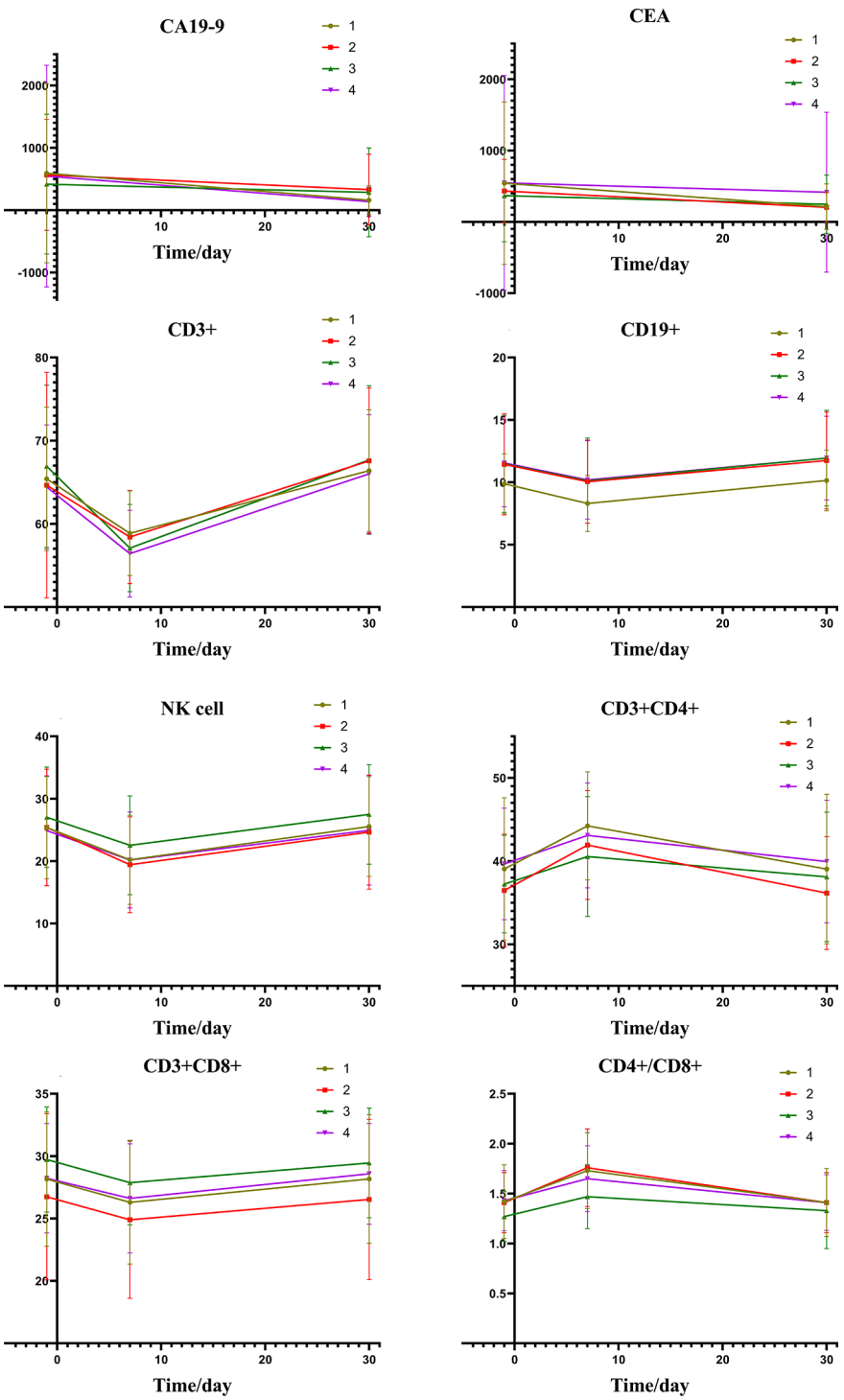

FIGURE 2 | Group1: low pre-expression of HMGB1 with c-TACE; Group2: high pre-expression of HMGB1 with c-TACE; Group3: low pre-expression of HMGB1 with DEB-TACE; Group4: high pre-expression of HMGB1 with DEB-TACE.

TABLE 4 | Expression of HMGB1 group, common adverse reactions treatment outcomes.

	DEB-TACE			c-TACE		
	High HMGB1 expression (34)	Low HMGB1 expression (22)	*p*-value	High HMGB1 expression (28)	Low HMGB1 expression (22)	*p*-value
fever	19	5	0.01	20	9	0.03
vomit	15	6	0.20	11	5	0.22
nausea	20	9	0.19	15	8	0.23
abdominal pain	17	7	0.19	12	6	0.26
hepatic failure	0	0	0	0	0	0
CR [a]	2	4		1	3	
PR [b]	16	14		14	15	
SD [c]	14	3		10	4	
PD [d]	2	1		3	0	
ORR [e]	18	18	0.02	15	18	0.03
Pre-score of Qol[f]	32.76	33.10		31.72	20.91	
Post-score of Qol	47.91	48.32		48.29	47.86	

[a]*CR, complete response.*
[b]*PR, partial response.*
[c]*SD, stable disease.*
[d]*PD, progressive disease.*
[e]*ORR, Objective response rate; ORR = CR + PR.*
[f]*Qol, quality of life.*

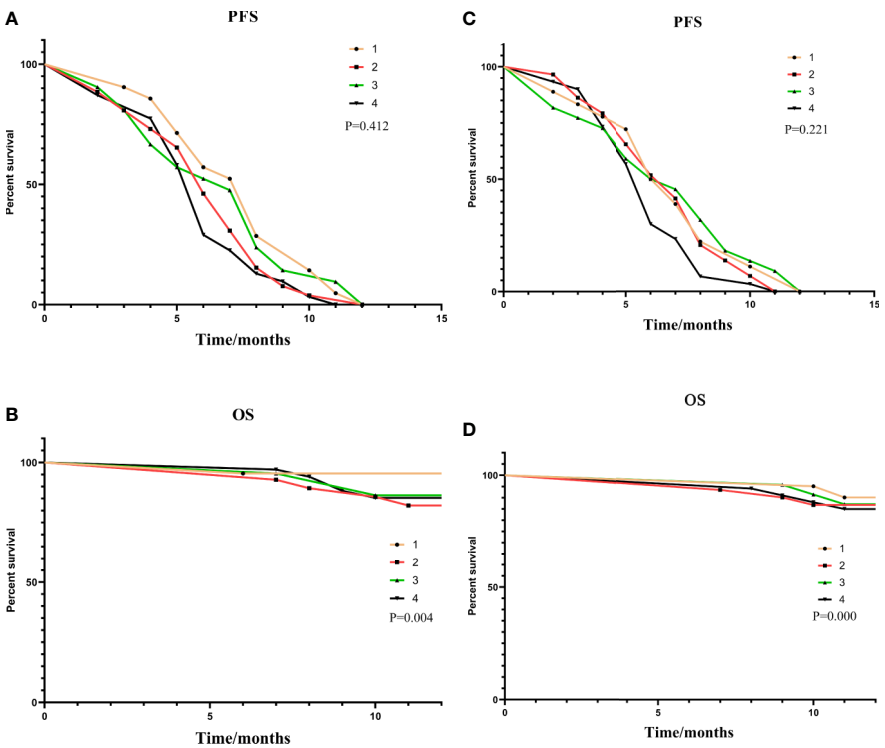

FIGURE 3 | **(A, B)** were subgroup analyses of HMGB1 expression before TACE, and **(C, D)** were subgroup analyses based on HMGB1 changes after TACE. In **(A, B)**: Group1: low pre-expression of HMGB1 with c-TACE; Group2: high pre-expression of HMGB1 with c-TACE; Group3: low pre-expression of HMGB1 with DEB-TACE; Group4: high pre-expression of HMGB1 with DEB-TACE. In **(C, D)**:Group1: low post-expression of HMGB1 with c-TACE; Group2: high post-expressions of HMGB1 with c-TACE; Group3: low post-expressions of HMGB1 with DEB-TACE; Group4: high post-expression of HMGB1 with DEB-TACE.

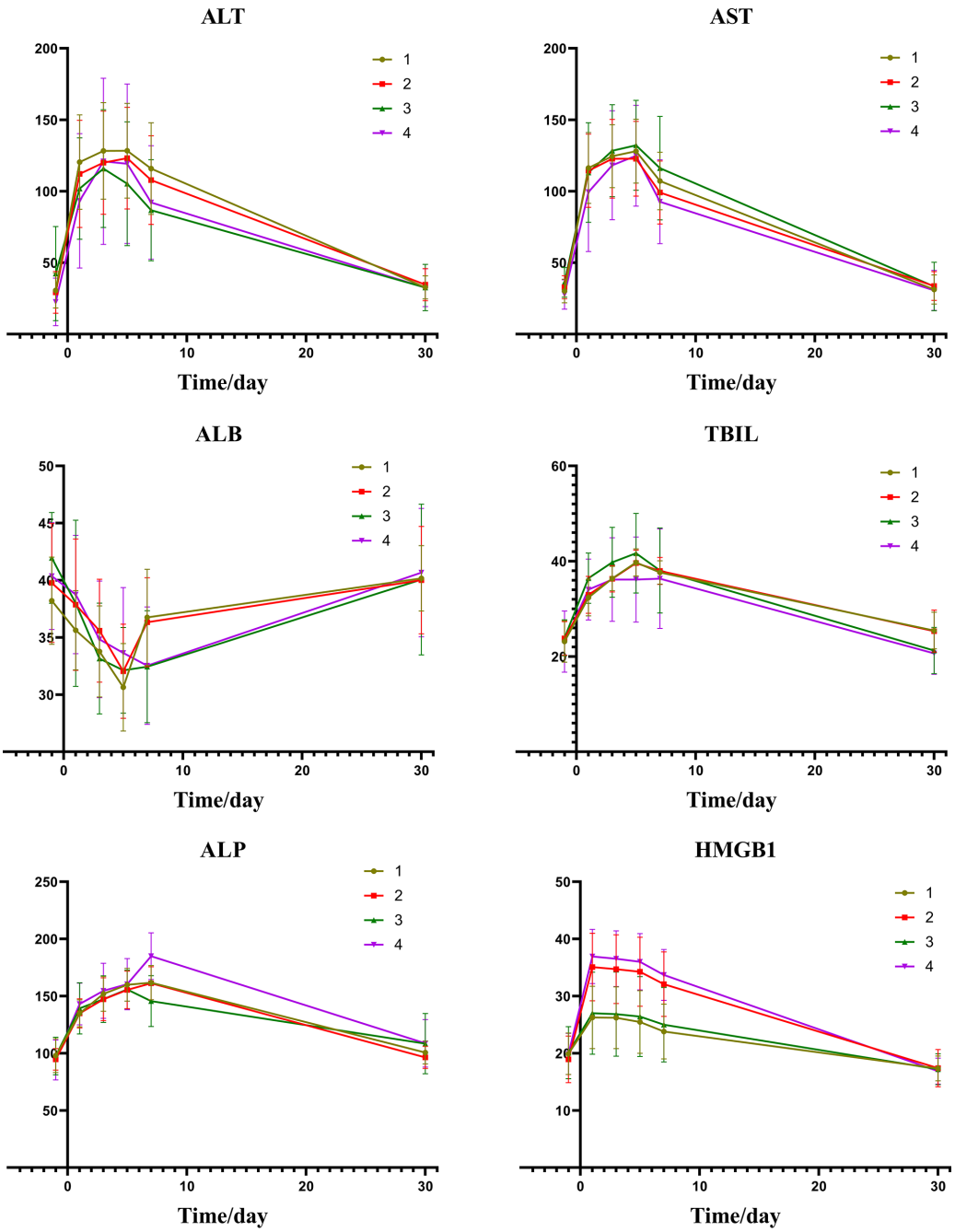

FIGURE 4 | Group1: low post-expression of HMGB1 with c-TACE; Group2: high post-expression of HMGB1 with c-TACE; Group3: low post-expression of HMGB1 with DEB-TACE; Group4: high post-expression of HMGB1 with DEB-TACE.

ischemia. However, the embolic material in the blood vessel is rinsed off by the blood, and the rapid formation of collateral vessels makes the ischemic area quickly regain blood supply. The large amount of HMGB1 produced in this process not only increased in the ischemic area but also reached the whole liver and the whole body by means of blood circulation, which leads to local and systemic inflammation of the liver (32). During ischemia–reperfusion in the liver, HMGB1 plays an important mediating role: within 1– h after TACE, cells in the ischemic area begin to necrotize and rupture, and release HMGB1. Induced by extracellular HMGB1 and other inflammatory factors, it leads to the release of more HMGB1 from the cells in the non-embolized area and mediates severe inflammatory response in the ischemic area (31, 33). Meanwhile, HMGB1 acetylated during the reperfusion phase can stimulate aggregated macrophages and monocytes to actively acetylate and release more HMGB1 (34).

TABLE 5 | Post-expression of HMGB1 group and changes of liver function, tumor marker and immune after TACE.

	DEB-TACE				c-TACE			
	High-change(n = 33)	Low-change (n = 23)	*p*-value	95%CI	High-change (n = 30)	Low-change (n = 20)	*p*-value	95%CI
Pre-ALT	22.57 ± 16.67	42.38 ± 32.99	0.01	19.82(4.68–34.95)	29.28 ± 14.62	30.68 ± 12.30	0.73	1.40 (–6.58–9.38)
1st post-ALT	93.37 ± 47.00	101.89 ± 35.49	0.44	8.52(–13.60–30.64)	112.22 ± 37.52	120.41 ± 33.07	0.43	8.19(–12.40–28.77)
3rd post-ALT	120.93 ± 58.19	115.92 ± 41.25	0.72	–5.01(–33.30–23.29)	120.06 ± 36.13	128.26 ± 33.78	0.42	8.21(–12.24–28.65)
5th post-ALT	119.32 ± 55.90	105.26 ± 43.34	0.32	–14.05(–41.91–13.81)	123.15 ± 35.59	128.37 ± 33.11	0.60	5.22 (–14.88–25.32)
7th post-ALT	92.20 ± 39.77	86.80 ± 35.37	0.60	–5.40(–26.12–15.32)	107.82 ± 31.02	115.91 ± 32.15	0.38	8.09(10.18–26.35)
30th post-ALT	32.54 ± 13.20	32.69 ± 16.20	0.97	0.15(–7.75–8.04)	34.65 ± 11.30	32.80 ± 8.05	0.53	–1.84 (–7.73–4.04)
Pre-AST	27.75 ± 10.27	36.26 ± 10.36	0.00	8.51(2.90–14.12)	32.84 ± 8.07	30.19 ± 8.28	0.27	–2.64 (–7.38–2.09)
1st post-AST	99.52 ± 41.65	113.17 ± 34.92	0.20	13.65(–7.62–34.91)	114.42 ± 25.65	116.34 ± 24.81	0.79	1.92 (–12.78–16.62)
3rd post-AST	118.17 ± 38.13	128.34 ± 32.24	0.30	10.17(–9.35–29.69)	122.80 ± 27.52	124.55 ± 22.01	0.81	1.75(–13.04–16.54)
5th post-AST	124.91 ± 35.29	132.18 ± 31.47	0.43	7.27(–11.13–25.67)	122.78 ± 26.18	128.07 ± 22.38	0.46	5.29 (–9.07–19.65)
7th post-AST	92.71 ± 29.33	116.25 ± 36.31	0.01	23.54(5.92–41.16)	99.11 ± 22.03	107.20 ± 20.07	0.19	8.09(–4.26–20.44)
30th post-AST	30.60 ± 14.00	33.42 ± 16.91	0.50	2.82(–5.48–11.13)	33.67 ± 10.08	31.29 ± 10.18	0.42	–2.38(–8.25–3.50)
Pre-ALB	40.37 ± 4.68	41.96 ± 3.97	0.19	1.59(–0.81–3.99)	39.78 ± 5.20	38.22 ± 3.82	0.26	–1.56 (–4.29–1.16)
1st post-ALB	38.76 ± 5.18	37.99 ± 7.27	0.64	–0.77(–4.10–2.56)	37.87 ± 5.75	35.63 ± 3.47	0.13	–2.24(–5.12–0.65)
3rd post-ALB	34.84 ± 5.10	33.16 ± 4.84	0.22	–1.68(-4.40–1.04)	35.60 ± 4.51	33.78 ± 3.98	0.15	–1.83(–4.32–0.67)
5th post-ALB	33.65 ± 5.72	32.13 ± 3.75	0.27	–1.52(–4.25–1.21)	32.05 ± 4.12	30.64 ± 3.83	0.23	–1.41(–3.74–0.92)
7th post-ALB	32.54 ± 5.14	32.45 ± 4.90	0.95	–0.09(–2.84–2.65)	36.34 ± 3.89	36.76 ± 4.21	0.72	0.43(–1.91–2.76)
30th post-ALB	40.67 ± 5.62	40.07 ± 6.59	0.71	–0.60(–3.89–2.68)	40.02 ± 4.71	40.17 ± 2.86	0.90	0.15(–2.22–2.51)
Pre-TBIL	23.14 ± 6.42	23.94 ± 3.80	0.56	0.80(–1.95–3.54)	23.82 ± 3.54	23.18 ± 4.35	0.57	–0.64 (–2.90–1.61)
1st post-TBIL	34.07 ± 6.38	36.46 ± 5.30	0.15	2.39(–0.85–5.64)	32.95 ± 3.89	32.38 ± 3.83	0.61	–0.57 (–2.81–1.67)
3rd post-TBIL	36.17 ± 8.78	39.79 ± 7.34	0.11	3.62(–0.86–8.09)	36.31 ± 2.78	36.40 ± 2.56	0.91	0.09 (–1.47–1.65)
5th post-TBIL	36.15 ± 8.93	41.70 ± 8.36	0.02	5.54(0.80–10.28)	39.61 ± 2.73	39.76 ± 2.87	0.85	0.16(–1.46–1.77)
7th post-TBIL	36.35 ± 10.44	38.07 ± 8.93	0.52	1.71(–3.65–7.08)	37.98 ± 2.85	37.66 ± 2.46	0.68	–0.32 (–1.89–1.25)
30th post-TBIL	20.65 ± 4.41	21.27 ± 4.88	0.62	0.62(–1.89–3.13)	25.34 ± 4.41	25.47 ± 3.86	0.91	0.13 (–2.31–2.57)
Pre-ALP	94.15 ± 17.51	97.56 ± 16.39	0.47	3.40(–5.89–12.69)	94.67 ± 9.37	97.99 ± 14.90	0.34	3.32 (–3.57–10.21)
1st post-ALP	143.07 ± 18.30	139.31 ± 22.43	0.49	–3.76(–14.69–7.18)	134.86 ± 12.76	134.98 ± 11.87	0.97	0.12(–7.09–7.32)
3rd post-ALP	154.71 ± 24.12	147.41 ± 20.49	0.24	–7.29(–19.66–5.07)	147.28 ± 18.80	151.99 ± 15.26	0.36	4.72(–5.43–14.87)
5th post-ALP	160.50 ± 22.40	155.82 ± 16.97	0.40	–4.68(–15.77–6.42)	155.41 ± 16.49	159.97 ± 14.39	0.32	4.57(–4.55–13.67)
7th post-ALP	184.98 ± 204.77	145.59 ± 22.23	0.36	–39.40(–125.59–46.80)	161.20 ± 14.43	161.80 ± 15.00	0.89	0.61(–7.90–9.11)
30th post-ALP	108.72 ± 20.79	108.44 ± 26.29	0.96	–0.28(–12.91–12.34)	96.42 ± 9.67	100.69 ± 9.97	0.14	4.27 (–1.41–9.96)
Pre-CEA	605.42 ± 1539.29	294.36 ± 561.91	0.36	–311.28(–985.27–363.15)	494.96 ± 1014.14	461.57 ± 391.46	0.89	–33.39(–512.73–445.95)
30th post-CEA	263.92 ± 472.67	193.69 ± 289.73	0.53	–70.23(–292.51–152.04)	205.88 ± 304.64	213.35 ± 176.62	0.92	7.48(–144.34–159.30)
Pre-CA19-9	569.49 ± 1806.03	390.58 ± 1089.37	0.67	–178.90(–1025.43–667.63)	666.36 ± 1466.61	440.06 ± 307.87	0.50	–228.30(–899.45–442.85)
30th post- CA19-9	160.49 ± 253.45	246.37 ± 694.45	0.52	85.89(–177.85–349.62)	286.07 ± 569.82	210.82 ± 190.05	0.57	–75.24(–341.52–191.04)
Pre-CD3+	65.77 ± 9.25	64.94 ± 7.30	0.72	–0.83(–5.47–3.80)	63.71 ± 12.82	66.91 ± 9.33	0.34	3.20(–3.52–9.91)
7th post-CD3+	56.30 ± 4.72	57.24 ± 5.85	0.51	0.94(–1.90–3.77)	58.46 ± 5.41	59.87 ± 5.32	0.79	0.41(–2.71–3.53)
30th post-CD3+	66.64 ± 8.39	66.72 ± 7.14	0.97	0.08(–4.23–4.38)	66.65 ± 8.34	67.67 ± 7.91	0.67	1.02(–3.72–5.76)
Pre-CD19+	11.72 ± 3.71	11.24 ± 3.60	0.64	–0.47(–2.47–1.52)	10.60 ± 3.30	10.98 ± 3.81	0.71	0.38(–1.66–2.42)
7th post–CD19+	10.32 ± 3.26	9.94 ± 3.24	0.67	–0.38(–2.15–1.39)	9.10 ± 2.84	9.56 ± 3.31	0.60	0.46(–1.30–2.23)
30th post–CD19+	12.13 ± 3.62	11.66 ± 3.44	0.62	–0.47(–2.41–1.46)	10.91 ± 3.34	11.25 ± 3.55	0.73	0.35(–1.64–2.34)
Pre-NK cell	25.72 ± 8.83	25.75 ± 8.20	0.99	0.03(–4.64–4.70)	25.41 ± 9.44	23.98 ± 7.85	0.58	–1.43(–6.56–3.70)
7th post-NK cell	20.92 ± 7.88	21.37 ± 7.85	0.84	0.45(–3.84–4.73)	20.37 ± 8.14	18.87 ± 6.14	0.46	–1.51(–5.57–2.57)
30th post-NK cell	25.95 ± 8.75	25.93 ± 8.28	0.99	–0.03(–4.69–4.63)	25.58 ± 8.94	24.26 ± 8.18	0.60	–1.31(–6.33–3.71)
Pre-CD3+CD4+	38.23 ± 6.50	39.41 ± 6.43	0.51	1.17(–2.36–4.70)	37.21 ± 6.97	38.23 ± 8.71	0.65	1.02(–3.45–5.49)
7th post-CD3+CD4+	42.00 ± 6.69	42.24 ± 6.95	0.90	0.24(–3.46–3.95)	42.97 ± 6.04	42.93 ± 7.44	0.98	–0.04(–3.89–3.81)
30th post-CD3+CD4+	38.47 ± 7.26	40.31 ± 7.91	0.37	1.85(–2.26–5.95)	37.22 ± 7.42	37.74 ± 8.71	0.82	0.52(–4.09–5.14)

(Continued)

TABLE 5 | Continued

	DEB-TACE				c-TACE			
	High-change(n = 33)	Low-change (n = 23)	*p*-value	95%CI	High-change (n = 30)	Low-change (n = 20)	*p*-value	95%CI
Pre-CD3+CD8+	29.19 ± 3.94	28.28 ± 4.89	0.44	−0.91(−3.28–1.46)	26.94 ± 6.05	28.02 ± 6.30	0.55	1.08(−2.49–4.65)
7th post-CD3+CD8+	27.37 ± 3.54	26.76 ± 4.70	0.58	−0.61(−2.82–1.60)	25.33 ± 6.05	25.81 ± 6.03	0.77	0.48(−2.87–3.84)
30th post-CD3+CD8+	29.12 ± 3.77	28.67 ± 4.74	0.70	−0.45(−2.73–1.84)	26.87 ± 5.82	27.85 ± 6.13	0.57	0.98(−2.47–4.43)
Pre-CD4/CD8	1.32 ± 0.21	1.44 ± 0.37	0.17	0.12(−0.05–0.29)	1.44 ± 0.40	1.28 ± 0.20	0.46	−0.06(−0.23–0.11)
7th post-CD4/CD8	1.55 ± 0.27	1.63 ± 0.41	0.40	0.08(−0.11–0.26)	1.77 ± 0.42	1.71 ± 0.33	0.62	−0.06(−0.28–0.17)
30th post-CD4/CD8	1.33 ± 0.24	1.45 ± 0.40	0.21	0.12(−0.07–0.31)	1.43 ± 0.36	1.37 ± 0.23	0.48	−0.07(−0.25–0.12)

According to the statistics and analysis of common adverse reactions after TACE, patients with low pre-TACE expression of HMGB1 had a lower incidence of fever than those with higher expression. In addition, patients with a lower postoperative increase in HMGB1 expression had a lower risk of developing a fever. The results suggest that there is a correlation between the expression of HMGB1 and fever. On the one hand, post-TACE fever is due to the absorption of necrotic material at the site of embolization, which is the classic "absorption heat". On the other hand, HMGB1 can lead to the release of pro-inflammatory factors and excitatory amino acids in the microenvironment, which also promotes the development of fever in the body (35). Therefore, the high expression of HMGB1 in the serum before and after treatment may predict a higher risk of fever in patients.

DEB-TACE was modified from c-TACE. The classical c-TACE is to infuse chemotherapeutic drugs into the blood vessels of tumors, and then embolize the blood vessels with insoluble materials. The microspheres used in DEB-TACE can both adsorb drugs and serve as materials for embolization of blood vessels. Although there are some differences in the surgical procedures, the principles of the two treatments for tumors are consistent. For ischemia and hypoxia caused by c-TACE and DEB-TACE, the reperfusion injury of vascular recanalization after two TACE are consistent, and the change of HMGB1 expression level after treatment has the same trend.

During the follow-up of this subject, we found that tumor markers decreased significantly at one month after TACE. The expression changes of tumor markers in the two groups were similar, and the difference was not statistically significant, which showed that both treatment methods had good effects on liver metastasis of colorectal cancer.

Analysis of the quality of life data showed that the QoL scores increased substantially after TACE. The results show that TACE can significantly improve the quality of life.

The results of the present study showed that patients with dramatically increased HMGB1 level after TACE had a relatively poor outcome. Patients whose HMGB1 expression increased more than 50% after TACE had shorter PFS than those whose expression smaller. From the principle of TACE, the most ideal result is that the blood supply of tumors is permanently and completely blocked, combined with the killing effect of anti-tumor drugs, achieved the therapeutic purposes. However, some tumor cells survive and continue to grow after TACE due to the rapid emergence of collateral circulation and the existence of some unembolized micro-vessels. According to related studies, HMGB1 can promote the formation and development of new blood vessels (36). HMGB1 is also associated with cancer progression and immune escape, which is able to induce angiogenesis, metastasis (37). At present, HMGB1 is known to accelerate angiogenesis by 1) acting on vascular endothelium to promoting the formation of new blood vessels and neovascular network by promoting the synthesis of endothelial growth factor. 2) Acetylated HMGB1, which has been released, activates macrophages to upregulate nuclear factor kappa B, thereby promoting the synthesis and secretion of vascular endothelial growth factor and indirectly promoting the formation of new blood vessels (38, 39). 3) HMGB1 can upregulate the expression of fibroblast growth factor (FGF) (40), stimulate the

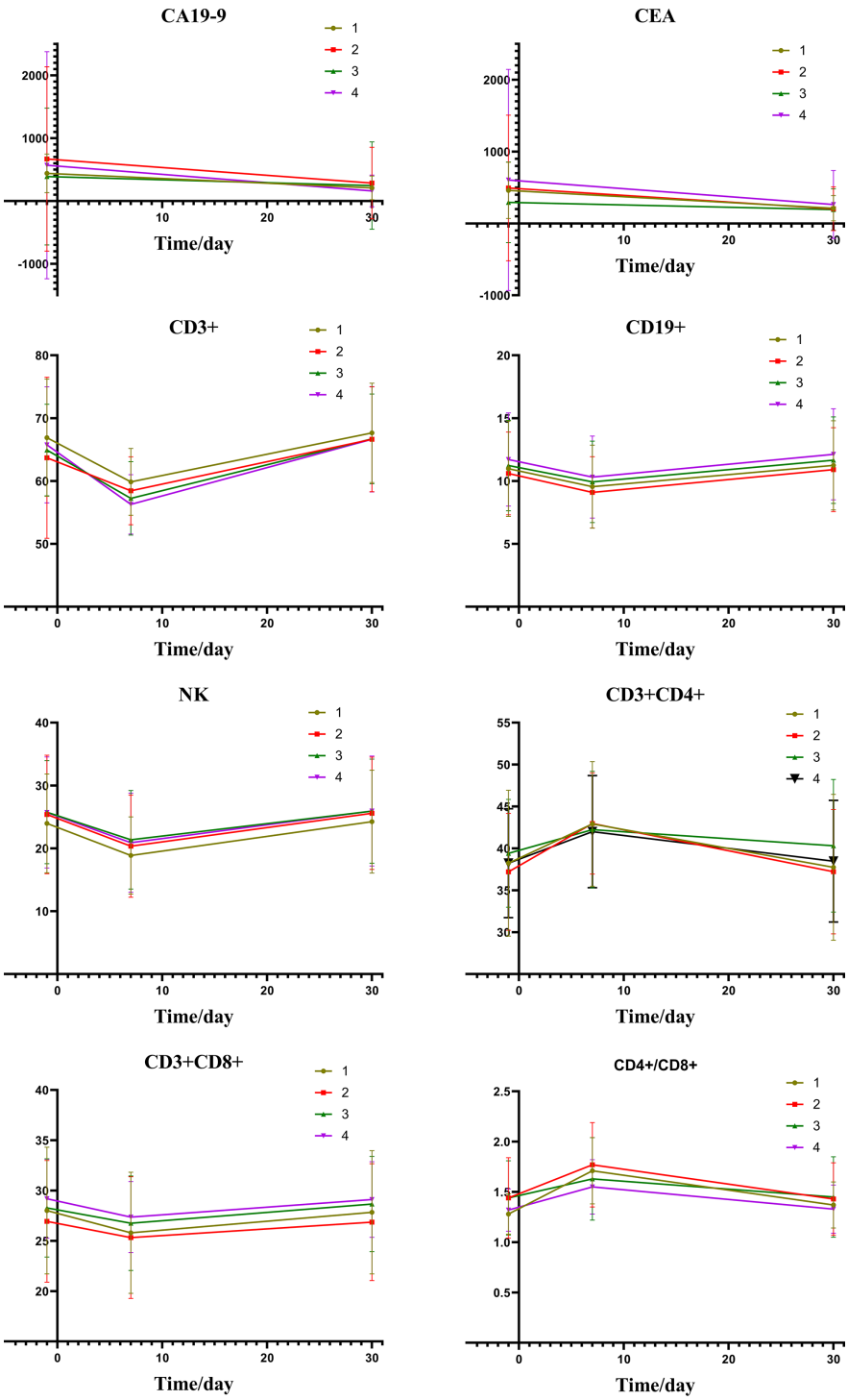

FIGURE 5 | Group1: low post-expression of HMGB1 with c-TACE; Group2: high post-expression of HMGB1 with c-TACE; Group3: low post-expression of HMGB1 with DEB-TACE; Group4: high post-expression of HMGB1 with DEB-TACE.

TABLE 6 | Post-expression of HMGB1group, common adverse reactions and treatment outcomes.

	DEB-TACE			c-TACE		
	High-change (33)	Low-change (23)	p-value	High-change (30)	Low-change (20)	*p*-value
fever	18	6	0.04	21	8	0.03
vomit	11	10	0.45	10	6	0.81
nausea	18	11	0.63	11	12	0.11
abdominal pain	14	10	0.94	10	8	0.64
hepatic failure	0	0	0	0	0	0
CR [a]	3	3		2	2	
PR [b]	13	17		14	15	
SD [c]	15	2		12	2	
PD [d]	2	1		2	1	
ORR [e]	16	20	<0.01	16	17	0.01
Pre-score of Qol [f]	32.64	33.26		31.27	31.50	
Post-score of Qol	47.58	48.78		48.37	47.70	

[a]*CR, complete response.*
[b]*PR, partial response.*
[c]*SD, stable disease.*
[d]*PD, progressive disease.*
[e]*ORR, Objective response rate; ORR = CR + PR.*
[f]*Qol, quality of life.*

secretion of platelet-derived growth factor (PDGF) (41, 42), and greatly enhance the proliferation and migration ability of endothelial cells.

Receptor for advanced glycation end products (RAGE) plays a role in tumor metastasis after binding to HMGB1 (43, 44). The C-terminus of HMGB1 can specifically bind to RAGE binding, triggering cytoplasmic signaling required for cell movement regulation and opening the molecular switches that control cytoskeletal organization (45). HMGB1/RAGE cannot only regulate the cytoskeleton to achieve cell movement, but also attract and aggregate other cells, and enhance the ability of cell aggregation and adhesion, which plays an important role in the formation of new collateral circulation after TACE. The combination of HMGB1/RAGE makes peripheral cells and smooth muscle cells aggregate to the high expression site and promotes the formation of the vascular structure. In addition, HMGB1/RAGE can regulate the expression of the BCL-2 gene (B-cell lymphoma/leukemia-2 gene) (46, 47), which is a cancer gene with the effect of inhibiting apoptosis. The anti-apoptotic effect of HMGB1/RAGE is directly related to the expression of BCL-2 (48). The multiple effects of HMGB1 enable the tumor to rapidly establish collateral circulation after the original blood supply is interrupted by embolization, which leads to the tumor to regain some vessels after TACE. When many tumor cells are necrotic, the remaining tumor cells with blood supply at the edge grow and proliferate rapidly, which leads to the progress and recurrence of local lesions.

The small size of patients' sample in this study may have some impact on the accuracy of the results. A larger number of patients can undoubtedly increase the accuracy of the results. The results of this study need to be verified by the analysis of large samples in the future, and we hope to promote a multicenter study with a larger sample size in the next time, which can verify the predictive role of HMGB1.

Most of the patients who had better outcomes in this study are still alive. Three-year and five-year survival rates were not analyzed due to time constraints. In the future, we will continue to follow up on the enrolled patients in order to obtain complete survival data and compare their long-term survival rates.

In conclusion, the results of this study suggest that HMGB1 may serve as a marker for predicting liver injury and long-term efficacy after TACE in patients with LMCRC. The change of HMGB1 before and after TACE is significantly associated with PFS. With the help of monitoring the change of HMGB1 expression, patients' PFS can be effectively predicted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shandong Cancer Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-jH and Y-dS made the study concept and design. Y-qC, C-xW, and J-bZ acquired the data. HZ, J-zL and H-rX made the interpretation of data. HZ and Y-dS made the analysis and drafted the manuscript. All authors contributed to the article and approved the submitted version. J-jH is the guarantor.

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Role of Preoperative Chemoradiotherapy in Clinical Stage II/III Rectal Cancer Patients Undergoing Total Mesorectal Excision: A Retrospective Propensity Score Analysis

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Background: Although the current standard preoperative chemoradiotherapy (PCRT) for stage II/III rectal cancer decreases the risk of local recurrence, it does not improve survival and increases the likelihood of preoperative overtreatment, especially in patients without circumferential resection margin (CRM) involvement.

Methods: Stage II/III rectal cancer without CRM involvement and lateral lymph node metastasis was radiologically defined by preoperative magnetic resonance imaging (MRI). Patients who received PCRT followed by total mesorectal excision (TME) (PCRT group) and upfront surgery (US) with TME (US group) between 2010 and 2016 were analyzed. We derived cohorts of PCRT group versus US group using propensity-score matching for stage, age, and distance from the anal verge. Three-year relapse-free survival rate, disease-free survival (DFS), and overall survival (OS) were compared between the two groups.

Results: A total of 202 patients were analyzed after propensity score matching. There were no differences in baseline characteristics. The median follow-up duration was 62 months (interquartile range, 46–87). There was no difference in the 3-year disease-free survival rate between the PCRT and US groups (83 vs. 88%, respectively; p=0.326). Likewise, there was no significant difference in the 3-year OS (89 vs. 91%, respectively; p=0.466). The 3-year locoregional recurrence rates (3 vs. 2% with US, p=0.667) and

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distant metastasis rates (16 vs. 11%, p=0.428) were not significantly different between the two groups. Time to completion of curative treatment was significantly shorter in the US group (132 days) than in the PCRT group (225 days) (p<0.001).

Conclusion: Using MRI-guided selection for better risk stratification, US without neoadjuvant therapy can be considered in early stage patients with good prognosis. PCRT may not be required for all stage II/III rectal cancer patients, especially for the MRI-proven intermediate-risk group (cT1-2/N1, cT3N0) without CRM involvement and lateral lymph node metastasis. Further prospective studies are warranted.

Keywords: stage II/III, rectal cancer, total mesorectal excision, upfront surgery, chemoradiotherapy

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and the second cause of cancer related death (1). The incidence of CRC is rising globally due to increase in western diet (2). Currently, the standard treatment for stage II/III rectal cancer is preoperative chemoradiotherapy (PCRT) followed by surgery and adjuvant chemotherapy (3). PCRT is effective in reducing local recurrence and down staging locally advanced rectal cancer (4, 5). However, it is associated with complications such as bowel (6), anorectal (7), and sexual dysfunctions (8, 9) and delay from surgical recovery (10). Although many patients benefit from local control of PCRT, there is still debate over whether it improves overall survival (11, 12). About one-third of patients relapse with metastasis despite treatment with PRCT and surgery (13).

Currently, National Comprehensive Cancer Network (NCCN) guidelines recommend preoperative chemoradiotherapy for tumors that are 1) T3, any N with clear CRM and 2) T1-2, N1-2 (14). In recent years, the incorporation of magnetic resonance imaging (MRI) in the preoperative setting has helped in better identification of tumor characteristics that dictate treatment strategies (15). Identification of features such as negative CRM, substaging T3, extramural venous invasion and nodal status in rectal cancer has helped treatment decisions in rectal cancer. In addition, the limitations of PRCT have led investigators to design trials that may omit PRCT in treatment of rectal cancer. Both the MERCURY (16) and OCUM (17) studies showed that rectal MRI could be used as an indicator to predict prognosis prior to surgery, thereby adjusting treatment according to patient's prognosis. The QuickSilver study further addressed this issue by selecting MRI-predicted good prognosis subjects that resulted in a low rate of positive circumferential resection margin (CRM) (18). This study suggested the possibility of omitting PCRT in subjects with a negative CRM and no lateral lymph node metastasis in stage II/III rectal cancer.

The identification of subjects who do not need PCRT in an important issue in stage II/III rectal cancer. If adequate local control can be achieved by surgery alone, omitting PRCT may save patients from unnecessary chemotherapy and radiotherapy, thereby decreasing the time from diagnosis to surgery and without the associated complications from PRCT. Whether upfront surgical resection without PCRT is feasible for the above-defined subset of patients warrants further evidence.

In this study, we retrospectively selected MRI-proven intermediate-risk group (cT1-2/N1, cT3N0) patients without CRM involvement or lateral lymph node metastasis. The patients either received PRCT followed by TME (PCRT group) or underwent upfront radical surgery (US group). After selecting patients using propensity score analysis, we assessed the clinicopathological characteristics, disease free survival (DFS), overall survival (OS), and cumulative incidence of local and distant recurrence between the two groups. The primary objective of our study was to evaluate the 3-year DFS and OS between these two groups. Our hypothesis was that DFS and OS of upfront surgical resection are non-inferior to those of PCRT.

MATERIALS AND METHODS

Study Design and Population

This was a retrospective study of stage II and III rectal cancer patients who received either PCRT followed by total mesorectal excision (TME) or underwent upfront radical surgery in Yonsei Cancer Center. Standardized rectal cancer MRI protocol was used for assessment of patients initially diagnosed with rectal cancer (18). Key inclusion criteria included (1) histologically confirmed stage II/III rectal adenocarcinoma with distance from anal verge ≤ 10 cm, (2) MRI predicted circumferential resection margin (CRM) greater than 1mm away from primary tumor, (3) extramural depth of invasion (EMD) ≤5mm, (4) absent extramural venous invasion (EMVI), (5) without pelvic lymph node involvement, (6) without distant metastasis, (7) surgery with TME, and (6) 3-year surveillance period after surgical resection. Patients with T1 and T2 tumors with N0 status, and patients requiring intersphicteric resection or abdominal perineal resection (APR) were excluded from the study.

The clinicopathologic variables such as age, gender, tumor grade, preoperative and postoperative MRI, clinical staging, types of surgery, pathologic staging, toxicity profiles of radiotherapy, and patterns of recurrence were collected. Staging was determined using the 8th edition of the American Joint Committee on Cancer guideline of tumor, node, and metastasis (TNM) classification (19). CRM negative was defined as distance to the mesorectal fascia greater than 1 mm from the primary tumor (18).

Propensity score method was used to balance covariates and minimize bias (20, 21). Covariates included 1) clinical T stage 2) clinical N stage 3) primary location from the anal verge and 4) carcinoembryonic antigen (CEA) levels. Patients with missing data were excluded from the analysis.

This study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. The study was approved by institution review board of Yonsei Cancer Center (IRB 4-2020-0209).

Treatment and Assessment

Baseline and follow up MRI were acquired on a 3.0-T system (MAGNETOM TrioTim; Siemens, Erlangen, Germany), and T2-weighted images of sagittal, axial, and oblique view were assessed (22). Further details of MRI techniques are discussed in previous protocols (23).

Patients diagnosed with stage II/III rectal cancer were either treated with upfront radical surgery only or PCRT followed by surgery. The 3-dimensional conformal radiotherapy (3D-CRT) was given at 45 Gy in 25 fractions over the course of 5 weeks, preoperatively for the PCRT group, and postoperatively for US group. During treatment, the following chemotherapy agents were given: intravenous 5-fluorouracil (425 mg/m^2) and leucovorin (20mg/m^2) were given as bolus on weeks 1 and 5, or capecitabine 1,650mg twice a day throughout radiation treatment. Interim assessment after PCRT was assessed with MRI for adaptation of surgical plan, and surgery was planned 6–8 weeks after completion of PCRT. In the US group, MRI was done at 4–6 weeks after surgery.

For both the PCRT and US group, patients were treated with adjuvant chemotherapy regimen such as FOLFOX (bolus and infused fluorouracil with oxaliplatin) and CAPOX (capecitabine and oxaliplatin) over the course of 6 months. Patients treated with FOLFOX were given oxaliplatin 85mg/m^2 for 2 h with leucovorin 350mg intravenously, followed by bolus of fluorouracil 400mg/m^2 on day 1 and infusion of fluorouracil 2,400mg/m^2 over 2 days. Treatment was repeated every 2 weeks with total of 12 cycles in 6 months. CAPOX was given with 1,000mg/m^2 of capecitabine twice per day for the first 14 days, and intravenous oxaliplatin 85mg/m^2 over 2 h on day 1. The cycle was repeated every 3 weeks with a total of 8 cycles over 6 months.

Six weeks after surgery, patients who were candidates for adjuvant chemotherapy and radiotherapy received treatment accordingly. All patients followed up with computed tomography (CT) scans and CEA levels every 3 months for the first 2 years, and every 6 months for the next 3 years thereafter. Follow up colonoscopy was done at 1 year, 3 years and 5 years after surgery. Local recurrence was defined as tumor relapse within pelvis and perineum. Distant metastasis was defined as tumor recurrence outside locoregional area.

Outcomes

Primary endpoints were 3-year DFS and OS in patients of rectal cancer with stage II/III disease who received either PCRT or underwent upfront radical surgery. Secondary endpoint was recurrence rate between two groups.

Statistical Analysis

We analyzed data using Statistical Package for the Social Sciences (SPSS) version 25 (IBM, Chicago, IL) and the statistical software R (https://www.r-project.org, v3.5.0). Propensity score matching was performed to control potential confounding bias (20). The matching was constructed using clinical T stage, clinical N stage, primary location from the anal verge and CEA levels using the "MatchIt" R package. The nearest neighbor method was used with a caliper of 0.20 (**Supplementary Figures 1A, B**). Further, we conducted a sensitivity analysis for the matching estimate using "rbounds" R package, suggested by Rosenbaum (23). Briefly, sensitivity analysis for matched data evaluated the magnitude of potential bias using the Wilcoxon signed rank test. When gamma Γ (log odds of differential assignment to treatment due to unobserved factors) = 1, it holds assuming there is no hidden bias due to an unobserved confounder (**Supplementary Figure 1C**).

The correlations between variables were analyzed using Fischer's exact test for categorical variables and sample *t*-test for continuous variables. Kaplan Meier with log-rank test was used to analyze survival difference between the two groups. Disease-free-survival (DFS) was defined as the time interval between surgery and tumor recurrence or last follow-up. Overall survival (OS) was defined as the time interval between the surgery and death or last follow-up.

RESULTS

Patient Characteristics

Between January 2010 to June 2016, a total of 354 patients were diagnosed with rectal cancer in Yonsei Cancer Center. After excluding patients who had metastatic sites (n=43), double primary cancer (n=5), and incomplete data set due to follow up loss (n=37), data of 269 patients [PCRT, n=160 (59%); US, n=109(41%)] were collected. Since PRCT patients had relatively poorer prognostic factors such as high CEA and advanced clinical stage as compared to the US group, we used propensity score to adjust baseline characteristics between the two groups. After propensity score matching of 1:1 ratio, a total of 202 patients were selected for analysis with 101 patients from each group (**Figure 1**). All of the patients in the PCRT group completed planned cycles of pre-operative chemoradiotherapy without dose modifications.

MRI-Based Tumor Characteristics and Histopathological Tumor Staging

Preoperative clinicopathological characteristics such as age, clinical stage, CEA level, and tumor location were well balanced between the two groups (**Table 1**). Most of the patients underwent lower anterior resection (LAR) [PCRT, n=96 (96%); US, n=96 (96%)]. Other surgical methods included ultralow anterior resection [PCRT, n=5(5%); US, n=3(3%)], transanal endoscopic operation (US, n=1) and total colectomy (US, n=1).

In the PRCT group, there was significant down staging of postoperative pathologic stage (ypStage) after pre-operative

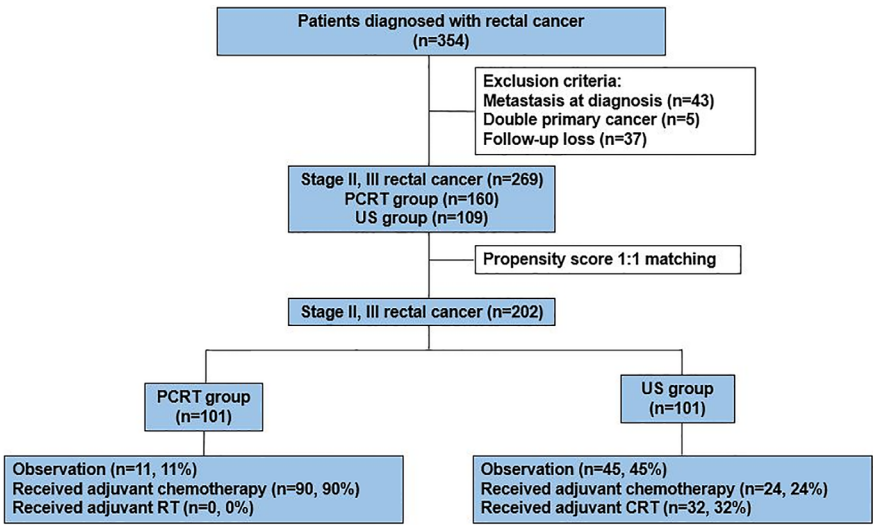

FIGURE 1 | Study scheme. PCRT, Preoperative chemoradiotherapy; US, Upfront surgery.

chemoradiotherapy. A total of 23 patients (23%) in this group had a complete response (pT0) (p=0.000) and 83 patients (83%) with negative lymph nodes (p=0.001). Overall, PRCT group had higher proportion of stage I (n=58, 58%, p=0.000) and lower percentage of stage III (n=18, 18%) compared to the US group of stage I (n=33, 33%) and stage III (n=39, 39%), respectively.

Overall, there was no difference in recurrence at 3-years between the two groups [PRCT, n=17 (17%); US, n=12 (12%)] (p=0.316). Patterns of recurrence were also similar between the two groups, indicating that although PCRT group may have lower TNM stages, it had no effect on local [PCRT, n=3 (3%); US, n=0], distant [PCRT, n=13(13%); US, n=11(11%)] and combined local and distant recurrence [PCRT, n=1(1%); US, n=1(1%)] at 3 years (p=0.364). After TME, majority (n=83, 83%) of the PRCT patients did not receive treatment while 56% of US group required further treatment such as adjuvant chemoradiotherapy (CRT) (n=32, 32%) and adjuvant chemotherapy (n=24, 24%).

Disease Free Survival, Overall Survival, and Local Recurrence Rate

At the data cut-off date of September 21, 2019, the median follow-up duration was 62 months (interquartile range, 46–87 months). Seventeen (17%) patients in the PRCT group and 8 patients (8%) in the US group had died. There was no difference in the 3-year DFS rate between PRCT group (83%) and US group (88%) (p=0.328) (**Figure 2**). No statistical difference in overall survival was seen between two groups; 3-year OS was 91 vs. 89% in US and PCRT group, respectively (p=0.466). Likewise, there was no difference in the rates of local and distant metastases. The rates of locoregional recurrence and distant metastasis at 3 years in PRCT and US groups were 3 vs. 2% (p=0.667) and 16 vs. 11% (p=0.428), respectively (**Figure 3**).

Toxicity Profile

Among the PCRT group, 73 patients (73%) experienced adverse events of any grade (**Table 2**). The most common adverse events related to CRT were fatigue (n=42, 42%), diarrhea (n=42, 42%), and poor oral intake (n=41, 41%). In addition, fecal incontinence and tenesmus were seen in 18 patients (18%) and 9 patients (9%), respectively. The most common grade 3 adverse event was diarrhea (n=16, 16%). Of note, 1 patient (1%) had anastomotic leakage which required surgical intervention.

In the US group, 32 patients (32%) received adjuvant CRT, and 26 (79%) experienced adverse events of any grade including fatigue (n=16, 48%), diarrhea (n=11, 33%), and fecal incontinence (n=5, 15%). Grade 3 adverse event was only seen in diarrhea (n=5, 15%). Although the US group had higher incidence of adverse events of any grade, there was less grade 3 adverse events, and adverse events were manageable with supportive care. There were no adverse events of ≥ G4 or death due to CRT complications in both groups.

DISCUSSION

In our single center, retrospective study using propensity score analysis, we assessed the 3-year DFS and OS of preoperative chemotherapy followed by surgery versus upfront surgery in MRI proven, CRM negative stage II and III rectal cancer. Our study showed that although PCRT had significantly down-staged from the earlier postoperative pathologic stage, there was no difference in 3-year DFS, OS and cumulative incidence of local and distant recurrence between PRCT and US group. In addition, the retrospective analysis of adverse events due to CRT reflect that US groups had manageable toxicity of mostly grade 1–2. Compared to the PRCT group, the US group had less grade 3 adverse events which led to hospitalization, and surgical

TABLE 1 | Baseline patient characteristics.

Characteristics No. (%)		PCRT Group n = 101	US Group n = 101	p value
Age, years, Median (IQR)				
	< 70	55 (51–63)	57 (49–62)	0.87
	≥ 70	73 (70–76)	74 (72–78)	
Gender				
	Male	73 (73%)	65 (65%)	0.226
	Female	28 (28%)	36 (36%)	
CEA, ng/dl				
	< 5	64 (64%)	76 (76%)	0.067
	≥ 5	37 (37%)	25 (25%)	
Tumor location, from anal verge, cm				
	≤ 5	12 (12%)	12 (12%)	1
	> 5	89 (89%)	89 (89%)	
Tumor grade				
	WD	14 (14%)	9 (9%)	*0.743
	MD	83 (83%)	87 (87%)	
	PD	2 (2%)	3 (3%)	
	Unknown	2 (2%)	2 (2%)	
MRI findings				
	cT stage			
	cT1, 2	15 (15%)	23 (23%)	0.132
	cT3	86 (86%)	78 (78%)	
	cN stage			
	N0	28 (28%)	39 (39%)	0.182
	N+	73 (73%)	62 (62%)	
	pT category			
	pT0	23 (23%)	0 (0%)	0
	pTis	2 (2%)	1 (1%)	
	pT1	7 (7%)	8 (8%)	
	pT2	35 (35%)	36 (36%)	
	pT3	34 (34%)	56 (56%)	
	pN category			
	pN0	83 (83%)	62 (62%)	0.001
	pN1	14 (14%)	32 (32%)	
	pN2	4 (4%)	7 (7%)	
	pStage			
	Stage I, T1-T2, N0	58 (58%)	33 (33%)	0
	Stage II, T3-4, N0	25 (25%)	29 (29%)	
	Stage III, any T, N1-N2	18 (18%)	39 (39%)	
Type of surgery				
	LAR	96 (96%)	96 (96%)	*0.499
	ULAR	5 (5%)	3 (3%)	
	Other surgery§	0 (0%)	2 (2%)	
Recurrence				
	No	84 (84%)	89 (89%)	0.316
	Yes	17 (17%)	12 (12%)	
Pattern of recurrence				
	Local recurrence	3 (3%)	0 (0%)	*0.364
	Distant	13 (13%)	11 (11%)	
	Local + Distant	1 (1%)	1 (1%)	
Surgery (TME)				
	Complete	101 (100%)	101 (100%)	
	Incomplete	0 (0%)	0 (0%)	

§Patients who received with "Other surgery" include 1 transanal endoscopic operation, 1 total colectomy.

**Fisher's exact test.*

PCRT, preoperative chemoradiotherapy; US, upfront surgery; IQR, interquartile range; CEA, carcinoembryonic antigen; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; MRI, magnetic resonance imaging; cT, clinical tumor; cN, clinical node; pT, pathologic tumor; pN, pathologic node; pStage, pathologic stage; LAR, lower anterior resection; ULAR, ultralow anterior resection; TME, total mesorectal excision.

intervention for anastomotic leakage. Taken together, the oncologic outcomes of upfront-surgery group are comparable to those of PRCT group. With MRI directed patient stratification, it is possible for patients in intermediate-risk group (cT1-2/N1, cT3N0) without CRM involvement and lateral lymph node metastasis to omit PRCT, thereby avoiding overtreatment and reducing treatment duration.

Previously, there have been conflicting reports about the role of PCRT in locally advanced rectal cancer (5, 24–28). The Dutch trial showed that PRCT reduced the rate of local recurrence but had no impact on overall survival rate (26). Similarly, studies by German Rectal Cancer Study Group and Medical Research Council (MRC)-07 showed that although PRCT has a role in improving local control, there was no impact in overall survival (5, 24). Few studies have even pointed out that PCRT is unnecessary and may possibly be an overtreatment for some patients with stage II disease (27, 28).

Thereafter, several studies have proved that high-resolution rectal MRI has optimized the selection of CRM negative patient (16, 17). Good prognosis tumors treated with upfront surgery resulted in 2–5% of positive CRM patients (18). Even with upfront TME surgery alone, the 5-year local recurrences rates were as low as 4.4% (29). These findings are encouraging since patients without CRM involvement may selectively omit PCRT, thereby avoiding unnecessary complications from radiotherapy, save medical costs, and receive surgery without delay (30, 31).

Very few retrospective studies have addressed whether PCRT is essential in locally advanced rectal cancer. In a study comparing surgery alone and PRCT in recto-sigmoid junction cancer, PRCT was associated with 5% improvement of 5-year OS (32). However, that study included recto-sigmoid colon cancer. In contrast to the distal tumors that respond more favorably to PCRT, tumors located proximal from the anal verge respond less to PCRT (26). Therefore, the results with improved OS with PRCT in recto-sigmoid colon cancers must be interpreted with caution. In another study, early T3 rectal cancer patients who were either treated with surgery alone versus PRCT showed that 5-year local recurrence rate was 2% for both groups, and 5-year DFS were not statistically different (87% in surgery alone versus 88% in PRCT group) (33). Recently, a meta-analysis on total neoadjuvant therapy (TNT) addressed that TNT increases pathological down staging compared with surgery and adjuvant CRT (34). Despite higher rate of pathological complete response (pCR) by 39% in the TNT group, there was no difference in DFS and OS was noted between two groups.

Similarly, our study results proved that there was no difference 3-year OS between PRCT and US group. In addition, there was no difference in 3-year DFS and incidence of both local and distant recurrence between the two groups. Whether PCRT is a prerequisite in stage II/II rectal cancer, especially for MRI proven intermediate-risk group (cT1-2/N1, cT3N0) without CRM involvement and lateral lymph node metastasis, should be validated with prospective, randomized controlled trials in the future.

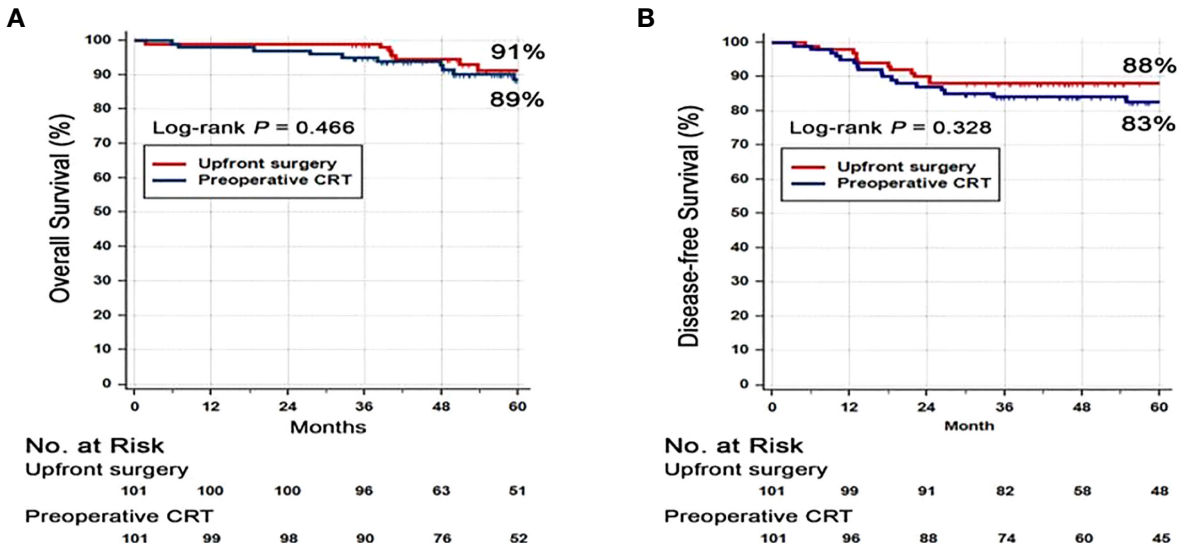

FIGURE 2 | Kaplan-Meier survival curves for **(A)** disease free survival (DFS) and **(B)** overall survival (OS). Abbreviation: CRT, Chemoradiotherapy.

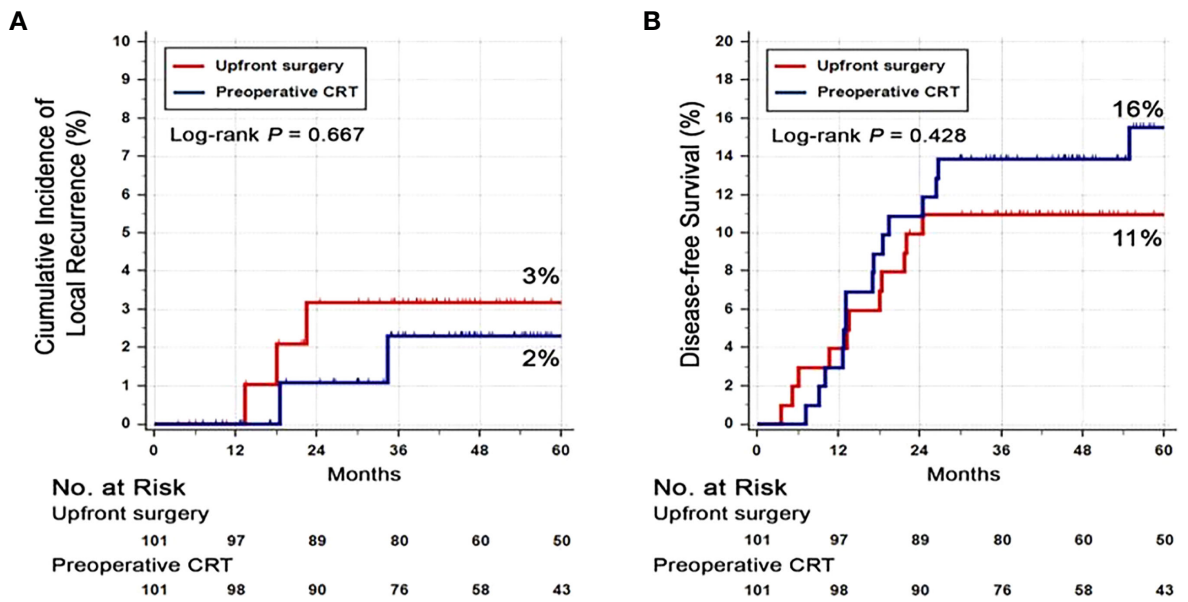

FIGURE 3 | Cumulative incidence of **(A)** local recurrence and **(B)** distant recurrence. Abbreviation: CRT, Chemoradiotherapy.

There are few limitations to our study. First, our study collected data retrospectively from a single center. Although we used propensity score matching to minimize cofounding covariates, variables such as physician's choice for upfront surgery or PCRT, and patients' treatment preferences may have inadvertently affected the allocation between two groups. Second, only the data from our institution was collected. Larger patient sampling from multi-centers in randomized controlled study (RCT) may provide additional information to clarify whether PCRT may be selectively avoided.

In conclusion, omitting PRCT and treatment with upfront surgery alone in CRM negative rectal cancer stage II/III patients may be considered as future treatment options. To further validate our retrospective results, a phase 2, randomized controlled trial of upfront surgery versus PRCT followed by surgery is currently ongoing (ClinicalTrials.gov, NCT02167321) (35).

TABLE 2 | Adverse events of PCRT and adjuvant CRT.

Adverse events		Any grade		Grade 3*	
		PCRT n=101	Adjuvant CRT n=32	PRCT n=101	Adjuvant CRT n=32
No. (%)		73 (73%)	26 (79%)	19 (19%)	5 (15%)
General					
	Fatigue	42 (42%)	16 (48%)	1 (1%)	0 (0%)
	Nausea	1 (1%)	0 (0%)	0 (0%)	0 (0%)
	Poor oral intake	41 (41%)	0 (0%)	1 (1%)	0 (0%)
Genitourinary Toxicity					
	Cystitis	2 (2%)	0 (0%)	0 (0%)	0 (0%)
	Urinary incontinence	3 (2%)	0 (0%)	0 (0%)	0 (0%)
	Erectile dysfunction	4 (2%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal Toxicity					
	Abdominal pain	3 (3%)	1 (3%)	0 (0%)	0 (0%)
	Anal pain	3 (3%)	0 (0%)	0 (0%)	0 (0%)
	Diarrhea	42 (42%)	11 (33%)	16 (16%)	5 (15%)
	Tenesmus	9 (9%)	1 (3%)	0 (0%)	0 (0%)
	Fecal incontinence	18 (18%)	5 (15%)	0 (0%)	0 (0%)
	Anastomotic leakage	1 (1%)	0 (0%)	1 (1%)	0 (0%)

PCRT, preoperative chemoradiotherapy; CRT, chemoradiotherapy; No., number.
**Grade ≥4 adverse events were not seen in both groups.*

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by institution review board of Yonsei Cancer 112 Center (IRB 4-2020-0209). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL, HK, AH, JC, SS, S-HB, WK, TK, YH, DH, HH, BSM, KL, YP, and NK participated in the collection of data. JSL reviewed radiologic findings, and HK and YP analysed the data. JL and HK drafted the manuscript. All the authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.609313/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Propensity score matching. **(A)** Distribution of propensity scores. **(B)** Histograms of propensity scores before and after matching. **(C)** Sensitivity analysis with matched data.

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Turmeric Is Therapeutic *in Vivo* on Patient-Derived Colorectal Cancer Xenografts: Inhibition of Growth, Metastasis, and Tumor Recurrence

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Colorectal cancer is the third most frequently diagnosed cancer worldwide. Clinically, chemotherapeutic agents such as FOLFOX are the mainstay of colorectal cancer treatment. However, the side effects including toxicity of FOLFOX stimulated the enthusiasm for developing adjuvants, which exhibit better safety profile. Turmeric extract (TE), which has been previously shown to suppress the growth of human and murine colon xenografts, was further demonstrated here for its inhibitory effects on colon cancer patient-derived xenografts (PDX). PDX models were successfully established from tissues of colon cancer patients and the PDX preserved the heterogeneous architecture through passages. NOD/SCID mice bearing PDX were treated either with TE or FOLFOX and differential responses toward these treatments were observed. The growth of PDX, metastasis and tumor recurrence in PDX-bearing mice were suppressed after TE treatments with 60% anti-tumor response rate and 83.3% anti-metastasis rate. Mechanistic studies showed that TE reduced tumor cell proliferation, induced cell apoptosis, inhibited metastasis *via* modulating multiple targets, such as molecules involved in Wnt and Src pathways, EMT and EGFR-related pathways. Nevertheless, FOLFOX treatments inhibited the PDX growth with sharp decreases of mice body weight and only mild anti-metastasis activities were observed. Furthermore, in order to have a better understanding of the underlying mechanisms, network pharmacology was utilized to predict potential targets and mechanism. In conclusion, the present study demonstrated for the first time that oral TE treatment was effective to suppress the growth of colon PDX and the recurrence of colon tumors in mice. The findings obtained from this clinically relevant PDX model would certainly provide valuable information for the potential clinical use of TE in colorectal cancer patients. The application of PDX model was well illustrated here as a good platform to verify the efficacy of multi-targeted herbal extracts.

Keywords: colorectal cancer, patient-derived xenografts, turmeric, tumor recurrence, herbal medicines, network pharmacology

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy and is the second leading cause of cancer death worldwide (1). The incidence and death rates of CRC decreased among individuals aged ≥ 50 years, but increased by 13% in those aged less than 50 years (2). Metastasis results in nearly 90% of all cancer deaths (3) and it occurs in 20%–30% of CRC patients (2, 4). Poor prognosis of patients with non-resectable stage III–IV metastatic CRC (mCRC) provoked the development of therapeutics with novel mechanisms of action. Nonetheless, adverse events were reported in some targeted therapies (5, 6). Hence, there remains high unmet needs for safer adjuvant and/or therapeutic agents for mCRC patients, which could be searched from natural sources.

In the progress of development of anti-tumor and anti-metastatic agents, preclinical models play vital roles in translating preclinical data to clinical efficacy. However, high failure ratio of novel anti-tumor therapies in clinical trials partly results from the inability of conventional cell line xenograft models in reliably predicting the clinical efficacy (7, 8). In fact, cell lines used in xenograft models have adapted to the passaging on plastic outside of a natural tumor environment, whereas cancer cell-stromal cell interactions should have taken place in the presence of human extracellular matrix component. Hence, the cell line xenograft models may not be accurate enough to reflect interaction with the tumor microenvironment and tumor heterogeneity (8). In contrast, patient-derived xenograft (PDX) models, which have been established and adapted in recent years, are shown to preserve the intratumoral cell heterogeneity, histopathological and genetic alterations (9, 10). The PDX models are now regarded as effective, reproducible and clinically relevant preclinical models for testing the efficacy of new target-directed therapies, validating biomarkers of drug response, and evaluating preclinical drugs or therapeutic agents (11–13).

Along this line, natural product curcumin has been proven to have multiple molecular targets in cancer (14), and other components such as turmerones and polysaccharides have also been shown to possess anti-proliferative and immune-stimulatory effects (15, 16). However, poor bioavailability and low solubility limit curcumin to achieve its satisfactory therapeutic outcome in clinical trials. Previous studies suggested that turmeric (main ingredient in curry and a source of curcumin) may contribute to the lower CRC incidence in Indians (17, 18). Our previous studies also demonstrated that the whole complex of turmeric extract (TE) rather than curcumin alone could exert better inhibitory effects on colon cancer cell line generated xenografts growth in mice (19). Xenografts derived from cell lines generally exhibit poorly differentiated carcinomas that lack resemblance to the original patient tumor in terms of molecular characteristics (20). In order to confirm the benefits arising from the use of turmeric in clinical-relevant animal models, which would be a crucial step before launching clinical trial for this health supplement, we established a panel of PDX of CRC. The efficacies of TE on tumor growth, metastasis and tumor recurrence were then investigated in this panel of PDX for the first time.

Systems biology approach, in particular network pharmacology enable the paradigm shift from "one-target, one-drug" to a "network-target, multiple-component-therapeutics" mode, which is a powerful tool for the analysis of drug combinations, especially for traditional Chinese medicines (21, 22). Network pharmacology is a new method based on "disease-gene-drug" network which provides a new way to explore the mechanism of drug action at molecular, cellular, tissue and biological levels (23). The multi-dimensional mechanism of drug action is evaluated by target prediction, pharmacokinetic measurement and network analysis. More importantly, network pharmacology has been successfully applied to predict/identify the molecular mechanisms of traditional Chinese medicine in the treatment of various diseases (21), including cardiovascular diseases (24), neurodegenerative diseases (25), cancer (26), etc. Here, we have identified potential active components of TE against CRC targets, and predicted their network, suggested the potential pathways and key regulatory genes.

METHOD AND MATERIALS

Reagents

Turmeric ethanolic extract (TE) was prepared as previously reported (19, 27). In brief, the dried rhizome of *Curcuma longa* was extracted under reflux using 95% ethanol and evaporated under reduced pressure at 60°C to dryness. Quantification of two commercially available chemical markers, curcumin and Ar-turmerone (Sigma-Aldrich, MO, USA), in turmeric ethanolic extract was performed using by UPLC and the chemical profiles were registered (27). The contents of curcumin and Ar-turmerone present in turmeric ethanolic extract were 18.7% (w/w) and 5.3% (w/w), respectively. Standardization of turmeric ethanolic extract used in the present study was same as the method reported previously, in which the 3-D chromatograms of the extract as well as the extracted ion chromatograms (EIC) and MS spectra of the marker compounds were also well reported previously (27).

Chemotherapeutics FOLFOX consisted of fluorouracil, folinic acid and oxaliplatin, were obtained from Sigma-Aldrich (MO, USA). Primary antibodies against RhoA, β-catenin, Src, p-Src, and GAPDH were purchased from Cell Signaling (MA, USA); Ki-67 from Abcam (MA, USA); CD31 from Dako (CA, USA); mouse/rabbit specific HRP/DAB (ABC) Detection IHC kit from Abcam Inc (Cambridge, UK). The *in situ* Cell Death Detection kit for TUNEL staining was from Roche (IN, USA). The Trizol reagent was from Thermo Fisher Scientific (MA, USA) and QuantiFast® SYBR® Green PCR mini kit was from Qiagen (CA, USA).

Establishment of Colorectal Cancer Patient Derived Xenograft Mouse Model

Experimental protocols and procedures were reviewed and approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC Ref No.: 2016.444) and the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong (Ref No. 15-164-MIS).

Freshly resected tissues (tumor and adjacent normal tissues) from consented CRC patients were collected after colon surgery. Half portion of resected tumor tissues were stored in −80°C freezer for further histological and molecular analysis and another half portion of tumors were transferred at 4°C into RPMI-1640 medium with antibiotics. Within 24 h of surgical resection, tumor tissues were trimmed, cut into 3- to 5-mm sizes and inoculated subcutaneously on the back of 6- to 8-week-old NOD/SCID mice, which were supplied by the Laboratory Animal Services Centre of The Chinese University of Hong Kong. Mice were maintained in pathogen-free conditions in specifically designed air-controlled rooms with a 12-h light/dark cycle. When the tumors reached 1,000–1,500 mm^3, mice with the first generation of xenografts (P1) were sacrificed and then the xenografts were isolated and expanded for two more generations (P2 and P3). When P3 xenografts reached an average volume of 50 mm^3, mice were then subjected to TE or FOLFOX treatments (**Figure 1A**).

Administration of Turmeric Extract or FOLFOX to PDX-Bearing Mice

In total, seven batches of PDX samples were established for the following pharmacological experiments. Six batches of PDX-bearing mice were treated with TE (200 mg/kg, orally administered) or vehicle control 6 days a week for 2–8 weeks depending on the tumor sizes and the well-being of mice. FOLFOX (15 mg/kg fluorouracil and 5 mg/kg folinic acid daily plus 5 mg/kg oxaliplatin once a week) was administered intraperitoneally for 2 or 4 weeks depending on the well-being of mice. The treatments and assessment details of the PDX samples were listed in **Table 1**. In our previous study, TE at 400 mg/kg has been shown to inhibit growth of human colon HT29 subcutaneous xenografts (27). Thus, both 200 and 400 mg/kg TE have been tested in our pilot studies in PDX-bearing mice. Results showed that the inhibitory activities of these two doses of TE were comparable and without significant difference. Hence, the minimum effective dose (200 mg/kg) was chosen for the subsequent PDX experiments in the present study.

Tumor volumes and body weights were measured once a week until the end of experiments. Tumor volumes were calculated using the formula: length × width × depth/2 (mm^3) as described previously (28). Tumor growth inhibition was calculated using the formula: (TGI= Average tumor weight in control group − Average tumor weight in treatment group)/ Average tumor weight in control group × 100%. Relative tumor volume was equaled to tumor volume (on the measuring day) divided by tumor volume (on day 0).

Histological Assessments

PDX samples were collected at the end of experiments and separated into two halves, one half was snap frozen at −80°C for further Western blot/RT-PCR analysis and the other half was fixed in 10% formalin. Lungs and livers were also fixed in 10% formalin, embedded in paraffin and then sectioned for hematoxylin and eosin (H&E) staining. Tumor area in liver and lung H&E sections were used to assess the level of metastasis. The metastatic area was calculated as percentage of metastatic area in whole view of photo. Embedded PDX tissues from each group were sectioned and stained with H&E or subjected to IHC staining against β-catenin and Ki 67 antibodies. The TUNEL assay was performed to assess apoptosis in tumor sections as per kit's instruction. H&E and IHC staining were performed as previously described (28). Immunoreactivity was analyzed in 5 random areas for each tumor tissue and was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining), and 4 (very strong staining) (29).

Western Blotting

Western blot was performed using the lysates of PDX samples. Twenty to sixty micrograms of protein were loaded to denaturing gel electrophoresis. After transfer to PVDF membrane, membranes were blocked with 5% BSA and incubated overnight with primary antibodies, RhoA, p-Src, Src, β-catenin, cleaved caspase-3, and GAPDH in 1:1,000 dilutions. Blots were then incubated with secondary antibodies (1:2,000). Quantification was performed using Image J software as described previously (28).

RNA Extraction and RT-PCR

Total RNA was extracted from three batches (CC15OT, CC15PT, and CC15ZT) of PDX samples using Trizol reagent. The reverse transcription and quantification were performed as previously described (30). The gene expression of Wnt signaling elements (AXIN2, DKK1, and SMAD7), EMT pathway related molecules (N-cadherin, Snail and Vimentin, and EpCAM), growth factor receptors [platelet-derived growth factor receptor B (PDGFRB), epidermal growth factor receptor (EGFR)], asparagine synthetase (ASNS), Rho signaling (RTKN2), ATP-binding cassette transporters (ABCA13), and iNOS were evaluated using qPCR. Besides, total RNA of CC15OT primary tumor and adjacent normal tissue samples were extracted and subjected to qPCR for the expressions of KRAS and EGFR. In addition, total RNA of lungs CC16AET were extracted and the expressions of human-specific 850-bp fragment of the α-satellite DNA on human chromosome 17 (Ch17) were detected by qPCR (31, 32). In brief, real time semi-quantitative PCR of cDNA samples were performed in Bio-Rad CFX96™ Real-time system C1000 Thermal cycler using the QuantiFast SYBR Green RT-PCR kit (Qiagen, USA). The primers sequences were listed in **Table S1** (in supplementary information), which were synthesized by Thermo Fisher Scientific (Hong Kong). The specific gene mRNA levels (including Ch17 expression level) were normalized to GAPDH and expressed as a fold change compared to the vehicle control group.

Data Preparation and Analysis of Network Pharmacology

The potential active compounds and putative targets of turmeric were searched using Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, http://tcmspw.com/tcmsp.php), and The Encyclopedia of Traditional Chinese Medicine (ETCM, http://www.tcmip.cn/ETCM/index.php/Home/Index/index.html). The optimal cutoff values of OB and DL in

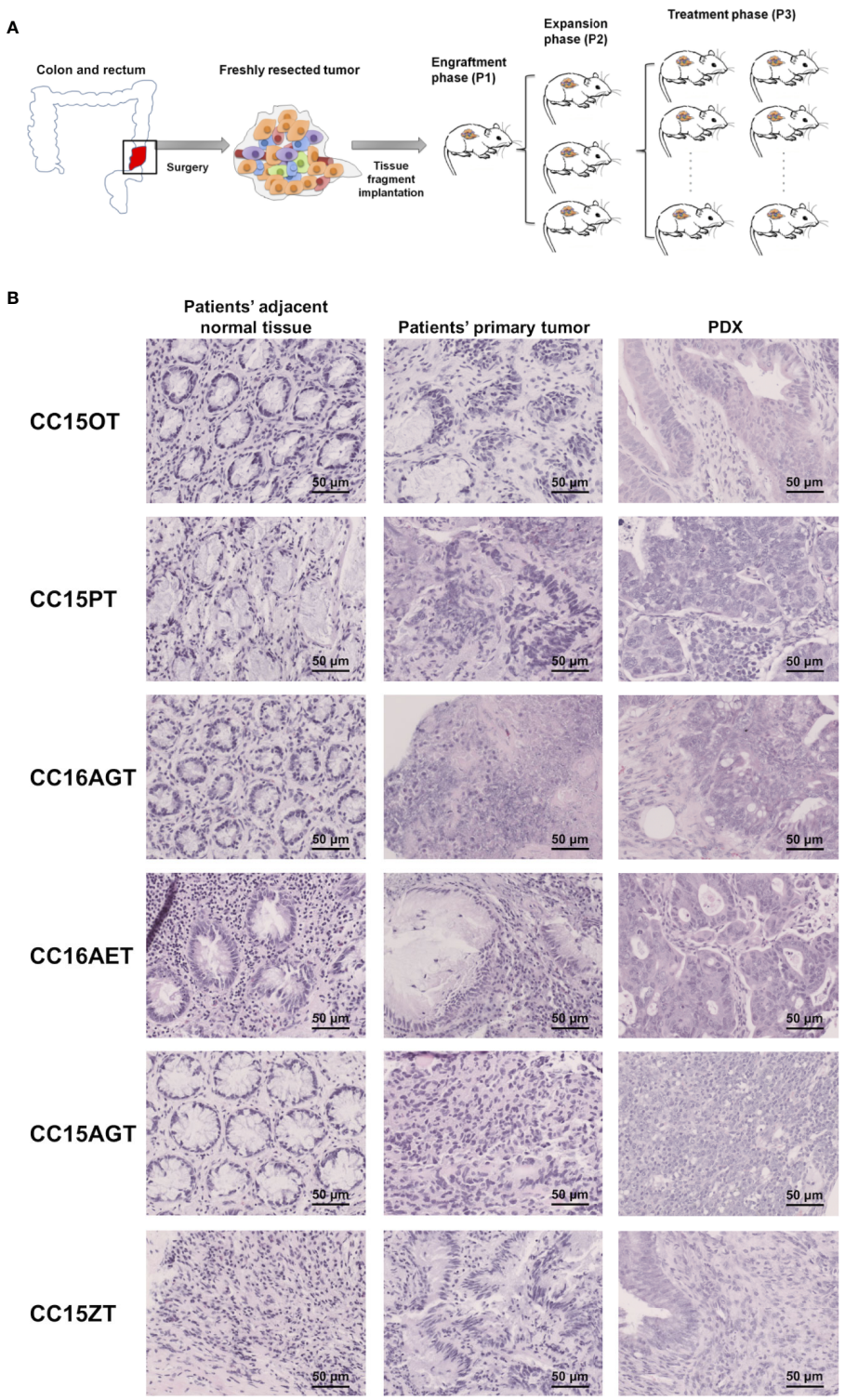

FIGURE 1 | Schematic diagram showing the establishment of PDX samples and histological assessment of PDX samples. **(A)** Patients' tumors were obtained, inoculated subcutaneously on the back of NOD-SCID mice (P1). When the size of PDX samples reached 1,000 mm^3, PDX samples were sub-cultured into the next batch of mice (P2), which subsequently expanded to the third passage (P3). Treatments of turmeric extract or FOLFOX or vehicle control were applied in the mice bearing P3 PDX samples. **(B)** The histology of representative patients' adjacent normal tissues, patients' primary tumors and PDX were determined using H&E staining. Bar = 50 μm.

TABLE 1 | Treatment and assessment details of PDX-bearing mice.

(A) Treatment and assessment details of TE-treated PDX-bearing mice.

PDX samples	Treatments	Duration	Tumor collection after treatments	Tumor size changes shown in Figure 2	Lung and liver metastasis assessment
CC15OT	TE or vehicle control	8 weeks	Yes	Yes	Yes
CC15PT	TE or vehicle control	2 weeks	Yes	Yes	Yes
CC16AGT	TE or vehicle control	5 weeks	Yes	Yes	Yes
CC16AET	TE or vehicle control	4 weeks	Yes	No[a]	Yes

(B) Treatment and assessment details of FOLFOX-treated PDX-bearing mice.

PDX samples	Treatments	Duration	Tumor collection after treatments	Tumor size changes shown in Figure 7	Lung and liver metastasis assessment
CC16AGT	FOLFOX or vehicle control	2 weeks	Yes	Yes	Yes
CC15AGT	FOLFOX or vehicle control	4 weeks	Yes	Yes	Yes

(C) Treatment and assessment details of TE-treated PDX-bearing mice with surgical removal of original PDX.

