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High expression of RRM2 mediated by non-coding RNAs correlates with poor prognosis and tumor immune infiltration of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is known to have a poor prognosis. Accumulating evidence indicates that RRM2 plays a critical role in the occurrence and progression of multiple human cancers. However, the knowledge about RRM2 in HCC is still insufficient, and further research is needed. Here, we first analyzed the expression and prognosis of RRM2 using TCGA and GTEx data, and found that RRM2 may play a potential carcinogenic role in HCC. Then, through a series of comprehensive analysis, including expression analysis, correlation analysis or survival analysis, non-coding RNAs (ncRNAs) that regulate RRM2 overexpression were identified. Finally, MIR4435-2HG/CYTOR were observed to be the most promising upstream lncRNAs for the miR-125b-5p/RRM2 axis in HCC. In addition, RRM2 expression was significantly positively related to immune cell infiltration, immune cell biomarker or immune checkpoint expression in HCC. Altogether, the upregulation of RRM2 mediated by ncRNAs correlates with poor prognosis and tumor immune infiltration of HCC.

KEYWORDS

HCC, RRM2, ncRNAs, regulatory mechanism, immune cell infiltration

Introduction

Hepatocellular carcinoma (HCC) is a common cause of cancer-related deaths with increasing mortality worldwide, and has very poor prognosis with an incidence rate almost equal to the mortality rate (1, 2). Risk factors associated with the etiology of HCC include hepatitis type B and C, cirrhosis, alcoholism, diabetes, non-alcoholic fatty liver disease (NAFLD) and toxin exposure (3, 4). Despite these encouraging advancements in prevention, diagnosis, prognosis, and treatment, the options and outcome for this deadly cancer remain very limited. For example, sorafenib, a protein tyrosine kinase inhibitor for the treatment of HCC, which can only prolong the survival time by about 3 months. Thus, there are urgent calls for effective diagnostic and therapeutic regimens.

Ribonucleotide reductase M2 subunit (RRM2), a small subunit of the ribonucleotide reductase complex (5), is a rate-limiting enzyme responsible for DNA synthesis and DNA repair by producing dNTP (6). Ribonucleotide reductase (RNR) activity consists of two subunits (regulatory subunit RRM1 and catalytic subunit RRM2), which are coordinated with the process of cell cycle to maintain a fine balance between dNTP production and DNA replication (7). Unlike the tumor suppressor of RRM1 (8), RRM2 has carcinogenic activity and is widely involved in tumor growth, metastasis and drug resistance in different types of cancer (9–11). During the cell cycle, the expression of RRM2 depends on the cell cycle and reaches the highest level in S-phase (12). RRM2 is also related to poor prognosis and overexpressed in a variety of human cancers, such as breast cancer (13), lung cancer (14), colorectal cancer (15), glioma (16), renal cell carcinoma (17), and prostate cancer (10). RRM2 can be used as a prognostic biomarker to predict the survival and potential therapeutic target in these cancer patients. Growing evidence suggests that RRM2 may be a promising cancer therapeutic target. For instance, a RRM2 inhibitor, COH29, can inhibit the growth of gemcitabine resistant cancer cells (10). In addition, another RRM2 inhibitor, GW8510, has shown to inhibit the growth of breast cancer (18) and lung cancer (19). Although there are some reports of RRM2 regulation in lung and breast cancer, there is no comprehensive molecular understanding of how RRM2 is regulated by key upstream biological processes. Especially, very little is known about the regulatory mechanism and function of RRM2 in HCC. In addition, the correlation between RRM2 and immune infiltration in HCC remain unclear.

Here, we first investigated RRM2's expression and survival analysis across multiple cancer types. Subsequently, the regulation mechanism of RRM2 related to non-coding RNA (ncRNA) involving small RNA (miRNA) and long non-coding RNA (lncRNA) was also discussed in HCC. Lastly, we identified the correlation between RRM2 expression and immune cell infiltration, biomarkers and immune checkpoints in HCC. In conclusion, our findings suggest that the upregulation of RRM2 mediated by ncRNAs correlates with poor prognosis and tumor immune infiltration of HCC patients.

Abbreviations: HCC, Hepatocellular carcinoma; RRM2, Ribonucleotide reductase M2 subunit; TCGA, the cancer genome atlas; ncRNAs, non-coding RNAs; NAFLDL, non-alcoholic fatty liver disease; miRNA, microRNA; lncRNA, long non-coding RNA; ceRNA, competing endogenous RNA; OS, overall survival; RFS, disease-free survival; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

Results

RRM2 expression analysis in pan-cancer

As an initial investigation of RRM2 in carcinogenesis, we evaluated RRM2 expression in 18 human cancer types. As shown in **Figure 1A**, we found that there was no significant change in RRM2 expression in KICH compared with normal tissues, but RRM2 expression was significantly increased in the remaining 17 cancer types, including BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Additionally, to further confirm the expression of RRM2 in multiple cancers, we carried out RRM2 expression analysis using GEPIA. As shown in **Figures 1B–P**, we observed consistent results of elevated RRM2 expression in BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, and UCEC. Data from the pan-cancer analysis revealed significant upregulation of RRM2 across a variety of cancer types, suggesting a specific function of RRM2 in these 15 types of cancer related to tumorigenesis.

The prognostic value of RRM2 in human cancer

Here, the GEPIA was used to explore the prognostic significance of RRM2 expression in various tumors, including BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, and UCEC. Study metrics included disease-free survival (RFS) and overall survival (OS). In RFS analysis, high expression of RRM2 in LIHC and PRAD was associated with poor prognosis (**Figure 2**). In OS analysis, high RRM2 expression in LIHC and LUAD predicts poor outcomes (**Figure 3**). The correlation between RRM2 and prognosis was not observed in other tumors. Finally, we conclude that RRM2 predicted poor prognosis in patients with HCC, indicating its unique prognostic biomarker in HCC patients.

