



Comprehensive Analyses of the Expression, Genetic Alteration, Prognosis Significance, and Interaction Networks of m⁶A Regulators Across Human Cancers

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Accumulating lines of evidence indicate that the deregulation of m⁶A is involved in various cancer types. The m⁶A RNA methylation is modulated by m⁶A methyltransferases, demethylases, and reader proteins. Although the aberrant expression of m⁶A RNA methylation contributes to the development and progression of multiple cancer types, the roles of m⁶A regulators across numerous types of cancers remain largely unknown. Here, we comprehensively investigated the expression, genetic alteration, and prognosis significance of 20 commonly studied m⁶A regulators across diverse cancer types using TCGA datasets via bioinformatic analyses. The results revealed that the m⁶A regulators exhibited widespread dysregulation, genetic alteration, and the modulation of oncogenic pathways across TCGA cancer types. In addition, most of the m⁶A regulators were closely relevant with significant prognosis in many cancer types. Furthermore, we also constructed the protein–protein interacting network of the 20 m⁶A regulators, and a more complex interacting regulatory network including m⁶A regulators and their corresponding interacting factors. Besides, the networks between m⁶A regulators and their upstream regulators such as miRNAs or transcriptional factors were further constructed in this study. Finally, the possible chemicals targeting each m⁶A regulator were obtained by bioinformatics analysis and the m⁶A regulators–potential drugs network was further constructed. Taken together, the comprehensive analyses of m⁶A regulators might provide novel insights into the m⁶A regulators' roles across cancer types and shed light on their potential molecular mechanisms as well as help develop new therapy approaches for cancers.

Keywords: m⁶A methylation, bioinformatics, TCGA, cancer, comprehensive analyses

INTRODUCTION

A variety of biological processes are orchestrated by post-transcriptional modifications including RNA modifications (Zhao et al., 2017). As the most common type of RNA methylation modifications, N⁶-methyladenosine (m⁶A), first unraveled in 1970s, modulates the corresponding target RNAs *via* influencing RNA translation, degradation, splicing, folding, or stability (He and He, 2021). Although studies had revealed that one to two m⁶A residues were found in an average of one thousand nucleotides, nearby the 3' untranslated region (UTR), stop codon as well as long internal exon might exhibit the relatively richer m⁶A in mRNAs (Meyer et al., 2012). In addition to mRNAs, m⁶A RNA methylation was also found to be distributed in other RNAs, such as ribosomal RNA (rRNA) and RNAs of bacteria and viruses (Ma et al., 2019).

The regulators involved in modulating m⁶A methylation include three types of proteins called “writer”, “eraser”, and “reader”, respectively (Zaccara et al., 2019). The writers consisting of m⁶A methyltransferases such as METTL3, METTL14, and their corresponding cofactors like RBM15 and WTAP exhibit in cellular nuclei and increase the m⁶A levels (Meyer and Jaffrey, 2017). On the contrary, the erasers, also being discovered in the cellular nuclei, are m⁶A demethylase enzymes such as FTO and ALKBH5, which remove the m⁶A and thus result in reducing the m⁶A levels (Zhang et al., 2017). Moreover, the readers such as IGF2BP1 and RBMX, distributing in both cellular nuclei and cytoplasm, can decode the m⁶A methylation information *via* binding to the m⁶A sites and further initiate the different downstream signals (Sun et al., 2019). The processes of m⁶A methylation are reversible and dynamic, which are homeostatically modulated by these writers, erasers, and readers (Chen et al., 2019).

Since the m⁶A regulators acted as crucial roles in a variety of biological processes, the abnormalities of m⁶A methylation might lead to multiple kinds of diseases including neuronal diseases, diabetes, immunological disorders, liver metabolic disorders, and numerous cancer types (He et al., 2019). For example, recent studies had demonstrated that the decreased RNA methylation of critical genes in β -cell markedly contributed to the pathophysiology of human T2D (De Jesus et al., 2019). Additionally, METTL3 was found to have dramatical overexpression in hepatocellular carcinoma (HCC), and the depletion of METTL3 contributed to the significant suppression of the HCC growth and metastasis (Chen et al., 2018). Besides, findings had uncovered that YTHDF2, an m⁶A reader, was markedly upregulated in human acute myeloid leukemia (AML), and targeting YTHDF2 might compromise the cancer stem cells in AML (Paris et al., 2019).