PDX samples	Treatments	Duration	Tumor size changes shown in Figure 6	Recurrent tumors collection	Lung and liver metastasis assessment
CC15ZT	TE or vehicle control	7 weeks	Yes	Yes, 4 weeks after surgery	Yes
CC16ANT	TE or vehicle control	4 weeks	Yes	Yes, 5 weeks after surgery	Yes

[a]*Tumor sizes of TE-treated and vehicle control groups were not significantly different so that the results have been shown in* ***Supplementary Information Fig. S4****.*

TCMSP were set to 30% and 0.18. Information on associated target genes of CRC was obtained from GeneCards (https://www.genecards.org/), and the top 1,000 genes were reserved for further analysis. The String database (http://string-db.org/) was utilized to obtain the data of protein-protein interaction (PPI) with the species limited to "Homo sapiens". The Cytoscape 3.7.2 software was applied for constructing the Compound-target Network, Compound-candidate target Network, PPI Network, and Hub Genes Network. GO Slim of candidate targets was analyzed by WebGestalt (http://www.webgestalt.org/), and the biological processes and KEGG pathway were analyzed by DAVID 6.8 (https://david.ncifcrf.gov/).

Statistical Analysis

All quantitative data were expressed as mean ± standard error of the mean (SEM). The unpaired Student's *t*-test was conducted to determine statistical significant differences. $p < 0.05$ was considered as statistically significance.

RESULTS

Generation of PDX From Colon Cancer Patients' Tumor Samples

PDX samples for colon cancer were established from seven patients' tumor samples. PDX were successfully grown through three serial passages in mice. The latency time from initial inoculation time to post-treatment varied from 6 to 8 months. Clinical characteristics including tumor size, origin of samples, tumor/node/metastasis stage and survival time, etc. were listed in **Table 2**. All patients' tumors included in this study were moderately differentiated without metastasis. Histological examination of the H&E stained PDX sections was conducted to compare the similarity of PDX with the original patients' tumors. Both the primary tumors and PDX show features of adenocarcinoma (**Figure 1B**). PDX could represent diverse clinical characteristics typically observed in patients according to pathologist's assessment. Meanwhile, the adjacent normal tissues were also stained by H&E to illustrate the differences between normal tissues and tumor tissues. Most of the adjacent tissues have well differentiated histomorphology, which were apparently different from tumor tissues.

Turmeric Extract Inhibited Tumor Growth in Colon PDX

Mice bearing 4 batches of PDX samples (CC15OT, CC15PT, CC16AGT, and CC16AET) were received TE treatments. Since the size of the original patients' tumors varied, the number of mice in each batch of PDX samples were different. Besides, the duration of treatment was determined by the tumor size and/or the well-being of mice. Some of the experiments were terminated when the tumor size of untreated control group was large (~600–700 mm^3) or the PDX-bearing mice became very weak (even the PDX size was not large), as it was unethical to continue the experiements.

TABLE 2 | Characteristics of the patients for CRC PDX model.

Sample codes	CC15OT	CC15PT	CC16AGT	CC15AGT	CC15ZT	CC16ANT	CC16AET
Range of age	70-74	80-84	50-54	65-69	80-84	65-69	60-64
Metastases at presentation (Y/N)	N	N	N	N	N	N	N
Tumor length (cm)	2.5	4	6	3.5	9	4	6
Origin of sample	sigmoid	rectum	ascending colon	rectum	ascending colon	splenic flexure	transverse colon
Number of lymph nodes removed	23	13	43	32	20	29	31
Tumor/node (TN) staging	T3N0	T2N0	T3N0	T3N0	T2N0	T3N0	T3N0
Tumor differentiation (well/moderate/poor)	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Lymphovascular permeation (Y/N)	N	N	N	N	N	N	N
Adjuvant chemotherapy (Y/N)	N	N	N	N	N	N	N
Adjuvant radiotherapy (Y/N)	N	N	N	N	N	N	N
Follow up time from surgery (months)	42	41	30	35	37	23	32
Recurrence (Y/N)	N	N	N	N	N	N	N

Nevertheless, our results could show that oral TE treatment decreased the size of PDX in three batches (except CC16AET) with different extent. As shown in **Figure 2**, all tumors in TE-treated groups were smaller than those in vehicle control groups, with tumor growth inhibition for CC15OT, CC15PT, and CC16AGT at 79.1%, 36.7%, and 51.5%, respectively. The inhibitory effects of TE were shown to be the most effective in CC15OT sample, the final tumor weight of TE-treated group was 54.9 ± 7.43 g versus that of control group 263.2 ± 67.3 g ($p < 0.01$, **Figure 2A**). Body weights of mice did not significantly differ between treatment and control groups in these three tested batches, indicating TE did not cause systemic toxicity in mice.

Anti-Metastatic Effect of TE Treatment in Colon PDX Models

The efficacy of TE treatment on tumor metastasis in PDX models was reflected by metastasis area in livers and lungs. Levels of metastasis varied among batches, metastatic area ranged from 7.8% to 20.9% in lungs, and from 6.3% to 17.7% in livers. The highest tumor burden was observed in CC16AET sample, with the metastatic area as 17.7% and 20.9% in liver and lung, respectively. The liver metastasis in four batches of PDX samples (CC15OT, CC15PT, CC16AGT, and CC16AET) could be significantly decreased after TE treatment (**Figure 3A**).Whereas TE treatment significantly reduced the lung

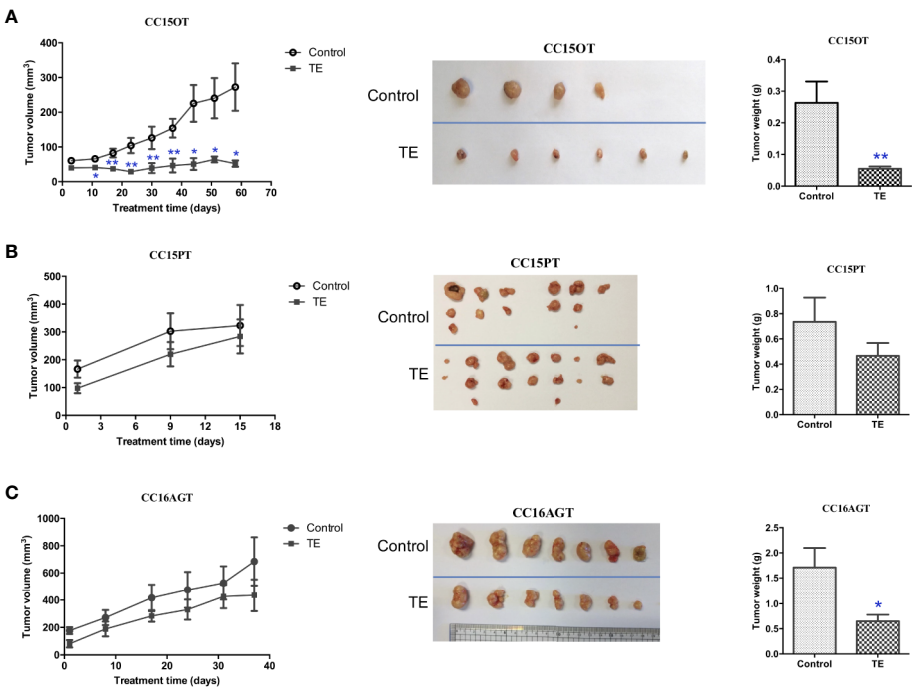

FIGURE 2 | Effects of TE treatment in 3 PDX samples CC15OT, CC15PT, and CC16AGT. The tumor volume and weight in the control group and TE treatment groups were compared using **(A)** CC15OT (n = 4–6), **(B)** CC15PT (n = 6–7), and **(C)** CC16AGT (n = 7). Tumor volume was measured once a week while the final tumor weight was measured at the end of the experiment. Representative photos for tumors from 4 to 7 mice in each PDX sample. Data were presented as mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle control at the same time point.

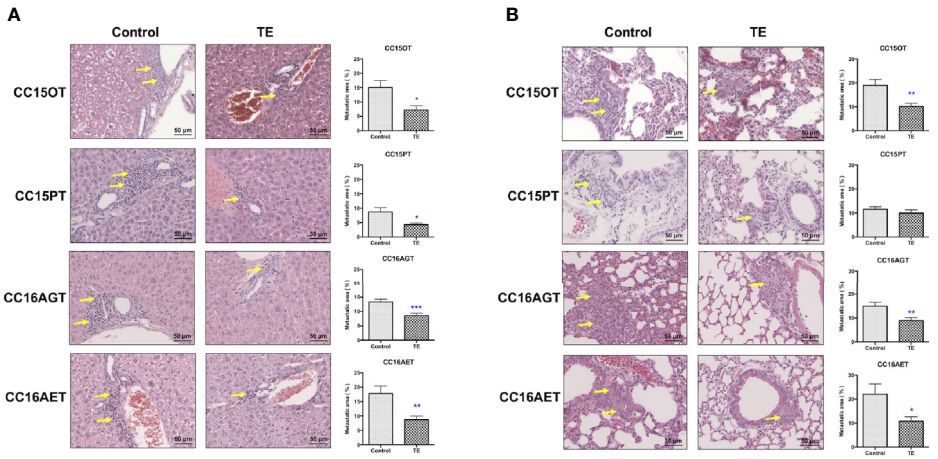

FIGURE 3 | Effects of TE treatment on liver and lung metastasis of PDX samples. **(A)** Livers and **(B)** lungs of mice bearing CC15OT, CC15PT, CC16AGT, and CC16AET PDX samples were collected and subjected to histological analysis. Liver and lung sections were stained with H&E and the metastatic area (shown by yellow arrows) was assessed. Representative photos for 4-8 livers or lungs in each group. Quantitative results of liver and lung metastasis were shown on the right panel. Data shown were mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle control.

metastatic area in CC15OT, CC16AGT, and CC16AET samples (**Figure 3B**). Importantly, most of the metastasis occurred near blood vessels where the tumor cells permeated from and then colonized. As the highest inhibition rate (51.0%) of lung metastasis by TE treatment was observed in CC16AET sample, the expression of the human-specific 850-bp fragment of the α-satellite DNA on human Ch17 (31, 32) was determined in lung tissues. Results showed that the relative Ch17 expression (after normalization with housekeeping gene GAPDH) in TE-treated group was 1.04 ± 0.70, while that in control group was 2.80 ± 1.56. There was significantly decreased of the Ch17 expression in TE-treated mice ($p < 0.05$), suggesting that the inhibitory effect of TE treatment on the metastasis and/or colonization of human xenograft cells in mice lungs.

Underlying Mechanisms of Anti-Tumor and Anti-Metastatic Effects of TE

As the tumor sizes were reduced after TE treatment, the tumor sections were subjected to TUNEL assay for evaluating apoptosis in tumors. As shown in **Figure 4A**, TE treatment could significantly induce apoptosis in tumors in CC15PT and CC16AGT samples as shown by TUNEL asssy and the expression of cleaved caspase-3. Besides, TE treatment decreased the expression of Ki 67 (proliferation marker, **Figure 4B**) in tumor sections. On the other hand, β-catenin, involving in tumor cell proliferation (33) and EMT process (34) which is considered as the first step in tumor metastasis, was also assessed in tumor sections. In CC15PT and CC16AGT samples (**Figure 4C**), β-catenin expression was shown to be significantly decreased in tumors. As the sizes of TE-treated tumors in CC15OT batch were small, tumors were subjected to the extraction of proteins and RNA instead of immunohistochemical analysis. The protein expression of β-catenin in CC15OT samples was examined using Western blot as shown in **Figure 5A**, and it was apparently reduced after TE treatment.

Furthermore, TE treatment significantly reduced the protein expressions of β-catenin, Src and RhoA in CC15OT ($p < 0.05$) and slightly reduced the protein expressions of RhoA in CC15PT samples ($p = 0.074$, **Figure 5A**). As the RNA of CC15OT and CC15PT samples were extracted, the gene expression of Wnt signaling elements (*AXIN2*, *DKK1*, and *SMAD7*), EMT pathway related molecules (N-cadherin, Snail and Vimentin, and EpCAM), growth factor receptors (*PDGFRB*), asparagine synthetase (*ASNS*), Rho signaling (*RTKN2*), ATP-binding cassette transporters (*ABCA13*), and *iNOS* were evaluated using qPCR. As shown in **Figure 5B**, mRNA expressions of Wnt signaling elements (*AXIN 2*), EMT pathway related molecules (Vimentin and EpCAM), *EGFR*, and *RTKN2* were all down-regulated in TE-treated tumors of CC15OT samples, whereas *DKK1* was significantly up-regulated by TE treatment. On the other hand, the expressions of *KRAS* and *EGFR* in primary tumor tissues were shown to be significantly higher than those in adjacent normal tissues ($p < 0.001$, **Figure 5C**, left panel). While the CC15OT PDX-bearing mice treated with TE, the expressions of these two genes in the PDX samples were significantly decreased when compared with untreated control ($p < 0.05$, **Figure 5C**, right panel).

Furthermore, in the samples of CC15PT, the expressions of *AXIN2*, *SMAD7*, N-cadherin, vimentin, *ASNS*, *EGFR*, *PDGFRB*, *ABCA13*, and *DKK1* were all significantly down-regulated after TE treatment (**Figure 5D**). These results suggested the multi-targeted activities of TE treatment on the colon PDX.

Novel Finding of TE in Reducing Colon Tumor Recurrence

Since the anti-tumor and anti-metastatic efficacies were observed in PDX-bearing mice treated with TE, the potential inhibitory effect of TE treatment on tumor recurrence was further investigated in another 2 batches of PDX samples. Mice bearing PDX samples CC15ZT and CC16ANT were treated with TE for

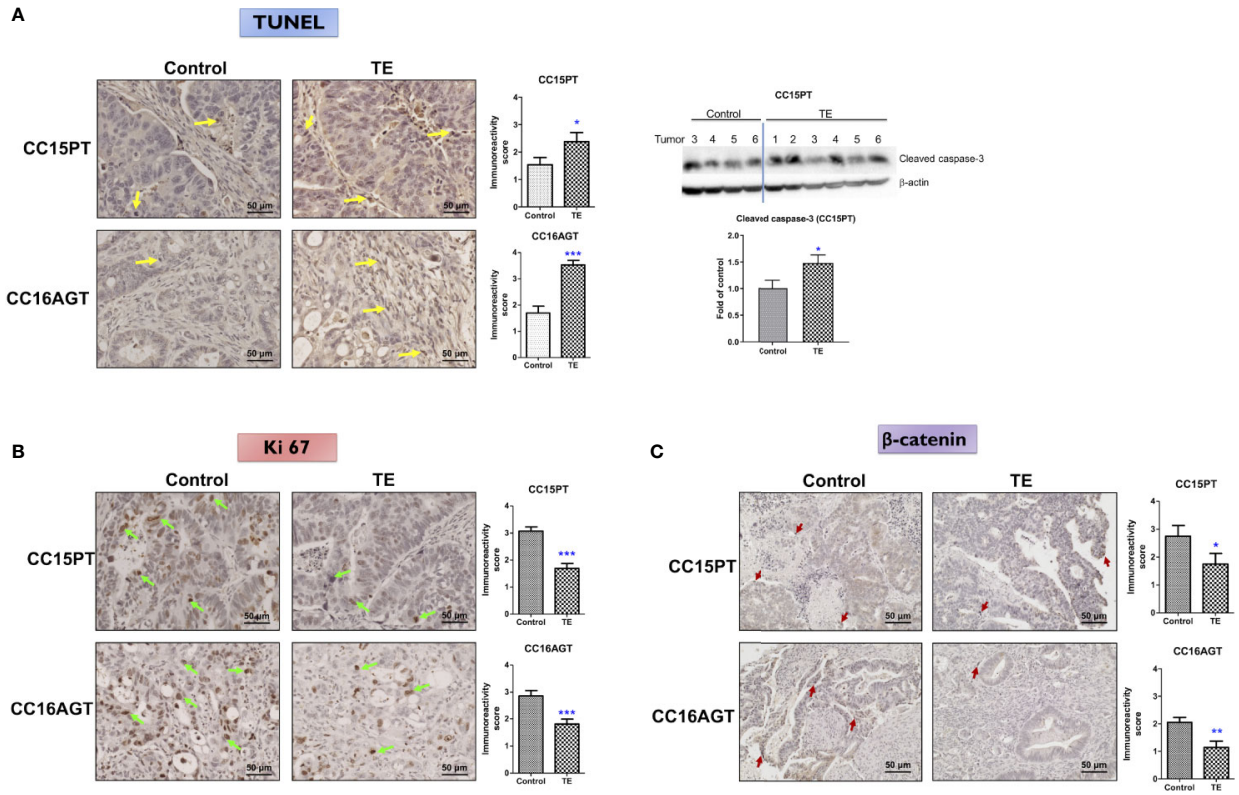

FIGURE 4 | Mechanism of the inhibitory effects of TE treatment on PDX samples. Representative images of **(A)** TUNEL, IHC staining against **(B)** Ki 67, and **(C)** β-catenin in tumor sections from two batches (CC15PT and CC16AGT) of PDX-bearing mice. Bar = 50 μm. Quantitative results of positive-stained cells in the tumor sections were shown on the right panel. Western blots of cleaved caspase-3 in CC15PT were shown in **(A)**. Data were presented as mean ± SEM. n = 20–25 sections in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle control.

7 and 4 weeks, respectively. Then, the xenografts were removed by surgery. The primary tumor volumes of TE-treated groups were smaller than control group, and the average tumor weight in control group was 1.47 ± 0.39 g versus 0.74 ± 0.23 g in TE-treated group (**Figure 6A**). After the mice recovered from surgery, they were maintained without any treatment in the following 4 weeks. The recurrent tumors could be observed in mice of vehicle control group. The mice were sacrificed when the largest recurrent tumors reached the size of 1,000 mm^3, and the recurrent tumors, livers and lungs were collected for analysis. The tumor incidence after surgery and recurrent tumor weight could indicate the inhibitory effect of TE on tumor recurrence. The incidence of tumor in vehicle control group was 100% (4/4), while that in TE treatment group was only 50% (2/4) (**Figure 6A**). The average weight of recurrent tumors in TE treatment group (0.018 ± 0.012 g) were lower than that in control group (0.393 ± 0.207 g). TUNEL assay for the primary xenografts and recurrent tumors revealed that TE treatment induced apoptosis in tumors (**Figure 6B**). The protein expressions of β-catenin, Src and pSrc were suppressed in the tumors in TE-treated group (**Figure 6C**). The gene expressions of EMT pathway related molecules (N-cadherin, EpCAM), *EGFR*, *ASNS*, and *RTKN2* were also down-regulated after TE-treatment (**Figure 6D**). All these molecular alterations in the primary xenografts might contribute to the inhibitory effects of TE on the growth of primary and recurrent tumors. Lastly, lung metastasis was found to be significantly reduced after TE treatment (**Figure 6E**).

The inhibitory effect of TE on tumor recurrence was also demonstrated in mice bearing PDX sample CC16ANT, which were treated with TE for 4 weeks, and the primary xenografts were removed by surgery. The average tumor weight in vehicle control group was 1.82 ± 0.32 g while that in TE-treated group was 0.95 ± 0.19 g, which was significantly decreased (**Figure 6F**, left panel). Five weeks after the removal of the primary xenografts, recurrent tumor was found in 1 out of 3 mice treated with TE (i.e., tumor incidence: 1/3). While in vehicle control group, five mice out of seven were found to have recurrent tumors (**Figure 6F**, right panel). Similarly, the significant reduction of metastasis in liver and lung could be observed in this set of experiment (**Figure 6G**).

Efficacy of FOLFOX in Colon PDX Model

Beyond surgery or in metastatic phase, FOLFIRI (fluorouracil, folinic acid, and irinotecan) or FOLFOX (plus oxaliplatin) are utilized as system treatment (35). Previous studies demonstrated the side effect of FOLFOX including neurotoxicity, immune suppression, hair loss, nausea, etc. (28, 36). Therefore, there is a

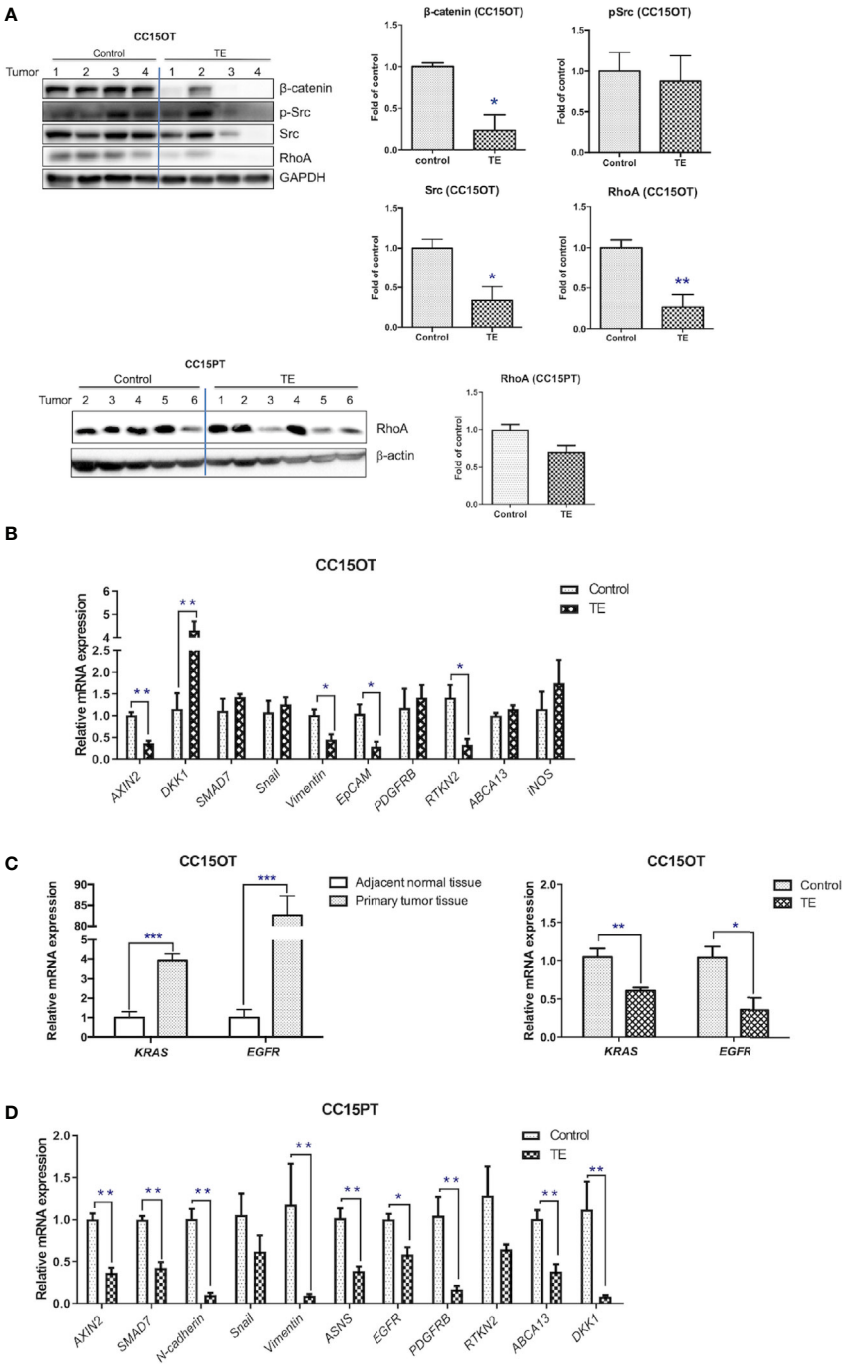

FIGURE 5 | Modulatory effects of TE treatment on protein and gene expressions in PDX samples. **(A)** Western blot of tumor protein against β-catenin, p-Src/Src, RhoA, GAPDH in CC15OT, and CC15PT PDX samples. The quantitative data presented as fold of vehicle control. **(B–D)** Real-time PCR of selected gene expressions in CC15OT and CC15PT PDX samples. GAPDH was used for normalization. n = 4–6 tumors in each groups. Data were shown as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle control.

strong desire for developing potent and safe anti-tumor and anti-metastatic therapeutics. In order to show the potential superior efficacy of TE over the conventional therapeutics, the efficacy of FOLFOX was also examined in the present study. Chemotherapeutics FOLFOX was administered to mice bearing CC16AGT and CC15AGT PDX samples. Surprisingly, the mice cannot even bear long time FOLFOX treatment. After 2 and 4 weeks of FOLFOX treatments, gradually decreases (>20%) of body weights (**Figures 7A, D**), the treatments were observered and the treatments were terminated at 2 and 4 weeks in CC16AGT and

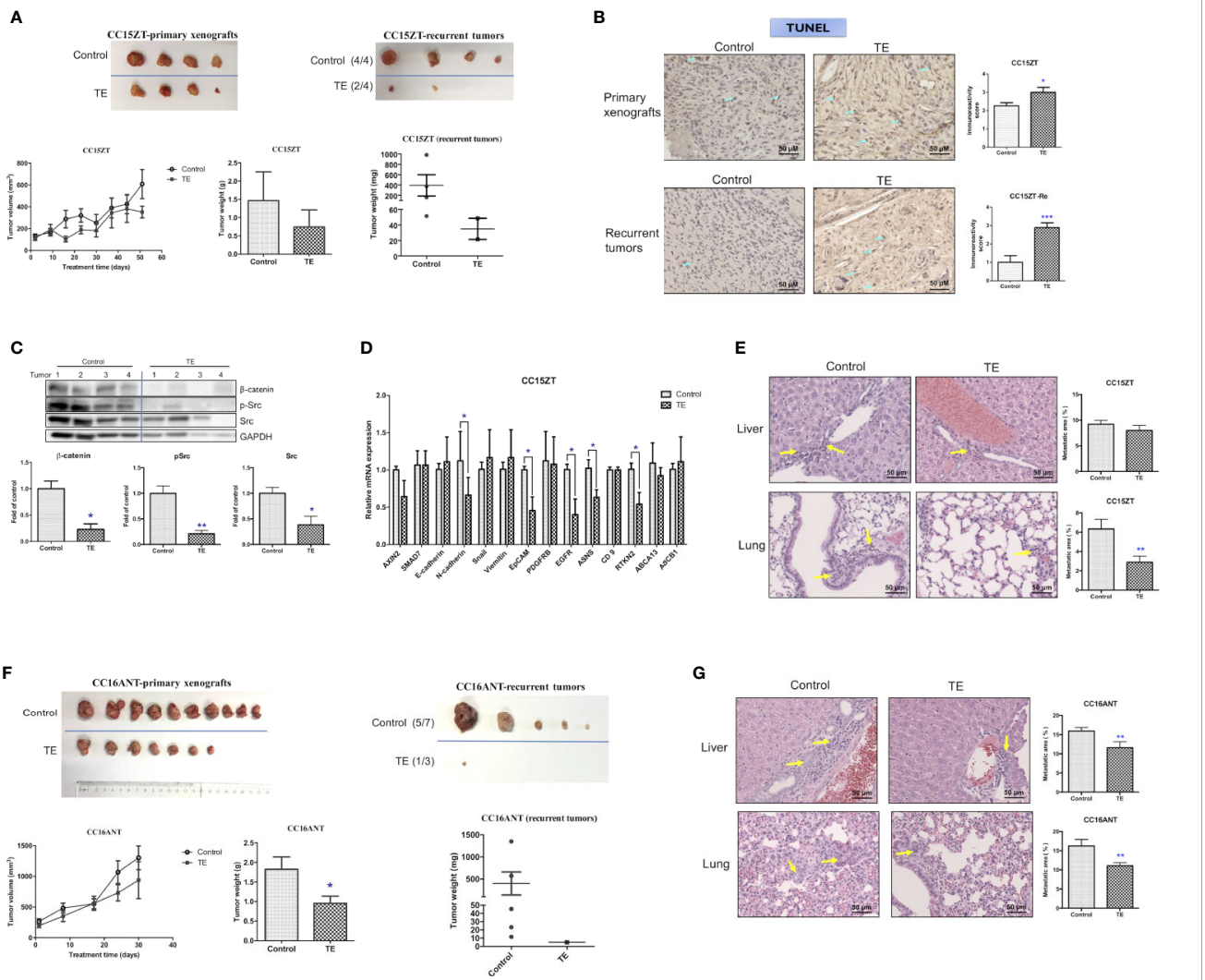

FIGURE 6 | Effects of TE treatment on the growth of PDX and the recurrence of tumor. Mice bearing PDX samples CC15ZT and CC16ANT were treated with TE and the primary xenografts were removed by surgery. For the CC15ZT PDX sample, **(A)** tumor volume changes, final tumor weight and photo of primary xenografts were shown on the left panel. The final tumor weight and photo of recurrent tumors were shown on the right panel. The tumor incidence was listed in the brackets next to the photo. **(B)** Representative images of TUNEL in primary xenografts and recurrent tumor sections and quantitative results of positive-stained cells in the tumor sections were shown. Bar = 50 μm. Data were presented as mean ± SEM. n = 20 sections in each group. **(C)** Western blot of primary xenograft protein against β-catenin, p-Src, Src, and GAPDH and the quantitative data presented as fold of vehicle control. **(D)** Real-time PCR of selected gene expressions in the primary xenografts. GAPDH was used for normalization. n = 4 tumors in each group. **(E)** Liver and lung sections were stained with H&E and the metastatic area (shown by yellow arrows) was assessed. n = 20 sections in each group. Quantitative results of liver and lung metastasis were shown on the right panel. For the CC16ANT PDX sample, **(F)** tumor volume changes, final tumor weight and photo of primary xenografts were shown on the left panel. The final tumor weight and photo of recurrent tumors were shown on the right panel. The tumor incidence was listed in the brackets next to the photo. **(G)** Liver and lung sections were stained with H&E and the metastatic area (shown by yellow arrows) was assessed. n = 15–25 sections in each group. Data were shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle control.

CC15AGT PDX samples, respectively. The mice were maintained without any treatment until the end of experiments.

In the CC16AGT PDX samples, the tumor volume was smaller in FOLFOX treatment group when compared with vehicle control group (days 14–21). However, the final tumor volume and weight were similar in FOLFOX-treated group and the vehicle control group (**Figure 7B**), which might due to the termination of FOLFOX treatment on day 15. Nonetheless, the anti-tumor activities of FOLFOX could still sustain as the apoptotic area in tumors was higher in FOLFOX-treated group than that in vehicle control group (**Figure 7C**). The metastasis in liver and lung were also examined after FOLFOX treatment, and results showed that the metastatic area was slightly increased in FOLFOX-treated group (**Figure 7C**).

For the CC15AGT PDX samples, which should be sensitive to FOLFOX treatment because the tumor growth was suppressed by

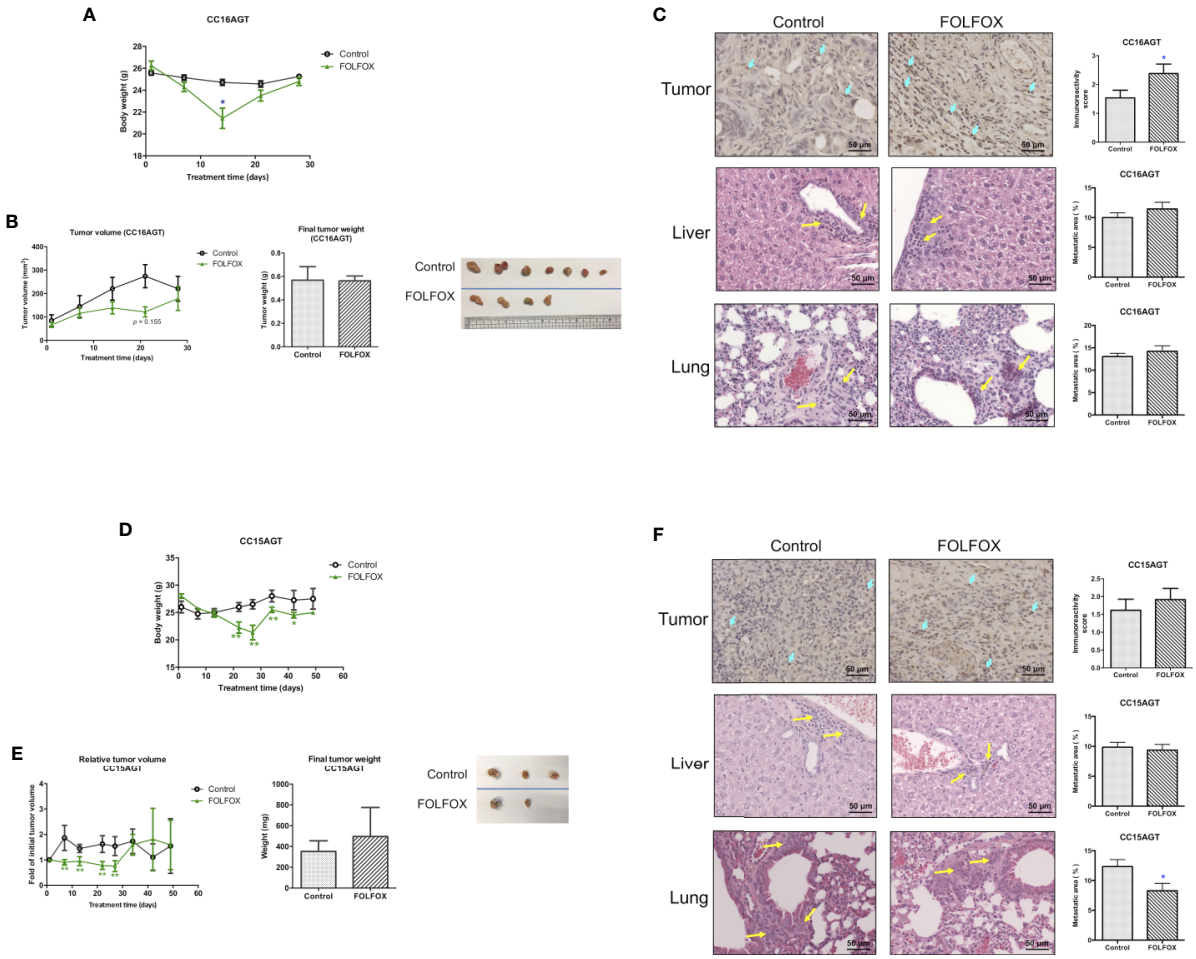

FIGURE 7 | Effects of FOLFOX treatment on the tumor growth and metastasis in PDX samples. Mice bearing PDX samples CC16AGT and CC15AGT were treated with FOLFOX. **(A, D)** Body weight changes during treatment and final body weight. **(B, E)** Tumor volume changes/relative tumor volume changes, final tumor weight and photo of tumors. Data were presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle control at the same time point. Representative images of TUNEL of tumor sections, H&E stained lung and liver sections of **(C)** CC16AGT samples and **(F)** CC15AGT samples. Quantitative results of positive-stained (TUNEL) cells in the tumor sections and the metastatic area were shown on the right panel. Bar = 50 μm. n = 10–35 sections in each group. Data were shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. vehicle control.

FOLFOX treatment as shown in the tumor growth curve (**Figure 7E**), meanwhile the body weight was decreased gradually (**Figure 7D**). After termination of FOLFOX treatment, the tumor volume of this group increased sharply (**Figure 7E**), resulting in comparable tumor size with vehicle control group. The apoptotic area of tumors were slightly higher in FOLFOX treatment group than that in vehicle control group (**Figure 7F**). Nevertheless, the metastasis in lung was found to be suppressed by FOLFOX treatment in this batch of PDX-bearing mice, further suggesting the diverse responses of PDX samples toward chemotherapeutics.

Network Pharmacology Predicts Potential Mechanism of Turmeric Extract Treatment in Colorectal Cancer

A total of 18 candidate targets for treating CRC were collected from 14 potential active compounds (**Table S2**) of TE. The PPI network of candidate targets contained 38 nodes and 202 edges, in which average node degree is 10.6 (**Figure 8A**). The cytoHubba plugin of the Cytoscape software was used to analyze the 38 nodes and found that 3 targets belonged to the hub genes in candidate targets according to rank by Degree, Closeness, and Betweenness (**Figure 8B**). Hence, we suspected that the following three genes encode proteins in pivotal roles: AKT1, ESR1, and PTGS2. We then performed a GO Slim analysis by WebGestalt, and it was suggested that the candidate targets participated in protein binding, ion binding, and lipid binding in membrane, protein-containing complex, and nucleus (**Figure 8C**). To further examine the signaling pathways and functions of these candidate targets, functional enrichment analysis was carried out using DAVID 6.8. Candidate targets were found involved in biological processes such as signal transduction, inflammatory response, and positive

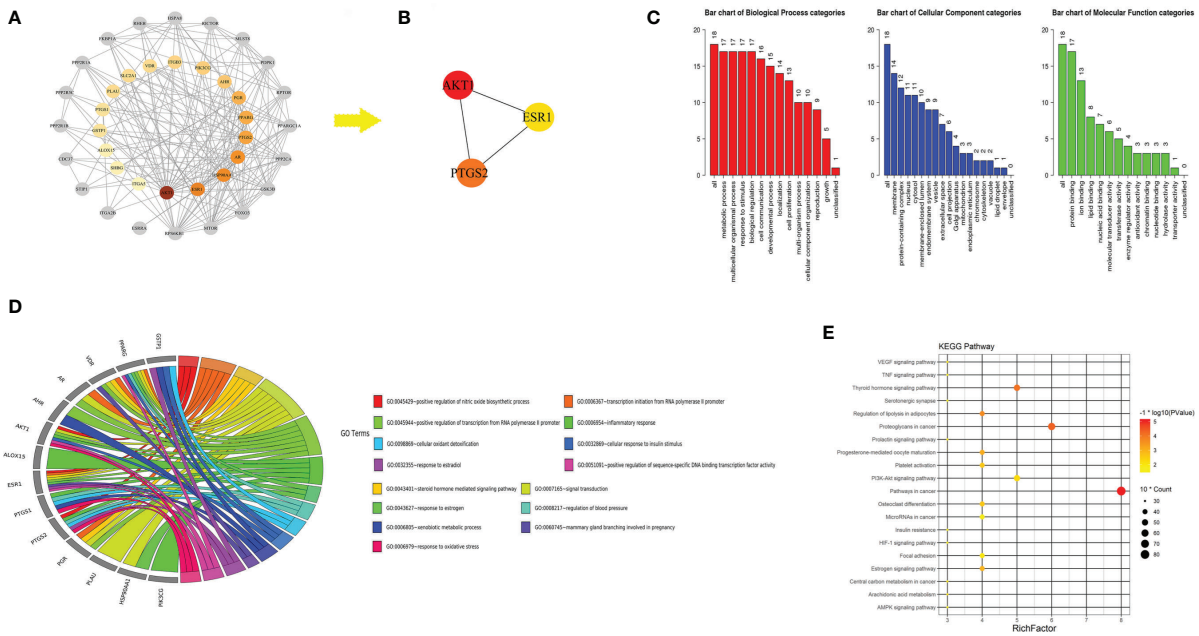

FIGURE 8 | Protein-protein interaction (PPI) network of turmeric extract treated colorectal cancer. **(A)** Node representation of candidate target with greater degree was described by darker color. Nodes in grey was ranking neighbors of candidate targets. **(B)** Hub genes in candidate targets. **(C)** GO slim analysis of candidate targets from WebGestalt. **(D)** Biological process (BP) from DAVID 6.8 showing the top 15 BPs of turmeric extract against colorectal cancer. **(E)** KEGG analysis of candidate targets from DAVID 6.8.

regulation of nitric oxide biosynthetic process (**Figure 8D**). The results of KEGG analysis indicated that candidate targets were intensively associated with PI3K-Akt signaling pathway and thyroid hormone signaling pathway (**Figure 8E**). In fact, previous studies have shown that these signaling pathways are related to regulating cancer cell growth and proliferation (33, 34), which indicates a possible direction for further cancer related research. Except three hub genes and pathways in cancer, other targets of TE which could be potential targets in treating other diseases were also proposed.

DISCUSSION

The *in vivo* efficacies of turmeric ethanolic extract, which contains absorbable curcumin, were demonstrated in mice bearing colon PDX for the first time in the present study. Seven PDX samples were established from the moderately differentiated colorectal tumors from consented patients and the comprehensive evaluation on anti-tumor (including tumor recurrence) and anti-metastatic effects of TE and chemotherapeutics FOLFOX have been conducted. In our established PDX samples, histological features were well preserved through passages, despite the high degree of heterogeneous architecture and molecular characteristics, they could recapitulate *in vivo* tumor biology and be employed in the accurate preclinical drug evaluation (8). The responses of these PDX samples toward therapeutic agents (natural or synthetic) would be more clinically relevant than those from cell-derived xenograft models.

In view of the multiple entities of pathology of CRC, PDX model would be the most appropriate model to allow accurate testing of potential multi-targeted therapeutics. Recent studies demonstrated the use of combination of targeted drugs, e.g., ABT-263 plus YM-155 (37), panitumumab plus TAS-102 (38) in colon PDX models. In fact, apart from those novel synthetic small molecules and biomolecules (antibodies) for cancer treatment, herbal medicines are certainly alternative source of therapeutic agents since herbal medicines have long been used for chronic disorders management. The multi-components properties of herbal medicines would match the multiple combination treatment approaches in many diseases, such as cancer.

To prove this concept, a well-known Chinese and Ayurvedic herbal medicine, turmeric, the dried rhizome of the plant *Curcuma longa* Linn. was chosen to be verified of its anti-tumor effects in colon cancer using PDX models. One of the major components of turmeric, curcumin, has been shown to be anti-tumor in various cancer types in preclinical studies (39, 40). However, low water solubility, poor bioavailability, rapid metabolism, and systemic elimination of curcumin hinder its pharmacological application (41). Nevertheless, our previous studies demonstrated that turmeric ethanolic extract, which contains curcuminoids, turmerones, and other components, exerted potent anti-tumor and anti-metastatic effects as well as augmented anti-tumor activities of bevacizumab in colon cancer

xenograft-bearing mice (19, 27, 42). Despite the mice models we used which covered both immuno-deficient nude mice and immuno-competent Balb/c mice, the xenografts were formed by homogeneous cell lines. Hence, the efficacy of TE treatment in colon cancer has been further verified using the PDX model in the present study, which would reveal the actual potential responders toward the treatments.

The present study demonstrated the anti-tumor and anti-metastatic effects of TE in six PDX samples. The responsive rate of anti-tumor effect toward TE was 50.0% (significantly reduced tumor weight in three out of six samples), and that of anti-metastatic effect was 83.3% responsive rate (five out of six) in both lungs and livers. Although the direct anti-tumor efficacy of TE was not as potent as that observed in cell-derived tumor-bearing model, the present findings further confirmed the potent anti-metastatic activities of TE treatment in PDX models, in which colon cancer cells from different patients metastasized to distal organs from the original xenografts. A recent study reported the inhibition of curcumin on tumor growth and aggressiveness using xenograft model with patient-derived oral squamous cell carcinoma cells (43). Nonetheless, the inhibitory effects of TE treatment toward lung and liver metastasis of colon PDX have not yet been reported. Interestingly, in our project, we did see all of moderately differentiated CRC tumors developed metastasis in PDX model. In fact, in clinical situation, around 70% of CRC tumor are moderately differentiated (44). For the hypothesis that moderately differentiated CRC tumors result in liver and lung metastasis, in our PDX model, we inoculated primary tumors into SCID mice, in which the immune surveillance to tumor metastasis is lacking. Meanwhile, not all primary tumors from patients could develop into PDX. Hence, the successful established PDX needs to be aggressive and may have potential to develop metastasis in patients as well. So, it is understandable for all models here turned out to be metastatic.

The samples of PDX after treatments were subjected to protein and molecular analysis in order to determine the mechanisms of action. Wnt signaling pathway controls cell fate, regulates homeostasis and is closely associated with malignant transformation (45). β-catenin is a key element in Wnt pathway and the loss of degradation of β-catenin in cytoplasm results in the transcription of cell proliferative, survival genes such as MYC, cyclin D (46). DKK1 encodes a Wnt suppressor gene which exerts pro-apoptotic and strong anti-proliferative activities in CRC cell lines (47, 48). TE treatment significantly up-regulated DKK1 expression in CC15OT sample (**Figure 5B**) and CC16ANT (data not shown) which might account for its anti-tumor effects. AXIN2 encoding a Wnt signaling component and promotes colon carcinoma oncogenic activity (49, 50). SMAD7 expression can affect β-catenin levels and lead to increased Wnt signaling (51). Results from IHC, Western blot and q-PCR showed the down-regulating effects of TE on Wnt signaling pathway, which involves in the CRC progression, including tumor initiation, growth and metastasis (52). In addition, KRAS and EGFR gene expressions were down-regulated by TE treatment in all tested PDX samples. EGFR signal pathway involves in angiogenesis, cancer cell proliferation, migration, invasion as well as inhibition to apoptosis (53). Furthermore, protein expression of Src was also down-regulated after TE treatment, indicating that TE also exerted anti-tumor and anti-metastatic effects through influencing cell growth, adhesion and migration (54). Vimentin, EpCAM, and N-cadherin (belong to EMT pathway) were all down-regulated in TE treatment group in tested PDX samples. These alternations of gene or protein expressions strongly suggested the multiple targets of TE treatment in colon cancer, such targets are also consistent with other studies regarding curcumin (55, 56).

On the other hand, the potential of TE treatment on inhibiting tumor recurrence in colon PDX model was firstly revealed in this study. Although the direct anti-tumor effect of TE treatment may not be as good as the first-line chemotherapeutics, the results of reduced numbers and sizes of recurrent tumors shown here should provide evidences on the "chemopreventive" efficacy of TE treatment. The molecular alterations after TE treatment may also explain the inhibition of tumor recurrence observed in the present PDX model. The results from the present study are of clinical significance as PDX model mimics clinical situation and predict the tumor metastasis. To the best of our knowledge, this is possibly the first study of herbal extract on PDX model of colon cancer.

The first-line chemotherapy for CRC in clinics is based on fluorouracil (5-FU) alone or in combination as FOLFOX (5-FU, folinic acid and oxaliplatin)/FOLFIRI (5-FU, folinic acid and irinotecan) for long time (35, 57). Unfortunately, despite effectiveness of the chemotherapeutics, the incidence of side effects is as high as 98% (58), including neurotoxicity, immune system suppression, hair loss, nausea and vomiting, severely affected the quality of life of patients (59). In the present study, two batches of mice bearing PDX samples were used to evaluate the efficacy of FOLFOX, which has seldom been reported. Similar to the side effects observed in patients, the body weight of mice bearing PDX received human equivalent dosages of FOLFOX decreased gradually (**Figure 7**). Meanwhile, the tumor growth was suppressed by FOLFOX treatment. The termination of treatment resulted in tumor volume increases. The overall outcomes of the incomplete FOLFOX treatment were not that positive, although the apoptotic activities retained (shown by $TUNEL^+$ stained cells in **Figure 7C**). FOLFOX treatment did not effectively suppress liver metastasis in the mice-bearing PDX (**Figures 7C, F**) and it did not affect the Ki 67 and β-catenin expressions in tumor sections (data not shown). Taken together, the observed differential responses toward TE and FOLFOX treatments were as expected because the study subjects (i.e., each batch of PDX samples) were heterogeneous. In general, TE treatment might exert superior anti-metastasis efficacy than FOLFOX treatment in the PDX samples tested in this study.

Beyond the molecular mechanisms examined here, we utilized system biology method especially network pharmacology approach to predict the potential targets of TE based on its main components. Collectively, we constructed the protein-protein network of the 18 candidate targets of TE treated CRC in which the hub genes were revealed: AKT1, ESR1, and

PTGS2. Furthermore, we discovered that the candidate targets may involve in signal transduction, inflammatory response, and positive regulation of nitric oxide biosynthetic process. Moreover, as the result of KEGG analysis, multiple pathways like PI3K-Akt signaling pathway and thyroid hormone signaling pathway, may be the potential pathways of turmeric in the treatment of CRC. We postulated that patients may benefit from the combination use of PI3K-Akt and/or thyroid hormone signaling pathway inhibitors together with TE treatment.

In conclusion, we have successfully established PDX model from a panel of CRC patients' tumors which was a good platform for evaluating the multi-targeted herbal medicines. The preclinical anti-tumor and anti-metastatic activities of turmeric ethanolic extract were firstly demonstrated in individual colon PDX. To make this study more informative, we utilized network pharmacology to figure out more potential drug targets and working mechanisms. This precision medicine approach can potentially provide crucial information regarding preclinical translational evidence to clinical trials and together with network pharmacology to illustrate the potential therapeutic values of TE in CRC prevention and treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Animal Experimentation Ethics Committee of the Chinese University of Hong Kong. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CL and GY conceived the study. ML and GY performed the *in vitro* and *in vitro* studies and analyzed the data. LL performed the network pharmacology analysis. SN provided colon cancer patients' tissue samples. ST, SN, and K-PF provided advices on study design and data interpretation. CL and K-PF contributed essential reagents and tools, ML, GY, and CL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.574827/full#supplementary-material

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Hemicolectomy Does Not Provide Survival Benefit for Right-Sided Mucinous Colon Adenocarcinoma

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Background: The extent of bowel resection is widely debated in colon cancer surgery. Right hemicolectomy (RHC) and partial colectomy (PC) are the most common operation options for right-sided colon cancer (RCC). However, there are still no treatment guidelines or published studies to guide surgical options for mucinous adenocarcinoma (MAC) of RCC.

Methods: Patients with MAC and non-specific adenocarcinoma (AC) of RCC who underwent RHC and PC from 2010 to 2015 in the Surveillance, Epidemiology, and End Results (SEER) database were retrieved. The general characteristics and survival were compared and analyzed.

Results: A total of 27,910 RCC patients were enrolled in this study, among them 3,413 were MAC. The results showed that race, carcinoembryonic antigen (CEA) level, perineural invasion (PNI), tumor size, tumor location, TNM stage, liver metastasis, chemotherapy were significantly different between MAC and AC groups. The MAC group had similar dissected lymph nodes, but more positive lymph nodes than the AC group. The overall survival (OS) of the MAC group was poorer than that of the AC group, but cancer-specific survival (CSS) was similar between the two groups. The RHC subgroup of the MAC group had more patients of age ≤60 years, larger tumor size, cecum/ascending colon location and dissected lymph nodes than the PC subgroup, but similar positive lymph nodes, perioperative mortality, OS and CSS as the PC subgroup. Moreover, the univariate and multivariable analyses for the survival of RCC patients with MAC showed that RHC might not be a superior predictor for OS and CSS compared with PC.

Conclusions: RHC could not dissect more positive lymph nodes or provide long-term survival benefits for RCC patients with MAC compared with PC. This study could provide some evidence for surgery treatment selection for MAC of RCC, which has important clinical value in individual management of colon cancer patients.

Keywords: right-sided colon cancer, mucinous adenocarcinoma, partial colectomy, right hemicolectomy, survival

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death in the world (1). Surgical resection is the predominant and standard therapy option for CRC (2, 3). Right-sided colon cancer (RCC) occurs in the cecum ascending colon, hepatic flexure and/or transverse colon, and its long-term survival after curative surgery is worse than that of left-sided colon cancer (4). Mucinous adenocarcinoma (MAC) is the second most common histopathological type of CRC, which more often occurs in the RCC (1, 2, 5). MAC is different from non-specific adenocarcinoma (AC) of CRC in the oncologic behavior, genomics, and clinicopathological characteristic (6), and it has a worse prognosis than AC of CRC based on previous studies (7, 8).

The extent of bowel resection for CRC is widely debated, especially in RCC (9). Right hemicolectomy (RHC) and partial colectomy (PC) are the most common operation options for RCC (10, 11). The main difference among the surgery options is the range of bowel resection, and all of them would perform adequate lymph node dissection for RCC treatment. PC means colectomy with longitudinal resection margins are within 10 cm beyond the tumor because lymph node metastases are rarely greater than 10 cm (12). RHC means all of right colon, and a portion of transverse was removed. However, most surgeons generally intend to choose RHC instead of PC for the following potential subjective reasons: first, extensive resection would remove more lymph nodes and supply vessels of the tumor; second, extensive resection might provide better survival but similar complications than PC; the third possible reason is that the operative technique of RHC is not difficult to master, and the process of RHC is more easily and widely publicized for surgeons with pride (3, 4, 10, 13).

However, there is no high-level evidence to show that RHC has any specific benefits for the long-term survival of RCC, as well as MAC of RCC. Instead, it might increase the perioperative complications and mortality as well as reduce the quality of life for RCC patients (14). In addition, studies showing that the number of lymph node metastases of MAC is relatively fewer than that of non-MAC (15). Moreover, there are no study or treatment guidelines to specifically recommend surgical options for RCC according to the histopathological subtype. These thought-provoking studies caused the rethinking of the value of RHC for MAC of RCC.

In this study, we performed a retrospective population-based investigation to explore whether RHC is justified for MAC of RCC based on overall survival (OS) and cancer-specific survival (CSS).

METHODS

Data Source

We collected data from the SEER cancer registry, which covers approximately 28% of the United States (US) population. SEER is an open and reliable database that provides demographic, epidemiological, tumor location and size and survival data. We required cases from 18 SEER registries in the anonymous data and obtained permission to download the data from the SEER database, which did not require informed patient consent.

Patient Selection

We accessed the SEER database by the SEER software (SEER*Stat 8.3.6), and patients who were diagnosed with RCC from 2010 to 2015 were enrolled (**Figure 1**). The study included RCC patients according to the following criteria: 1) treatment with surgical resection, surgical types including RHC (code 40) or PC (code 30); 2) the primary tumor sites were categorized as cecum, ascending colon, hepatic flexure, and transverse colon; 3) the patients had positive histology, and the morphology ICD-0–3 codes of MAC were limited to mucinous adenocarcinoma (8,480/3), the control AC group codes were limited to adenocarcinoma NOS (8,140/3); and 4) exact and complete follow-up information was included. The exclusion criteria: the stage, tumor size, carcinoembryonic antigen (CEA), perineural invasion (PNI), tumor differentiation were unknown, and patients who accepted preoperative chemoradiotherapy were also excluded. Furthermore, the other baseline data were extracted for all patients in the SEER database: race, age, sex, tumor location, tumor number, distant metastasis, perioperative mortality, and postoperative chemotherapy.

Statistical Analysis

Descriptive statistics of patient characteristics were summarized, and we compared differences in baseline characteristics between the MAC and AC groups in the RCC patients, as well as between the PC and RHC subgroups in the MAC of RCC patients. Continuous data were compared using the one-way ANOVA test, and categorical variables were compared using the chi-square test. For each patient, the survival outcomes were analyzed: 1) overall survival (OS), which was represented as the time from the date of diagnosis to death from any cause; 2) cancer-specific survival (CSS), which was defined as the time from the date of diagnosis until cancer metastasis or recurrence, cancer-associated death and the end of follow-up. Both OS and CSS were estimated using Kaplan–Meier survival curves, and the log-rank test was used to compare the differences among groups. The prognostic factors associated with OS and CSS were analyzed by univariate and multivariable Cox proportional regression. All statistical analyses were performed with the software package SPSS version 22.0 (SPSS Inc., Chicago, IL, USA), and a P value <0.05 was considered statistically significant.

RESULTS

General Characteristics and Survival of Right-Sided Colon Cancer Patients With Mucinous Adenocarcinoma

The baseline demographic, clinicopathological, and surgery features of RCC patients were analyzed and compared in **Table 1**, including

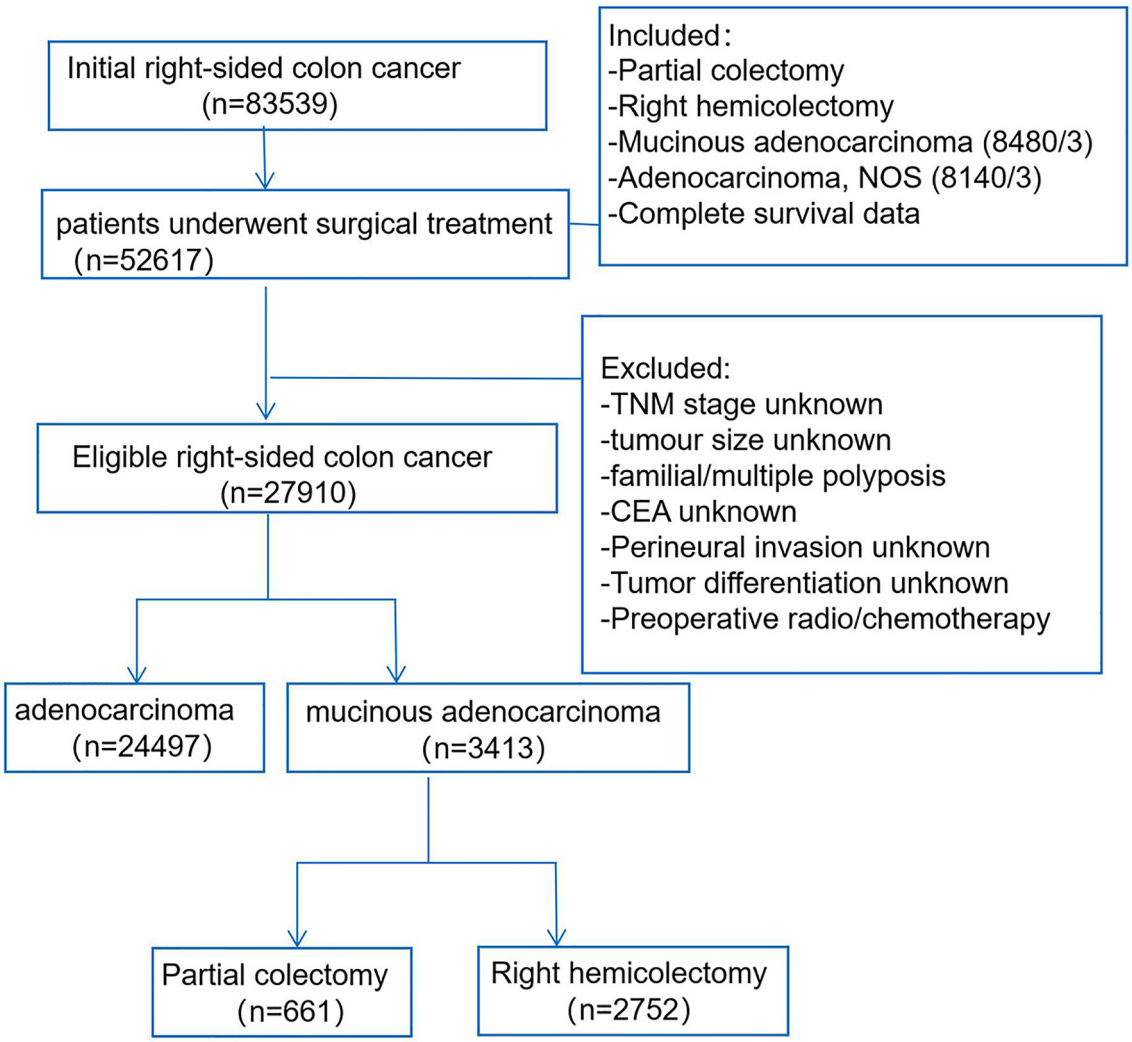

FIGURE 1 | Patient selection flowchart.

3,413 (16.2%) patients with MAC and 24,497 (83.8%) patients with AC. The results showed that the MAC group had a higher proportion of white patients, elevated CEA level, tumor size over 5 cm, tumor location at the cecum, positive lymph nodes, liver metastases, postoperative chemotherapy, and advanced TNM stage than the AC group ($P < 0.05$). However, the surgery type, dissected lymph nodes, perioperative mortality were similar between the two groups (both $P > 0.05$).

Then, the survival between the two groups was also compared using Kaplan–Meier curves. The results showed that OS of the MAC group was poorer than that of the AC group ($P = 0.012$, **Figure 2A**), but the CSS was comparable between the two groups ($P = 0.139$, **Figure 2B**). These results included the general characteristics and long-term survival of MAC of RCC, which indicated that MAC was different from AC.

Patient Characteristics of Right-Sided Colon Cancer With Mucinous Adenocarcinoma According To Surgery Type

Then, we explored the characteristics of RCC with MAC according to surgery type. The results are shown in **Table 2**, including 661 patients who underwent PC and 2,752 patients who underwent RHC. The RHC group of MAC had a higher proportion of age ≤60 years, tumor size >5 cm and tumor location at cecum/ascending colon than the PC group ($P < 0.05$, respectively). There were no significant differences for race, sex, CEA, PNI, tumor differentiation, stage, or postoperative chemotherapy ($P > 0.05$, respectively). More interestingly, the number of dissected lymph nodes in the RHC group was more than that in the PC group ($P < 0.001$), but the number of positive lymph nodes between the two groups was not significantly different ($P = 0.130$). What's more, the perioperative

TABLE 1 | The baseline demographic, clinicopathological and surgery features of mucinous adenocarcinoma (MAC) and non-specific adenocarcinoma (AC) of right colon cancer (RCC) patients.

Variables	MAC (3,413)	AC (24,497)	P value
Race			
White	2,839(83.2%)	19,457(79.4%)	
Black	356(10.4%)	3,054(12.5%)	
Others	218(6.4%)	1,986(8.1%)	<0.001
Age (years)			
≤60	795(23.3%)	5572(22.7%)	
>60	2618(76.7%)	18,925(77.3%)	0.475
Sex			
Female	1,869(54.8%)	13,050(53.3%)	
Male	1,544(45.2%)	11,447(46.7%)	0.102
Surgery type			
RHC	2,752(80.6%)	19,583(79.9%)	
PC	661(19.4%)	4,914(20.1%)	0.343
CEA			
Normal	1,773(50.7%)	14,186(57.9%)	
Elevated	1,640(48.1%)	10,311(42.1%)	<0.001
PNI			
Absent	3,100(90.8%)	21,398(87.3%)	
Present	313(9.2%)	3,099(12.7%)	<0.001
Size (cm)			
≤5	1,485(43.5%)	14,776(60.3%)	
>5	1,928(56.5%)	9,721(39.7%)	<0.001
Tumor number			
Solitary	2,396(70.2%)	17,583(71.8%)	
Multiple	1,017(29.8%)	6,914(28.2%)	0.056
Location			
Cecum	1,417(41.5%)	9,373(38.3%)	
Ascending Colon	1,185(34.7%)	8,688(35.5%)	
Hepatic Flexure	274(8.0%)	2,068(8.4%)	
Transverse Colon	537(15.7%)	4,368(17.8%)	0.001
Differentiation			
Grade I/II	2,603(76.3%)	18,372(75.0%)	
Grade III/IV	810(23.7%)	6,125(25.0%)	0.108
Stage (TNM 7ed)			
I	381(11.2%)	3,954(16.1%)	
II	1,364(40.0%)	8,961(36.6%)	
III	1,185(34.7%)	8,005(32.7%)	
IV	483(14.2%)	3,577(14.6%)	<0.001
Bone metastases			
No	3,386(99.2%)	24,324(99.3%)	
Yes	8(0.2%)	73(0.3%)	
Unknown	19(0.6%)	100(0.4%)	0.374
Brain metastases			
No	3,396(99.5%)	24,372(99.5%)	
Yes	1(0.0%)	16(0.1%)	
Unknown	16(0.5%)	109(0.4%)	0.713
Liver metastases			
No	3,134(91.8%)	21,839(89.1%)	
Yes	273(8.0%)	2,593 (10.6%)	
Unknown	6(0.2%)	65 (0.3%)	<0.001
Lung metastases			
No	3,338(97.8%)	23,841(97.3%)	
Yes	57(1.7%)	539(2.2%)	
Unknown	18(0.5%)	117(0.5%)	0.124
Perioperative mortality			
Yes	82(2.4%)	680(2.8%)	
No	3,331(97.6%)	23,817(97.2%)	0.210
Postoperative chemotherapy			
Yes	1,278(37.4%)	8,523(34.8%)	
No/Unknown	2,135(62.6%)	15,974(65.2%)	0.002
Dissected lymph nodes	21.08 ± 10.120	20.72 ± 9.996	0.059
Positive lymph nodes	2.41 ± 4.690	2.01 ± 3.923	<0.001

CEA, carcinoembryonic antigen; PNI, perineural invasion; TNM, tumor-node-metastasis; RHC, right hemicolectomy; PC, partial colectomy.

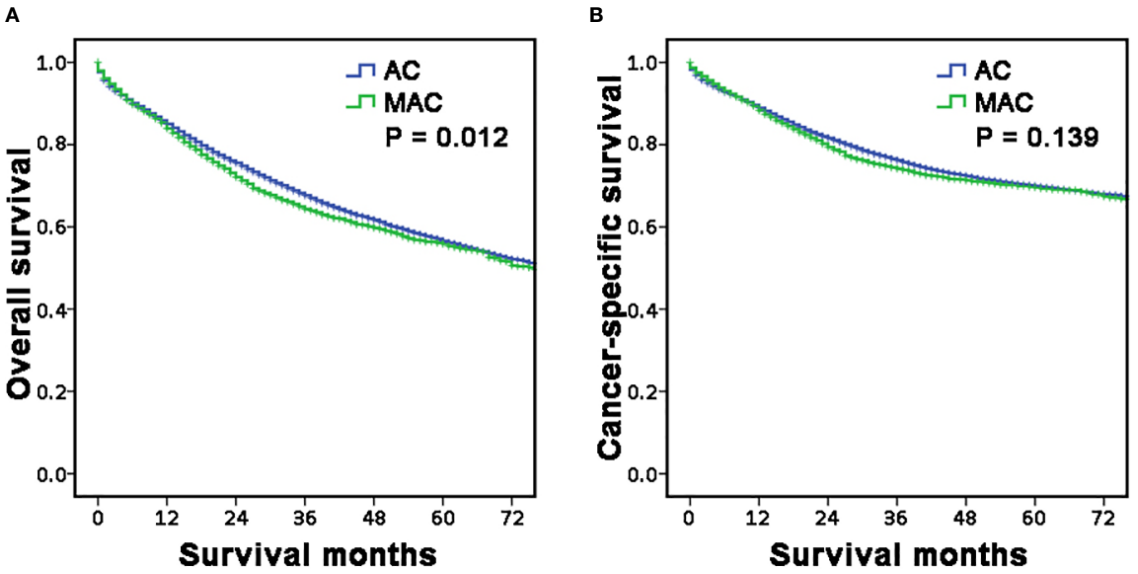

FIGURE 2 | Long-term survival of RCC according to histopathology type. **(A, B)** The survival curves showed that the MAC of RCC group had worse OS **(A)** but similar CSS **(B)** with the AC of RCC group. RCC, right-sided colon carcinoma; OS, overall survival; CSS, cancer-specific survival; MAC, mucinous adenocarcinoma; AC, non-specific adenocarcinoma.

mortality is similar between the two groups (P = 0.272). These results showed the different characteristics of MAC patients who underwent PC or RHC, which indicated the surgeon selection preferences and no benefit of positive lymph node removal.

Risk Factors for Long-Term Survival of of Right-Sided Colon Cancer With Mucinous Adenocarcinoma

Next, the risk factors for survival of RCC with MAC who accept PC or RHC were analyzed by univariate or multivariable analyses (**Tables 3**, **4**). The results showed that age, CEA level, PNI, tumor size, tumor number, differentiation, and TNM stage were significant prognostic factors for OS in MAC of RCC in univariate analyses (P < 0.05). The association remained significant in multivariable analyses that excluded tumor size (P = 0.863), but included postoperative chemotherapy (P < 0.001). However, tumor location and surgery type were not significant for OS (both P > 0.05, **Table 3**).

The analyses for CSS showed age, CEA level, PNI, tumor size, differentiation, TNM stage, and postoperative chemotherapy were significant prognostic factors in univariate analyses (P < 0.05, respectively); the multivariable analyses excluded tumor size (P = 0.076). The tumor location and surgery type were neither significant for CSS (both P > 0.05, **Table 4**).

These analyses indicated age, CEA level, PNI, differentiation, and TNM stage were independent prognostic risk factors for both OS and CSS of RCC with MAC. However, RHC surgery type was not a superior prognostic risk factor for OS and CSS compared with PC.

Long-Term Survival of of Right-Sided Colon Cancer With Mucinous Adenocarcinoma According to Surgery Type

After we concluded the different characteristics and risk factors of RCC with MAC, we intended to explore the long-term survival of the MAC group and the subgroup based on surgery type. First, the OS and CSS of all MAC and AC of RCC patients were analyzed according to surgery type (**Figures 3A–D**). The results showed that OS (P = 0.285, **Figure 3A**) and CSS (P = 0.682, **Figure 3B**) of the MAC group were both comparable with those of the AC group when stratified by PC. However, the OS of the MAC group in RHC sub-hierarchy was worse than that of the AC group (P = 0.023, **Figure 3C**), but the CSS was similar between the two group (P = 0.153, **Figure 3D**).

Then, the OS and CSS of RCC with MAC patients were analyzed according to surgery type. The results showed that there was no significant difference in OS (P = 0.597, **Figure 3E**) and CSS (P = 0.405, **Figure 3F**) between the PC and RHC groups. These results indicated that histological subtype was the decisive factor for OS of RCC, especially in RHC sub-hierarchy.

Additional Sub-Hierarchy Analyses for Long-Term Survival of Right-Sided Colon Cancer

Because the multivariable analyses indicated age, CEA level, PNI, differentiation, and TNM stage were independent prognostic risk factors for both OS and CSS of RCC with MAC. Then we further compared the survival between PC and RHC groups stratified by

TABLE 2 | The demographic and clinicopathological features of MAC of RCC patients according to surgery type.

Variables	PC (661)	RHC (2752)	P value
Race			
White	550(83.2%)	2,289(83.2%)	
Black	70(10.6%)	286(10.4%)	
Others	41(6.2%)	177(6.4%)	0.969
Age (60 years)			
≤ 60	131(19.8%)	664(24.1%)	
>60	530(80.2%)	2,088(75.9%)	0.019
Sex			
Female	374(56.6%)	1,495(54.3%)	
Male	287(43.4%)	1,257(45.7%)	0.295
CEA			
Normal	361(54.6%)	1,412(51.3%)	
Elevated	300(45.4%)	1,340(48.7%)	0.127
PNI			
Absent	600(90.8%)	2,500(90.8%)	
Present	61(9.2%)	252(9.2%)	0.954
Size (cm)			
≤5	325(49.2%)	1,160(42.2%)	
>5	336(50.8%)	1,592(57.8%)	0.001
Tumor number			
Solitary	453(68.5%)	1943(70.6%)	
Multiple	208(31.5%)	809(29.4%)	0.296
Location			
Cecum	231(34.9%)	1,186(43.1%)	
Ascending Colon	172(26.0%)	1,013(36.8%)	
Hepatic Flexure	40(6.1%)	234(8.5%)	
Transverse Colon	218(33.0%)	319(11.6%)	<0.001
Differentiation			
Grade I/Grade II	502(75.9%)	2,101(76.3%)	
Grade III/Grade IV	159(24.1%)	651(23.7%)	0.829
Stage(TNM 7ed)			
I	90(13.6%)	291(10.6%)	
II	268(40.5%)	1,096(39.8%)	
III	212(32.1%)	973(35.4%)	
IV	91(13.8%)	392(14.2%)	0.101
Perioperative mortality			
Yes	12(1.8%)	70(2.5%)	
No	649(98.2%)	2,682(97.5%)	0.272
Postoperative chemotherapy			
Yes	228(34.5%)	1,050(38.2%)	
No/Unknown	433(65.5%)	1,702(61.8%)	0.081
Dissected lymph nodes	18.96 ± 9.477	21.58 ± 10.206	<0.001
Positive lymph nodes	2.160 ± 4.479	2.47 ± 4.737	0.130

these risk factors. Results showed that the PC group had a similar OS (P > 0.05, respectively, **Figures 4A–L**)and CSS (P > 0.05, respectively, **Supplementary Figures A–L**) with RHC group, no matter which risk factors were sub-hierarchically analyzed.

DISCUSSION

Surgical resection plays a fundamental role in treating RCC, of which PC and RHC are the most common options (5). However, surgical decision-making for RCC is still controversial, especially in the range of bowel resection (16). Today, many surgeons tend to select RHC for many reasons; the predominant causes are oncology concerns but ignorance of bowel preservation. Moreover, the surgical decision is not specified clearly enough in existing guidelines, especially in the context of histopathology classification. MAC is a specific but not rare histopathological subtype of CRC that has unique demographic and clinicopathological features and potential poor survival according to previous studies as well as this study (17). However, there are still no treatment guidelines or published studies to guide the management of MAC of RCC (6, 10).

Although MAC of RCC has relatively poor survival, surgery is still the key treatment method, but the selection strategy of surgery type is rarely known (10, 18). Interestingly, we found MAC of RCC accepted RHC always had younger age, larger tumor size and cecum or ascending colon location in this study, which indicated a selection tendency of decreasing operating difficulty and increasing patient safety for surgeons but not based on oncology status. In other words, these differences came from the inherent characteristics of surgery types, but didn't reflect the survival advantage. These findings were also supported by some

TABLE 3 | Univariate and multivariable analysis of factors associated with overall survival of MAC of RCC.

Variable	Univariate		Multivariable	
	RR(95%CI)	*P*	RR(95%CI)	*P*
Race				
White	1	0.215		
Black	0.970(0.809–1.164)			
Others	0.800(0.623–1.028)			
Age (years)				
≤60	1	<0.001	1	<0.001
>60	1.672(1.443–1.938)		1.783(1.525–2.084)	
Sex				
Female	1	0.707		
Male	0.979(0.876–1.094)			
CEA				
Normal	1	<0.001	1	<0.001
Elevated	1.927(1.722–2.157)		1.467(1.300–1.656)	
PNI				
Absent	1	<0.001		0.001
Present	2.034(1.734–2.386)		1.312(1.110–1.551)	
Size (cm)				
≤5	1	0.003	1	0.863
>5	1.183(1.058–1.324)		1.010(0.897–1.138)	
Tumor number				
Solitary	1	<0.001	1	<0.001
Multiple	1.237(1.101–1.388)		1.246(1.107–1.403)	
Location				
Cecum	1	0.779		
Ascending	1.012(0.892–1.148)			
Hepatic Flexure	0.903(0.726–1.123)			
Transverse Colon	1.014(0.862–1.193)			
Differentiation				
Grade I/Grade II	1	<0.001	1	0.011
Grade III/Grade IV	1.492(1.323–1.683)		1.175(1.038–1.330)	
Stage(TNM 7ed)				
I	1	<0.001	1	<0.001
II	1.127(0.890–1.428)		1.091(0.855–1.392)	
III	1.981(1.573–2.495)		2.872(2.241–3.681)	
IV	6.599(5.214–8.352)		9.854(7.526–12.902)	
Surgery type				
PC	1	0.599		
RHC	1.039(0.902–1.197)			
Postoperative chemotherapy				
No/Unknown	1	0.073	1	<0.001
Yes	1.108(0.990–1.240)		0.480(0.419–0.551)	

former studies (19, 20). These findings suggested more evidence-based medicine studies were needed for surgery selection guidance in MAC of RCC.

According to this study, we also concluded that patients could obtain similar OS and CSS from PC or RHC in MAC of RCC. This finding was consistent with a few previous studies with relatively small sample size, in which extensive operation would not be beneficial for CRC patients, especially in RCC (19, 21). We further confirmed that PC could achieve similar long-term survival as RHC, regardless of stratification analysis of the demographic and clinicopathological factors in RCC of MAC. This conclusion overturned the traditional concept that RHC would provide better survival benefits than PC for RCC and also provided the first evidence that PC would be a non-inferiority selection for MAC of RCC. There are some possible explanations for this in the following. First, PC could provide an effective and appropriate extent of colectomy which is consistent with the criteria in the existing guidelines, so extensive colectomy, such as RHC, maybe unnecessary (22). Second, some surgeons suggest the purpose of RHC is to dissect more lymph nodes, but several studies have found that MAC patients had a lower lymph nodes' metastatic rate than AC patients, thus RHC might not be necessary (23). Third, previous studies suggested that PC had the advantages of a smaller resection range and lower operation stress than RHC, which could potentially provide survival benefit (24). These possible reasons indicated that surgeons have to make the appropriate choice in surgical option decision-making and should not be too eager to perform the extensive operation.

In fact, the extent of positive lymph node dissection is the key to obtaining favorable long-term survival in CRC (2, 8–11, 25). Some research claimed that RHC could dissect more lymph nodes than PC (26, 27). This study showed that the number of

TABLE 4 | Univariate and multivariable analysis of factors associated with cancer-specific survival of MAC of RCC.

Variable	Univariate		Multivariate	
	RR(95%CI)	*P*	RR(95%CI)	*P*
Race				
White	1	0.201		
Black	1.154(0.935–1.425)			
Others	0.846(0.624–1.148)			
Age (years)				
≤ 60	1	0.010	1	<0.001
>60	1.244(1.053–1.470)		1.603(1.342–1.916)	
Sex				
Female	1	0.415		
Male	0.944(0.823–1.084)			
CEA				
Normal	1	<0.001	1	<0.001
Elevated	2.291(1.986–2.643)		1.428(1.225–1.664)	
PNI				
Absent	1	<0.001	1	<0.001
Present	2.864(2.404–3.412)		1.436(1.196–1.726)	
Size (cm)				
≤5	1	<0.001	1	0.076
>5	1.428(1.239–1.645)		1.143(0.986–1.326)	
Tumor number				
Solitary	1	0.454		
Multiple	0.944(0.812–1.098)			
Location				
Cecum	1	0.269		
Ascending	0.875(0.747–1.025)			
Hepatic Flexure	0.844(0.644–1.107)			
Transverse Colon	0.999(0.820–1.217)			
Differentiation				
Grade I/Grade II	1	<0.001	1	0.001
Grade III/Grade IV	1.870(1.620–2.158)		1.288(1.112–1.493)	
Stage(TNM 7ed)				
I	1	<0.001	1	<0.001
II	2.239(1.331–3.765)		2.002(1.183–3.385)	
III	7.720(4.674–12.751)		9.293(5.553–15.554)	
IV	31.162(18.860–51.489)		36.099(21.298–61.187)	
Surgery type				
PC	1	0.407		
RHC	1.078(0.903–1.287)			
Postoperative chemotherapy				
No/Unknown	1	<0.001	1	<0.001
Yes	0.534(0.466–0.612)		0.571(0.488–0.669)	

dissected lymph nodes in RHC was indeed more than that in PC, but the dissected positive lymph nodes were comparable in the PC and RHC groups. There are also several small sample studies that came to similar conclusions (19, 28). The probable causes of the similar rate of positive lymph node dissection in PC and RHC were as follows: first, the present surgical technique allows most patients to be dissected with sufficient lymph nodes, even though patients undergo relatively less bowel resection range. Second, the lymph node metastases are mainly located in the D1 and D2 groups, which are always along the mesentery and no more than 5 to 10 cm from the primary tumor (29). In addition, D3 lymph node dissection mainly focuses on the central vascular ligation and central lymph node dissection, but does not involve excess resection of the bowel (30). These results further suggest that PC is still an alternative treatment for positive lymph node dissection in patients with MAC of RCC.

There are also several limitations to the present study. First, this is a retrospective study of public databases, which limits the data source due to a lack of homogeneity. Second, due to limitations of the SEER database, we were unable to assess some information such as vascular-lymphatic invasion, postoperative complications, as well as hospital stay time; with these data we could obtain more information, for instance, the operation stress. Lacking support of large multicenter prospective randomized controlled trials is another weakness of the research.

In conclusion, this large population-based study provides a new perspective in the treatment of patients with MAC of RCC and finds that RHC could not dissect more positive lymph nodes, or provide any long-term survival benefit. Moreover, this study could provide some evidence for an update of guidelines for MAC of RCC, which have important clinical value in individual management of colon carcinoma patients.

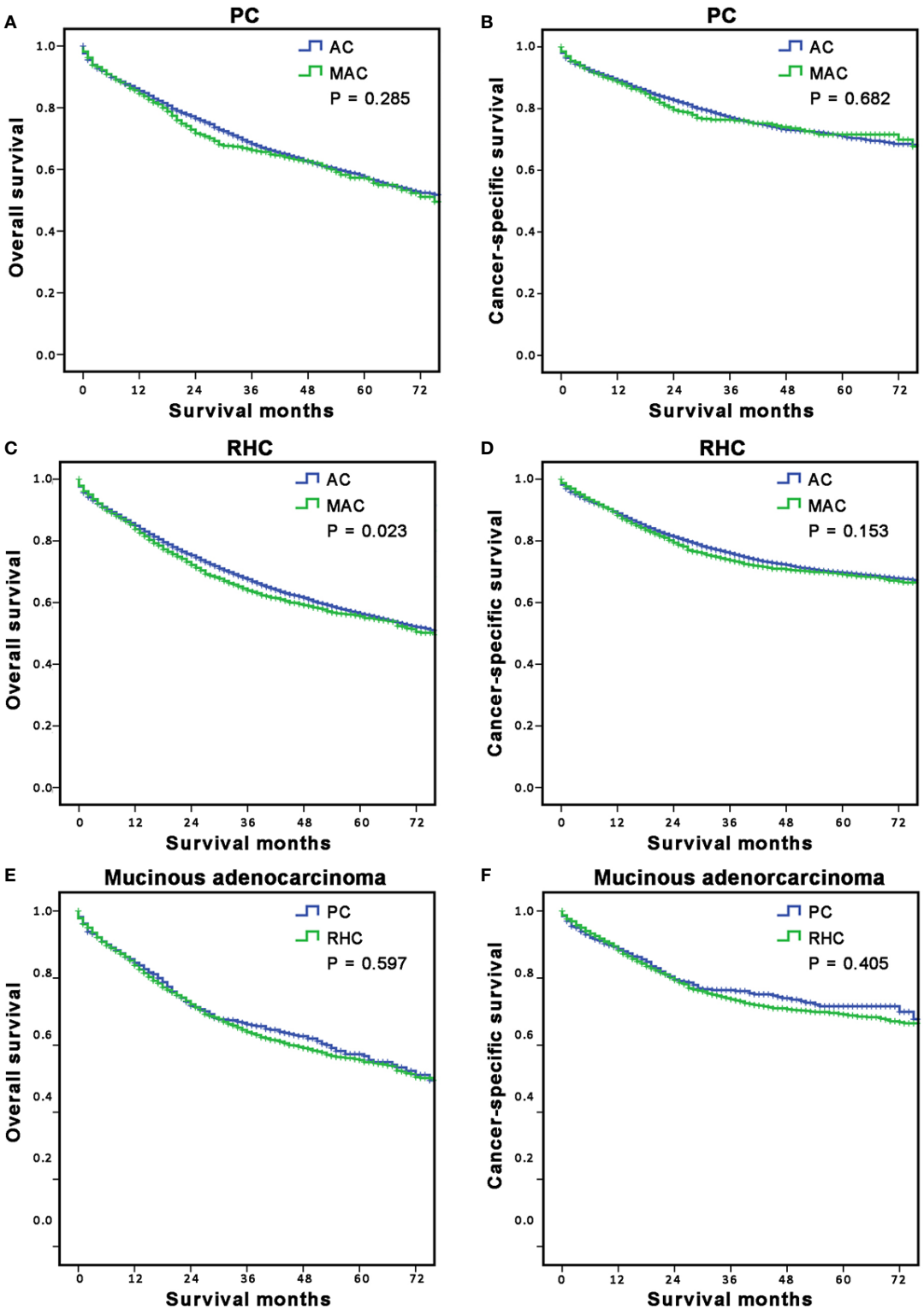

FIGURE 3 | Long-term survival of MAC of RCC according to surgery options. **(A, B)** The stratified analysis survival curves showed that the MAC of RCC group who underwent PC had comparable OS **(A)** and CSS **(B)** with the AC of RCC group; **(C, D)** The stratified analysis survival curves showed that the MAC of RCC group who underwent the RHC had worse OS **(C)** but similar CSS **(D)** with the AC of RCC group. **(E, F)** The survival curves showed that the MAC of RCC patients in the RHC group had similar OS **(E)** and CSS **(F)** as the PC group. PC, partial colectomy; RHC, right hemicolectomy.