Prediction and analysis of upstream miRNAs of RRM2

The most widely known ncRNA system is post-transcriptional regulation of gene expression by miRNAs. We first performed prediction of the upstream miRNAs that might bind RRM2, and found 29 candidate miRNAs. We also used Cytoscape software based interaction maps to represent the miRNA-RRM2 regulatory network (**Figure 4A**). Next, based on the negative regulation mechanism of miRNA, RRM2 should

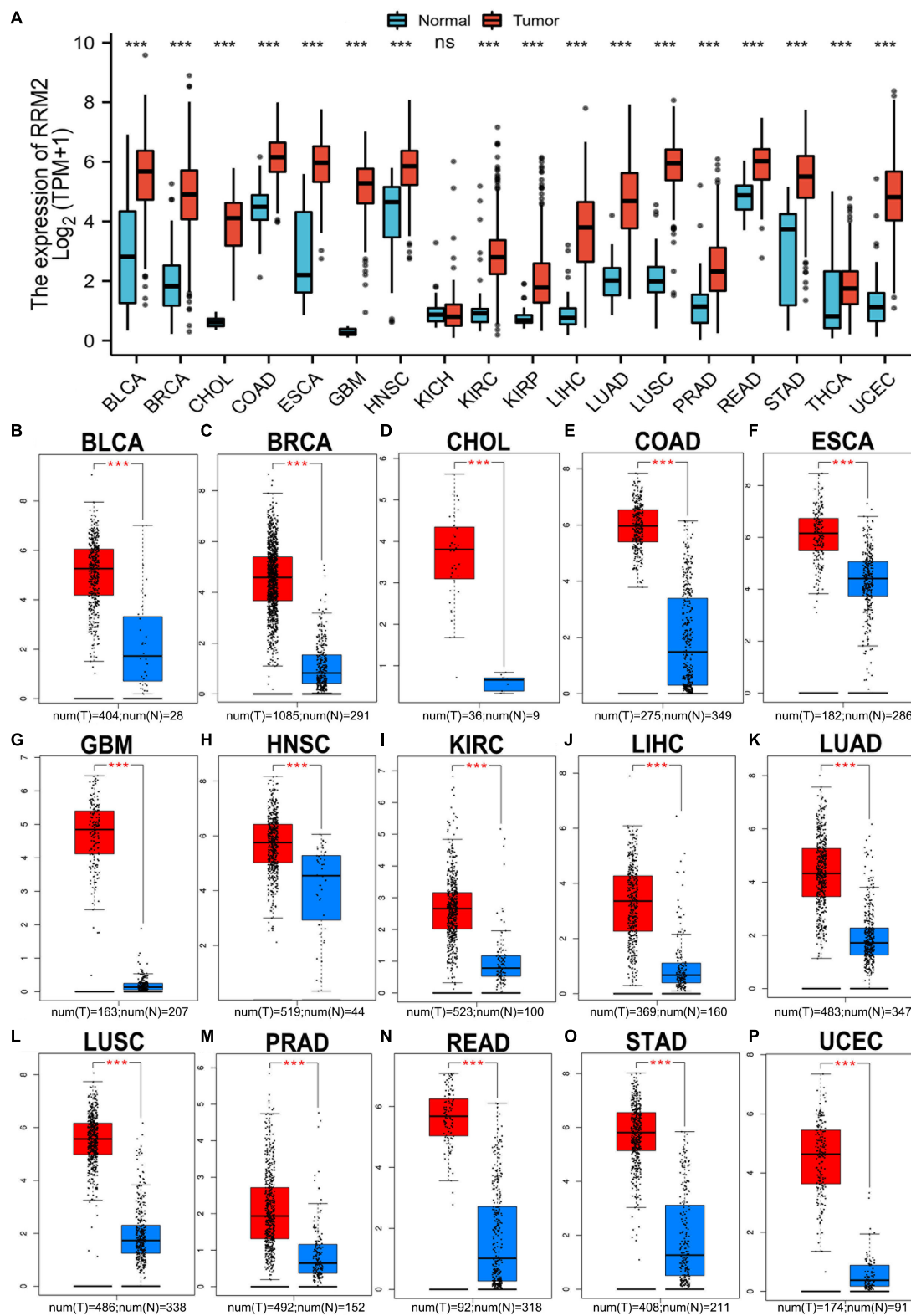


FIGURE 1
Pan-cancer analysis of RRM2 expression (A) RRM2 expression analysis across cancer types (18) using TCGA database. (B–P) RRM2 expression analysis using GEPIA database in BLCA (B), BRCA (C), CHOL (D), COAD (E), ESCA (F), GBM (G), HNSC (H), KIRC (I), LIHC (J), LUAD (K), LUSC (L), PRAD (M), READ (N), STAD (O), and UCEC (P). TPM, transcripts per million; Red, tumor group; Blue, normal group; ns, no significance; ***p value < 0.001.

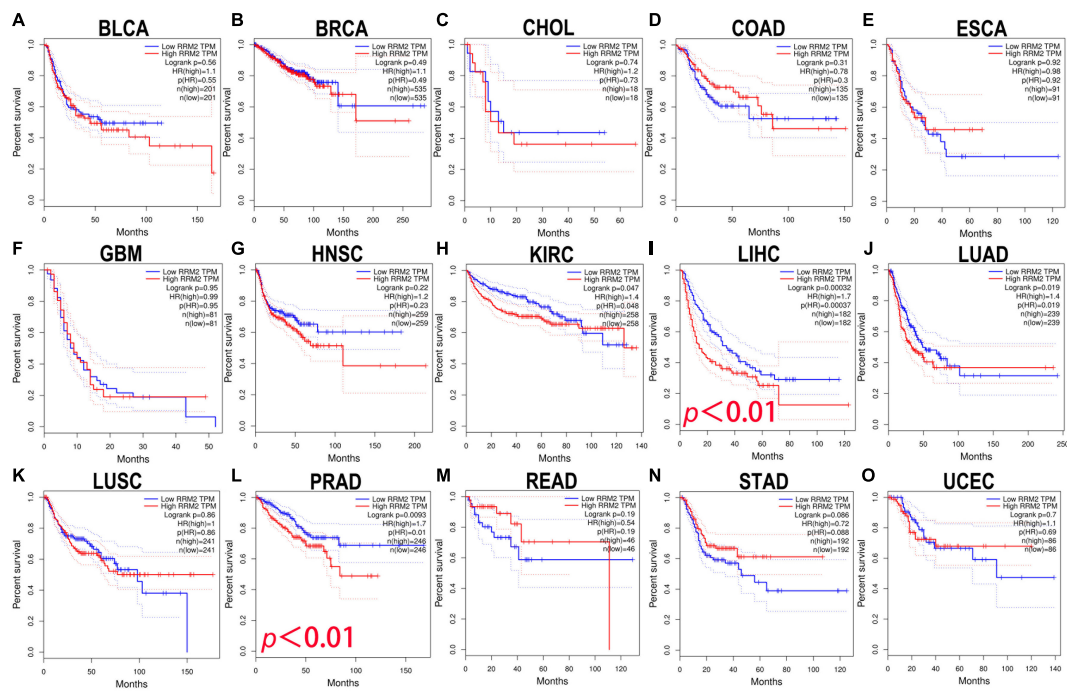


FIGURE 2

The prognostic analysis of RRM2 expression in disease-free survival (RFS) of various tumors using GEPIA. (A–O) The RFS curve of RRM2 in BLCA (A), BRCA (B), CHOL (C), COAD (D), ESCA (E), GBM (F), HNSC (G), KIRC (H), LIHC (I), LUAD (J), LUSC (K), PRAD (L), READ (M), STAD (N), and UCEC (O).