Although the m⁶A methylation has been identified as the most abundant modification of RNAs, and served as crucial regulators in diverse biological processes and diseases including numerous types of cancers, the relevant factors involved in that modification are still not completely discovered, and their associated molecular mechanisms, expression, and interacting networks remain unclear. Therefore, in the present study, we comprehensively investigated the expression, genetic alteration, and prognosis significance of 20 commonly studied m⁶A regulators across diverse cancer types using TCGA *via*

bioinformatic analyses. In addition, we also constructed the networks between m⁶A regulators and potential chemical drugs, miRNAs, or upstream transcriptional factors. These comprehensive analyses of m⁶A regulators might provide novel understanding of these m⁶A regulators' roles across cancer types and shed light on their potential molecular mechanisms in cancers as well as helping developing new therapy approaches for cancers.

MATERIALS AND METHODS

The Gene Expression and Methylation Analyses of m⁶A Regulators

The expression of the gene set (the 20 m⁶A methylation regulators) and m⁶A regulators' interacting proteins across diverse cancer types based on TCGA data was analyzed using the GSCALite database (Liu et al., 2018). Besides, we also analyzed the expression of 20 m⁶A methylation regulators through R software package using microarray data (GSE11969, GSE63898, GSE37182, GSE22820, GSE54129, GSE53757, GSE23036, GSE33630, and GSE11024) from the Gene Expression Omnibus (GEO) datasets. The heatmaps of these GEO data were displayed by the R software package pheatmap. The expression of IGF2BP1, IGF2BP2, IGF2BP3, SP1, ELK1, and EGR1 across diverse TCGA cancer types was analyzed using the UALCAN database (Chandrashekar et al., 2017). In addition, the methylation of the gene set, and the correlation between the methylation and m⁶A methylation regulators' gene expression were also analyzed using GSCALite database.

The Genetic Alteration Analyses of the m⁶A Regulators

The single nucleotide variations (SNVs) and copy number variations (CNVs) of the m⁶A regulators across cancer types were analyzed by the GSCALite database using TCGA data. The SNV-oncoplot and CNV-percent-profile (CNV pie plots) were also generated by GSCALite database. In addition, the bubble plots describing the correlation between CNV and m⁶A methylation regulators' mRNA expression were generated by GSCALite database based on TCGA data. Besides, the genetic alterations of the 20 m⁶A regulators were also analyzed by cBioportal database (Cerami et al., 2012).

The Oncogenic Pathway Analyses of the m⁶A Regulators and Protein-Protein Interaction Network Construction

The m⁶A methylation regulators-related oncogenic pathways were analyzed by GSCALite database. The pathway activity pie plots, and the interaction map of genes and pathways were also generated using the GSCALite database. The protein-protein interaction (PPI) networks were generated by STRING database (Szklarczyk et al., 2019).

The Overall Survivals Analyses

The overall survivals of the m⁶A methylation regulators across cancer types were analyzed by the GSCALite database. The overall survivals

of the m⁶A regulators in kidney renal clear cell carcinoma (KIRC) were evaluated by the GEPIA database based on TCGA data.

The m⁶A Regulators–Drug Interacting Network Construction

The potential chemicals targeting each m⁶A regulator were obtained by applying the comparative toxicogenomics database (CTD) (Davis et al., 2021). Thereafter, the chemicals and their corresponding m⁶A regulator were inputted into Cytoscape software (Shannon et al., 2003) to generate the m⁶A regulators–drug interacting network.

The Generation of the MiRNAs–m⁶A Regulators Network

First, the potential miRNAs targeting each m⁶A regulator were predicted by miRDB (Chen and Wang, 2020), targetScan (Agarwal et al., 2015), and starbase (Li et al., 2014). Subsequently, the overlapping miRNAs (commonly expressed in the prediction of miRDB, targetScan, and starbase) were obtained using the VENNY 2.1 database (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>). Then, the Cytoscape software was utilized for generating the miRNAs–m⁶A regulators networks.

Constructing the Transcription Factors–m⁶A Regulators Network

The potential TFs targeting each group of m⁶A regulators (writers, erases, and readers) were obtained by KnockTF database (Feng et al., 2020). The erasers–TFs interacting network, writers–TFs interacting network, and readers–TFs interacting network were also generated by KnockTF database. In addition, the top 25 potential TFs were achieved exhibited by doughnut plots using FunRich software (Pathan et al., 2015).