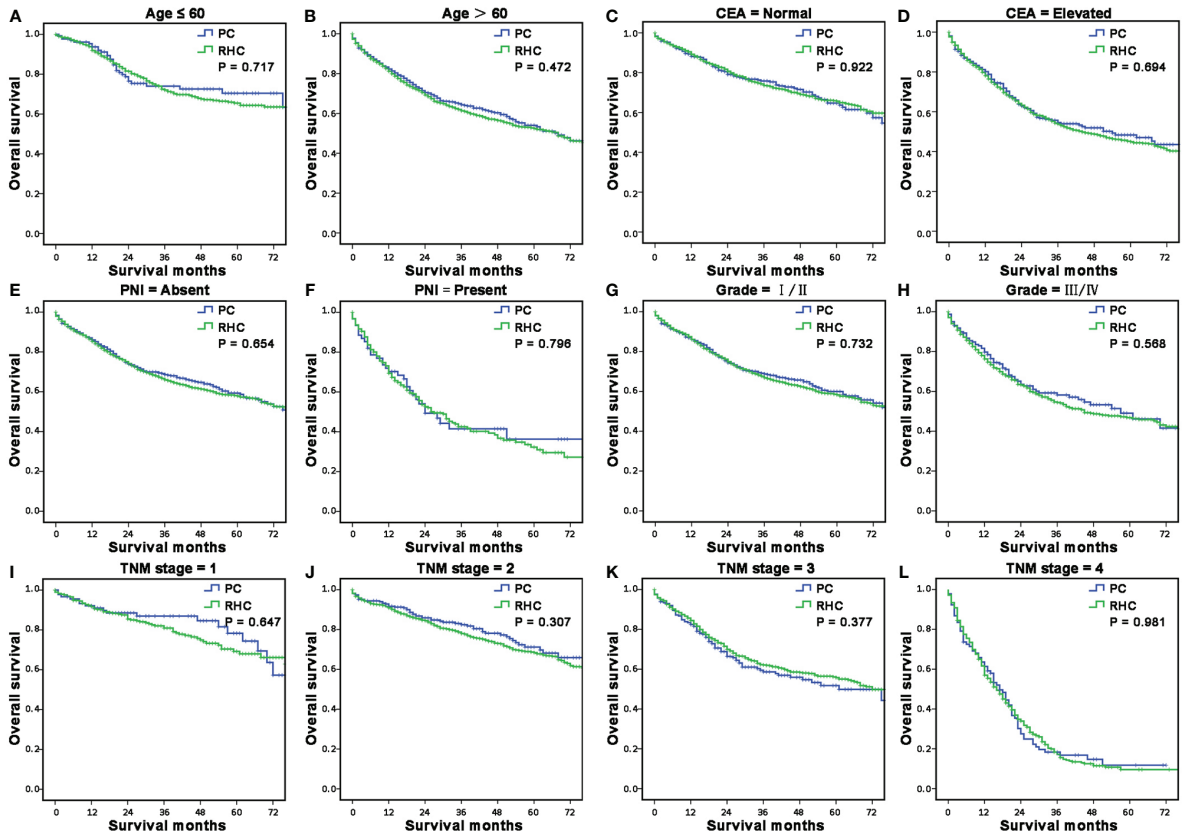

FIGURE 4 | The sub-hierarchy analyses for long-term survival of MAC of RCC according to independent risk factors. **(A, B)** The survival curves showed that the MAC of RCC patients in the RHC group had similar OS and CSS as the PC group when sub-stratified by age **(A, B)**, CEA level **(C, D)**, PNI **(E, F)**, differentiation **(G, H)** and TNM stage **(I–L)**.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov/).

ETHICS STATEMENT

Institutional review board approval and written informed consent are not required for publicly available data, and none of the potentially identifiable images or data is used in this article.

AUTHOR CONTRIBUTIONS

JH, KF, XP, and SX conceptualized the study, conducted the formal analysis, acquired funding, provided the software, and wrote, reviewed, and edited the manuscript. JH, QH, RT, GC, YZ, RH, XZ, KF, and SX performed the data curation, methodology, project administration. SX, KF, and XP: the manuscript revision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.608836/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Artificial Intelligence in Decision-Making for Colorectal Cancer Treatment Strategy: An Observational Study of Implementing Watson for Oncology in a 250-Case Cohort

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Background: Personalized and novel evidence-based clinical treatment strategy consulting for colorectal cancer has been available through various artificial intelligence (AI) supporting systems such as Watson for Oncology (WFO) from IBM. However, the potential effects of this supporting tool in cancer care have not been thoroughly explored in real-world studies. This research aims to investigate the concordance between treatment recommendations for colorectal cancer patients made by WFO and a multidisciplinary team (MDT) at a major comprehensive gastrointestinal cancer center.

Methods: In this prospective study, both WFO and the blinded MDT's treatment recommendations were provided concurrently for enrolled colorectal cancers of stages II to IV between March 2017 and January 2018 at Shanghai Minimally Invasive Surgery Center. Concordance was achieved if the cancer team's decisions were listed in the "recommended" or "for consideration" classification in WFO. A review was carried out after 100 cases for all non-concordant patients to explain the inconsistency, and corresponding feedback was given to WFO's database. The concordance of the subsequent cases was analyzed to evaluate both the performance and learning ability of WFO.

Results: Overall, 250 patients met the inclusion criteria and were recruited in the study. Eighty-one were diagnosed with colon cancer and 189 with rectal cancer. The concordances for colon cancer, rectal cancer, or overall were all 91%. The overall rates were 83, 94, and 88% in subgroups of stages II, III, and IV. When categorized by treatment

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strategy, concordances were 97, 93, 89, 87, and 100% for neoadjuvant, surgery, adjuvant, first line, and second line treatment groups, respectively. After analyzing the main factors causing discordance, relative updates were made in the database accordingly, which led to the concordance curve rising in most groups compared with the initial rates.

Conclusion: Clinical recommendations made by WFO and the cancer team were highly matched for colorectal cancer. Patient age, cancer stage, and the consideration of previous therapy details had a significant influence on concordance. Addressing these perspectives will facilitate the use of the cancer decision-support systems to help oncologists achieve the promise of precision medicine.

Keywords: Watson for Oncology, artificial intelligence, colorectal cancer, multidisciplinary team, concordance analysis

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in both men and women worldwide (1). Its incidence and mortality rates have been increasing in China for several decades (2). The rapid expansion of clinical databases and massive genetic profiling programs has raised tremendous challenges for oncologists where there is insufficient time for tracking the treatment-related information (3).

Clinical decision-support systems that have emerged in the early days, called expert systems (4), are computer programs that help clinicians manage the comprehensive demands of relevant information developments. These systems collect and analyze knowledge in ways that allow algorithms to simulate human reasoning to assist decision-making. AI systems in cancer care have generally focused on obtaining information from unstructured data such as text (using natural language processing) or large structured datasets (using machine-learning methods) (5). However, a cognitive-support computer program for cancer treatment has, as far as we know, not emerged until the development of IBM's Watson for Oncology (WFO).

Despite substantial computer science and clinical expertise, mainly from Memorial-Sloan-Kettering Cancer Centre (MSKCC), guided the development of IBM WFO, which holds promise for improving the value of cancer care delivery, the prospects for its use in patients outside the US have not been examined clearly. According to the reports from oncologists in China and other countries, concordance of treatment decisions made by physicians and WFO varies depending on cancer type, where outcomes in terms of breast cancer (5), lung cancer (6), and gastric cancer (7) were likely to be highly concordant, the results in other studies (8, 9) were not.

Hence, we carried out this prospective study to assess the level of agreement regarding colorectal cancer treatment between WFO and a multidisciplinary cancer team in a major comprehensive gastrointestinal cancer center in Shanghai, China. We report the results of decision concordance using the AI system and performed an in-depth analysis on patients where concordance was absent to update the AI model and discuss the potential value of the technology as a clinical adviser and a learning system in cancer treatment.

PATIENTS AND METHODS

Study Design

This is a prospective, double-blind, and self-controlled trial to evaluate the clinical conformance between WFO and the multidisciplinary team of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (henceforward the RJ MDT) in patients undergoing colorectal cancer therapy in the gastrointestinal center. The clinic information of patients was entered into WFO with patients' consent, and the results were compared with those of actual clinical treatment plans made by the RJ MDT (**Figure 1**). This study was approved by the ethics committee of Ruijin Hospital.

Patients

Patients admitted to Ruijin Hospital between March 2017 and January 2018 were eligible for the trial if they were aged between 18 years and 90 years and were diagnosed with colorectal cancer proven by colonoscopy biopsy. All patients provided informed written consent and were advised of their extensive rights to know about related information of the study.

Inclusion Criteria

1. Age between 18 and 90 years.
2. Diagnosis of colorectal adenocarcinoma proven by colonoscopic biopsy.
3. Clinical or radiological evidence of Stage II (T3-4, N0, M0), Stage III (T1-4, N1-2, M0), or Stage IV (T1-4, N0-2, M1) disease [according to the Eighth Edition Cancer Staging Manual of the American Joint Committee for Cancer (10)].
4. Could provide all the tumor-related information required by WFO.
5. Patients with recurrence or metastasis should provide the time of recurrence at least.
6. Have signed the informed consent.

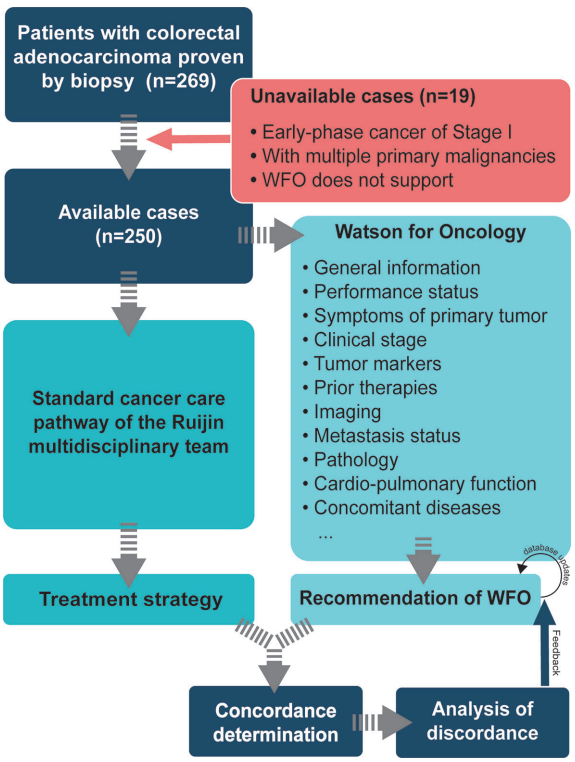

FIGURE 1 | Flow diagram of the study.

Exclusion Criteria

1. Multiple primary tumors.
2. Pregnancy.
3. Cases not supported by WFO.
4. Refusal to accept standardized therapy according to the guidelines.

Procedure

Strategy Determined by the RJ MDT Team

The clinical information of patients was analyzed by the RJ MDT, which includes multiple experts from the Departments of General Surgery (including experts specialized in gastrointestinal, hepatobiliary, and pancreatic surgery), Oncology, Radiology, Radiation Oncology, Intervention and Radiotherapy, and Pathology. The treatment plan for each patient was decided according to the guidelines of The National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO), and the Chinese Society of Clinical Oncology (CSCO). Clinical-experience-based treatment suggestion was given when the guidelines recommended various strategies. None of the clinical decisions by RJ MDT was influenced by WFO's recommendations.

Decision Made by WFO

Watson for Oncology (IBM Corporation, USA, version 17.3-17.11) used in our study was provided by Hangzhou Cognitive Network Technology Co., Ltd. (Hangzhou CognitiveCare). The database was updated every 1–2 months by the training team at MSKCC. The patients' clinical information entered into WFO included general information, performance status, tumor-related symptoms, clinical stage, laboratory examination, prior treatment, imaging, metastasis status, pathology, and other essential data. It generates patient-specific treatment recommendations in three categories: "Recommended" is strongly evidence-supported, "For consideration" is a potentially suitable evidence-based alternative considered by oncologists based on their clinical judgment, and "Not recommended" is treatment with contraindications or strong evidence against its use. If the recommendation involves drug treatment, WFO will mention the therapeutic dose and treatment mode, as well as adverse reactions, risks, and treatment measures for the adverse reactions. If WFO cannot give an accurate judgment, it could recommend global ongoing clinical trials suitable for this case.

Comparison

WFO and the doctors in charge of running the system were blinded to the treatment strategies that had been made by the RJ MDT. Concordance was assessed based on how the MDT's therapy strategy was categorized in WFO's recommendation list. If MDT's decision matched the "recommended" or "for consideration" categories, it was designated as concordant. If the decision was either in the "not recommended" table or not listed, the case was defined as non-concordant.

Statistics and Analysis

Descriptive statistics of patients' characteristics were presented using Microsoft Excel. Concordance was presented as percent agreement. Overall survival (OS) was calculated by the Kaplan-Meier method; the difference between survival curves was determined by the log-rank test. The difference was considered statistically significant when P value was less than 0.05. All analyses were conducted with IBM SPSS 22.0 for macOS (IBM, Chicago, USA).

RESULTS

Characteristics of Colorectal Cancer Cases

A majority of enrolled colorectal cancer patients were younger than 75 years old (217/250, 86.80%), while there were 14 (17.28%) and 19 (11.25%) patients over the age of 75 years in the colon and rectal cancer groups, respectively. Overall, 69.2% (173/250) were males, and 30.8% (77/250) were females (**Table 1**). In colon and rectal, phase III cases accounted for 48.15% (35/81) and 68.05% (115/169), respectively, while phase IV ranked second and phased II ranked last (**Table 1**). Categorized by final treatment strategy, adjuvant therapy was the most often implemented recommendation in both groups (40.74% in colon cancer and 39.05% in rectal cancer; **Table 1**), followed by surgery (33.33 and 18.93%, respectively; **Table 1**) and first-line treatment (22.22 and 20.71%, respectively;

TABLE 1 | Baseline characteristics of patients enrolled.

Characteristics	Cases, n (%)		
	Colon cancer	Rectal cancer	Total
	(n = 81)	(n = 169)	(n = 250)
Gender			
Male	54 (66.67)	119 (70.41)	173 (69.20)
Female	27 (33.33)	50 (29.59)	77 (30.80)
Age			
<75 years	67(82.72)	150 (88.76)	217 (86.80)
≥75 years	14(17.28)	19 (11.25)	33 (13.20)
Stage			
II	18 (22.22)	18 (10.65)	36 (14.40)
III	39 (48.15)	115 (68.05)	154 (61.60)
IV	24 (29.63)	36 (21.30)	60 (24.0)
Treatment strategy			
Adjuvant	33 (40.74)	66 (39.05)	99 (39.60)
Surgery	27 (33.33)	32 (18.93)	59 (23.60)
Neoadjuvant	0 (0.00)	30 (17.75)	30 (12.00)
First Line	18 (22.22)	35 (20.71)	53 (21.20)
Second Line	3 (3.70)	6 (3.55)	9 (3.60)

Table 1). There was also a relatively small proportion of cases who underwent neoadjuvant and second-line therapy (**Table 1**).

Concordance of WFO Treatment Recommendations With the RJ MDT's Opinions

Of the 250 patients treated by the RJ MDT experts and WFO in total, the overall concordance was 91% (**Figure 2A**). Subgroups based on the cancer phase showed concordance rates varied by the staging. Overall cases of Stage III exhibited higher concordance (94%) than stages II (83%) and IV cancers (88%; **Figure 2A**), while cases of stage II colon cancer exhibited higher concordance (94%) than stages III (92%) and IV colon cancers (88%; **Figure 2B**). In contrast, stage II rectal cancer cases showed a relatively lower concordance rate (72%) than stages III (94%) and IV rectal cancers (89%; **Figure 2C**).

When exploring the concordance based on different treatment strategies, we noticed that the second-line group had the highest concordance rate of 100% for both cancer types (**Figure 3A**). Furthermore, cases recommended undergoing neoadjuvant therapy and surgery had higher concordance (97, 93%, respectively; **Figure 3A**) than the other two, namely adjuvant and first-line groups. Similar results were seen for colon and rectal cancers, where concordance rates were 96 and 91% in surgery groups of colon cancer (**Figure 3B**) and rectal cancer (**Figure 3C**), respectively. Besides, adjuvant therapy for the two cancers showed a 91% concordance rate in colon cancer and 88% in rectal cancer (**Figures 3B, C**). The decisions and recommendations of second-line treatment displayed largely consistent rates of 100% in both cancers (**Figures 3B, C**).

Besides, we speculated if there was a difference in the situation of patients who had consistent or inconsistent results. Patients in the consistent group compared favorably to the inconsistent group (p = 0.0049), as shown in **Figure 4**. In the inconsistent group, we observed a median overall survival of 29 months, which was not yet available among the consistent group patients (**Figure 4**).

Factors Affect the Concordance and Corresponding Updates of WFO

Continuous training was thought to be fundamental to improve the capability of WFO. In applying WFO, we discussed the main reasons resulting in the discordance, and gave feedback to the platform accordingly. We suggested WFO to avoid adjuvant therapy in patients over 80 years in March 2017 and received positive responses from the supporter (**Table 2**). When treating postoperative high-risk stage II colorectal cancers, we found WFO recommended observing strategy, which was against the CSCO (Chinese Society of Clinical Oncology) guidelines. The reason might be the absence of high-risk factors evaluation in dealing with such cases. In spite of a few unresolved proposals, most problems we reported received feedback of update soon after (**Table 2**).

To evaluate the performance of WFO due to its continuous updating database, we analyzed the concordance rate in every 50 cases grouped by treatment strategy. Noticeable rising curves were found in most subgroups of various therapy strategies. Though the concordance met different levels of declines in the last 50 patients in neoadjuvant, surgery, and adjuvant groups, the overall rates were higher than the time applying earlier versions of WFO (**Figure 5**).

DISCUSSION

The validity and timeliness of clinical guidelines and other therapeutic information an oncologist uses in practice are critical to cancer treatment. With the trends of delegating information-intensive tasks to technologies such as machine learning algorithms, physicians and computer companies are seeking a balance in utilizing evidence-based decision-making support systems in modern clinical practice. While some physicians applied them as a powerful resource, others, especially patients, believed the recommendations they made were already equal to those of the experts. This reflected not only the perspectives and expectations of patients regarding these tools but, more importantly, indicated the concerns of oncologists regarding the validity of the AI-made options. It has been a long time since we introduced such decision-making support systems in real life (4), and the exploration of the most proper model has never ceased.

For such purposes, by examining the concordance between the advice made by WFO, a decision support tool to provide personalized medical recommendations, and an experienced multidisciplinary cancer team, we observed broad agreement and realized the unfulfilled potential of the self-learning machine, as prior studies (11, 12) have suggested. Nevertheless, as we expected, several aspects need to improve. In the early cases, we observed inconsistency in WFO's recommendations with respect to guidelines. As the classical chemotherapy regimen, FOLFIRI was no longer recommended for adjuvant

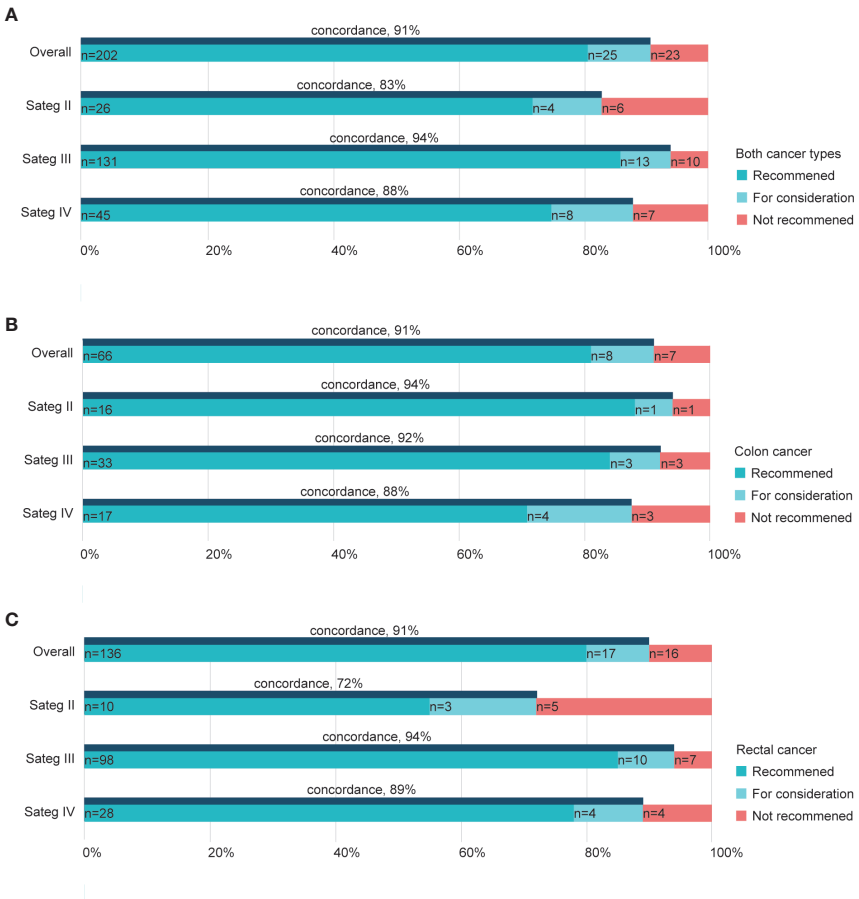

FIGURE 2 | Treatment concordance between WFO and the RJ MDT by stage. Concordance rate in both cancer types **(A)**, colon cancers **(B)**, and rectal cancers **(C)**. RJ MDT, multidisciplinary team of Shanghai Jiao Tong University Medical School affiliated Ruijin Hospital; WFO, Watson for Oncology.

therapy for stage II or III patients unless enrolled in trials in either the Chinese Society of Clinical Oncology (CSCO) or the National Comprehensive Cancer Network (NCCN) guidelines (13), WFO still listed irinotecan in treatment options. This situation was resolved in the later updated version of WFO. Factors resulting in non-concordance could also come from variations in the aggressiveness of treatment approaches in patient subpopulations based on age. We found in our trial that patients over 80, who were not recommended for aggressive strategies such as chemotherapy in our clinical practice, were likely to have discordance where WFO still recommended standard systemic therapy for this subpopulation. However, the health status of the patients at this age should be rigorously evaluated to manage the benefits and risks of chemotherapy.

Our study also demonstrated that inconsistency between WFO and the RJ MDT occurred in 9% of cases, where the main difference was deriving due to the availability of treatments in China that were not included in the oncology advisor.

China has the largest cancer population with a particular cancer spectrum. The different local conditions and customs of national medicine form different therapeutic experiences and considerations. Since WFO was NCCN guidelines-based and MSKCC experience-trained AI, inevitable deviation from therapeutic guidelines arose. We suggest that, in the process of localizing WFO or developing similar prospective products in China or places outside the US, it is necessary to take more diverse patients treated in varying care settings into consideration (14). In terms of the poor survival rate of patients with inconsistent results, the worse and more complex status of disease and older age probably have played a crucial role in causing the difference. But it also indicates a potential possibility that the AI-powered supporting system could be used as a clinical assistant to help make decisions with better outcome.

Despite the endless arguments towards the responsibility in AI-assisted clinical decision-making systems (15), the great potentials of computerized decision support tools have been demonstrated in medical practice, and many modern technologies are expanding into this area. Google has developed a deep learning machine that can detect diabetic retinopathy and diabetic macular edema (16). Microsoft is exploiting new technology for automated analysis of radiological images (17). The current and potential AI

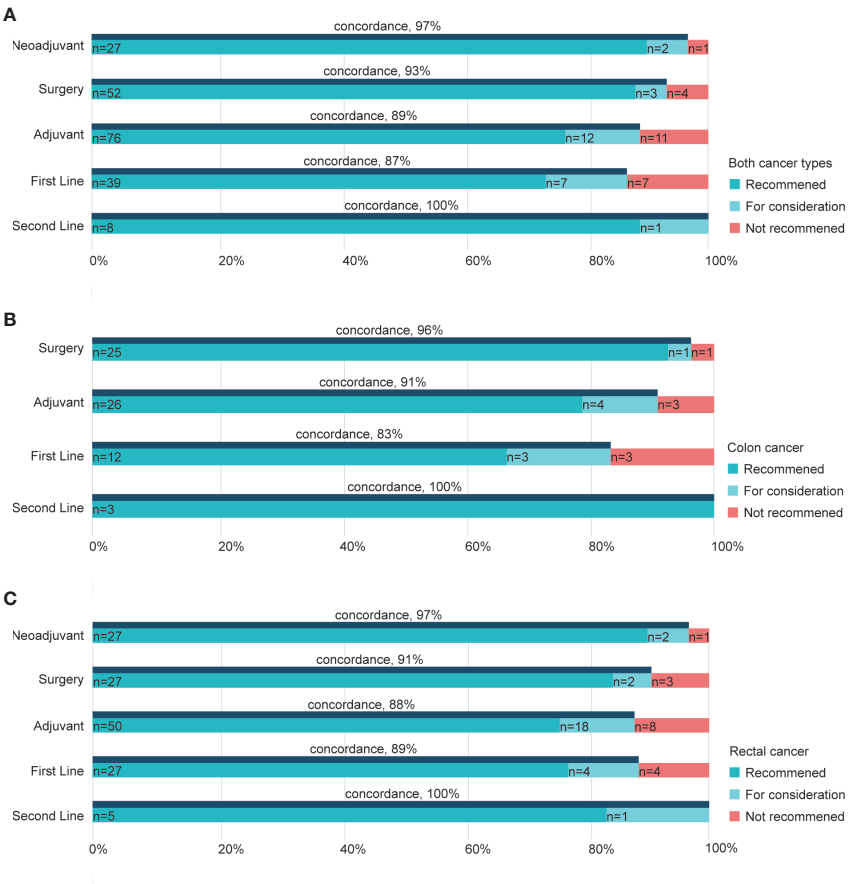

FIGURE 3 | Concordance between WFO and the RJ MDT by treatment strategy. Concordance in subgroups of different strategies in both cancer types **(A)**, colon cancers **(B)**, and rectal cancers **(C)**. RJ MDT, multidisciplinary team of Shanghai Jiao Tong University Medical School affiliated Ruijin Hospital; WFO, Watson for Oncology.

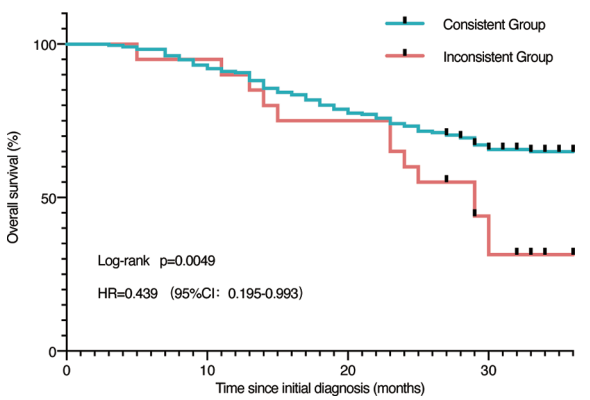

FIGURE 4 | Survival analysis. Survival analysis of all patients grouped by concordance and discordance.

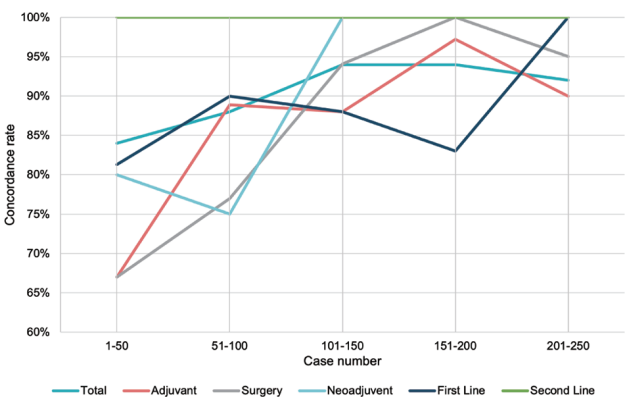

FIGURE 5 | Timeline visualization of the changes in concordance rate. Trend curve of concordance rate in every 50 patients grouped by treatment strategy.

TABLE 2 | Feedback given to WFO and the corresponding updated status.

Feedback time	Suggestion	Updated time
2017.3	Apply adjuvant therapy to patient <80 y	2017.3
2017.5	Genetic analysis should be mandatory	2017.5
2017.5	Stage II: High-Risk Evaluation + MSI test should be added	MSI (2017.9) Risk Evaluation (2018.1)
2017.5	Add previous therapy details	2017.5
2017.4	NCCN/ESMO Guidelines should list as the top priority as well; classic publications recommended	Updated every 3 months
2017.5	Indication for neoadjuvant therapy: CRM/EMVI evaluation	2017.9
2017.5	RFA should be considered as a candidate option for mCRC treatment	IBM feedback pending
2017.8	Evaluation of pCR and NED should be added	IBM feedback pending

MSI, Microsatellite instability; NCCN, National Comprehensive Cancer Network; ESMO, European Society for Medical Oncology; CRM, Circumferential resection margin; EMVI, Extramural vascular invasion; pCR, Pathological complete response; NED, No evidence of disease; RFA, Radio-frequency ablation; mCRC, Metastatic colorectal cancer.

applications cover not only clinical practice, such as diagnosis, robotic surgery, and translational research, such as drug discovery and repurposing, but also several basic biomedical research fields, including gene function annotation and automated experiments (18).

Multi-gene panel testing has been taken into consideration for prognostic cancer staging in conjunction with the American Joint Committee for Cancer (AJCC) staging (10). By combining genomic factors with conventional TNM staging, some anatomically classified groups (such as $T_2N_0M_0$, stage 2A) were down- or upgraded and were determined to be more suitable therapy in clinical practice. Because of the trends towards relying more on molecular characteristics, supplementary decision support might be needed (19). *KRAS*, which was involved in NCCN guidelines for colorectal cancer in 2008 for the first time, has proven to be a key biomarker in applying EGFR-targeted therapies. Though *KRAS* and *BRAF* mutations were considered optional considerations of WFO, the decision it made did not always match standard treatment well. In our study, metastatic rectal cancer cases with *RAS* wt were treated with cetuximab according to NCCN guidelines (Version 17.3), and this was absent in WFO's options. This may be due to the different treatment strategy of Memorial Sloan Kettering Cancer Center, where WFO has been trained.

Additionally, the evolving feature of the clinical value of genetic assays may cause an unprecedented condition in which a given mutation may not lead to actionable events at the time of initial diagnosis but may later become considerable as research progresses become available (20). Therefore, tracking cancer's somatic mutations and reanalyzing them in an updated data pool would seem to be a potential ability of AI-based technology such as WFO to achieve precision medicine.

Patient perspectives are integral for the advanced use of WFO in the clinical workflow. Though modern societies, especially those in China, hold optimistic views of applying cutting edge technology in life, it raises a concern regarding health care, involving both data security and decision precision. Therefore, achieving higher levels of patient acceptance of WFO through systematically upgrade will not only improve oncology practice but contribute to enhance the relationship of cancer patients and physicians as well. Given that WFO is not yet commonly used in practice at the hospital, future studies should exploit their findings with physicians, as well as patients, in using WFO in clinical practice.

There are notable limitations to this study. First, the study design was observational and self-controlled with a relatively small sample size that may cause the results potentially to be susceptible to the bias of unmeasured factors. Patients participated in our study were treated at one comprehensive gastrointestinal cancer center on China's east coast. Adding cases treated in community-based clinics might widen the gap between WFO and clinician responses and lower the concordance but improve the value of computer-aided decision support in minimizing the medical disparities across different regions.

Many who were glad to accept WFO as a resource to provide oncologists with cutting-edge medical research and knowledge believed the ideal model of such tools in clinical practice is to be used as "a tool, not a crutch" (21). By addressing such perspectives, we wish to facilitate the use of WFO and other decision support tools, to help realize the promise of more effective clinical and precision healthcare.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ruijin Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BA: Investigation, methodology, software, writing—original draft, and editing. PX: Resources, data curation, formal analysis, validation, investigation, and writing—original draft. HH: Resources, formal analysis, methodology, and editing. HJ: Resources, formal analysis, methodology, and editing. CW: Resources, software, formal analysis, and editing. SL: Resources and methodology. LH: Resources, formal analysis, and methodology. XD: Resources. HZ: Resources. GC: Resources. AL: Resources. LX: Resources, methodology, and formal analysis. MZ: Conceptualization, data curation, supervision,

acquisition, validation, methodology, writing—original draft, writing—review and editing. HL: Conceptualization, data curation, supervision, acquisition, validation, methodology, writing—original draft, writing—review and editing. JS: Conceptualization, data curation, formal analysis, supervision, acquisition, validation, methodology, investigation, writing—original draft, writing—review and editing. All authors contributed to the article and approved the submitted version.

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The Predictive and Guidance Value of Signet Ring Cell Histology for Stage II/III Colon Cancer Response to Chemotherapy

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Purpose: To evaluate the predictive and guidance value of signet-ring cell carcinoma for chemotherapy response in stage II/III colon cancer.

Methods: Eligible patients were recruited from the Surveillance, Epidemiology and End Results (SEER) database. The differences between adenocarcinoma (AD) and SRCC groups in the incidence of patients' demographic and clinical characteristics were analyzed by Pearson's chi-squared (×2) test. Survival was analyzed using the Kaplan–Meier method, and the differences were determined by the log-rank test. Some Cox regression models were built to assess hazard ratios (HRs) of different variables with 95% confidence intervals (95% CIs).

Results: In stage II AD, it was found that the receipt of chemotherapy had significantly 12.6% decreased risk of cancer-specific mortality (HR = 0.874, 95% CI = 0.825–0.927, P < 0.001). In stage II SRCC, however, the receipt of chemotherapy had significantly 70.00% increased risk of cancer-specific mortality (HR = 1.700, 95% CI = 1.032–2.801, P = 0.037). In stage III AD, it was found that the receipt of chemotherapy had significantly 45.3% decreased risk of cancer-specific mortality (HR = 0.547, 95% CI = 0.530–0.564, P < 0.001). In stage III SRCC, the receipt of chemotherapy had significantly 24.6% decreased risk of cancer-specific mortality (HR = 0.754, 95% CI = 0.632–0.900, P = 0.002).

Conclusions: The cancer-specific survival (CSS) difference between AD and SRCC was not statistically significant in stage II colon cancer. We provided the first compelling evidence that chemotherapy should not be treated in stage II SRCC, while stage III SRCC should be treated with chemotherapy.

Keywords: signet ring cell histology, stage II/III, colon cancer, chemotherapy, survival

INTRODUCTION

Colon cancer is one of the most common malignant tumors in clinical practice and among the leading causes of cancer-related deaths all over the world (1). The conventional adenocarcinoma (AD) characterized by glandular architecture accounts for more than 90% of cases according to the histologic analysis (2). Signet-ring cell carcinoma (SRCC), however, is a rare type of malignant dedifferentiated AD, accounts for only approximately 1% of colorectal cancer, and is defined as the presence of abundant intracellular mucin in more than 50% of its cells (3–7).

Given that SRCC is a rare disease in colon cancer and often not addressed in clinical trials, there is some debate about the prognostic value of this histologic subtype. SRCC had a distinct histologic appearance and underlying biologic behavior and some researchers reported that SRCC had higher pattern of peritoneal and ovarian metastasis and worse prognosis compared with AD (4, 5, 7–10). In addition, it is still unclear whether SRCC would influence clinical decision-making with the aggressive behavior (11–13). Stage II colon cancer with high-risk factors (including T4 status, poorly differentiated histology, vascular invasion, ileus, <12 lymph nodes examined, and neural invasion, as recommended by several treatment guidelines) and stage III cancer are usually treated with adjuvant chemotherapy after the resection of the primary tumor (14, 15).

Therefore, in this population-based study using a large cancer database, we aimed to evaluate the predictive and guidance value of SRCC for colon cancer response to chemotherapy in stage II and stage III colon cancer.

MATERIALS AND METHODS

Study Population

The Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute provided authoritative information on cancer statistics from 18 registries [San Francisco–Oakland, Connecticut, metropolitan Detroit, Hawaii, Iowa, New Mexico, Utah (since 1973), Seattle–Puget Sound (since 1974), metropolitan Atlanta (since 1975), Alaska, San Jose–Monterey, Los Angeles, rural Georgia (since 1992), greater California (excluding San Francisco, Los Angeles, and San Jose), Kentucky, Louisiana, New Jersey, and greater Georgia (excluding Atlanta and rural Georgia, since 2000)], and it covered approximately 28% of the total US population (https://seer.cancer.gov/) (16).

As a retrospective population-based study, the flowchart of the patient selection was shown in **Figure S1**. Using the SEER database through SEER*Stat software V.8.3.5, we then extracted 404189 colorectal cancer patients from the SEER database. However, patients satisfied one of the following conditions were excluded from the cohort: without active follow-up; rectal primary; with unknown race; with T.N.M stages unknown or without radical surgery of the primary tumor. Given our study focused on stage II/III colon cancer, we also excluded patients with distant metastases or stage I disease. Only patients diagnosed with stage II/III colon cancer were included in the present analyses, and all the patients were divided in two groups according to the histology: AD and SRCC groups. The following clinical features were acquired: T stage (T1, T2, T3, and T4), N stage (N0, N1, and N2), age at diagnosis (years), race (white, black, and other), gender (male and female), grade (grade I/II, grade III/IV, and unknown), the receipt of chemotherapy (no/unknown or yes), and histological type (AD and SRCC).

Statistical Analysis

The differences between AD and SRCC groups in the incidence of patients' demographic and clinical characteristics were analyzed by Pearson's chi-squared (×2) test. The primary outcome of the interest in the present study was the cancer-specific survival (CSS), which was calculated from the time of diagnosis to the time of death due to colon cancer. CSS was analyzed using the Kaplan–Meier method, and the differences were determined by the log-rank test.

Some Cox regression models were built to identify whether a pathological characteristic impacted the prognosis independently and assess hazard ratios (HRs) of different variables with 95% confidence intervals (95% CIs). Aimed to evaluate the predictive value of signet ring cell histology for stage II/III colon cancer response to chemotherapy, we then defined an interaction variable (combined by histology and chemotherapy). The common demographic and clinicopathological data, including T stage, N stage, age at diagnosis, race, gender, grade, the receipt of chemotherapy, and histological type were entered as covariates in univariate analyses and only those characteristics with a P value less than 0.20 in the univariable analyses would be considered as candidates for the multivariable analyses. Statistical significance was set as a two-sided P value less than 0.05. All the statistical analyses were performed using SPSS 23.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Demographic and Clinical Characteristics

A total of 142,983 patients diagnosed with stage II/III colon cancer were recruited from the SEER database, including 141,281 patients (98.8%) with AC and 1,702 patients (1.2%) with SRCC, 72,796 patients (50.9%) with stage II disease, and 70,187 patients (49.1%) with stage III disease, 69,248 males (48.4%) and 73,735 females (51.6%), most of them were white (80.4%). The median age of all the patients in SRCC histology was 71 years. Among these patients, the median follow-up time was 46 months, 84,903 (59.38%) patients with stage II/III colon cancer were followed up for at least 1 year.

The differences between AS and SRCC groups in the incidence of patients' demographic and clinical characteristics were shown in **Table 1**. It was found that SRCC histology was more likely to be related to T4 stage (P < 0.001), N2 stage (P < 0.001), and grade III/IV (P < 0.001), indicating that SRCC histology was more likely to be associated with adverse tumor pathology. We also noted that SRCC histology was more prone

TABLE 1 | Patient characteristics of stage II/III colon cancer.

	Patient characteristics		*P*
	Adenocarcinoma N = 141281 (%)	Signet ring cellcarcinoma N = 1702 (%)	
T stage			<0.001
T1	3131 (2.2)	24 (1.4)	
T2	6030 (4.3)	41 (2.4)	
T3	108556 (76.8)	1052 (61.8)	
T4	23564 (16.7)	585 (34.4)	
N stage			<0.001
N0	72305 (51.2)	491 (28.8)	
N1	46323 (32.8)	445 (26.1)	
N2	22653 (16.0)	766 (45.0)	
Age (years)			0.849
≤65	52144 (36.9)	632 (37.1)	
>65	89137 (63.1)	1070 (62.9)	
Race			<0.001
White	113441 (80.3)	1460 (85.8)	
Black	16444 (11.6)	133 (7.8)	
Other	11396 (8.1)	109 (6.4)	
Gender			0.183
Male	68451 (48.5)	797 (46.8)	
Female	72830 (51.5)	905 (53.2)	
Grade			<0.001
Grade I/II	108073 (76.5)	112 (6.6)	
Grade III/IV	30560 (21.6)	1495 (87.8)	
Unknown	2648 (1.9)	95 (5.6)	
Chemotherapy			<0.001
No/unknown	90587 (64.1)	913 (53.6)	
Yes	50694 (35.9)	789 (46.4)	

to white race (P < 0.001) and the receipt of chemotherapy (P < 0.001). But the difference between SRCC and AD histology in age of diagnosis (P = 0.849) and gender (P = 0.183) did not achieve statistical significance.

The Prognostic Value of SRCC in Stage II/III Colon Cancer

In **Table 2**, univariate and multivariate Cox analyses were conducted to evaluate the prognostic value of SRCC in stage II/III colon cancer. The univariate analysis produced seven variables that were then included in the multivariate analysis and the variable of gender was excluded. The results of multivariate analysis showed that SRCC histology was independently associated with 30.2% increased risk of colon cancer-specific mortality compared with AD histology (HR = 1.302, 95% CI = 1.196–1.417, P < 0.001, using AD histology as the reference). It was also found that higher T stage (P < 0.001), higher N stage (P < 0.001), older age (P < 0.001), black race (P < 0.001), and higher grade (P < 0.001) were more likely to be associated with worse CSS, and the receipt of chemotherapy was associated with 38.0% decreased risk of colon cancer-specific mortality (HR = 0.620, 95% CI = 0.603–0.637, P < 0.001, using no/unknown chemotherapy as the reference).

Stage II/III SRCC Response to Chemotherapy

The Kaplan-Meier CSS curves comparing the survival improvement offered by chemotherapy of SRCC and AD histology in stage II colon cancer were plotted in **Figure 1**. In stage II colon cancer with the histology of AD, it was found that the CSS of colon cancer patients with the receipt of chemotherapy was similar to those without the receipt of chemotherapy though survival difference was statistically significant and the five-year CSS rates of patients with and without the receipt of chemotherapy were 86.7 and 87.2%, respectively (P = 0.0078, **Figure 1A**); in stage II colon cancer with the histology of SRCC, however, it was found that the receipt of chemotherapy significantly decreased the CSS of colon cancer patients and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 74.9 and 87.2%, respectively (P = 0.045, **Figure 1B**).

TABLE 2 | Cox regression analyses of prognostic factors for CSS in stage II/III colon cancer.

Variable	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Histology		<0.001		<0.001
Adenocarcinoma			1	
Signet ring cell carcinoma			1.302 (1.196-1.417)	
T stage		<0.001		<0.001
T1			1	
T2			1.449 (1.251-1.679)	<0.001
T3			2.929 (2.575-3.330)	<0.001
T4			6.716 (5.899-7.645)	<0.001
N stage		<0.001		<0.001
N0			1	
N1			2.201 (2.134-2.270)	<0.001
N2			4.079 (3.947-4.216)	<0.001
Age (years)		<0.001		<0.001
≤65			1	
>65			1.482 (1.443-1.522)	
Race		<0.001		<0.001
White			1	
Black			1.333 (1.287-1.381)	<0.001
Other			0.936 (0.895-0.980)	0.005
Gender		0.515		
Male				
Female				
Grade		<0.001		<0.001
Grade I/II			1	
Grade III/IV			1.200 (1.167-1.234)	<0.001
Unknown			1.230 (1.134-1.334)	<0.001
Chemotherapy		<0.001		<0.001
No/unknown			1	
Yes			0.620 (0.603-0.637)	

In **Table 3** and **Table S1**, univariate and multivariate Cox analyses were conducted to evaluate the predictive value of histology for colon cancer response to chemotherapy. The univariate analysis produced five variables that were then included in the multivariate analysis and the variable of gender was excluded. In stage II AD, it was found that the receipt of chemotherapy had significantly 12.6% decreased risk of cancer-specific mortality (HR = 0.874, 95% CI = 0.825–0.927, P < 0.001, **Table 3**). In stage II SRCC, however, the receipt of chemotherapy had significantly 70.00% increased risk of cancer-specific mortality (HR = 1.700, 95% CI = 1.032–2.801, P = 0.037, **Table S1**). We also noted that the CSS difference between AD

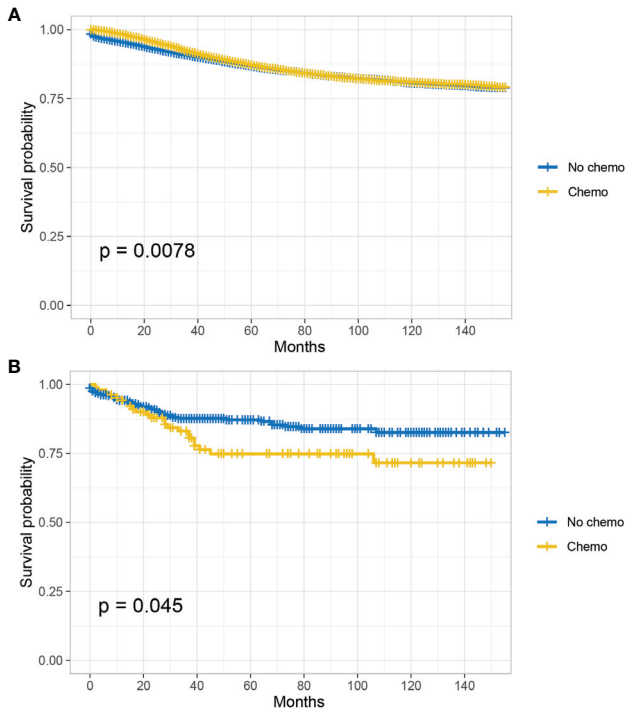

FIGURE 1 | CSS among AD and SRCC patients with or without chemotherapy in stage II colon cancer. **(A)** CSS of colon cancer patients with the receipt of chemotherapy was similar to those without the receipt of chemotherapy in stage II AD; **(B)** The receipt of chemotherapy significantly decreased the CSS of colon cancer patients in stage II SRCC.

and SRCC was not statistically significant in stage II colon cancer (P = 0.388).

The Kaplan-Meier CSS curves comparing the survival improvement offered by chemotherapy of SRCC and AD histology in stage III colon cancer were plotted in **Figure 2**. In stage III colon cancer with the histology of AD, it was found that the CSS of colon cancer patients with the receipt of chemotherapy was significantly better than those without the receipt of chemotherapy and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 77.5 and 64.4%, respectively (P < 0.001, **Figure 2A**); In stage III colon cancer with the histology of SRCC, it was found that the receipt of chemotherapy improved the CSS of colon cancer patients and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 53.1 and 49.3%, respectively (P < 0.001, **Figure 2B**).

In **Table 4** and **Table S2**, univariate and multivariate Cox analyses were conducted to evaluate the predictive value of histology for colon cancer response to chemotherapy. The univariate analysis produced six variables that were then included in the multivariate analysis and the variable of gender was excluded. In stage III AD, it was found that the receipt of chemotherapy had significantly 45.3% decreased risk of cancer-specific mortality (HR = 0.547, 95% CI = 0.530–0.564, P < 0.001, **Table 4**). In stage III SRCC, the receipt of chemotherapy had

TABLE 3 | Cox regression analyses of prognostic factors for CSS in stage II colon cancer.

Variable	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T3			1	
T4			2.786 (2.656-2.923)	
Age (years)		<0.001		<0.001
≤65			1	
>65			1.697 (1.616-1.781)	
Race		<0.001		<0.001
White			1	
Black			1.414 (1.331-1.502)	<0.001
Other			0.910 (0.937-0.990)	0.028
Gender		0.262		
Male				
Female				
Grade		<0.001		<0.001
Grade I/II			1	
Grade III/IV			1.071 (1.015-1.129)	0.012
Unknown			1.291 (1.129-1.477)	<0.001
Histology and chemotherapy		0.003		<0.001
Adenocarcinoma, No/unknown			1	
Adenocarcinoma, Yes			0.874 (0.825-0.927)	<0.001
SRCC, No/unknown			0.880 (0.659-1.175)	0.388
SRCC, Yes			1.497 (0.992-2.259)	0.055

significantly 24.6% decreased risk of cancer-specific mortality (HR = 0.754, 95% CI = 0.632–0.900, P = 0.002, **Table S2**). We also note that SRCC histology had 14.5% increased risk of cancer-specific mortality with the borderline statistical significance compared with the histology of AD (HR = 1.145, 95% CI = 0.998–1.313, P = 0.054).

DISCUSSION

SRCC was a very rare histological type of AD and the reported incidence ranged from 0.1 to 5% (4, 6, 9, 17–19). In our study, SRCC accounted for 1.20% of the colon cancer, which was consistent with the reported frequency. It was found that SRCC histology was more likely to be related to T4 stage, N2 stage, and grade III/IV, indicating that SRCC histology was more likely to be associated with adverse tumor pathology. SRCC was reported to be associated with peritoneal seeding and infiltration into lymphatics and nodes more frequently, which was attributed to the mucopolysaccharide nature of the colloid-type carcinoma which prevents discrimination of host immunocytes for tumor cells and thus allowing easier invasion into peri-intestinal tissue and subsequent lymphatics (4). Some previous studies indicated that primary colorectal signet-ring cell carcinoma tended to arise before 40 years of age (17, 20–22), and another series reported that the mean age varied from 52 to 67 years (6). The patients' mean age of SRCC histology in the present study was 68.51 years, which was not entirely consistent with previous studies.

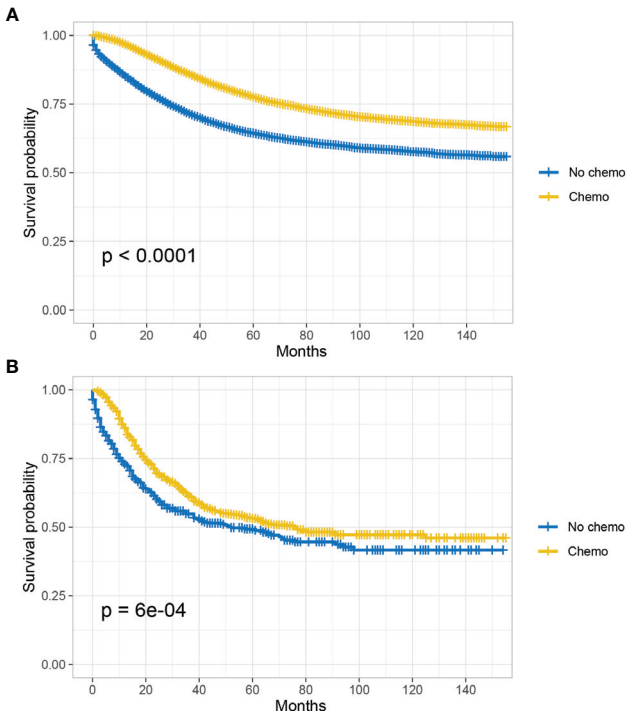

FIGURE 2 | CSS among AD and SRCC patients with or without chemotherapy in stage III colon cancer. **(A)** CSS of colon cancer patients with the receipt of chemotherapy was significantly better than those without the receipt of chemotherapy in stage II AD; **(B)** The receipt of chemotherapy improved the CSS of colon cancer patients.

TABLE 4 | Cox regression analyses of prognostic factors for CSS in stage III colon cancer.

Variable	Univariate analyses		Multivariate analyses	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T1			1	
T2			1.444 (1.246-1.673)	<0.001
T3			3.019 (2.654-3.434)	<0.001
T4			6.182 (5.426-7.044)	<0.001
N stage		<0.001		<0.001
N1			1	
N2			1.877 (1.821-1.935)	
Age (years)		<0.001		<0.001
≤65			1	
>65			1.406 (1.362-1.452)	
Race		<0.001		<0.001
White			1	
Black			1.292 (1.237-1.350)	<0.001
Other			0.947 (0.897-0.999)	0.048
Gender		0.963		
Male				
Female				
Grade		<0.001		<0.001
Grade I/II			1	
Grade III/IV			1.259 (1.218-1.300)	<0.001
Unknown			1.189 (1.074-1.316)	0.001
Histology and chemotherapy		<0.001		<0.001
Adenocarcinoma, No/unknown			1	
Adenocarcinoma, Yes			0.547 (0.530-0.564)	<0.001
SRCC, No/unknown			1.145 (0.998-1.313)	0.054
SRCC, Yes			0.863 (0.766-0.973)	0.016

Although some researchers reported that SRCC had worse prognosis compared with AD (4, 5, 7–10), disputes about the prognostic value of histology of SRCC still existed (11–13, 23, 24) and clearly more research was required to solve this controversial issue. In the present study, it was found that SRCC histology was independently associated with 30.2% increased risk of colon cancer-specific mortality compared with AD histology in the whole cohort. In subgroup analyses, however, results of multivariate analyses showed that the CSS difference between AD and SRCC was not statistically significant in stage II colon cancer (P = 0.388) and SRCC histology had 14.5% increased risk of cancer-specific mortality with the borderline statistical significance compared with the histology of AD in stage III colon cancer. In 2014, it was reported that SRCC was an independent prognostic marker for a bad prognosis in stage III colon cancer (11). Recently, a retrospective analysis also found that SRCC did not negatively impact survival in stage II colon cancer after risk-adjusting for other prognostic factors (10). The different roles of SRCC histology in stage II and stage III colon cancer might partly explain the controversy about the prognosis of SRCC histology in colon cancer.

Further, the subgroup analyses showed that, in stage II colon cancer with the histology of AD, the CSS of colon cancer patients with the receipt of chemotherapy was similar to those without the receipt of chemotherapy though survival difference was statistically significant and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 86.7 and 87.2%, respectively (P = 0.0078); after adjusting for many other factors that influenced the CSS of colon cancer, the receipt of chemotherapy had significantly 12.6% decreased risk of cancer-specific mortality (P < 0.001), showing that the receipt of chemotherapy independently improved CSS of stage II colon cancer with AD histology, which supported the chemotherapy use in stage II colon cancer reported by some previous studies (25, 26). In stage III colon cancer with the histology of AD, it was found that the CSS of colon cancer patients with the receipt of chemotherapy was significantly better than those without the receipt of chemotherapy and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 77.5 and 64.4% (P < 0.001); after adjusting for many other factors that influenced the CSS of colon cancer, the receipt of chemotherapy had significantly 45.3% decreased risk of cancer-specific mortality, showing that chemotherapy use had greatly improved patient outcomes in stage III colon cancer, which was consistent with previous studies (27–29).

In stage II colon cancer with the histology of SRCC, however, it was found that the receipt of chemotherapy significantly decreased the CSS of colon cancer patients and the 5-year CSS rates of patients with and without the receipt of chemotherapy

were 74.9 and 87.2%, respectively (P = 0.045); after adjusting for many other factors that influenced the CSS of colon cancer, the receipt of chemotherapy had significantly 70.00% increased risk of cancer-specific mortality. To the best of our knowledge, this was the first population-based study to focus on the efficacy of chemotherapy in stage II colon cancer with the histology of SRCC. Given the increased risk of colon cancer-specific mortality in stage II SRCC with the chemotherapy use, we held the view that chemotherapy should not be treated in stage II SRCC.

In stage III colon cancer with the histology of SRCC, it was found that the receipt of chemotherapy improved the CSS of colon cancer patients and the 5-year CSS rates of patients with and without the receipt of chemotherapy were 53.1 and 49.3%, respectively (P < 0.001), after adjusting for many other factors that influenced the CSS of colon cancer, the receipt of chemotherapy had significantly 24.6% decreased risk of cancer-specific mortality. Hugen et al. (11) reported that there was a comparable benefit from adjuvant chemotherapy in stage III AD and SRCC. In our study, combined the above analysis, however, we could find that SRCC had worse response to chemotherapy compared with AD in stage III colon cancer, we believed that it might account from the changes of treatment regimens during the past twenty years because cases selected in Hugen's study were from as early as 1989. The receipt of chemotherapy could also significantly improve the prognosis of SRCC, therefore, we still recommended the chemotherapy use in stage III SRCC.

There were some potential weaknesses in our study. Firstly, some information including the chemotherapy regimens, chemotherapy duration, and basic diseases were not available for the included patients due to the limitation of SEER database. Secondly, our research was a retrospective type of study and the inherent deficiencies could lead to confusion or observer bias which cannot be removed. However, SRCC was a very rare disease, only large cancer database like SEER would be suitable for the investigation of it, we believed that the large size and breadth of this database across the US could mitigate the drawbacks outlined above.

CONCLUSIONS

Using a large cancer database, we found that the CSS difference between AD and SRCC was not statistically significant in stage II colon cancer (P = 0.388) and SRCC histology had 14.5% increased risk of cancer-specific mortality with the borderline statistical significance compared with the histology of AD in stage III colon cancer. In addition, we provided the first compelling evidence that chemotherapy was associated with the increased risk of colon cancer-specific mortality and chemotherapy should not be treated in stage II SRCC. The receipt of chemotherapy could significantly improve the prognosis of stage III SRCC, therefore, we still recommended the chemotherapy use in stage III SRCC.

DATA AVAILABILITY STATEMENT

Publicly available data sets were analyzed in this study. The raw data supporting the conclusions of this article are available upon reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

YW: concept and design. HJ and DS: collection and assembly of data. PZ: data analysis and interpretation. HJ: results interpretation and manuscript writing. HJ and DS: manuscript revision. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.631995/full#supplementary-material

Supplementary Figure 1 | Flowchart for creation of the SEER patient dataset.

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Associations of P Score With Real-World Survival Improvement Offered by Adjuvant Chemotherapy in Stage II Colon Cancer: A Large Population-Based Longitudinal Cohort Study

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Background: Based on a prognostic scoring system (*P* score) proposed by us recently, this retrospective large population-based and propensity score-matched (PSM) study focused on predicting the survival benefit of adjuvant CT in stage II disease.

Methods: Patients diagnosed with stage II colon cancer (N = 73397) were identified from the Surveillance, Epidemiology, and End Results database between January 1, 1988 and December 31, 2005 and divided into the CT and non-CT groups. PSM balanced the patient characteristics between the CT and non-CT groups.

Results: The magnitude of CSS improvement among patients treated with adjuvant CT was significantly associated with the P score, score 8 [hazard ratio (HR) = 0.580, 95% confidence interval (CI) = 0.323–1.040, P = 0.067] was associated with a much higher increased CSS benefit among patients treated with adjuvant CT as compared to score 2* (*, including scores 0, 1, and 2; HR = 1.338, 95% CI = 1.089–1.644, P = 0.006).

Conclusions: High *P* scores were demonstrated to be associated with superior survival benefit of adjuvant CT. Therapy decisions of adjuvant CT in stage II colon cancer could be tailored on the basis of tumor biology, patient characteristics and the *P* score.

Keywords: prognostic scoring system, stage II, colon cancer, adjuvant chemotherapy, SEER

BACKGROUND

Colon cancer was the third most commonly diagnosed malignant tumor worldwide (1). Despite that adjuvant chemotherapy (CT) was widely applied clinically with clearly established evidence of survival benefit for stage III colon cancer, its efficacy for stage II colon cancer was yet controversial (2–5). The famous Quick, Simple, and Reliable (QUASAR) prospective trial reported a pool survival benefit for patients with stage I–III colorectal cancer after CT as compared to surgery alone; however, it failed to demonstrate the efficacy of CT among stage II colon cancer subgroup (3).

Although direct evidence of benefit was lacking, the American Society of Clinical Oncology (ASCO) clinical guidelines recommended adjuvant CT for high-risk stage II colon cancer (including patients with inadequately sampled nodes, T4 lesions, perforation, or poorly differentiated histology) (6). Also, the European Society for Medical Oncology (ESMO) proposed similar recommendations (7). However, the efficacy of adjuvant CT in stage II colon cancer with high-risk factors was still controversial (8). Two retrospective clinical studies reported the survival benefit of adjuvant CT in stage II colon cancer with high-risk factors (9, 10). But more clinical studies suspected the survival benefit of adjuvant CT in the so-called high-risk stage II colon cancer (8, 11–14).

A wide clinical application of adjuvant CT in high-risk stage II colon cancer in spite of the uncertainty of survival benefit makes the studies of adjuvant CT in stage II colon cancer quite necessary. Thus, the purpose of the study was to predict the survival effect among stage II colon cancer with the prognostic scoring system proposed in our previous study (15) in order to obtain an improved prognostic prediction of stage II colon cancer with different *P* scores after receiving adjuvant CT.

METHODS

Study Design and Data Source

In this study, patients were recruited from the Surveillance, Epidemiology, and End Results (SEER) Program of the United States National Cancer Institute, released in 2018. The SEER database was an authoritative and public source of information on cancer incidence, mortality, prevalence, lifetime risk statistics, and survival in the United States. We used SEER-Stat software (version 8.3.5) to get access in this study.

As shown in **Figure 1**, we identified 73,397 stage II colon cancer patients from January 1, 1988 to December 31, 2005 for the initial analysis. Next, patients diagnosed within these years were included in our study because the SEER database started recording detailed tumor size from 1988 (tumor size was essential for the prognostic scoring system) and we wanted to allow for 10 years of follow-up (the follow-up of the present study ended in 2015). We excluded patients with unknown information of some significant prognostic factors, such as tumor grade, tumor size, race, tumor location (appendix was not included from this study), and so on. Also, patients without surgery or adenocarcinoma histology or positive histology or active follow-up were excluded from our target population.

Prognostic Scoring System

To investigate the benefit of adjuvant CT after surgery, we used the newly proposed prognostic scoring system (*P* score) and the detailed scoring rules were showed in our previous study (15). Since only 457 patients (0.6%) were diagnosed with undifferentiated tumor grade (grade IV), grade III and grade IV were merged. As shown as **Figure 2**, *P* score (that is the prognostic scoring system) that was obtained based on the tumor size, tumor grade, and age at diagnosis ranged from 0–8 with a score of 0 indicating the best prognosis and those with a score of 8 indicating the poorest survival.

Statistical Analyses

In this study, different clinicopathologic factors were compared between the CT and non-CT groups using Pearson's chi-squared test for categorical variables. The primary endpoint used for comparison were cause-specific survival (CSS). We also constructed some multivariate Cox proportional hazard models to evaluate the survival benefit of adjuvant CT.

As an observational study, significant bias might be introduced by inherent differences between patients receiving or not receiving adjuvant CT. In addition, we defined the predicted probability of treatment as a propensity score to balance the clinicopathologic factors between the CT and non-CT groups in SEER cohort using the following baseline characteristics that strongly related to the survival but less strongly related to the treatment: year of diagnosis, race, gender, tumor location, histology, T stage (including T3, T4a or T4b), age at diagnosis, tumor size, and tumor grade (16). Patients receiving adjuvant CT were matched on a one-to-one basis with patients without receiving adjuvant CT (**Figure 1**). We performed the matching based on the nearest-neighbor methods. The propensity score indicated the probability of the patients receiving the adjuvant CT based on the baseline characteristics. In our study, we preformed the statistical analysis mainly using SPSS version 22 (IBM Corporation, Armonk, NY, USA), and two-sided P value < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

The median follow-up time of the censored patients in the SEER cohort was 9.67 years, following which, at the end of the follow-up time, 13,880 (18.9%) patients died because of colon cancer. Of the initial cohort, 61,015 patients (83.1%) were stratified into the non-CT group, and 12,382 patients (16.9%) were stratified into the CT group. **Table 1** summarized the patients' baseline demographic characteristics. All demographic characteristics were statistically related to the receipt of the adjuvant CT ($P < 0.001$). The patients diagnosed during later years, male patients, T4 stage, younger patients, patients with large tumor size, and patients with high tumor grade were more likely to receive adjuvant CT ($P < 0.001$).

Survival Benefit of Adjuvant Chemotherapy According to *P* score Before Propensity Score Matching

Considering that the scores 0 and 1 accounted for only <0.1 and 0.4% of the overall cohort, respectively, the scores 0, 1, and 2 were then classified as the same score. As shown in **Figure S1** after multivariate Cox and Kaplan–Meier analyses of CSS, the magnitude of CSS improvement among patients treated with adjuvant CT was significantly associated with the *P* score, score 8 [hazard ratio (HR) = 0.580, 95% confidence interval (CI) =

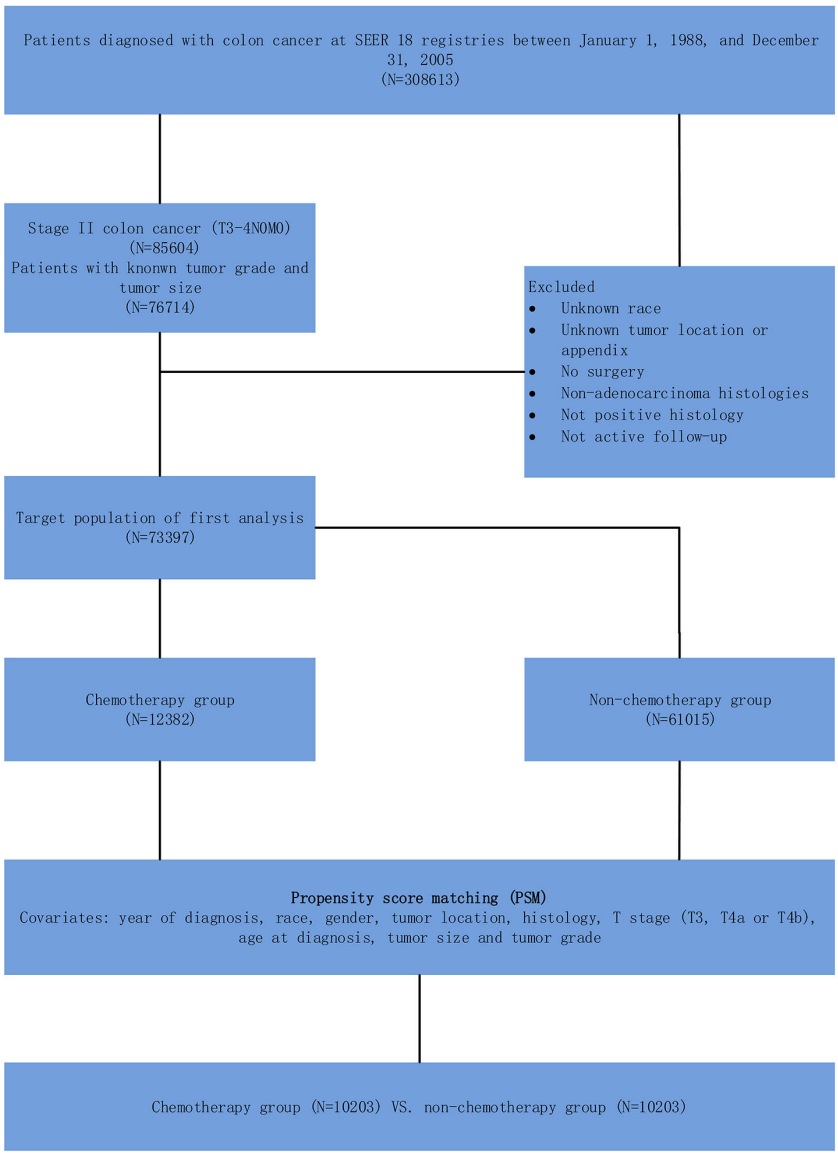

FIGURE 1 | Schematic representation of patient population selected from SEER database.

0.323–1.040, P = 0.067] was associated with a much higher increased CSS benefit among patients treated with adjuvant CT compared to score 2* (*, including scores 0, 1, and 2; HR = 1.338, 95% CI = 1.089–1.644, P = 0.006). In other words, the decrease of 10-year CSS rates among the non-CT group with the increase of *P* score was much faster than the CT group [the decrease of CSS with the increase of *P* score in colon cancer has been demonstrated in our previous study (15)]. In the CT group, the 10-year CSS rate decreased gradually as the score increased only with the exception that the 10-year CSS was higher in score 8 (78.7%) than that in score 7 (74.9%), and we thought it was plausible to conclude it was mainly due to the substantial survival benefit of adjuvant CT in score 8.

Survival Benefit of Adjuvant Chemotherapy According to *P* score After Propensity Score Matching

As shown in **Table 2**, PSM generated 10,203 patients in the CT group and 10,203 patients in the non-CT group. The median follow-up time among the censored patients was 11.83 years. At the end of the follow-up time, 3,844 (18.8%) patients died of colon cancer. As shown in **Figure 3A**, multivariate Cox and Kaplan–Meier analyses of CSS found that the magnitude of CSS improvement among patients treated with adjuvant CT was also significantly associated with the *P* score and the HRs between CT and non-CT groups decreased gradually when the score increased without exception. Score 8 (HR = 0.473, 95% CI =

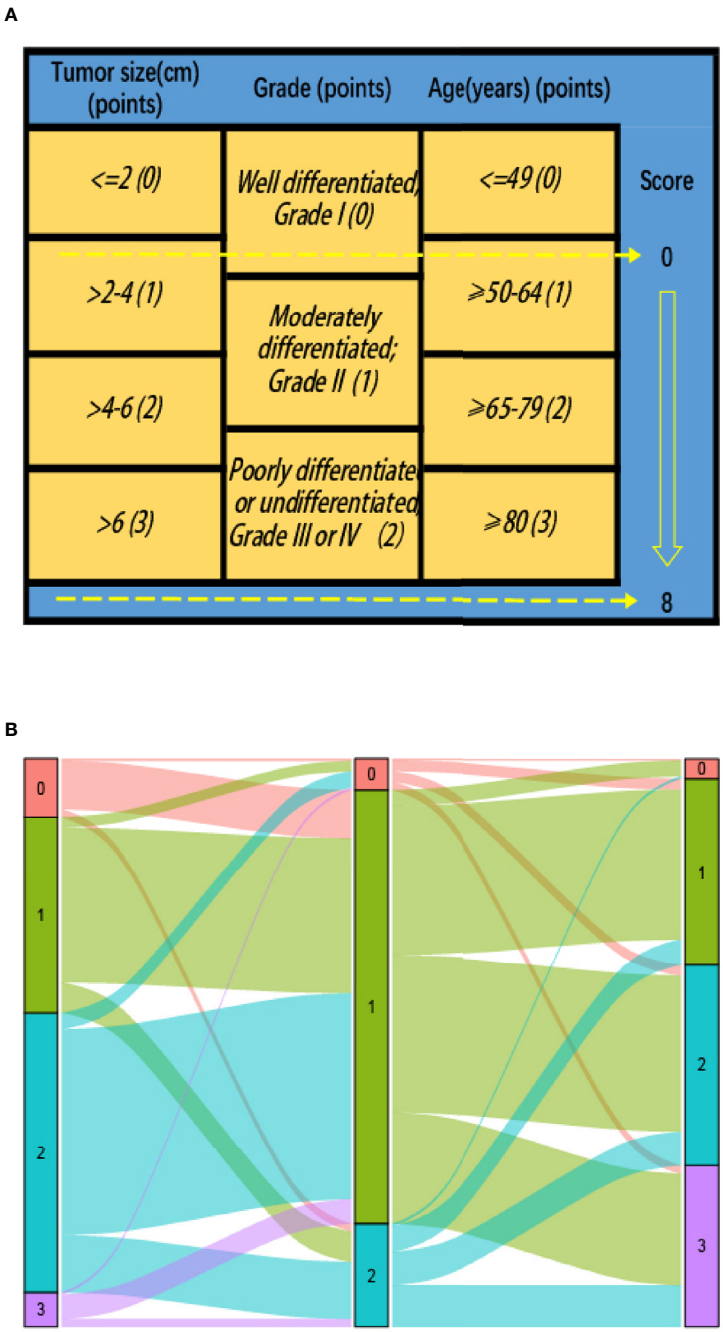

FIGURE 2 | **(A)** Modified prognostic scoring system (*P* score) in stage II colon cancer patients: risk-stratifications; **(B)** Graphical summary of tumor size, tumor grade and age, and their subgroup distribution.

0.188–1.191, P = 0.112) was associated with a much higher increased CSS benefit among patient with adjuvant CT as compared to that of score 2* (*, including scores 0, 1, and 2; HR = 1.516, 95% CI = 1.100–2.089, P = 0.011), and the phenomenon was more obvious than in the overall cohort before PSM. The decrease of 10-year CSS rate among the non-CT group with the increase of *P* score was much faster than that among the CT group [the decrease of CSS with the increase of *P* score in colon cancer has been demonstrated in our previous study (15)]; the 10-year CSS rate was even higher in score 8 (83.3%) than score 7 (76.7%) among the CT group, and we thought it was plausible to conclude it was mainly due to the substantial survival benefit of adjuvant CT in score 8.

Figure 3B showed that the overall survival (OS) benefit improved gradually when the score increased without exception, and the decline of 10-year OS rate among the non-

TABLE 1 | Baseline characteristics of the overall cohort by the receipt of adjuvant CT before PSM.

Characteristic	No. of Patients (%)		*P*
	CT Group (n = 12,382)	Non-CT Group (n = 61,015)	
Year of diagnosis			<0.001
1988–1992	1,282 (10.4)	10,735 (17.6)	
1993–1997	2,747 (22.2)	13,222 (21.7)	
1998–2001	3,791 (30.6)	16,143 (26.5)	
2002–2005	4,562 (36.8)	20,915 (34.3)	
Race			<0.001
White	10,237 (82.7)	51,884 (85.0)	
Black	1,121 (9.1)	5,404 (8.9)	
Other	1,024 (8.3)	3,727 (6.1)	
Gender			<0.001
Male	6,221 (50.2)	28,162 (46.2)	
Female	6,161 (49.8)	32,853 (53.8)	
Tumor location			<0.001
Cecum	2,728 (22.0)	15,141 (24.8)	
Ascending colon	2,085 (16.8)	12,174 (20.0)	
Hepatic flexure	782 (6.3)	4,393 (7.2)	
Transverse colon	1,391 (11.2)	7,074 (11.6)	
Splenic flexure	604 (4.9)	2,931 (4.8)	
Descending colon	935 (7.6)	3,983 (6.5)	
Sigmoid Colon	3,857 (31.2)	15,319 (25.1)	
Histology			<0.001
Adenocarcinoma	11,017 (89.0)	55,221 (90.5)	
Mucinous adenocarcinoma	1,279 (10.3)	5,474 (9.0)	
Signet ring cell carcinoma	86 (0.7)	320 (0.5)	
T stage			<0.001
T3	9,671 (78.1)	52,364 (85.8)	
T4a	1,253 (10.1)	5,817 (9.5)	
T4b	1,458 (11.8)	2,834 (4.6)	
Age at diagnosis (years)			<0.001
≤49	1,900 (15.3)	2,377 (3.9)	
>49–64	4,377 (35.3)	9,361 (15.3)	
>64–79	5,439 (43.9)	27,704 (45.4)	
>79	666 (5.4)	21,573 (35.4)	
Tumor size (cm)			<0.001
≤2	494 (4.0)	3,202 (5.2)	
>2–4	3,801 (30.7)	22,152 (36.3)	
>4–6	4,230 (34.2)	21,096 (34.6)	
>6	3,857 (31.2)	14,565 (23.9)	
Tumor grade			<0.001
Grade I	854 (6.9)	5,338 (8.8)	
Grade II	8,950 (72.3)	44,944 (73.7)	
Grade III/IV	2,578 (20.8)	10,732 (17.6)	

CT, chemotherapy; PSM, propensity score matching.

CT group was much faster than among the CT group, which further validated the above findings. In addition, the Kaplan–Meier CSS curves of different *P* scores were also plotted, which also demonstrated the increased survival benefit offered by adjuvant chemotherapy as *P* score increased ($P < 0.05$, **Figures 4A–C**).

Survival Benefit of Adjuvant Chemotherapy According to the *P* score Between T3 and T4 Groups

Next, we furtherly conducted the subgroup analyses and **Figure 5** showed the results of multivariate Cox and Kaplan-Meier analyses of CSS among both T3 and T4 subgroups. In the T3 subgroup analysis, it was also found that the 10-year CSS rate was higher in score 8 (86.3%) than that in score 7 (79.2%) among the CT group (**Figure 3A**). In the T4 subgroup analysis, a notable phenomenon we called "survival inversion" was that 10-year CSS rate increased gradually instead of decreasing when the score increased from 6 to 8 (**Figure 3B**). Thus, the "survival inversion" effect as *P* scores increased was even more pronounced among the T4 subgroup than among T3 subgroup. And the magnitude of CSS improvement offered by adjuvant CT was positively correlated with the *P* scores in both T3 and T4 subgroups. More importantly, more patients in the T4 subgroup favored adjuvant CT than in the T3 subgroup.

DISCUSSION

The majority of the randomized controlled trials (RCTs) regarding adjuvant CT in stage II colon cancer mixed the

TABLE 2 | Baseline characteristics of the overall cohort by the receipt of adjuvant CT after PSM.

Characteristic	No. of Patients (%)		*P*
	CT Group (n = 10,203)	Non-CT Group (n = 10,203)	
Year of diagnosis			0.943
1988–1992	1,063 (10.4)	1,083 (10.6)	
1993–1997	2,271 (22.3)	2,290 (22.4)	
1998–2001	3,115 (30.5)	3,091 (30.3)	
2002–2005	3,754 (36.8)	3,739 (36.6)	
Race			0.958
White	8,832 (86.6)	8,821 (86.5)	
Black	769 (7.5)	780 (7.6)	
Other	602 (5.9)	602 (5.9)	
Gender			0.966
Male	5,155 (50.5)	5,158 (50.6)	
Female	5,048 (49.5)	5,045 (49.4)	
Tumor location			1.000
Cecum	2,332 (22.9)	2,335 (22.9)	
Ascending colon	1,741 (17.1)	1,733 (17.0)	
Hepatic flexure	581 (5.7)	584 (5.7)	
Transverse colon	1,096 (10.7)	1,088 (10.7)	
Splenic flexure	418 (4.1)	424 (4.2)	
Descending colon	695 (6.8)	699 (6.9)	
Sigmoid Colon	3,340 (32.7)	3,340 (32.7)	
Histology			0.913
Adenocarcinoma	9,431 (92.4)	9,445 (92.6)	
Mucinous adenocarcinoma	748 (7.3)	733 (7.2)	
Signet ring cell carcinoma	24 (0.2)	25 (0.2)	
T stage			0.850
T3	8,668 (85.0)	8,641 (84.7)	
T4a	786 (7.7)	806 (7.9)	
T4b	749 (7.3)	756 (7.4)	
Age at diagnosis (years)			0.998
≤49	1,048 (10.3)	1,045 (10.3)	
>49–64	3,522 (34.5)	3,516 (34.5)	
>64–79	5,018 (49.2)	5,014 (49.1)	
>79	615 (6.0)	619 (6.1)	
Tumor size (cm)			0.999
≤2	352 (3.4)	355 (3.5)	
>2–4	3,358 (32.9)	3,351 (32.8)	
>4–6	3,594 (35.2)	3,596 (35.2)	
>6	2,899 (28.4)	2,901 (28.4)	
Tumor grade			0.952
Grade I	560 (5.5)	565 (5.5)	
Grade II	7,787 (76.3)	7,798 (76.4)	
Grade III/IV	1,856 (18.2)	1,840 (18.0)	

CT, chemotherapy; PSM, propensity score matching.

study population together with stage II and stage III diseases; only one RCT had focused on adjuvant CT in stage II colon cancer; however, the study found that high-risk stage II colon cancer did not benefit from 1-year adjuvant treatment with oral tegafur-uracil (UFT) (11, 17, 18). Although lack of sufficient evidence, ASCO and ESMO recommended the adjuvant chemotherapy in stage II colon cancer with the so-called high-risk prognostic factors (6, 7).

Furthermore, a unified definition of "high-risk" was absent as many countries had their different rules for risk assessment (19–22). In addition, ASCO (including inadequately sampled nodes, T4 lesions, perforation, or poorly differentiated histology) and ESMO (including lymph nodes sampling <12; poorly differentiated tumor; vascular or lymphatic or perineural invasion; tumor presentation with obstruction or tumor perforation and pT4 stage) clinical guidelines were different (6, 7). On the other hand, we could not quantify the necessity of adjuvant CT among stage II disease with high-risk factors considering they were only several independent prognostic factors (8).