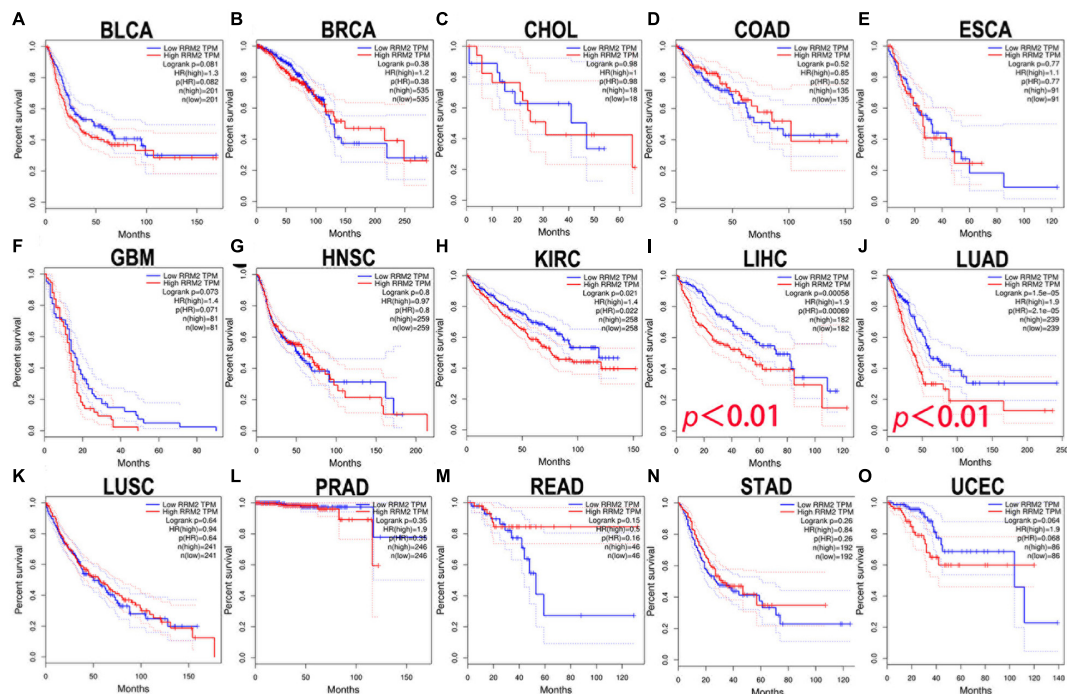


FIGURE 3

The prognostic analysis of RRM2 expression in overall survival (OS) of various tumors using GEPIA. (A–O) The OS curve of RRM2 in BLCA (A), BRCA (B), CHOL (C), COAD (D), ESCA (E), GBM (F), HNSC (G), KIRC (H), LIHC (I), LUAD (J), LUSC (K), PRAD (L), READ (M), STAD (N), and UCEC (O).