The Interacting Networks Construction and Gene Ontology and Biological Pathway Analyses

The m⁶A regulators' interacting proteins were obtained by FunRich software, and the interacting regulatory network including m⁶A regulators and their corresponding interacting proteins was then constructed by FunRich software. The FunRich software was also utilized for investigating the GO analyses and biological pathways of the m⁶A regulators interacting proteins. The column diagrams (exhibiting CC: cellular component; MF: molecular function; BP: biological process) and doughnut plots (exhibiting biological pathways) were also generated using FunRich software.

RESULTS

The Expression of m⁶A Methylation Regulators Across Cancer Types

The m⁶A methylation regulators could be clarified into three types: writers, erasers, and readers (Figure 1A). The reports

relevant with m⁶A methylation regulators in recent years were reviewed and a total of 20 genes (writers: 7; erasers: 2; readers: 11) were included in this study (Figure 1B). Next, we sought to evaluate the expressing levels of these 20 m⁶A methylation regulators across diverse cancer types using TCGA datasets. By searching the GSCALite database, we found that the expressions of many m⁶A methylation regulators (especially IGF2BP1, IGF2BP2, and IGF2BP3) were changed across multiple cancer types (Figure 1C). Furthermore, the detail expressing situations of IGF2BP1, IGF2BP2, and IGF2BP3 were evaluated by applying UALCAN algorithm. The results revealed that the levels of IGF2BP1, particularly IGF2BP2 and IGF2BP3, were remarkably upregulated in many cancer types such as bladder urothelial carcinoma (BLCA), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), and lung squamous cell carcinoma (LUSC) (Figure 1D). In addition, to further verify the above results using TCGA data, we also analyzed the expression of 20 m⁶A regulators using microarray data from GEO datasets in many cancer types including LUAD, LUSC, LIHC, colon adenocarcinoma (COAD), breast invasive carcinoma (BRCA), stomach adenocarcinoma (STAD), kidney renal clear cell carcinoma (KIRC), head and neck squamous cell carcinoma (HNSC), thyroid carcinoma (THCA), and kidney chromophobe (KICH). The results revealed that the expression of many m⁶A regulators including IGF2BP1, IGF2BP2, and IGF2BP3 were notably changed, which was consistent with the results from TCGA analysis (Supplementary Figure S1). Given that the methylation of genes was able to influence genes expression, we next attempted to investigate the methylation of the 20 m⁶A methylation regulators across cancer types. Through searching GSCALite database, we found that the majority of the 20 m⁶A regulators' methylation was lower in the tumor samples than that of the normal control samples in prostate adenocarcinoma (PRAD), LIHC, LUSC, KIRC, BLCA, and THCA (Figure 1E). Besides, the correlation between the methylation and m⁶A methylation regulators' gene expression was further analyzed using the GSCALite database. According to the data, we found that most of these m⁶A regulators' expression was negatively correlated with the methylation across diverse cancer types, which was consistent with the above finding that the majority of the 20 m⁶A regulators' methylation was downregulated in the tumor samples of many cancer types (Figure 1F). Overall, our data suggested that the expression and methylation of the 20 m⁶A regulators were remarkable dysregulation across many cancer types.

Genetic Alterations of m⁶A Regulators Across Cancer Types

Considering that the alteration of the genome might always affect the gene expression, we next attempted to explore the genetic alterations including single nucleotide variations (SNVs) and copy number variations (CNVs) of the m⁶A regulators across cancer types. First, the GSCALite database was utilized for analyzing the SNVs of the 20 m⁶A regulators. The results suggested that the SNVs of the 20 m⁶A regulators altered in 74.8% TCGA samples across cancer types, and the waterfall plots