Many clinical studies suspected the survival improvement of adjuvant CT in stage II colon cancer with high-risk factors (8, 11–14). In 2011, a large retrospective population-based clinical study found that adjuvant CT did not improve the overall survival substantially in stage II colon cancer either with or without high-risk prognostic features (including obstruction, perforation, emergent admission, T4-stage, resection of <12 lymph nodes, and poor histology) (14). A wide clinical application of adjuvant CT in stage II colon cancer with high-risk factors in spite of the uncertainty of survival benefit which could result in the overtreatment or undertreatment in stage II

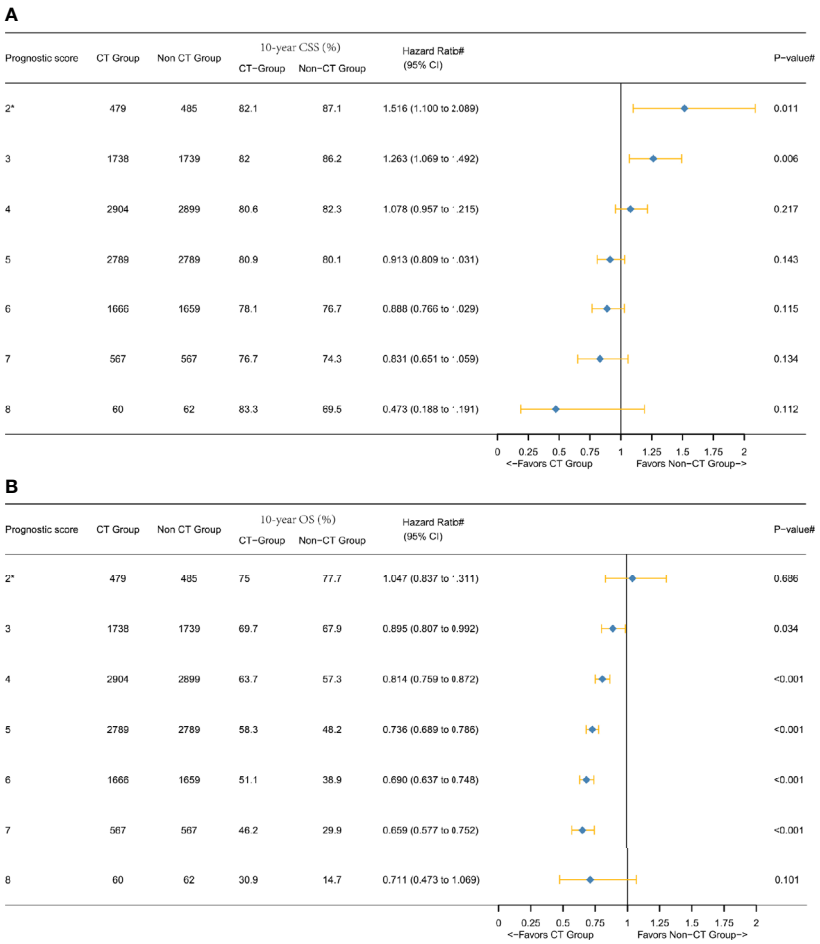

FIGURE 3 | Hazard ratios comparing the survival between CT and non-CT groups according to the *P* score in the overall cohort after PSM comparing **(A)** CSS and **(B)** overall survival (OS). (2*) Including *P* scores 0, 1, and 2. (#) Multivariate analysis adjusted by the year of diagnosis, race, gender, tumor location, histology, T stage (including T3, T4a, or T4b), age at diagnosis, tumor size, and tumor grade.

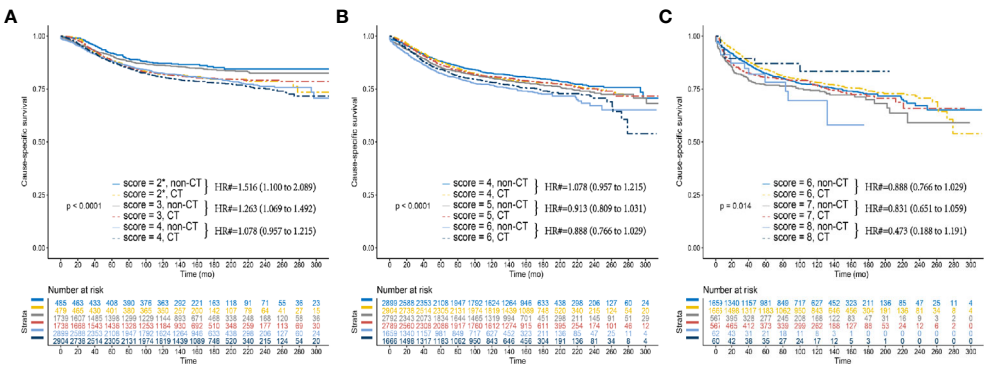

FIGURE 4 | Kaplan–Meier CSS curves between the CT and non-CT groups after PSM in **(A)** *P* scores 2, 3, and 4 **(B)** *P* scores 4, 5, and 6 **(B)** *P* scores 6, 7, and 8. **(**2*) Including *P* scores 0, 1, and 2. (#) Multivariate analysis adjusted by the year of diagnosis, race, gender, tumor location, histology, T stage (including T3, T4a, or T4b), age at diagnosis, tumor size, and tumor grade.

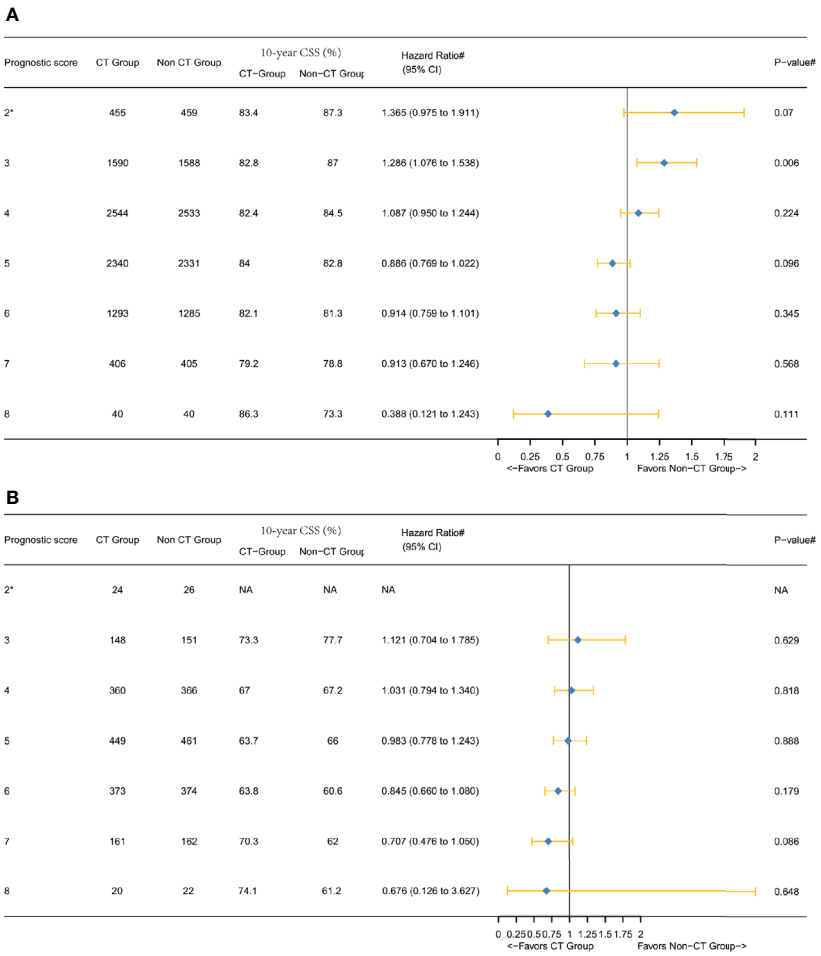

FIGURE 5 | Hazard ratios comparing the CSS between the CT and non-CT groups according to *P* score in the subgroups after PSM comparing **(A)** T3 subgroup and **(B)** T4 subgroup. (2*) Including *P* scores 0, 1, and 2. (#) Multivariate analysis adjusted by the year of diagnosis, race, gender, tumor location, histology, T stage (T4 subgroup analysis, including T4a or T4b), age at diagnosis, tumor size, and tumor grade. (NA) Not applicable.

colon cancer. In addition, a significant patient morbidity could result from toxicity and side effects caused by adjuvant chemotherapy of overtreatment (23).

In this large population-based and PSM study, the current findings indicated that stage II colon cancer with higher *P* score (older patients, higher tumor grade, and larger tumor size) might be associated with improved CSS benefit of adjuvant CT. This phenomenon is of great clinical significance as we can predict the survival benefit of adjuvant CT well in stage II colon cancer using a simple *P* score. Considering that the *P* score is based on the tumor size, age, and tumor grade, which could be acquired before the operation, we could predict the survival benefit of adjuvant CT well among stage II disease preoperatively. Also, this study showed a successful validation of OS benefit improvement with increasing *P* scores (**Figure 3B**).

Our previous study demonstrated incremental mortality risk with increasing *P* scores among stage II disease (15). And it was also observed in the non-CT group that could validate our previous finding, yet we also noted that the phenomenon was slightly different among the CT group: the highest *P* score did not generate the lowest CSS rate either in T3 or T4 subgroup (**Figures 3–5** and **Figure S1**). The different phenomenon was more distinct in T4 subgroup analysis of CT group as 10-year CSS rate increased gradually instead of decreasing when the score increased from 6 to 8 (**Figure 3B**). This phenomenon was termed as "survival inversion" that could be attributed to the improvement in the survival benefit offered by adjuvant CT, contrary to decreased survival when *P* scores increased in the non-CT group. Moreover, the "survival inversion" was evident T4 subgroup than in the T3 subgroup.

In 2014, Aalok et al. (24) reported that the survival benefit of adjuvant CT was primarily observed in the T4 disease, thereby suspected the effect of adjuvant CT in stage II colon cancer with non-T4 high-risk factors. The study indicated that the several high-risk factors were not equivalent. Moreover, Matsuda et al. (11) reported that lymphatic invasion and poorly differentiated histology did not have any impact on the relapse-free survival of stage II colon cancer though they were listed as "high-risk"

factors. Then, two studies from the United States and Netherlands proved that T4 had the maximum survival benefit with adjuvant therapy (8, 13). The results of the present study also showed that patients with lower *P* scores in the T4 subgroup were more likely to favor adjuvant CT as compared to the T3 subgroup in the prognostic scoring system, which was consistent with the previous studies, and it could lead to the speculation that *P* score might replace the role of high-risk factors in stage II disease.

The main strength of our study was the investigation of the survival benefit offered by adjuvant CT in stage II colon cancer according to the individualized patient risk factors. Based on the results of this large population-based and strictly PSM study with a long median follow-up time of about 10 years in the censored subjects, it was possible to guide the individual treatment decisions based on different *P* scores that could predict the survival benefit of adjuvant CT well in stage II disease. The "survival inversion" that reflected the association between tumor biology and clinical treatment also necessitates further exploration.

Nevertheless, the present study has some limitations. First, new biomarkers, such as RAS mutation, microsatellite instability, and carcinoembryonic antigen (CEA) level were studied intensively (18, 25–27). *P* score did not take other prognostic factors into account, indicating that *P* score requires further improvement. However, as a simple and convenient prognostic scoring system, *P* score could be obtained and calculated easily. Second, due to the limitation of SEER database, we cannot differentiate the chemotherapy regimens of CT, preoperative CT, postoperative CT, and the CT regimens. Considering it was not the standard therapy plan to treat stage II disease with preoperative CT, we can stratify the variable of "patient had chemotherapy" as "adjuvant CT." Third, the statistical power was limited because some individual subgroups, such as score 0 and 8, were small after stratifying in spite of a large initial study population from SEER database. And survival difference was not statistically significant in some P score subgroups, which was consistent with previous large population-based study (28). Forth, some factors, such as clinical presentation with obstruction or perforation and disease-free survival data, were not available in the SEER database, were therefore not included in the present study. Finally, because a very large sample size was required to validate the clinical value of *P* score, we cannot conduct relevant analyses in our center, and the value of *P* score needed to be confirmed in large multi-center studies, especially in prospective cohorts.

CONCLUSIONS

Here, based on the results of this large population-based and strictly PSM study with a long median follow-up time of about 10 years, our study demonstrated the improved survival benefit offered by adjuvant CT as *P* score increased, which can be used to guide the individual treatment decisions and predict the efficacy of adjuvant CT well in patients diagnosed with stage II colon cancer. In addition, *P* score was also easily obtained and calculated, meaning it could be of great clinical significance in therapy decisions in stage II colon disease. However, future studies focused on *P* score with prospective design were also essential.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: The Surveillance, Epidemiology, and End Results (SEER) Program (https://seer.cancer.gov/).

AUTHOR CONTRIBUTIONS

QLi and XL conceptualized and designed the study. QLiu and ZS conducted the analyses of the study. QLiu, ZS, and DL interpreted the data. QLiu drafted the manuscript. QLiu, ZS, and DL revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.574772/full#supplementary-material

Supplementary Figure 1 | Hazard ratio comparing the cause-specific survival (CSS) between chemotherapy (CT) and non-CT groups according to the *P* score in the overall cohort before propensity score matching (PSM). (2*) Including *P* scores 0, 1, and 2. (#) Multivariate analysis adjusted by the year of diagnosis, race, gender, tumor location, histology, T stage (including T3, T4a, or T4b), age at diagnosis, tumor size, and tumor grade.

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ORIGINAL RESEARCH
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Survival Benefit of Crossover Administration of Regorafenib and Trifluridine/Tipiracil Hydrochloride for Patients With Metastatic Colorectal Cancer: Exploratory Analysis of a Japanese Society for Cancer of the Colon and Rectum Multicenter Observational Study (REGOTAS)

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Background: The survival benefits of regorafenib (REG) and trifluridine/tipiracil hydrochloride (TFTD) have been demonstrated in chemorefractory patients with metastatic colorectal cancer (mCRC). However, the effects of crossover administration of REG and TFTD on patient survival remain unclear. The present study evaluated the

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association between exposure to REG and TFTD and overall survival (OS) in patients with mCRC using data from the REGOTAS study.

Patients and Methods: We analyzed patients registered in the REGOTAS study, which retrospectively compared the efficacy and safety of use of REG or TFTD as later-line chemotherapy for chemorefractory mCRC patients. We compared the survival outcomes of cohort A (treated using both REG and TFTD) and cohort B (treated using either REG or TFTD).

Results: A total of 550 patients (cohort A, $n = 252$; cohort B, $n = 298$) met the inclusion criteria. The median OS was significantly increased in cohort A compared with cohort B [9.6 months (95% confidence interval (CI), 8.9–10.9 months) vs. 5.2 months (95% CI, 4.4–6.0 months), $P < 0.001$]. Multivariate analysis revealed that cohort A was independently associated with a significant increase in OS [A vs. B: Hazard ratios (HR), 0.58; 95% CI, 0.47–0.72; $P < 0.001$]. Subgroup analysis adjusted using multivariate Cox model revealed a consistently better trend in most subgroups for cohort A compared with cohort B.

Conclusions: Our study revealed prolonged survival in patients treated with REG and TFTD. Therefore, all active agents, including REG and TFTD, should be made available to mCRC patients.

Keywords: regorafenib, trifluridine/tipiracil hydrochloride, colorectal cancer, prognosis, chemotherapy – oncology

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide (1). The development of combination chemotherapy regimens involving cytotoxic agents [such as fluoropyrimidine (FU), oxaliplatin (OX), and irinotecan (IRI)] and molecular targeted therapies (such as bevacizumab, ramucirumab, ziv-aflibercept, cetuximab, and panitumumab) has increased the survival of metastatic CRC (mCRC) patients by around 30 months (2–8). In the CORRECT and RECOURSE phase III trials, the active agents, regorafenib (REG) and trifluridine/tipiracil hydrochloride (TFTD), significantly improved overall survival (OS) in patients with chemorefractory mCRC (9, 10).

The strategic availability of the active ingredients FU, OX, and IRI for all mCRC patients suitable for systemic chemotherapy maximizes OS (11). However, there are few reports of the benefits of using both REG and TFTD as a salvage therapy to improve OS in mCRC patients (12).

We previously reported the REGOTAS study, which was a multicenter, large cohort, observational study, showed no significant difference in OS between treatment using REG and TFTD in patients with mCRC. The present study compared patients treated using both REG and TFTD with those treated with either REG or TFTD alone in the REGOTAS study to assess the effects of exposure to REG and TFTD on OS in patients with mCRC who received FU, OX, IRI, and bevacizumab, as well as anti-EGFR antibody (in patients with wild type *KRAS*/*NRAS* tumors).

METHODS

Patients

The present study retrospectively examined the clinical records of patients with mCRC treated with later-line chemotherapy comprising REG or TFTD during the period from June 1, 2014 to November 30, 2015 in the participating institutions. All patients were registered in the REGOTAS study, which is described in detail elsewhere (13). The main eligibility criteria were: (1) histologically confirmed colorectal adenocarcinoma; (2) no prior treatment using REG and TFTD; (3) previous treatment with FU, OX, IRI, bevacizumab, and anti-EGFR antibody (in patients with wild type *KRAS*/*NRAS* tumors); (4) Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; and (5) adequate organ function. The present study was approved by the ethics committees at each institution and was in accordance with the guidelines for biomedical research specified in the Declaration of Helsinki. The REGOTAS study was registered with the University Medical Information Network (number UMIN000020416). The requirement for informed consent was waived due to the retrospective design of this study.

Statistical Analysis

The exploratory primary endpoint was OS of all patients stratified by exposure to REG and/or TFTD as follows: cohort A (both REG and TFTD) and cohort B (either REG or TFTD). The following pretreatment clinical data and baseline laboratory values were used in the analysis as covariates: age, sex, body mass index, ECOG PS, primary tumor site, surgery on primary tumor, RAS status, metastatic tumor site (liver

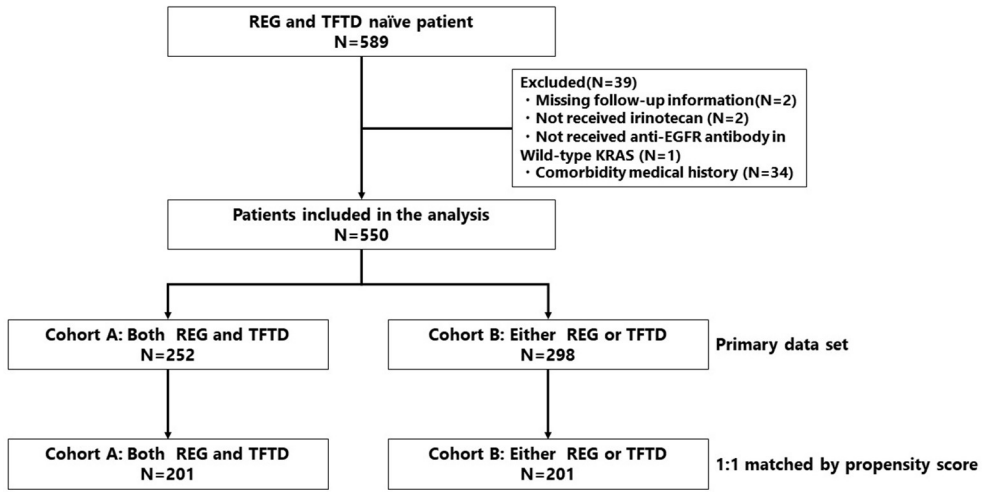

FIGURE 1 | Patient selection flow diagram.

metastasis, lung metastasis, lymph node metastasis, peritoneal dissemination, and bone metastasis), number of metastatic sites, pathologic type, time from initiation of first-line chemotherapy to initiation of later-line treatment, serum albumin, serum aspartate transaminase (AST), serum C-reactive protein (CRP), and serum carcinoembryonic antigen (CEA). Each cutoff value of quantitative data was set with reference to that of albumin, AST, CRP, AST, and CEA in the REGOTAS study (13).

OS was defined as the time from the start of initial REG or TFTD to death or last follow-up. Quantitative data are expressed as median and interquartile range (IQR). The Mann–Whitney *U*-test was used to compare the continuous variables, and Fisher's exact test was performed to compare the categorical variables. Survival curves were estimated using the Kaplan–Meier method, and differences between the groups were tested by the log-rank test. Hazard ratios (HRs) were estimated using the Cox proportional hazard model. OS was analyzed using univariate and multivariate Cox regression analyses. The backward selection method was conducted for the selection of factors retained ($P < 0.2$) in the multivariate analysis. The predictive factor for OS between each group was explored using subgroup analyses with the multivariate Cox model including interaction terms.

A 1:1 matching using the propensity score (propensity score-matched dataset) was performed as a sensitivity analysis. Patients in the two groups were matched by a difference of propensity score within 0.05. The propensity score was calculated with a multivariate logistic regression model including 19 prognostic variables (**Supplementary Table 1**). All *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients

Among 589 mCRC patients, 550 met the inclusion criteria (cohort A, $n = 252$; cohort B, $n = 298$) (**Figure 1**). The patient characteristics are summarized in **Table 1**. Significant differences between cohorts A and B were found for the following factors: ECOG PS 0 (49 vs. 33%, respectively; $P < 0.001$), lymph node metastasis (37 vs. 48%, respectively; $P = 0.012$), peritoneal dissemination (12 vs. 24%, respectively; $P < 0.001$), number of metastatic organ sites ≥ 2 (71 vs. 79%, respectively; $P = 0.007$), baseline serum albumin <3.5 g/dL (29 vs. 58%, respectively; $P < 0.001$), baseline serum AST ≥40 IU/L (20 vs. 42%, respectively; $P < 0.001$), and baseline serum CRP ≥1 mg/dL (34 vs. 52%, respectively; $P < 0.001$). All patients received FU, OX, IRI, and bevacizumab, and all patients with wild type *KRAS*/*NRAS* tumors received anti-EGFR antibody.

Efficacy

The median follow-up at the time of analysis was 17.3 months [95% confidence interval (CI), 16.1–18.0 months]. The median OS for all patients was 6.8 months (95% CI, 3.4–11.5 months), and 418 (76%) patients had died. The median follow-up was significantly longer in cohort A compared with cohort B (17.6 vs. 15.2 months, respectively; $P < 0.001$). The median OS was significantly greater in cohort A compared with cohort B [9.6 (95% CI, 8.9–10.9) months vs. 5.2 months (95% CI, 4.4–6.0 months), respectively; $P < 0.001$] (**Figure 2A**). There was no significant difference in OS between patients receiving REG followed by TFTD and TFTD followed by REG in cohort A [10.5 months (95% CI, 9.2–12.2 months) vs. 9.4 months (95% CI, 8.3–10.8 months), $P = 0.52$] (**Figure 2B**).

Table 2 shows the results of univariate and multivariate analyses for OS. In these analyses, the factors significantly

TABLE 1 | Patient characteristics.

	cohort A ($n = 252$)		cohort B ($n = 298$)		*P*-value*
Age, year					
Median (IQR)	65	(57–71)	64	(54–69)	0.066
≥65 years, *n* (%)	110	(44)	153	(51)	0.087
Sex, *n* (%)					0.543
Male	144	(57)	179	(60)	
Female	108	(43)	119	(40)	
Body mass index, *n* (%)					0.417
<18.5 kg/m^2	37	(17)	52	(17)	
≥18.5 kg/m^2	215	(83)	246	(83)	
ECOG PS, *n* (%)					<0.001
0	124	(49)	99	(33)	
1 or 2	128	(51)	199	(67)	
Primary tumor site, *n* (%)					0.758
Right	54	(21)	68	(23)	
Left	198	(79)	230	(77)	
Surgery on primary tumor site, *n* (%)					
Yes	45	(18)	74	(25)	0.061
Pathologic type, *n* (%)					0.334
Well-moderately differentiated adenocarcinoma	226	(90)	268	(90)	
Others	14	(5)	22	(7)	
Missing data	12	(5)	8	(3)	
***RAS* status, *n* (%)**					0.261
WT	131	(52)	137	(46)	
MT	118	(47)	152	(51)	
Missing data	3	(1)	9	(3)	
Metastatic organ site, *n* (%)					
Liver	158	(63)	184	(62)	0.860
Lung	168	(67)	195	(65)	0.787
Lymph node	94	(37)	143	(48)	0.012
Peritoneal dissemination	30	(12)	72	(24)	<0.001
Bone	17	(7)	36	(12)	0.049
Number of metastatic organ site(s), *n* (%)					0.055
1	72	(29)	63	(21)	
≥2	180	(71)	235	(79)	
Drug exposure, *n* (%)					1.000
Fluoropyrimidine	252	(100)	298	(100)	
Oxaliplatin	252	(100)	298	(100)	
Irinotecan	252	(100)	298	(100)	
Bevacizumab	252	(100)	298	(100)	
Anti-EGFR antibody (in patients with wild type *KRAS/NRAS* tumors)	131	(100)	137	(100)	
Intolerable drug, *n* (%)					
Any drugs	79	(31)	96	(32)	0.900
Fluoropyrimidine	4	(2)	22	(7)	0.003
Oxaliplatin	66	(26)	77	(26)	1.000
Irinotecan	8	(3)	28	(9)	0.006
Bevacizumab	11	(4)	31	(10)	0.013
Anti-EGFR antibody (in patients with wild type *KRAS/NRAS* tumors)	5	(2)	10	(3)	0.471
Prior regimens, *n* (%)					0.892
≥3	125	(50)	145	(49)	
Time since initiation of first-line chemotherapy, *n* (%)					0.152
<18 months	59	(23)	87	(29)	
≥18 months	193	(77)	211	(71)	
Baseline albumin, *n* (%)					
<3.5 g/dL	72	(29)	172	(58)	<0.001
Missing	6	(2)	9	(3)	
Baseline serum AST, *n* (%)					
≥40 IU/L	50	(20)	107	(36)	<0.001
Missing data	2	(1)	1	(0.5)	
Baseline CRP, *n* (%)					
≥1 mg/dL	85	(34)	156	(52)	<0.001
Missing data	7	(3)	9	(3)	
Baseline serum CEA, *n* (%)					
≥5 ng/mL	218	(87)	270	(91)	0.253
Missing data	3	(1)	4	(1)	

**P-values were calculated by Fisher's exact probability test for categorical variables. IQR, interquartile range; EGFR, epidermal growth factor receptor; RAS, rat sarcoma; AST, aspartate transaminase; CEA, carcinoembryonic antigen; WT, wild-type; MT, mutant.*

associated with OS were cohort (A vs. B: HR, 0.58; 95% CI, 0.47–0.72; $P < 0.001$), ECOG PS (1–2 vs. 0: HR, 1.44; 95% CI, 1.16–1.78; $P = 0.001$), albumin (≥3.5 vs. <3.5 g/dL: HR, 0.77; 95% CI, 0.63–0.94; $P = 0.012$); AST (≥40 vs. <40 IU/L: HR, 1.48; 95% CI, 1.24–1.76; $P < 0.001$), CRP (≥1.0 vs. <1.0 mg/dL: HR, 1.84; 95% CI, 1.46–2.32; $P < 0.001$), CEA (≥ 5 vs. <5 ng/mL: HR, 1.69; 95% CI, 1.16–2.47; $P = 0.006$), liver metastasis (Yes vs. No: HR, 1.51; 95% CI, 1.17–1.94; $P = 0.001$), peritoneal dissemination (Yes vs. No: HR, 1.38; 95% CI, 1.05–1.82; $P = 0.023$), and time since initiation of first-line chemotherapy (≥18 vs. <18 months: HR, 0.64; 95% CI, 0.50–0.81; $P < 0.001$).

In the subgroup analysis adjusted using the multivariate Cox model, cohort A demonstrated consistently better trends in almost all subgroups examined compared with cohort B (**Figure 3**).

Safety and Toxicity

Safety and toxicity are shown in **Table 3**. There was no significant difference in incidence of grade ≥3 hematologic toxicities between cohorts A and B, except for anemia (5 vs. 11%, respectively; $P = 0.019$). Additionally, for nonhematologic toxicities, incidence of grade ≥3 anorexia was higher in cohort B than cohort A (2 vs. 8%, respectively; $P = 0.001$), whereas the incidence of hand–foot skin reaction was higher in cohort A than cohort B (13 vs. 4%, respectively; $P < 0.001$).

TABLE 2 | Univariate and multivariate analyses of overall survival (OS).

Variable	Category	Univariate				Multivariate			
		HR	Lower	Upper	*P*-value*	HR	Lower	Upper	*P*-value*
Treatment	Cohort A vs. cohort B	0.48	0.40	0.59	<0.001	0.58	0.47	0.72	<0.001
Age	≥65 vs. <65	1.22	1.01	1.48	0.044	1.17	0.96	1.43	0.127
Gender	Male vs. Female	0.95	0.79	1.16	0.634				
Body mass index	<18.5 kg/m^2 vs. ≥18.5	0.94	0.72	1.22	0.623				
ECOG PS	1 or 2 vs. 0	1.64	1.35	2.01	<0.001	1.44	1.16	1.78	0.001
Primary tumor site	Left vs. Right	0.79	0.63	0.99	0.042	0.88	0.69	1.13	0.315
Surgery on primary tumor site	Yes vs. No	0.60	0.48	0.76	<0.001	0.80	0.62	1.02	0.071
Pathologic type	Well-moderately differentiated vs. others	0.97	0.64	1.46	0.874				
Baseline serum albumin	>3.5 g/dL vs. <3.5	0.51	0.43	0.62	<0.001	0.77	0.63	0.94	0.012
Baseline serum AST	≥40 IU/L vs. <40	1.84	1.53	2.22	<0.001	1.48	1.24	1.76	<0.001
Baseline serum CRP	≥1.0 mg/dL vs. <1.0	1.68	1.44	1.95	<0.001	1.84	1.46	2.32	<0.001
Baseline serum CEA	≥5 ng/mL vs. <5	1.85	1.38	2.49	<0.001	1.69	1.16	2.47	0.006
Liver metastasis	Yes vs. No	1.65	1.35	2.03	<0.001	1.51	1.17	1.94	0.001
Lung metastasis	Yes vs. No	0.84	0.69	1.03	0.089	0.95	0.75	1.49	0.691
Lymph node metastasis	Yes vs. No	1.40	1.15	1.70	<0.001	1.18	0.94	1.49	0.150
Peritoneal dissemination	Yes vs. No	1.52	1.20	1.93	<0.001	1.38	1.05	1.82	0.023
Bone	Yes vs. No	0.96	0.70	1.34	0.829				
Number of metastatic organ sites	≥2 vs. 1	1.48	1.18	1.87	0.001	1.05	0.76	1.43	0.777
Prior regimens	≥3 *vs.* <3	0.85	0.70	1.04	0.108	0.79	0.61	1.03	0.078
Time since initiation of first-line chemotherapy	≥18 months vs. <18	0.63	0.51	0.78	<0.001	0.64	0.50	0.81	<0.001
RAS status	MT vs. WT	1.18	0.99	1.41	0.067	0.91	0.71	1.16	0.455

**P-values were calculated using the Cox proportional-hazards model.*

RAS, rat sarcoma; AST, aspartate transaminase; CEA, carcinoembryonic antigen; WT, wild-type; MT, mutant.

Sensitivity Analysis

A total of 201 patients per group were matched by propensity score. Patients' characteristics were well-balanced between the two groups (**Supplementary Table 2**), and the median OS was found to be significantly longer in cohort A compared with that of cohort B [9.3 months (95% CI, 8.2–10.5 months) vs. 5.3 months (95% CI, 4.8–6.7 months), $P < 0.001$] as in the observational dataset (**Supplementary Figure 1**). The incidence of grade ≥3 toxicity was also similar to that in the observational dataset, except for the incidence of hand–foot skin reaction (**Supplementary Table 3**).

DISCUSSION

To the best of our knowledge, this is the first study to report the beneficial effects of the crossover administration of REG and TFTD on survival of patients with chemorefractory mCRC. Although increased exposure to standard chemotherapy agents, such as FU, OX, and IRI, and molecular targeting agents, including bevacizumab and anti-EGFR antibodies, contribute to a prolongation of OS (11, 14), our findings suggested that making all key active agents, including REG and TFTD, available could further improve OS in mCRC patients.

It has not previously been shown that treatment with both REG and TFTD contributes to longer OS compared with use of either REG or TFTD alone in patients with chemorefractory mCRC. The CORRECT trial did not include patients who had previously received TFTD, and the RECOURSE trial included only 18% of patients who had previously received REG (9, 10). Furthermore, details of post-study treatments were not reported in either of these phase III studies. Our findings indicated that treatment with both REG and TFTD improved OS compared with use of either REG or TFTD alone in mCRC patients who were refractory or intolerant to standard chemotherapy, irrespective of the subgroup. While the optimal sequential order of REG and TFTD therapy remains unclear, there was no significant difference in OS between the patients in cohort A who received TFTD followed by REG or REG followed by TFTD. These data support the findings from an Italian retrospective study in which patients received both REG and TFTD that showed that the median OS was 12.8 months (95% CI, 10.2–14.4 months) for REG followed by TFTD and 10.3 months (95% CI, 8.7–14.4 months) for TFTD followed by REG (15).

The differences observed in patients' characteristics indicate the requirement for both REG and TFTD to be made available to patients with chemorefractory mCRC. In the present study, patients with ECOG PS 1 or 2, peritoneal dissemination, albumin <3.5 g/dL, AST ≥40 IU/L, or CRP ≥1 mg/dL had fewer chances to receive both REG and TFTD. The consistent efficacy of crossover administration of REG and TFTD irrespective of the subgroup highlights that exposure to both REG and TFTD contributes to improved OS in patients with poor prognostic factors.

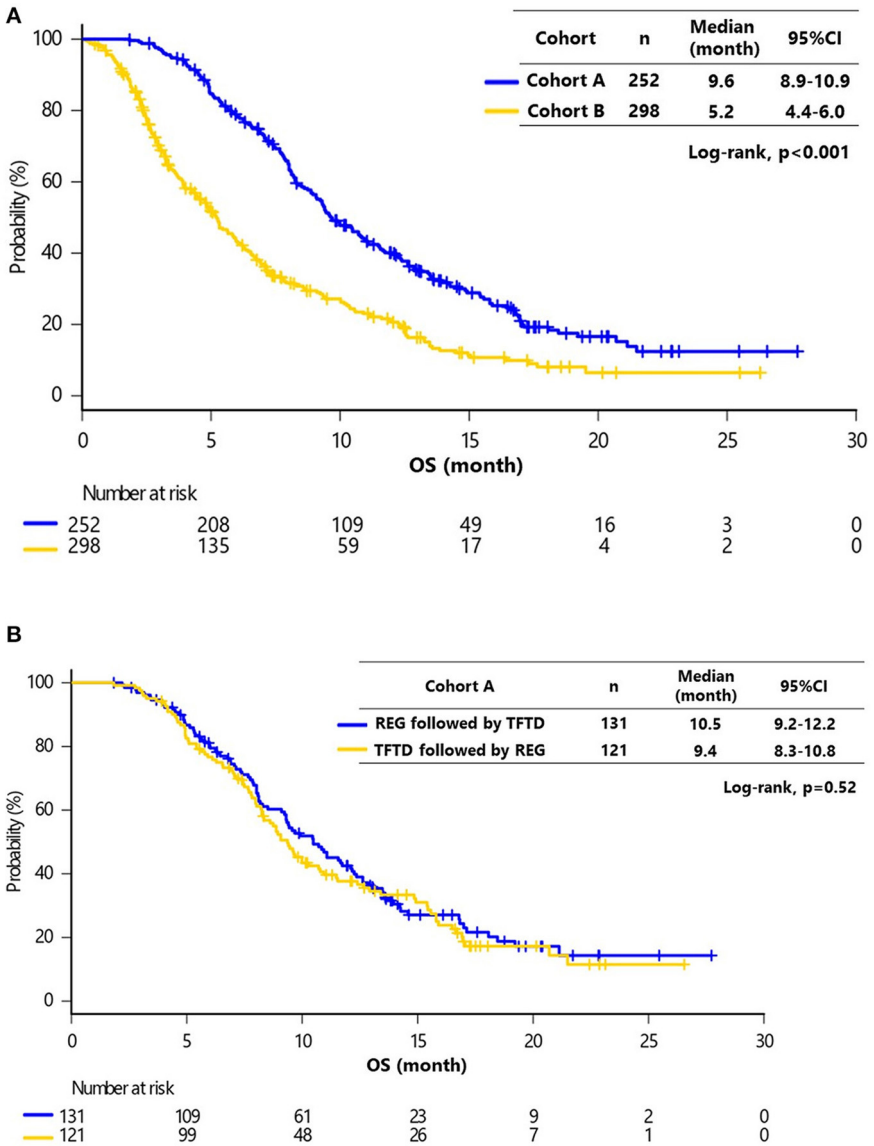

FIGURE 2 | (A) Kaplan–Meier curves of overall survival (OS) in cohorts A and B. The median OS of cohorts A and B were 9.6 months (95% CI, 8.9–10.9) and 5.2 months (95% CI, 4.4–6.0), respectively (Log-rank, $P < 0.001$). **(B)** Kaplan–Meier curves of OS in cohort A (REG followed by TFTD vs. TFTD followed by REG). The median OS values of REG followed by TFTD and TFTD followed by REG were 10.5 months (95% CI, 10.2–16.1) and 9.4 months (95% CI, 7.3–17.3), respectively (Log-rank, $P = 0.52$).

It is important to note that the nature of this analysis may have led to an inherent bias. In particular, patients who live longer have a greater opportunity to be treated with more lines of chemotherapies. Furthermore, patients with poor ECOG PS or a shorter life expectancy may have been excluded from receiving the salvage-line chemotherapy of REG and/or TFTD. Therefore, we only analyzed patients who were refractory or intolerant to standard chemotherapies [FU, OX, IRI, bevacizumab, and anti-EGFR antibody (if the patients had wild type *KRAS*/ *NRAS* tumor)] in order to minimalize the inherent bias. Multivariate analysis of prognostic factors also demonstrated that crossover administration of REG and TFTD was independently associated with significant OS prolongation. In addition, subgroup analysis adjusted by the multivariate Cox model revealed that cohort A consistently demonstrated better trends in almost all subgroups examined compared with cohort B. These findings highlight the importance of making active agents, including REG and TFTD, available to all patients.

However, while making these active agents available is a valuable treatment strategy, the OS of these patients remains unsatisfactory and warrants further improvement. A promising

efficacy of TFTD with bevacizumab was previously reported in a phase I–II trial (C-task force) (16), and was recently replicated in retrospective and prospective studies (17, 18). In addition, the combination of REG with nivolumab showed manageable toxicities and encouraging antitumor activity in microsatellite stable mCRC patients (19). We believe that these combination therapies are effective strategies to prolong OS in patients with chemorefractory mCRC.

The present study had some limitations that should be considered when interpreting the results. First, this was a non-randomized retrospective study with a limited sample size. Second, all patients enrolled in this study were Japanese. However, the absence of ethnic differences in the analysis of the efficacy of REG and TFTD in the phase III trials could enable the results to be applied to all patients, regardless of ethnicity (9, 10, 20, 21).

TABLE 3 | Frequency of treatment-related grade ≥3 adverse events (AE).

Variable	cohort A (n = 252)		cohort B (n = 298)		P-value*
Hematologic toxicities, *n* (%)					
Any	67	(27)	91	(31)	0.355
Neutropenia	50	(20)	63	(21)	0.674
Anemia	13	(5)	33	(11)	0.019
Thrombocytopenia	12	(5)	13	(4)	0.985
Non-hematologic toxicities, *n* (%)					
Any	65	(26)	80	(27)	0.856
Fatigue	3	(1)	12	(4)	0.076
Anorexia	4	(2)	24	(8)	0.001
Febrile neutropenia	3	(1)	6	(2)	0.674
Hand-foot skin reaction	32	(13)	12	(4)	<0.001
Liver dysfunction	14	(6)	14	(5)	0.794

**P-values were calculated by Fisher's exact probability test for categorical variables.*
AE, adverse event.

CONCLUSIONS

Our multicenter retrospective study revealed the survival benefits of crossover administration of REG and TFTD. Our findings highlight the importance of making all active agents, including REG and TFTD, available to patients with mCRC.

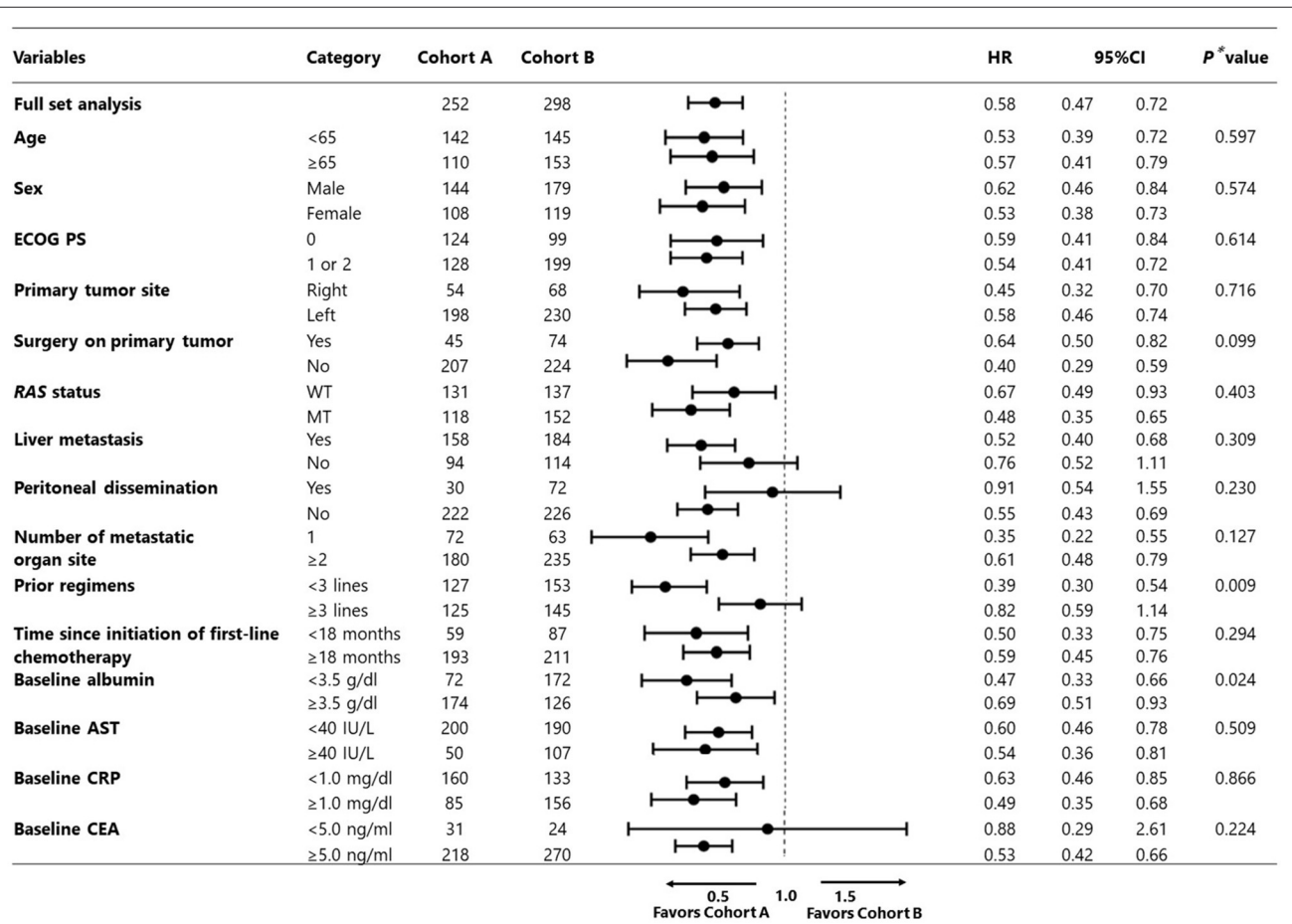

FIGURE 3 | Subgroup analysis adjusted by multivariate Cox model (cohort A vs. B).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Cancer Center Hospital East and each participating facilities. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KC, DK, and YS: conception and design and development of methodology. SF, TMo, AT, YK, TKaj, KYamaz, MK, AM, TD, YH, TS, NS, ME, TI, TKas, KA, SY, YO, HK, DS, KO, TT, and KYamas: acquisition of data. KC, DK, TMo, and MG: analysis and interpretation of data. KC, DK, and TMo: writing, review and/or revision of the manuscript. TMo and YS: study supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.576036/full#supplementary-material

Supplementary Figure 1 | Kaplan–Meier curves of overall survival (OS) in the propensity score-matched dataset (cohort A vs cohort B). The median OS of cohort A and B were 9.3 months (95% CI, 8.2–10.5 months) and 5.3 months (95% CI, 4.8–6.7 months), respectively ($P < 0.001$).

Supplementary Table 1 | Variables used for calculating propensity score.

Supplementary Table 2 | Comparison of patient characteristics between regorafenib and TFTD groups in the propensity score-matched dataset.

Supplementary Table 3 | Frequency of treatment-related grade≥3 AE in the propensity score-matched dataset.

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Neoadjuvant Chemoradiotherapy Does Not Contribute to Worse Survival in Pathological Node-Negative Rectal Cancer

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Purpose: The prognostic significance of ypN0 rectal cancer with comparison to pN0 disease still remains poorly defined. This study aimed to compare the prognosis of ypN0 and pN0 rectal cancer.

Methods: Eligible patients were identified from the SEER18 registries research database (the latest data up to date was on April 15, 2019). Propensity score (PS) matching was usually performed to reduce the imbalance and potential confounding that were introduced by inherent differences between the groups. The cause-specific survival (CSS) was analyzed to evaluate the prognostic prediction of ypN0 and pN0 groups using the Kaplan–Meier method with the log-rank test. Cox proportional hazard model was also used to identify independent prognostic variables.

Results: In total, 26,832 patients diagnosed with pN0 or ypN0 rectal cancer were confirmed as the final cohort, including 7,237 (27.0%) patients with radiation and 19,595 (73.0%) patients without radiation prior to surgery. The median follow-up time was up to 81 months. After adjusting for other prognostic factors, neoadjuvant radiotherapy was not an independent prognostic variable of CSS (HR = 1.100, 95%CI = 0.957–1.265, $P = 0.180$, using pN0 group as the reference).

Conclusions: ypN0 rectal cancer was strongly associated with worse pathological diagnoses compared with pN0 rectal cancer, contributing to worse oncologic outcomes. However, the receipt of neoadjuvant chemoradiotherapy was not an independent prognostic factor of worse prognosis in pathological node-negative patients. Our study could give guidance to the treatment of ypN0 rectal cancer.

Keywords: neoadjuvant chemoradiotherapy, rectal cancer, ypN0, pN0, propensity score matching

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INTRODUCTION

Colorectal cancer was one of the most frequently diagnosed malignances around the world (1, 2). Due to the different anatomical location characteristics of the rectum from colon, the treatment of rectal cancer is more complex.

Currently, neoadjuvant chemoradiotherapy followed by total mesorectal excision (TME) has been widely accepted as the standard treatment for locally advanced rectal cancer (3, 4). And the histopathological evaluation of TME resection specimens played a vital role in evaluating the prognosis of rectal cancer after neoadjuvant chemoradiotherapy, which was highly dependent on the accurate assessment of postoperative lymph node status (5).

Previous studies had shown that lymph node-negative rectal cancer after neoadjuvant chemoradiation therapy (ypN0) was associated with an excellent prognosis, and the 5-year disease-free survival ranged from 79.8 to 87% (6–8). Later in 2014, with a retrospective analysis of a total of 473 patients diagnosed with rectal cancer, Erlenbach-Wünsch et al. (9) found that ypN0 rectal cancer could achieve similar oncologic results compared with pN0 disease, which suggested that adjuvant chemotherapy for ypN0 might result in overtreatment. However, this study had just a small sample size and needs to be validated in other studies, and the prognostic significance of ypN0 rectal cancer with comparison to pN0 disease still remains poorly defined (9). Here, therefore, using the newly released large population-based cancer database, we conducted this propensity score (PS) matched study to compare the prognosis of ypN0 and pN0 rectal cancer.

METHODS

Ethics

The present study complied with the Declaration of Helsinki. All authors reviewed and approved the final edition of this manuscript. The US Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute (NCI) was an open public database, and the release of data from the SEER database did not require informed patient consent because cancer was a reportable disease in every state of the USA.

Patients

As a population-based cancer registration system, the US SEER database of the NCI provides different datasets on cancer demographic information and survival, covering approximately 28% of US populations. Using the SEER* Stat 8.3.5 software, we identified patients from the SEER18 registries research database (the latest data up to date was on April 15, 2019). The SEER18 database contained data from the SEER9 registries, the SEER13 registries (SEER 9 plus Los Angeles, San Jose-Monterey, Rural Georgia, and the Alaska Native Tumor Registry), and the registries of Greater California, Kentucky, Louisiana, New Jersey, and Greater Georgia (10). Patients' characteristics including No. of LNs dissected, American Joint Committee on Cancer *T*-stage (T1, T2, T3, and T4), age at diagnosis (years), race (white, black, and other), gender (male and female), year of diagnosis (2004–2011), tumor site (rectosigmoid primary and rectal primary), grade (well/Moderate, poor/anaplastic, and unknown), chemotherapy, serum carcinoembryonic antigen (CEA) level (negative, positive, and unknown), tumor size ($\leq$5, $>$5 cm, and unknown) and perineural invasion (no, yes, and unknown) were obtained from the SEER database.

As shown in **Figure 1**, at first, a total of 74,688 patients diagnosed with rectal cancer between 2004 and 2011 were identified from the Surveillance, Epidemiology, and End Results (SEER) database. Then, patients with surgery performed, active follow-up, positive histological confirmation, and pathological N0 status were included into our analyses. Those with non-adenocarcinoma histologies, unknown TNM stage, unknown

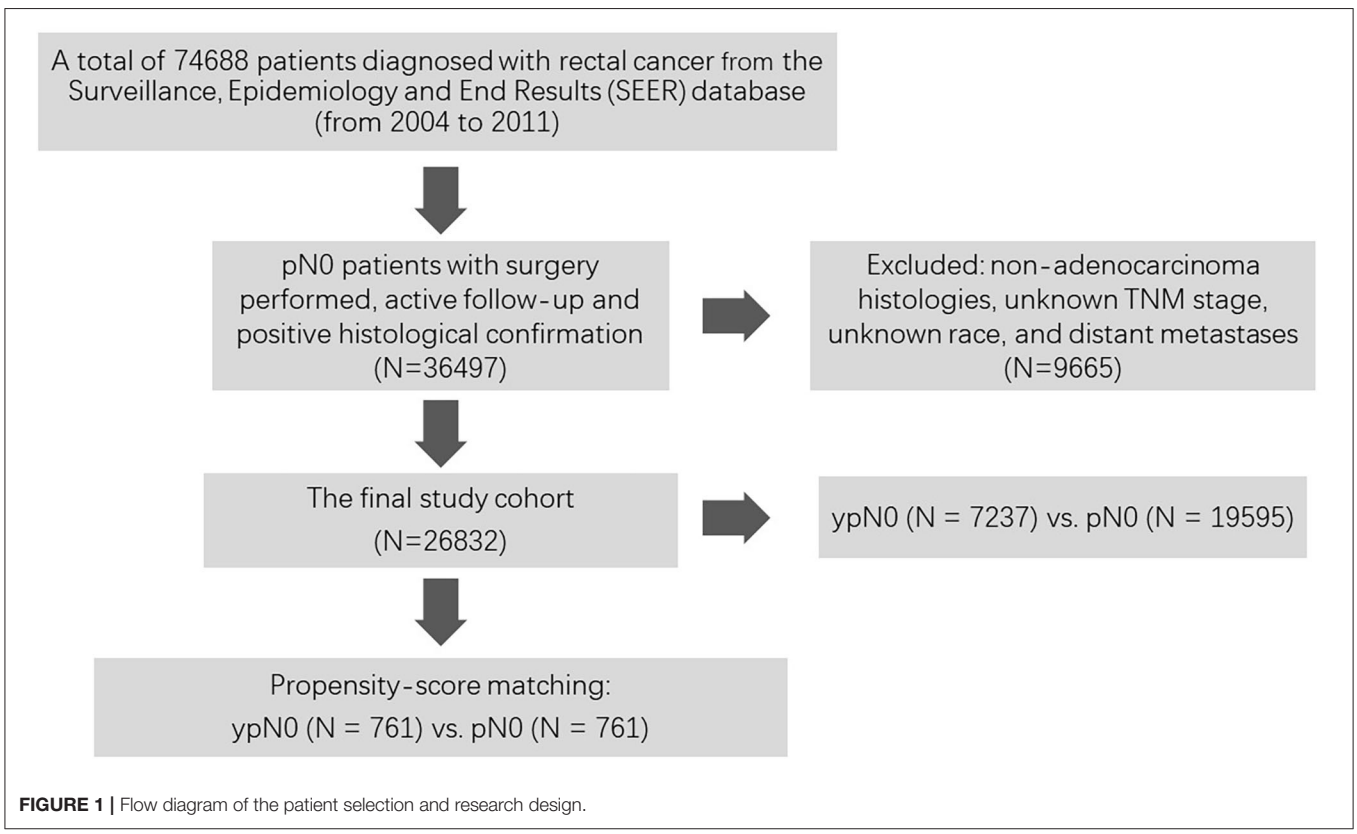

FIGURE 1 | Flow diagram of the patient selection and research design.

race, and distant metastases were excluded from the present study. Among them, patients with (n = 7,237) or without (n = 19,595) radiation prior to surgery were confirmed as the final cohort.

Propensity-Score Matching

In the analyses of retrospective cohort without randomization, propensity score (PS) matching was usually performed to reduce the imbalance and potential confounding that were introduced by inherent differences between the groups (11). In the present study, one to one PS matching was also used to reduce selection bias in patient characteristics between ypN0 and pN0 groups based on the following covariates: No. of LNs dissected, American Joint Committee on Cancer T stage (T1, T2, T3, and T4), age at diagnosis (years), race (white, black, and other), gender (male and female), year of diagnosis (2004–2011), tumor site (rectosigmoid primary, and rectal primary), grade (well/Moderate, poor/anaplastic, and unknown), chemotherapy, serum carcinoembryonic antigen (CEA) level (negative, positive, and unknown), tumor size ($\leq$ 5, $>$ 5 cm, and unknown) and perineural invasion (no, yes, and unknown). PS matching was performed based on nearest-neighbor matching, propensity scores reflected the probability that patients would be in ypN0 and pN0 groups based on their baseline characteristics. Once the propensity scores were estimated, patients in the pN0 group were matched to patients with radiation prior to the surgery. The histograms of propensity score before and after PS matching were shown in **Figure 2**. Finally, 761 matched pairs (761 patients in ypN0 group and 761 patients in pN0 group) were selected from the whole cohort (n = 26,832).

Statistical Analyses

The differences in the baseline characteristics between the ypN0 and pN0 groups were analyzed using the Pearson's chi-square test. The causes of death in the present study were categorized as rectal cancer specific or non–rectal cancer related. Rectal cancer cause-specific survival (CSS) was calculated from the date of diagnosis to the date of death due to rectal cancer. However, patients who died of other causes were censored at the date of death.

In our analyses, the CSS was analyzed to evaluate the prognostic prediction of ypN0 and pN0 groups using the Kaplan–Meier method with the log-rank test. The prognostic variables were entered in the multivariable analyses using the Cox proportional hazard model to identify independent prognostic variables. All the hazard ratios (HRs) were shown with 95% confidence intervals (CI). All tests were two sided, and two-sided P-values < 0.05 were considered to be statistically significant in

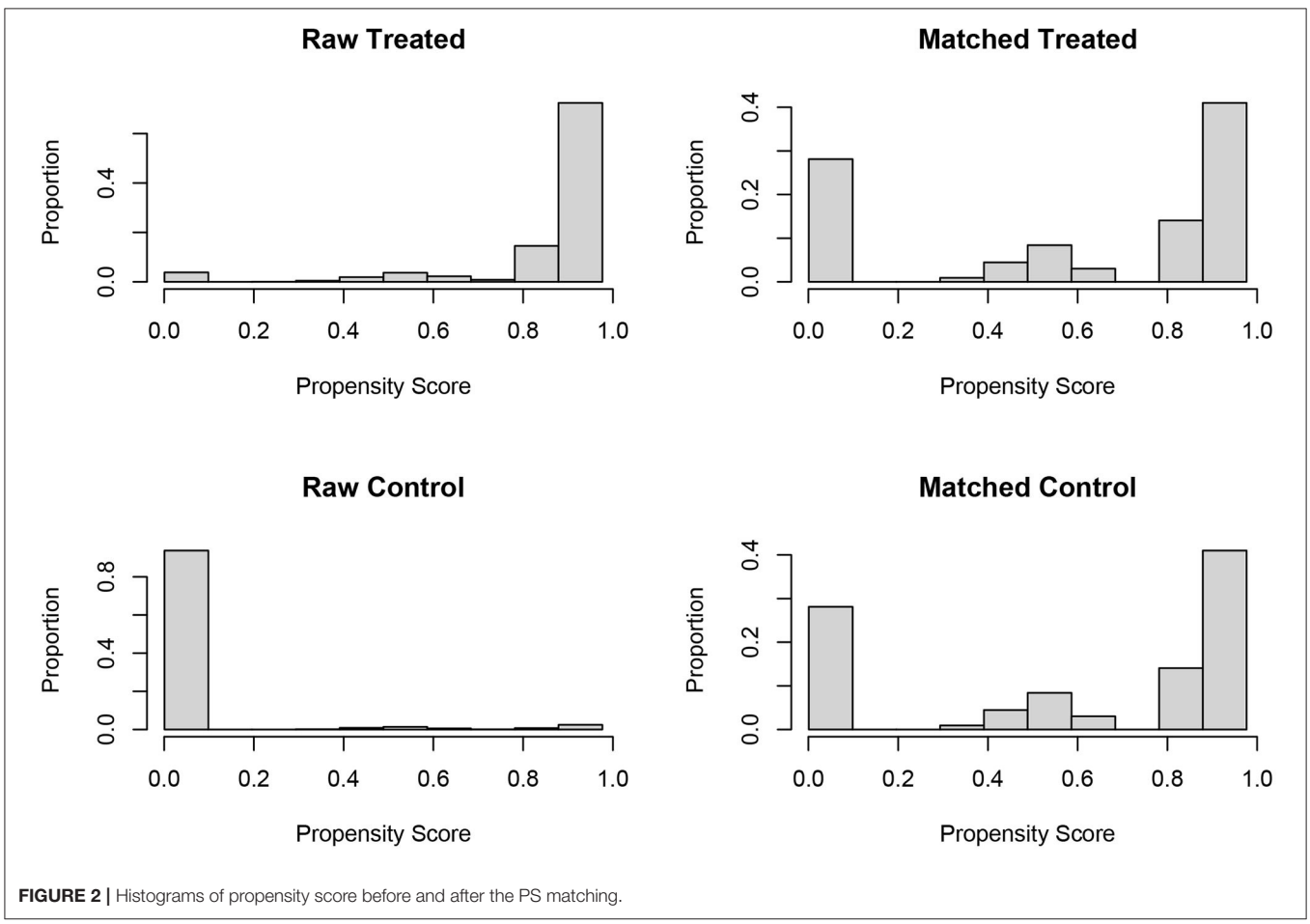

FIGURE 2 | Histograms of propensity score before and after the PS matching.

TABLE 1 | Patients' baseline characteristics before PSM.

Variables	No. of Patients (%)		*P*
	pN0 (19,595)	ypN0 (7,237)	
No. of LNs dissected			0.014
<12	10,961 (55.9)	4,169 (57.6)	
≥12	8,634 (44.1)	3,068 (42.4)	
T-stage			<0.001
T1	8,588 (43.8)	936 (12.9)	
T2	5,351 (27.3)	1,439 (19.9)	
T3	5,085 (26.0)	4,374 (60.4)	
T4	571 (2.9)	488 (6.7)	
Age at diagnosis (years)			<0.001
≤65	8,490 (43.3)	4,366 (60.3)	
>65	11,105 (56.7)	2,871 (39.7)	
Race			0.018
White	16,390 (83.6)	5,951 (82.2)	
Black	1,521 (7.8)	596 (8.2)	
Other	1,684 (8.6)	690 (9.5)	
Gender			<0.001
Male	10,872 (55.5)	4,555 (62.9)	
Female	8,723 (44.5)	2,682 (37.1)	
Year of diagnosis			<0.001
2004	2,620 (13.4)	703 (9.7)	
2005	2,598 (13.3)	766 (10.6)	
2006	2,481 (12.7)	877 (12.1)	
2007	2,480 (12.7)	987 (13.6)	
2008	2,490 (12.7)	920 (12.7)	
2009	2,358 (12.0)	1,022 (14.1)	
2010	2,349 (12.0)	1,051 (14.5)	
2011	2,219 (11.3)	911 (12.6)	
Tumor site			<0.001
Rectosigmoid primary	7,483 (38.2)	691 (9.5)	
Rectal primary	12,112 (61.8)	6,546 (90.5)	
Grade			<0.001
Well/moderate	16,300 (83.2)	5,675 (78.4)	
Poor/anaplastic	1,656 (8.5)	733 (10.1)	
Unknown	1,639 (8.4)	829 (11.5)	
Chemotherapy			<0.001
No/unknown	18,377 (93.8)	278 (3.8)	
Yes	1,218 (6.2)	6,959 (96.2)	
Serum CEA level			<0.001
Negative	6,566 (33.5)	2,631 (36.4)	
Positive	2,437 (12.4)	1,744 (24.1)	
Unknown	10,592 (54.1)	2,862 (39.5)	
Tumor size			<0.001
≤5 cm	12,156 (62.0)	4,141 (57.2)	
>5 cm	2,701 (13.8)	1,120 (15.5)	
Unknown	4,738 (24.2)	1,976 (27.3)	
Perineural invasion			<0.001
No	3,598 (18.4)	1,508 (20.8)	
Yes	129 (0.7)	91 (1.3)	
Unknown	15,868 (81.0)	5,638 (77.9)	

our analyses. Statistical analyses were mainly performed using SPSS version 23 (IBM, Armonk, NY, USA).

RESULTS

Patient Characteristics Before PS Matching

In total, 26,832 patients diagnosed with pN0 or ypN0 rectal cancer were confirmed as the final cohort, including 7,237 (27.0%) patients with radiation and 19,595 (73.0%) patients without radiation prior to surgery. 8,177 (30.5%) patients received chemotherapy and 18,655 (69.5%) patients did not. The median follow-up time was up to 81 months, which was more than 5 years. At the end of follow-up time, 3,453 (12.9%) patients died of rectal cancer. The 5-year CSS rate of the whole cohort was 89.8%. The median ages of ypN0 group and pN0 group were 68 and 62 years old, respectively.

Shown as **Table 1**, patient demographics and pathological features between ypN0 and pN0 groups were summarized. For the number of lymph nodes dissected in total, patients in the ypN0 group were more likely to be associated with <12 lymph nodes dissected than patients in the pN0 group ($P = 0.014$); for American Joint Committee on Cancer (AJCC) *T*-stage, patients in the ypN0 group were more likely to be associated with higher *T*-stage than patients in the pN0 group ($P < 0.001$); for postoperative tumor grade, patients in the ypN0 group were more likely to be associated with higher postoperative tumor grade than patients in the pN0 group ($P < 0.001$). The above findings showed that ypN0 was strongly associated with advanced postoperative clinicopathological characteristics.

In addition, postoperative lymph node negative patients who were aged <65 years old, black, male, diagnosed in later years, rectal primary and received chemotherapy correlated with higher probability to have received neoadjuvant treatment.

Prognosis of ypN0 and pN0 Groups Before PS Matching

Using Kaplan–Meier estimates, we analyzed the CSS between ypN0 and pN0 groups. Patients in the ypN0 group had significantly worse survival compared with patients in the pN0 group: the 5-year CSS rate of ypN0 and pN0 were 86.6 and 91.1%, respectively, ($P < 0.001$, **Figure 3**). Then, results of multivariable analyses using the Cox proportional hazard were summarized in **Table 2**. No. of LNs dissected <12 (HR =0.700, 95%CI = 0.650–0.753, $P < 0.001$ for No. of LNs dissected ≥ 12, using No. of LNs dissected < 12 as the reference), higher *T*-stage (HR = 1.518, 95%CI = 1.359–1.695, $P < 0.001$ for T2 stage; HR = 2.439, 95%CI = 2.194–2.712, $P < 0.001$ for T3 stage; HR = 5.353, 95%CI = 4.619–6.204, $P < 0.001$ for T4 stage; using T1 stage as the reference), aged than 65 years old (HR = 1.697, 95%CI = 1.582–1.820 for age at diagnosis > 65, $P < 0.001$, using age at diagnosis ≤ 65 as the reference), black (HR = 1.457, 95%CI = 1.305–1.626, $P < 0.001$ for black race, using white race as the reference), rectal primary (HR = 1.159, 95%CI = 1.068–1.257, $P < 0.001$ for rectal primary, using rectosigmoid primary as the reference), and higher tumor grade (HR = 1.360, 95%CI = 1.228–1.506,

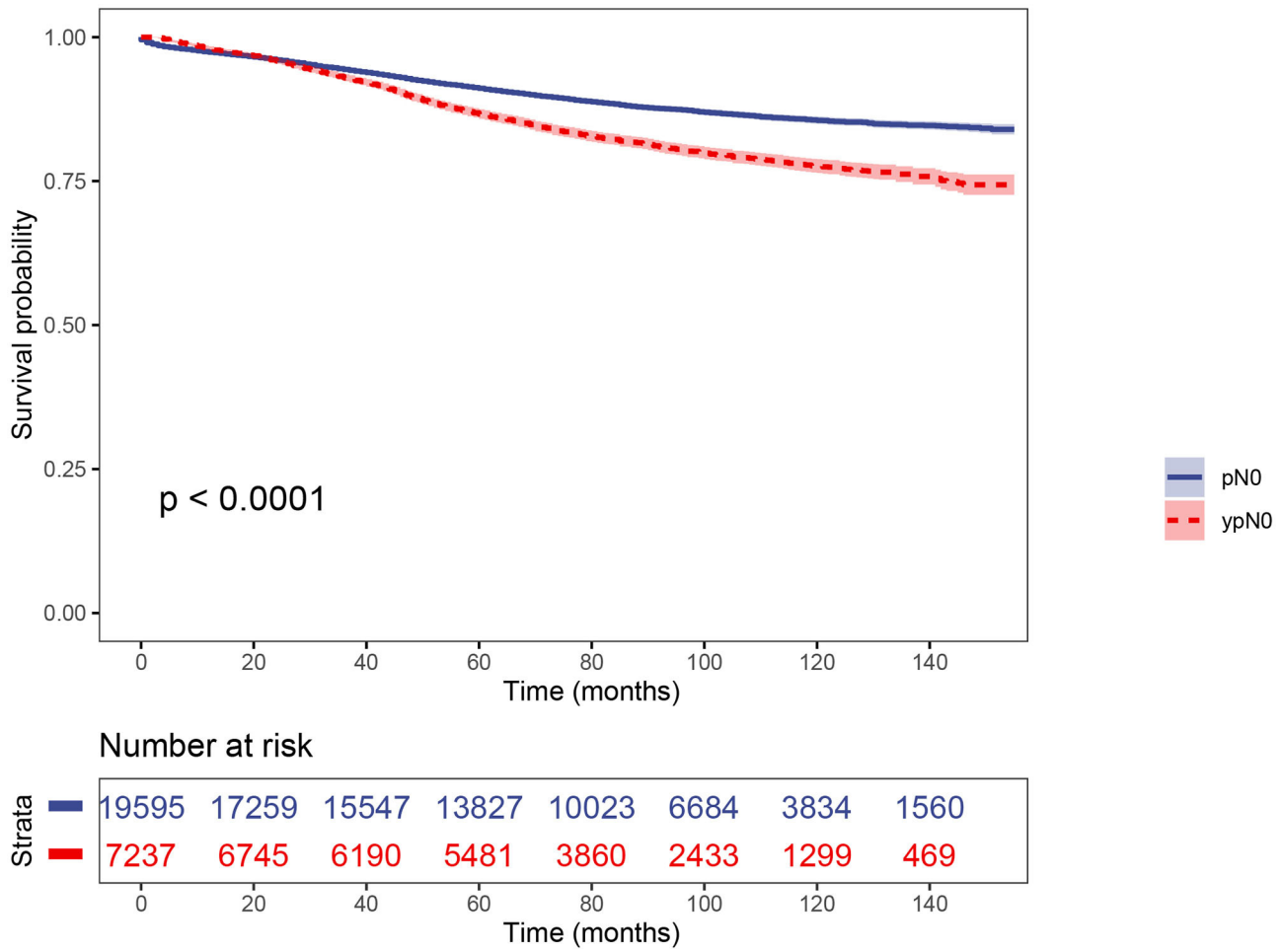

FIGURE 3 | The CSS curves of ypN0 and pN0 groups using Kaplan-meier estimates before PSM.

$P < 0.001$ for poor/anaplastic grade, using well/moderate grade as the reference) were independently associated with significantly worse CSS. With regards to neoadjuvant radiotherapy, however, after adjusting for other prognostic factors, it was not an independent prognostic variable of CSS (HR = 1.095, 95%CI = 0.952–1.260, $P = 0.205$, using pN0 group as the reference).

Patient Characteristics and Prognosis of ypN0 and pN0 Groups After PS Matching

PS matching created 761 matched pairs, including 761 patients in the ypN0 group and 761 patients in the pN0 group. The comparison of baseline characteristics between the two groups were summarized in **Table 3**. All the tumor and patient characteristics except year of diagnosis showed no statistically significant differences between ypN0 and pN0 groups ($P > 0.05$, **Table 3**). Then, we also conducted CSS analyses using the Kaplan–Meier method, which indicated that there was no statistically significant CSS difference between the two groups, the 5-year CSS rates of the ypN0 and pN0 groups were 88.2 and 86.2%, respectively, ($P = 0.84$; **Figure 4**).

DISCUSSION

The use of neoadjuvant chemoradiotherapy in advanced rectal cancer could result in pathologic response of the primary tumor, and many studies demonstrated that tumor response of neoadjuvant treatment was significantly associated with the prognosis of rectal cancer (12–16). According to the clinical guidelines of National Comprehensive Cancer Network (NCCN), patients who had received neoadjuvant chemoradiotherapy followed by surgery were recommended to receive adjuvant chemotherapy (17). However, the use of adjuvant chemotherapy in ypN0 rectal cancer was still controversial and some researchers questioned the clinical value of adjuvant chemotherapy in ypN0 patients (6, 7, 17). As early as in 2006, the study Fietkau et al. reported that disease-free survival (36 months) for rectal cancer without lymph node metastases (ypN0) was excellent, independent of whether they had received postoperative chemotherapy (6). Then in 2010, after identifying randomized studies exploring adjuvant chemotherapy against observation in patients with rectal cancer previously treated with preoperative radio(chemo)therapy, Bujko et al. (18). concluded

TABLE 2 | Multivariate Cox regression analyses of the clinicopathological characteristics concerning CSS.

Variable	Reference	Characteristic	Cause-specific survival		
			HR (95%CI)	SE	*P*-value
Neoadjuvant radiotherapy					0.205
	No	Yes	1.095 (0.952–1.260)	0.072	
No. of LNs dissected					<0.001
	<12	≥12	0.700 (0.650–0.753)	0.037	
T stage					<0.001
	T1	T2	1.518 (1.359–1.695)	0.056	<0.001
		T3	2.439 (2.194–2.712)	0.054	<0.001
		T4	5.353 (4.619–6.204)	0.075	<0.001
Age at diagnosis (years)					<0.001
	≤65	>65	1.697 (1.582–1.820)	0.036	
Race					<0.001
	White	Black	1.457 (1.305–1.626)	0.056	<0.001
		Other	0.884 (0.782–1.000)	0.063	0.051
Gender					
	Male	Female	0.956 (0.893–1.023)	0.035	0.195
Year of diagnosis					0.706
	2004	2005	1.083 (0.957–1.225)	0.063	0.205
		2006	1.017 (0.896–1.155)	0.065	0.791
		2007	1.042 (0.918–1.184)	0.065	0.523
		2008	1.009 (0.884–1.152)	0.068	0.897
		2009	0.994 (0.868–1.139)	0.069	0.931
		2010	1.035 (0.842–1.271)	0.105	0.746
		2011	0.923 (0.736–1.157)	0.116	0.486
Tumor site					<0.001
	Rectosigmoid primary	Rectal primary	1.159 (1.068–1.257)	0.042	
Grade					<0.001
	Well/moderate	Poor/Anaplastic	1.360 (1.228–1.506)	0.052	<0.001
		Unknown	0.859 (0.751–0.982)	0.069	0.027
Chemotherapy					0.736
	No/unknown	Yes	0.977 (0.852–1.120)	0.070	
Serum CEA level					<0.001
	Negative	Positive	1.646 (1.498–1.808)	0.048	<0.001
		Unknown	1.225 (1.131–1.327)	0.041	<0.001
Tumor size					<0.001
	≤5 cm	>5 cm	1.274 (1.163–1.395)	0.046	<0.001
		Unknown	1.123 (1.027–1.228)	0.045	0.011
Perineural invasion					0.008
	No	Yes	1.648 (1.205–2.254)	0.160	0.002
		Unknown	1.037 (0.849–1.265)	0.102	0.723

that delivery of adjuvant chemotherapy in patients undergoing preoperative radio(chemo)therapy was not evidence based. Later, after comparing the prognosis of ypN0 patients who had received adjuvant chemotherapy and those who had not, Kiran et al. (7) found that ypN0 rectal cancer, whether or not the patient had received adjuvant chemotherapy, showed similar local recurrence, disease-free survival, and overall survival after prolonged follow-up. The famous EORTC 22921 trial's long-term results also showed that adjuvant fluorouracil-based chemotherapy after preoperative radiotherapy (with or without chemotherapy) does not affect either 10-year overall survival or disease-free survival of rectal cancer (19). Therefore, it was quite necessary to examine the long-term oncologic results of ypN0 disease.

To the best of our knowledge, the present population-based study was the largest study to compare the oncologic outcomes of ypN0 and pN0 rectal cancer. In the present study, at first, shown as the results of Kaplan–Meier estimates, patients in the ypN0 group had significantly worse survival compared with patients in the pN0 group. However, after adjusting for other known

TABLE 3 | Patients' baseline characteristics after PSM.

Variables	No. of Patients (%)		*P*
	pN0 (761)	ypN0 (761)	
No. of LNs dissected			1.000
<12	395 (51.9)	395 (51.9)	
≥12	366 (48.1)	366 (48.1)	
T stage			0.888
T1	121 (15.9)	112 (14.7)	
T2	148 (19.4)	157 (20.6)	
T3	469 (61.6)	470 (61.8)	
T4	23 (3.0)	22 (2.9)	
Age at diagnosis (years)			0.756
≤65	428 (56.2)	434 (57.0)	
>65	333 (43.8)	327 (43.0)	
Race			0.864
White	667 (87.6)	671 (88.2)	
Black	45 (5.9)	46 (6.0)	
Other	49 (6.4)	44 (5.8)	
Gender			0.316
Male	479 (62.9)	460 (60.4)	
Female	282 (37.1)	301 (761)	
Year of diagnosis			0.944
2004	81 (10.6)	95 (12.5)	
2005	97 (12.7)	96 (12.6)	
2006	99 (13.0)	106 (13.9)	
2007	107 (14.1)	101 (13.3)	
2008	104 (13.7)	102 (13.4)	
2009	98 (12.9)	101 (13.3)	
2010	91 (12.0)	83 (10.9)	
2011	84 (11.0)	77 (10.1)	
Tumor site			0.950
Rectosigmoid primary	158 (20.8)	159 (20.9)	
Rectal primary	603 (79.2)	602 (79.1)	
Grade			0.221
Well/moderate	686 (90.1)	666 (87.5)	
Poor/anaplastic	50 (6.6)	59 (7.8)	
Unknown	25 (3.3)	36 (4.7)	
Chemotherapy			1.000
No/unknown	214 (28.1)	214 (28.1)	
Yes	547 (71.9)	547 (71.9)	
Serum CEA level			0.890
Negative	276 (36.3)	267 (35.1)	
Positive	153 (20.1)	156 (20.5)	
Unknown	332 (43.6)	338 (44.4)	
Tumor size			0.721
≤5 cm	516 (67.8)	511 (67.1)	
>5 cm	125 (16.4)	119 (15.6)	
Unknown	120 (15.8)	131 (17.2)	
Perineural invasion			0.738
No	153 (21.1)	141 (18.5)	
Yes	8 (1.1)	8 (1.1)	
Unknown	600 (78.8)	612 (80.4)	

prognostic factors, the results of multivariate analyses showed that the prognostic difference between ypN0 and pN0 groups was not statistically significant. More importantly, PS matching was also used to validate our results and we found that there was no statistically significant CSS difference between the two groups after PS matching. Therefore, we held the belief that ypN0 status could achieve similarly good oncologic outcomes compared with pN0 disease. Therefore, we strongly believed that having received neoadjuvant chemoradiotherapy should not be the reason for adjuvant chemotherapy in pathological node-negative patients.

Although the nature of the retrospective design and small sample size were considered to be potential limitations, two previous studies questioned the routine use of adjuvant chemotherapy for ypN0 rectal cancer patients who had undergone curative surgery following neoadjuvant chemoradiation (6, 7). What is more, a recent analysis of the SEER database found that rectal cancer patients with ypTis-2N0 did not benefit from adjuvant chemotherapy after neoadjuvant treatment followed by radical surgery (20). Therefore, our research could add new evidence supporting the above findings.

Why did the Kaplan–Meier survival analyses, before adjusting for other prognostic variables or PS matching, show worse survival of ypN0 disease? In our analyses of differences in the baseline characteristics between the ypN0 and pN0 groups, we could easily find that, compared with pN0 rectal cancer, ypN0 status was strongly associated with poorer postoperative pathological diagnoses: ypN0 was more likely to be associated with <12 lymph nodes dissected, higher T stage and higher postoperative tumor grade. Before adjusting for other prognostic factors, therefore, it was normal to find that ypN0 disease was more likely to be associated with worse oncologic outcomes compared with pN0 rectal cancer.

In 2014, Erlenbach-Wünsch et al. (9) retrospectively analyzed the prognosis of 132 rectal cancer patients who underwent standard TME surgery after neoadjuvant chemoradiotherapy (ypN0) and those of 341 patients diagnosed with pN0 rectal disease without neoadjuvant chemoradiotherapy, showing a similar oncologic outcome between the two groups, which was consistent with our analyses. However, the sample size of this study was still too small for any general recommendation. Maybe limited to the sample size, they did not find that ypN0 status was strongly associated with poorer postoperative pathological diagnoses (less lymph nodes dissected, higher T stage and higher postoperative tumor grade) compared with pN0 rectal cancer, which contributed to the phenomenon that ypN0 disease was more likely to be associated with worse oncologic outcomes than pN0 rectal cancer before adjusting for other prognostic factors.

Although previous research had showed that the histological lymph node status after chemoradiotherapy seemed to be the only significant prognostic parameter of oncologic outcomes, to our knowledge, few studies were reported to study on the prognostic value of ypN0 status (6). In 2007, with the analyses of 35 patients who underwent neoadjuvant chemoradiotherapy followed by excisional surgery with TEM for rectal cancer, Caricato et al. (21) reported the effect of preoperative chemoradiotherapy on postoperative lymph node status, though

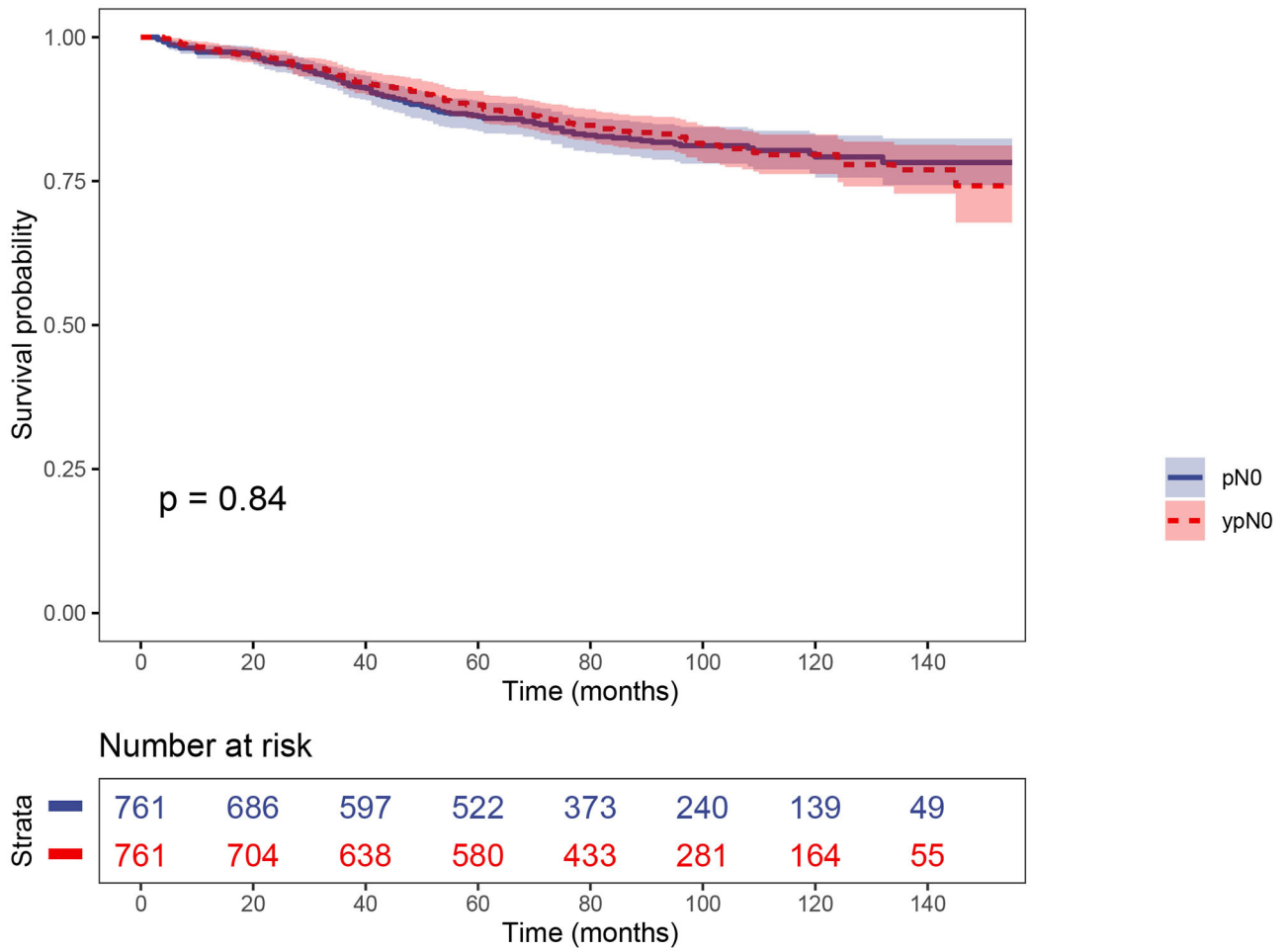

FIGURE 4 | The CSS curves of ypN0 and pN0 groups using Kaplan-meier estimates after PSM.

the prognostic assessment was not performed due to the low case number.

Lindebjerg et al. (22) reported that rectal cancer patients with a major tumor response and no lymph node metastases after treatment had a survival rate of 100% compared to 60% in the group of patients with major response but lymph node metastases after surgery. Like ypCR patients, ypN0 patients were reported to achieve significantly better oncologic outcomes compared with lymph node-positive patients (17). Sprenger et al. (23) shared the similar view that residual nodal status was the most important predictor of individual outcome after analyzing the effect of preoperative and pathological nodal status on disease-free and overall survival in 496 patients with rectal adenocarcinoma identified from a prospective database.