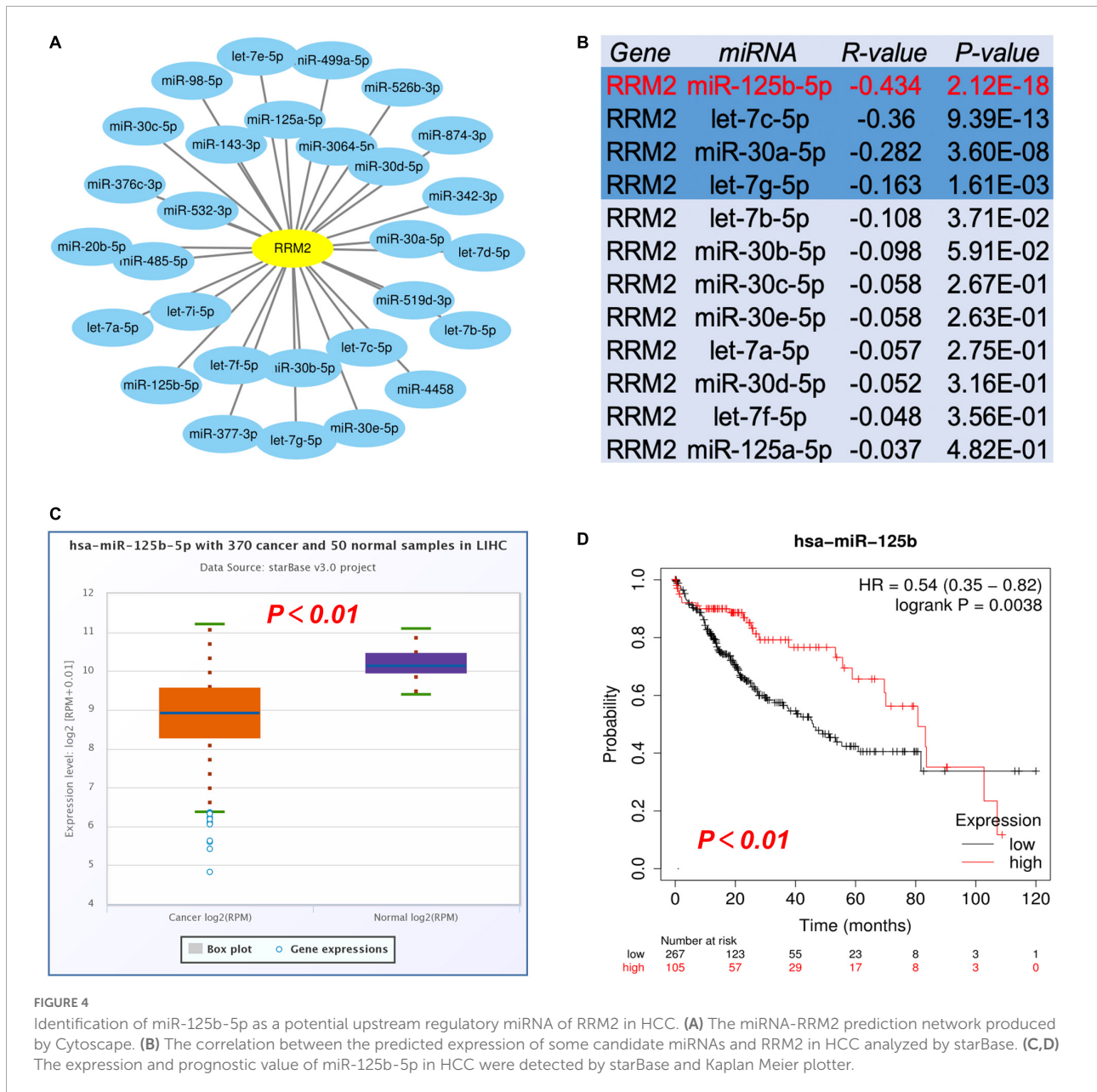


FIGURE 4 Identification of miR-125b-5p as a potential upstream regulatory miRNA of RRM2 in HCC. (A) The miRNA-RRM2 prediction network produced by Cytoscape. (B) The correlation between the predicted expression of some candidate miRNAs and RRM2 in HCC analyzed by starBase. (C,D) The expression and prognostic value of miR-125b-5p in HCC were detected by starBase and Kaplan Meier plotter.

be negatively related to miRNA. Therefore, we performed correlation analysis. As shown in **Figure 4B**, there was a statistically significant negative correlation between RRM2 and four miRNAs in HCC, including miR-125b-5p ($r = -0.434$, $p = 2.12e-18$), let-7c-5p ($r = -0.36$, $p = 9.39e-13$), miR-30a-5p ($r = -0.282$, $p = 3.60e-8$) and let-7g-5p ($r = -0.163$, $p = 1.61e-03$). The other miRNAs did not enter the next research step. Finally, we evaluated the expression and prognostic value of these four miRNAs in HCC. All four miRNAs were down regulated in HCC, only the expression of miR-125b-5p ($p = 0.0038$) was statistically correlated with the prognosis of HCC by Kaplan-Meier plotter analysis (**Figures 4C,D** and **Supplementary Figure 1**). Taken these together, these results

indicated that miR-125b-5p may be the most potential upstream regulator of RRM2 in HCC.

Prediction and analysis of upstream lncRNAs of miR-125b-5p

Firstly, we used starBase to predict the upstream lncRNAs of miR-125b-5p in HCC. As a result, a total of 47 possible lncRNAs were included. The visualization of miR-125b-5p regulatory network was plotted with Cytoscape software (**Supplementary Figure 2**). Then, we used GEPIA to evaluate the expression of these lncRNAs in HCC. Among all 47

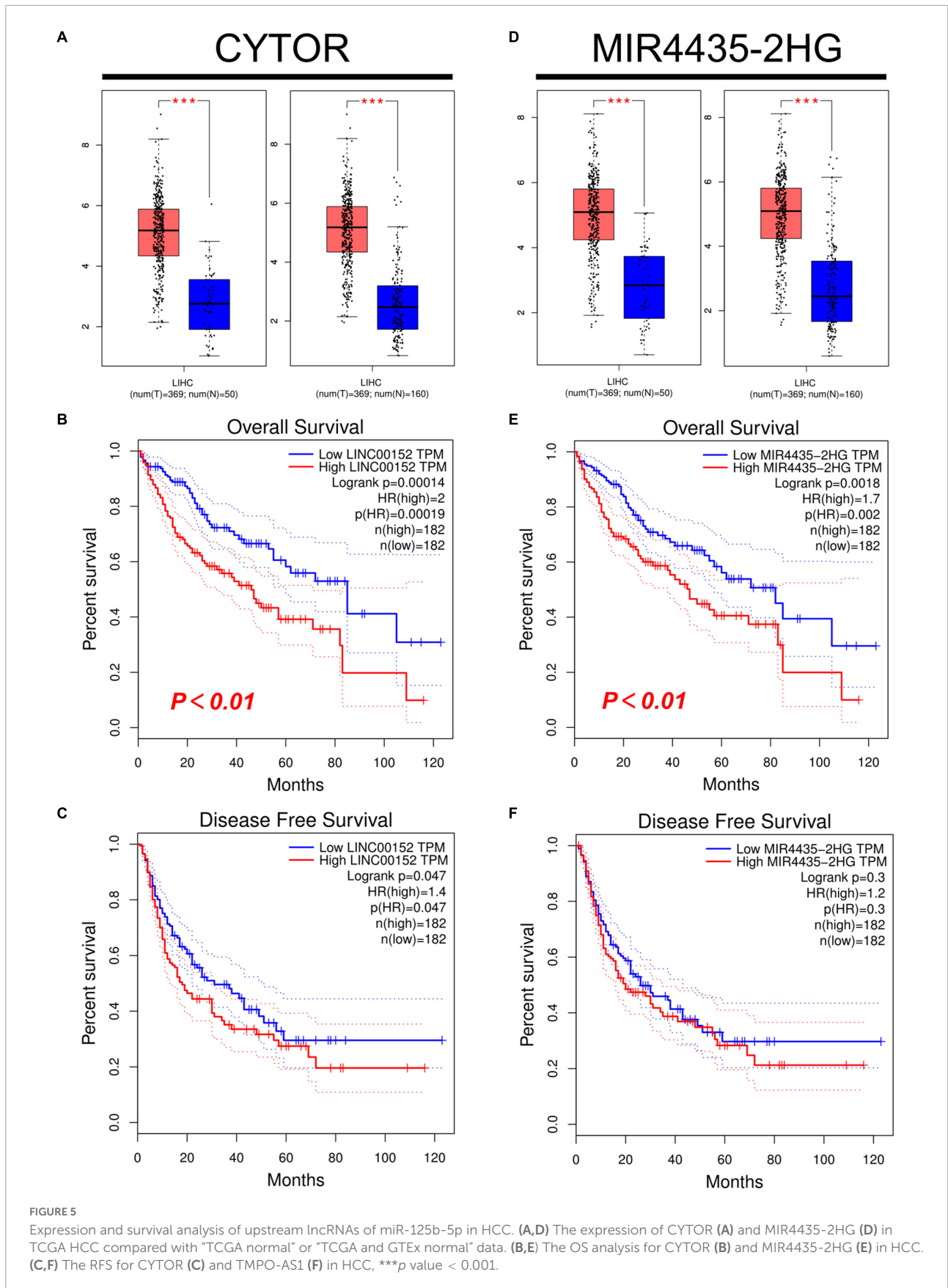


TABLE 1 Association between lncRNA and miR-125b-5p or lncRNA and RRM2 in HCC analyzed by starBase.