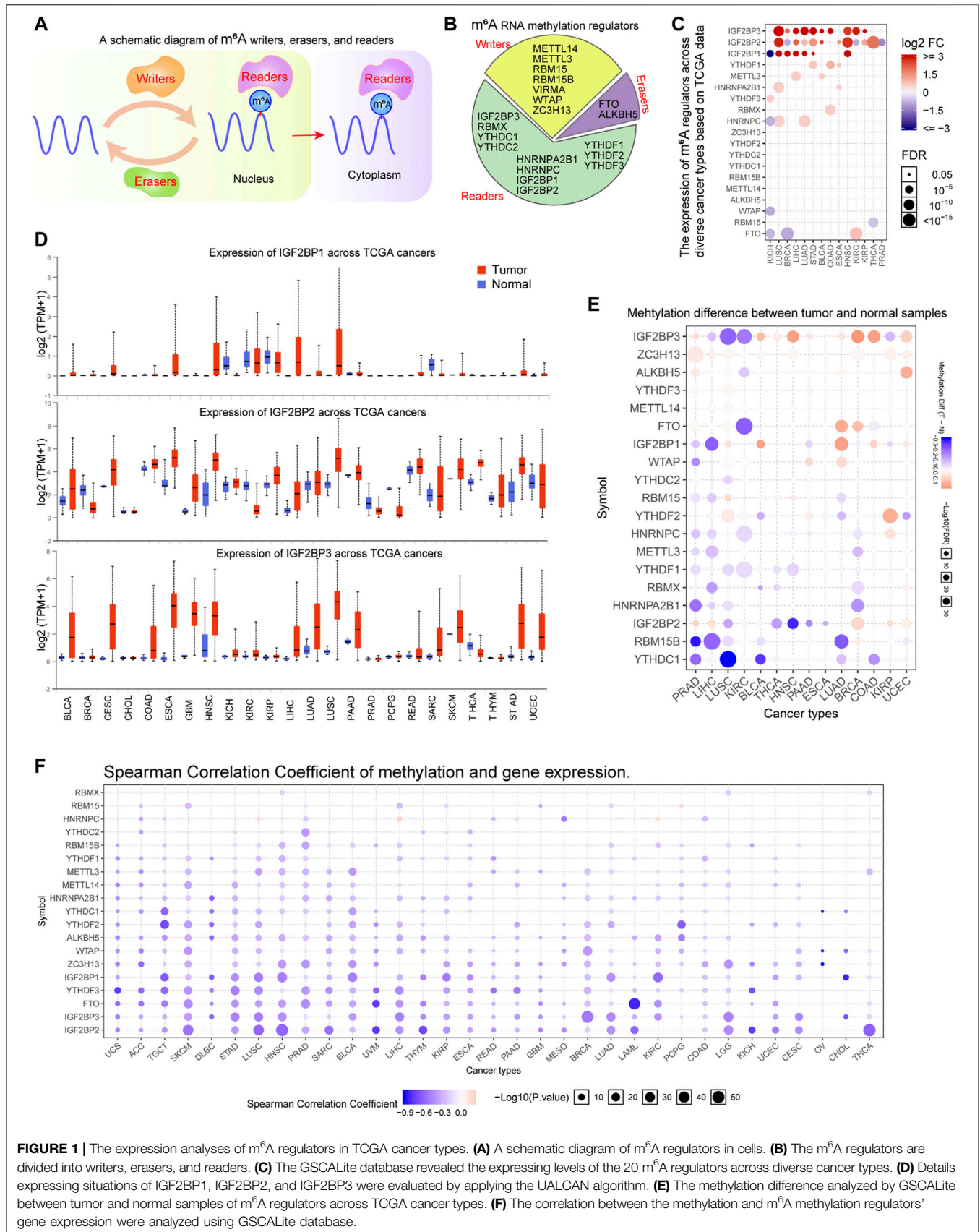


FIGURE 1 | The expression analyses of m⁶A regulators in TCGA cancer types. **(A)** A schematic diagram of m⁶A regulators in cells. **(B)** The m⁶A regulators are divided into writers, erasers, and readers. **(C)** The GSCALite database revealed the expressing levels of the 20 m⁶A regulators across diverse cancer types. **(D)** Details expressing situations of IGF2BP1, IGF2BP2, and IGF2BP3 were evaluated by applying the UALCAN algorithm. **(E)** The methylation difference analyzed by GSCALite between tumor and normal samples of m⁶A regulators across TCGA cancer types. **(F)** The correlation between the methylation and m⁶A methylation regulators' gene expression were analyzed using GSCALite database.