Our research, therefore, as the largest one focused on the comparison of prognosis between ypN0 and pN0 rectal cancer, could add strong evidence that the receipt of neoadjuvant chemoradiotherapy was not an independent prognostic factor in rectal cancer patients with negative pathological nodal status. However, ypN0 status was strongly associated with worse postoperative pathological diagnoses compared with pN0 rectal cancer: ypN0 was more likely to be associated with <12 lymph nodes dissected, higher T stage higher postoperative tumor grade, contributing to the phenomenon that ypN0 disease was more likely to be associated with worse oncologic outcomes than pN0 rectal cancer before adjusting for other prognostic factors.

However, this study was only a retrospective one, we hope further randomized prospective study could be conducted to provide higher grade evidence to guide the treatment of rectal cancer with negative pathological nodal status who had received neoadjuvant chemoradiotherapy followed by total mesorectal excision (TME). Moreover, regimens used in the present study were not available in SEER database, which was also a limitation of our research.

In summary, our study showed that ypN0 rectal cancer was strongly associated with worse postoperative pathological diagnoses compared with pN0 rectal cancer, contributing to worse oncologic outcomes. After adjusting for other known prognostic factors, however, the prognostic difference between

ypN0 and pN0 groups was not statistically significant, which could give guidance to the treatment of ypN0 rectal cancer.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: seer.cancer.gov.

AUTHOR CONTRIBUTIONS

YH and GF: conceptualization. YH and WW: data curation and writing—original draft. YH, WW, and ZW: data analysis. TL and ST: visualization. GF: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Perineural Invasion Is a Strong Prognostic Factor but Not a Predictive Factor of Response to Adjuvant Chemotherapy in Node-Negative Colon Cancer

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Purpose: To validate the prognostic value and evaluate the predictive value of response to adjuvant chemotherapy of perineural invasion (PNI) in node-negative colon cancer using the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) 18 tumor registry database.

Methods: Patients diagnosed with colon cancer from the SEER database between January 1, 2010 and December 31, 2015 were identified. Chi-square analysis was performed to evaluate different demographic and clinical features of patients between PNI-negative (PNI (–)) and PNI-positive (PNI (+)) groups. Univariate and multivariate Cox proportional hazard regression models were built to examine the relationship of demographic and clinical features and survival outcomes with the hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: In total, 57,255 node-negative colon cancer patients were extracted from the SEER database. The receipt of chemotherapy was not an independent prognostic factor for CSS in T3 colon cancer with or without the presence of PNI (P >0.05). The receipt of chemotherapy was independently associated with 34.0% decreased risk of cancer-specific mortality compared with those without the receipt of chemotherapy in T4 colon cancer without the presence of PNI (HR = 0.660, 95%CI = 0.559–0.779, P <0.001); the receipt of chemotherapy was independently associated with 36.0% decreased risk of cancer-specific mortality compared with those without the receipt of chemotherapy in T4 colon cancer with the presence of PNI (HR = 0.640, 95%CI = 0.438–0.935, P = 0.021).

Conclusions: The present study demonstrated the poor prognosis of PNI (+) in both stage I and II colon cancer. However, the presence of PNI was not a predictive factor of response to adjuvant chemotherapy in node-negative colon cancer.

Keywords: perineural invasion, prognostic, adjuvant chemotherapy, node-negative, colon cancer

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INTRODUCTION

As one of the most commonly diagnosed malignant tumors, colon cancer is an important public health issue worldwide (1). Currently, the current standards for clinical treatment and prognostic prediction of survival and recurrence in colon cancer are principally based on pathological staging of the American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) staging system. According to the clinical guidelines of National Comprehensive Cancer Network, stage III colon cancer deserve adjuvant chemotherapy for better prognosis (2–8). However, the TNM staging is not accurate enough to stratify those node-negative (stage I/II) colon cancer patients, and previous studies have indicated the prognostic implications of various histopathological factors (9–12).

In addition to direct growth, tumor cells can disseminate through the blood and lymph channels or grow along the nerves. Positive perineural invasion (PNI) is therefore defined as the invasion spreading in or around the neural tissue and/or spread along nerve sheaths, even in the absence of lymphovascular invasion (LVI) or lymph node metastasis (12–16). PNI would finally occur after changes in nerve cells and supporting cells, changes and metastasis of the perineural matrix, injury and regeneration of nerves; adhesion of nerve cells and tumor cells; and escape, autophagy and apoptosis of tumor cells and so on (17). It has been widely reported that the presence of PNI would indicate more aggressive clinicopathological features, resulting in poor prognosis in colorectal cancer, and some previous studies found that PNI could be an indicator for the receipt of chemotherapy in colon cancer (12, 18–22). The prognostic value of PNI in colorectal cancer has been widely recognized, however, its predictive role for the receipt of adjuvant chemotherapy is less clear (23–25). Therefore, the present large population-based study was to validate the prognostic value and evaluate the predictive value of response to adjuvant chemotherapy of PNI in node-negative colon cancer.

MATERIALS AND METHODS

Patients

Data used in the present study were retrieved from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) 18 tumor registry database. The SEER database, which emphasized quality control and stipulates a less than five percent error rate, contained approximately 28% of the US population and included population demographic information, clinicopathological characteristics, treatment and survival information from more than three million patients (26). Using SEER*Stat version 8.3.6, patients diagnosed with colon cancer between January 1, 2010 and December 31, 2015 were identified. Personal information of patients was not involved in the present study, therefore, the requirement for informed consent was waived.

The patient characteristics extracted from the SEER database included T stage, age at diagnosis, race, sex, year of diagnosis, tumor grade, histological type, total number of lymph nodes examined, the receipt of chemotherapy and perineural invasion status. The exclusion criteria were (1) lack of positive histological confirmation, (2) race was unknown, (3) non-adenocarcinoma histologies, (4) not active follow-up, and (5) lack of radical surgery. In addition, only those patients without lymph node or distant metastasis and with known perineural invasion status were included into our analyses.

Statistical Analysis

In the present study, Cancer-specific survival (CSS) was used as the survival endpoint and analyzed using the Kaplan–Meier method with log-rank test to evaluate the outcomes of different groups. Kaplan–Meier curves were often used to visually summarize time-to-event data, in which y axis indicated the proportion of individuals under risk of an event, and the x axis indicated time. The curves were often presented with 95% confidence intervals and a difference between curves can be tested statistically, most commonly using the log rank test (27). CSS was defined as the time between the diagnosis of colon cancer and cancer-specific death or the last follow up, mortality cases resulted from other causes were censored.

Chi-square analysis was performed to evaluate different demographic and clinical features of patients between PNI-negative (PNI (–)) and PNI-positive (PNI (+)) groups. The chi-square test commonly either compared the distribution of a categorical variable to a hypothetical distribution or tested whether the two categorical variables were independent. In our analyses, the chi-square test was used to evaluate the null hypothesis that two categorical variables (e.g., treatment group [male versus female] and outcome [PNI (–) versus PNI (+)]) were not associated with each other (28, 29).

Univariate and multivariate Cox proportional hazard regression models were built to examine the relationship of demographic and clinical features and survival outcomes with the hazard ratios (HRs) and 95% confidence intervals (CIs). The Cox proportional hazard regression model was to determine the extent to which changed in the risk factors affect the survival of colon cancer. The HR of each demographic or clinical feature in the model can be estimated according to the minimum, maximum, or standard deviation of the values of the demographic and clinical features (30). P values less than 0.05 were considered statistically significant. Statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) Statics software (version 23; IBM Corporation, NY, USA).

RESULTS

Patient Characteristics

In total, 57,255 node-negative colon cancer patients satisfying the inclusion and exclusion criteria were extracted from the SEER database. Among the whole cohort, 25,450 (44.5%) patients were diagnosed with stage I disease, 21,090 (26.8%) patients aged less than 65 years old, 28,369 (49.5%) patients were male, 2,372 (4.1%) patients were diagnosed with the presence of PNI. The median follow-up time for patients alive at last follow-up time was 37 months.

Different demographic and clinical features of patients between PNI (–) and PNI (+) groups were compared in **Table 1**. It was found that high T stage (4.4% VS. 24.7% for stage T1, 9.2% VS. 21.1% for stage T2, 62.4% VS. 46.4% for stage T3, 23.9% VS. 7.8% for stage T4, P <0.001), later year of diagnosis (13.7%

TABLE 1 | Demographic and clinical features of the patients according to perineural invasion status.

Feartue	No. of Patients (%)		*P*
	PNI (–) (N = 54,883)	PNI (+) (N = 2,372)	
T stage			<0.001
T1	13,552 (24.7)	104 (4.4)	
T2	11,575 (21.1)	219 (9.2)	
T3	25,459 (46.4)	1,481 (62.4)	
T4	4,297 (7.8)	568 (23.9)	
Age at diagnosis			0.672
≤65	20,226 (36.9)	864 (36.4)	
>65	34,657 (63.1)	1,508 (63.6)	
Race			0.199
White	44,381 (80.9)	1,895 (79.9)	
Black	6,219 (11.3)	297 (12.5)	
Other	4283 (7.8)	180 (7.6)	
Sex			0.418
Male	27,213 (49.6)	1,156 (48.7)	
Female	27,670 (50.4)	1,216 (51.3)	
Year			0.003
2010	8,980 (16.4)	326 (13.7)	
2011	9,185 (16.7)	366 (15.4)	
2012	9,267 (16.9)	415 (17.5)	
2013	9,107 (16.6)	428 (18.0)	
2014	9,192 (16.7)	415 (17.5)	
2015	9,152 (16.7)	422 (17.8)	
Grade			<0.001
I	6,003 (10.9)	116 (4.9)	
II	40,089 (73.0)	1,630 (68.7)	
III	5,567 (10.1)	505 (21.3)	
IV	1,156 (2.1)	88 (3.7)	
Unknown	2,068 (3.8)	33 (1.4)	
Histological type			0.042
Adenocarcinoma	50,600 (92.2)	2,214 (93.3)	
Mucinous/signet-ring cell carcinoma	4,282 (7.8)	158 (6.7)	
Total number of lymph nodes examined			<0.001
<12	10,487 (19.1)	259 (10.9)	
≥12	44,396 (80.9)	2,113 (89.1)	
Chemotherapy			<0.001
No	50,305 (91.7)	1,886 (79.5)	
Yes	4,578 (8.3)	486 (20.5)	

VS. 16.4% for 2010, 15.4% VS. 16.7% for 2011, 17.5% VS. 16.9% for 2012, 18.0% VS. 16.6% for 2013, 17.5% VS. 16.7% for 2014, 17.8% VS. 16.7% for 2015, P = 0.003); higher tumor grade (4.9% VS. 10.9% for grade I, 68.7% VS. 73.0% for grade II, 21.3% VS. 10.1% for grade III, 3.7% VS. 2.1% for grade IV, P <0.001); adenocarcinoma (93.3% VS. 92.2% for adenocarcinoma, 6.7% VS. 7.8% for mucinous/signet-ring cell carcinoma, P = 0.042) and more lymph nodes examined (10.9% VS. 19.1% for less than 12 lymph nodes examined, 89.1% VS. 80.9% for more than 12 lymph nodes examined, P <0.001) were more likely to be associated with the presence of PNI. In addition, the presence of PNI was more likely to correlate with the receipt of chemotherapy (P <0.001). The associations of age at diagnosis, race and sex between PNI (–) and PNI (+) groups did not reach statistical significance (P >0.05).

Prognostic Significance of PNI in Node-Negative Colon Cancer

Shown as **Figures 1A, B**, we plotted the Kaplan–Meier CSS curves of node-negative colon cancer patients with the presence of PNI compared to those without the presence of PNI. Kaplan–Meier analyses showed that PNI (+) patents (5-year CSS rate = 93.6%) were significantly associated with poorer CSS compared with PNI (–) patents (5-year CSS rate = 96.2%) in stage I colon cancer (P = 0.025, **Figure 1A**). It was also found that PNI (+) patents (5-year CSS rate = 77.5%) were significantly associated with poorer CSS compared with PNI (–) patents (5-year CSS rate = 87.9%) in stage II colon cancer and the survival difference was widened in stage II colon cancer than in stage I colon cancer (P <0.0001, **Figure 1B**).

In addition, Cox proportional hazard regression models were completed to assess the independent prognostic factors for CSS in node-negative colon cancer, including T stage, age at diagnosis, race, sex, year of diagnosis, tumor grade, histological type, total number of lymph nodes examined and perineural invasion status (**Tables 2, 3**). Only the predictors with P values less than 0.20 in the univariate analysis were entered into a multivariate Cox model. Multivariate survival analyses showed that T stage (P <0.001), age at diagnosis (P <0.001), race (P <0.001), sex (P <0.028) and total number of lymph nodes

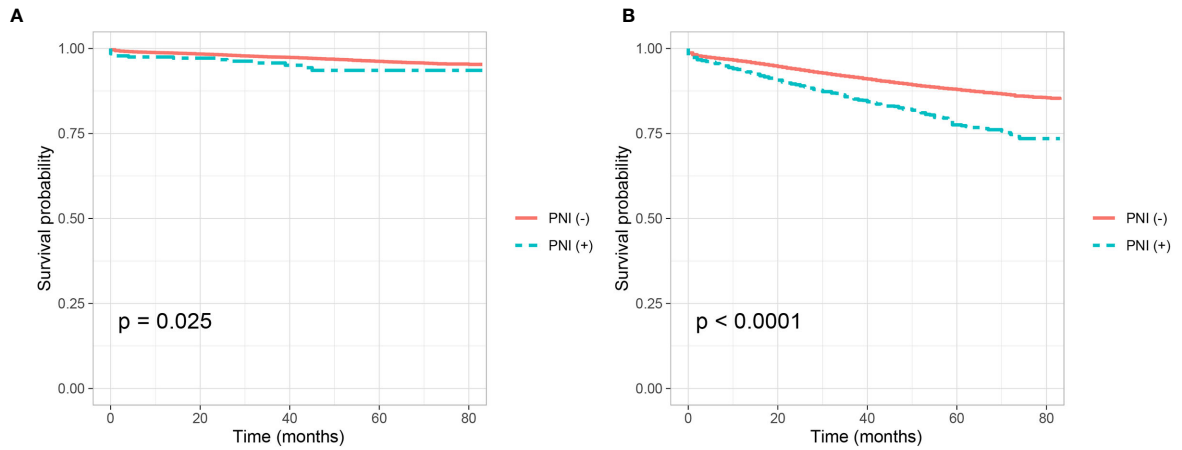

FIGURE 1 | Kaplan–Meier curves for cancer-specific survival according to the perineural invasion status (PNI (–) VS. PNI (+) in **(A)** stage I colon cancer (P = 0.025) and **(B)** stage II colon cancer (P <0.001).

TABLE 2 | Univariate and multivariate survival analyses of stage I colon cancer.

Variable	Univariate		Multivariate	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T1	Reference		Reference	
T2	1.550 (1.336–1.798)		1.552 (1.324–1.819)	
Age at diagnosis		<0.001		<0.001
≤65	Reference		Reference	
>65	2.276 (1.913–2.707)		2.281 (1.913–2.720)	
Race		<0.001		<0.001
White	Reference		Reference	
Black	1.493 (1.224–1.822)	<0.001	1.703 (1.393–2.081)	<0.001
Other	0.804 (0.589–1.097)	0.168	0.874 (0.641–1.193)	0.397
Sex		0.151		0.028
Male	Reference		Reference	
Female	0.897 (0.774–1.040)		0.847 (0.730–0.982)	
Year		0.106		0.122
2010	Reference		Reference	
2011	0.929 (0.745–1.160)	0.517	0.929 (9.744–1.160)	0.516
2012	0.900 (0.709–1.143)	0.388	0.912 (0.718–1.159)	0.451
2013	1.195 (0.941–1.517)	0.143	1.223 (0.963–1.553)	0.100
2014	0.885 (0.669–1.172)	0.394	0.921 (0.695–1.219)	0.564
2015	0.808 (0.582–1.120)	0.200	0.852 (0.614–1.182)	0.337
Grade		0.009		0.085
I	Reference		Reference	
II	1.444 (1.146–1.820)	0.002	1.360 (1.076–1.718)	0.010
III	1.583 (1.117–2.243)	0.010	1.459 (1.026–2.075)	0.035
IV	1.413 (0.684–2.919)	0.351	1.319 (0.637–2.730)	0.456
Unknown	1.041 (0.704–1.540)	0.839	1.085 (0.732–1.610)	0.685
Histological type		0.999		
Adenocarcinoma	Reference			
Mucinous/signet-ring cell carcinoma	1.000 (0.694–1.442)			
Total number of lymph nodes examined		<0.001		<0.001
<12	Reference		Reference	
≥12	0.726 (0.622–0.848)		0.614 (0.521–0.722)	
Perineural invasion		0.028		0.077
None	Reference		Reference	
Present	1.777 (1.066–2.964)		1.590 (0.951–2.658)	

TABLE 3 | Univariate and multivariate survival analyses of stage II colon cancer.

Variable	Univariate		Multivariate	
	HR (95%CI)	*P*	HR (95%CI)	*P*
T stage		<0.001		<0.001
T3	Reference		Reference	
T4	2.847 (2.631–3.079)		2.806 (2.591–3.039)	
Age at diagnosis		<0.001		<0.001
≤65	Reference		Reference	
>65	1.833 (1.683–1.995)		1.894 (1.738–2.064)	
Race		<0.001		<0.001
White	Reference		Reference	
Black	1.146 (1.027–1.279)	0.015	1.268 (1.135–1.417)	<0.001
Other	0.785 (0.673–0.915)	0.002	0.825 (0.707–0.962)	0.014
Sex		0.005		0.149
Male	Reference		Reference	
Female	1.111 (1.033–1.196)		1.056 (0.981–1.136)	
Year		0.136		0.426
2010	Reference		Reference	
2011	0.994 (0.980–1.109)	0.909	0.993 (0.890–1.108)	0.901
2012	0.957 (0.853–1.073)	0.449	0.961 (0.857–1.078)	0.499
2013	0.926 (0.818–1.048)	0.223	0.947 (0.836–1.072)	0.386
2014	0.883 (0.771–1.012)	0.074	0.917 (0.800–1.051)	0.212
2015	0.824 (0.704–0.966)	0.017	0.855 (0.729–1.002)	0.053
Grade		<0.001		0.020
I	Reference		Reference	
II	1.018 (0.877–1.183)	0.810	1.023 (0.881–1.188)	0.764
III	1.356 (1.147–1.603)	<0.001	1.203 (1.016–1.424)	0.032
IV	1.244 (0.974–1.590)	0.080	1.110 (0.868–1.419)	0.404
Unknown	1.313 (0.954–1.807)	0.095	1.169 (0.849–1.610)	0.337
Histological type		0.682		
Adenocarcinoma	Reference			
Mucinous/signet-ring cell carcinoma	1.025 (0.911–1.154)			
Total number of lymph nodes examined		<0.001		<0.001
<12	Reference		Reference	
≥12	0.525 (0.479–0.576)		0.541 (0.493–0.594)	
Perineural invasion		<0.001		<0.001
None	Reference		Reference	
Present	1.841 (1.636–2.072)		1.607 (1.426–1.812)	

examined (P <0.001) were independent prognostic factors in stage I colon cancer (**Table 2**). After adjusting for other prognostic factors, more importantly, it was found that PNI (+) patients were independently associated 59.0% increased risk of colon cancer-specific mortality compared with PNI (−) patients in stage I colon cancer though the P value did not reach statistical significance, which might result from the small sample size (n = 323) of stage I colon cancer patients with the presence of PNI (HR = 1.590, 95%CI = 0.951–2.658, P = 0.077, using no PNI as the reference; **Table 2**).

In **Table 3**, multivariate survival analyses showed that T stage (P <0.001), age at diagnosis (P <0.001), race (P <0.001), tumor grade (P = 0.020) and total number of lymph nodes examined (P <0.001) were independent prognostic factors in stage II colon cancer. After adjusting for other prognostic factors, more importantly, it was found that PNI (+) patients were independently associated 60.7% increased risk of colon cancer-specific mortality compared with PNI (−) patients in stage II colon cancer (HR = 1.607, 95%CI = 1.426–1.812, P <0.001, using no PNI as the reference; **Table 3**).

PNI Is Not a Predictive Factor of Response to Adjuvant Chemotherapy in Stage II Colon Cancer

Adjuvant chemotherapy was not traditionally used in stage I colon cancer, we then evaluate whether PNI is a predictive factor of response to adjuvant chemotherapy in stage II colon cancer. Shown as **Figures 2A, B**, we plotted the Kaplan–Meier CSS curves of T3 colon cancer patients with the receipt of chemotherapy compared to those without the receipt of chemotherapy. Kaplan–Meier analyses showed that patents with the receipt of chemotherapy (5-year CSS rate = 91.1%) were significantly associated with better CSS compared to those without the receipt of chemotherapy (5-year CSS rate = 90.0%) in T3 colon cancer without the presence of PNI (P = 0.004, **Figure 2A**). It was also found that the receipt of chemotherapy (5-year CSS rate = 83.6%) was associated with better CSS compared with those without the receipt of chemotherapy (5-year CSS rate = 81.4%) in T3 colon cancer with the presence of PNI, but the P value did not reach statistical significance (P = 0.096, **Figure 2B**).

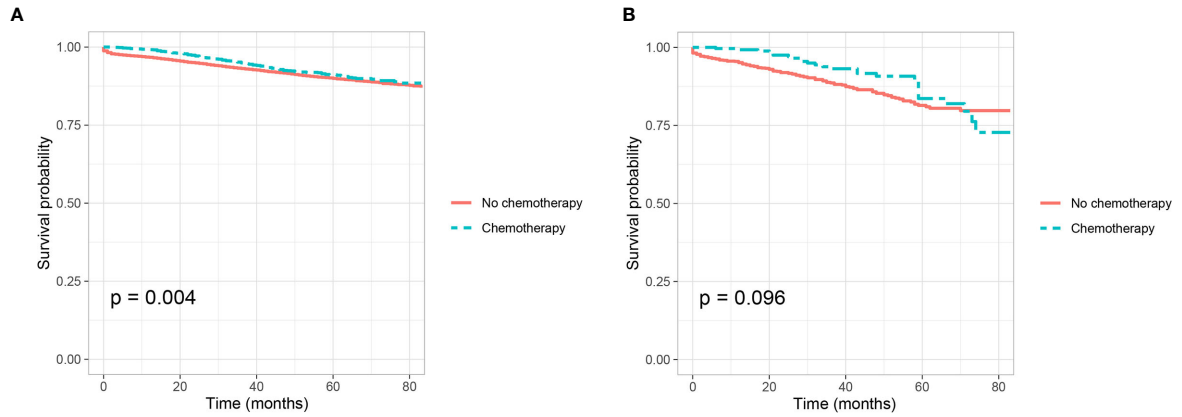

FIGURE 2 | Kaplan–Meier curves for cancer-specific survival according to the receipt of chemotherapy (no chemotherapy VS. chemotherapy) in stage IIA colon cancer **(A)** with the presence of perineural invasion (P = 0.004) and **(B)** without the presence of perineural invasion (P = 0.096).

Cox proportional hazard regression models were completed to assess the independent prognostic factors for CSS in T3 colon cancer (**Table 4** and **Table S1**). After adjusting for other prognostic factors, it was found that the receipt of chemotherapy was not an independent prognostic factor for CSS in T3 colon cancer without the presence of PNI (HR = 0.943, 95%CI = 0.802–1.108, P = 0.473, using no PNI and no chemotherapy as the reference; **Table 4**); the receipt of

TABLE 4 | Univariate and multivariate survival analyses of T3N0M0 colon cancer.

Variable	Univariate		Multivariate	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age at diagnosis		<0.001		<0.001
≤65	Reference		Reference	
>65	2.072 (1.863–2.306)		2.038 (1.825–2.276)	
Race		0.001		<0.001
White	Reference		Reference	
Black	1.138 (0.997–1.299)	0.056	1.260 (1.102–1.440)	0.001
Other	0.744 (0.616–0.899)	0.002	0.786 (0.650–0.950)	0.013
Sex		0.292		
Male	Reference			
Female	1.048 (0.960–1.145)			
Year		0.068		0.204
2010	Reference		Reference	
2011	0.957 (0.840–1.091)	0.512	0.965 (0.847–1.099)	0.591
2012	0.946 (0.825–1.084)	0.424	0.958 (0.836–1.099)	0.539
2013	0.871 (0.750–1.012)	0.072	0.878 (0.755–1.020)	0.089
2014	0.851 (0.721–1.003)	0.055	0.892 (0.756–1.052)	0.175
2015	0.758 (0.623–0.922)	0.006	0.794 (0.652–0.966)	0.021
Grade		0.192		0.406
I	Reference		Reference	
II	1.050 (0.878–1.254)	0.595	1.057 (0.885–1.263)	0.542
III	1.210 (0.985–1.485)	0.069	1.186 (0.965–1.457)	0.105
IV	1.063 (0.773–1.462)	0.708	1.058 (0.768–1.455)	0.731
Unknown	1.220 (0.812–1.834)	0.339	1.139 (0.758–1.713)	0.531
Histological type		0.459		
Adenocarcinoma	Reference			
Mucinous/signet-ring cell carcinoma	0.944 (0.811–1.099)			
Total number of lymph nodes examined		<0.001		<0.001
<12	Reference		Reference	
≥12	0.539 (0.482–0.604)		0.561 (0.501–0.629)	
Perineural invasion, chemotherapy		<0.001		<0.001
None, no/unknown	Reference		Reference	
None, yes	0.793 (0.678–0.928)	0.004	0.943 (0.802–1.108)	0.473
Present, no/unknown	1.749 (1.476–2.073)	<0.001	1.761 (1.485–2.088)	<0.001
Present, yes	1.269 (0.868–1.856)	0.219	1.632 (1.113–2.391)	0.012

chemotherapy was not an independent prognostic factor for CSS in T3 colon cancer with the presence of PNI (HR = 0.927, 95%CI = 0.613–1.400, P = 0.717, using the presence of PNI and no chemotherapy as the reference; **Table S1**).

Shown as **Figures 3A, B**, we plotted the Kaplan–Meier CSS curves of T4 colon cancer patients with the receipt of chemotherapy compared to those without the receipt of chemotherapy. Kaplan–Meier analyses showed that patents with the receipt of chemotherapy (5-year CSS rate = 80.1%) were significantly associated with better CSS compared to those without the receipt of chemotherapy (5-year CSS rate = 71.2%) in T4 colon cancer without the presence of PNI (P <0.0001, **Figure 3A**). It was also found that the receipt of chemotherapy (5-year CSS rate = 73.3%) was significantly associated with better CSS compared with those without the receipt of chemotherapy (5-year CSS rate = 62.7%) in T4 colon cancer with the presence of PNI (P = 0.001, **Figure 3B**).

In addition, Cox proportional hazard regression models were completed to assess the independent prognostic factors for CSS in T4 colon cancer **Table 5** and **Table S2**). After adjusting for other prognostic factors, it was found that the receipt of chemotherapy was independently associated with 34.0% decreased risk of cancer-specific mortality compared with those without the receipt of chemotherapy in T4 colon cancer without the presence of PNI (HR = 0.660, 95%CI = 0.559–0.779, P <0.001, using no PNI and no chemotherapy as the reference; **Table 5**); the receipt of chemotherapy was independently associated with 36.0% decreased risk of cancer-specific mortality compared with those without the receipt of chemotherapy in T4 colon cancer with the presence of PNI (HR = 0.640, 95%CI = 0.438–0.935, P =0.021, using the presence of PNI and no chemotherapy as the reference; **Table S2**).

DISCUSSION

PNI was reported in head and neck cancers by Russian and French researchers since the 1800s (12). Subsequently, the prognostic value was then reported by Bataskis until the 1970s, described as "tumor invasion in, around, and through the nerves" (31). PNI would finally occur after changes in nerve cells and supporting cells, changes and metastasis of the perineural matrix, injury and regeneration of nerves; adhesion of nerve cells and tumor cells; and escape, autophagy and apoptosis of tumor cells and so on (17). In addition, slug was reported to promote PNI and distant metastasis of tumor cells through the MAPK signal pathway (32, 33). And the expression of the L1 cell adhesion molecule could promote the occurrence of PNI by influencing the migration of nerve cells (34).

Some studies have reported that the presence of perineural invasion would indicate more aggressive clinicopathological features (35). In this study, we have showed that the presence of PNI was significantly correlated with high T stage, later year of diagnosis, higher tumor grade, adenocarcinoma, the receipt of chemotherapy and more lymph nodes examined.

It has been widely reported that the presence of PNI was a poor prognostic factor in colorectal cancer (9, 10, 12, 15, 22). In 2000, PNI was adopted as a negative prognostic factor by AJCC and the College of American Pathologists (CAP) has recommended categorization of PNI in the pathology reports for a decade (36, 37). We then validated the prognostic value of PNI in node-negative colon cancer, it was found that PNI (+) patients were independently associated 59.0 and 60.7% increased risk of colon cancer-specific mortality compared with PNI (–) patients in stage I and stage II colon cancer, respectively. In addition, the 5-year CSS rates of PNI (+) patents and PNI (–) patents were 93.6 and 96.2% in stage I colon cancer, 77.5 and 87.9% in stage II colon cancer, respectively. Thus, the poor prognosis of PNI (+) has been demonstrated in both stage I and II colon cancer in the current study.

Some previous studies reported the poor prognosis of PNI (+) might need to be mitigated by adjuvant chemotherapy in node-negative colon cancer, however, the predictive role of PNI (+) for the receipt of adjuvant chemotherapy is not yet established (7, 22, 23, 25, 38). Adjuvant chemotherapy was not traditionally used in stage I colon cancer, we then evaluated whether PNI was a

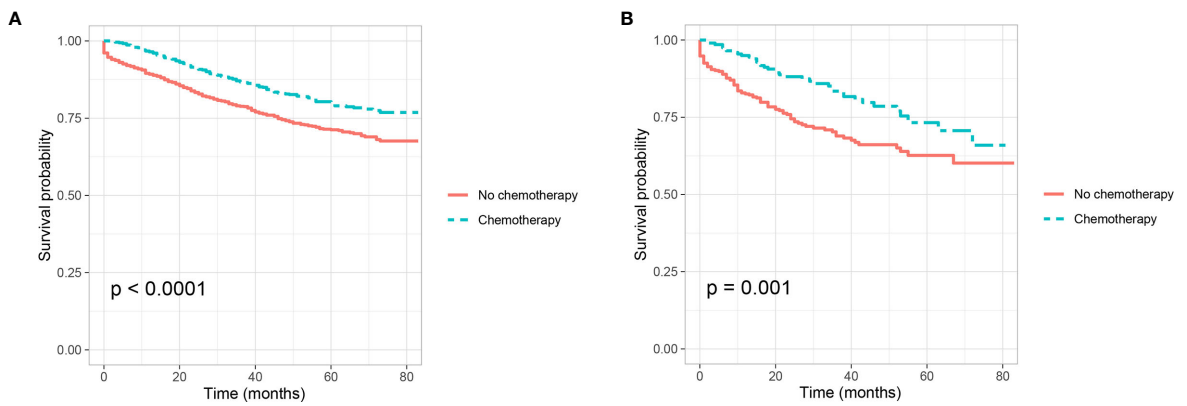

FIGURE 3 | Kaplan–Meier curves for cancer-specific survival according to the receipt of chemotherapy (no chemotherapy VS. chemotherapy) in stage IIB colon cancer **(A)** with the presence of perineural invasion (P <0.001) and **(B)** without the presence of perineural invasion (P = 0.001).

TABLE 5 | Univariate and multivariate survival analyses of T4N0M0 colon cancer.

Variable	Univariate		Multivariate	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age at diagnosis		<0.001		<0.001
≤65	Reference		Reference	
>65	1.655 (1.437–1.908)		1.417 (1.216–1.651)	
Race		0.153		0.014
White	Reference		Reference	
Black	1.183 (0.971–1.440)	0.095	1.324 (1.085–1.616)	0.006
Other	0.897 (0.689–1.167)	0.419	0.913 (0.701–1.189)	0.500
Sex		0.003		0.051
Male	Reference		Reference	
Female	1.223 (1.072–1.395)		1.141 (0.999–1.303)	
Year		0.690		
2010	Reference			
2011	1.068 (0.873–1.308)	0.522		
2012	0.946 (0.765–1.169)	0.606		
2013	1.048 (0.841–1.307)	0.676		
2014	0.909 (0.715–1.157)	0.439		
2015	0.927 (0.707–1.216)	0.483		
Grade		0.001		0.016
I	Reference		Reference	
II	0.928 (0.706–1.221)	0.595	0.950 (0.722–1.252)	0.717
III	1.289 (0.960–1.730)	0.092	1.246 (0.925–1.678)	0.147
IV	1.170 (0.788–1.736)	0.436	1.168 (0.786–1.736)	0.442
Unknown	1.097 (0.652–1.847)	0.726	1.183 (0.702–1.993)	0.528
Histological type		0.508		
Adenocarcinoma	Reference			
Mucinous/signet-ring cell carcinoma	0.938 (0.775–1.134)			
Total number of lymph nodes examined		<0.001		<0.001
<12	Reference		Reference	
≥12	0.521 (0.443–0.612)		0.508 (0.431–0.598)	
Perineural invasion, chemotherapy		<0.001		<0.001
None, no/unknown	Reference		Reference	
None, yes	0.583 (0.499–0.683)	<0.001	0.660 (0.559–0.779)	<0.001
Present, no/unknown	0.802 (0.577–1.114)	0.188	1.458 (1.174–1.811)	0.001
Present, yes	1.497 (1.207–1.855)	<0.001	0.933 (0.668–1.33)	0.684

predictive factor of response to adjuvant chemotherapy in stage II colon cancer. Kaplan–Meier analysis showed that the 5-year CSS rates of patents with and without the receipt of chemotherapy were 91.1 and 90.0% in T3 colon cancer without the presence of PNI, respectively; and the 5-year CSS rates of patents with and without the receipt of chemotherapy were 83.6 and 81.4% in T3 colon cancer with the presence of PNI, respectively. However, after adjusting for other prognostic factors, it was found that the receipt of chemotherapy was not an independent prognostic factor for CSS neither in T3 colon cancer without the presence of PNI nor in T3 colon cancer with the presence of PNI.

Kaplan–Meier analysis showed that patents with the receipt of chemotherapy were significantly associated with better CSS compared to those without the receipt of chemotherapy in T4 colon cancer without the presence of PNI (80.1% VS. 71.2% for 5-year CSS rate, P <0.0001); the receipt of chemotherapy was significantly associated with better CSS compared with those without the receipt of chemotherapy in T4 colon cancer with the presence of PNI (73.3% VS. 62.7% for 5-year CSS rate, P = 0.001). Moreover, after adjusting for other prognostic factors, it was found that the receipt of chemotherapy was independently associated with 34.0 and 36.0% decreased risk of cancer-specific mortality compared with those without the receipt of chemotherapy in T4 colon cancer without and with the presence of PNI, respectively. Therefore, adjuvant chemotherapy was found to provide a survival benefit in stage IIB colon cancer but not in stage IIA colon cancer, irrespective of presence of PNI. We then believed that the presence of PNI was not a predictive factor of response to adjuvant chemotherapy in node-negative colon cancer.

In 2016, a retrospective analysis was conducted by Dr. Cienfuegos and his colleagues (25), which identified 507 patients with stage I–II colon cancer from January 2000 and December 2012. They reported adjuvant chemotherapy could improve the prognosis in PNI (+) patients but not in PNI (−) patients. However, the sample size of this study was very small (n = 57 for PNI (+)), and the authors did not conduct subgroup analyses in stage IIA and stage IIB colon cancer patients. Then in 2019, Leijssen et al. (23) aimed to establish the predictive value of PNI in stage I to III colon cancer and included 1,222 pathological stage I to III colon cancer patients from a prospectively maintained survival and outcomes database. Consistent with our findings, their work also showed that a significant predictive response with adjuvant chemotherapy was not found in PNI (+) node-negative colon cancer.

The present research to the best of our knowledge is the first large population-based study to evaluate the predictive value of response to adjuvant chemotherapy of PNI in the subgroups of stage IIA and stage IIB colon cancer. We have demonstrated the poorer prognosis of PNI (+) in both stage I and II colon cancer. More importantly, in the present study, adjuvant chemotherapy was found to provide a survival benefit in stage IIB colon cancer but not in stage IIA colon cancer, irrespective of presence of PNI. The large sample size made it convincing to conclude that the presence of PNI was not a predictive factor of response to adjuvant chemotherapy in node-negative colon cancer.

Three limitations need to be addressed in this study. First, the retrospective design of this study was subject to its inherent limitations, and the results of the present study warranted replication in larger prospective studies. Second, in the era of individualized and precision medicine, the prognostic values of some biomarkers were widely recognized in colorectal cancer, but they were not included into our analyses due to the limitations of the database (39–43). Third, the detailed chemotherapy regimens were not available from the SEER database.

CONCLUSION

We have demonstrated the poor prognosis of PNI (+) in both stage I and II colon cancer. More importantly, adjuvant chemotherapy was found to provide a survival benefit in stage IIB colon cancer but not in stage IIA colon cancer, irrespective of presence of PNI. The presence of PNI was not a predictive factor of response to adjuvant chemotherapy in node-negative colon cancer.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov).

AUTHOR CONTRIBUTIONS

YL and YX were responsible for the conception and design the study. JT, ZY and WW performed the study selection, data extraction and statistical analyses. JT and JJ were responsible for the draft of the manuscript. JT and ZY contributed to a critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.663154/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Mutated DNA Damage Repair Pathways Are Prognostic and Chemosensitivity Markers for Resected Colorectal Cancer Liver Metastases

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Deficiency of the DNA damage repair (DDR) signaling pathways is potentially responsible for genetic instability and oncogenesis in tumors, including colorectal cancer. However, the correlations of mutated DDR signaling pathways to the prognosis of colorectal cancer liver metastasis (CRLM) after resection and other clinical applications have not been fully investigated. Here, to test the potential correlation of mutated DDR pathways with survival and pre-operative chemotherapy responses, tumor tissues from 146 patients with CRLM were collected for next-generation sequencing with a 620-gene panel, including 68 genes in 7 DDR pathways, and clinical data were collected accordingly. The analyses revealed that 137 of 146 (93.8%) patients had at least one mutation in the DDR pathways. Mutations in BER, FA, HRR and MMR pathways were significantly correlated with worse overall survival than the wild-types ($P < 0.05$), and co-mutated DDR pathways showed even more significant correlations ($P < 0.01$). The number of mutated DDR pathways was also proved an independent stratifying factor of overall survival by Cox multivariable analysis with other clinical factors and biomarkers (hazard ratio = 9.14; 95% confidence interval, 1.21–68.9; $P = 0.032$). Additionally, mutated FA and MMR pathways were positively and negatively correlated with the response of oxaliplatin-based pre-operative chemotherapy ($P = 0.0095$ and 0.048, respectively). Mutated DDR signaling pathways can predict pre-operative chemotherapy response and post-operative survival in CRLM patients.

Keywords: colorectal cancer liver metastasis, DNA damage repair, next-generation sequencing, prognosis, chemo-sensitivity

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths (1). Approximately 50% of patients diagnosed with colorectal cancer will develop liver metastases during their disease. The liver is the most common site of dissemination and causes two thirds of death. Surgical resection of colorectal liver metastases (CRLM) remains the only potentially curative therapy, with 5-year survival rates exceeding 50% in many series. Unfortunately, of patients who undergo liver resection, 50% to 75% will develop disease recurrence within 2 years after resection (2, 3). Therefore, accurate prognostic markers are needed for risk stratification and optimization of patient selection for hepatic resection. However, the prognostic landscape for predicting long-term outcomes in patients undergoing CRLM resection is changing (4–8). In the past 20 years, clinicopathological factors had been gradually established and applied. Recent studies have focused on molecular alterations in CRLM for risk stratification. Specifically, some tumor-related genomic alterations, such as RAS/RAF, are necessary to guide patient selection not only for target therapies but also for hepatic resection and related treatments to achieve the best clinical benefit (5–8). As our understanding, the molecular and genetic determinants of metastatic colorectal cancer's outcomes continue to expand, the importance of these molecular biomarkers in the personalized management of CRLM will only continue to increase.

Since next-generation sequencing (NGS) technology has been widely applied, it is now possible to evaluate a large number of genes and samples extensively and rapidly for prognostic and therapeutic response potentials. Previously integrative genomics analysis has revealed that colorectal cancer usually starts from benign lesions, and accumulation of DNA damage leads to cancer progression to more metastatic and invasive forms (9–11). Seven functional signaling pathways are involved in DNA damage repair (DDR): homologous recombination (HRR), mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), nonhomologous end-joining (NHEJ), checkpoint factors (CPF), and Fanconi anemia (FA) (10, 11), with defective MMR being established as an essential factor in colorectal cancer pathogenesis, treatment, and outcome (12). However, the mutational landscape of DDR pathways and their clinical implications of pre-operative chemotherapy sensitivity and post-operative prognosis has not yet been systematically explored in CRLM. Therefore, in the present study, we aimed to investigate the DDR mutational profile and its impacts on the outcome of patients undergoing liver resection for CRLM.

MATERIALS AND METHODS

Patients and Sample Collection

One hundred forty-six patients who underwent liver resection for CRLM with curative intent at The Beijing Cancer Hospital between January 2015 and February 2017 were included in this study. formalin-fixed paraffin-embedded (FFPE) tissue samples from metastatic liver lesions were collected. Peripheral blood or adjacent healthy tissues were collected from each patient as controls for genomic profiling. Hematoxylin and eosin-stained sections from each tissue sample were subjected to independent pathological reviews to confirm that the tumor specimen was histologically consistent with metastatic tumors (>20% tumor cells) and that the adjacent tissue specimen contained no tumor cells. Demographic and clinicopathologic characteristics and outcomes were collected. This study was conducted in accordance with the Declaration of Helsinki. All patients were acknowledged of the study with informed consent and had granted permission to being included. For survival analyses, overall survival (OS) was examined from liver resection to date of death. Disease-free survival (DFS) was calculated from the date of liver resection until tumor recurrence.

Next-Generation Sequencing

DNA from FFPE tumor tissue samples and patient-matched adjacent healthy tissues or normal blood samples were extracted using the DNA Extraction Kit (QIAamp DNA FFPE Tissue Kit or CWBio Blood Genomic DNA Mini Kit [CW2087M]). Then the DNA was sheared into 150 to 200 bp fragments with Bioruptor®Pico Instrument (Diagenode, Seraing, Belgium). Fragmented DNA libraries were constructed by The KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA) following manufacturer's instruction. DNA libraries were captured with a designed panel of 620 key cancer-related genes (GloriousMed, Shanghai, China). The captured samples were subjected to Illumina HiSeq X-Ten for sequencing. Sequencing adapters were trimmed by Trimmomatic from the raw data (13). Duplicated reads were removed by Picard (http://broadinstitute.github.io/picard/). Mapped reads were also realigned to the genome by Genome Analysis Tool Kit 3.7 (14). Somatic mutations were called by Mutect2 and GATK's HaplotypeCaller (3.7) with a paired workflow and GATK (3.7) respectively (14). Variants were then annotated by ANNOVAR (v-xxx) and self-development code (15). An in-house script was used to verify the human identity concordance of paired samples, and known germline alternations in dbSNP were excluded. Mutations were then filtered with the threshold of 2% in allele frequencies and >8 mutant reads for hotspot mutations, and 5% in allele frequencies, >10 mutant reads for non-hotspot mutations (16).

Statistical Analysis

For comparison of genomic alterations, targeted sequencing data of 195 samples from stage IV liver biopsy and metastasectomy was selected from an 1134 metastatic colorectal tumor/normal pairs database downloaded from cBioPortal (17–19). Sequencing results were trimmed to fit the Memorial Sloan Kettering (MSK)-IMPACT 341 gene assay for comparison of mutation consistency between the two datasets using two-sided Fisher's exact test. Kaplan–Meier survival curves were generated and compared using the log-rank test. Multivariable survival models were computed using Cox proportional hazards regression.

Correlation of DDR mutations with pre-operative chemosensitivity was analyzed by Fisher's exact test. Statistical significance thresholds were set to a two-tailed 0.05 value. R software (version 3.6.1) was used for statistical analyses.

RESULTS

Study Populations

A total of 146 patients with CRLMs underwent hepatectomy between January 4, 2015, and February 24, 2017, in the Hepatopancreatobiliary Surgery Department I at the Beijing Cancer Hospital and Institute (Beijing, China). 29 (19.8%) of patients went directly to surgery, 117 (80.1%) had pre-operative chemotherapy (**Supplementary Figure 1**). Demographic and clinicopathologic characteristics of all patients were summarized in **Table 1**. All patients provided written informed consent, and the ethical review board committee approved the study of the Beijing Cancer Hospital and Institute. Information on specific regimens and efficacy evaluation of pre-operative chemotherapy with or without target agents were collected in 112 of 146 patients. According to the World Health Organization criteria, the response to chemotherapy was classified, which agrees with the Response Evaluation Criteria in Solid Tumors (RECIST). Treatment response was evaluated to assess the possibility of through surgery in a multidisciplinary discussion. Numerous studies have demonstrated that a tumor's response to pre-operative chemotherapy (TRC) is an important predictive factor for evaluating long term survival in patients with CRLMs (17–20). The good TRC group (response to pre-operative chemotherapy) included 66 patients with a complete or partial response and those with a response within a stable disease status (a reduction in the sum of tumor diameters of <30%), while the bad TRC group comprised of 41 patients with progressive disease or progression within a stable disease status (an increase in the sum of the diameters of the target lesion of <20%). The median duration of follow-up was 39.5 months (range, 7–64 months). During the follow-up period, 73 (50.0%) patients died and 108 (74.0%) patients experienced recurrence.

TABLE 1 | Clinical characteristics and pre-operative plans of the study population.

Characteristics	Number of concerns
Gender	
Male	94
Female	52
Age, median (range)	58 (37–80)
Primary site	
Right colon	20
Transverse colon (counted right)	4
Left colon	48
Sigmoid colon (counted left)	14
Rectum	60
Liver metastases occurrence	
Synchronous	93
Metachronous	53
Direct surgery	29
Pre-hepatectomy CEA level, median (IQR), ng/mL	7.01 (0.613 – 651.5)
Number of metastases, median (range)	2 (1–25)
Size of largest liver metastasis	
<5	132
≥5	14
Resection margin	
R0	115
R1	31
Pre-operative therapy (regimen specified)	112
Oxaliplatin-based	79
Irinotecan-based	32
Other	1

Mutation Profile and Survival Analyses for Key Genes in Our Cohort

The mutation profile of our data and the mutation profile comparison with the MSK CRLM dataset were shown in **Figure 1**. The gene distributions were similar in important oncogenic genes between the MSK CRLM and our dataset. The most frequently mutated genes in our cohort were *TP53* (82.9%), *APC* (69.9%), *KRAS* (43.2%), *SMAD4* (17.8%), *CHEK2* (13.0%), *ARID1A* (11.0%), *PIK3CA* (10.3%), *FBXW7* (10.3%), *AMER1* (10.3%), *BRCA2* (5.5%), *CTNNB1* (5.5%), etc.

The DDR-Related Pathway Mutation

Despite the consistency in genes with high mutation occurrences, the DDR-related genes, such as *CHEK2* and *ARID1A*, appear to be significantly more frequently mutated in our population than that in the MSK CRLM population (**Supplementary Table S1**). To depict the profile of DDR pathway mutations in our cohort, we referred to a category including 68 genes in 7 DDR pathways: MMR, BER, CPF, FA, HRR, NER, and NHEJ, according to Wang et al. (20) (**Supplementary Table S2**). 137 of 146 (93.8%) patients had at least one mutation in genes of the covered DDR signaling pathways. The most frequently mutated individual DDR gene was *TP53* (82.9%), followed by *CHEK2* (13.0%), *BRCA2* (5.5%), *FANCM* (5.5%), *PRKDC* (4.8%), *ATM* (4.8%), *ATR* (4.8%), *FANCD2* (3.4%), *BRCA1* (2.7%), *POLE* (2.7%), *BLM* (2.7%), *MLH1* (2.7%) and *POLD1* (2.0%), *etc.* (**Figure 2A**). The signal pathway with the most mutations detected was the CPF signal pathway, in which 88.4% (129/146) of patients carried mutations. This high proportion might be caused by the high frequency of mutations in the *TP53* gene belonging to this pathway. The ranking of the mutation ratios of other DDR pathways were shown in **Figure 2B**.

Mutated DDR Pathways Predicted Worse OS After CRLM Resection

As most DDR genes have not yet been well studied, we defined mutations in DDR pathways as any mutations in the corresponding pathways, including missense, nonsense, insertion, deletion, splice, and multi-hit mutations. A significant difference of OS was found between the patients with or without any DDR pathway mutation (P = 0.039), but the disparity of sample sizes with a wild-type subgroup of only nine patients might have compromised the statistic power. Therefore, further evaluations were conducted separately in the seven specific DDR pathways. The correlations between

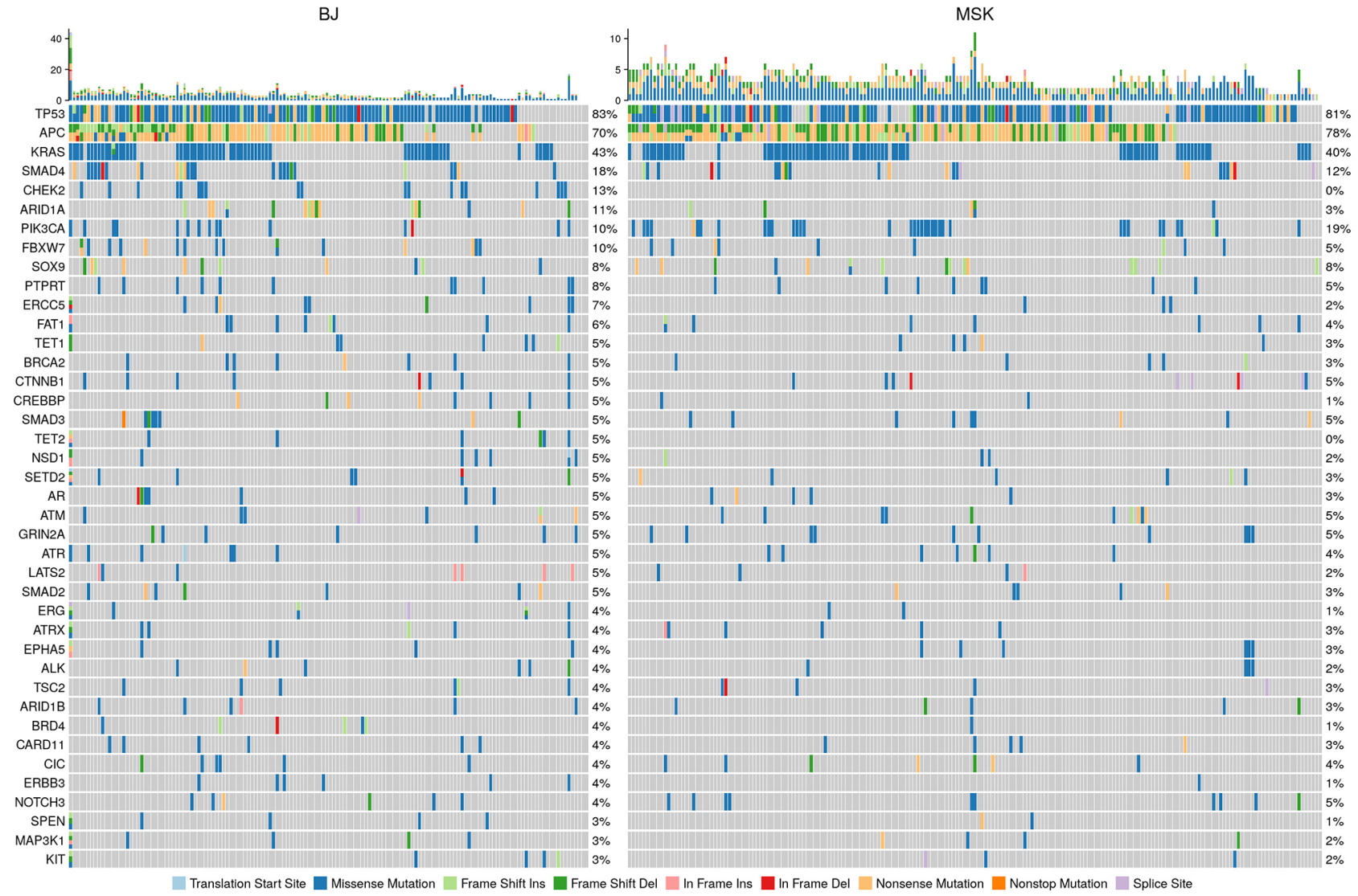


FIGURE 1 | Mutation spectrum comparison of our cohort with the 195 CRLM samples of the MSK data set (Yaeger et al. 2018). Our sequencing results were trimmed according to the standards and gene panel of the other dataset to maintain comparability. The distribution of genes and mutation were consistent between the two datasets, especially for the essential genes with high occurrences, such as TP53, KRAS, APC and PIK3CA.

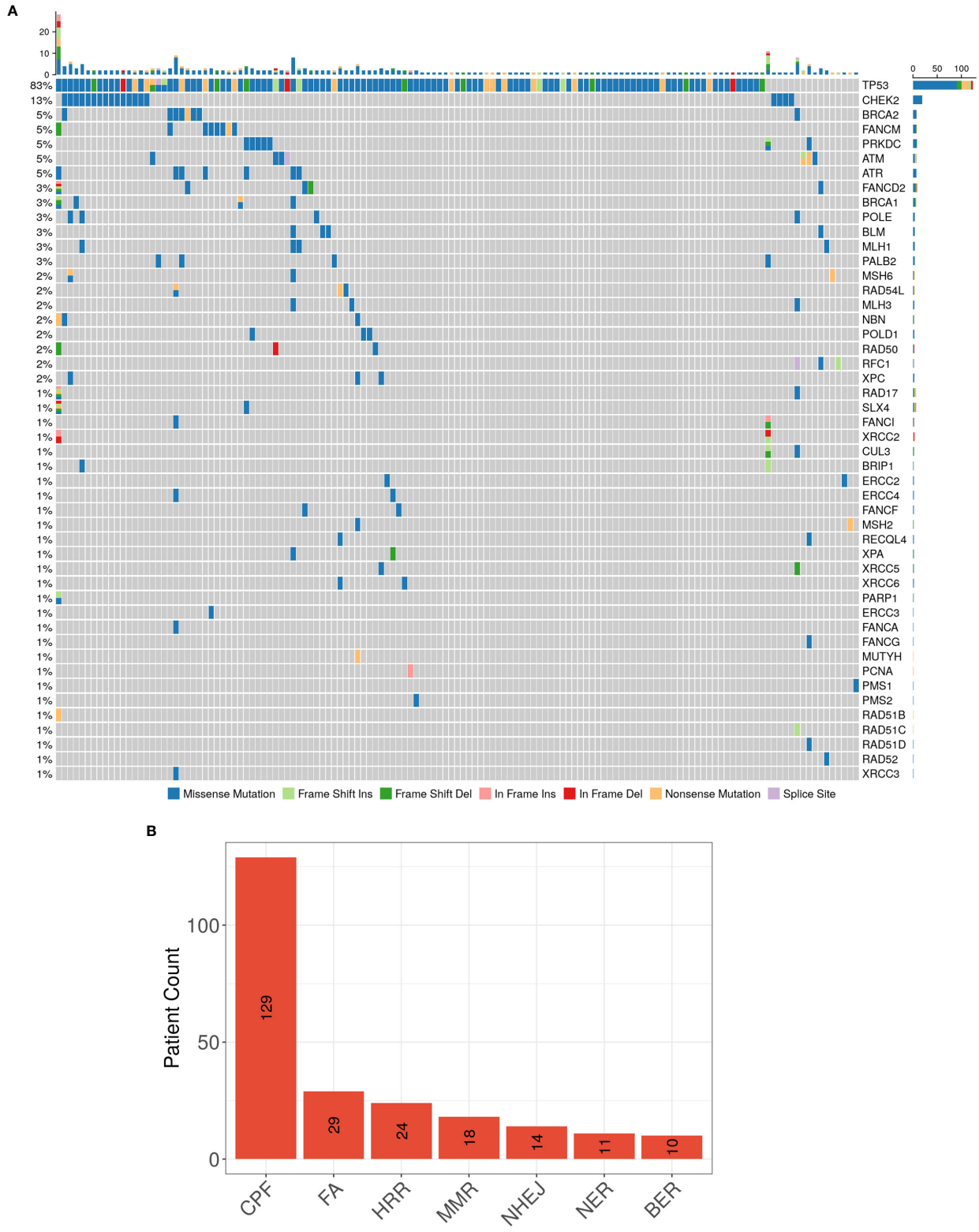


FIGURE 2 | **(A)** The spectrum of DDR genes with detected somatic mutations, and **(B)** the ranking of patients carrying mutations in the 7 DDR signaling pathways in our CRLM cohort NGS results.

mutations and OS after resection of CRLM are shown in **Figure 3**, that mutations in BER, FA, HRR and MMR pathways were significantly associated with shorter OS (mOS: BER mutation [mut] vs. wild-type [wt], 22 months vs. not reached [NR], P = 0.014; FA mut vs. wt, 27 months vs. NR, P = 0.021; HRR mut vs. wt, 28.5 months vs. NR, P = 0.047; MMR mut vs. wt, 26 months vs. NR, P = 0.038). DFS also distinguishably differed between mutated and wild-type subgroups of the above pathways, but the difference appeared significant only concerning the FA pathway (mDFS FA mut vs. wt, 4 vs. 11 months, P = 0.016). Additionally, no significant difference in either OS or DFS outcomes was found in patients with CPF, NER and NHEJ pathway alterations and the wild-types (**Supplementary Figure 2**).

DDR Co-Mutations and Quantity of Mutated DDR Pathways Predicted Better Stratification of Post-Operative Survival in CRLM Patients

To investigate whether co-mutations of specific DDR pathways could have combined and more significant effect than single DDR pathway mutations on the patients' survival, we compared the survival data of subgroups with and without co-mutations in every two of the seven DDR pathways. Co-mutations in the pathways of CPF + FA and FA + HRR, in which the difference showed particular significance between the mutated and the wild-types (mOS: CPF + FA co-mut vs wt, 27 months vs NR, P = 0.045; FA + HRR co-mut vs wt, 25 months vs NR, P = 0.018; mDFS FA + HRR co-mut vs wt, 2 vs 11 months, P = 0.0058), and the lower P value also demonstrated more significance in stratifying OS or DFS than the two single pathways considered independently (**Figure 4A; Supplementary Table 3**).

Additional analyses on the correlation between numbers of mutated DDR signaling pathways with survival also revealed that subgroups with higher amount of mutated DDR signaling pathways had significantly worse OS (P = 0.01). The patients carrying mutations in genes in more than one DDR pathway had a mOS of 29.5 months, while the ones with 1 or 0 mutated DDR pathway showed mOS not yet reached. The DFS of these three subgroups were also distinguishable, but with less significance (median DFS [mDFS]: 8.0 vs 10.5 vs 30.0 months, respectively, P = 0.2; **Figure 4B**).

Multivariable Hazard Ratio Revealed the Correlation of DDR Pathway Mutations and Other Biomarkers in This Cohort

Clinical factors previously reported independently associated with CRC prognosis were entered in a Cox proportional hazards regression model: age, gender, primary tumor sites, metastatic synchronicity, metastatic lesion number, metastatic tumor size, surgical margin, pre-operative carcinoembryonic antigen (CEA), together with the number of mutated DDR signaling pathways. The known prognostic biomarkers, *KRAS* and *PIK3CA* (21–25), which were consistently proved significantly correlated with worse OS in our study population (**Supplementary Figure 3**), were also taken into analysis. Carrying more than one mutated DDR pathways maintained significant negative correlation with OS (HR, 9.14; 95% CI, 1.21–68.9), but not with DFS. Primary site in right colon (HR, 2.325; 95% CI, 1.178–4.588), larger tumor size (HR, 1.17; 95% CI, 1.02–1.3) and *KRAS* mutation (HR, 1.73; 95% CI, 1.03–2.8) were also significantly correlated with OS. No other factor was found significantly associated with either OS or DFS in the Cox regression model (**Figure 4C**; **Supplementary Figure 4**).

The FA and MMR Signaling Pathways Showed Correlations With Efficacy of Oxaliplatin-Based Pre-Operative Therapies

We analyzed whether the mutations in each DDR pathway were related to the efficacy of oxaliplatin- and irinotecan-based pre-operative treatments. The subgroup of patients in the irinotecan subgroup is too small (32/146) and thus the analyses showed low statistical power. In the 79 patients experienced oxaliplatin-based pre-operative treatment, the efficacy of oxaliplatin-based treatment was positively correlated with FA pathway mutations (good TRC% of FA-mutated group: 31.0%, of FA-wild-type group: 6.3%), while negatively correlated with MMR pathway mutations (good TRC% of MMR-mutated group: 7.1%, of MMR-wild-type group: 25.0%). The correlations were both significant (**Figure 5**).

DISCUSSION

Regular functions of DDR are essential to regular replication and metabolism for cells. Mutations that may influence the functions of DDR signaling pathways would cause genomic instability and thus the accumulation of mutations, DNA base mismatches, and chromosomal abnormalities. Although there are already several studies about the clinical significance of specific DDR genes, such as *BRCA1/2*, *POLE*, *POLD1*, and *MLH1* (26–31), studies about correlations of DDR pathway somatic mutations with the prognosis of CRC that consider the DDR pathways as a whole are still lacking. Herein, we investigated the mutational distribution and clinical significance of DDR signaling pathways in 146 patients with CRLM after resection. We demonstrated that the existence and quantity of mutated DDR pathways might correlate with survival after liver resection and pre-operative chemotherapy response for CRLM patients.

Single gene biomarkers of CRC, such as *TP53*, *APC*, *KRAS*, and *PIK3CA*, have already been well-recognized of their high populational mutation occurrences, as well as their significant correlations with CRC prognosis (32–36). Previous study on Chinese CRC patients with brain metastases also reveals modified DDR gene signature, homologous recombination deficiency and mismatch repair deficiency in brain metastases than the primary lesions (37). Therefore, considering the mutational status of DDR pathways, which is possibly unique to metastatic CRC patients, may help provide a more comprehensive reference for treatment and surveillance.

Different DNA damage forms evoke responses by different repair-related signaling pathways (38, 39). Alterations in DDR

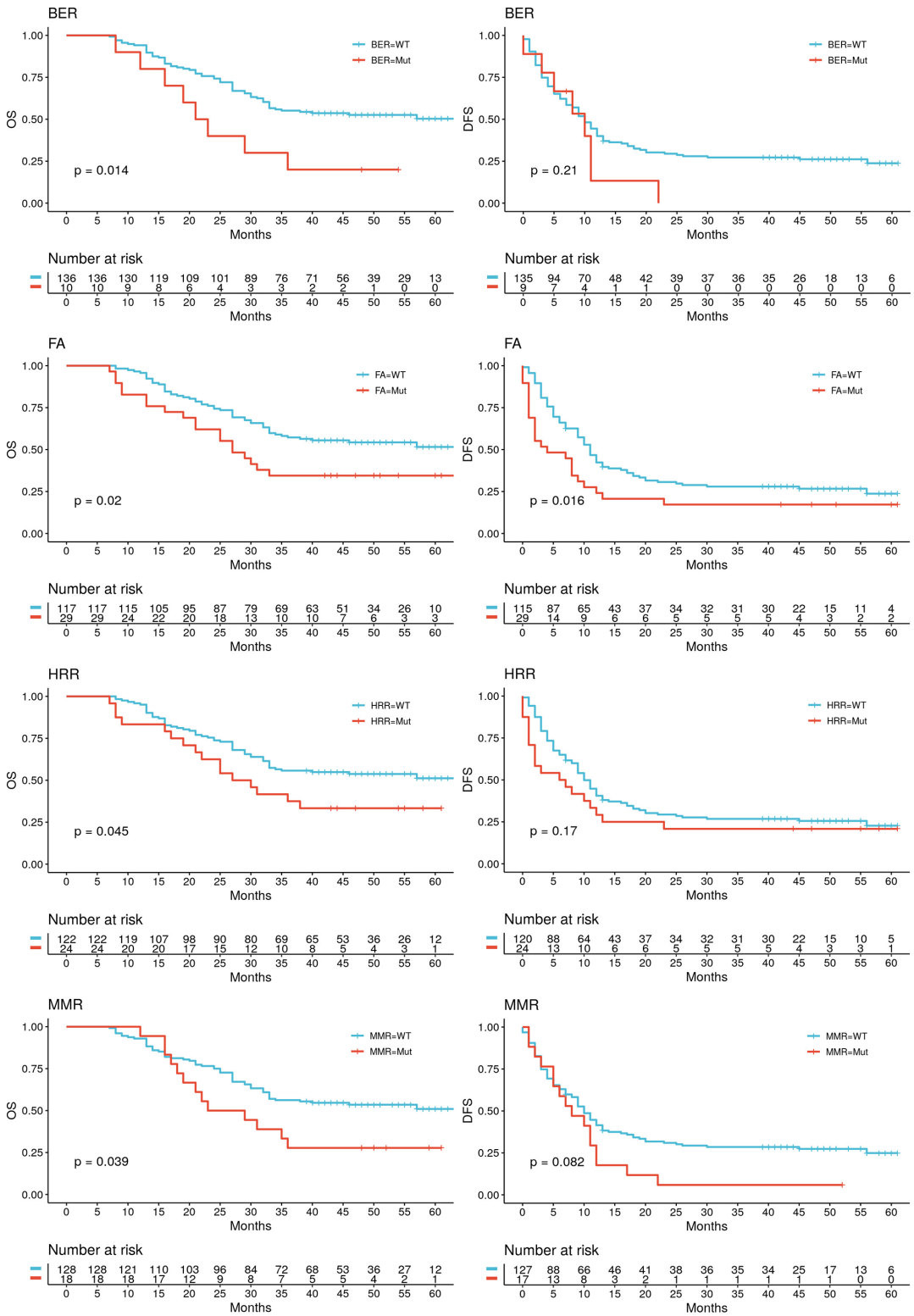


FIGURE 3 | Kaplan-Meier curves of survival differences in patients with or without mutations in certain DDR pathways. OS in four of the DDR pathways showed significant differences between the mutated and wildtype patients: BER, FA, HRR and MMR. The patients carrying mutations in these four pathways are statistically having shorter OS and thus poorer prognosis than the wildtype ones. DFS in patients with or without mutations in the above pathways showed no significant difference, except for the FA pathway. The curves of other pathways without any significance in results are attached in **Supplementary Figure 3**.

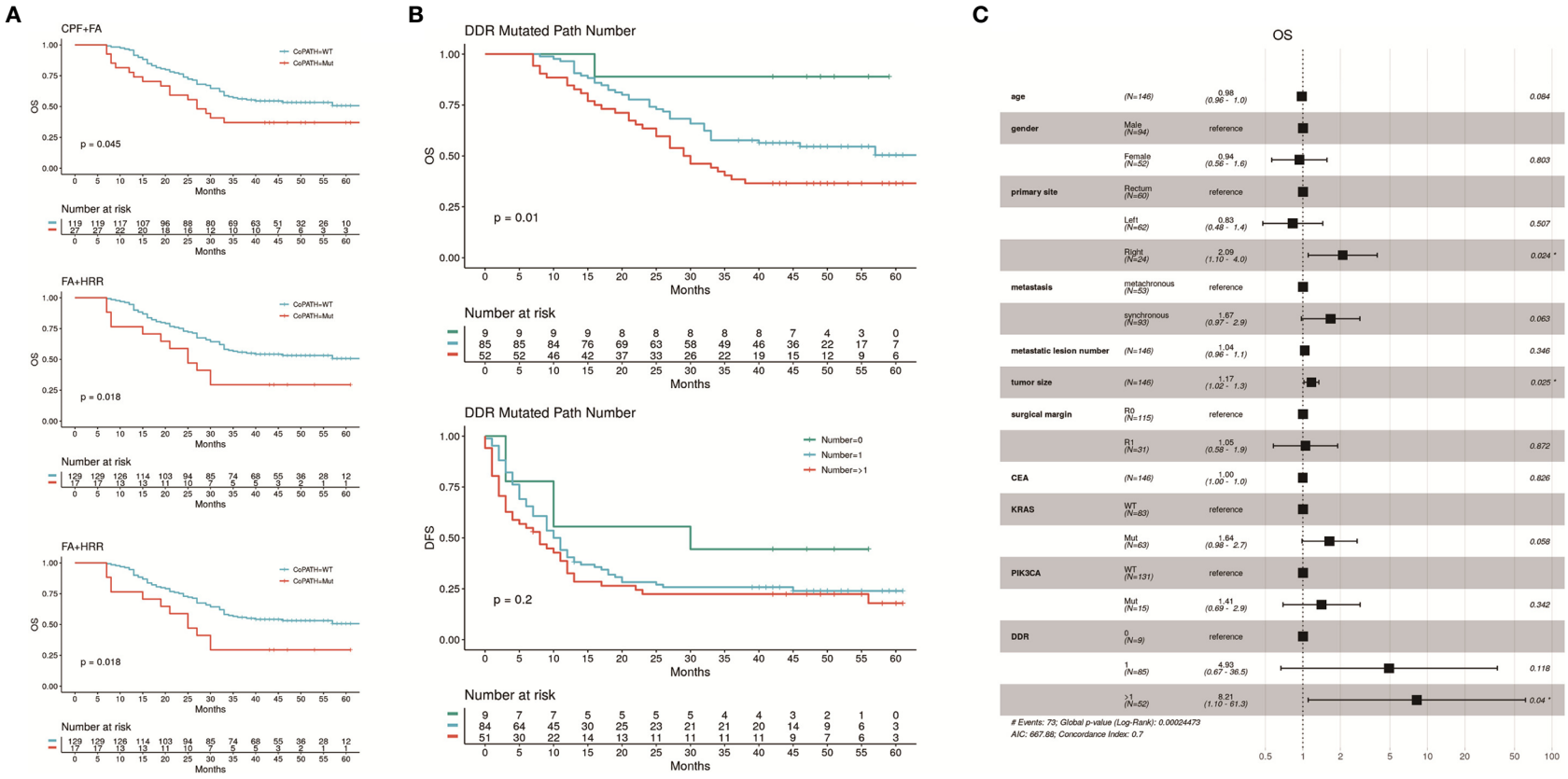
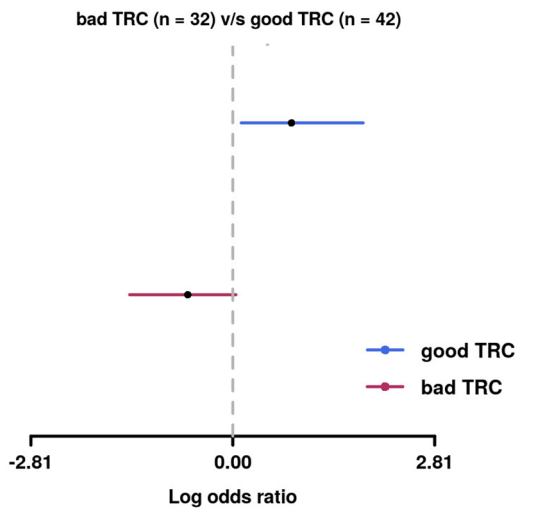


FIGURE 4 | **(A)** Kaplan-Meier curves of OS and DFS showing differences between subgroups with and without specific co-mutations of two DDR pathways. The subgroups carrying co-mutations in CPF + FA or FA + HRR pathways had significantly worse OS, and the FA + HRR subgroup also had significantly worse DFS. The results with insignificant correlations were shown in **Supplementary Table 3**. **(B)** the patients carrying mutations in more than one DDR pathway had the worst OS comparing with those with 1 or 0 mutated DDR pathway mutations. The DFS of these three subgroups were also distinguishable, but with less significance. **(C)** Result of multivariable Cox proportional hazards regression analysis of OS, including the number of mutated DDR pathways, as well as clinical factors previously reported independently associated with CRC prognosis. Carrying more than one mutated DDR pathways maintained significant negative correlation with OS (HR = 9.14, 95% CI: 1.21 – 68.9). The analysis of DFS showed no significance, and result was attached in **Supplementary Figure 4**.



	good TRC	bad TRC	p-value	95% CI-low	95% CI-high
FA	13/42	2/32	**	1.312	65.144
MMR	3/42	8/32	*	0.034	1.103

FIGURE 5 | The efficacy of oxaliplatin-based treatment was positively correlated with FA pathway mutations, while negatively correlated with MMR pathway mutations. The correlations were both significant.

pathways could hinder the DNA repair capacity, inducing those that confer genetic and chromosomal instability, and each of the DDR pathways possesses a specific function and collaborate in DNA repairment. The BER pathway is mainly responsible for DNA single-strand breaks, which are the most common type of DNA damage (40–42). The HRR pathway answers DNA double-strand breaks (39, 42), and the FA pathway aims for DNA inter-strand crosslinks (39, 43, 44). Although the loss of function in one or more DDR signaling pathways can, to some extent, be compensated by other pathways (44), due to the generally considered mutually exclusive and distinct functions of each, the outcomes could potentially accumulate the influence on survival, causing significant damage. Mice embryo studies have shown synthetic lethality of HRR and NHEJ pathways (45, 46). Defective variants in *POLD1* and *POLE*, essential genes in the BER pathway, are related to significantly higher mutational burden and malignancy through BER's correlation to the MMR pathway (47). Co-mutations in the MMR and HRR pathways may also be related to hypermutated CRC with worse survival, via interruption of DNA binding and replication (48). Our study reveals that beyond each single DDR pathway mutations, the co-mutations and the number of mutated DDR pathways are also significantly related to post-operative survival, and the correlations were independent of other clinical traits. Even though the sparsity of patients with mutations possibly influenced the statistical power in each of the overlaps, these results indicated that not only mutations in separate DDR pathways are prognostic-related in our cohort, but the effect could also act additively with possibly better stratification power when considered together.

Beyond mutations in DDR pathways, multivariate Cox analysis also indicates that other known prognostic biomarkers, such as right colon-primary, larger tumor size and *KRAS* mutations, could act accumulatively with DDR pathway mutations on influencing the OS, enlightening further clinical explorations of for stratification of risks of CRLM patients. According to previous studies, DDR mutations are more frequently detected in right colon-primary sites than left colon-primary cases (49), indicating probable developmental differences. Molecular analysis has shown that *POLE* damaging variants may influence the oncogenesis through the RAS/RAF signaling pathway (50). *KRAS* activating mutations also present augmentation to the expression of HRR signaling pathway in *in vitro* study (51). However, the mechanistic details and specific molecular collaborations concerning clinical application may still require further researches.

The effects of platinum-based chemotherapy on DNA are mainly intra-strand crosslink and inter-strand crosslink (46, 52), which are primarily repaired by the FA/BRCA pathway. The normal or overexpression of the FA pathway has been discovered to be one of the mechanisms of platinum resistance in various cancers, including ovarian cancer. Multiple studies on ovarian cancer cell lines have shown that FA-deficiency induced by FA pathway inhibitors, such as bortezomib and curcumin, can sensitize the cell line to cisplatin treatment (39, 43, 52, 53). Other studies also showed that the MMR pathway's normal function is necessary for detecting and repairing DNA damages caused by platinum-based chemotherapy. With MMR defective, tumor cells can resist DNA damage caused by platinum and continue to proliferate. MMR deficiency has been considered as a related pathway of cisplatin resistance in many studies. Ovarian adenocarcinoma cell line research has revealed that loss of hMLH1 or hMSH2 can lead to an approximately two-fold increase in cisplatin, and a 1.3-fold increase in carboplatin resistance (53, 54). Studies on ovarian cancer cell lines have also shown that the MMR pathway's inactivation can reduce the sensitivity to cisplatin and carboplatin, yet has no significant effect on oxaliplatin (55). With no confirmed results concerning DDR pathway mutations and the efficacy of platinum-based therapies in CRLM, our results were mostly consistent with other cancers' existing studies, while also called on more specific and CRLM-related studies. Moreover, instead of focusing on merely the essential genes, we considered FA and MMR pathways as a whole, which may have better coverage for clinical application. However, our study has inevitable limitations that the tumor tissues are sampled from resections after the neo-adjuvant or conversion chemotherapy, and the number of patients in each subgroup is small. This may have caused the controversy that patients with FA pathway mutations present better TRC to oxaliplatin-based pre-operative treatment but worse OS than the FA wildtypes. As shown in **Supplementary Table 4**, among all patients carrying FA pathway mutations, the subgroup showing good

oxaliplatin TRC appeared to have more metastatic lesions and synchronous metastases. Both factors have been reported to correlate significantly independent of treatment with shorter OS in mCRC (56, 57). On the other hand, the higher pre-operative CEA levels and more patients undergoing direct surgeries presented in the subgroup without good oxaliplatin TRC are also negatively correlated with the survival of mCRC (58–61). Therefore, when all patients carrying FA pathway mutations were considered as a whole in survival analyzes, the positive effect of chemotherapeutic response may have been compromised by other negative factors listed above, especially in small populations as in this study. Further verifications would be needed to avoid the above compromising factors.

In conclusion, mutations in DDR signaling pathways may predict worse post-operative survival in our CRLM patients. Nevertheless, studies with larger sample sizes and better coverage of DDR-related genes are pivotal for further verifications. Clinical explorations are also ongoing to use the poly (ADP-ribose) polymerase (PARP) inhibitors in colorectal cancer patients carrying DDR inactivation and have benefited from previous platinum chemotherapy (62, 63). These findings may be useful for clinical decisions in patients with tumor characteristics associated with poor prognosis and risk stratification of patients in future clinical studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Peking University Cancer Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

B-CX and KW conceived and designed the study. ML, H-WW, K-MJ, QB, DX, and L-JW contributed to the clinical samples and informed consents collection. JL and L-JL provided clinical information. C-HY and X-LY conducted the bioinformatics analyses. X-YZ wrote the first draft of the manuscript. B-CX, KW, ML, and GJ reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.643375/full#supplementary-material

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Identification of Hub Genes Associated With Sensitivity of 5-Fluorouracil Based Chemotherapy for Colorectal Cancer by Integrated Bioinformatics Analysis

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Colorectal cancer (CRC) is one of the most common malignant tumors. 5-fluorouracil (5-FU) has been used for the standard first-line treatment for CRC patients for several decades. Although 5-FU based chemotherapy has increased overall survival (OS) of CRC patients, the resistance of CRC to 5-FU based chemotherapy is the principal cause for treatment failure. Thus, identifying novel biomarkers to predict response to 5-FU based chemotherapy is urgently needed. In the present study, the gene expression profile of GSE3964 from the Gene Expression Omnibus database was used to explore the potential genes related to intrinsic resistance to 5-FU. A gene module containing 81 genes was found to have the highest correlation with chemotherapy response using Weighted Gene Co-expression Network Analysis (WGCNA). Then a protein-protein interaction (PPI) network was constructed and ten hub genes (*TGFBI*, *NID*, *LEPREL2*, *COL11A1*, *CYR61*, *PCOLCE*, *IGFBP7*, *COL4A2*, *CSPG2*, and *VTN*) were identified using the CytoHubba plugin of Cytoscape. Seven of these hub genes showed significant differences in expression between chemotherapy-sensitive and chemotherapy-resistant samples. The prognostic value of these seven genes was evaluated using TCGA COAD (Colorectal Adenocarcinoma) data. The results showed that TGFBI was highly expressed in chemotherapy-sensitive patients, and patients with high TGFBI expression have better survival.

Keywords: colorectal cancer, 5-fluorouracil, chemotherapy sensitive, weighted gene co-expression network analysis, biomarker

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INTRODUCTION

CRC is one of the most common malignant tumors and the second cause of tumor-related mortality worldwide (1, 2). By 2030, the global burden of CRC is expected to increase by 60%, with 2.2 million new cases and 1.1 million deaths (3). The 5-year survival for CRC patients with local tumor is 90.3%, and 70.4% for patients with locally advanced disease, which declines to 12.5% for patients with metastatic disease (4). Surgery is highly recommended for early CRC and locally advanced CRC (5, 6). However, half of the patients treated with surgery will suffer a recurrence within 3 years after surgery (7). For patients with stage III and some stage II CRC, chemotherapy followed by surgery is given for about six months to reduce the risk of recurrence (8).

Over the last few decades, substantial progress has been made in the development of new treatment regimens that fundamentally increase the overall survival (OS) of CRC patients. Patients with stage III or high-risk stage II CRC benefit from the use of adjuvant chemotherapy with5-FU-based regimens (9, 10). Although most patients can benefit from chemotherapy, others may suffer ineffective chemotherapy for several cycles until the treatment effects are determined, which usually leads to adverse, life-threatening side effects (11, 12). The resistance of CRC to 5-FU based chemotherapy is the principal cause for treatment failure. Thus, the stratification of chemotherapy response based on biological characteristics is critical for individualized treatment. Identifying novel biomarkers to predict response to 5-FU based chemotherapy is urgently needed.

Human tumors become resistant to treatment in the presence of a drug, that is, tumors possessing innate resistance to drugs. Innate resistance is usually detected in the early stages of drug development or early clinical trials of biological effects. However, sometimes innate resistance can't be found until retrospective analysis of *in vivo* studies (13).

Some biomarkers that predict the response of 5-FU based therapy for CRC patients have been identified. Low expression of thymidylate synthase (TS), an enzyme encoded by *TYMS* gene, was associated with increased sensitivity to 5-FU based therapy (14, 15). Several studies have indicated that the expression of dihydropyridine dehydrogenase (DPD) which is encoded by *DPYD* gene is a predictive marker for both the effectiveness and toxicity of 5-FU treatment (16). High DPD activity in tumor tissue might be associated with the drug resistance by reducing the cytotoxic effects of 5 FU (16). In addition, DPD level affects 5-FU catabolism, low DPD level leading to an effective accumulation of the drug inside cell through reducing 5-FU catabolism (17). It has been reported the range of *DPYD* expression in CRC tissues which were nonresponsive to 5-FU was much broader than that of the responding CRC tissues (18). Thymidine phosphorylase (TP), encoded by *TYMP* gene, has been found to be a useful marker for predicting the effectiveness of 5-FU based chemotherapy (19). There is a correlation between low TP expression and improved treatment outcomes, low TP expression predicting a good response to 5-FU chemotherapy (20, 21). However, some other studies indicated the opposite conclusion. The cells with higher TP expression may be related to increased sensitivity to 5-FU (16). Besides, membrane transporter proteins are involved in chemoresistance mechanisms by transporting drugs out of the cell, thereby resulting in chemotherapy failure. ATP-binding cassette (ABC) transporters belong to membrane transporter proteins. Several ABC transporters related to 5-FU resistance of CRC patients have been identified, such as ABCB5 (22), ABCC11 (23). Although some proteins and mechanisms associated with 5-FU resistance have been reported, more biomarkers and related mechanisms of 5-FU resistance remain to be further studied. In the present study, we aimed to explore novel biomarkers for predicting intrinsic resistance of CRC patients to 5-FU.