lncRNA	miRNA	R-value	P-value
CYTOR	miR-125b-5p	-0.372 ^a	1.35e-13***
MIR4435-2HG	miR-125b-5p	-0.307 ^a	1.70e-09***
lncRNA	mRNA	R-value	P-value
CYTOR	RRM2	0.342 ^a	1.11e-11***
MIR4435-2HG	RRM2	0.239 ^a	3.08e-06***

^ameans statistically significant.

***p value < 0.001.

lncRNAs, only the expression of CYTOR and MIR4435-2HG increased significantly upregulated in HCC compared with normal samples (Figure 5A, $p < 0.01$, Figure 5D, $p < 0.01$). Finally, we evaluated the prognostic value of these two lncRNAs in HCC. Patients with higher expression of CYTOR and MIR4435-2HG showed worse OS rather than RFS in HCC (Figures 5B,C,E,F).

The competing endogenous RNA (ceRNA) hypothesis was presented to explain the relationship among various RNAs. According to the ceRNA hypothesis, there should be positive association between lncRNA expression and mRNA expression or negative correlation between lncRNA and miRNA. We then used the starBase database to analyze the correlation between lncRNA and RRM2 or lncRNA and miR-125b-5p in HCC. The results were in line with ceRNA theory (Table 1). As shown in Table 1, we found that lncRNAs (CYTOR and MIR4435-2HG) were negatively correlated with miR-125b-5p ($r = -0.372$, $p = 1.35e-13$; $r = -0.307$, $p = 1.70e-09$, respectively), but positively correlated with RRM2 ($r = 0.342$, $p = 1.11e-11$; $r = 0.239$, $p = 3.08e-06$, respectively). Combined with all the above analysis, it is finally concluded that lncRNA CYTOR and MIR4435-2HG may be the two most potential upstream regulators of the miR-125b-5p/RRM2 axis in HCC.

Correlation of RRM2 with immune cell infiltration in hepatocellular carcinoma

To verify the correlation between RRM2 and immune cell infiltration, we tested whether RRM2-expressing cancers exhibited gene expression signatures of high immune infiltration. First, we observed that the level of immune cell infiltration did not change with the change of RRM2 copy number in HCC (Figure 6A). Correlation analysis can provide important clues to reveal the function and mechanism of RRM2. Therefore, we evaluated the correlation between RRM2 expression level and immune cell infiltration level. As presented in Figures 6B–G, our results showed that the expression of RRM2 was significantly positively correlated with all analyzed immune cells, including B cells ($r = 0.48$, $p = 3.06e-21$), CD8 + T cells ($r = 0.336$, $p = 1.78e-10$), CD4 + T

cells ($r = 0.257$, $p = 1.31e-06$), macrophages ($r = 0.394$, $p = 4.05e-14$), neutrophils ($r = 0.37$, $p = 1.19e-12$) and dendritic cells ($r = 0.456$, $p = 7.06e-19$) in HCC.

Association between RRM2 and immune cell biomarkers in hepatocellular carcinoma

In this part, we used the GEPIA to analyze the correlation between RRM2 and the expression of immune cell biomarkers in HCC. As presented in Table 2, RRM2 was significantly positively related to dendritic cell's biomarkers (HLA-DPB1, HLA-DPA1, HLA-DRA, ITGAX, and NRP1), B cell's biomarkers (CD79A and CD19), M2 macrophage's biomarkers (MS4A4A and VSIG4), CD4 + T cell's biomarker (CD4), CD8 + T cell's biomarkers (CD8A and CD8B), neutrophil's biomarkers (ITGAM) and M1 macrophage's biomarkers (IRF5) in HCC. These results further support the positive correlation between RRM2 and immune cell infiltration in HCC.

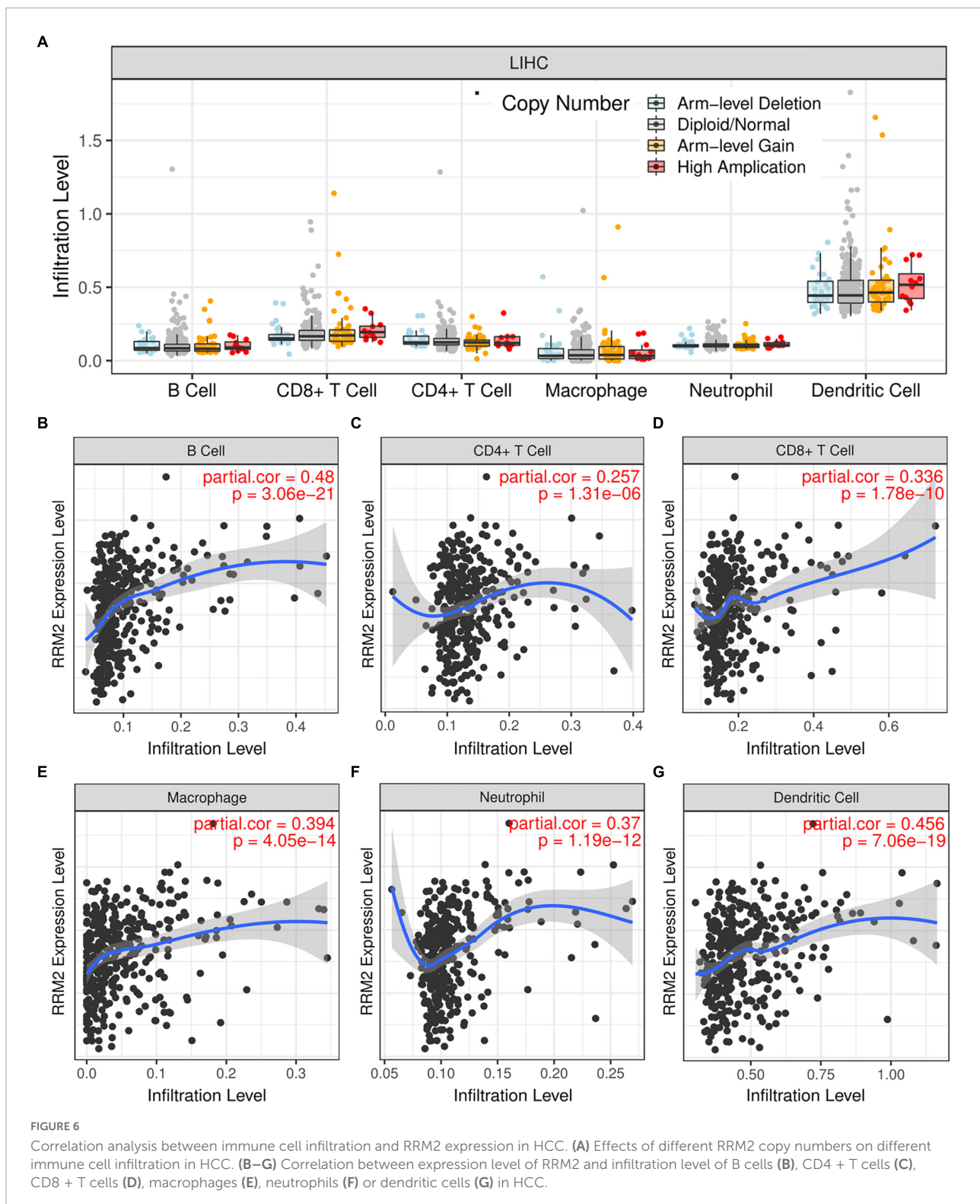
Expression correlation of RRM2 and immune checkpoints in hepatocellular carcinoma