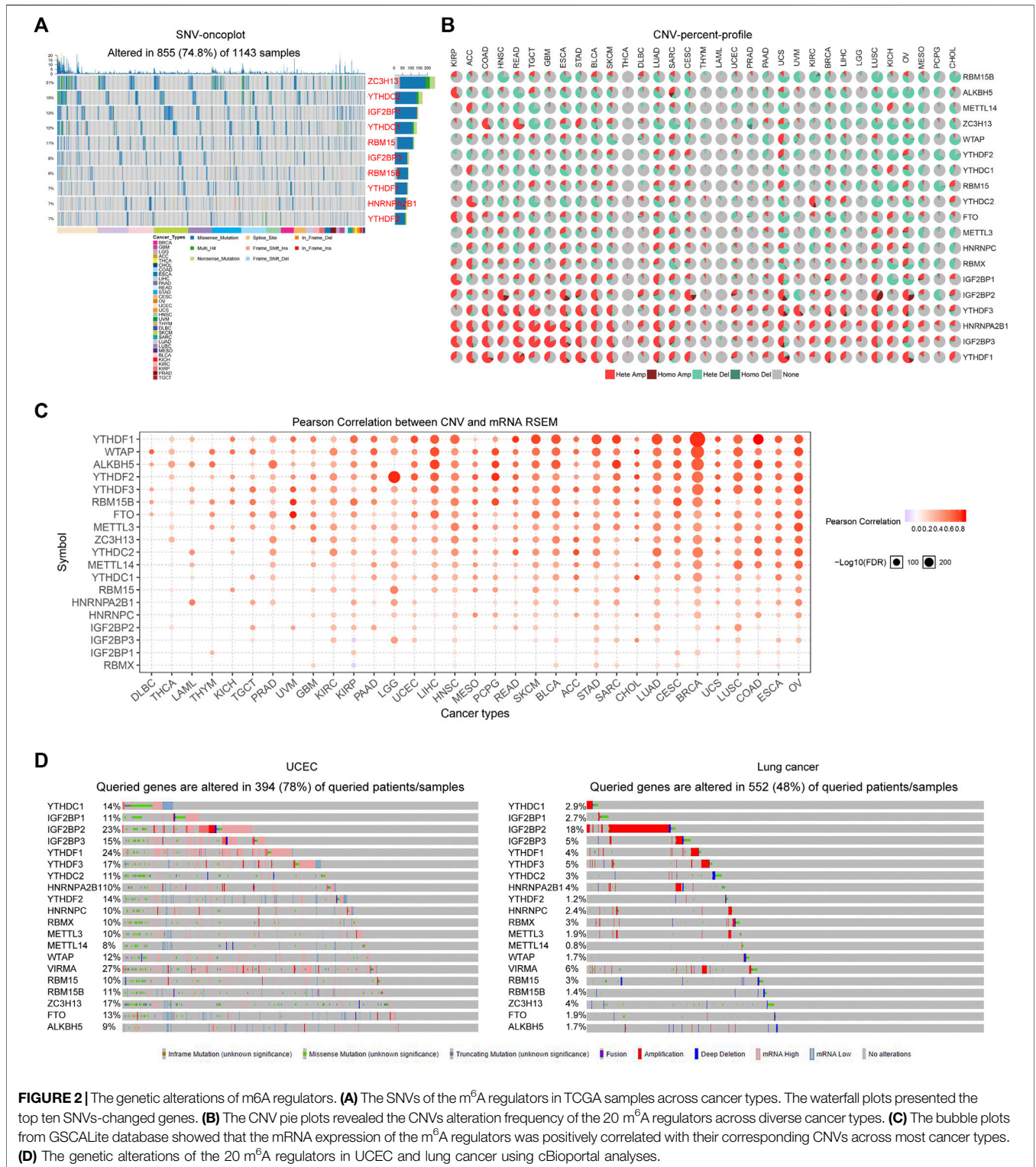
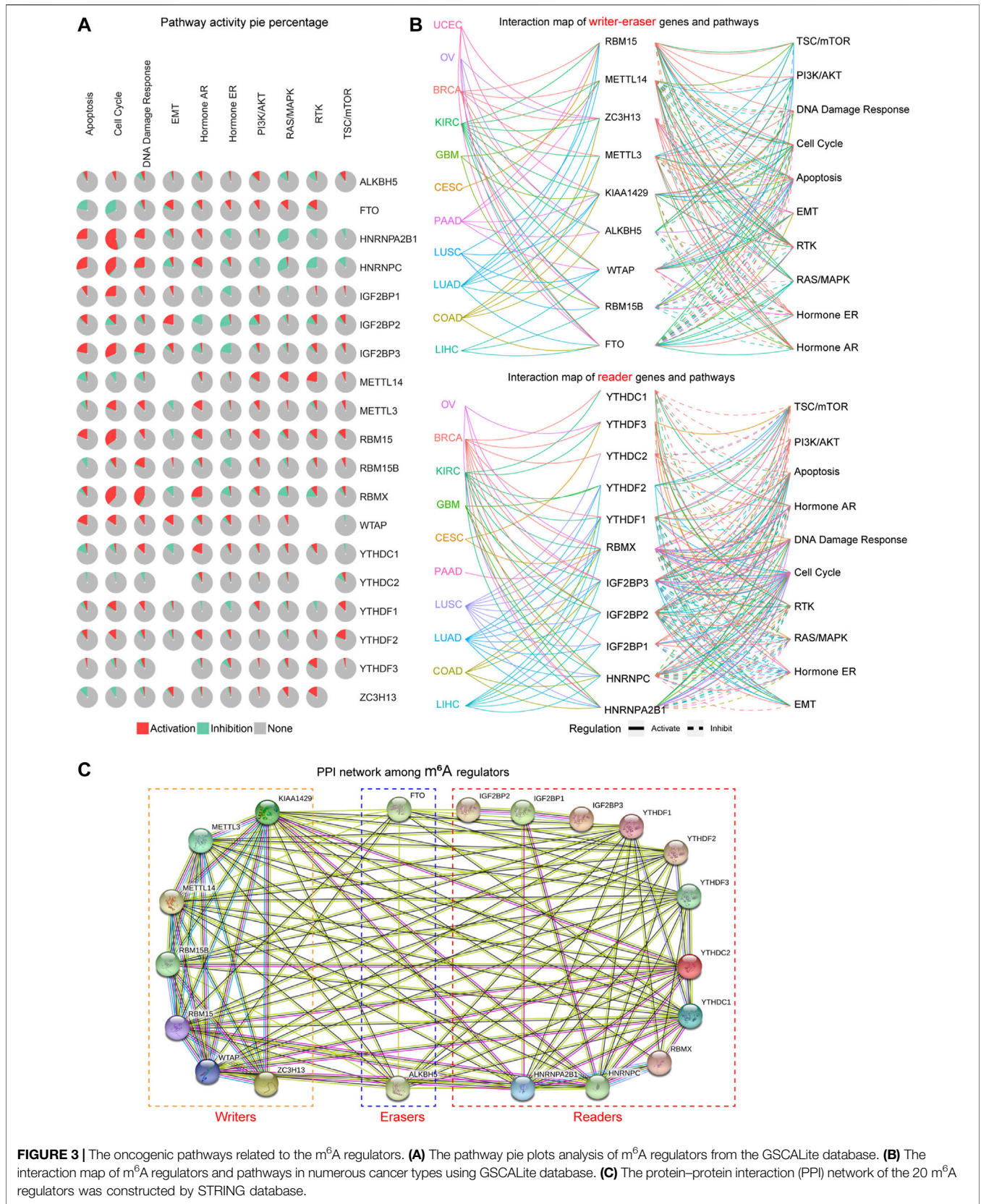