Abbreviations: ABC transports, ATP-binding cassette (ABC) transports; BP, biological processes; CC, cellular compartments; CRC, colorectal cancer; DPD, dihydropyridine dehydrogenase; FDR, false discovery rate; GEO, gene expression omnibus; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular functions; OS, overall survival; PPI, protein-protein interaction; TGFB, transforming growth factor β; TOM, topological overlap matrix; TP, thymidine phosphorylase; TS, thymidylate synthase; WGCNA, weighted gene co-expression network analysis; 5-FU, 5-fluorouracil.

MATERIALS AND METHODS

Data Collection

A flowchart of this study is presented in **Figure 1**. Gene expression profiles of Dataset GSE3964 were downloaded from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3964). This dataset contains expression profiling of clinical samples collected from CRC patients before the exposure to 5-FU based combined chemotherapy. Analysis of gene expression profiles between chemotherapy-sensitive patients and chemotherapy-resistant patients may identify biomarkers associated with innate tumor drug responses. Another gene expression profiles of CRC patients undergoing chemotherapy and corresponding clinical information were downloaded from TCGA COAD (https://portal.gdc.cancer.gov/). Gene expression profiles of Dataset GSE19860 without z-score normalized (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19860) was used to verify the expression patterns of the screened hub genes. This dataset contains responders and non-responders who received modified FOLFOX6 therapy.

Weighted Gene Co-Expression Network Analysis (WGCNA)

The gene expression profile of GSE3964 was constructed to gene co-expression networks using the WGCNA package in R to explore the modules of highly correlated genes among samples for relating modules to external sample traits (24). A weighted adjacency was constructed through calculating Pearson correlations of all gene pairs. Soft power $\beta = 4$ was selected to construct a standard scale-free network. The similarity matrix which is done by Pearson correlation of all gene pairs was transformed into a topological overlap matrix (TOM) as well as the corresponding dissimilarity (1- TOM). Then a hierarchical clustering dendrogram of the 1-TOM matrix was used to classify

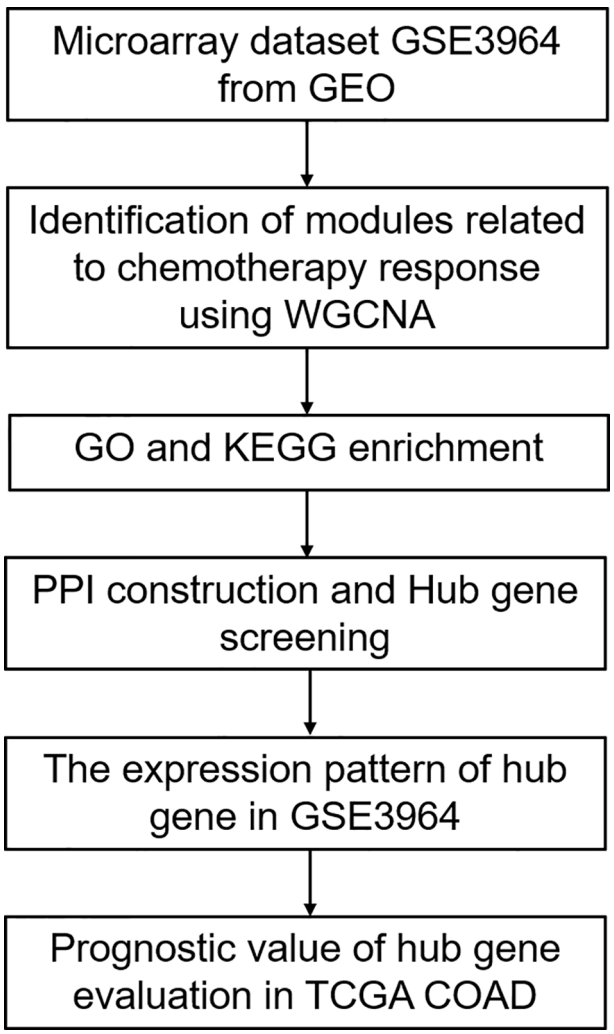

FIGURE 1 | The workflow for analyzing the gene related to chemotherapy response in CRC.

the similar gene expression into different gene co-expression modules. Afterward, the module-clinical trait association was calculated to identify functional modules in a co-expression network. The module with a high correlation coefficient was regarded to be associated with clinical traits and was selected for further analysis.

GO and KEGG Functional Enrichment Analyses

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for genes in functional modules were performed using an R package "clusterProfiler". GO annotation is based on three categories, including biological processes (BP), cellular compartments (CC), and molecular functions (MF). Terms in GO and KEGG with a false discovery rate (FDR) < 0.05 were considered significantly enriched and were visualized by R package "ggplot2".

Protein-Protein Interaction Network Construction and Hub Gene Screening

The protein-protein interaction (PPI) network of genes in functional modules was constructed with an online STRING database (https://string-db.org), and an interaction with a combined score > 0.4 was considered as statistical significance. Cytoscape, an open-source bioinformatics software platform, was used to visualize molecular interaction networks. Hub genes were identified using the DMNC algorithm of cytoHubba plugin in Cytoscape.

Verification of the Expression Patters and Prognostic Values of Hub Genes

The expression of the top ten hub genes between chemotherapy-sensitive and chemotherapy-resistant samples was analyzed. Gene with $p < 0.05$ is considered a significantly differentially expressed gene between the chemotherapy-resistant and chemotherapy-sensitive group. Then prognostic values of significantly differentially expressed hub genes were evaluated in CRC patients undergoing chemotherapy from TCGA COAD. Kaplan-Meier survival analysis was performed using the "survival" R package based on the median value of each gene. $p < 0.05$ is considered a statistically significance.

Cell Culture and Transfection

HCT116 and DLD1 cells (purchased from ATCC) were cultured in DMEM medium (Cellmax) containing 10% fetal calf serum (BI), and 100 U/ml each of penicillin and strepcomycin (BI) at 37°C with 5% CO_2. Cells were transfected with si-RNAs or expression plasmids using Lipofectamine 2000 reagent (Invitrogen, USA) according to the manufacturer's instructions.

Establishment of 5-FU Resistant Cells

To establish 5-FU resistant cells, cells were treated with a high concentration of 5-FU for 24h, then the media was replaced with fresh media containing a low concentration of 5-FU. After 2 weeks of treatment at the low concentration, increase 1.5 times of the dose, and repeat the same. 5-FU resistant HCT116 cells were generated by treating its parental cells with 40μM 5-FU for 24h, then treating the cells with 0.3125 μM 5-FU and increasing 1.5 times of the dose. 5-FU resistant DLD1 cells were generated by treating its parental cells with 100μM 5-FU for 24h, then treating the cells with 1.25μM 5-FU and increasing 1.5 times of the dose. The 5-FU resistant HCT116 cells and 5-FU resistant DLD1 cells were obtained by continuous exposure to gradually increased concentrations of 5-FU for four months.

MTT Assay

HCT116 or DLD1 cells were washed with DMEM without phenol red and incubated with MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide) at the concentration of 0.5 mg/ml in DMEM without phenol red. Four hours after incubation, the media were dumped off and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The optical density (OD) was measured by a photometer at 490 nm. The data were normalized to control and the ratios were presented as mean ± SE with three experiments.

Immunoblot Analysis

Proteins were separated by 9% SDS-PAGE and then transferred onto a nitrocellulose membrane (pall). The following primary antibodies were used: anti-Beta Actin (Proteintech), anti-TGFBI (Proteintech). The following secondary antibody was used: Goat anti-mouse IgG-HRP antibody (Proteintech). The proteins were visualized using an ECL detection kit (GE).

Plasmids

Full-length TGFBI DNA was amplified by PCR with the primer 5'-GATCTCGAGCTCAAGCTTCGAATTCCATGGCGCTCTTCGTGCGG-3' and 5'-GATCTCGAGCTCAAGCTTCGAATTCCATGGCGCTCTTCGTGCGGTCAGTTATCTAGATCCGGTGGATCCCTAATGCTTCATCCTCTCTAATAACTTTTGATAGACAG-3' using pOTB7-TGFBI (P14682, www.miaolingbio.com). pEGFP-C1 was linearized through EcoRI and BamHI. Then the TGFBI DNA was cloned into pEGFP-C1 through Gibson Assembly.

RESULTS

Weighted Gene Co-Expression Network Analysis and Key Modules Identification

To explore the functional clusters related to chemotherapy response, the weighted gene co-expression network was constructed from GSE3964 datasets which containing 10 chemotherapy-sensitive and 13 chemotherapy-resistant samples. The included samples were clustered with the average linkage hierarchical clustering method. The power of $\beta = 4$ was selected as the soft-thresholding parameter to conduct a scale-free network (**Figure 2**). A total of 12 modules were identified with the average linkage hierarchical clustering (**Figure 3**). The size of each identified module was listed in **Supplementary Table 1**. To evaluate the association between each module and two clinical traits (chemotherapy-sensitive and chemotherapy-resistant), the heatmap of the module-trait relationship was plotted (**Figure 4**). The red module was found to have the highest correlation with chemotherapy response (**Figure 4**, r = 0.58, $p = 0.004$). The genes in the red module were highly correlated with the module (**Figure 5**, r = 0.62, $p = 6.7\times10^{-10}$).

Functional and Pathway Enrichment Analysis

To explore the potential function of the red module which had the highest correlation with chemotherapy response, GO and KEGG enrichment analysis was performed. For BP enrichment, the genes in the red module were mostly enriched in extracellular matrix organization and extracellular structure organization (**Figure 6A**). For CC enrichment, these genes were mainly involved in collagen-containing extracellular matrix and collagen trimer (**Figure 6A**). For MF enrichment, these genes were mainly enriched in extracellular matrix structural constituent and extracellular matrix binding (**Figure 6A**). For KEGG enrichment, these genes were mainly enriched in focal adhesion pathway and ECM-receptor interaction pathway (**Figure 6B**).

PPI Network Construction and Hub Genes Screening

PPI network of the genes in the red module was constructed through the STRING database and visualized with Cytoscape software. The PPI network and hub genes identified from the

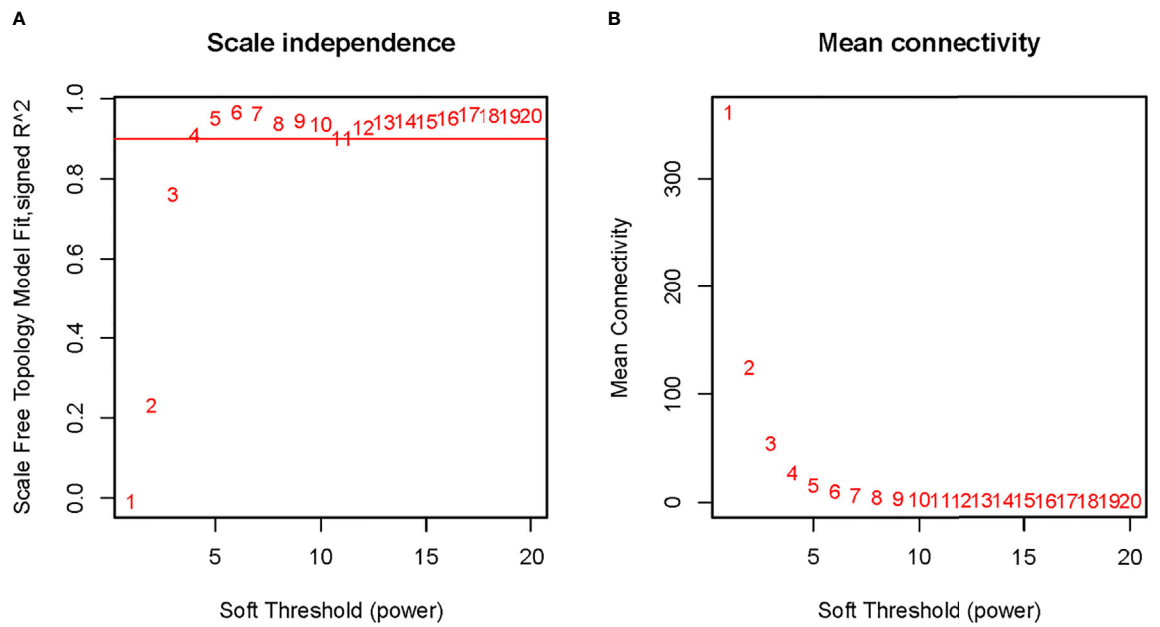

FIGURE 2 | Determination of soft-thresholding power in weighted gene co-expression network analysis (WGCNA). **(A)** The scale-free fit index for various soft-thresholding powers; **(B)** The mean connectivity for various soft-thresholding powers.

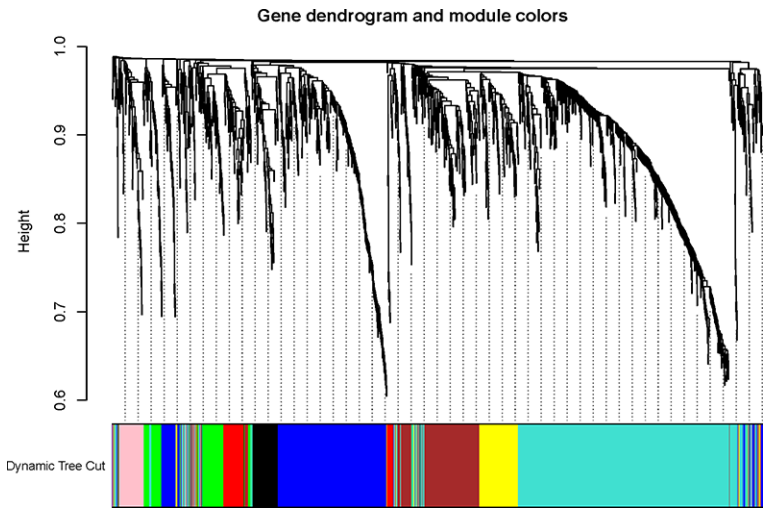

FIGURE 3 | The Cluster dendrogram of co-expression network modules was ordered by a hierarchical clustering of genes based on the 1-TOM matrix. Each module was assigned to different colors.

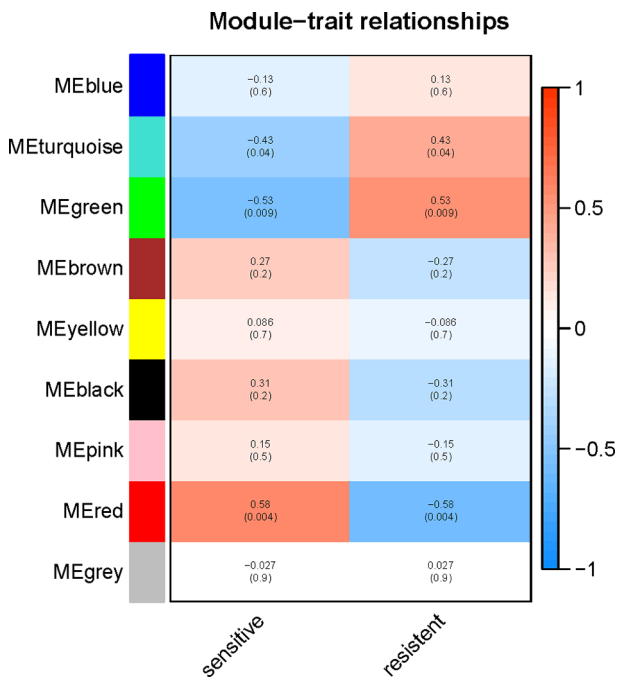

FIGURE 4 | Relationships between the module and clinical traits. Each row represents a color module and column corresponds to a clinical trait (chemotherapy-sensitive or chemotherapy-resistant). Each cell contains the corresponding correlation and *p*-value.

network through the DMNC algorithm of CytoHubba plugin were shown in **Figure 7**. According to the DMNC scores, the top ten highest-scored genes, including *TGFBI, NID, LEPREL2, COL11A1, CYR61*, *PCOLCE*, *IGFBP7*, *COL4A2*, *CSPG2*, and *VTN*, were regarded as hub genes.

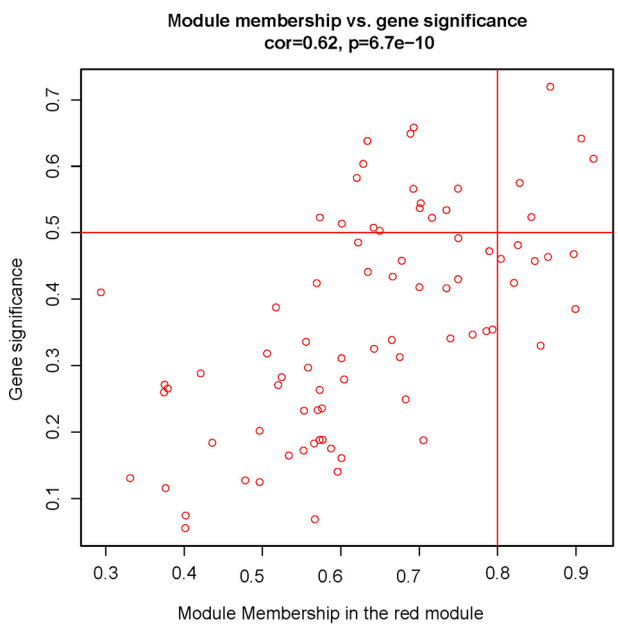

FIGURE 5 | Scatter plots of the red module.

Verification of the Expression Patterns and Prognostic Values of Hub Genes

To confirm the reliability of the hub genes, the expression of the top ten hub genes between chemotherapy-sensitive and chemotherapy-resistant samples was plotted as a box plot graph. Seven hub genes showed significant differences between chemotherapy-sensitive and chemotherapy-resistant samples (**Figure 8**). To evaluate the prognostic value of the seven genes, the chemotherapy patients from TCGA COAD were

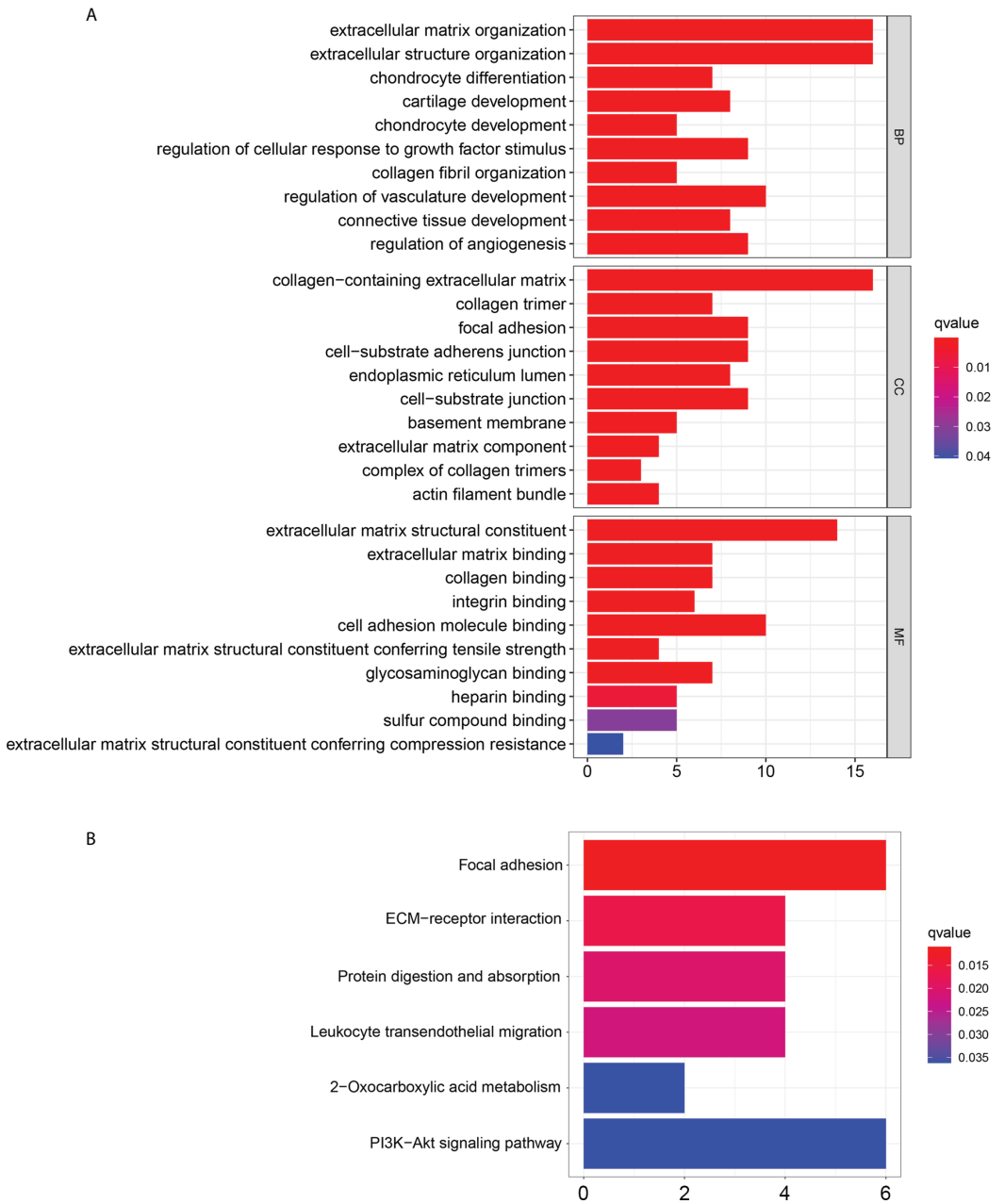

FIGURE 6 | GO and KEGG enrichment analysis of the genes in the red module. **(A)** GO enrichment analysis based on biological processes (BP), cellular compartments (CC), and molecular functions (MF). **(B)** KEGG enrichment analysis.

stratified into a high-expression group and a low-expression group based on the median value of each gene. As shown in **Figure 9**, The survival of patients with high expression of TGFBI is better than those patients with low expression of TGFBI.

Verification of the Relationship Between TGFBI level and 5-FU Sensitivity in Datasets and Cells

To further verify the relationship between TGFBI level and 5-FU sensitivity, samples from the GEO dataset (GSE19860) were analyzed. In line with our expectations, among the CRC patients who received modified FOLFOX6 therapy, responders also showed a higher level of TGFBI than non-responders (**Supplementary Figure 1**).

To evaluate whether TGFBI mediates the response of CRC cells to 5-FU treatment. We generated the 5-FU resistant cells from the parental HCT116 cells and DLD1 cells, respectively by continuous exposure to gradually increased concentrations of 5-FU. Then the TGFBI levels were detected in the parental cells and 5-FU resistant cells. Cell viability of the parental cells and 5-FU

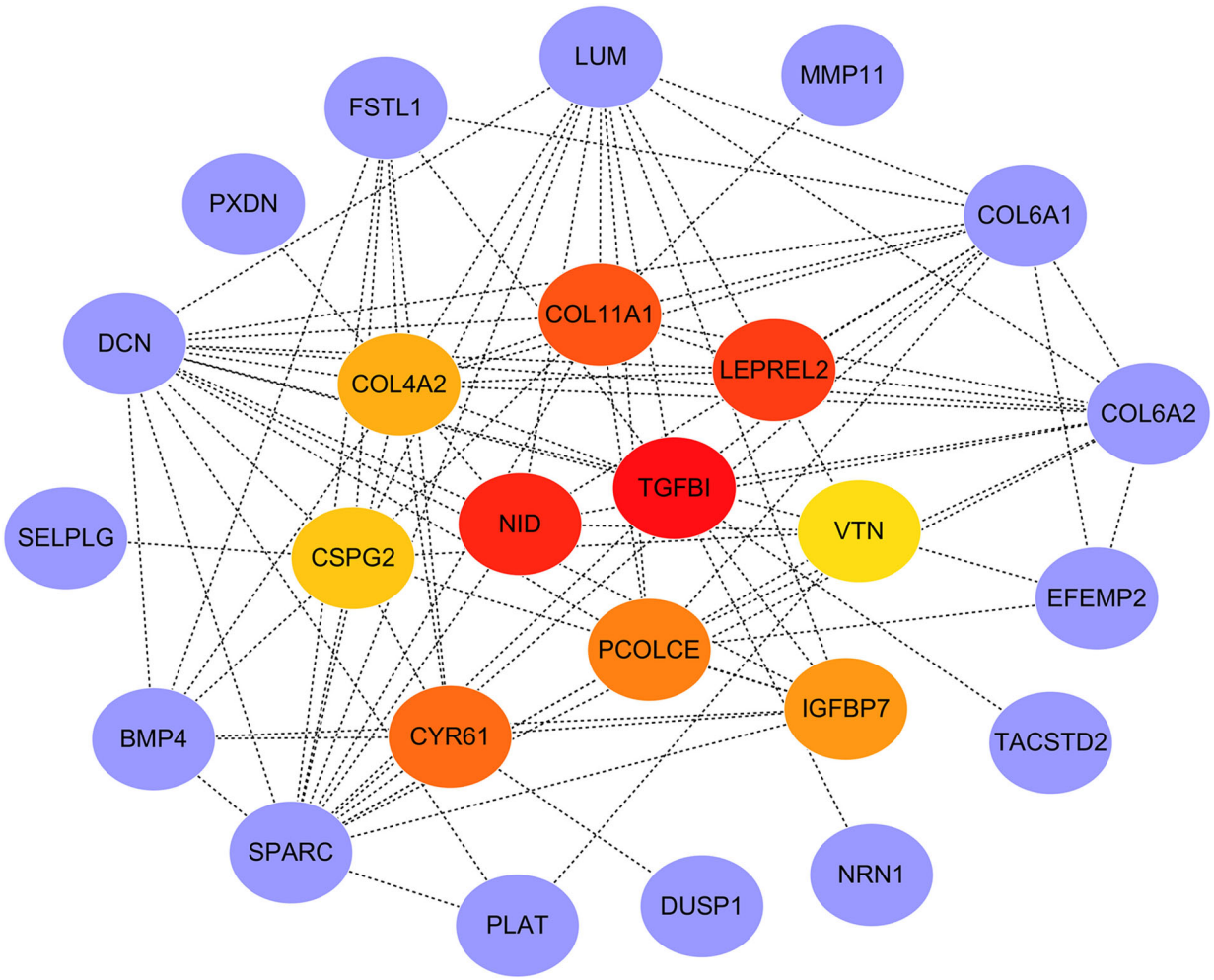

FIGURE 7 | PPI network of genes in the red module and hub gene screening.

resistant cells was detected using MTT assay. The IC_{50} of 5-FU was 4.032 μM, 40.085μM, 2.091μM, 70.820μM for the parental HCT116 cells, the 5-FU resistant HCT116 cells, the parental DLD1 cells, and the 5-FU resistant DLD1 cells (**Figure 10A**), which suggested that the resistant cells we obtained indeed more resistant to 5-FU. Consistent with our analysis, TGFBI levels are dramatically decreased in 5-FU resistant cell lines than that in the corresponding parental cells, both in HCT116 cells and DLD1 cells (**Figure 10B**). As shown in **Figures 10C, D**, knocking down TGFBI in HCT116 cells and DLD1 cells both led to decreased sensitivity to 5-FU treatment compared with control cells. To further verify our conclusion, a complementary experiment was carried out. GFP-TGFBI or GFP were transfected into 5-FU resistant HCT116 cells respectively, then the cell viability was detected after being treated with different concentrations of 5-FU. As we expected, compared with 5-FU resistant HCT116 cells overexpressing GFP, 5-FU resistant HCT116 cells overexpressing GFP-TGFBI showed increased sensitivity to 5-FU (**Figures 10E, F**).

DISCUSSION

5-FU has been used as the standard first-line treatment for CRC patients for several decades. To improve the anti-tumor activity of 5-FU and reduce drug resistance, some optimizing strategies have been adopted including 5-FU based combination therapy. Despite the encouraging progress in CRC treatment to data, failure of chemotherapy due to 5-FU resistance still occurs frequently. In the present study, the gene expression profile of CRC patients before their exposure to 5-FU based combined chemotherapy were analyzed to identify biomarkers related to intrinsic resistance to 5-FU based chemotherapy.

Potential gene modules related to response to 5-FU based chemotherapy were identified with WGCNA analysis. The red module was found to have the highest correlation with chemotherapy response. To further understand the potential function of genes among the red module, GO and KEGG enrichment analysis was performed. Functional and pathway enrichment analysis results showed the genes in the red module

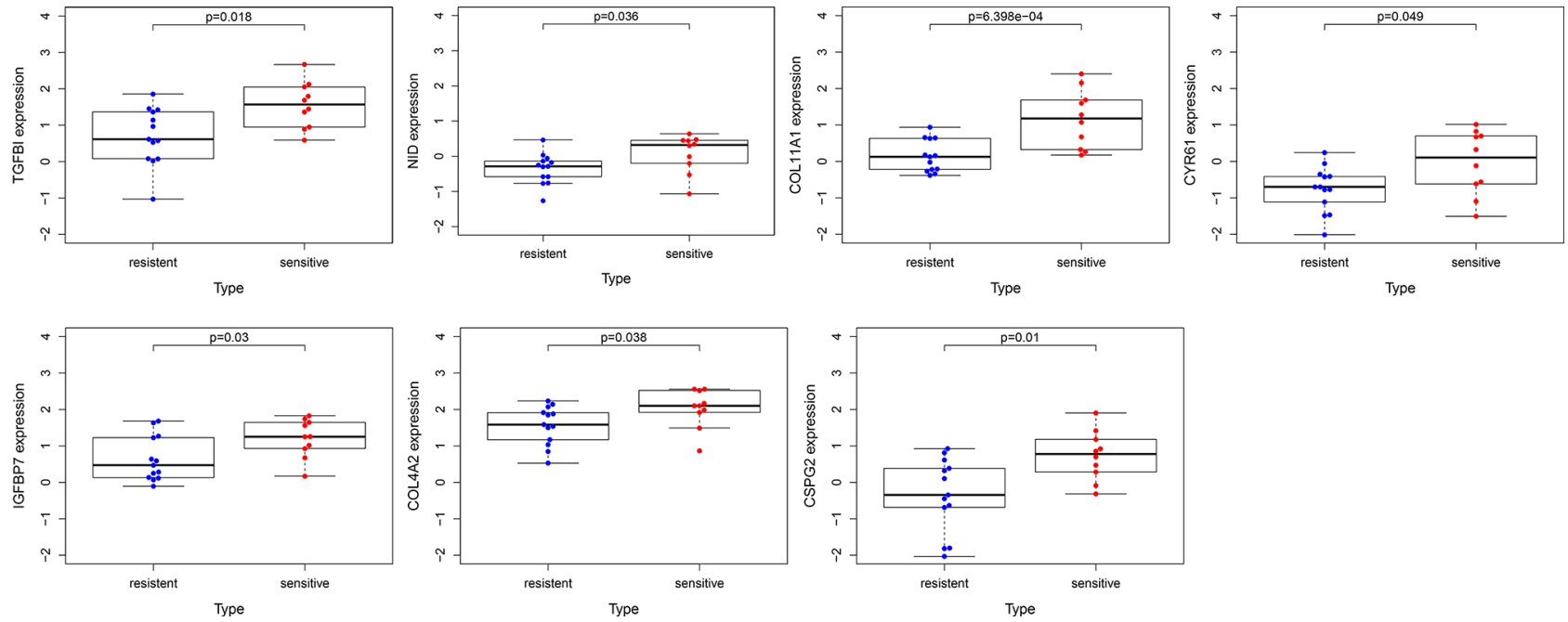

FIGURE 8 | The expression of hub genes between chemotherapy-resistant samples and chemotherapy-sensitive samples.

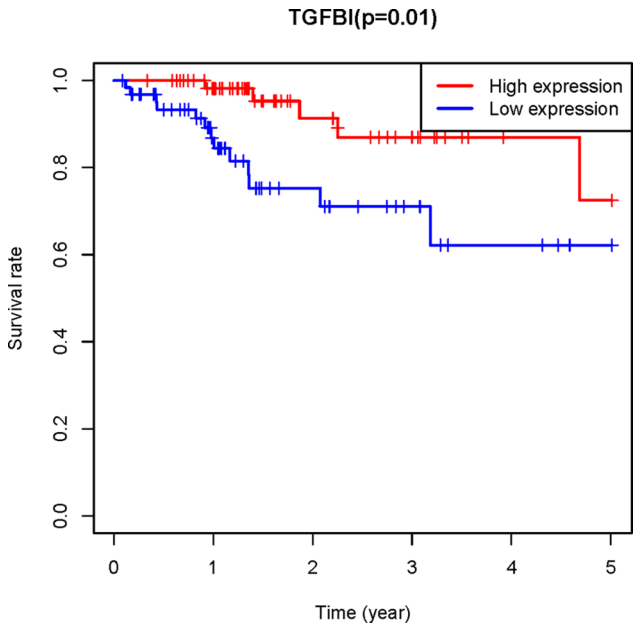

FIGURE 9 | The Kaplan–Meier analysis. the Kaplan–Meier analysis of the patients from TCGA COAD receiving chemotherapy showed the survival of patients with high expression of TGFBI is better than those patients with low expression of TGFBI.

were mainly enriched in extracellular matrix organization and ECM-receptor interaction pathway.

PPI network of the genes in the red module was constructed and ten hub genes were screened through CytoHubba plugin in Cytoscape. Then the expression of the hub genes was confirmed in GSE3964. Among the ten hub genes, the expression of seven genes (*TGFBI*, *NID*, *COL11A1*, *CYR61*, *IGFBP7*, *COL4A2*, and *CSPG2*) showed significant differences between chemotherapy-sensitive and chemotherapy-resistant samples. To explore the prognostic value of these seven genes, Kaplan-Meier survival analysis was performed in CRC patients treated with 5-FU based chemotherapy from TCGA COAD. *TGFBI* was identified as a prognostic gene ($p = 0.01$), a high expression of which indicates a good prognosis (**Figure 8**). Also, the expression of TGFBI is higher in CRC patients who are sensitive to 5-FU based chemotherapy than those resistant to 5-FU based chemotherapy (**Figure 7**). These results suggested that TGFBI may act as a biomarker for predicting the response of 5-FU based chemotherapy for CRC patients.

TGFBI (transforming growth factor β-induced protein), encoded by *TGFBI* gene, was first identified in a human lung adenocarcinoma cell line A549 treated with TGFB (transforming growth factor β), it contains an RGD (Arg-Gly-Asp) motif that can serve as a ligand recognition site for integrins (25). TGFBI mediates cell adhesion to extracellular proteins including collagen, fibronectin, and laminins through integrin binding (26). Many reports have indicated that TGFBI functioned as a tumor suppressor. Down-regulation of TGFBI has been observed in various tumors. Immunochemistry results showed the expression of TGFBI in lung carcinomas was lower than normal tissues (27). TGFBI level got down-regulated due to promoter hypermethylation in ovarian carcinoma tissues (28). TGFBI promoter hypermethylation also occurs in lung and prostate cancer specimens (29). Overexpression of TGFBI in Chinese hamster ovary (CHO) cells resulted in a significant decrease in cell growth and tumor-forming ability of these cells in nude mice (30). TGFBI expression also reduced the metastatic potential of lung and breast tumor cells (31). TGFBI can facilitate TGFB-induced apoptosis through releasing RGD peptides when TGFBI normally undergoes carboxy-terminal processing (32). TGFBI deficiency predisposed mice to spontaneous tumor development (33). Besides, recovery of TGFBI expression in lung cancer cell H522 lacking endogenous TGFBI protein leads to a significant decrease in cell growth and a significantly higher sensitivity to apoptotic induction (27). These studies support that TGFBI functions as a tumor suppressor. However, controversy has arisen to the role of TGFBI in tumorigenesis. Multiple studies report a tumor-promoting function of TGFBI. TGFBI increased the metastatic potential of ovarian cancer cells, and TGFBI may be a potential therapeutic target against ovarian cancer (34). It has been suggested that TGFBI plays a dual role in ovarian cancer and can act both as a tumor suppressor or tumor promoter depending on the tumor microenvironment (35). A study suggested TGFBI may play a pro-tumor or anti-tumor role, depending on the integrins to which it binds on the cell surface (36).

In addition to the dual role in tumor progression, the expression of TGFBI has also been associated with chemotherapeutic drug sensitivity. Loss of TGFBI induced specific resistance to paclitaxel in ovarian cancer cells, and paclitaxel-resistant cells treated with recombinant TGFBI protein show restoration of paclitaxel sensitivity (37). Immunohistochemistry results in non-small cell lung cancer (NSCLC) clinical samples suggested there was a strong association between elevated TGFBI expression and the response to chemotherapy (38). Human NSCLC cells overexpressing TGFBI displayed increased sensitivity to etoposide, paclitaxel, cisplatin, and gemcitabine (38). High TGFBI level was associated with longer survival in lung squamous cell carcinomas patients received adjuvant platinum-based chemotherapy (39). The overexpression of TGFBI sensitized the nasopharyngeal carcinoma (NPC) cells to cisplatin (40). It has been reported that the TGF-β pathway is activated by 5-FU treatment in drug-resistant colorectal carcinoma cells (41). In the present study, we associated the expression level of TGFBI with the sensitivity of 5-FU based chemotherapy for CRC for the first time. Our results suggested that CRC patients with high expression of TFGBI indicated increased sensitivity of 5-FU based chemotherapy and improved survival. The conclusion was further confirmed in an independent dataset (GSE19860). Furthermore, experiments *in vitro* also support the conclusion. TGFBI levels were dramatically decreased in 5-FU resistant cell lines than that in the corresponding parental cells, both in HCT116 cells and DLD1 cells. Knocking down TGFBI in HCT116 cells led to increased resistance to 5-FU treatment compared with control cells. GFP-TGFBI overexpression dramatically restored the

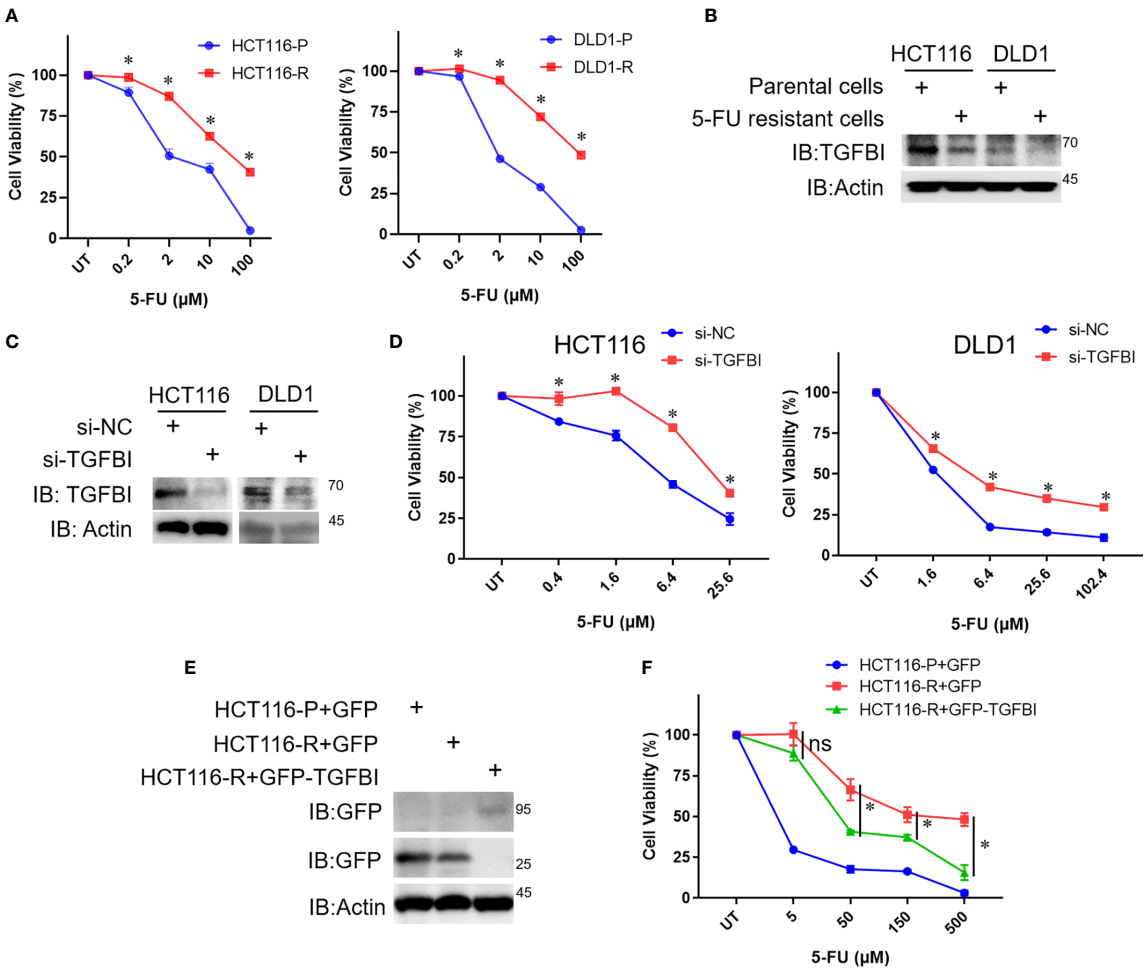

FIGURE 10 | The relationship between TGFBI level and 5-FU sensitivity of CRC cells. **(A)** The parental cells and 5-FU resistant cells of HCT116 and DLD were treated with different concentrations of 5-FU for 72h, then the media were dumped off, cells were subjected to the 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) assay. Data were calculated from three independent experiments and analyzed by one-way analysis of variance (data shown are the means ± s.e.m., $^*p < 0.05$). **(B)** Whole-cell lysates were derived from the parental cells and 5-FU resistant cells and immunoblotted (IB) for TGFBI, with actin as a loading control. **(C)** HCT116 cells or DLD1 cells were transfected with si-NC or si-TGFBI in day 0 and day 2, 8 h after the second transfection, some of the cells were harvested to detect TGFBI knockdown efficiency. The remaining cells were used for **(D)**. **(D)** Cells from **(C)**, which were transfected with si-NC or si-TGFBI twice, were treated with different concentrations of 5-FU for 72h, then cells were subjected to MTT assay. Data were calculated from three independent experiments and analyzed by one-way analysis of variance (data shown are the means ± s.e.m., $^*p < 0.05$). **(E)** The parental cells of HCT116 were transfected with pEGFP, the resistant cells of HCT116 were transfected with pEGFP and pEGFP-TGFBI, respectively. Half of the cells were harvested to detect the expression of TGFBI using GFP-antibody after 24 h transfection. The remaining cells were used for **(F)**. **(F)** Cells from **(E)**, which were transfected with pEGFP or pEGFP-TGFBI were treated with different concentrations of 5-FU for 72h, then cells were subjected to MTT assay. Data were calculated from three independent experiments and analyzed by one-way analysis of variance (data shown are the means ± s.e.m., ns, no statistical significance by one-way analysis of variance, $^*p < 0.05$).

sensitivity of resistant HCT116 cells to 5-FU treatment compared with GFP overexpression.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3964 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19860.

ETHICS STATEMENT

This study was approved by the Ethical Committee of Central South University (China).

AUTHOR CONTRIBUTIONS

YW and KF conceived the concept, instructed data analysis, and revised manuscript. YW and QW conducted most data analysis and preparation of figures and table and wrote manuscript draft. YY,

YC, and SL helped with some analysis and interpretation of data. KF reviewed the manuscript with input from all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.604315/full#supplementary-material

Supplementary Figure 1 | The expression of TGFBI between responders and non-responders who receiving modified FOLFOX6 therapy in GSE19860 (*p<0.05).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Clinical Impact of Primary Tumor Location in Metastatic Colorectal Cancer Patients Under Later-Line Regorafenib or Trifluridine/Tipiracil Treatment

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Background: Primary tumor location (PTL) is an important prognostic and predictive factor in the first-line treatment of metastatic colorectal cancer (mCRC). Although regorafenib (REG) and trifluridine/tipiracil (FTD/TPI) have been introduced recently, the clinical impact of PTL in these treatments is not well understood.

Materials and Methods: We retrospectively evaluated patients with mCRC who were registered in a multicenter observational study (the REGOTAS study). The main inclusion

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criteria were Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, refractory or intolerant to fluoropyrimidines, oxaliplatin, irinotecan, angiogenesis inhibitors, anti-epidermal growth factor receptor therapy (if RAS wild-type), and no prior use of REG and FTD/TPI. The impact of PTL on overall survival (OS) was evaluated using Cox proportional hazard models based on baseline characteristics.

Results: A total of 550 patients (223 patients in the REG group and 327 patients in the FTD/TPI group) were included in this study, with 122 patients with right-sided tumors and 428 patients with left-sided tumors. Although the right-sided patients had significantly shorter OS compared with the left-sided patients by univariate analysis ($p = 0.041$), a multivariate analysis revealed that PTL was not an independent prognostic factor (hazard ratio, 0.95; $p = 0.64$). In a subgroup analysis, the OS was comparable between the REG and FTD/TPI groups regardless of PTL (p for interactions = 0.60).

Conclusions: In the present study, PTL is not a prognostic and predictive factor in patients with mCRC under later-line REG or FTD/TPI therapy.

Keywords: regorafenib, trifluridine/tipiracil, colorectal cancer, primary tumor location, biomarker

INTRODUCTION

The standard of care for patients with metastatic colorectal cancer (mCRC) has evolved with combination chemotherapy regimens, including cytotoxic agents (e.g., fluoropyrimidine [FU], oxaliplatin [OX], and irinotecan [IRI]), angiogenesis inhibitors (e.g., bevacizumab, aflibercept, and ramucirumab), and anti-epidermal growth factor receptor (EGFR) antibodies (e.g., cetuximab, and panitumumab) for patients with RAS wild-type tumors. (1–8) In recent years, regorafenib (REG) and trifluridine/tipiracil (FTD/TPI) significantly improved the overall survival (OS) in patients with chemorefractory mCRC compared with placebo (9–12) and have been available in clinical practice.

Accumulating evidence indicates that primary tumor location (PTL) is an important prognostic factor in mCRC, as right-sided tumors are associated with poorer outcomes than left-sided tumors, especially after first-line treatments (13–17). Retrospective analyses of randomized trials in first-line settings indicate that right-sided primary tumors were negative predictive markers for the efficacy of anti-EGFR therapy. (13, 14) Therefore, anti-EGFR-based first-line treatment was only recommended for patients with left-sided primary tumors in several international guidelines. (15–17) Thus, treatment stratification based on PTL is one of the critical aspects of standard care for mCRC.

However, the clinical impact of PTL in patients with mCRC under later-line REG or FTD/TPI treatment is not well understood. Although a subgroup analysis of these pivotal trials showed a survival benefit of REG and FTD/TPI regardless of PTL, (9, 11, 12) no randomized study has compared REG and FTD/TPI directly. Thus, the optimal treatment sequence of REG and FTD/TPI according to PTL remains unclear.

We previously reported that the multicenter, large cohort, and observational REGOTAS study showed no significant difference in OS between REG and FTD/TPI treatments in patients with mCRC. (18) The present study investigated the prognostic and predictive values of PTL in mCRC patients under later-line REG and FTD/TPI treatment in the REGOTAS study.

MATERIALS AND METHODS

Patients

The present study retrospectively examined the clinical records of patients with mCRC treated with later-line REG or FTD/TPI chemotherapy during the period from June 1, 2014, to November 30, 2015. All the patients were registered in the REGOTAS study, which is described in detail elsewhere. (18) The main eligibility criteria were as follows: (1) histologically confirmed colorectal adenocarcinoma; (2) no prior treatment using REG and FTD/TPI; (3) previous treatment with FU, OX, IRI, bevacizumab, and anti-EGFR antibody (in patients with RAS wild-type tumor); (4) Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; and (5) adequate organ function. Patients who could receive only a specific drug treatment, either REG or FTD/TPI, due to comorbidity and/or medical history. The primary tumors were classified as right-sided tumors if located between the cecum and the splenic flexure of the transverse colon. Others, from the descending colon to the rectum, were defined as left-sided tumors.

The present study was approved by the ethics committees at each institution and was in accordance with the guidelines for biomedical research specified in the Declaration of Helsinki. The REGOTAS study was registered with the University Medical Information Network (number UMIN000020416). The requirement for informed consent was waived due to the retrospective nature of this study.

Statistical Analysis

The exploratory primary endpoint was OS, defined as the time from the start of REG or FTD/TPI treatment to death or last

follow-up. The following pretreatment clinical data and baseline laboratory values were used in the analysis as covariates: age, sex, body mass index, ECOG PS, surgery on primary tumor, histological grade, RAS status, metastatic tumor site (liver metastasis, lung metastasis, lymph node metastasis, and peritoneal dissemination), number of metastatic organ sites, and treatment duration from initiation of first-line chemotherapy.

The Mann-Whitney U test was used to compare the continuous variables, and Fisher's exact test to compare the categorical variables. Survival curves were estimated using the Kaplan-Meier method, and differences between the groups were analyzed with the log-rank test. Hazard ratios (HRs) were estimated using the Cox proportional hazard model. OS was analyzed using univariate and multivariate Cox regression analyses. The backward selection method was performed to select covariates retained ($p < 0.1$) in the multivariate analysis.

Primary analysis was conducted using all patients with sufficient information. A 1:1 matching using the propensity score (the propensity-score-matched cohort) was performed as a sensitivity analysis. The details of the propensity-score-matched cohort were described elsewhere. (18) All p values < 0.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing).

RESULTS

Patients

Among 589 mCRC patients, 550 met the inclusion criteria (the observational cohort), including 223 patients in the REG group and 327 patients in the FTD/TPI group (**Figure 1**). Sixty patients (27%) in the REG group and 62 patients (19%) in the FTD/TPI group had right-sided tumors ($p = 0.029$). Patient characteristics are summarized in **Table 1**. More patients with right-sided tumors had lower BMI, RAS mutations, lung metastases, and less than three prior lines of chemotherapy than those with left-sided tumors in both the REG and FTD/TPI groups. The patients' follow-up was until September 2016. The median follow-up at the time of analysis was 17.2 months, and 418 (76%) patients had died at the time of analysis.

Efficacy

Prognostic Value of PTL

In the observational cohort (the REG and FTD/TPI groups), the median OS was 5.9 months (95% CI 5.3–7.1) in the right-sided tumors and 8.0 months (7.3–9.1) in the left-sided tumors (unadjusted HR 0.79 [95% CI 0.63–0.99], log-rank $p = 0.041$; **Supplemental Figure 1A**). The subgroup analysis of each treatment group also demonstrated that the OS was shorter in right-sided tumors (**Supplemental Figures 1B, C**). **Table 2** showed the results of univariate and multivariate analyses of OS. Multivariate analysis revealed that PTL was not significantly associated with OS (adjusted HR 0.95, [95% CI 0.75–1.20], $p = 0.64$).

Predictive Value of PTL

In the right-sided tumors, the median OS was 5.7 months (4.5–7.8) in the REG group and 6.0 months (5.3–7.7) in the FTD/TPI group (unadjusted HR 0.93 [95% CI 0.62–1.39], log-rank $p = 0.71$; **Figure 2A**). In the left-sided tumors, the median OS was 8.5 months (7.3–10.2) in the REG group and 7.8 months (6.9–8.9) in the FTD/TPI group (unadjusted HR 1.07 [95% CI 0.85–1.34], log-rank $p = 0.56$; **Figure 2B**). Interactions between treatment groups and PTL were not significant (p for interactions = 0.60). In the right-sided tumors, the progression-free survival (PFS)

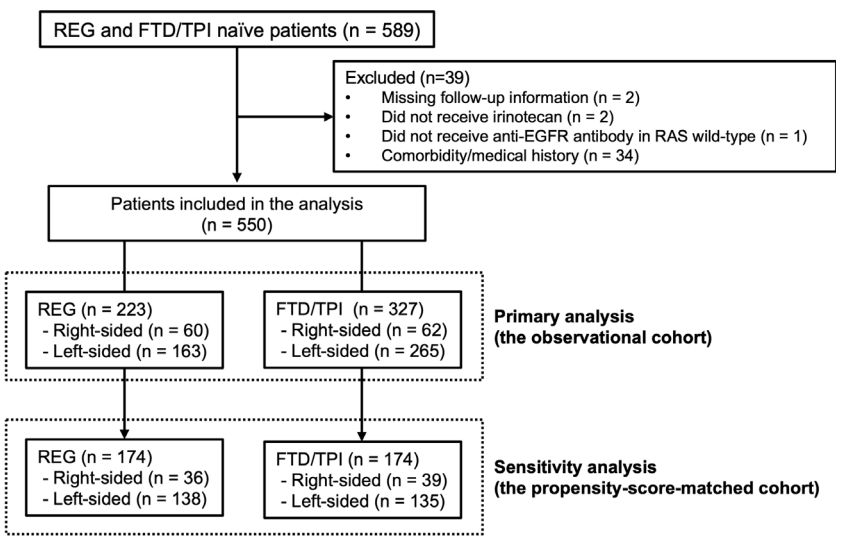

FIGURE 1 | Patient selection flow diagram.

TABLE 1 | Patient characteristics.

	REG group		*p* value*	FTD/TPI group		*p* value*
	Right (n = 60)	Left (n = 163)		Right (n = 62)	Left (n = 265)	
Age, years						
Median (IQR)	65 [58–71]	64 [55–71]	0.30	65 [59–72]	64 [55–70]	0.17
≥ 65, n (%)	31 (51.7)	76 (46.6)	0.55	33 (53.2)	123 (46.4)	0.40
Sex, n (%)			0.17			0.89
Male	29 (48.3)	97 (59.5)		38 (61.3)	159 (60.0)	
Female	31 (51.7)	66 (40.5)		24 (38.7)	106 (40.0)	
BMI, n (%)			0.025			0.046
≥ 18.5	47 (78.3)	147 (90.2)		45 (72.6)	222 (83.8)	
ECOG PS, n (%)			0.086			0.41
PS0 or 1	56 (93.3)	160 (98.2)		56 (90.3)	248 (93.6)	
PS2	4 (6.7)	3 (1.8)		6 (9.7)	17 (6.4)	
Surgery on primary tumor, n (%)			0.20			0.31
Yes	51 (85.0)	125 (76.7)		45 (72.6)	210 (79.2)	
Histological grade, n (%)			0.01			0.35
Well/mod	48 (80.0)	149 (91.4)		55 (88.7)	242 (91.3)	
Others	10 (16.7)	7 (4.3)		3 (4.8)	16 (6.0)	
Missing	2 (3.3)	7 (4.3)		4 (6.5)	7 (2.6)	
RAS status, n (%)			< 0.001			0.013
Mutant	42 (70.0)	67 (41.1)		37 (59.7)	124 (46.8)	
Missing	0 (0.0)	6 (3.7)		3 (4.8)	3 (1.1)	
Metastasis, n (%)						
Liver	40 (66.7)	101 (62.0)	0.54	40 (64.5)	161 (60.8)	0.66
Lung	29 (48.3)	51 (31.3)	0.027	28 (45.2)	79 (29.8)	0.024
Lymph node	26 (43.3)	68 (41.7)	0.88	21 (33.9)	122 (46.0)	0.089
Peritoneum	15 (25.0)	20 (12.3)	0.036	26 (41.9)	41 (15.5)	< 0.001
Number of metastatic organ site(s), n (%)			0.17			
≥ 3	11 (18.3)	46 (28.2)		25 (40.3)	103 (38.9)	0.89
Duration from initiation of 1st line chemotherapy, n (%)			0.13			0.078
≥ 18 months	39 (65.0)	124 (76.1)		40 (64.5)	201 (75.8)	
Prior regimens, n (%)			0.024			< 0.001
≥ 3	21 (35.0)	85 (52.1)		17 (27.4)	147 (55.5)	

**The p values were calculated using the Mann-Whitney U test for continuous variable and Fisher's exact probability test for categorical variables.*
BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; RAS, rat sarcoma; REG, regorafenib; FTD/TPI, trifluridine/tipiracil.

TABLE 2 | Univariate and multivariate analyses of overall survival (OS) in the observational cohort.

Variable	Category	Univariate	*p* value*	Multivariate	*p* value*
		HR (95% CI)		HR (95% CI)	
PTL	Left *vs.* Right	0.79 (0.63–0.99)	0.042	0.95 (0.75–1.20)	0.64
Treatment group	FTD/TPI *vs.* REG	1.02 (0.84–1.25)	0.80		
Age	≥ 65 *vs.* < 65	1.22 (1–1.48)	0.044	1.32 (1.08–1.61)	< 0.001
Sex	Female *vs.* Male	1.05 (0.86–1.27)	0.63		
BMI	≥ 18.5 *vs.* 18.5	0.94 (0.72–1.22)	0.62		
ECOG PS	PS2 *vs.* PS1 or 2	1.48 (0.99–2.21)	0.059	1.57 (1.03–2.39)	0.036
Surgery on primary resection	Yes *vs.* No	0.60 (0.48–0.76)	< 0.001	0.74 (0.58–0.94)	0.014
Histology	Others *vs.* well/mod	1.03 (0.68–1.56)	0.87		
RAS status	Mutant *vs.* Wild	1.18 (0.99–1.41)	0.067	1.10 (0.91–1.33)	0.33
Liver metastasis	Yes *vs.* No	1.65 (1.35–2.03)	< 0.001	1.59 (1.252.01)	< 0.001
Lymph node metastasis	Yes *vs.* No	1.40 (1.15–1.7)	< 0.001	1.34 (1.03–1.73)	0.026
Lung metastasis	Yes *vs.* No	0.84 (0.69–1.03)	0.089	0.92 (0.72–1.18)	0.52
Peritoneal metastasis	Yes *vs.* No	1.52 (1.2–1.93)	< 0.001	1.52 (1.13–2.04)	0.0051
Number of metastatic organ site(s)	≥ 3 *vs.* < 3	1.57 (1.28–1.92)	< 0.001	1.10 (0.80–1.52)	0.55
Duration from initiation of 1st line chemotherapy	≥ 18 months *vs.* < 18 months	0.63 (0.51–0.78)	< 0.001	0.65 (0.52–0.81)	< 0.001
Prior regimens	≥ 3 *vs.* < 3	0.85 (0.7–1.03)	0.11		

** p values were calculated using the Cox proportional-hazards model.*
BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; RAS; rat sarcoma; PTL, primary tumor location; REG, regorafenib; FTD/TPI, trifluridine/tipiracil.

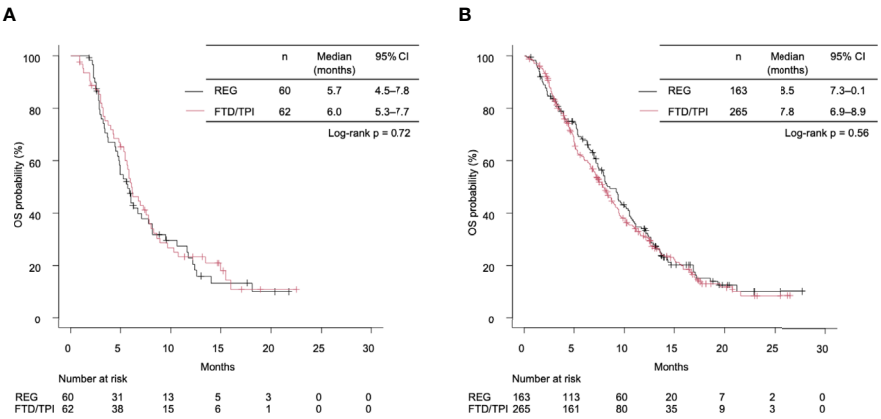

FIGURE 2 | **(A)** Kaplan-Meier curves of overall survival (OS) stratified by treatment group in right-sided tumors. The median OS times of the REG and FTD/TPI groups were 5.7 months (95% CI 4.5–7.8) and 6.0 months (5.3–7.7), respectively (log-rank p = 0.72). **(B)** Kaplan-Meier curves of OS stratified by treatment for left-sided tumors. The median OS times of the REG and the FTD/TPI groups were 8.5 months (95% CI 7.3–10.1) and 7.8 months (6.9–8.9), respectively (log-rank p = 0.56). REG, regorafenib; FTD/TPI, trifluridine/tipiracil.

tended to be longer in the FTD/TPI group (unadjusted HR 0.71 [95% CI 0.48–1.05], log-rank p = 0.086; **Supplemental Figure 2A**), while in the left-sided tumors, the result was comparable between the treatment groups (unadjusted HR 1.05 [95% CI 0.85–1.29], log-rank p = 0.64; **Supplemental Figure 2B**). The interactions between the treatment groups and PTL were not significant (p for interactions = 0.072). Among patients with target lesions (112 patients in the right-sided tumors and 407 patients in the left-sided tumor), no complete responses were observed, and partial response was found in 3 patients who received FTD/TPI in the left-sided tumors. The disease control rate was comparable between the treatment groups in each PTL (**Supplemental Table 1**).

Sensitivity Analysis

A total of 174 patients per treatment group were matched by propensity score. The details of this cohort were described in the previous report. (18) Multivariate analysis revealed that PTL was not an independent prognostic factor (adjusted HR 0.97, [95% CI 0.72–1.33], p = 0.87; **Supplemental Table 2**). In the subgroup analysis, the OS and PFS were similar between the treatment groups regardless of PTL (**Supplemental Figures 3A, B**). Moreover, there were no significant interactions between the treatment groups and PTL in OS and PFS (p for interactions = 0.82 and 0.37, respectively).

DISCUSSION

To the best of our knowledge, this is the first study to assess PTL as a prognostic or predictive factor during later-line REG and FTD/TPI treatments in patients with chemorefractory mCRC. As described above, there were several differences in patient characteristics according to PTL, such as RAS status and lung metastasis incidence. Nevertheless, PTL was not an independent prognostic factor in the multivariate analysis in the cohort treated with REG or FTD/TPI. Moreover, no interactions were observed between the treatment groups and PTL in terms of OS and PFS, which suggests that the efficacy of REG and FTD/TPI is not influenced by PTL.

Recent investigations revealed differences in epidemiological, clinical, and molecular-pathological profiles between the right-sided (between the cecum and transverse colon) and left-sided tumors (between the descending colon and rectum), (19–21) and patients with right-sided tumors had poorer survival than patients with left-sided tumors. (13, 14, 22, 23) However, most of the evidence on the prognostic value of PTL was based on first-line data, and few later-line data are available. In *post hoc* analyses of data from phase III studies evaluating the efficacy of later-line panitumumab, RAS wild-type patients with right-sided tumors had significantly shorter OS and PFS than those with left-sided tumors, while no clear prognostic impact of PTL was found in RAS mutant patients. (24) By contrast, in the large-scale, prospective, observational study (CORRELATE), the REG treatment outcome was comparable across the different PTLs, similar to our results. (25) Although the reasons for the different outcomes according to PTL remain unclear, different molecular profiles related to sensitivity or resistance to anti-EGFR antibodies could be responsible. (26, 27) In the CORRELATE and our study, most patients with RAS wild-type had already been treated with anti-EGFR therapy. A possible explanation for the difference in the prognostic value of PTL among studies is whether the anti-EGFR therapy-naive and RAS wild-type/left-sided patients, who would benefit more from anti-EGFR therapy, were included or not.

In the pivotal trials of REG and FTD/TPI, subgroup analyses of PTL have been reported only according to the classification of the colon and rectum. In the CORRECT trial, which compared REG with placebo, the HR for OS was 0.70 in the colon group and 0.95 in the rectum group. (9) By contrast, in the RECOURSE

trial, which compared FTD/TPI with placebo, the HR for OS was 0.68 in the colon group and 0.64 in the rectum group. (11) Although these results were seemingly considered less survival benefits of REG in patients with rectal cancers, the HRs for PFS were similar between the colon and rectum groups (0.55 *vs* 0.45); therefore, we speculated that the clinical benefits of REG and FTD/TPI are similar regardless of the colon or rectum. In fact, our results support the hypothesis that the classification of the PTL had no predictive value in later-line treatment with REG or FTD/TPI.

To date, novel molecular biomarkers that predict the effectiveness of REG and FTD/TPI have been investigated. Small studies suggest that APC mutations or FGFR1 amplification in tumor tissue were more enriched in REG patients with a clinical benefit than those without, (28) and plasma VCAM-1 was potentially predictive of OS benefit in REG treatment. (29) A survival benefit of FTD/TPI was observed regardless of KRAS status. (11, 12) High thymidine kinase 1 (TK1) expression level correlated with a larger survival benefit in FTD/TPI treatment, (30) although no significant difference in TKI expression according to PTL was reported. Moreover, evolving technologies of liquid biopsies using circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) analysis have accelerated research on the dynamism of clonal evolution, enabling us to reveal molecular profiling, monitor clonal dynamics, and identify resistance mechanism by longitudinal biopsies (31–33). The subgroup and exploratory biomarker analyses in the CORRECT trial suggested that a survival benefit was observed regardless of RAS or PIK3CA mutational status in ctDNA. (34) Amatu et al. reported baseline and dynamic circulating methylated DNA as prognostic and predictive in patients treated with REG (35). The TACT-D trial (NCT03844620) is currently conducting to validate changes in ctDNA to predict resistance early and limit toxicities in mCRC patients who receive REG or FTD/TPI. More comprehensive molecular analyses in a larger cohort of patients treated with REG or FTD/TPI may be needed to clarify the exact biomarkers to predict outcomes.

It is essential to describe the limitations of this observational study. First, this is not a randomized study to directly compare REG and FTD/TPI. Treatment selection was mainly based on the patient's request or investigator's decision as previously described (18), which led to an inherent bias. The proportion of patients with right-sided tumors was higher in the REG than in the FTD/TPI group. The exact reasons for treatment selection were not collected in the study, but FTD/TPI may be more favored in patients with skin toxicity due to previous anti-EGFR therapies, which are used for longer in patients with left-sided tumors in early treatment settings. Second, all patients enrolled in this study were Japanese. However, the absence of ethnic differences in the analysis of the efficacy of REG and FTD/TPI in phase III trials could enable the results to be applied to all patients regardless of ethnicity. (9–12) Third, death events were observed in 76% of patients, but the follow-up period might have been relatively short. Finally, biomarkers other than RAS status (e.g., BRAF and microsatellite instability) and detailed clinical outcomes of previous treatments were not collected in this study. These limitations encourage us to conduct a prospective study with sufficient statistical power to confirm the findings of this study.

CONCLUSIONS

Our multicenter retrospective study revealed that PTL is not a prognostic factor in patients with mCRC under later-line REG or FTD/TPI treatment. No significant difference in OS was observed between the REG and FTD/TPI groups, irrespective of PTL. Our findings highlight the importance of selecting later-line treatments regardless of PTL for patients with mCRC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committees at each institution. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Concept/design: HN, SF, and TMo. Provision of study materials or patients: SF, TMa, AT, YK, TaK, KeY, YN, MK, AM, TD, YH, TS, NS, ME, TI, ToK, KA, SY, HO, HK, DS, KO, TT, KiY, and TMo. Analysis and interpretation of data: HN, SF, TMo, and MG. Manuscript writing: HN, SF, and TMo. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.688709/full#supplementary-material

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Development and Validation of a Prognostic Nomogram for Colorectal Cancer Patients With Synchronous Peritoneal Metastasis

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Purpose: Synchronous peritoneal metastasis (S-PM) is considered a poor prognostic factor for colorectal cancer (CRC) and there is no nomogram to predict the survival of these patients. In this study, we aimed to use a multicenter data to identify the factors associated with S-PM of CRC to construct a nomogram for predicting the overall survival (OS) of these patients.

Methods: CRC patients with S-PM from two medical centers were enrolled between September 2007 and June 2017. Multivariate analysis was used to identify independent factors associated with OS for the nomogram to predict the 1-, 2-, and 3-year OS rates in the development group. The concordance index (C-index), calibration plot, relative operating characteristic (ROC) curve with area under the curve (AUC) were calculated to evaluate the performance of the nomogram in both the development and an external validation group.

Results: 277 CRC patients with S-PM in the development group and 68 patients in the validation group were eligible for this study. In multivariate analysis of development group, age, carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), cytoreductive surgery (CRS), hyperthermic intraperitoneal chemotherapy (HIPEC), and chemotherapy were independent variables for OS, based on which the nomogram was built. The C-index of the nomogram in the development and validation group was 0.701 (95% CI, 0.666–0.736) and 0.716 (95% CI, 0.622–0.810); demonstrating good discriminative ability. The calibration plots showed satisfactory consistency between actual observation and nomogram-predicted OS probabilities in the development and external validation group. The nomogram showed good predictive accuracy for 1-, 2-, and 3-year OS rates in both groups with AUC >0.70. An online dynamic webserver was also developed for increasing the ease of the nomogram.

Conclusions: We developed and validated a predictive nomogram with good discriminative and high accuracy to predict the OS in CRC patients with S-PM.

Keywords: colorectal cancer, nomogram, prognosis, peritoneal metastasis (PM), synchronous peritoneal metastasis

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant tumor worldwide, with 1.8 million cases and 881,000 deaths registered globally, in 2018 (1). It is ranked third in morbidity and fifth in mortality in China (2, 3). Currently, radical resection combined with neo-/adjuvant chemotherapy, radiotherapy, or targeted therapy has been shown to be associated with a promising 5-year OS rate of >70% in non-metastatic CRC, and >90% for early CRC (4).

The peritoneum is the third most frequent site for metastasis in CRC, secondary to the liver and lung (5, 6). In regard to synchronous metastatic CRC, the peritoneum is the second most common metastatic site, secondary to the liver (7). Peritoneal metastasis (PM) is associated with poorer progression-free survival and OS, as compared to other CRC metastatic sites (8–10). In the 8th edition of the American Joint Committee on Cancer (AJCC) TNM Classification for CRC, patients with PM are separately classified into an M1c group since they were found to have the worst prognosis compared to patients in the M1a (metastases to one organ) and M1b (metastases to more than one organ) groups (11, 12). At initial diagnosis, 1–13% of CRC patients often present with synchronous peritoneal metastasis (S-PM) (12–14). The prognosis of S-PM has been found to be poorer than metachronous PM (15–17). Once S-PM develops, without active treatment, the patients' median OS can range between 4 and 7 months (18–21).

According to the Japanese Society for Cancer of the Colon and Rectum (JSCCR) (22) and the National Comprehensive Cancer Network (NCCN) guidelines (23) for the treatment of CRC patients with PM, if complete cytoreduction can be achieved, resection of the isolated peritoneal lesion could be recommended but is advisable to be performed in an experienced cancer center. For incomplete cytoreductive surgery (CRS), the combination of HIPEC with systematic chemotherapy could improve the patient's survival (24, 25).

Clinically, clinicians need to comprehensively evaluate the imaging findings of PM, tumor marker levels, surgical skill level, development of treatment platform, patient's symptoms, nutrition condition, patient's willingness and financial situation, and multiple disciplinary team (MDT) advices, then decide whether to recommend CRS, HIPEC, or palliative chemotherapy. As there is no standard tools to weigh the benefits of these factors for an individualized treatment approach, oncologists can only rely on their clinical experience and judgment; possibly leading to a certain level of bias in selecting treatment methods. Thus, in this study, we aimed to develop and validate a nomogram able to predict the survival of S-PM CRC patients as a tool to help oncologists to make better treatment selection decisions.

MATERIALS AND METHODS

Patients and Study Criteria

S-PM was defined as PM which was concurrently identified at the time of initial primary CRC diagnosis (23). The inclusion criteria for patient selection were: (1) a pathological diagnosis of CRC with S-PM between September 2007 and June 2017; (2) no history of other primary malignant tumors; (3) had complete clinical and follow-up data. Patients were excluded if the clinicopathological information was incomplete or died from other diseases. Clinicopathological parameters included sex, age, body mass index (BMI), carcinoembryonic antigen (CEA), CA19-9, CA125, computed tomography (CT) findings of PM, other organ-invasion, other metastasis, tumor location, digestive obstruction, fistulation or bypass, CRS, HIPEC, chemotherapy, and differentiation grade. The patients were classified in a development group, comprising of patients from The Sixth Affiliated Hospital of Sun Yat-sen University (Guangzhou, China), and a validation group, which comprised of patients from the Guangdong Provincial People's Hospital (Guangzhou, China).

Treatment Approaches: CRS, HIPEC, and Chemotherapy

In case that the tumor burden was deemed resectable or caused severe perforation, bleeding or obstruction, CRS was performed using primary tumor removal, invaded-organ resection, and/or peritonectomy techniques. The degree of CRS was evaluated by the completeness of cytoreduction score (CCR score) after surgery. CCR0 was assigned for no remaining visible cancer lesion after the CRS. CCR1, 2, and 3 were assigned if the remaining lesions were less than 2.5 mm, 2.5 to 2.5 cm, and greater than 2.5 cm, respectively.

HIPEC was conducted using the closed abdomen technique after surgery. Briefly, four tubes (two for the inflow of chemotherapy reagents and saline solution at 42 °C and two for outflow liquid) were inserted into the abdomen. Several HIPEC chemotherapy regimens (i.e. 5-fluorouracil, Cisplatin, 5-fluorouracil plus Cisplatin, Paclitaxel, Oxaliplatin, 5-fluorouracil plus Lobaplatin) were used. The duration of each HIPEC treatment was at least 1 h. In addition, all cases underwent at least two courses of HIPEC during the first 24–72 h after CRS.

Chemotherapy included perioperative chemotherapy (neo- and adjuvant chemotherapy) or palliative chemotherapy. In some cases, targeted therapy was added. The chemotherapy regimens were 5-fluorouracil based chemotherapy (i.e. FOLFOX, FOLFIRI, or XELOX). Targeted therapy contained Cetuximab or Bevacizumab. All patients with chemotherapy received at least four courses of continuous therapy.

Follow-Up, Univariate and Multivariate Analysis

The last follow-up time of all the patients was on May 2019 or the date of registered death prior to May 2019. The endpoint of this study was OS, calculated from the date of initial biopsy diagnosis to death. Survival curves were drawn using the Kaplan–Meier plots. Univariate analyses were conducted to identify prognostic factors associated with OS in the development group. Factors with a P <0.05 in the univariate analysis were selected for multivariate Cox regression analysis.

Development and Validation of the Nomogram

Independent variables from multivariate analyses with a P <0.05 were used to develop a nomogram able to predict the 1-, 2-and 3-year OS rate of CRC patients with S-PM. To decrease the risk of bias, an internal validation using the development group and an external validation using the validation group were performed. The interpretation of the probability of C-index between predicted and actual outcome was used to evaluate the predictive ability and discriminative ability of the nomogram model of the development and validation groups. The value of the C-index should be 0.5–1.0. 0.5 of C-index to indicate random chance, and 1.0 indicated a perfect discriminative ability. The fitting degree of the nomogram was assessed in the development and validation groups using calibration plots. The Relative Operating Characteristic (ROC) curve with the area under the curve (AUC) was used to evaluate the discriminative and predictive ability in both groups. A user-friendly webserver was then built based on the validated nomogram to facilitate the use of the nomogram.

Statistical Analysis

Statistical analyses were performed using the R (www.R-project.org, version 3.6.3), SPSS (version 22.0 for Windows; SPSS, Chicago, IL, USA), and GraphPad Prism (version 8.2.1) software. The R statistical packages "rms", "survival", "foreign", "survivalROC", "DynNom", "shiny", and "rsconnect" were used to calculate the C-index, plot the calibration and ROC curve, construct the nomogram and build the webserver. Chi-squared test, Kaplan–Meier plot, univariate, and multivariate Cox regression analysis were calculated by using SPSS software. The Forest plot was drawn by GraphPad Prism software. A p value <0.05 was considered statistically significant.

RESULTS

Patients and OS

A total of 345 patients, 277 patients from the development group and 68 from the validation group, with pathologically diagnosed CRC with S-PM were included in the study (**Figure 1**). The clinicopathological factors and the therapeutic details of the patients are shown in **Table 1** and **Table S1**. Among the 277 patients from the development group, 198 patients (71.5%) received CRS. Of them, 54 (19.5%) patients were classified as CCR0-1 and 144 (52.0%) as CCR2-3. Further, 61 patients (22.0%) received HIPEC and 149 patients (53.8%) received chemotherapy.

In the validation group, 48 patients (70.6%) received CRS, of whom 12 (17.6%) and 36 (53.0%) patients were classified as CCR0-1 and CCR2-3, respectively. Also, 38 patients (55.9%) received HIPEC and 49 patients (72.1%) received chemotherapy.

Significant differences in terms of CT findings (p = 0.001), other organ-invasion (p <0.001), digestive obstruction (p <0.001), HIPEC (p = 0.001) and chemotherapy (p = 0.006) were observed between the development and validation groups (**Table 1** and **Table S1**). The mean OS for all patients was 16 (1–119) months and the 1-, 2-, and 3-year OS rates were 59.8, 37.7, and 27.0%, respectively. In the development group, the 1-, 2-, and 3-year OS rates were 54.9, 33.2, and 23.1%, respectively, and in the validation group, they were 66.7, 44.5, and 33.3%, respectively.

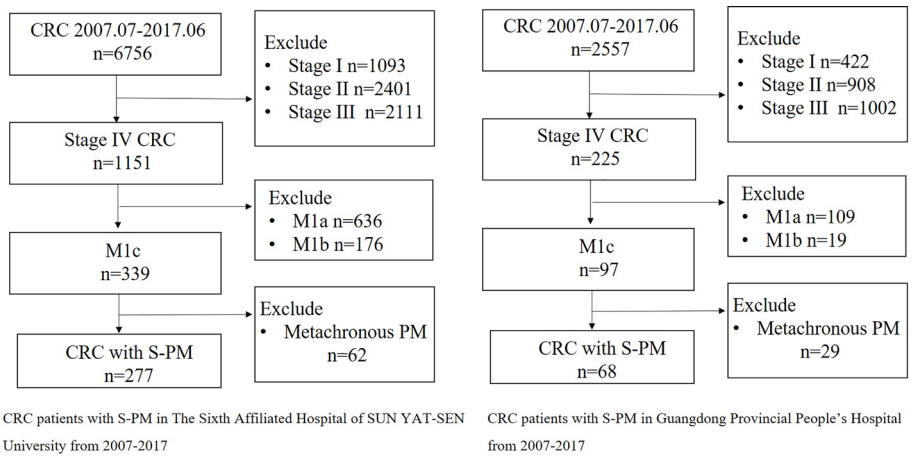

FIGURE 1 | Flow chart of patient selection in two medical centers.

TABLE 1 | A part of characteristics of CRC patients with S-PM in the development and validation groups.

Variables	All patients N = 345	Development group N = 277	Validation group N = 68	*p*-value
Age (years)				0.762
≤65	253 (73.3%)	204 (73.6%)	49 (72.1%)	
>65	92 (26.7%)	73 (26.4%)	19 (27.9%)	
CA19-9 (u/ml)				0.684
≤37	191 (55.4%)	155 (56.0%)	36 (52.9%)	
>37	154 (44.6%)	122 (44.0%)	32 (47.1%)	
CA125 (U/ml)				0.412
≤35	143 (41.4%)	118 (42.6%)	25 (36.8%)	
>35	202 (58.6%)	159 (57.4%)	43 (63.2%)	
Fistulation or bypass				0.625
No	270 (78.3%)	215 (77.6%)	55 (80.9%)	
Yes	75 (21.7%)	62 (22.4%)	13 (19.1%)	
Other metastasis				0.207
Absent	221 (64.1%)	182 (65.7%)	39 (57.4%)	
Present	124 (35.9%)	95 (34.3%)	29 (42.6%)	
CRS				0.117
No	99 (28.7%)	79 (28.5%)	20 (29.4%)	
CCR 0-1	94 (27.2%)	54 (19.5%)	12 (17.6%)	
CCR 2-3	152 (44.1%)	144 (52.0%)	36 (53.0%)	
HIPEC				0.001
No	246 (71.3%)	216 (78.0%)	30 (44.1%)	
Yes	99 (28.7%)	61 (22.0%)	38 (55.9%)	
Chemotherapy				0.006
No	147 (42.6%)	128 (46.2%)	19 (27.9%)	
Yes	198 (57.4%)	149 (53.8%)	49 (72.1%)	

CA19-9, carbohydrate antigen 19-9; CA125, carbohydrate antigen 125; CRS, cytoreductive surgery; CCR, completeness of cytoreduction; HIPEC, hyperthermic intraperitoneal chemotherapy.

Univariate and Multivariate Cox Regression Analyses in the Development Group

For the development group, univariate analyses identified age ≤65 years (p <0.001), CA19-9 ≤37 u/ml (p <0.001), CA125 ≤35 U/ml (p <0.001), absence of fistulation or bypass (p <0.001), absence of distant metastasis (p = 0.015), CRS (p <0.001), HIPEC (p = 0.001) and chemotherapy (p = 0.004) as factors that were associated with better prognosis in CRC patients with S-PM (**Table 2**). Of them, age, CA19-9, CA125, CRS, HIPEC, and chemotherapy were found to be independent prognostic factors for OS in multivariate analyses (**Table 2**) and were therefore used for building the nomogram.

Construction and Validation of the Nomogram

The forest plot and survival curves of six independent factors were shown in **Figure S1**. The 1-, 2-, 3-year survival-predicting nomograms in the development group were presented in **Figure 2**.

As shown in **Figure 3A**, in the internal validation cohort (development group), the C-index for the nomogram to predict the OS of CRC patients with S-PM was 0.701 (95% Cl, 0.666–0.736). For the calibration plot, the dotted line represents the predicted values of the nomogram, while the colorful line represents the actual values of the 1-, 2-, and 3-year OS rates. The less discrepant they are, the more precise the predictive capability of the nomogram was. In the external validation cohort (validation group), the C-index was 0.716 (95% Cl, 0.622–0.810). This was higher than the development group, which indicated the nomogram model obtained an ideal predictive accuracy. The external calibration plot for the nomogram showed good agreement between the predicted and actual survival rates (**Figure 3B**).

For the internal calibration, the colorful lines fluctuated above and below the dotted line, to identify a reliable predictive capability of the nomogram. The AUC of 1-, 2-, and 3-year OS predictions of the development group were 0.793, 0.775, and 0.766, respectively. These results indicated favorable discrimination of this proposed nomogram (**Figure 3C**). The AUC of 1-, 2-, and 3-year survival predictions of the validation group were 0.754, 0.765, and 0.714 (**Figure 3D**). Favorable discrimination was shown and the results were very close to that of the development group.