PD1, PD-L1, and CTLA-4 were found to be immune checkpoints responsible for tumor immune escape and key targets of immunotherapy. We further evaluated the relationship between RRM2 and PD1, PD-L1 or CTLA-4 in HCC using TIMER and GEPIA. As presented in Figure 7, the analysis results of the two databases were consistent, showing that the expression of RRM2 in HCC was significantly positively related to the expression of PD1, PD-L1, and CTLA-4 in HCC. Altogether, our findings suggest that tumor immune escape may be involved in hepatocarcinogenesis mediated by RRM2.

Discussion

Hepatocellular carcinoma is known to have a poor prognosis. Various mechanisms have proved to be involved in the occurrence of HCC. Clarifying the molecular mechanism of HCC is helpful for the development of therapeutic targets, or the discovery of new and ideal prognostic clinical markers. A growing body of evidence shows that RRM2 plays a key role in the occurrence and progression of a variety of human cancers, including HCC. Growing evidence points to the RRM2 as a key role in the occurrence and progression of a variety of human cancers, including HCC. Yet, the knowledge about RRM2 in HCC is still insufficient, and further research is needed.

In this study, we first used TCGA to perform a pan-cancer expression analysis of RRM2, and then further used GEPIA



to confirm the expression of RRM2. Our results showed that RRM2 expression is elevated in most cancer types (including HCC), and this high expression indicates a poor prognosis in HCC patients. Studies have found that RRM2 showed

specifically elevated levels in HCC and inhibits ferroptosis by stimulating glutathione synthesis via glutathione synthetase, thus participating in the occurrence of HCC (20). Pei et al. (21) have proved that sorafenib can inhibit RRM2 and exert

TABLE 2 Association between RRM2 and immune cell biomarkers in HCC analyzed by GEPIA.

Immune cell	Biomarker	R-value	P-value
B cell	CD19	0.27 ^a	8.9E-08***
	CD79A	0.16 ^a	2.4E-03**
CD8 + T cell	CD8A	0.22 ^a	1.8E-05***
	CD8B	0.21 ^a	4.7E-05***
CD4 + T cell	CD4	0.32 ^a	3.3E-10***
M1 macrophage	NOS2	0.057	2.7E-01
	IRF5	0.37 ^a	1.1E-13***
	PTGS2	0.13	1.2E-02
M2 macrophage	CD163	0.12	1.7E-02*
	VSIG4	0.17 ^a	1.1E-03**
	MS4A4A	0.19 ^a	2.9E-04***
Neutrophil	CEACAM8	0.13	1.0E-02
	ITGAM	0.37 ^a	1.1E-13***
	CCR7	0.12	2.6E-02
Dendritic cell	HLA-DPB1	0.22 ^a	2.7E-05***
	HLA-DQB1	0.12	2.4E-02
	HLA-DRA	0.25 ^a	1.1E-06***
	HLA-DPA1	0.22 ^a	3.0E-05***
	CD1C	0.12	2.2E-02
	NRP1	0.18 ^a	3.6E-04***
	ITGAX	0.37 ^a	2.5E-13***

^ameans statistically significant.

*p-value < 0.05; **p-value < 0.01; ***p-value < 0.001.

anticancer activity. Together with our results, these reports show the carcinogenic effect of RRM2 in HCC.

A growing number of ncRNAs are being considered as regulators of various cellular processes, including cancer, and play a regulatory role through ceRNA mechanism (22–26). Given the involvement of ncRNAs in carcinogenesis and tumor heterogeneity, a deeper understanding of its molecular function is imperative. In this study, we searched for upstream miRNAs that might bind to RRM2 using starBase software. Through analysis, we finally obtained 29 candidate miRNAs, most of which were found to play a role as tumor suppressor in HCC. For instance, let-7c-5p increased the antitumor effect of 5-FU by inhibiting the expression of ABCC5/MRP5 (27). miR-30a-5p can reverse the resistance of sorafenib in HCC cells by directly targeting CLCF1, thereby exerting an anti-tumor effect (28). Let-7b-5p can slow down G2/M cell cycle transition by directly target CDC25B/CDK1 axis, and inhibit HCC cell metastasis and EMT progression by targeting HMGA2 (29). MiR-30b-5p acts as a tumor suppressor and inhibits cell proliferation and cell cycle in HCC by targeting DNMT3A and USP37 (30). Combined with our results, we believe that miR-125b-5p may be the most potential upstream regulator of RRM2 in HCC.