FIGURE 2 | The genetic alterations of m⁶A regulators. **(A)** The SNVs of the m⁶A regulators in TCGA samples across cancer types. The waterfall plots presented the top ten SNVs-changed genes. **(B)** The CNV pie plots revealed the CNVs alteration frequency of the 20 m⁶A regulators across diverse cancer types. **(C)** The bubble plots from GSCALite database showed that the mRNA expression of the m⁶A regulators was positively correlated with their corresponding CNVs across most cancer types. **(D)** The genetic alterations of the 20 m⁶A regulators in UCEC and lung cancer using cBioportal analyses.

presented the top ten SNVs-changed genes, such ZC3H13, YTHDC2, and IGF2BP1 (Figure 2A). Thereafter, we sought to investigate the CNVs alteration frequency for the 20 m⁶A regulators using the GSCALite database. The CNV pie plots revealed that several readers (YTHDF1, YTHDF3, IGF2BP1,

IGF2BP2, IGF2BP3, and HNRNPA2B1) exhibited very high percentages of heterozygous CNVs, particularly amplification (Hete Amp) in multiple cancer types, while genes like RBM15B, ALKBH5, METTL14, ZC3H13, and WTAP had high percentages of heterozygous CNVs with depletion (Hete Del)



(**Figure 2B**). Afterwards, we attempted to explore whether these m⁶A regulators' CNVs alterations were able to influence the expression of their mRNA expression. The bubble plots from GSCALite database demonstrated that the mRNA expression of the majority of the m⁶A regulators was positively correlated with their corresponding CNVs across most cancer types, which indicated that CNVs alterations could remarkably promote m⁶A regulators' expression (**Figure 2C**). In addition, the genetic alterations of the 20 m⁶A regulators in TCGA cancer types were also investigated using cBioportal database. The data suggested that the genetic alterations of the m⁶A regulators were remarkably high in tumor specimens of many cancer types, particularly uterine corpus endometrial carcinoma (UCEC; with 78% genetic alterations) and lung cancer (with 48% genetic alterations) (**Figure 2D**). Taken together, these results revealed that the m⁶A regulators exhibited widespread genetic alterations across cancer types, and these genetic alterations could significantly affect their expression.