Implementation of the Webserver

An online dynamic platform (https://younghone.shinyapps.io/NomogramCRCSPM/) was developed to increase the applicability of the proposed nomogram (**Figure 4**). It can assist researchers and clinicians to more easily obtain the survival probability of their patients by inputting their corresponding clinical factors, after which the webserver will generate the output read in the forms of figures and tables.

TABLE 2 | Univariate and multivariate analyses of the 277 CRC patients with S-PM in the development group.

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Age (≤65 years)	1.766 (1.326–2.352)	<0.001	1.445 (1.070–1.951)	0.016
CA19-9 (u/ml) (≤37)	1.663 (1.279–2.163)	<0.001	1.447 (1.085–1.929)	0.012
CA125 (U/ml) (≤35)	1.658 (1.268–2.168)	<0.001	1.375 (1.040–1.819)	0.026
Fistulation or bypass (No)	2.269 (1.673–3.077)	<0.001	1.206 (0.794–1.898)	0.394
Other metastasis (Absent)	1.397 (1.067–1.749)	0.015	1.098 (0.851–1.518)	0.529
CCR0-1	0.277 (0.184–0.419)	<0.001	0.407 (0.241–0.685)	0.001
CCR2-3	0.486 (0.362–0.653)	<0.001	0.613 (0.422–0.934)	0.023
HIPEC (No)	0.561 (0.398–0.790)	0.001	0.659 (0.464–0.935)	0.020
Chemotherapy (No)	0.680 (0.523–0.883)	0.004	0.702 (0.537–0.919)	0.010

CA19-9, carbohydrate antigen 19-9; CA125, carbohydrate antigen 125; PM, peritoneal metastasis; CRS, primary tumor resection; HIPEC, hyperthermic intraperitoneal chemotherapy.

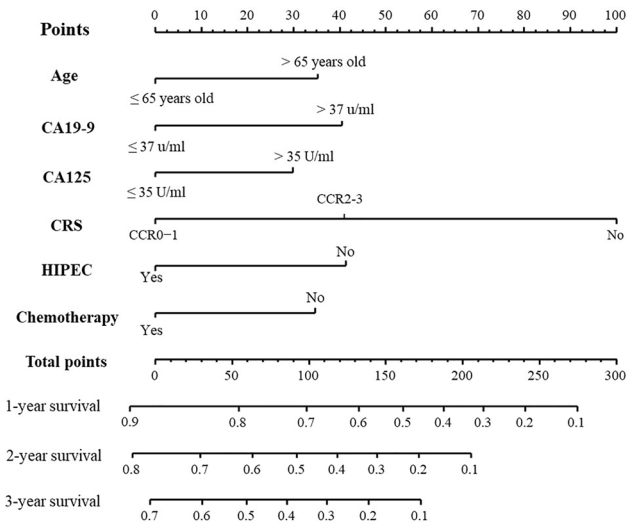

FIGURE 2 | Nomogram for predicting the OS of CRC patients with S-PM. The C-index of this nomogram is 0.701 (95% CI, 0.666–0.736).

DISCUSSION

In this study, the incidence of CRC with S-PM was found to be 4.1% (277/6,756) in the development group and 2.7% in the validation group; within the range of 1–13% in previous studies (14, 19, 26). The primary tumor was mainly located in the right side of the colon (44.3%, 153/345). Left-sided (34.2%, 118/345) and rectal tumors accounted for only 21.4% (74/345), which was similar to some previous studies (13, 19, 27). The rate of S-PM with distant metastases (liver or lung are the most common site) was 36.2–74.1% in previous studies (11, 13, 26, 27), while it was 35.9% (124/345) in our study. The diagnosis of CRC with S-PM was often made at an advanced stage due to the lack of specific symptoms of peritoneal involvement and the low sensitivity of current imaging techniques in detecting PM (14, 27). In this study, only 41.4% (143/345) could be diagnosed by contrast-enhanced CT scans, so the diagnostic technology still needs to be improved.

Laparoscopic exploration or laparotomy is considered the gold standard for the diagnosis of PM (28). In this study, there were significant differences in imaging diagnosis, other organ-invasion, digestive obstruction, HIPEC, and chemotherapy

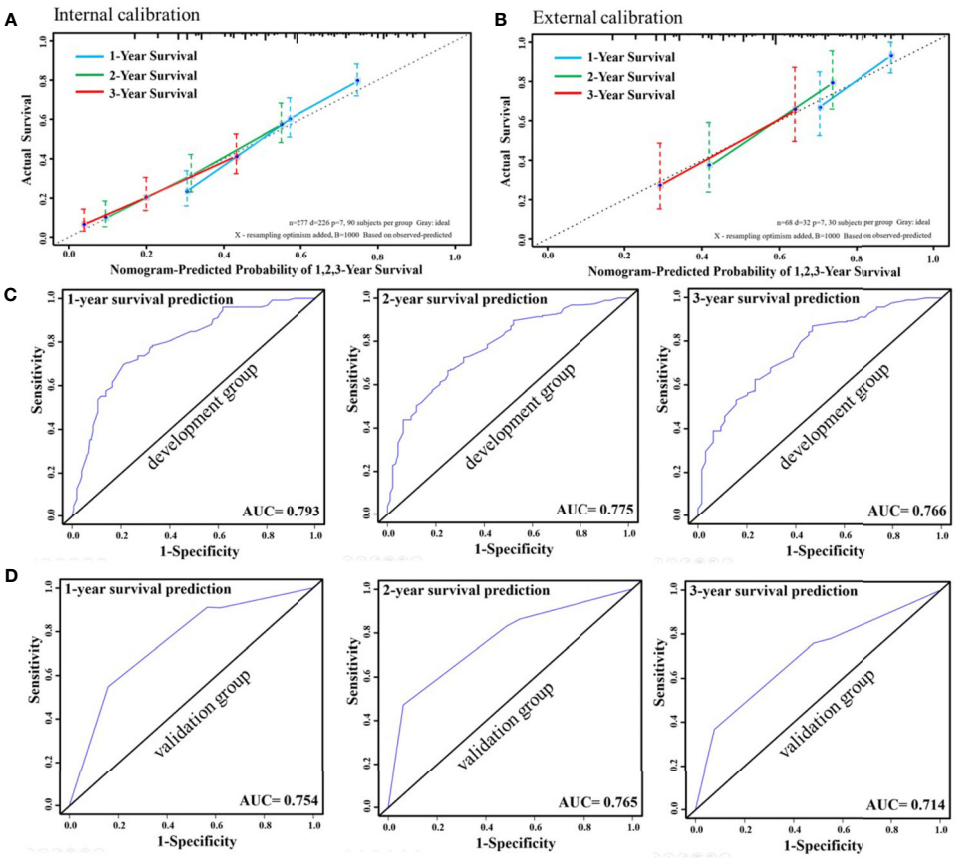

FIGURE 3 | Internal calibration curve to validate nomogram model for 1-, 2-, and 3-year survival and its C-index was 0.701 (95% CI, 0.666–0.736) **(A)**. External calibration curve to validate nomogram model for 1-, 2-, and 3-year survival and its C-index was 0.716 (95% CI, 0.622–0.810) **(B)**. ROC curve of 1-, 2-, and 3-year survival prediction in the development group **(C)**. ROC curve of 1-, 2-, and 3-year survival prediction in the validation group **(D)**.

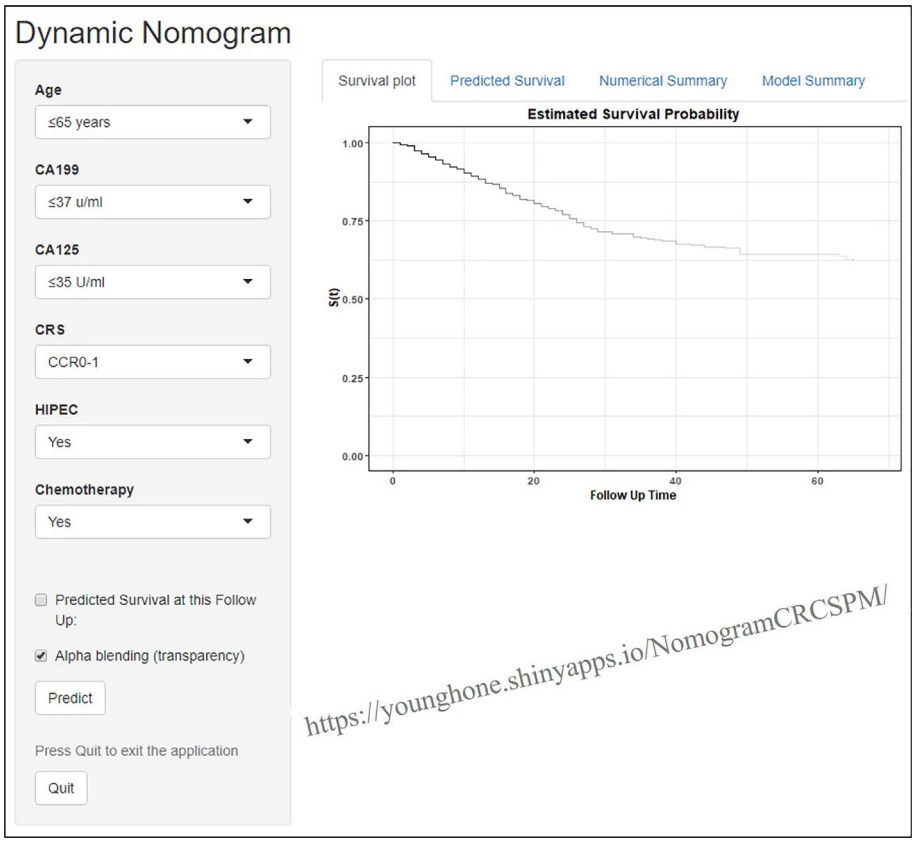

FIGURE 4 | Webserver display of the online dynamic nomogram.

between the development group and the validation group ($p < 0.05$). This might be related to the degree of peritoneal invasion, the local invasion of the primary tumor, the differences in diagnostic criteria, therapeutic level, and the treatment concept of the MDT in different medical centers (14, 17). The 1-, 2-, and 3-year OS rates were different in the two groups, possibly due to more cases in the validation group who underwent HIPEC and chemotherapy, as HIPEC and chemotherapy were identified as independent factors for good survival. Then, we performed univariate analysis in the development group and identified prognostic factors of survival including age, the levels of CA19-9 and CA125, fistulation or bypass, distant metastasis, CRS, chemotherapy, and HIPEC. The elderly patients were usually presented with a poor prognosis with poor physical function, lower immune function, and lack of sufficient treatment (29–31). Furthermore, we built a nomogram to predict prognosis for CRC patients with S-PM, which may be used to guide clinical practice.

Previous studies have shown that CA19-9 and CA125 were independent factors of prognosis in CRC patients with PM (32, 33). In this study, the overall positive rate (>37 u/ml) of CA19-9 was 49.5%, compared to 45.6–62.7% in previous studies (27). Meanwhile, CA19-9 was an independent prognostic factor and could be used during surveillance for early detection of disease recurrence or aggravation (24, 31, 33, 34). CA125 was identified as a sensitive tumor marker for ovarian tumors. However, a recent study on 853 patients demonstrated that CA125 could be more significant in predicting the prognosis of PM in CRC in both males and females than CEA (35). Huo (33) and Chuk (21) also found that CA125 was an independent risk factor of prognosis and could be used as a prognostic predictor.

Current guidelines and related studies confirm that CRS, HIPEC, and systematic chemotherapy in selected CRC with S-PM cases could significantly improve the long-term survival of these patients (17, 22, 36). However, to comprehensively evaluate and consider the patients' physical function, nutrition level, willingness, economic situation, tumor marker value, surgical skill level, development of treatment platform, and multiple disciplinary team (MDT) discussion results in the busy daily clinical practice is quite laborious and could vary from clinicians to clinicians (14). Therefore, not all CRC patients with S-PM have the opportunity to simultaneously undergo CRS, HIPEC, and systematic chemotherapy. In our study, 62.8% (199/317) of CRC patients with S-PM underwent CRS (CCR0-3), while in Wang (27) and Tanaka's (12) study, the proportion of CRS was

45.6 and 88.4%. Previous literature reports showed that the HIPEC treatment rates of CRC patients with S-PM were 21% (27) and 73% (37), while only 38.2% (121/317) CRC patients with S-PM received HIPEC treatment in our study. Further, in this study, a total of 54.9% (174/317) of the patients underwent perioperative chemotherapy, compared to 53.3–70.1% in other studies (10, 11, 16, 27). Meanwhile, as a relatively mature and effective treatment, previous studies have shown that high-quality CRS, standard HIPEC treatment and systematic perioperative chemotherapy could improve the prognosis of CRC patients with PM (14, 23). It is important to note that, for selective PM cases, on the basis of CRS combined with HIPEC, standard perioperative chemotherapy could better improve the prognosis (22, 23).

Complete CRS plus HIPEC, and systematic perioperative chemotherapy can improve the prognosis of CRC with S-PM (13, 22, 25). HIPEC comprises of intraperitoneal perfusion of chemotherapy reagents, heated to 42 °C to eliminate microscopic disease. The HIPEC technique is currently controversial for drug regimens, volume of infusion, duration, and concentrations in S-PM (38). A study from Australia showed that oxaliplatin offers a survival advantage when used for HIPEC in CRC with PM (39). As for stage IV CRC patients, whether or not it requires CRS, the NCCN guidelines (22) and relevant studies (40, 41) recommend 5-fluorouracil or capecitabine-based systemic chemotherapy to improve the prognosis. In this study, the HIPEC and chemotherapy were independent factors affecting the prognosis.

Although several nomograms had been developed to predict the OS for PM or stage IV CRC (8, 29, 42), no nomogram for predicting the OS of CRC with S-PM has yet been reported. In this study, an OS-predicting nomogram for S-PM was established and had a promising C-index of 0.701, signifying decent discriminatory ability of the nomogram. We used an independent cohort from other medical center for external validation and similarly observed a promising C-index of 0.716, further validating the good predictive performance of this proposed nomogram. Further, the calibration plot for 1-, 2-, and 3-year survival showed a first-rank consistency between the predicted and actual observation in both the development and validation group; indicating the reproducibility and reliability of this nomogram. Next, to make this nomogram easy to use in clinical practice, we developed an online time-saving dynamic nomogram, https://younghone.shinyapps.io/NomogramCRCSPM/, which can output a prognostic predictive value by inputting corresponding indicators.

Despite the interesting findings showed in this study, there were several potential limitations worth mentioning. First, this was a retrospective study and the cohort size could be considered limited; thus, potential selection bias might have existed. Second, the details of the peritoneal cancer index (PCI), the most widely used index to predict the survival of patients with PM (16, 17, 43), was unavailable due to the retrospective nature or incomplete data, and was thereby not calculated in this study. Third, the developed and validated data came from different medical centers and might differ in treatment concepts and details, and the number of cases in the external verification group is small. Therefore, we plan to conduct a prospective trial to validate our nomogram and its applicability in the clinic in the future.

CONCLUSIONS

We constructed and validated a nomogram able to predict the 1-, 2-, and 3-year OS for CRC patients with S-PM with good discriminative and high accuracy. The proposed nomogram could be used as a tool for more accurate prediction of individual prognosis and improve oncologists' clinical decision-making when formulating personalized treatments of CRC patients with S-PM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committees of The Sixth Affiliated Hospital (No. 2020ZSLYEC-109). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZfY, DqW, YL, and HW contributed to the idea and design. ZfY, XsQ, ZxY, HmW, and ZjL contributed to the data acquisition and analysis. ZfY, DyW, XsQ, and ZxY contributed to the manuscript writing and revision. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.615321/full#supplementary-material

Supplementary Figure 1 | Forest plot showed multivariate analyses of OS in the development group **(A)**. OS curve of age, CA19-9, CA125, CRS, HIPEC and chemotherapy in the development group **(B)**.

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Association Between Chemotherapy and Survival in T1 Colon Cancer With Lymph Node Metastasis: A Propensity-Score Matched Analysis

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This study aimed to comprehensively examine the efficacy of chemotherapy in T1 colon cancer patients with lymph node metastasis.

Methods: The differences in categorical variables in colon cancer patients according to lymph node status were evaluated by Pearson's chi-square test. The Kaplan-Meier method was used to assess Cancer-specific survival (CSS) and overall survival (OS) with the log-rank test. Cox proportional hazards models were built, multivariate Cox regression analyses were performed with the hazard ratio (HR) and 95% confidence interval (CI) to identify the potential independent prognostic factors. Propensity score matching was also undertaken to adjust for treatment bias due to measured confounders.

Results: Younger age (52.2% VS. 43.0% for ≤ 65 years old, $p < 0.001$), female gender (50.3% VS. 46.8% for female, $p < 0.001$), more lymph nodes harvested (68.1% VS. 46.6% for ≥12 lymph nodes harvested, $p < 0.001$), Black race (13.6% VS. 12.0% for the Black race, $p < 0.001$), and higher tumor grade (14.2% VS. 5.6% for grade III/IV, $p < 0.001$) were more prone to be diagnosed with lymph node involvement. The receipt of adjuvant chemotherapy following radical surgery significantly reduced the risk of colon cancer-specific mortality by 33.9% after propensity-score matching (HR = 0.661, 95%CI = 0.476-0.917, $p = 0.013$).

Conclusions: Younger age, female gender, more lymph nodes harvested, Black race, and higher tumor grade were more prone to be diagnosed with lymph node involvement. The receipt of adjuvant chemotherapy following radical surgery also significantly decreased the risk of colon cancer-specific mortality by 33.9% in T1 colon cancer with lymph node involvement.

Keywords: chemotherapy, survival, T1, colon cancer, propensity score

INTRODUCTION

Colon cancer is among the most common causes of cancer and cancer-related death (1). T1 colon cancer refers to carcinoma with invasion confined to the submucosa (2, 3). As reported, however, approximately 10% of T1 colon cancer patients experience lymph node metastases and require radical intestinal resection with lymph node dissection (4). Although the risk factors for lymph node metastasis in T1 colon cancer have been widely reported, differences in opinion do exist (5, 6).

The oncological outcomes of stage I colon cancer patients are generally excellent following curative surgery; however, the presence of lymph node metastasis represents a prognostic feature in poor prognosis. Among treatments, 5-FU-based chemotherapy has been demonstrated to have significant survival benefits for patients with lymph node metastasis (7–9). Despite this, some patients do not receive further chemotherapy following radical surgery (10). The available data of oncological outcomes in T1 node-positive (N+) patients is lacking. For example, the well-known MOSAIC study, assessing the impact of adjuvant chemotherapy for stage III colon cancer, did not include T1 disease (9).

T1 disease is relatively rare and represents a small proportion of cases of colon cancer. It has been reported that such patients account for 2 to 12 percent of all cases of colon cancer in colonoscopic studies (11–15). Therefore, a large population-based cohort is needed to evaluate the predictors for lymph node metastasis in T1 colon cancer following curative surgery. The present study aimed to comprehensively examine the efficacy of chemotherapy in T1 colon cancer patients with postoperative lymph node metastasis.

MATERIAL AND METHODS

Sponsored by the National Cancer Institute (NCI), the Surveillance, Epidemiology, and End Results (SEER) database included both the incidence, clinicopathological information, and survival characteristics of malignant tumors, and covered 28% of the US population from 18 established cancer registries across the USA. The SEER*Stat software, version 8.3.8 (Surveillance Research Program, National Cancer Institute) was utilized to acquire data for this population-based study from the SEER database. Because data from the SEER database were anonymous and publicly available, ethical approval was waived and informed consent was unnecessary in this study.

The baseline covariates included the year of diagnosis, tumor location, age at diagnosis, the number of lymph nodes harvested, race, gender, grade, and chemotherapy based on the postcode of patients. As seen in **Figure S1**, we identified patients diagnosed with colon cancer between 2004 and 2015. Patients who met the following criteria were excluded: ① patient race was unknown, ② no positive histological confirmation, ③ non-adenocarcinoma histologies, ④ lack of active follow-up, and ⑤ without radical surgery. Finally, the targeted population was patients diagnosed with stage T1NanyM0 colon cancer, who were included in our analyses. Further analysis was conducted in stage T1N+M0 colon cancer patients.

Statistical Analysis

Patients' demographic and clinical characteristics were included as follows: year of diagnosis (2004-2007, 2008-2011 and 2012-2015), tumor location [right-sided colon (from caecum to transverse colon) and left-sided colon (from splenic flexure to rectosigmoid junction)], age at diagnosis (≤65 years old and >65 years old), the number of lymph nodes harvested (≤11 and ≥12), race (white, Black and other), gender (male and female), grade (I/II, III/IV and unknown), and chemotherapy (no chemotherapy and chemotherapy).

Cancer-specific survival (CSS) and overall survival (OS) served as the endpoints. The differences of the categorical variables in colon cancer patients according to the lymph node status were analyzed by Pearson's chi-square test. The Kaplan-Meier method was used to assess the survival with the log-rank test. Cox proportional hazards models were built and multivariate Cox regression analyses were performed with hazard ratio (HR) and 95% confidence interval (CI) to identify the potential independent prognostic factors from the variables examined, with P value less than 0.20 in univariate analyses.

Patient demographic and clinicopathological features were not balanced due to the inherent deficits of the retrospective cohort. Propensity score matching, a statistical normalization method for analyzing observational data by estimating the effects of a large number of factors that could affect treatment allocation, were then generated to balance covariates in different groups and reduce selection bias due to confounding variables (16). To provide a more robust assessment of survival outcomes, propensity score matching was performed between stage T1N+M0 colon cancer patients with and without the receipt of chemotherapy using a 1:1 nearest neighbor matching algorithm. The following variables were used to calculate propensity to receive chemotherapy: year of diagnosis, tumor location, age at diagnosis, the number of lymph nodes retrieved, patient race, gender, and tumor grade. Statistically significant levels were two-tailed and set at a P value of less than 0.05. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 23.0 software package for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patient Characteristics

In total, 36595 eligible colon cancer patients met the inclusion criteria, of which 33633 (91.9%) patients were diagnosed with lymph node-negativity and 2962 (8.1%) patients were diagnosed with lymph node positivity; 12428 (34.0%) patients were diagnosed between 2004 to 2007, 12496 (34.1%) patients were diagnosed between 2008 to 2011 and 11671 (31.9%) patients were diagnosed between 2012 to 2015; 17379 (47.5%) patients were right-sided colon cancer and 19216 (52.5%) patients were left-sided colon cancer; 15999 (43.7%) patients were less than 65 years old and 20596 (56.3%) patients were over 65 years old; 18891 (51.6%) patients had less than 12 lymph nodes retrieved and 17704 (48.4%) patients had more than 12 lymph nodes retrieved; 29051 (79.4%) patients were of white race, 4437

(12.1%) patients were of Black race and 3107 (8.5%) patients were other races; 19371 (52.9%) patients were male and 17224 (47.1%) patients were female; 29071 (79.4%) patients were diagnosed with grade I/II and 2320 (6.3%) patients were diagnosed with grade III/IV; 2414 (6.6%) patients received chemotherapy and 34181 (93.4%) patients did not. The demographic and clinical characteristics among the whole cohort are shown in **Table 1**. Our study found that those who were younger in age (52.2% VS. 43.0% for ≤ 65 years old, $p < 0.001$), had female gender (50.3% VS. 46.8% for female, $p < 0.001$), more lymph nodes harvested (68.1% VS. 46.6% for ≥12 lymph nodes harvested, $p < 0.001$), were of Black race (13.6% VS. 12.0% for Black race, $p < 0.001$), and who had a higher tumor grade (14.2% VS. 5.6% for grade III/IV, $p < 0.001$) were more prone to be diagnosed with lymph node involvement.

The Efficacy of Chemotherapy in T1N+ Colon Cancer Patients Before Propensity Score Matching

We then included 2962 (8.1%) T1N+ colon cancer patients in further analyzes. As shown as **Figure 1**, the CSS curves of T1N+ colon cancer patients with and without the receipt of chemotherapy were generated using the Kaplan-Meier method. The CSS of T1N+ colon cancer patients with the receipt of chemotherapy was significantly better than those without the receipt of chemotherapy (94.3% VS. 89.3% for 5-year CSS rate, $p < 0.001$).

In an unadjusted Cox proportional hazards analysis, the cancer-specific mortality risk in patients with the receipt of chemotherapy was reduced by 48.1% (HR = 0.519, 95%CI =0.397-0.678, $p < 0.001$). Only variables with a P value less than 0.20 in unadjusted Cox analyses were then entered into multivariate Cox analyses, including information on the year of diagnosis, tumor location, age at diagnosis, patient race, gender, tumor grade, and whether they received chemotherapy. The results of multivariate analyses also showed that the cancer-specific mortality risk in patients with the receipt of chemotherapy was independently decreased by 46.0% (HR = 0.540, 95%CI =0.409-0.712, $p < 0.001$; **Table 2**).

The Efficacy of Chemotherapy in T1N+ Colon Cancer Patients After Propensity-Score Matching

As shown in **Table 3**, the clinicopathologic characteristics of T1N+ colon cancer patients were compared according to the receipt of chemotherapy before propensity-score matching. The year of diagnosis (35.2% VS. 29.3% for 2012-2015, $p < 0.001$), left-sided colon cancer (56.3% VS. 48.4% for left-sided colon, $p < 0.001$), younger age (62.5% VS. 34.8% for ≤ 65 years old, $p < 0.001$), more lymph nodes harvested (71.5% VS. 62.4% for ≥12 lymph node harvested, $p < 0.001$), and higher tumor grade (15.0% VS. 13.0% for grade III/IV, $p < 0.001$) were more prone to be associated with receipt of adjuvant chemotherapy in T1N+ colon cancer patients.

In evaluating the effect of chemotherapy on the survival of T1N+ colon cancer patients, to avoid the bias introduced by the retrospective design, we balanced the above demographic and clinical characteristics mentioned with propensity score matching. After matching by the ratio of 1:1, a total of 890

TABLE 1 | Clinicopathologic characteristics of T1 colon cancer patients according to the lymph node status.

Variables	N0 (N=33,633)	N+ (N=2,962)	*P value*
Year of diagnosis			0.063
2004-2007	11,480 (34.1%)	948 (32.0%)	
2008-2011	11,459 (34.1%)	1,037 (35.0%)	
2012-2015	10,694 (31.8%)	977 (33.0%)	
Tumor location			0.344
Right-sided colon	15,997 (47.6%)	1,382 (46.7%)	
Left-sided colon	17,636 (52.4%)	1,580 (53.3%)	
Age at diagnosis (years)			<0.001
≤65	14,453 (43.0%)	1,546 (52.2%)	
>65	19,180 (57.0%)	1,416 (47.8%)	
Number of lymph nodes harvested			<0.001
≤11	17,946 (53.4%)	945 (31.9%)	
≥12	15,687 (46.6%)	2,017 (68.1%)	
Race			<0.001
White	26,799 (79.7%)	2,252 (76.0%)	
Black	4,033 (12.0%)	404 (13.6%)	
Other	2,801 (8.3%)	306 (10.3%)	
Gender			<0.001
Male	17,898 (53.2%)	1,473 (49.7%)	
Female	15,735 (46.8%)	1,489 (50.3%)	
Grade			<0.001
I/II	26,754 (79.5%)	2,317 (78.2%)	
III/IV	1,898 (5.6%)	422 (14.2%)	
Unknown	4,981 (14.8%)	223 (7.5%)	
Chemotherapy			<0.001
No chemo	33,077 (98.3%)	1,104 (37.3%)	
Chemo	556 (1.7%)	1,858 (62.7%)	

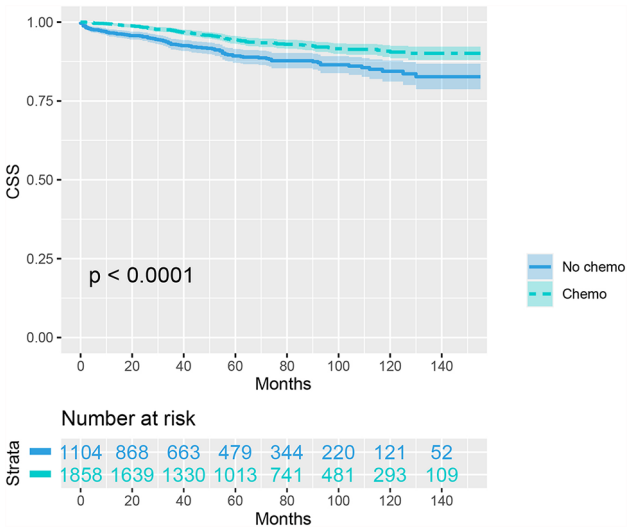

FIGURE 1 | Kaplan-Meier plot, revealing cancer-specific survival differences based on the receipt of adjuvant chemotherapy in T1N+ colon cancer patients before propensity score matching.

T1N+ colon cancer patients with the receipt of chemotherapy were matched to 890 T1N+ colon cancer patients without the receipt of chemotherapy. The distribution histograms before and after propensity-score matching are illustrated in **Figure 2**.

As indicated by **Table 4**, the clinicopathologic characteristics of T1N+ colon cancer patients were compared according to the receipt of chemotherapy after propensity-score matching. Our study found that there was no difference between both groups with regards to year of diagnosis (p = 1.000), tumor location (p = 1.000), age at diagnosis (p = 1.000), number of lymph nodes harvested (p = 1.000), patient race (p = 1.000), gender (p = 1.000) and tumor grade (p = 1.000). The receipt of adjuvant chemotherapy treatment following radical surgery did significantly decrease the risk of colon cancer-specific mortality by 33.9% after propensity-score matching (HR = 0.661, 95%CI = 0.476-0.917, p = 0.013). The Kaplan-Meier CSS curves of T1N+ colon cancer patients with and without the receipt of chemotherapy after propensity score matching are shown in **Figure 3**. The CSS of T1N+ colon cancer patients who received chemotherapy was significantly better than those who did not receive chemotherapy (93.5% VS. 89.9% for 5-year CSS rate, p = 0.013). Moreover, as seen in **Figure 4**, the OS of T1N+ colon cancer patients who received chemotherapy was significantly better than those who did not (84.8% VS. 66.3% for 5-year OS rate, p < 0.001).

DISCUSSION

In colon cancer, the presence of lymph node metastasis is a prognostic feature in poor prognosis configuration. In theory, lymph node metastasis should not occur when the tumor is confined to the mucosal layer because this layer is devoid of

TABLE 2 | Cox regression analysis of prognostic factors for cancer-specific survival in T1N+ colon cancer.

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P value*	HR (95%CI)	*P value*
Year of diagnosis		0.097		0.179
2004–2007	1		1	
2008–2011	0.918 (0.682–1.236)	0.573	0.990 (0.735–1.335)	0.950
2012–2015	0.613 (0.393–0.957)	0.031	0.668 (0.427–1.046)	0.078
Tumor location		0.006		0.037
Right-sided colon	1		1	
Left-sided colon	0.686 (0.525–0.897)		0.744 (0.564–0.983)	
Age at diagnosis (years)		0.009		0.275
≤65	1		1	
>65	1.429 (1.093–1.869)		1.173 (0.881–1.561)	
Number of lymph nodes harvested		0.370		
≤11	1			
≥12	0.881 (0.669–1.161)			
Race		0.051		0.031
White	1		1	
Black	1.430 (1.004–2.036)	0.048	1.552 (1.086–2.218)	0.016
Other	0.741 (0.443–1.238)	0.252	0.825 (0.492–1.381)	0.464
Gender		0.127		0.102
Male	1		1	
Female	0.811 (0.620–1.061)		0.798 (0.609–1.045)	
Grade		0.044		0.035
I/II	1		1	
III/IV	1.490 (1.064–2.088)	0.020	1.482 (1.056–2.081)	0.023
Unknown	0.837 (0.475–1.473)	0.536	0.760 (0.431–1.340)	0.343
Chemotherapy		<0.001		<0.001
No chemo	1		1	
Chemo	0.519 (0.397–0.678)		0.540 (0.409–0.712)	

TABLE 3 | Clinicopathologic characteristics of T1N+ colon cancer patients according to the receipt of chemotherapy before propensity score matching.

Variables	N0 chemo (N=1104)	Chemo (N = 1858)	*P value*
Year of diagnosis			<0.001
2004-2007	411 (37.2%)	537 (28.9%)	
2008-2011	370 (33.5%)	667 (35.9%)	
2012-2015	323 (29.3%)	654 (35.2%)	
Tumor location			<0.001
Right-sided colon	570 (51.6%)	812 (43.7%)	
Left-sided colon	534 (48.4%)	1046 (56.3%)	
Age at diagnosis (years)			<0.001
≤65	384 (34.8%)	1,162 (62.5%)	
>65	720 (65.2%)	696 (37.5%)	
Number of lymph nodes harvested			<0.001
≤11	415 (37.6%)	530 (28.5%)	
≥12	689 (62.4%)	1,328 (71.5%)	
Race			0.090
White	864 (78.3%)	1,388 (74.7%)	
Black	137 (12.4%)	267 (14.4%)	
Other	103 (9.3%)	203 (10.9%)	
Gender			0.821
Male	552 (50.0%)	921 (49.6%)	
Female	552 (50.0%)	937 (50.4%)	
Grade			<0.001
I/II	849 (76.9%)	1,468 (79.0%)	
III/IV	143 (13.0%)	279 (15.0%)	
Unknown	112 (10.1%)	111 (6.0%)	

lymphatic vessels. T1 colon cancer, which refers to carcinoma with invasion confined to the submucosa, however, had an approximately 10% probability of experiencing lymph node metastases and therefore requires radical intestinal resection with lymph node dissection. Many studies have previously evaluated the risk factors for lymph node metastasis in T1 colon cancer, however, differences in opinion have always existed (3, 5, 17–21).

In our analyses, younger age, female gender, more lymph nodes harvested, Black race and higher tumor grade were more prone to be diagnosed with lymph node involvement. Two recent studies have reported that young age at diagnosis could be associated with an increased risk of lymph node involvement and more aggressive screening and postoperative treatments should be considered for young patients with T1 colon adenocarcinoma/ (22, 23) This phenomenon might be due to the potential genetic differences between young and elderly patients, as young patients are more likely to present with more aggressive features and adverse histological grades (24–26) In line with the results of previous studies, we found that Black race had a risk factor of developing metastasis (23). The higher rate of lymph node metastasis in female colon cancer patients diagnosed with T1 disease might result from the sex

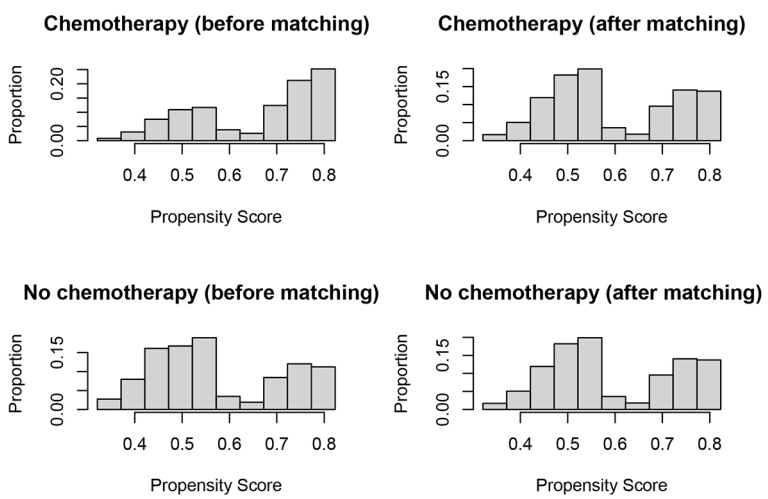

FIGURE 2 | Distribution histograms before and after propensity score matching (treated = no surgery; control = radical surgery).

TABLE 4 | Clinicopathologic characteristics of T1N+ colon cancer patients according to the receipt of chemotherapy after propensity score matching.

Variables	N0 chemo (N = 890)	Chemo (N = 890)	*P value*
Year of diagnosis			1.000
2004-2007	322 (36.2%)	322 (36.2%)	
2008-2011	287 (32.2%)	287 (32.2%)	
2012-2015	281 (31.6%)	281 (31.6%)	
Tumor location			1.000
Right-sided colon	440 (49.4%)	440 (49.4%)	
Left-sided colon	450 (50.6%)	450 (50.6%)	
Age at diagnosis (years)			1.000
≤65	356 (40.0%)	356 (40.0%)	
>65	534 (60.0%)	534 (60.0%)	
Number of lymph nodes harvested			1.000
≤11	282 (31.7%)	282 (31.7%)	
≥12	608 (68.3%)	608 (68.3%)	
Race			1.000
White	715 (80.3%)	715 (80.3%)	
Black	106 (11.9%)	106 (11.9%)	
Other	69 (7.8%)	69 (7.8%)	
Gender			1.000
Male	445 (50.0%)	445 (50.0%)	
Female	445 (50.0%)	445 (50.0%)	
Grade			1.000
I/II	717 (80.6%)	717 (80.6%)	
III/IV	121 (13.6%)	121 (13.6%)	
Unknown	52 (5.8%)	52 (5.8%)	

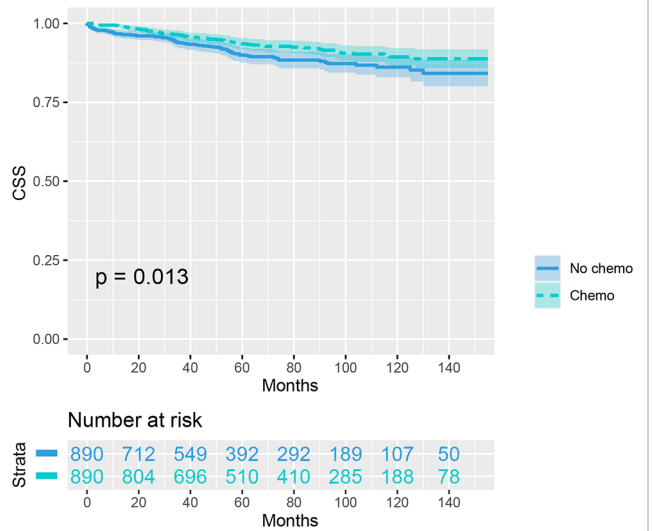

FIGURE 3 | Kaplan-Meier plot, revealing the cancer-specific survival differences based on the receipt of adjuvant chemotherapy in T1N+ colon cancer patients after propensity score matching.

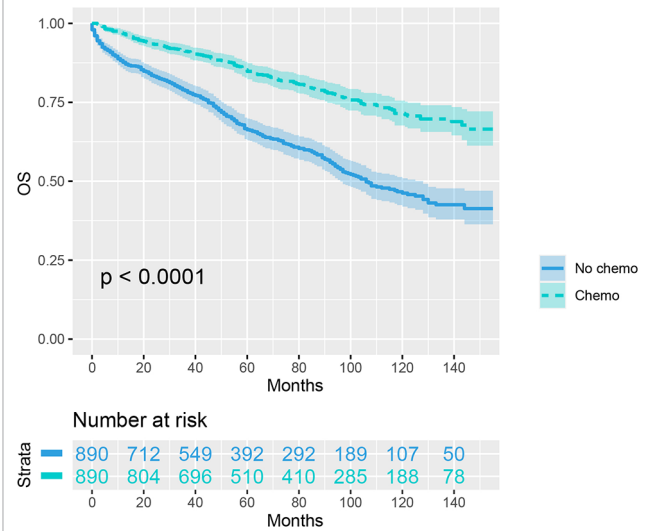

FIGURE 4 | Kaplan-Meier plot, revealing the overall survival differences based on the receipt of adjuvant chemotherapy in T1N+ colon cancer patients after propensity score matching.

hormones between male and female patients (27, 28). It has also been observed that T1 carcinoma located in the left-sided colon shows higher rates of lymph node metastasis than right-sided colon, though it was not statistically significant in our study (29,30).

According to current clinical guidelines, T1 colon cancer patients with lymph node metastasis should receive adjuvant chemotherapy following radical surgery. Moreover, 5-FU-based chemotherapy has been demonstrated to have significant survival benefits for patients with lymph node metastasis (31–33). Early in 1990, Moertel and collaborators (8) demonstrated an improved prognosis of chemotherapy in colon carcinoma with lymph node metastasis following radical resection. Later in 2004, the famous MOSAIC study proposed that adding oxaliplatin to a regimen of fluorouracil and leucovorin provides improved efficacy in the adjuvant treatment of colon

cancer (9). Cases of T1 disease are relatively few and account for a small proportion of colon cancer. It has been reported that such patients account for 2 to 12 percent of all colon cancer patients in colonoscopic studies (11–15). It is important to note that the above studies evaluating the efficacy of adjuvant chemotherapy for stage III colon cancer did not include T1 disease. However, T1 colon cancer patients with lymph node involvement following radical resection often did not receive further chemotherapy after surgery and the available data of oncological outcomes in T1 node-positive (N+) patients is lacking (10). In 2005, Wang et al. (34) evaluated the prognosis of T1 colorectal cancer in a small population (n = 159) and found that predictive factors for the risk of lymph node metastasis in T1 colorectal cancer after radical resection do not impact the long-term prognosis.

Despite findings such as these, studies on T1 colon cancer patients are mostly focused on the predictive factors for the risk of lymph node metastasis following radical resection (5, 6). T1 disease with lymph node involvement is much rarer than without lymph node metastasis, therefore, a large cancer database was required to examine the efficacy of chemotherapy in such patients. More importantly, our study has shown that adjuvant chemotherapy treatment could provide significantly better oncological outcomes in T1 colon cancer patients with lymph node involvement following radical surgery. In an unadjusted Cox proportional hazards analysis, the cancer-specific mortality risk in patients with the receipt of chemotherapy was reduced by 48.1% ($p < 0.001$). The results of multivariate analyses also showed that the cancer-specific mortality risk in patients who received chemotherapy was independently reduced by 46.0% ($p < 0.001$). In addition, to reduce the bias introduced by the retrospective design, we balanced the demographic and clinical characteristics with propensity-score matching. The receipt of adjuvant chemotherapy treatment following radical surgery significantly reduced the risk of colon cancer-specific mortality by 33.9%, even after propensity-score matching. Kaplan-Meier analysis also showed that the CSS of T1N+ colon cancer patients with the receipt of chemotherapy was significantly better than those without the receipt of chemotherapy after propensity score matching, and the 5-year CSS rates were 93.5%, and 89.9%, respectively ($p = 0.013$).

The major strengths of the current study are that it used a large cohort, and that we validated that younger age, female gender, more lymph nodes harvested, Black race, and higher tumor grade are more prone to be diagnosed with lymph node involvement. More importantly, by employing propensity score matching, the study provides a high level of evidence that the receipt of adjuvant chemotherapy following radical surgery significantly decreases the risk of colon cancer specific mortality by 33.9% in T1 colon cancer with lymph node involvement.

Several limitations of the current study should also be noted. First, the information on postoperative complications, which could negatively affect the prognosis of colon cancer patients after radical resection was not included in the database, and could cause potential systematic bias. Second, the drawbacks introduced by the retrospective design could not be avoided, even though propensity-score matching was used. Finally, this database did not provide information on specific chemotherapy regimens, and further large-scale studies evaluating the effect of different chemotherapy regimens on survival in T1 colon cancer patients with lymph node metastasis are required.

CONCLUSIONS

Patients of younger age, female gender, more lymph nodes harvested, Black race, and higher tumor grade are more prone to be diagnosed with lymph node involvement. Using propensity-score matching, this study has provided important evidence that the receipt of adjuvant chemotherapy following radical surgery could significantly decrease the risk of colon cancer-specific mortality by 33.9% in T1 colon cancer with lymph node involvement.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/.

AUTHOR CONTRIBUTIONS

WY and QL conceived the project and wrote the manuscript. SS and JL collected the data. WY, HZ, and SS undertook the analysis. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.699400/full#supplementary-material

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Can Elevated Pretreatment Serum Carcinoembryonic Antigen Levels Serve as a Potential Biomarker Guiding Adjuvant Chemotherapy in Rectal Cancer Patients With ypTis-3N0 After Neoadjuvant Radiotherapy and Surgery?

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Survival benefit of adjuvant chemotherapy (ACT) remained controversial in patients with stage II/III rectal cancer (RC) who received neoadjuvant therapy and surgery. This study aimed to investigate the guiding role of elevated pretreatment serum carcinoembryonic antigen (CEA) levels for receiving ACT in yield pathological Tis-3N0 (ypTis-3N0) RC patients after neoadjuvant radiotherapy and surgery. Between 2004 and 2015, 10,973 RC patients with ypTis-3N0 who received neoadjuvant radiotherapy and radical surgery were retrospectively analyzed using the Surveillance, Epidemiology, and End Results (SEER) database. Compared with CEA-normal group, elevated-CEA patients had worse 5-year CSS rate (90.1 *vs* 83.5%). The 5-year CSS rates were 86.3 and 87.4% for ypTis-3N0M0 patients with or without ACT, respectively. Patients receiving ACT had a comparable 5-year CSS rate compared to those who did not regardless of CEA levels in ypTis-3N0M0 RC patients (CEA elevation group: 76.4 *vs*. 83.5%, P = 0.305; CEA normal group: 90.0 *vs*. 90.1%, P = 0.943). Intriguingly, ypT3N0M0 RC patients with elevated CEA levels may benefit from ACT (5-year CSS: 69.1 *vs*. 82.9%, P = 0.045), while those with normal CEA levels did not (5-year CSS: 89.3 *vs*. 89.3%, P = 0.885). Multivariate Cox analysis demonstrated that ACT tended to be a protective factor in elevated-CEA ypT3N0M0 RC patients (HR = 0.633, 95% CI = 0.344–1.164, P = 0.141), while ACT was not associated with improved CSS in normal-CEA ypT3N0M0 RC patients (HR = 1.035, 95% CI = 0.487–2.202, P = 0.928). Elevated pretreatment serum CEA levels may serve as a promising biomarker guiding ACT in rectal cancer patients with ypT3N0M0.

Keywords: neoadjuvant radiotherapy, rectal cancer, serum carcinoembryonic antigen, ypTis-3N0, surgery

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INTRODUCTION

Based on the results from the German Rectal Cancer Study Group (the CAO/ARO/AIO-94 trial) that demonstrated preoperative chemoradiotherapy (CRT) could decrease local recurrence among patients with locally advanced rectal cancer compared to postoperative chemoradiotherapy (1, 2), neoadjuvant CRT followed by radical resection has been established as a standard strategy for locally advanced rectal cancer.

Satisfactory regression has often been observed after neoadjuvant radiotherapy (RT), and some patients even achieved clinical complete response (CCR) or pathological complete response (PCR), which brings debates to the choice of adjuvant chemotherapy (ACT) (3). ACT could reduce the risk of recurrence and mortality for patients with locally advanced rectal cancer (4). However, ACT could also bring systemic toxicity problems. Conclusive data on the use of ACT depending on pretreatment clinical stage or yield pathological stage are lacking. Patients with rectal cancer were often excluded from phase III studies due to the potential impact of RT or CRT. For colon cancer, survival benefit of ACT has been observed for patients with 'high-risk' stage II and stage III disease (5). According to yield pathological stage, ACT will no longer be needed in patients with ypTis-2N0 and "low-risk" ypT3N0. Besides, it is hard to determine real 'high-risk' stage II after preoperative CRT. Evidence from some studies indicated that patients with pathological complete response (pCR) did not benefit from ACT (6, 7), while other studies had come to the opposite conclusion (8, 9). However, the National Comprehensive Cancer Network (NCCN) recommended use of ACT for patients with stage II/III rectal cancer regardless of postoperative yield pathology if the patient did not receive neoadjuvant chemotherapy. The European Society for Medical Oncology (ESMO) indicated that it was reasonable to consider ACT in rectal cancer patients after preoperative chemoradiotherapy with yp stage III and "high-risk" yp stage II. In fact, the role of ACT in patients after neoadjuvant CRT and surgery has not been well established.

Previous studies reported the use of serum carcinoembryonic antigen (CEA) levels to guide ACT for stage IIA colon cancer (10). Patients with elevated pretreatment CEA levels should be grouped into 'high-risk' stage II disease. Inspired from these points of view, we have evaluated the guiding role of elevated pretreatment serum CEA levels for use of ACT in ypTis-3N0 rectal cancer using the Surveillance, Epidemiology, and End Results (SEER) database.

METHODS

Patient Selection

Patients with ypTis-3N0 rectal cancer who received neoadjuvant radiotherapy and underwent definitive/curative surgery were included and retrospectively analyzed from the SEER database (2004–2015): pretreatment serum CEA information was available starting from 2004. This study was approved by the Institutional Review Board of Affiliated Hospital of Nanjing University of Chinese Medicine. The inclusion criteria were listed as follows: the site code represented "rectum (130)"; patients received "radiation before surgery" (2, preoperative radiotherapy); surgery was performed in primary site; patients with ypTis-3N0M0; information about cancer-specific survival (CSS), and survival months were available. All patients were enrolled in the current analysis according to the American Joint Committee on Cancer staging system. Preoperative radiotherapy is mainly beam radiotherapy, and a few patients used radioactive implants or radioisotopes; the main methods of operation were abdomen perineal reservation (APR) and anterior resection (AR), and the specific chemotherapy regimen was unknown; according to the SEER database.

Data Collection

The following data were gathered: gender, age at diagnosis, marital status, race, tumor size, T stage, histologic type, differentiation status, pretreatment serum CEA levels, CSS, and survival months. CSS represented the time from the date of initial diagnosis to the date of death resulting from rectal cancer. Among them, the age should be over 18 years old, rectal cancer was primary, and the cut-off value of CEA level was selected as 5 ng/ml. It should be noted that the study lacked data on complications in patients with rectal cancer, which is an important factor for survival.

Statistical Analysis

The differences between two groups (the CEA-normal group and CEA-elevated group regardless of whether having received ACT, the receiving ACT group and not receiving ACT group regardless of the level of CEA, the receiving ACT group and not receiving ACT group in condition of CEA-elevated and CEA-normal, respectively.) were compared using $\chi 2$ test. The Kaplan–Meier method was adopted to evaluate CSS and to estimate relative 5-year survival rate. The difference was compared with log-rank test. Cox proportional hazards regression models were performed to screen out independent factors which were associated with CSS. To minimize the risk of biased estimates of treatment effect, propensity score matching (PSM) at a 1:2 ratio was performed. The PSM model included gender, age, marital status, race, tumor size, T stage, histologic type, and differentiation status. All statistical analyses were performed with SPSS 25.0 and R (version 3.6.0).

RESULTS

Pretreatment Serum CEA Levels Is an Independent Prognostic Factor in ypTis-3N0M0 Rectal Cancer

A total of 6,806 ypTis-3N0M0 rectal cancer patients with known pretreatment serum CEA levels were identified from the SEER database. Among them, 4,190 patients were grouped into the CEA-normal group, 2,616 patients were grouped into the CEA-elevated group. Compared with the CEA-normal group, patients with elevated pretreatment serum CEA levels had worse 5-year cancer-specific survival (CSS) rate (90.1 *vs.* 83.5%) (**Figure 1**). Multivariate Cox analysis demonstrated that elevated pretreatment serum CEA level was an independent risk factor in the cohort (HR = 1.597, 95%

CI = 1.385–1.841, P < 0.001) (**Table 1**). Intriguingly, multivariate Cox analyses showed that pretreatment serum CEA elevation in stage ypTis-1N0M0 group presented the most remarkable increased risk of CSS compared with stage ypT2N0M0 or ypT3N0M0 group (ypTis-1N0M0: HR = 1.891, 95% CI = 1.286–2.781, P = 0.001; ypT2N0M0: HR = 1.465, 95% CI = 1.008–2.129, P = 0.045; ypT3N0M0: HR = 1.570, 95% CI = 1.325–1.861, P < 0.001) (**Table 2**).

ACT Was Not Associated With Improved CSS in Patients With ypTis-3N0M0 Rectal Cancer

A total of 10,973 patients with ypTis-3N0M0 rectal cancer were identified from the SEER database. Among them, 10,594 patients received ACT, 379 patients did not receive ACT. Kaplan–Meier survival curves revealed that patients with ypTis-3N0M0 rectal cancer may not benefit from ACT (**Figure 2A**). The 5-year CSS estimates were 86.3 and 87.4 for patients with ACT and without ACT, respectively. Multivariate Cox analysis demonstrated that ACT was not associated with improved CSS in patients with ypTis-3N0M0 rectal cancer (HR = 0.971, 95% CI = 0.731–1.288, P = 0.836). Subgroup analysis revealed that patients with ypTis-2N0M0 or ypT3N0M0 rectal cancer may not benefit from ACT (**Figures 2B, C**). Similarly, multivariate Cox analysis also revealed that ACT was not associated with improved CSS in patients with ypTis-2N0M0 or ypT3N0M0 rectal cancer (ypTis-2N0M0: HR = 1.012, 95% CI = 0.638–1.606, P = 0.958; ypT3N0M0: HR = 0.906, 95% CI = 0.632–1.298, P = 0.590).

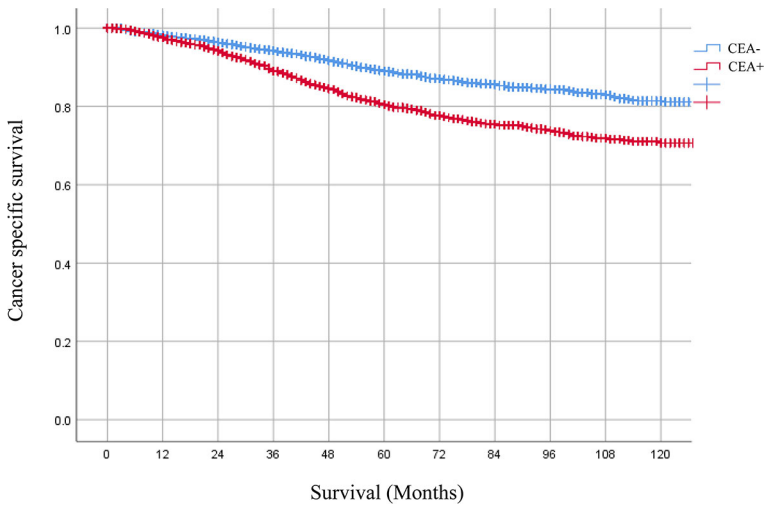

FIGURE 1 | Kaplan–Meier CSS curves of patients with elevated or normal pretreatment serum. CEA levels were 90.1 and 83.5%, respectively. HR = 1.597, 95% CI = 1.385–1.841, P < 0.001.

TABLE 1 | Multivariate Cox regression analyses of CSS in ypTis-3N0M0 rectal cancer patients with pretreatment serum CEA level.

Covariate	Reference	Characteristic	Cancer-specific survival		
			HR(95%CI)	SE	P value
Age (year)	≤60	>60	1.323 (1.145–1.527)	0.073	<0.001*
Race	White	Black	1.214 (0.964–1.528)	0.117	0.099
		Other	0.785 (0.605–1.020)	0.133	0.070
Marital status	Unmarried	Married	0.727 (0.627–0.843)	0.075	<0.001*
		Unknown	0.589 (0.345–1.007)	0.273	0.053
Gender	Male	Female	0.814 (0.699–0.947)	0.078	0.008*
Grade	G1 + G2	G + G4	1.565 (1.341–1.828)	0.079	<0.001*
		Unknown	0.787 (0.657–0.943)	0.092	0.009*
Histology	Adenocarcinoma	Mucinous adenocarcinoma	1.362 (1.034–1.794)	0.140	0.028*
		Signet ring cell carcinoma	6.202 (3.519–10.928)	0.289	<0.001*
		Other	1.114 (0.902–1.376)	0.108	0.314
Tumor size	<5.0 cm	≥5.0 cm	1.161 (0.979–1.377)	0.087	0.087
		Unknown	1.169 (1.983–1.390)	0.088	0.078
CEA level	Normal	Elevated	1.597 (1.385–1.841)	0.073	<0.001*

**P < 0.05 was considered significant.*

TABLE 2 | Multivariate Cox regression analyses of the role of pretreatment serum CEA level on CSS in patients with different ypT stage.

ypT stage	Reference	Characteristic	Cancer-specific survival		
			HR(95%CI)	SE	P value
ypTis-1	Normal	Elevated	1.891 (1.286–2.781)	0.197	0.001*
ypT2			1.465 (1.008–2.129)	0.191	0.045*
ypT3			1.570 (1.325–1.861)	0.087	<0.001*

**P < 0.05 was considered significant.*

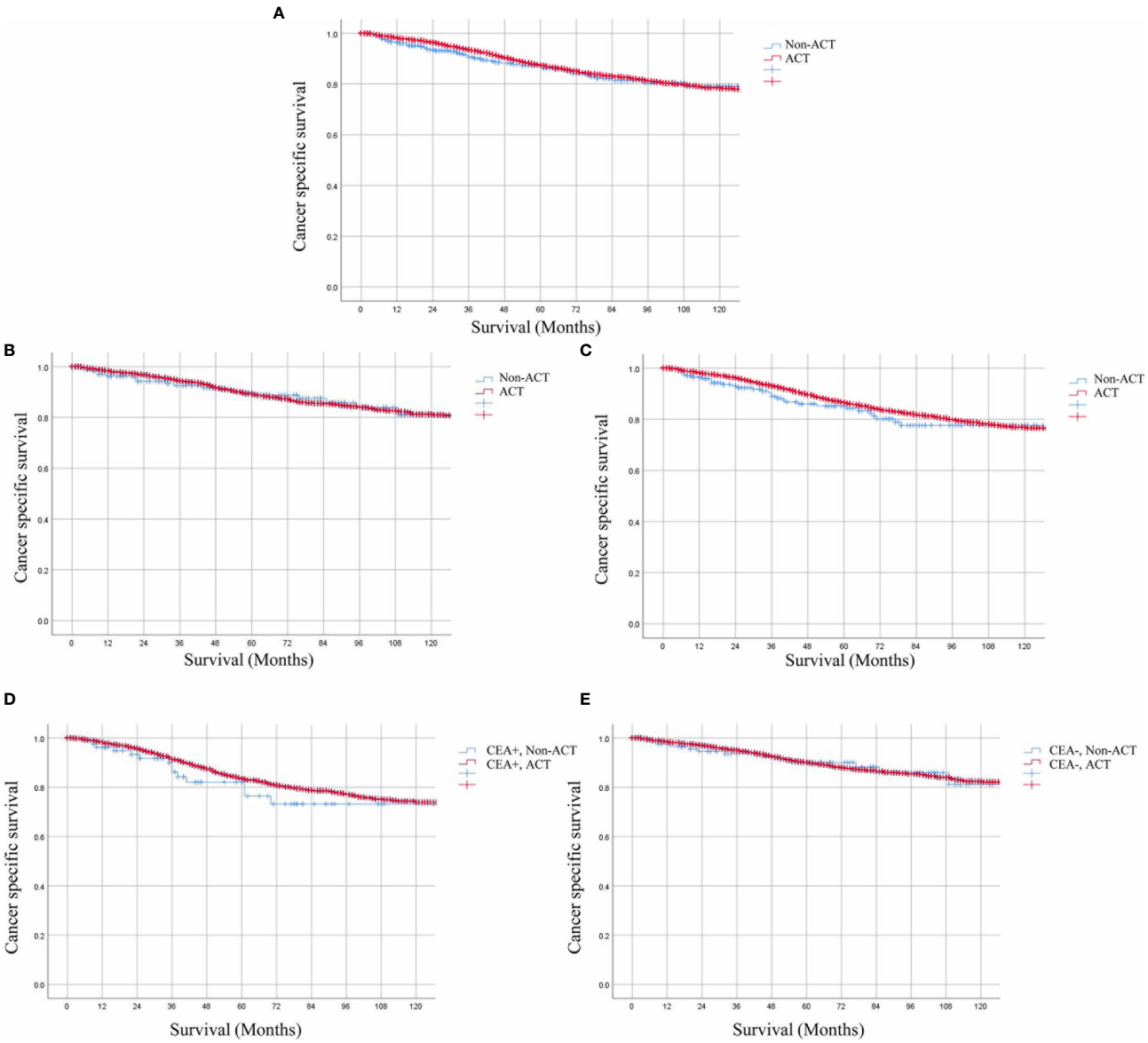

FIGURE 2 | Kaplan–Meier CSS curves of patients receiving or not receiving ACT. **(A)** The 5-year CSS estimates were 86.3 and 87.4% for ypTis-3N0M0 rectal cancer patients with ACT and without ACT. **(B, C)** Patients with ypTis-2N0M0 (HR = 1.012, 95% CI = 0.638–1.606, P = 0.958.) or ypT3N0M0 (HR = 0.906, 95% CI = 0.632–1.298, P = 0.590). Rectal cancer may not benefit from ACT. **(D, E)**. Kaplan–Meier analysis demonstrated that the 5-year CSS rate of ypTis-3N0M0 rectal cancer patients receiving ACT and those without receiving ACT were 76.4 and 83.5% (HR = 0.830, 95% CI = 0.484–1.422, P = 0.497), respectively in the setting of elevated pretreatment serum CEA levels; 90.0 and 90.1% (HR = 0.966, 95% CI = 0.554–1.684, P = 0.904), respectively, in the setting of normal pretreatment serum CEA levels.

Evaluating Associations of the Pretreatment Serum CEA Levels and ACT on the Basis of CSS

For ypTis-3N0M0 rectal cancer patients, Kaplan–Meier analysis demonstrated that patients receiving ACT had comparable 5-year CSS rate as compared to those not receiving ACT in the setting of elevated pretreatment serum CEA levels (76.4 *vs* 83.5%, P = 0.305) (**Figure 2D**). In the setting of normal pretreatment serum CEA levels, 5-year CSS rate of patients receiving ACT was similar to those not receiving ACT (90.0 *vs*. 90.1%, P = 0.943) (**Figure 2E**). Multivariate Cox analysis also revealed that ACT was not associated with improved CSS regardless of pretreatment serum CEA levels in ypTis-3N0M0 rectal cancer patients (elevated-CEA group: HR = 0.830, 95% CI = 0.484–1.422, P = 0.497; CEA normal group: HR = 0.966, 95% CI = 0.554–1.684, P = 0.904). Intriguingly, ypT3N0M0 rectal cancer patients with elevated pretreatment serum CEA levels may benefit from ACT (5-year CSS: 69.1 *vs*. 82.9%, P = 0.045) (**Figure 3A**), while ypT3N0M0 rectal cancer patients with normal pretreatment serum CEA levels did not benefit from ACT (5-year CSS: 89.3 *vs*. 89.3%, P = 0.885) (**Figure 3B**). Multivariate Cox analysis demonstrated that ACT tended to be a protective factor in ypT3N0M0 rectal cancer patients with elevated pretreatment serum CEA levels (HR = 0.633, 95% CI = 0.344–1.164, P = 0.141), while ACT was not associated with improved CSS in ypT3N0M0 rectal cancer patients with normal pretreatment serum CEA levels (HR = 1.035, 95% CI = 0.487–2.202, P = 0.928).

CSS of ACT in ypT3N0M0 Rectal Cancer Patients With Elevated Serum CEA Levels After PSM

After PSM, 147 ypT3N0M0 rectal cancer patients with elevated serum CEA levels were involved. 98 patients received ACT and 49 patients did not receive ACT; no characteristics showed statistical differences between the two groups. However, Kaplan–Meier analysis revealed that patients receiving ACT had comparable 5-year CSS rate as compared to those without receiving ACT (69.1 *vs*. 77.4%, P = 0.216) (**Figure 4**).

DISCUSSION

Rectal cancer is a malignant tumor that originates in the epithelium of the rectal mucosa. It is asymptomatic in the early stage and has stool characteristics and changes in bowel habits in the late stage. According to the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) eighth edition colorectal cancer TNM staging system, rectal cancer can be divided into stages 0–IV according to the severity. The treatment of rectal cancer is a comprehensive treatment based on surgery, including chemotherapy and radiotherapy. Surgical methods include classic Miles surgery, Dixon surgery, *etc.*, which specifically refer to NCCN rectal cancer treatment guidelines (11).

Before neoadjuvant radiotherapy had been adopted as a routine clinical practice in locally advanced middle and low rectal cancer, several studies demonstrated that ACT could improve the prognosis of patients with Dukes' B and Dukes' C stages (12). A systematic review including 21 eligible randomized controlled trials (RCTs) showed a reduction in the risk of mortality (17%) and disease recurrence (25%) of ACT in rectal cancer (13). However, two limitations need attention. The patients who received neoadjuvant radiotherapy were enrolled in only two RCTs. No modern drugs, such as oxaliplatin, were included in the ACT. The adoption of ACT largely depended on pathological TNM stage. Neoadjuvant radiotherapy resulted in tumor down-sizing and down-staging; some patients (ypTis-3N0M0) no longer needed ACT according to previous criteria. However, clinicians would prefer to adopt ACT in clinical practice despite the lack of high-level evidence.

The EORTC 22921 study randomly assigned patients with clinical stage T3 or T4 resectable rectal cancer to receive

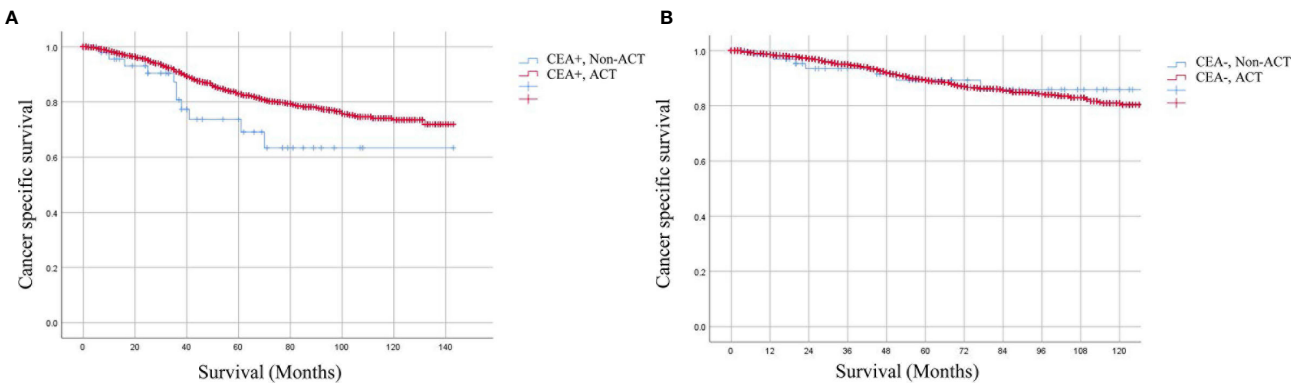

FIGURE 3 | Kaplan–Meier CSS curves stratified by the combination of pretreatment serum CEA levels and receiving ACT in different stages. **(A)** The 5-year CSS rates of ypT3N0M0 rectal cancer patients with elevated pretreatment serum CEA levels were 69.1 and 82.9% (HR = 0.633, 95% CI = 0.344–1.164, P = 0.141), respectively, **(B)** while ypT3N0M0 rectal cancer patients with normal pretreatment serum CEA levels were 89.3 and 89.3% (HR = 1.035, 95% CI = 0.487–2.202, P = 0.928), respectively.

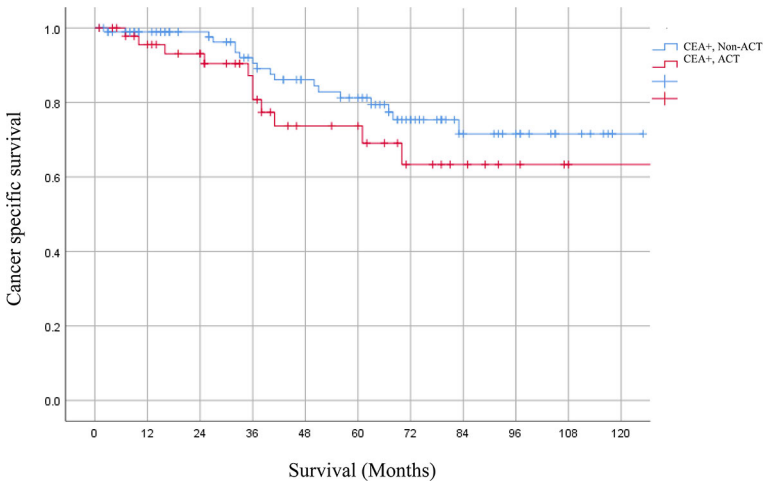

FIGURE 4 | The 5-year CSS rates of ypT3N0M0 rectal cancer patients with elevated serum CEA levels receiving or not receiving ACT after PSM were 69.1 and 77.4% (P = 0.216), respectively.

preoperative radiotherapy with or without concomitant chemotherapy before surgery followed by either ACT or surveillance. With regret, ACT after preoperative radiotherapy was not associated with improved DFS or OS after a median follow-up of 10.4 years (14). Similarly, another three trials did not classify the value of ACT (15–17). Based on the four trials, a systematic review and meta-analysis yielded the same results (18). However, the limitations of the above trials are obvious. The major problem was poor adherence to ACT. The value of ACT may be partially impaired. Evidence from ADORE trial indicated that adjuvant FOLFOX was associated with improved DFS compared with fluorouracil plus leucovorin in patients with locally advanced rectal cancer after neoadjuvant chemoradiotherapy and surgery (19). At present, there are still disputes about the value of ACT for locally advanced rectal cancer patients who received neoadjuvant CRT and surgery.

Serum CEA is the most important tumor marker for the presence of subclinical hepatic or pulmonary metastases, and elevated pretreatment serum CEA levels were significantly associated with poor prognosis in rectal cancer patients (20, 21). Besides, serum CEA levels could predict PCR after neoadjuvant therapy for rectal cancer (22). The American Joint Committee on Cancer (AJCC) had suggested that serum CEA levels serve as an additional factor for clinical care. Combination of carcinoembryonic antigen with the AJCC TNM staging system could improve prognostic precision for rectal cancer (23). Recently, several studies have reported the use of serum CEA levels to guide ACT in stage II colon cancer patients (10, 24, 25). However, another study found that stage IIA colon cancer patients with elevated pretreatment serum CEA levels did not show survival benefit from ACT. Can elevated pretreatment serum carcinoembryonic antigen levels guide ACT in rectal cancer patients with ypTis-3N0 after neoadjuvant radiotherapy and surgery? No previous studies explored the predictive value of pretreatment serum CEA levels to adaptation of ACT in rectal cancer patients after neoadjuvant radiotherapy and surgery. For patients with yp T4 or yp stage III, adoption of ACT is well-accepted, while the value of ACT in patients with ypTis-3N0 is full of controversy.

In the present study, we first found that pretreatment serum CEA levels were an independent prognostic factor in ypTis-3N0M0 rectal cancer. Intriguingly, multivariate Cox analyses showed that pretreatment serum CEA elevation in stage ypTis-1N0M0 group presented the most remarkable increased risk of CSS compared with stage ypT2N0M0 or ypT3N0M0 group. Early stage rectal cancer with elevated serum CEA levels presented with more aggressive behavior and unexpected poor prognosis. This subgroup needed more intensive follow-up and intervention.

To the best of our knowledge, this is the first study to investigate the value of pretreatment serum CEA levels for guiding ACT in rectal cancer patients with ypTis-3N0 after neoadjuvant radiotherapy and surgery. To evaluate the value of ACT, multivariate Cox analysis demonstrated that patients with ypTis-3N0M0 rectal cancer did not benefit from ACT. Further, we evaluated associations of the pretreatment serum CEA levels and ACT on the basis of CSS. Similarly, ACT was not associated with improved CSS regardless of pretreatment serum CEA levels in ypTis-3N0M0 rectal cancer patients. However, ypT3N0M0 rectal cancer patients with elevated pretreatment serum CEA levels who received ACT had superior 5-year CSS than those who did not receive ACT, while ypT3N0M0 rectal cancer patients with normal pretreatment serum CEA levels did not benefit from ACT. Although multivariate Cox analysis did not confirm the value of ACT in ypT3N0M0 rectal cancer patients with elevated pretreatment serum CEA levels, a trend toward a protective factor of ACT was observed. A relatively small sample size may result in insufficient power in our study. Especially, a large cohort is needed to verify the value of pretreatment serum CEA levels for guiding ACT in rectal cancer patients with ypT3N0M0. The results will have a profound effect on clinical practice.

Several limitations are inevitable in our present study. First, the lack of serum CEA levels after neoadjuvant CRT made it impossible to compare with pretreatment serum CEA levels, resulting in insufficient evaluation of the value of serum CEA levels. Second, the SEER database did not include other important prognostic factors, such as the regime and course of chemotherapy, and the adherence to ACT. Third, clinical staging, which is indispensable for selection of neoadjuvant CRT, was unavailable.

In conclusion, elevated pretreatment serum carcinoembryonic antigen levels may serve as a promising biomarker guiding ACT in rectal cancer patients with stage ypT3N0M0. Further study with larger sample size is needed to verify our results.

DATA AVAILABILITY STATEMENT

The datasets are available in the SEER repository and can be obtained from https://seer.cancer.gov.

AUTHOR CONTRIBUTIONS

XH and XM conceived this study. CH and MJ improved the study design and contributed to the interpretation of results. YL collected the data. CT performed data processing and statistical analysis. XH and XM wrote the manuscript. CH and MJ revised the manuscript. All authors contributed to the article and approved the submitted version.

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Clinicopathological Features of Stage I–III Colorectal Cancer Recurrence Over 5 Years After Radical Surgery Without Receiving Neoadjuvant Therapy: Evidence From a Large Sample Study

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Late recurrence (5 or more years) after radical resection of colorectal cancer (CRC) is rare. This study aims to investigate the features of late recurrence in stage I–III CRC. A total of 9,754 stage I–III patients with CRC who underwent radical surgery without receiving neoadjuvant therapy, at the Fudan University Shanghai Cancer Center (FUSCC), were enrolled in this study. These patients were divided into three groups: early recurrence (3 months−2 years), intermediate recurrence (2–5 years), and late recurrence (over 5 years). The median duration of follow-up was 53.5 ± 30.1 months. A total of 2,341 (24.0%) patients developed recurrence. The late recurrence rate was 11.7%. Patients with a higher risk of late recurrence were more likely to be older, to be at the T4 stage, to have a higher degree of colon cancer, to have a lower frequency of signet ring cell carcinoma, to have fewer poorly differentiated tumors, to be at the early stage of CRC, along with less perineural and vascular invasions. Multivariate logistic regression analysis identified age, differentiation, T stage, N stage, perineural, and vascular invasions as independent factors for late recurrence. Late recurrent CRC has some distinctive characteristics. Although recurrence over 5 years after surgery is infrequent, an enhanced follow-up is still needed for the selected patients after 5 years.

Keywords: late recurrence, colorectal cancer, radical surgery, early recurrence, clinicopathological features

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy and the second most common cause of cancer-related mortality worldwide (1). For resectable non-metastatic CRC, surgery with bowel resection and removal of the regional lymph nodes is preferred. Adjuvant therapy is administrated according to the postoperative pathological stage. Posttreatment surveillance is regularly performed to identify a recurrence that is potentially resectable for the cure. Although receiving standard treatment, about 25–40% of patients still suffer tumor recurrence during follow-up due to high spatiotemporal heterogeneity (2–4).

The risk of relapse largely depends on the tumor, node, metastases (TNM) stage, and several other important clinicopathological factors (5). Previous evidence indicated that 80% of recurrences occurred in the first 3 years and 95% of them occurred in the first 5 years after curative surgery (6–8). In general, early recurrence was defined as recurrence within 2 years of surgery. Early recurrence is majorly ascribed to adverse clinicopathological characteristics and resistance to adjuvant chemotherapy. Most surveillances compromise over 5 years after curative surgery. However, some relapses were detected after 5 years. It is necessary to identify the characteristics of recurrent CRC that occurred >5 years and to implement enhanced follow-up programs.

MATERIALS AND METHODS

Study Population

During the months between January 2008 and May 2018, a total of 13,765 patients with CRC were identified from the Fudan University Shanghai Cancer Center (FUSCC) database. In this study, the inclusion criteria were as follows: (1) patients had stage I–III diseases, patients in the T stage or the undetermined TNM stage were excluded; (2) patients had undergone curative surgery; (3) patients did not undergo neoadjuvant therapy; (4) the histology presented with adenocarcinoma, mucinous adenocarcinoma, or signet ring cell carcinoma; (5) survival information was available; and (6) the disease-free, survival period was longer than 3 months. A total of 4,011 patients were excluded due to their unknown pathological stage, receiving salvage surgery for local excision without the evidence of tumor cells, receiving neoadjuvant therapy, or without active follow-up. The following clinicopathological characteristics were extracted from the FUSCC database: age at diagnosis, gender, tumor location, histologic type, histological differentiation, T stage, N stage, pathological stage (AJCC 8th Edition), perineural invasion, vascular invasion, and survival information. This study was approved by the Ethics Committee and Institutional Review Board of the FUSCC and written informed consent was obtained from all the patients.

Treatment and Follow-up

All patients underwent standard curative surgery in accordance with the clinical guidelines. Total mesorectal excision or complete mesocolic excision was performed. Adjuvant therapy was adopted in selected patients with stage II and in all stage III patients who were capable of tolerating the treatment. In general, patients with low-risk stage II disease can be considered for adjuvant therapy with capecitabine alone or with observation, while patients with high-risk stage II and stage III disease can be considered for adjuvant chemotherapy with CapeOX (oxaliplatin and capecitabine). Recurrence includes local recurrence and distant metastasis. Clinical or radiological detection was accepted and histopathological confirmation was not mandatory. The diagnosis should be evaluated by the multidisciplinary team. Review of the medical records, follow-ups *via* telephone, and data linkage of the death registry were employed for collecting the survival data. The last follow-up date was November 30, 2019.

TABLE 1 | Clinicopathological features of stage I–III colorectal cancer (CRC) recurrence according to postoperative time.

Variables	Recurrence		
	<2 years ($N = 1187$)	2–5 years ($N = 849$)	>5 years ($N = 274$)
Age			
≤60	544 (45.8%)	326 (38.4%)	83 (30.6%)
>60	643 (54.2%)	523 (61.6%)	188 (69.4%)
Gender			
Male	715 (60.2%)	514 (60.7%)	164 (59.9%)
Female	472 (39.8%)	334 (39.3%)	110 (40.1%)
Location			
Colon cancer	629 (53.0%)	390 (45.9%)	133 (48.5%)
Rectal cancer	558 (47.0%)	459 (54.1%)	141 (51.5%)
Histologic type			
Adenocarcinoma	983 (82.8%)	721 (84.9%)	230 (83.9%)
Mucinous	149 (12.6%)	112 (13.2%)	42 (15.3%)
Signet ring cell	55 (4.6%)	16 (1.9%)	2 (0.7%)
Differentiation			
Poor	411 (34.6%)	200 (23.6%)	47 (17.2%)
Moderate	730 (61.5%)	617 (72.7%)	210 (76.6%)
Well	6 (0.5%)	13 (1.5%)	5 (1.8%)
Unknown	40 (3.4%)	19 (2.2%)	12 (4.4%)
T stage			
T1	24 (2.0%)	27 (3.2%)	12 (4.4%)
T2	113 (9.5%)	143 (16.8%)	57 (20.8%)
T3	379 (31.9%)	143 (16.8%)	3 (1.1%)
T4	671 (56.5)	536 (63.1%)	202 (73.7%)
N stage			
N0	368 (31.0%)	368 (43.3%)	154 (56.2%)
N1	395 (33.3%)	297 (35.0%)	87 (31.8%)
N2	424 (35.7%)	184 (21.7%)	33 (12.0%)
TNM stage			
I	90 (7.6%)	125 (14.7%)	52 (19.0%)
II	278 (23.4%)	243 (28.6%)	102 (37.2%)
III	819 (69.0%)	481 (56.7%)	120 (43.8%)
Perineural invasion			
Negative	723 (60.9%)	620 (73.0%)	239 (87.1%)
Positive	464 (39.1%)	229 (27.0%)	35 (12.8%)
Vascular invasion			
Negative	659 (55.5%)	600 (70.7%)	227 (82.8%)
Positive	528 (44.5%)	249 (29.3%)	47 (17.2%)

Statistical Analysis

The patients were divided into three periods of recurrences, namely early recurrence (3 months−2 years), intermediate recurrence (2–5 years), and late recurrence (over 5 years). The categorical variables were analyzed by the chi-squared test. Univariate and multivariate ordinal logistic regression models or multinomial logistic regression models were used to evaluate the potential factors associated with the recurrence time. The patients were also divided into two groups (<2 years, >2 years or <5 years, > 5 years), and univariate and multivariate binary logistic

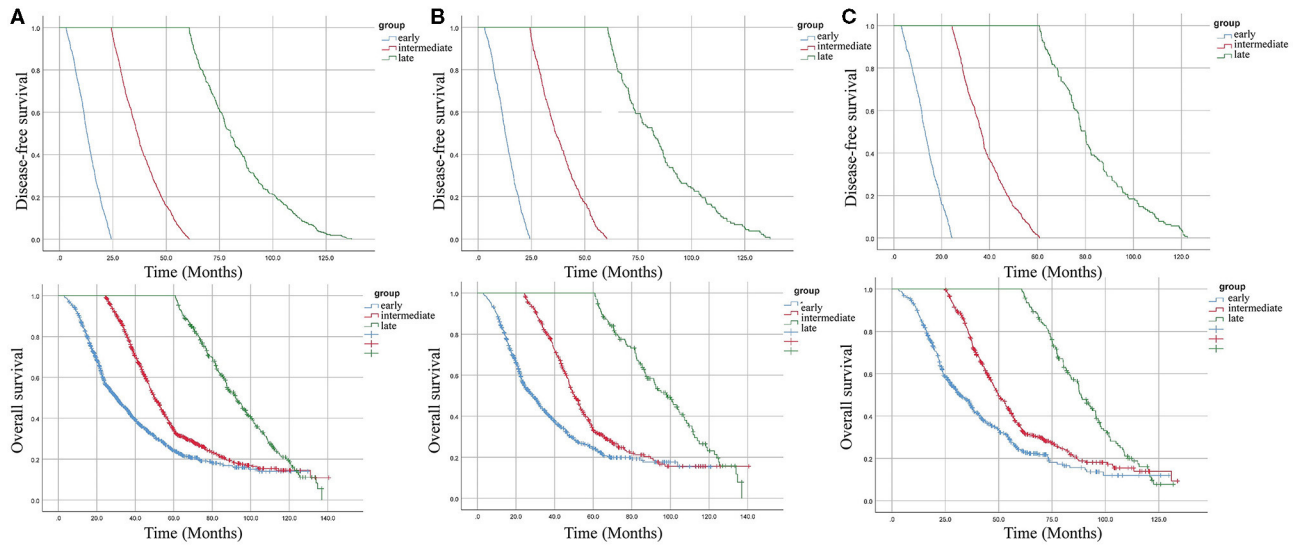

FIGURE 1 | Kaplan–Meier curves for disease-free survival (DFS) and overall survival (OS) according to the time taken for recurrence. **(A)** overall cohort. **(B)** patients with colon cancer. **(C)** patients with rectal cancer.

regression models were adopted to identify the potential factors associated with the recurrence time. The Kaplan–Meier method was utilized to plot the survival curves, and the survival difference was determined using the log-rank test. All statistical analyses were performed with SPSS 25.0.