According to the ceRNA hypothesis, the potential upstream lncRNAs of miR-125b-5p/RRM2 axis should be carcinogenic

in HCC. Subsequently, we predicted the upstream lncRNAs of this axis and found 47 possible lncRNAs. Through a series of comprehensive analysis, two most potential candidate up-regulated lncRNAs were finally determined, namely CYTOR and MIR4435-2HG. Combined with literature reports, these two lncRNAs play a role as oncogenes in a variety of malignant tumors, including HCC. For example, CYTOR promotes the proliferation and cell cycle of hepatoma cells and inhibits the apoptosis of hepatoma cells through miR-125b-5p/KIAA1522 axis (31). MIR4435-2HG promotes HCC proliferation and metastasis through the miR-22-3p/YWHAZ axis (32) and miRNA-487a (33). Therefore, we identified lncRNAs (CYTOR and MIR4435-2HG) may be the two most potential upstream regulators of the miR-125b-5p/RRM2 axis in HCC.

Infiltration of solid tumors by immune cells is a hallmark of cancer and plays a key role in tumor progression (34). The tumor microenvironment may reprogram tumor-infiltrating immune cells to obtain tumor-promoting functions that promote tumor growth (35, 36). In addition to the characteristics of tumor autonomy, the pattern of invasive immune cell types is also related to tumor progression and patient prognosis (37, 38). This study showed that RRM2 was significantly positively related to most immune cells in HCC. In addition, RRM2 was also significantly positively associated with these biomarkers infiltrating immune cells. These results suggest that tumor immune infiltration could play a vital role in RRM2-mediated development of HCC. Based on our results, it seems that immune-infiltrating cells are negatively correlated with the prognosis of HCC patients, but we did not study the prognostic correlation between immune-infiltrating cells and HCC patients. This also reflects the complex role of immune infiltrating cells in HCC, which need further exploration.

The expression of immune checkpoint molecules in tumor tissues is very important for the efficacy of immune checkpoint blockade drugs (39–42). Immune checkpoint receptors can either inhibit or enhance immune response pathways (43). Therefore, we evaluated the correlation between RRM2 and immune checkpoints. Our findings showed that the high expression of RRM2 in HCC was closely related to PD1, PD-L1, and CTLA-4, suggesting that tumor immune escape may be involved in RRM2-mediated hepatocarcinogenesis, and RRM2 may be a synergistic therapeutic target related to immunotherapy in HCC.

In conclusion, we clarified that RRM2 is up-regulated in a variety of human cancers (including HCC), and is positively related to the poor prognosis of HCC. We suggested that lncRNAs (CYTOR and MIR4435-2HG) may be the two most potential upstream regulators of the miR-125b-5p/RRM2 axis in HCC (Figure 8). In addition, our current results also suggest that RRM2 may play a carcinogenic role by increasing immune cell infiltration and immune checkpoint expression. However, these findings still need to be further proved with more benchwork studies and clinical studies.

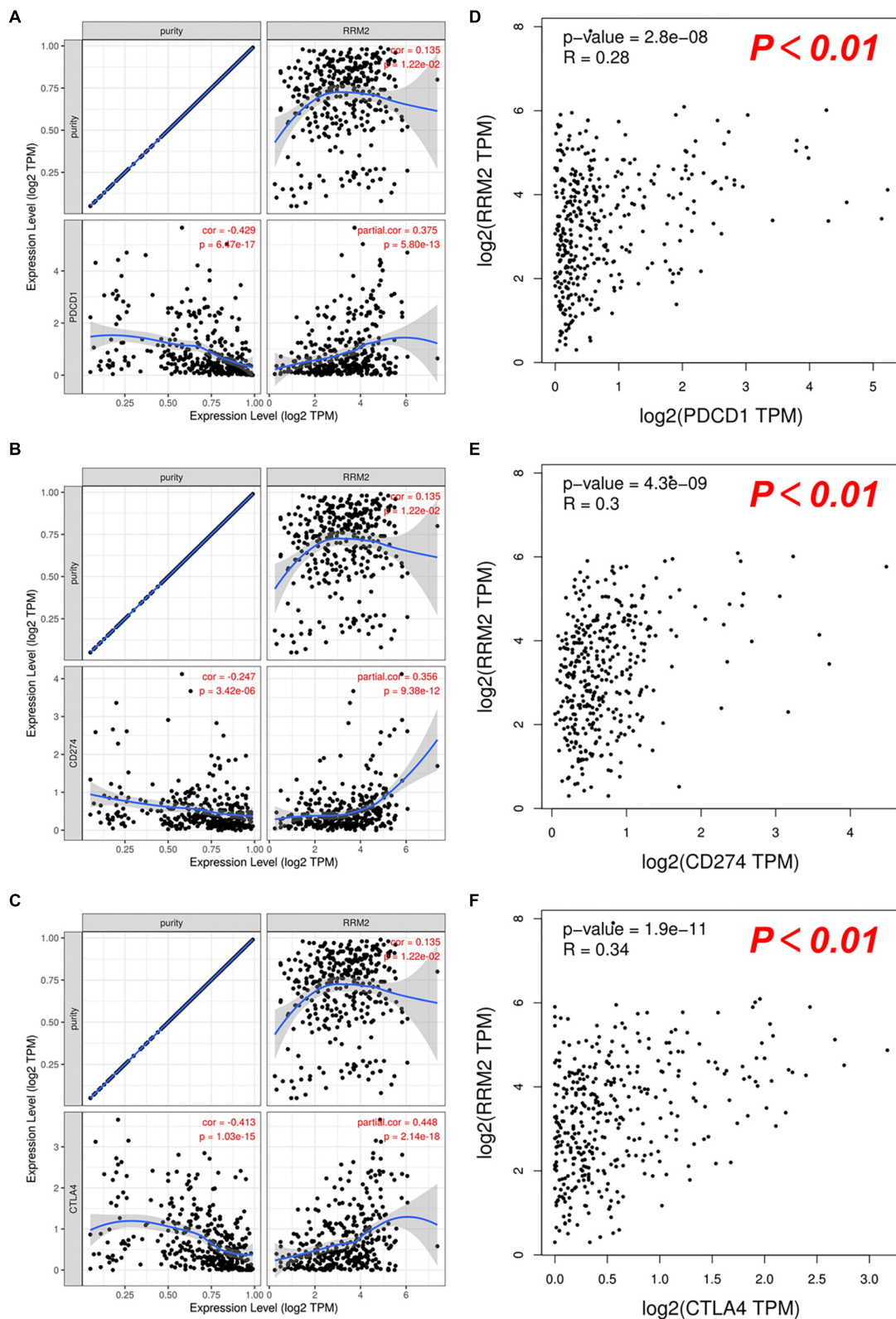
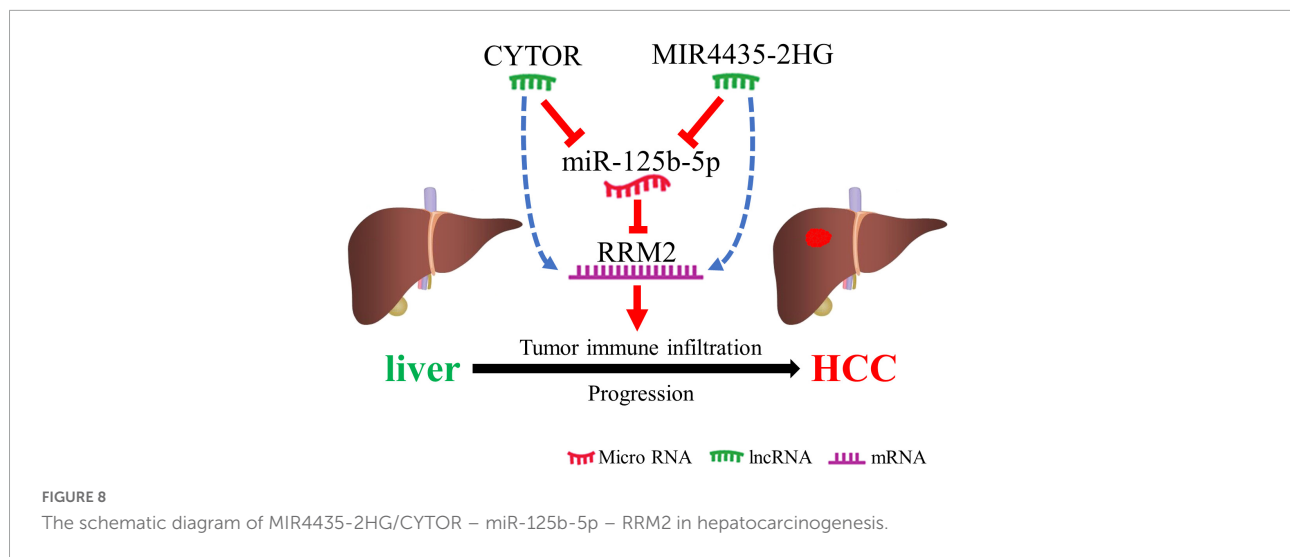