The Analyses of the Oncogenic Pathways Relevant With the m⁶A Regulators

Next, we attempted to investigate whether these m⁶A regulators were associated with oncogenic pathways. According to the results of the pathway pie plots from GSCALite database, we found that HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP3, RBM15, and RBMX were markedly related with the activation of the cell cycle (**Figure 3A**). In addition, the pathway pie plots also showed that FTO was relevant with the inhibition of apoptosis and cell cycle; HNRNPA2B1 and HNRNPC were significantly correlated with the inhibition of the RAS/MAPK pathway; RBMX was also related with the activation of DNA damage response pathway (**Figure 3A**). Besides, the 20 m⁶A regulators were divided into two groups (writer–eraser genes and reader genes) to respectively construct the interaction map of genes and pathways using the GSCALite database, and the results further confirmed the above findings that many m⁶A regulators were associated with the activation or inhibition of these famous cancer-related pathways across TCGA cancer types (**Figure 3B**). Furthermore, considering that genes always exerted their functions *via* interacting with other genes, we thereby next sought to investigate the interaction among these writers, erasers, and readers. The protein–protein interaction (PPI) network of the 20 m⁶A regulators was constructed by the STRING database, and the PPI network demonstrated that the m⁶A regulators interacted with each other with very high frequency, which indicated that the m⁶A methylation in cancers might be regulated by collaboration among writers, erasers, and readers (**Figure 3C**). Collectively, these data validated that the m⁶A regulators could modulate the oncogenic pathways *via* collaboration.

Prognosis Significance of the m⁶A Regulators Across Cancer Types

Since the m⁶A regulators were dysregulated in many cancer types and several of them were closely relevant with oncogenic pathways, we next sought to explore whether the aberrant expression of the m⁶A regulators was associated with