RESULTS

Characteristics and Survival in Early Recurrence, Intermediate Recurrence, and Late Recurrence

A total of 9,754 eligible patients were identified in the study. The median duration of follow-up was 53.5 ± 30.1 months. During the surveillance, 2,341 patients experienced recurrence. These patients were divided into three groups: early recurrence (3 months−2 years, $N = 1{,}187$), intermediate recurrence (2–5 years, $N = 849$), and late recurrence (over 5 years, $N = 274$), after initial surgery. In this study, the overall recurrence rate after curative surgery was 24.0% (2341/9754) in stage I–III CRC without neoadjuvant therapy. The early recurrence rate was 50.7% (1187/2341) and the late recurrence rate was 11.7% (274/2341). The clinicopathological features in the different groups are shown in **Table 1**. Clinicopathological features of different time intervals to recurrence were shown in **Supplementary Tables 1**, **2** according to tumor location. Kaplan–Meier curves were plotted based on three groups (**Figure 1**). As expected, a significantly increased 5-year overall survival (OS) rate was observed with prolonged recurrence time (colon cancer: 24.4, 33.2, 100%, $p < 0.001$; rectal cancer: 23.8, 34.8, 100%, $p < 0.001$; overall cohort: 24.2, 34.3, 100%, $p < 0.001$).

Recurrence Pattern of Patients With Late Recurrence

Most patients in the late recurrence group were over 60 years of age. Colon cancer, non-signet ring cell carcinoma, tumors with a well-differentiated histological type, lack of lymph node metastasis, stage I disease, and no evidence of perineural and vascular invasions were the more frequently demonstrated characteristics in the late recurrence group. Compared with patients with T4, patients with T2 had a higher risk of developing late recurrence, while patients with T3 had a lower risk. There was no difference between the three groups in terms of gender (**Table 2**). Multivariate logistic regression analysis identified age, differentiation, T stage, N stage, perineural and vascular invasions as independent factors for late recurrence (**Table 3**).

DISCUSSION

Local recurrence and distant metastasis after curative surgery in patients with CRC remain a major concern and are associated with dismal prognosis (9). Regular posttreatment surveillance of patients with CRC is conducive to identify a recurrence. Patients will benefit more from early detection and management of disease recurrence. For the heterogeneity of CRC, the relapse varies significantly with comparable clinicopathological features. Great efforts have been made to explore novel strategies which could predict early relapse by integrating clinicopathological characteristics and multigene expression patterns (10, 11). As more than 70% recurrences occurred within 2 years and over 90% occurred within 5 years after curative surgery, the frequency of follow-up gradually decreases after 5 years of curative resection. However, some patients with CRC still developed relapse after 5 years of curative surgery. It questions whether a follow-up

TABLE 2 | Univariate ordinal logistic regression or multinomial logistic regression analysis of the factors associated with recurrences <2 years, 2–5 years, and >5 years after radical surgery (<2 years as a reference).

Variables	Crude OR (95%CI)	*P* value
Age		
≤60	0.664 (0.566–0.781)	<0.001
>60	Reference	
Gender		
Male	1.003 (0.855–1.178)	0.967
Female	Reference	
Location		
Colon cancer	1.246 (1.065–1.458)	0.006
Rectal cancer	Reference	
Histologic type		
Adenocarcinoma	3.015 (1.758–5.170)	<0.001
Mucinous	3.297 (1.857–5.854)	<0.001
Signet ring cell	Reference	
Differentiation		
Poor	0.243 (0.113–0.521)	<0.001
Moderate	0.462 (0.218–0.981)	0.044
Well	Reference	
T stage		
2–5 years		
T1	1.408 (0.803–2.469)	0.232
T2	1.584 (1.207–2.079)	0.001
T3	0.472 (0.378–0.591)	<0.001
T4	Reference	
>5 years		
T1	1.661 (0.816–3.380)	0.162
T2	1.676 (1.175–2.390)	0.004
T3	0.026 (0.008–0.083)	<0.001
T4	Reference	
N stage		
N0	2.904 (2.365–3.566)	<0.001
N1	1.923 (1.557–2.375)	<0.001
N2	Reference	
TNM stage		
I	2.612 (2.038–3.349)	<0.001
II	1.777 (1.483–2.130)	<0.001
III	Reference	
Perineural invasion		
<2 years		
Negative	1.738 (1.435–2.104)	<0.001
Positive	Reference	
2–5 years		
Negative	4.382 (3.017–6.366)	<0.001
Positive	Reference	
Vascular invasion		
Negative	2.321 (1.955–2.754)	<0.001
Positive	Reference	

program should last beyond 5 years to improve prognosis by identifying recurrences and metastases early when they are at a curable stage. Previous studies reported that recurrence rates

TABLE 3 | Multivariate multinomial logistic regression analysis of the factors associated with recurrences <2 years, 2–5 years, and >5 years after radical surgery (>5 years as a reference).

Variables	Adjusted OR (95%CI)	*P* value
Age		
<2 years		
≤60	1.804 (1.336–2.440)	<0.001
>60	Reference	
2–5 years		
≤60	1.354 (1.001–1.832)	0.049
>60	Reference	
Location		
<2 years		
Colon cancer	0.830 (0.621–1.108)	0.206
Rectal cancer	Reference	
2–5 years		
Colon cancer	1.084 (0.813–1.445)	0.582
Rectal cancer	Reference	
Histologic type		
<2 years		
Adenocarcinoma	0.323 (0.042–2.493)	0.279
Mucinous	0.221 (0.028–1.713)	0.148
Signet ring cell	Reference	
2–5 years		
Adenocarcinoma	0.374 (0.047–2.979)	0.353
Mucinous	0.347 (0.043–2.780)	0.319
Signet ring cell	Reference	
Differentiation		
<2 years		
Poor	4.737 (1.228–18.271)	0.024
Moderate	2.773 (0.753–10.211)	0.125
Well	Reference	
2–5 years		
Poor	1.411 (0.436–4.563)	0.566
Moderate	1.129 (0.369–3.456)	0.831
Well	Reference	
T stage		
<2 years		
T1	0.892 (0.337–2.356)	0.817
T2	0.692 (0.371–1.292)	0.248
T3	38.721 (12.237–122.527)	<0.001
T4	Reference	
2–5 years		
T1	0.777 (0.301–2.011)	0.604
T2	0.841 (0.454–1.557)	0.582
T3	17.864 (5.617–56.819)	<0.001
T4	Reference	
N stage		
<2 years		
N0	0.409 (0.250–0.667)	<0.001
N1	0.601 (0.380–0.951)	0.030
N2	Reference	

(Continued)

TABLE 3 | Continued

Variables	Adjusted OR (95%CI)	*P* value
2–5 years		
N0	0.617 (0.376–1.014)	0.057
N1	0.819 (0.513–1.308)	0.404
N2	Reference	
TNM stage		
<2 years		
I	1.765 (0.830–3.751)	0.140
II	/	/
III	Reference	
2–5 years		
I	1.674 (0.802–3.492)	0.170
II	/	/
III	Reference	
Perineural invasion		
<2 years		
Negative	0.456 (0.304–0.683)	<0.001
Positive	Reference	
2–5 years		
Negative	0.561 (0.372–0.844)	0.006
Positive	Reference	
Vascular invasion		
<2 years		
Negative	0.536 (0.362–0.794)	0.002
Positive	Reference	
2–5 years		
Negative	0.709 (0.476–1.056)	0.091
Positive	Reference	

were 1.2–11.6% after 5 years (12–14). No clinical guidelines are available for the effective detection of late recurrences. This study reported a higher rate of recurrence over 5 years (11.7%) than previous studies. The variations in the late recurrence rate in different studies may result from different inclusion criteria. Patients who received neoadjuvant therapy were excluded from this study. These patients were more likely to have an advanced stage of the disease and were likely to experience early relapse. Besides that, metastatic patients with CRC who are more likely to experience early relapse were not included in the study.

Age is a well-established prognostic factor in CRC (15, 16). (13) found that median ages of recurrence were higher in the late recurrence group than in the early and intermediate recurrence groups. In another study, there were no differences between the early and late recurrence groups in terms of age (8). In this study, older patients were associated with an increased risk of late recurrence. Elderly patients tend to have more indolent cells. It is hard to detect relapse in the early period because the lesions grow slowly.

Evidence from the Surveillance, Epidemiology, and End Results Program database indicated that features and survival between colon and rectal cancer were different (17). (8) found that a higher proportion of late recurrence was observed in rectal cancer as compared with colon cancer, although the difference was not statistically significant. The results were consistent with their study. For the histological grade, tumors that recurred after 5 years were more likely to be well-differentiated. It seems to be consistent with the findings of previous studies (6, 8). Indeed, patients with poorly differentiated tumors were associated with shorter disease-free survival (DFS). Patients in the late recurrence group were less likely to have signet ring cell carcinoma, which indicated a worse prognosis.

Pathological staging is the most important prognostic factor in CRC. As recurrence time is prolonged, the proportion of T3 and stage III patients are on a remarkable decline. Patients without lymph node involvement experienced more later recurrence than patients with lymph node involvement. Vascular and perineural invasions are prognostic markers of tumor aggressiveness and poor outcomes in CRC (18, 19). In patients with CRC with stage IIA disease, vascular and perineural invasions are robust indicators for implementing adjuvant chemotherapy. Fewer vascular and perineural invasions were observed in patients with recurrence after 5 years. Unfortunately, there is still no clear consensus on the mechanisms underlying such late recurrence. Evidence indicated that patients with early recurrence were more likely to have adenomatous polyposis coli mutations (20). More biomarkers are urgently needed to identify early and late relapse. In general, patients with well-differentiated pathological features are associated with an increased risk of late recurrence. Tumors with clear pathological features are more sensitive to adjuvant chemotherapy and tend to make slow progress after relapse.

The main strength of this study is that it provides large population-based evidence for the characteristics of late recurrence after radical surgery in stage I–III CRC. The results contribute to predicting patients with a high risk of late recurrence and provide personalized follow-up strategies for the selected patients. Several limitations should be addressed. The "MSI" status, the recurrence site of CRC, pretreatment CEA levels, and tumor size were not recorded in detail in the FUSCC database. The recurrence patterns (local and/or distant) were not included in the study. Additionally, data regarding the course of adjuvant chemotherapy and therapeutic regimen after recurrence were unavailable as many patients returned to the local hospital for further adjuvant treatment after surgery. Generally, patients with high-risk stage II and stage III disease received adjuvant chemotherapy with CapeOX for 6 months. In patients who had low rectal cancer with lymph node metastasis, adjuvant radiotherapy will be advised. Patients received chemotherapy with FOLFIRI after recurrence.

In conclusion, the late recurrence of CRC was associated with certain specific clinicopathological features. After 5 years of follow-up, an enhanced follow-up is still needed for the selected patients with a high risk of late recurrence.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee and Institutional Review

Board of the Fudan University Shanghai Cancer Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL and QL conceived this study. DL, YY, and QL improved the study design and contributed to the interpretation of results. YY and ZS collected the data. SC performed data processing and statistical analysis. DL and YY wrote the manuscript. ZS and QL revised the manuscript. All authors approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2021.666400/full#supplementary-material

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Single-Incision vs. Conventional Laparoscopic Surgery for Colorectal Cancer: An Update of a Systematic Review and Meta-Analysis

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Background: Although the advantages of single-incision laparoscopic surgery have been reported in several meta-analyses, the low quality of studies included in the meta-analyses limits the reliability of such a conclusion. In recent years, the number of randomized controlled trials on the efficacy of SILS in colorectal cancer has been on the rise. This update systematic review and meta-analysis of RCTs aims to compare efficacy and safety of SILS and CLS in the patients with colorectal cancer.

Methods: Relevant data was searched on the CNKI, Wanfang, VIP, Sinomed, PubMed, Embase, and Cochrane CENTRAL databases from inception until February 5th, 2021. All RCTs comparing SILS and CLS were included. The main outcomes were 30 days of mortality, postoperative complications, intraoperative complications, whereas secondary outcomes were the number of lymph nodes removed, duration of hospital stay, intraoperative blood loss, abdominal incision length, reoperation, readmission, conversion to laparotomy, operation time and anastomotic leakage.

Results: A total of 10 RCTs were included, involving 1,133 participants. The quality of the included studies was generally high. No significant difference was found between SILS and CLS in the 30 days mortality rate. The results showed that SILS group had a lower rate of postoperative complications (RR = 0.67, 95% CI: 0.49–0.92), higher rate of intraoperative complications (RR = 2.26, 95%CI: 1.00–5.10), shorter length of abdominal incision (MD = −2.01, 95% CI:−2.42–1.61) (cm), longer operation time (MD = 11.90, 95% CI: 5.37–18.43) (minutes), shorter hospital stay (MD = −1.12, 95% CI: −1.89–0.34) (days) compared with CLS group. However, intraoperative blood loss (MD = −8.23, 95% CI: −16.75–0.29) (mL), number of lymph nodes removed (MD = −0.17, 95% CI: −0.79–0.45), conversion to laparotomy (RR=1.31, 95% CI: 0.48–3.60), reoperation (RR = 1.00, 95% CI: 0.30–3.33) and readmission (RR =1.15, 95% CI: 0.12–10.83) and anastomotic leakage were not significantly different between the two groups.

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Conclusion: These results indicate that SILS did not has a comprehensive and obvious advantage over the CLS. Surgeons and patients should carefully weigh the pros and cons of the two surgical procedures. Further RCTs are needed to prove long-term outcomes of SILS in colorectal cancer.

Keywords: single-incision laparoscopic surgery, conventional laparoscopic surgery, colorectal cancer, randomized controlled trials, meta-analysis, systematic review

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world (1). Surgical resection is the only curative treatment for CRC (2, 3). In the past 60 years, general surgery has radically changed to minimally invasive surgery techniques to enhance the recovery rate (4). Since the first laparoscopic surgery was performed over 100 years ago by Jacobaeus (5), minimally invasive surgery has continued to play an important role as an alternative to traditional open surgery. Laparoscopic surgery demonstrates faster functional recovery rates, fewer postoperative complications, shorter length of the incision, and shorter hospital stay when compared with open surgery. Therefore, laparoscopic surgery is gaining acceptance as an alternative treatment option for colorectal cancer (6).

Recent innovations in surgical techniques such as robot-assisted laparoscopic surgery (RALS), single-incision laparoscopic surgery (SILS), and natural orifice transluminal endoscopic surgery (NOTES), etc, have greatly benefited patients with colorectal cancer (7–9). NOTES has gained significant attention because it offers the possibility of "scarless" surgery (10). However, the clinical application of NOTEs has been limited due to several unresolved problems such as limitations of surgical techniques and equipment, unregulated insufflations and narrow working angles, etc., (11). SILS is regarded as an alternative surgical technique for NOTES and the next major advance in minimally invasive surgical methods for colorectal cancer (12). In SILS, the surgeon operates through a single incision, and it is generally considered to have the following advantages, less postoperative pain, better cosmetic effect, less postoperative complications, less intraoperative blood loss, shorter hospital stay and shorter length of skin incision, etc., when compared with conventional laparoscopic surgery (CLS) (13, 14). However, SILS presents some new technical challenges compared with CLS (15, 16), such as (1) the limited number of working instruments which makes it difficult to achieve correct exposure and the necessary traction to tissues; (2) Limited external working space: multiple instruments and laparoscopies required for a procedure compete for the same space at the entry port, leading to external hand collisions and difficulty in internal manipulation of the instrument tip compared with CLS; (3) difficult to maintain pneumoperitoneum; (4) Requirement of training and adjustment. The skills required for SILS differ from those required for CLS, including laparoscopic surgeons' experienced, and skills. Besides, colorectal surgery magnifies all the challenges of SILS. Unlike laparoscopic cholecystectomy or appendectomy, which only involves surgery in one abdominal quadrant, single-incision laparoscopic colectomy requires operating in different abdominal quadrants. However, there is no clinical evidence confirming the feasibility and safety of SILS for colorectal cancer.

The number of studies on SILS for colorectal cancer has increased seven-fold between 2010 and 2021. Randomized controlled trials (RCTs) comparing single-incision vs. conventional laparoscopic surgery for colorectal cancer are reported. Consequently, this systematic review and meta-analysis aims to compare efficacy and safety of SILS and CLS in the patients with colorectal cancer. The study included only randomized controlled trials.

MATERIALS AND METHODS

Study Design

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (17) (PRISMA) and was registered in PROSPERO, Registration number: CRD42021232237.

Search Strategy

PubMed, Embase, Cochrane CENTRAL, CNKI, Sinomed, and Wan Fang databases were searched through February 5th, 2021 by two independent researchers. The Chinese search terms used were "jiechangai" "zhichangai" "jiezhichangai" "dachangai" "changzhongliu" "fuqiangjing" "dankong" "danqiekou". The English search terms used were "colon cancer" "colorectal cancer" "rectal cancer" "sigmoid cancer" "single-incision laparoscopic surgery" "conventional laparoscopic surgery" and "randomized controlled trials". Different search strategies were adapted for each database (**Table 1**). References of included studies were also examined to find relevant studies.

Inclusion and Exclusion Criteria

Inclusion criteria: (1) Patients were diagnosed with colon cancer or rectal cancer; (2) Patients not less than 18 years old; (3) UICC stage 0-III or Dukes stage A-C; (4) The intervention in the experimental group was single-incision laparoscopic surgery, and conventional laparoscopic surgery used in the control group; (5) Main outcomes: 30 days of mortality, postoperative complications, intraoperative complications; (6) Secondary outcomes: number of lymph nodes removed, hospital stay, intraoperative blood loss, abdominal incision length, reoperation, readmission, conversion to laparotomy and operation time; (7) If there were multiple reports that came from the same study, the latest report were included; (8) Randomized controlled

TABLE 1 | PubMed search strategy of single-incision vs. conventional laparoscopic surgery for colorectal cancer study.

Number	Search terms
#1	"colon neoplasm" [MeSH] OR "colon carcinoma" [Title/Abstract] OR "colon cancer" [Title/Abstract] OR "colon tumor" [Title/Abstract] OR "colonic neoplasm" [Title/Abstract] OR "colonic carcinoma" [Title/Abstract] OR "colonic cancer" [Title/Abstract] OR "colonic tumor" [Title/Abstract] OR "colorectal neoplasm" [Title/Abstract] OR "colorectal carcinoma" [Title/Abstract] OR "colorectal cancer" [Title/Abstract] OR "colorectal tumor" [Title/Abstract]
#2	"rectal neoplasm" [MeSH] OR "rectal carcinoma" [Title/Abstract] OR "rectal cancer" [Title/Abstract] OR "rectal tumor" [Title/Abstract]
#3	"sigmoid neoplasm" [MeSH] OR "sigmoid carcinoma" [Title/Abstract] OR "sigmoid cancer" [Title/Abstract] OR "sigmoid tumor" [Title/Abstract]
#4	"single-incision" [Title/Abstract] OR "single-site" [Title/Abstract] OR "single-port" [Title/Abstract]
#5	"laparoscopy" [Title/Abstract] OR "surgery" [Title/Abstract] OR "laparoscopic" [Title/Abstract] OR "laparoscopic surgery" [Title/Abstract]
#6	#1 OR #2 OR #3
#7	#4 AND #5 AND #6

trials; (9) Type of surgery is resection. Exclusion criteria**:** (1) Patients with other malignancies diagnosed within the past 5 years; (2) Pregnant or lactating patients; (3) American Society of Anesthesiologists (ASA) class>III; (4) Emergency cancer surgery due to perforation or obstruction; (5) No outcomes available in the studies.

Data Extraction and Management

A predefined data extraction table was used by two independent researchers to extract relevant information, including (publication year, author, title), demographic information (the number of participants in the treatment group and the control group, gender, average age, diagnosis method, inclusion and exclusion criteria), intervention feature information (explanation of the surgical procedure) and methodological elements (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias). Any disagreements on information extracted by the two researchers were resolved by a third researcher after discussion with the two researchers.

Assessment of Risk of Bias

A 7-point Jadad scale (18) was used to assess the quality of the identified studies and includes four assessment items: randomization (2 points), allocation concealment (2 points), blinding methods (2 points), and withdrawals (1 point). A score of 0 to 7 was assigned, and higher scores indicated higher quality. Any study scoring at least 4 was considered to have high methodology quality, and disagreements between the two researchers were resolved by a third researcher who would make the final decision.

Statistical Analysis

RevMan 5.3 and R 4.02 were used to perform all the statistical analyses. Similar populations, interventions, and outcomes were combined in the study. For dichotomous data and continuous variables, the inverse variance method and the Mantel-Haenszel method were used. Otherwise, relative risk (RR) or mean difference (MD) were used for both types of data to compare the treatment results with a 95% confidence interval (95% CI). We assessed heterogeneity by χ^2 test and I^2 statistics. $I^2 \geq 0.1$ and $P \leq 50\%$ indicated acceptable heterogeneity, using a fixed-effect model to analyze effect quantities. Conversely, $I^2 < 0.1$ or $P > 50\%$ suggests significant heterogeneity, using a random-effect model to analyze effect quantities. Sensitivity analysis was used to identify clinical heterogeneity, after excluding studies with obvious clinical heterogeneity, the fixed-effects model was used to calculate the combined effect or qualitative description. Subgroup analysis was performed according to the cancer type and previous history of major abdominal surgery in patients in both groups. The funnel plot and egger's test were used to test for publication bias of main outcomes.

Quality of Evidence Assessment

Use GRADE profiler 3.6 was used to evaluate the quality of the study results. The evaluation criteria included five aspects: risk bias, inconsistency, indirectness, imprecision, and publication bias. Finally, the quality of evidence was divided into four levels: high quality, medium quality, low quality, and very low quality.

RESULTS

Study Characteristics

A total of 2,356 entries were obtained by searching the Chinese and English databases and 1,629 entries were obtained after excluding duplicate entries. Further, 1,605 entries were excluded by reading the titles and abstracts. The full text of the remaining 24 articles was downloaded and 11 articles were included in this systematic review and meta-analysis after reading the entire article. Finally, ten RCTs with 1,133 participants were included in this study, 566 participants were enrolled in the SILS group and 567 participants were enrolled in the CLS group. All included RCTs were published between 2012 and 2020. **Figure 1** shows this study's literature searching and screening process and **Table 2** presents a summary of the included studies.

Quality Assessment of Included Studies

Only one single study (25) did not mention random sequence generation and allocation concealment, while nine studies (19–24, 26–28) indicated the use of random number tables or other random allocation schemes. The double blind method was not adopted in any of the studies. All studies indicated the reasons

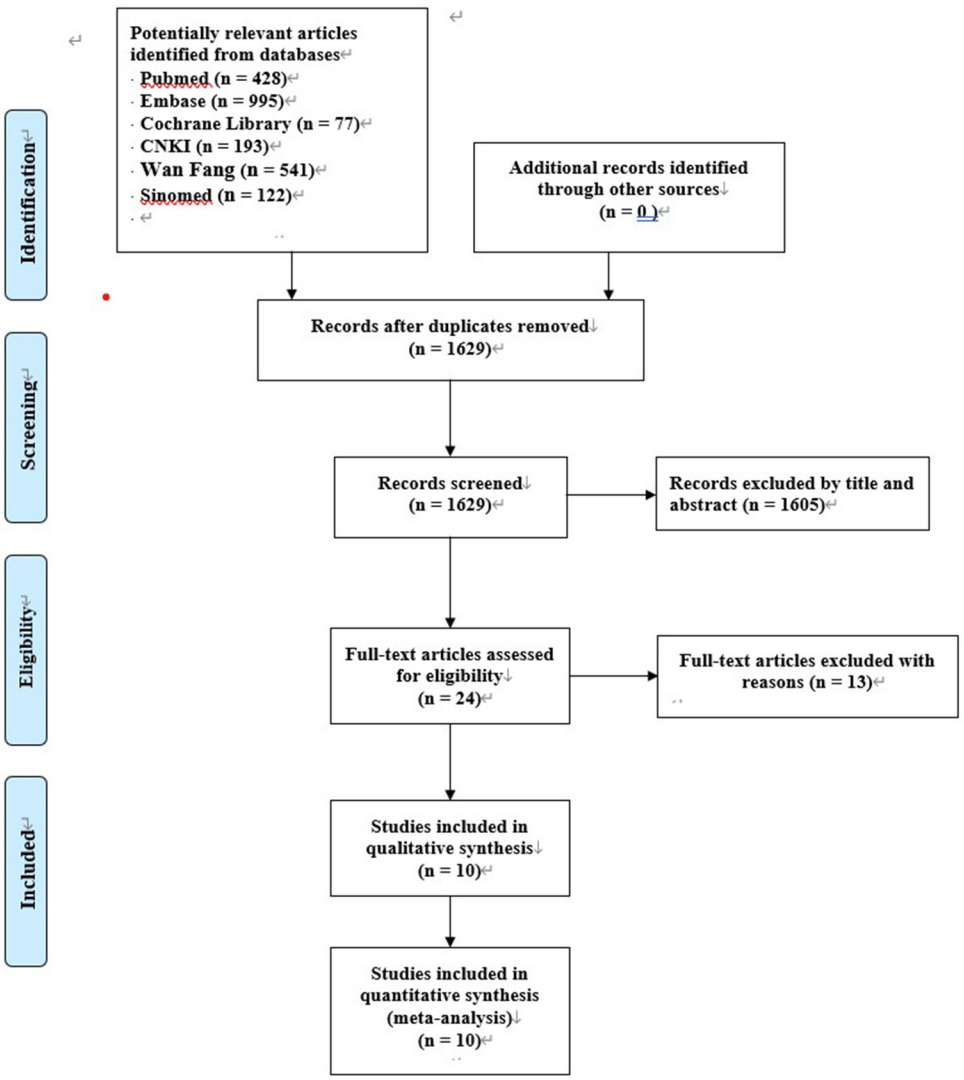

FIGURE 1 | PRISMA diagram of literature searching and screening process.

for and numbers of withdrawals. The quality of included studies was considered high (**Table 2**).

Meta-Analysis Results

Main Outcomes

The Thirty-Day Mortality

Three studies (21, 22, 26), with 294 patients showed no significant difference between SILS and CLS in 30-day mortality. No deaths were reported in two studies within 30 days (21, 26). Kang et al. (22) reported the death of 1 SILS patient within 30 days.

Postoperative Complications

A total of nine studies (20–28) with 1,035 patients reported a high rate of postoperative complications. Heterogeneity test: $P = 0.54$, $I^2 = 0$, showed no heterogeneity. Fixed-effect model was applied in the analyses. The SILS group showed lower rate of postoperative complications compared with the CLS group [RR = 0.67, 95% CI: 0.49–0.92, $P = 0.01$] (**Figure 2**).

Subgroup analysis: The patients were divided into three subgroups according to the cancer type. Colorectal cancer: The effects of three studies (25–27) including 406 patients were combined to perform meta-analysis. Heterogeneity test: $P = 0.38$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 0.61, 95% CI: 0.34–1.07, $P = 0.09$], showed no statistical significance (**Figure 2**) Colon cancer: The effects of four studies (21–24), including 653 patients were combined to perform meta-analysis. Heterogeneity test: $P = 0.58$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 0.80, 95% CI: 0.54–1.18, P = 0.26], showed no statistical significance (**Figure 2**) Rectal cancer: The effects of two studies (20, 28), including 126 patients were combined to perform

TABLE 2 | Main characteristics of the selected studies.

Reference	Country/Area	Year	Sample size		Age		Gender (male/female)		BMI		Tumor diameter(cm)		Disease	Outcome	Jadad score
			SILS	CLS	SILS	CLS	SILS	CLS	SILS	CLS	SILS	CLS			
Wu et al. (19)*	China	2020	49	49	61.89 ± 7.50	62.04 ± 7.2	29/20	31/18	23.11 ± 2.69	23.05 ± 2.81	3.95 ± 0.40	3.88 ± 0.49	Colorectal cancer	defghi	4
Bulut et al. (20)*	Denmark	2014	20	20	69 (50–86)	73 (50–84)	8/12	8/12	24 (16–32)	24 (19–29)	/	/	Rectal cancer	bgjk	4
Huscher et al. (21)*	Italy	2012	16	16	70 ± 11	70 ± 13	6/10	9/7	/	/	/	/	Colon cancer	abcfhi	5
Kang et al. (22)#	Korea	2016	31	31	63.2–11.4	63.2–11.4	19/12	16/15	24.0 ± 3.0	24.5 ± 3.0	5.3 ± 2.0	4.5 ± 2.9	Colon cancer	abcdfghik	5
Lee et al. (23)#	Korea	2020	179	180	63.4 (34–84)	62.6 (28–85)	97/82	99/81	24.3 (17.0–32.0)	24.3 (18.0–35.0)	3.7 (0–9.0)	3.5 (0–9.5)	Colon cancer	bcgk	4
Poon et al. (24)#	Hong Kong	2012	25	25	67 (37–83)	67 (57–81)	14/11	18/7	23.2 (16.9–28.8)	23.6 (16.5–28.2)	3.5 (1–7)	4 (1–7)	Colon cancer	b	4
Chen et al. (25)*	China	2017	43	43	54.39 ± 11.66	54.87 ± 10.98	27/16	25/18	22.01 ± 2.10	21.87 ± 2.02	3.31 ± 0.31	3.40 ± 0.45	Colorectal cancer	bdefhi	2
Watanabe et al. (26)*	Japan	2016	100	100	66.7	66.6	56/44	56/44	23.1	23.2	2.75	2.77	Colorectal cancer	abgj	4
Wang et al. (27)*	China	2018	60	60	55.24 ± 7.88	55.86 ± 7.28	32/28	36/24	26.02 ± 2.84	25.38 ± 2.64	3.62 ± 1.48	3.58 ± 1.65	Colorectal cancer	bdefhi	4
Xu (28)	China	2019	43	43	47.92 ± 5.58	47.89 ± 5.61	27/16	25/18	/	/	/	/	Rectal cancer	bdefhi	4

**: Major abdominal surgery history; #: No major abdominal surgery history; a: 30 days of mortality; b: Postoperative complications; c: Intraoperative complications; d: Length of abdominal incision; e: Intraoperative blood loss; f: Number of lymph nodes removed; g: Conversion to laparotomy; h: Operation time; i: Hospital stay; j: Reoperation; k: Readmission; SILS: single-incision laparoscopic surgery; CLS: conventional laparoscopic surgery.*

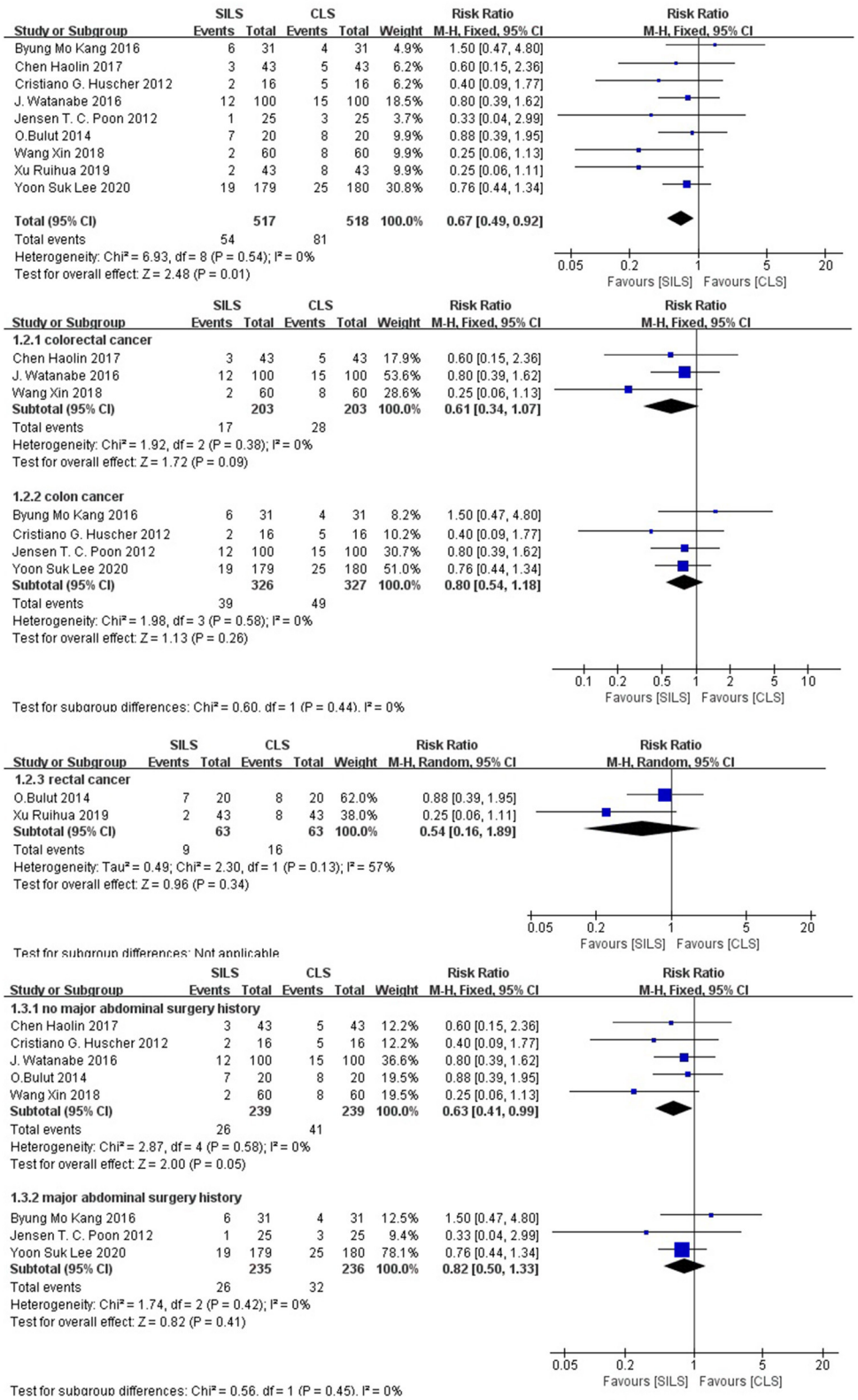

FIGURE 2 | Meta-analysis of postoperative complications. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

meta-analysis. Heterogeneity test: $P = 0.13$, $I^2 = 57\%$, showed substantial heterogeneity. Random-effect model was applied in the analyses. Meta-analysis result [RR = 0.54, 95% CI: 0.16–1.89, $P = 0.34$], showed no statistical significance (**Figure 2**).

The patients were divided into two subgroups according to their previous history of major abdominal surgery. No major abdominal surgery history was reported: The effects in five studies (20, 21, 25–27), including 478 patients were combined to perform the meta-analysis. Heterogeneity test: $P = 0.58$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis result [RR = 0.63, 95% CI: 0.41–0.99, $P = 0.05$] in the SILS group showed lower rates of postoperative complications than the CLS group. Major abdominal surgery history: The effects in three studies (22–24), including 471 patients were combined to perform the meta-analysis. Heterogeneity test: $P = 0.42$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis result [RR = 0.82, 95% CI: 0.50–1.33, $P = 0.41$] showed no statistical significance (**Figure 2**).

Intraoperative Complications

Three studies (21–23) with a total of 453 patients reported the rate of intraoperative complications. Heterogeneity test: $P = 0.68$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. The SILS group showed higher rate of intraoperative complications compared with the CLS group [RR = 2.26, 95% CI: 1.00–5.10, $P = 0.05$] (**Figure 3**).

Secondary Outcomes

Anastomotic Leakage

Anastomotic leakage may be a postoperative complication of interest to surgeons. In the included studies, the incidence of anastomotic leakage was low. We did not perform quantitative synthesis, but used a qualitative description of the outcome. Eight studies (20–26, 28),with 915 patients showed no significant difference between SILS and CLS in anastomotic leakage. We found that nine patients with anastomotic leakage were found in SILS group with 457 patients and 11 patients with anastomotic leakage were found in CLS group with 458 patients. No anastomotic leakages were reported in three studies (22, 24, 28). Bulut et al. (20) reported that there were four patients with anastomotic leakage in SILS group and CLS group separately. Huscher et al. (21) reported that no patient with anastomotic leakage was found in SILS group and one patient with anastomotic leakage was found in CLS group. Lee et al. (23) reported that two patients with anastomotic leakage were found in SILS group and one patient with anastomotic leakage was found in CLS group. Chen et al. (25) reported that there was one patient with anastomotic leakage in SILS group and CLS group separately. Watanabe et al. (26) reported that two patients with anastomotic leakage were found in SILS group and four patients with anastomotic leakage were found in CLS group.

Length of Abdominal Incision (cm)

Five studies (19, 22, 25, 27, 28) with a total of 452 patients reported the length of abdominal incision. Heterogeneity test: $P = 0.002$, $I^2 = 76\%$, showed high heterogeneity. The random-effect model was applied in the analyses. The SILS group showed shorter length of abdominal incision compared with the CLS group [MD = −2.01, 95% CI: −2.42 to −1.61, $P < 0.00001$] (**Figure 4**).

Intraoperative Blood Loss (mL)

Four studies (19, 25, 27, 28) with a total of 390 patients reported intraoperative blood loss. Heterogeneity test: $P < 0.00001$, $I^2 = 89\%$, showed high heterogeneity. The random-effect model was applied in the analyses. Meta-analysis result [MD = −8.23, 95% CI: −16.75–0.29, $P = 0.06$] showed no statistical significance (**Figure 5**).

Number of Lymph Nodes Removed

Six studies (19, 21, 22, 25, 27, 28) with a total of 484 patients reported the number of lymph nodes removed. Heterogeneity test: $P = 0.35$, $I^2 = 10\%$, showed low heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis result [MD = −0.17, 95%CI: −0.79–0.45, $P = 0.58$] showed no statistical significance (**Figure 6**).

Conversion to Laparotomy

Five studies (19, 20, 22, 23, 26) with a total of 759 patients reported the rate of conversion to laparotomy. Heterogeneity test: $P = 0.41$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis result [RR = 1.31, 95% CI: 0.48–3.60, $P = 0.60$] showed no statistical significance (**Figure 7**).

Subgroup analysis: The patients were divided into two subgroups according to the cancer type. Colorectal cancer: Two studies (19, 26) with a total of 298 patients reported the rate of conversion to laparotomy. Heterogeneity test: $P = 1.00$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 0.5, 95% CI: 0.09–2.68, $P = 0.42$] showed no statistical significance. Colon cancer: Two studies (22, 23) with a total of 421 patients reported the operation time. Heterogeneity test: $P = 0.87$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 6.02, 95% CI: 0.74–49.24, $P = 0.09$] showed no statistical significance (**Figure 7**).

The patients were divided into two subgroups according to their previous history of major abdominal surgery. No major abdominal surgery history: The effects of three studies (19, 20, 26), with a total of 338 patients were combined to perform the meta-analysis. Heterogeneity test: $P = 0.98$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 0.45, 95% CI: 0.10–1.99, $P = 0.30$], showed no statistical significance. Major abdominal surgery history: The effects of two studies (22, 23), with a total of 421 patients were combined to perform the meta-analysis. Heterogeneity test: $P = 0.87$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses, showed no statistical significance (**Figure 7**).

Operation Time (Minutes)

Six studies (19, 21, 22, 25, 27, 28) with a total of 484 patients reported the operation time. Heterogeneity test: $P = 0.93$, $I^2 = 0$,

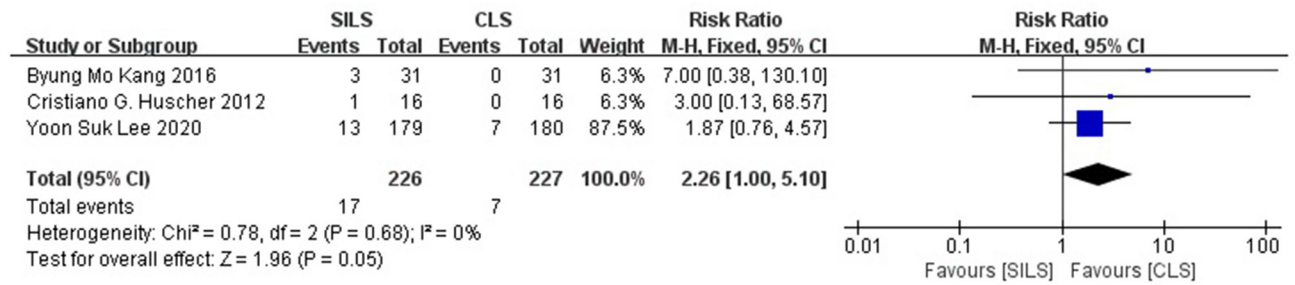

FIGURE 3 | Meta-analysis of intraoperative complications. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

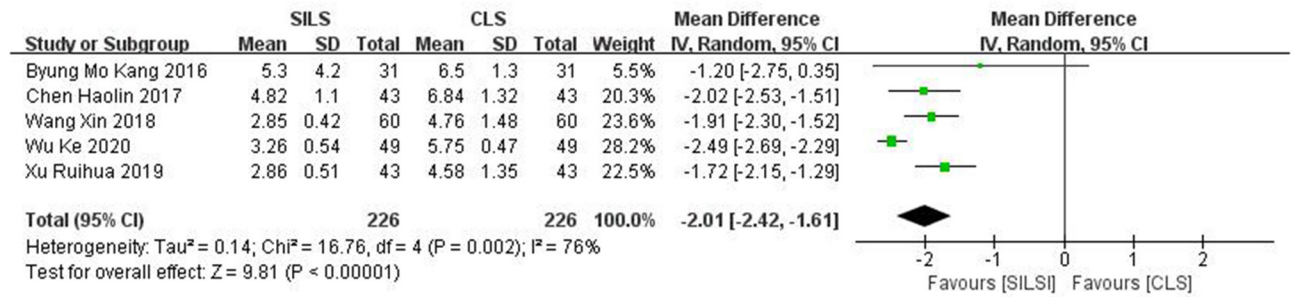

FIGURE 4 | Meta-analysis of length of abdominal incision. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

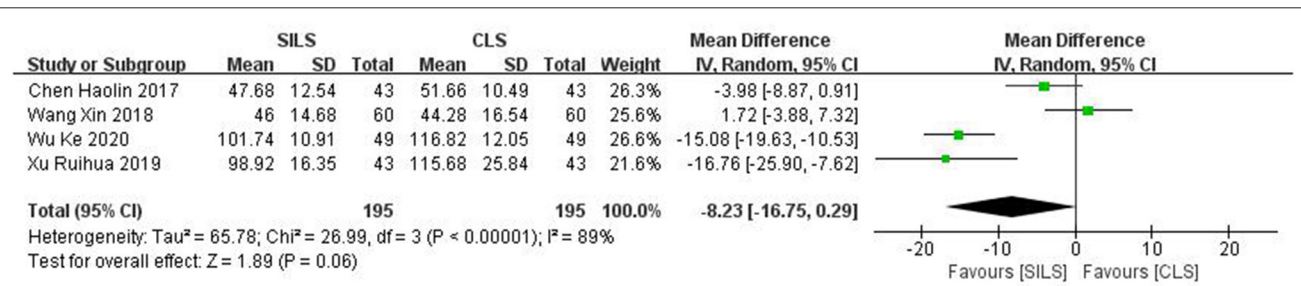

FIGURE 5 | Meta-analysis of intraoperative blood loss. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

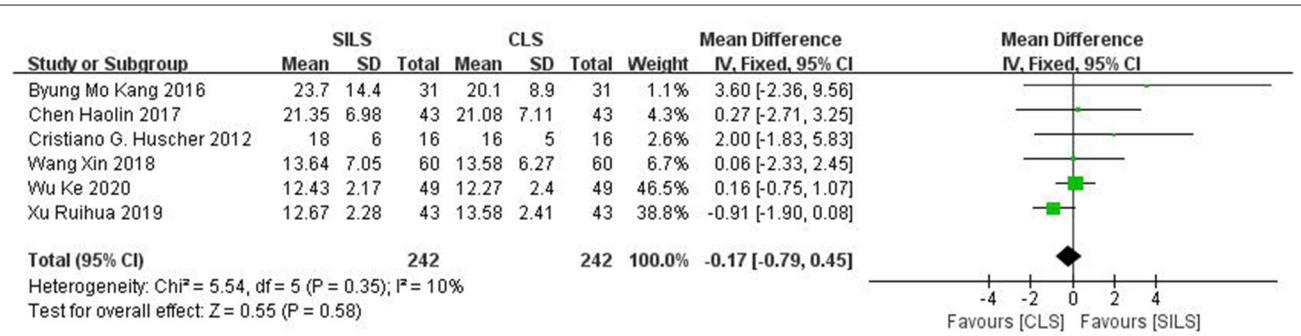

FIGURE 6 | Meta-analysis of number of lymph nodes removed. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

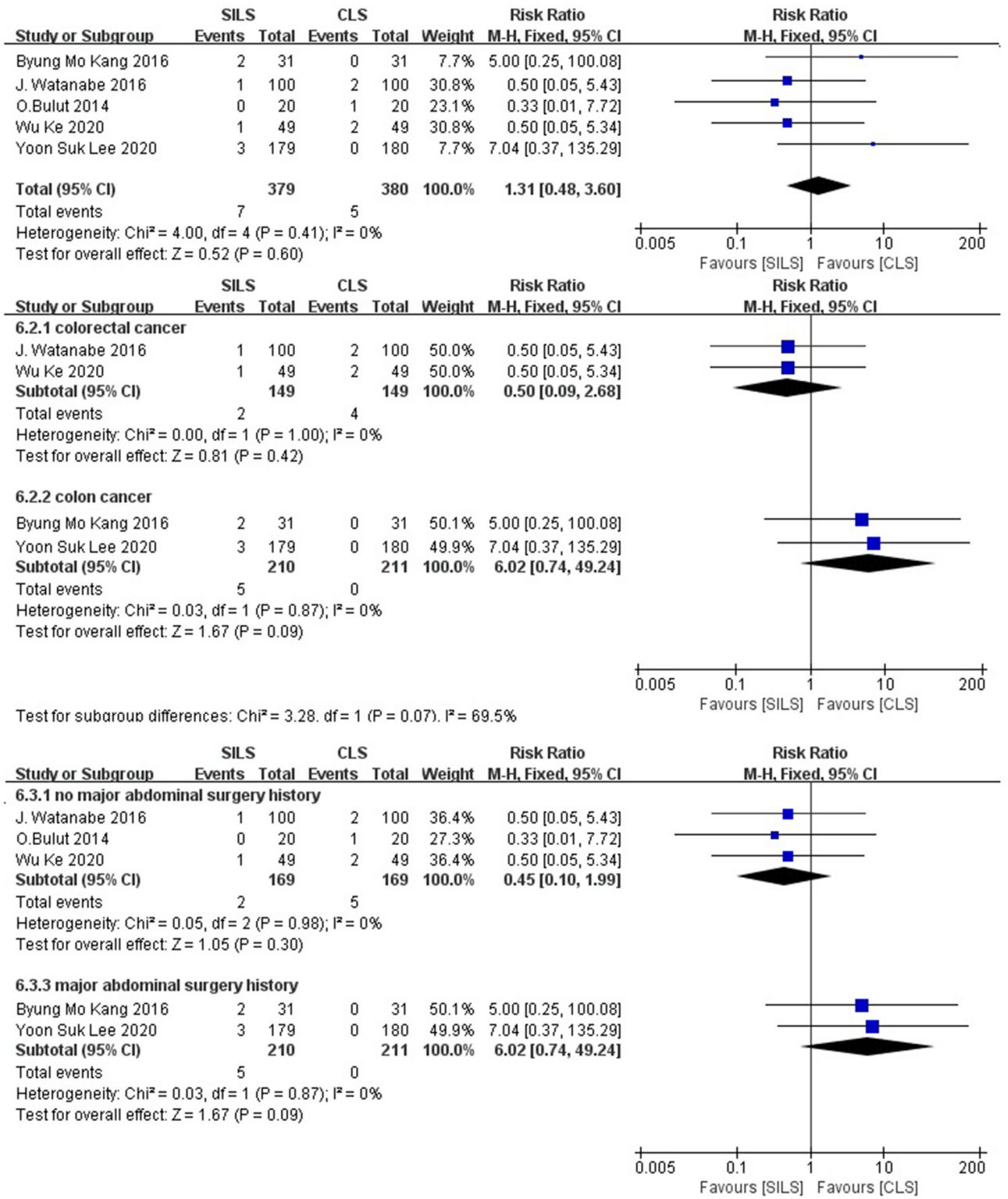

FIGURE 7 | Meta-analysis of conversion to laparotomy. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

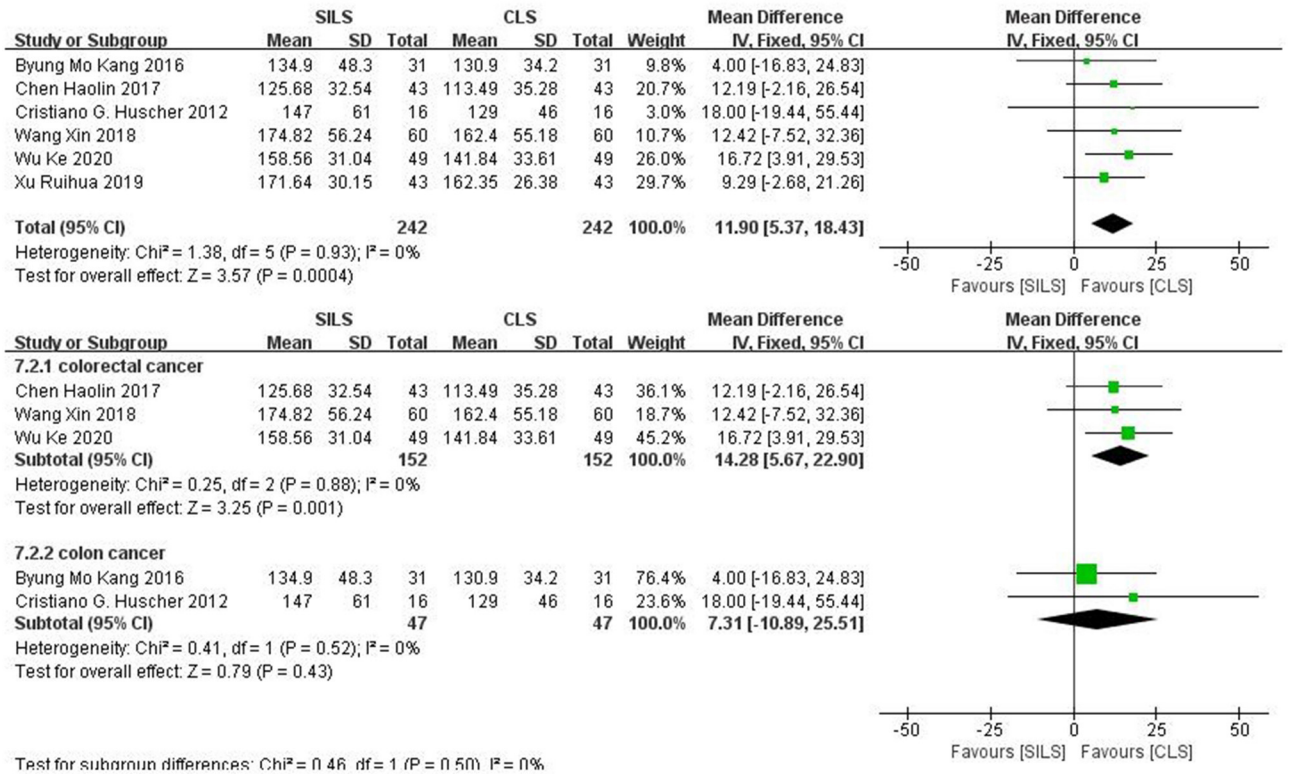

FIGURE 8 | Meta-analysis of operation time. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

showed no heterogeneity. The fixed-effect model was applied in the analyses. The SILS group showed longer operation time compared with the CLS group [MD = 11.9, 95% CI: 5.37–18.43, $P = 0.0004$] (**Figure 8**).

Subgroup analysis: The patients were divided into two subgroups according to the cancer type. Colorectal cancer: Three studies (19, 25, 27) including 304 patients reported the operation time. Heterogeneity test: $P = 0.88$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. The SILS group showed longer operation time compared with the CLS group [MD = 14.28, 95% CI: 5.67–22.9, $P = 0.001$]. Colon cancer: Two studies (21, 22) including 94 patients reported the operation time. Heterogeneity test: $P = 0.52$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis result [MD = 7.31, 95% CI: −10.89–25.51, $P = 0.43$] showed no statistical significance (**Figure 8**).

Length of Hospital Stay (Days)

Six studies (19, 21, 22, 25, 27, 28) with a total of 484 patients reported the length of hospital stay. Heterogeneity test: $P = 0.0001$, $I^2 = 80\%$, showed high heterogeneity. The random-effect model was applied in the analyses. The SILS group showed shorter hospital stay compared with the CLS group [MD = −1.12, 95%CI: −1.89 to −0.34, $P = 0.005$] (**Figure 9**).

Subgroup analysis: The patients were divided into two subgroups according to the cancer type. Colorectal cancer: Three studies (19, 25, 27) with a total of 304 patients reported the length of hospital stay. Heterogeneity test: $P = 0.0008$, $I^2 = 86\%$, showed high heterogeneity. The random-effect model was applied in the analyses. SILS group shows shorter hospital stay compared with the CLS group [MD = −1.84, 95%CI: −3.30 to −0.38, P = 0.01] (**Figure 9**). Colon cancer: Two studies (21, 22) with a total of 94 patients reported the length of hospital stay. Heterogeneity test: $P = 0.19$, $I^2 = 41\%$, showed moderate heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [MD = 0.06, 95%CI: −0.69–0.82, $P = 0.87$] showed no statistical significance (**Figure 9**).

Reoperation

Two studies (20, 26) with a total of 240 patients reported the rate of reoperation. Heterogeneity test: $P = 1.00$, $I^2 = 0$, showed no heterogeneity. The fixed-effect model was applied in the analyses. Meta-analysis results [RR = 1.00, 95% CI: 0.30–3.33, $P = 1.00$] showed no statistical significance (**Figure 10**).

Readmission

Three studies (20, 22, 23) with a total of 461 patients reported the rate of readmission. Heterogeneity test: $P = 0.09$, $I^2 = 65\%$, showed high heterogeneity. The random-effect model was applied in the analyses. Meta-analysis results [RR =1.15, 95% CI: 0.12–10.38, $P = 0.90$] showed no statistical significance (**Figure 11**).

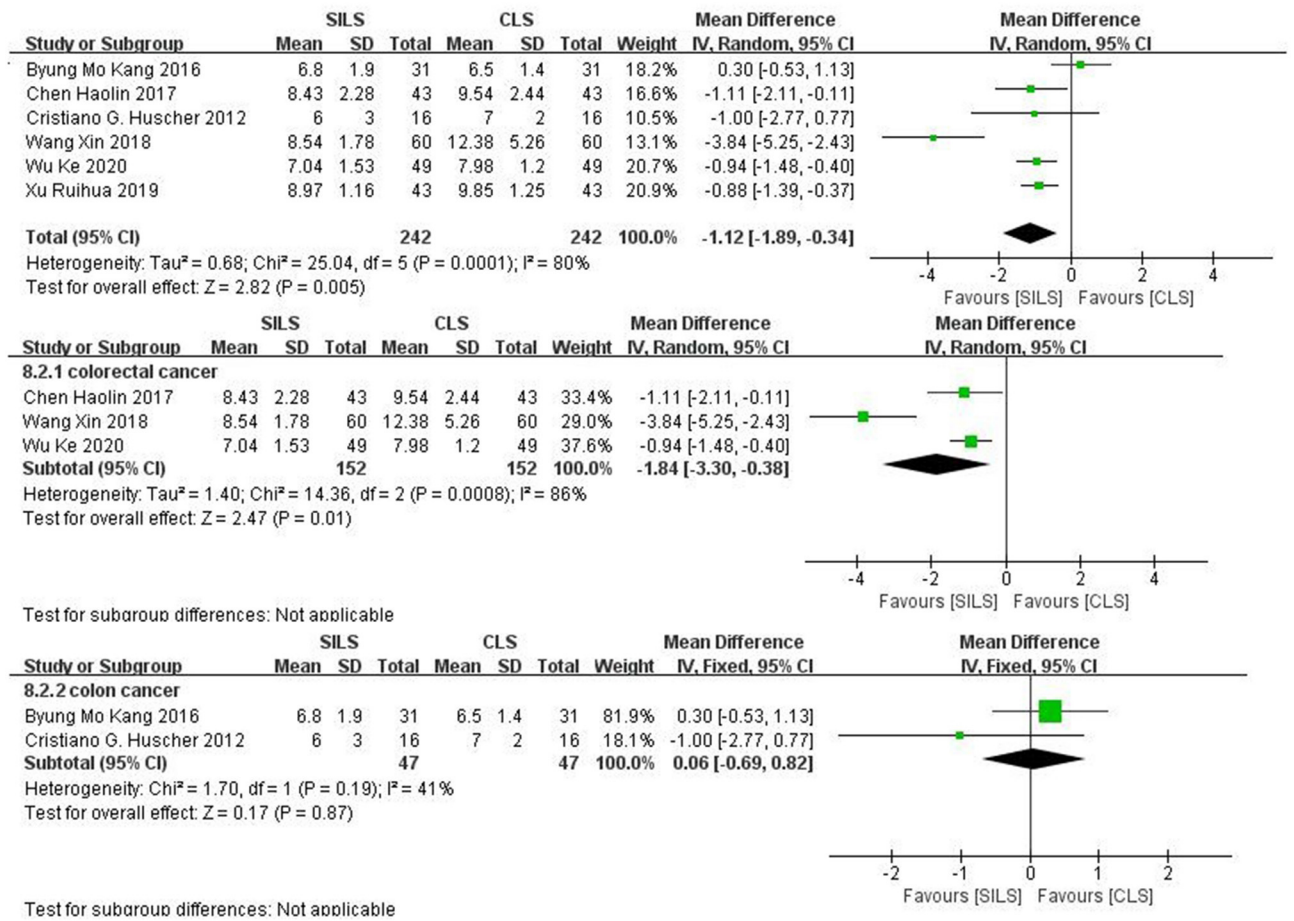

FIGURE 9 | Meta-analysis of length of hospital stay. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

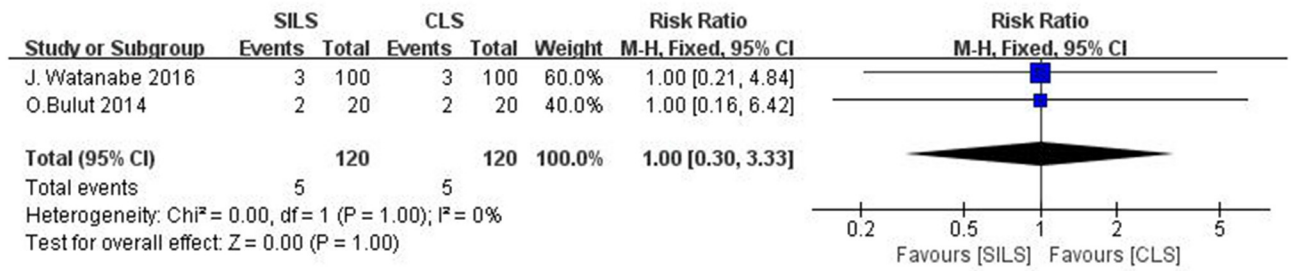

FIGURE 10 | Meta-analysis of reoperation. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

Publication Bias and Sensitivity Analysis

Publication bias was detected for the main outcomes. An asymmetrical inverted funnel plot for postoperative complications from egger's test ($t = 1.78$, $p = 0.33$), publication bias were not detected as a result of postoperative complications (**Figure 12**) (20, 28). Publication bias were not detected on intraoperative complications using the egger's test ($t = 2.41$, $p = 0.14$, $p > 0.05$) (21–23). The heterogeneity test for length of abdominal incision showed ($P = 0.002$, $I^2 = 76\%$), high heterogeneity. After excluding a study with low methodological quality (19), there was no observed heterogeneity ($P = 0.67$, $I^2 = 0$). Therefore, it was concluded that this study was the source of heterogeneity. After deleting the source of heterogeneity, the result of length of abdominal incision using the fixed effects model showed little difference with the previous result, [MD = −1.85, 95%CI: −2.10 to −1.61, $P < 0.00001$].

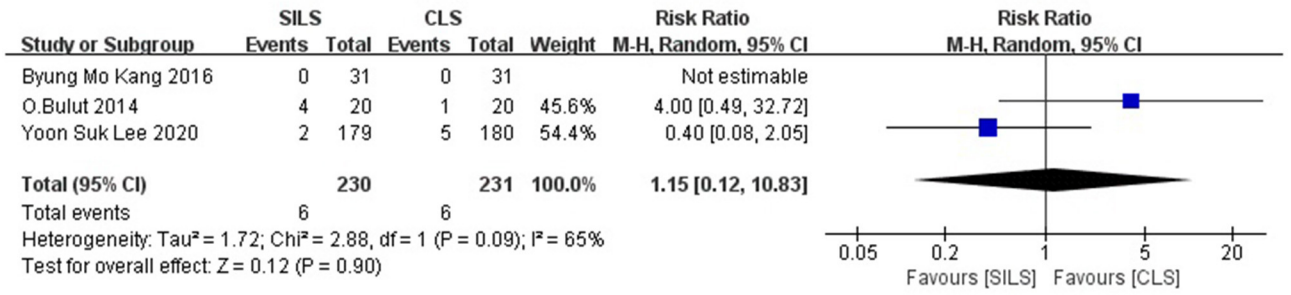

FIGURE 11 | Meta-analysis of readmission. SILS, single-incision laparoscopic surgery; CLS, conventional laparoscopic surgery.

Heterogeneity test ($P < 0.00001$, $I^2 = 89\%$) of intraoperative blood loss showed high heterogeneity. After excluding two studies with low methodological quality (25, 27), there was no observed heterogeneity ($P = 0.75$, $I^2 = 0$). Thus, these two studies were considered to be the source of heterogeneity. After deleting the source of heterogeneity, the result of intraoperative blood loss using the fixed effects model showed difference with the previous result, [MD = −15.41, 95% CI: 19.49 to −11.34, $P < 0.00001$]. Heterogeneity test of hospital stay showed high heterogeneity ($P = 0.0001$, $I^2 = 80\%$,). After excluding a study with low methodological quality (27), moderate heterogeneity ($P = 0.11$, $I^2 = 46\%$), was observed. Thus, this study was considered a source of heterogeneity. After deleting the source of heterogeneity, the result of hospital stay using the fixed effects model showed little difference with the previous result, [MD = −0.71, 95% CI: −1.19 to −0.24, $P = 0.0033$]. Sensitive analysis found that the exclusion of any single study did not affect the pooled results and heterogeneity in the meta-analysis (**Table 3**).

Assessment of the Quality of Evidence

A total of 10 outcome measures were evaluated. Risk bias: Jadad's score of the included studies was ≥4, and were considered high-quality studies. Thus, all outcomes had no risk of bias. However, studies without allocation concealment were considered to have a serious risk of bias. Inconsistency: Due to the high heterogeneity, outcomes of length of abdominal incision, intraoperative blood loss, and length of hospital stay were considered to have serious inconsistencies. Indirectness: All studies were direct comparisons, so indirectness was not significant. Imprecision: The sample size was large enough for outcomes of postoperative complications, conversion to laparotomy, and readmission, thus no imprecision was considered. Other outcomes were assessed serious imprecision due to their small sample size. Publication bias: Evidence of publication bias was detected in the outcome of postoperative complications. Overview: The quality of evidence of the length of abdominal incision, intraoperative blood loss, and length of hospital stay was low. The quality of evidence of intraoperative complications, postoperative complications, number of lymph nodes removed, operation time, and reoperation was moderate. The quality of evidence of conversion to laparotomy and readmission was high (**Table 4**).

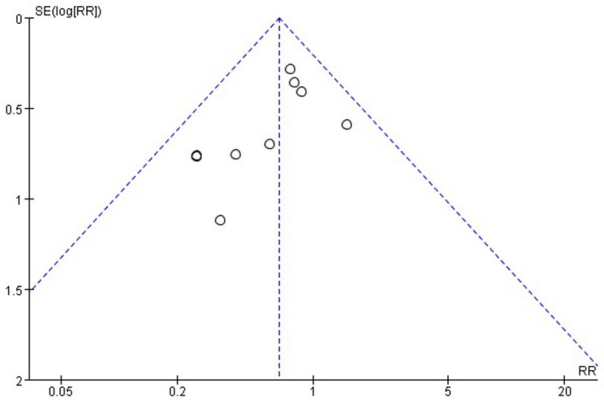

FIGURE 12 | Funnel plot of the studies reporting postoperative complications (20, 21, 24–30).

DISCUSSION

SILS, as an emerging minimally invasive technique, attracts a lot of attention from patients and surgeons, because of its potential advantages such as smaller incision length, lower rate of intraoperative complications, and so on. After analyzing several clinical controlled trials, the European Association of Endoscopic Surgery (EAES) pointed out that SILS also has the advantages of better aesthetics and reduced postoperative pain (29). Although high-quality visualization has brought many benefits, single-incision laparoscopic surgery has some weaknesses. Poor ergonomics and technical difficulty are the most important reasons why this technology has not been rapidly adopted. Some scholars (30) believe that SILS has no obvious advantages over CLS, the operation time is longer, and the difficulty in the operation is greatly increased. Therefore, this study analyzed the efficacy of SILS and CLS in the treatment of colorectal cancer based on randomized controlled trials.

A total of 10 RCTs with 1,133 participants were included in this study. No significant difference was found in the mortality of 30 days between SILS and CLS. The meta-analysis results showed that SILS could reduce postoperative complications, length of abdominal incision, and length of hospital stay compared with

TABLE 3 | Sensitive analysis.

References	No. of patients	SILS	CLS	RR or MD (95% CI)	*P*-value	I2 (%)
Postoperative complications						
Kang et al. (22)	62	31	31	0.62 [0.45; 0.87]	0.0057	0.0
Chen et al. (25)	86	43	43	0.67 [0.48; 0.93]	0.0175	0.0
Huscher et al. (21)	32	16	16	0.69 [0.49; 0.95]	0.0235	0.0
Watanabe et al. (26)	200	100	100	0.64 [0.45; 0.91]	0.0136	0.0
Poon et al. (24)	50	25	25	0.68 [0.49; 0.94]	0.0193	0.0
Bulut et al. (20)	40	20	20	0.65 [0.46; 0.91]	0.0127	0.0
Wang et al. (27)	120	60	60	0.71 [0.51; 0.99]	0.0436	0.0
Xu (28)	86	43	43	0.71 [0.51; 0.99]	0.0438	0.0
Lee et al. (23)	359	179	180	0.63 [0.42; 0.92]	0.0176	0.0
Pooled estimate	1035	517	518	0.67 [0.49; 0.92]	0.0130	0.0
Intraoperative complications						
Kang et al. (22)	62	31	31	1.94 [0.82; 4.59]	0.1297	0.0
Huscher et al. (21)	32	16	16	2.21 [0.95; 5.14]	0.0651	0.0
Lee et al. (23)	359	179	180	5.00 [0.61; 41.30]	0.1352	0.0
Pooled estimate	453	226	227	2.26 [1.00; 5.10]	0.0494	0.0
Length of abdominal incision						
Kang et al. (22)	62	31	31	−2.06 [−2.47; −1.65]	< 0.0001	80.0
Chen et al. (25)	86	43	43	−2.00 [−2.50; −1.50]	< 0.0001	81.2
Wang et al. (27)	120	60	60	−2.03 [−2.54; −1.52]	< 0.0001	77.7
Wu et al. (19)	98	49	49	−1.85 [−2.10; −1.61]	< 0.0001	0.0
Xu (28)	86	43	43	−2.11 [−2.54; −1.69]	< 0.0001	71.0
Pooled estimate	452	226	226	−2.01 [−2.42; −1.61]	< 0.0001	76.1
Intraoperative blood loss						
Chen et al. (25)	86	43	43	−9.83 [−21.98; 2.31]	0.1124	91.5
Wang et al. (27)	120	60	60	−11.56 [−19.97; −3.14]	0.0071	84.0
Wu et al. (19)	98	49	49	−5.59 [−14.27; 3.09]	0.2069	82.5
Xu (28)	86	43	43	−5.88[−15.63; 3.88]	0.2375	91.3
Pooled estimate	390	195	195	−8.23 [−16.75; 0.29]	0.0583	88.9
Number of lymph nodes removed						
Kang et al. (22)	62	31	31	−0.21 [−0.83; 0.41]	0.5008	0.0
Chen et al. (25)	86	43	43	−0.19 [−0.82; 0.44]	0.5507	26.6
Huscher et al. (21)	32	16	16	−0.23 [−0.86; 0.40]	0.4705	6.3
Wang et al. (27)	120	60	60	−0.19 [−0.83; 0.45]	0.5624	27.3
Wu et al. (19)	98	49	49	−0.46 [−1.31; 0.38]	0.2844	12.6
Xu (28)	86	43	43	0.30 [−0.49; 1.09]	0.4632	0.0
Pooled estimate	484	242	242	−0.17 [−0.79; 0.45]	0.5845	9.8
Conversion to laparotomy						
Kang et al. (22)	62	31	31	1.00 [0.33; 3.07]	0.9974	0.0
Watanabe et al. (26)	200	100	100	1.67 [0.53; 5.30]	0.3849	12.6
Bulut et al. (20)	40	20	20	1.60 [0.53; 4.85]	0.4040	10.8
Wu et al. (19)	98	49	49	1.67 [0.52; 5.31]	0.3857	12.2
Lee et al. (23)	359	179	180	0.83 [0.26; 2.69]	0.7605	0.0
Pooled estimate	759	379	380	1.31 [0.48; 3.60]	0.6011	0.1
Operation time						
Kang et al. (22)	62	31	31	12.76 [5.89; 19.64]	0.0003	0.0
Chen et al. (25)	86	43	43	11.83 [4.49; 19.16]	0.0016	0.0
Huscher et al. (21)	32	16	16	11.71 [5.08; 18.34]	0.0005	0.0
Wang et al. (27)	120	60	60	11.84 [4.93; 18.75]	0.0008	0.0
Wu et al. (19)	98	49	49	10.21 [2.62; 17.80]	0.0084	0.0
Xu (28)	86	43	43	13.01 [5.22; 20.79]	0.0011	0.0

(Continued)

TABLE 3 | Continued

References	No. of patients	SILS	CLS	RR or MD (95% CI)	*P*-value	I2 (%)
Pooled estimate	484	242	242	11.90 [5.37; 18.43]	0.0004	0.0
Hospital stay						
Kang et al. (22)	62	31	31	−1.40 [−2.17; −0.63]	0.0003	74.5
Chen et al. (25)	86	43	43	−1.14 [−2.06; −0.22]	0.0155	83.9
Huscher et al. (21)	32	16	16	−1.14 [−1.99; −0.29]	0.0088	84.0
Wang et al. (27)	120	60	60	−0.71 [−1.19; −0.24]	0.0033	46.2
Wu et al. (19)	98	49	49	−1.21 [−2.29; −0.13]	0.0281	84.0
Xu (28)	86	43	43	−1.23 [−2.33; −0.13]	0.0281	84.0
Pooled estimate	484	242	242	−1.12 [−1.89; −0.34]	0.0048	80.0
Reoperation						
Watanabe et al. (26)	200	100	100	1.00 [0.16; 6.42]	1.0000	0.0
Bulut et al. (20)	40	20	20	1.00 [0.21; 4.84]	1.0000	0.0
Pooled estimate	240	120	120	1.00 [0.30; 3.33]	1.0000	0.0
Readmission						
Kang et al. (22)	62	31	31	1.15 [0.12; 10.83]	0.9044	65.2
Bulut et al. (20)	40	20	20	0.40 [0.08; 2.05]	0.2725	/
Lee et al. (23)	359	179	180	4.00 [0.49; 32.72]	0.1961	/
Pooled estimate	461	230	231	1.15 [0.12; 10.83]	0.9044	65.2

SILS, single incision laparoscopic surgery; CLS, conventional laparoscopic surgery; RR, relative risk; MD, mean difference; 95%CI, 95% confidence interval.

CLS. However, SILS had poorer intraoperative complications and operation time compared with CLS. In addition, no significant difference was found in intraoperative blood loss, number of lympg nodes removed, the rate of conversion to laparotomy, the rate of reoperation, the rate of readmission and the rate of anastomotic leakage between the two groups.

This meta-analysis confirmed that SILS reduced the rate of postoperative complications. Besides, we inferred that SILS reduced the length of abdominal incision and number of ports, which may be beneficial to wound care and cause less damage for patients (31). Moreover, some studies show that patients who undergo single incision laparoscopic surgery have lower levels of postoperative inflammation than patients who undergo conventional laparoscopic surgery (19, 28). This could be one of the reasons why fewer postoperative complications were reported in the SILS group. The length of abdominal incision in SILS is 2.01 cm shorter than CLS. Besides, SILS not only plays a cosmetic role but also makes the patients think that they are doing a "minor surgery", which is important for their postoperative mood adjustment. The postoperative recovery time depends on several factors including age, nutritional status, underlying disease, and scope of resection. SILS does not reduce the scope of resection compared with CLS, and apart from the aesthetic advantage, avoiding some small incisions may not affect the speed of recovery. In this meta-analysis, six studies provided data on the length of hospital stay. SILS's length of hospital stay was 1.12 days shorter compared with CLS. However, since the included studies did not use the same discharge standards, the difference in hospital stay has a low reference value. Moreover, the reduction in the length of hospital stay by 1.12 days may not have any clinical significance. This study confirmed that the SILS group had worse rates of intraoperative complications and operation time, compared with the CLS group. These may have been caused by several reasons. First, different levels of experience among surgeons may affect the operation time and rate of intraoperative complications. U-Syn Ha et al. found that the surgical skills acquired by traditional laparoscopic surgeons cannot be directly converted into SILS skills and that novices with laparoscopic surgery can obtain SILS skills similar to those of experienced surgeons through training (32). Another study found that in the absence of practice, SILS skills acquired at 8 weeks deteriorated, while conventional laparoscopic skills were well maintained during the entire 12-week observation period (33). This means that the maintenance of SILS skills differs from conventional laparoscopic surgery, and the maintenance of SILS is more difficult. Second, different specifications of surgical instruments, and inconsistent colorectal cancer surgical methods (such as low anterior resection of rectal cancer, radical resection of abdominal perineum combined with rectal cancer) may also affect the operation time and rate of intraoperative complications. Third, compared with CLS, SILS is an emerging technology, and surgeons require a certain degree of operation proficiency. SILS requires direct insertion of the operating instruments into the abdominal cavity through a single incision in the abdominal wall in a nearly parallel manner. Operating under the limited surgical view, lack of effective traction, equipment crowding, and collision during the operation, make SILS more difficult, resulting in prolonged operation time and increase the rate of intraoperative complications (34).

The rate of conversion to laparotomy is an outcome that surgeons may be interested in. For SILS surgery, there is a transition option: conversion to CLS, but for CLS, it can only be directly converted to open surgery, which makes it meaningless to compare the rate conversion to CLS between

TABLE 4 | GRADE evidence profile of outcomes.

Outcome	Number of studies	Assessment of evidence quality					Number of participants	Effect (95%CI)	Evidence quality
		Risk bias	Inconsistency	Indirectness	Imprecision	Publication bias			
Postoperative complications	9	No	No	No	No	Undetected	1,035	RR = 0.67 (0.49, 0.92)	High
Intraoperative complications	3	No	No	No	Serious	Undetected	453	RR = 2.26 (1.00, 5.10)	Moderate
Length of abdominal incision	5	No	Serious	No	Serious	Undetected	452	MD = −2.01 (−2.42, −1.29)	Low
Intraoperative blood loss	4	No	Serious	No	Serious	Undetected	390	MD = −8.23 (−16.75, 0.29)	Low
Number of lymph Nodes removed	6	No	No	No	Serious	Undetected	484	MD = −0.17 (−0.79, 0.45)	Moderate
Conversion to laparotomy	5	No	No	No	No	Undetected	759	RR = 1.31 (0.48, 3.60)	High
Operation time	6	No	No	No	Serious	Undetected	484	MD = 11.9 (5.37, 18.43)	Moderate
Hospital stay	6	No	Serious	No	Serious	Undetected	484	MD = −1.12 (−1.89, −0.34)	Low
Reoperation	2	No	No	No	Serious	Undetected	240	RR = 1 (0.3, 3.33)	Moderate
Readmission	3	No	Serious	No	Serious	Undetected	461	RR = 1.15 (0.12, 10.83)	Low

the two groups. In the studies we included, the definitions of conversion to CLS cannot be unified. Conversion to CLS was defined as the insertion of additional trocars during SILS in two studies (20, 22), but in other studies, conversion to CLS was defined as the addition of two or more trocars (23, 26). The definition of conversion to laparotomy was that a skin incision longer than designated incision was required to extract the resected specimen or to control intraoperative complications in two studies (20, 22), but in another study, conversion to laparotomy was defined by a wound length measuring 8 cm or greater (26). We believe that the definition of conversion to laparotomy between different studies has low clinical heterogeneity. Although the meta-analysis did not show a statistical difference between the two groups, the subgroup analysis suggested that in colon cancer patients, the rate conversion to open surgery of SILS was higher than that of CLS, and the data was consistent.

The long-term outcome from the SIMPLE study showed that SILS did not have an absolute advantage (23, 35). Although there were some statistical differences in the overall quality of life scores, functional scores, and symptom scores at different measurement points after surgery, these statistical differences do not always indicate that SILS has more advantages or disadvantages than CLS. Moreover, these differences can be explained by type I errors caused by multiple hypothesis tests.

To further reduce clinical heterogeneity, we performed subgroup analysis according to the cancer type and previous history of major abdominal surgery. The SILS group showed lower rates of postoperative complications compared with the CLS group in all subgroups. A comparison of the rate of postoperative complications in patients with colorectal cancer, colon cancer, and rectal cancer in the SILS and CLS group, we found that the relative risk (RR) of patients with colon cancer [RR = 0.80, 95% CI: 0.54–1.18, $P = 0.26$] was higher than that of colorectal cancer [RR = 0.61, 95% CI: 0.34–1.07, $P = 0.09$] and rectal cancer patients [RR = 0.54, 95% CI: 0.16–1.89, $P = 0.34$]. We hypothesized that the colon has more blood vessels, which may cause more vascular injury complications and increase the difficulty of surgery. Therefore, SILS for colon cancer can cause postoperative complications, thus increasing the RR of patients with colon cancer. The RR of postoperative complications in patients with major abdominal surgery history [RR = 0.82, 95% CI: 0.50–1.33, $P = 0.41$] was higher than the RR of postoperative complications in patients with no major abdominal surgery history [RR = 0.63, 95% CI: 0.41–0.99, $P = 0.05$]. We hypothesized that patients with major abdominal surgical history have a worse physical condition, and SILS may cause severe damage to these patients, thus, resulting in more postoperative complications. The SILS group showed longer operation time compared with the CLS group in patients with colorectal and colon cancer, and the MD of colorectal cancer patients [MD = 14.28, 95% CI: 5.67–22.9, $P = 0.001$] was higher than the MD of colon cancer [MD = 7.31, 95% CI: −10.89–25.51, $P = 0.43$]. We infer it is determined by the level of surgical skill in different countries. The included studies on colorectal cancer were all from China, while those on colon cancer were from Korea and Italy, which are considered to have a higher level of surgical skills compared with China. Moreover, the two

countries have a higher level of training for SILS. Therefore, the operation time of SILS and the MD of operation time in colorectal cancer patients were found to be longer than in the colon cancer group. The colorectal cancer subgroup analysis showed that, the SILS group had a shorter hospital stay than the CLS group [MD = −1.84, 95% CI: −3.30 to −0.38, $P = 0.01$], while colon cancer subgroup analysis showed that the SILS group had a similar length of hospital stay compared with the CLS group [MD = 0.06, 95% CI: −0.69–0.82, $P = 0.87$]. We hypothesized that colon cancer patients have higher rate of postoperative complications, which caused longer hospital stay. The above explanation may also be affected by the instability caused by the reduction in the sample size of the subgroup analysis.

A meta-analysis published by Gu et al. was the closest to our study in terms of structured clinical issues (PICO) (36). Our findings differed from that study in almost all outcomes. We carefully analyzed and speculated that the most likely reason for the difference is that our meta-analysis only included RCTs, and the above meta-analysis also included propensity-score matched studies. The apparently higher heterogeneity (I square) in the above meta-analysis supports our speculation. The randomized controlled trials included in the two meta-analysis are almost the same, and we have reason to believe that the meta-analysis results of the two based on the same randomized controlled trial should also be the same. Future research should focus on comparing data from randomized controlled trials with data from propensity-score matched studies.

Compared with other previous meta-analysis (37–40) including retrospective studies or clinical controlled trials (CCTs), this meta-analysis only included and analyzed all relevant RCTs in the present to ensure that the results were more reliable. However, this study has some limitations. First, the literature included in this study mostly comes from China and Korea, thus, the study results are poorly extrapolated. Secondly, the included literature lacks long-term follow-up results, including the rate of local tumor recurrence or distant metastasis, and survival rate. Thirdly, only two studies blinded the participants, while the others were open-labeled RCTs, which can lead to substantial implementation bias. None of the studies reported whether outcome evaluators were blinded, so measurement bias may also have influenced the results, especially in those subjective outcomes such as length of stay in the hospital. Finally, the sample size of included studies is generally small. Therefore, the above conclusions need to be verified using well-designed long-term large sample RCTs. This systematic review and meta-analysis did not prove that the SILS has a comprehensive and obvious advantage over the CLS. Although SILS for colorectal cancer showed advantages including shorter incision length, lower postoperative complication rates, and shorter hospital stay compared with CLS. Some poor short-term outcomes of SILS, such as longer operation time and more intraoperative complications, suggest that it should be considered carefully. Surgeons should fully discuss the pros and cons of the two surgical procedures with patients, and make a selection based on factors such as the surgeon's experience and training level, surgical facilities, and patient values. RCTs focusing on long-term outcomes are warranted to provide more information on clinical options.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

YY is the principal investigator with overall responsibility for the original draft, together with JJ and HJ wrote the draft and submitted the PROSPERO registration. LD performed searching for relevant studies, data collection, and data analysis. RY, XF, FY, and WL provided help in designing, data analysis, and editing of the manuscript. All authors read and approved the final manuscript.

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