FIGURE 7

Correlation between RRM2 expression and PD-1, PD-L1 or CTLA-4 expression in HCC. (A–C) Spearman correlation of RRM2 with expression of PD-1 (A), PD-L1 (B), or CTLA-4 (C) in HCC determined by TIMER. (D–F) The correlation of RRM2 with expression of PD-1 (D), PD-L1 (E), or CTLA-4 (F) in HCC determined by GEPIA.



Materials and methods

mRNA expression analysis

All available mRNA expression data was downloaded from the following projects: TCGA-ALL. Fragments per kilobase per million (FPKM) were subsequently transformed to transcripts per million (TPM). The data of RRM2 expression in 18 cancer types (BRCA, BLCA, COAD, CHOL, ESCA, HNSC, GBM, KICH, KIRP, KIRC, LIHC, LUSC, LUAD, PRAD, STAD, READ, UCEC, and THCA) were performed using a Mann-Whitney U test with the R package ggplot2. This study complies with the publication guidelines and access rules of TCGA.

GEPIA

GEPIA is an RNA sequencing database derived from analysis of 9,736 tumors and 8,587 healthy samples from TCGA and GTEx (44). RRM2 and lncRNA expression profiles in patients across cancers were matched with TCGA normal and GTEx data, and analyzed using GEPIA. GEPIA was also used to perform survival analysis of RRM2 and lncRNAs in HCC with log-rank test, including OS and RFS. The expression correlation between RRM2 and immune checkpoints in HCC was also determined using Spearman test within GEPIA. For correlation analysis, $|R| > 0.1$ and $p < 0.01$ were set as the criteria for identifying significant interactive pairs.

Identification of candidate novel miRNA

Candidate novel miRNAs to regulate RRM2 expression were predicted using following miRNA target prediction

databases: PITA, miRmap, RNA22, microT, PicTar, TargetScan, and miRanda. Only miRNAs that were predicted by at least two out of the seven databases and were then retained for subsequent steps. Finally, the filtered ones were marked as “candidate” miRNAs of RRM2.

Upstream lncRNAs prediction

starBase collects miRNA-ceRNA, miRNA-ncRNA, and protein-RNA interaction networks from large-scale CLIP-Seq data, and is usually used to study lncRNA-miRNA-mRNA networks (45). The starBase 3.0 software was used to identify potential complementary lncRNA binding partners for miR-125b-5p. Then, the starBase was employed to study the expression correlation between lncRNA and miR-125b-5p or lncRNA and RRM2 in HCC.

Kaplan-Meier plotter

Kaplan-Meier plotter¹, an *in silico* online tool was used to predict survival of cancer types patients depending on the different gene expression (46). Survival analysis for candidate ncRNAs (lncRNAs and miRNAs) in HCC was achieved using Kaplan-Meier plotter.

TIMER

TIMER database is a web tool for the comprehensive analysis and visualization of immune cells infiltration in tumors.

¹ <http://kmplot.com/analysis/>

It provides the infiltration of six kinds of immune cells (CD4 + T cells, CD8 + T cells, B cells, neutrophils, macrophages and dendritic cells). The correlation between RRM2 expression and tumor-infiltrating or immune checkpoint expression in HCC was analyzed and visualized using Spearman's rho value within TIMER.

Statistical analysis

The statistical analysis was automatically calculated by the online database mentioned above or via the R software (v.4.1.3). A p -value < 0.01 or log-rank p -value < 0.01 was considered to be statistically significant.

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

GM and SZ conceived and designed the research. CS performed the data collection, bioinformatics analysis, and wrote the initial manuscript. BL and LM analyzed the data and generated the figures and tables. GM and LL conceptualized and revised the manuscript. All authors approved the final manuscript.

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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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2022.833301/full#supplementary-material>

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