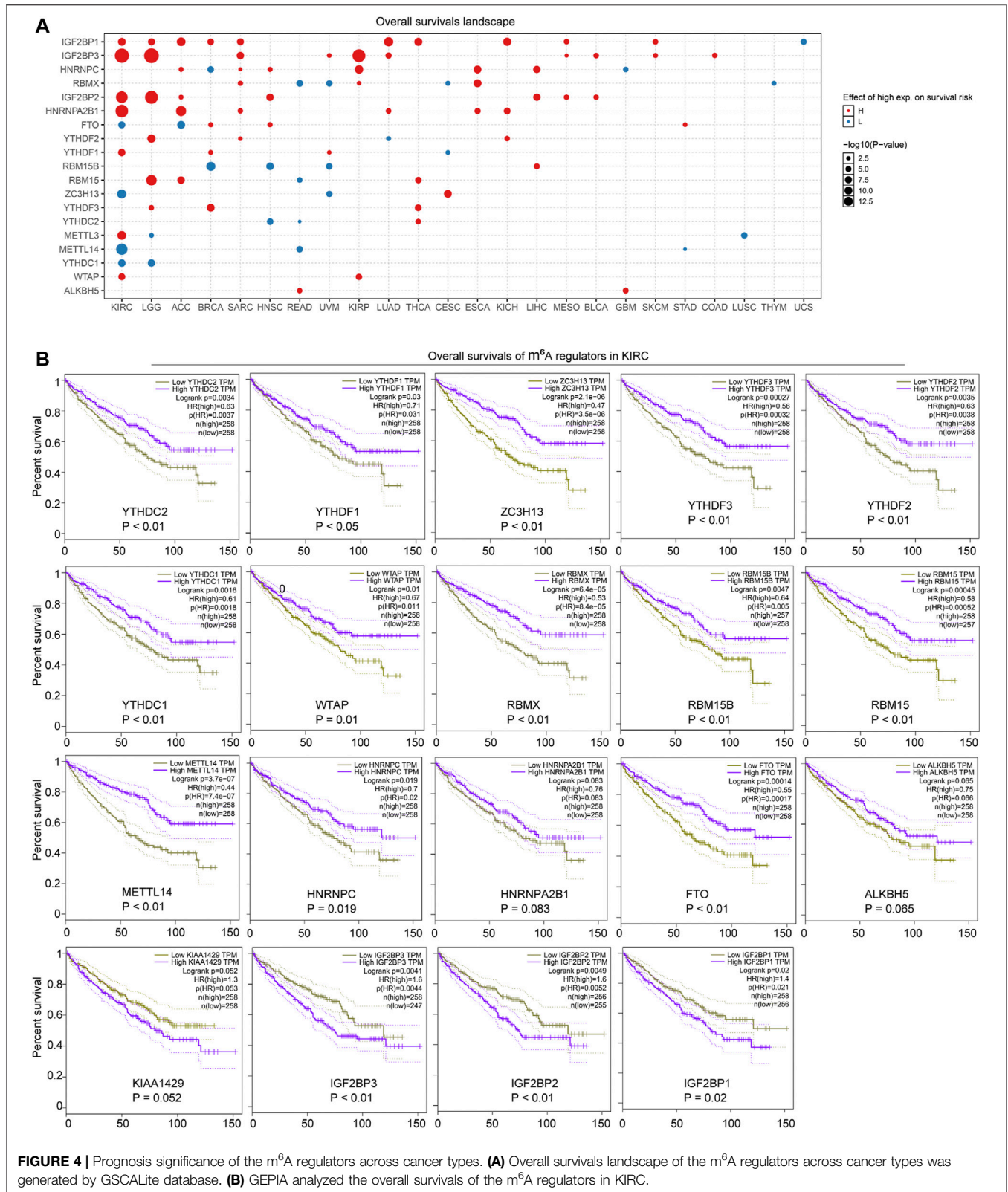
prognosis significance. After inputting the gene set of the m⁶A regulators into the GSCALite database, we found that most of the m⁶A regulators were associated with overall survivals across TCGA cancer types (**Figure 4A**). Particularly, more than half of the 20 m⁶A regulators were notably correlated with poor or good prognosis in multiple cancer types, such as KIRC, brain lower grade glioma (LGG), adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), and sarcoma (SARC). Therefore, we next attempted to investigate the detailed overall survivals of the m⁶A regulators (high or low expression) in KIRC. The overall survivals of the 20 m⁶A regulators in KIRC were analyzed by applying the GEPIA database. The results validated that 19 of the 20 m⁶A regulators were dramatically correlated with significantly good or poor prognosis (**Figure 4B**). Especially, high expression of all the erasers (FTO and ALKBH5) and most writers (METTL14, RBM15, RBM15B, WTAP, and ZC3H13) and readers (YTHDF1, YTHDF2, YTHDF3, HNRNPA2B1, HNRNPC, YTHDC1, YTHDC2, and RBMX) was significantly with poor prognosis, while the high expression of VIRMA (also named KIAA1429; a writer) and IGF2BPs (IGF2BP1, IGF2BP2, and IGF2BP3; readers) predicted good prognosis (**Figure 4B**). Therefore, these data revealed that the dysregulation of the m⁶A regulators was remarkably associated with significant prognosis in many cancer types, especially KIRC, which indicated that the aberrant expression of the m⁶A regulators might be a prognostic marker in cancers including KIRC.

The Construction of the m⁶A Regulators–Potential Drugs Network

Since the above findings revealed that the dysregulation of the m⁶A regulators might be correlated with tumor progression, we next thought to investigate whether there were some potential chemicals that could increase or decrease the expression of the m⁶A regulators. First, the comparative toxicogenomics database (CTD) was utilized for analyzing the possible chemicals targeting each m⁶A regulator. Afterwards, the 20 m⁶A regulators were divided into three groups (writers, erasers, and readers) and we subsequently drew three sub gene–drug interaction networks (writers–drugs interaction network, erasers–drugs interaction network, and readers–drugs interaction network), using Cytoscape software. The m⁶A regulators–potential drugs network is presented in **Figure 5**, and these chemicals were able to increase or decrease the expression of the m⁶A regulators (**Supplementary Table S1**). Therefore, the m⁶A regulators–potential drugs network provided benefits for potential drugs discovery to target specific m⁶A regulators.

The Upstream MiRNAs–m⁶A Regulators Network

Although our above findings demonstrated that the methylation and genetic alterations were capable to affect the expression of the m⁶A regulators across TCGA cancer types, there might be other factors such as miRNAs that could also contribute to the dysregulation of the m⁶A regulators in cancers. Therefore, we next sought to investigate the potential upstream miRNAs, which



were able to target these m⁶A regulators. First, we employed three classical miRNAs predicting databases—miRDB, targetScan, and starbase—to predict the possible miRNAs that could target each

m⁶A regulator. Thereafter, the common miRNAs targeting each m⁶A regulator in the three databases were selected. Subsequently, we applied the Cytoscape software to generate the miRNAs–m⁶A

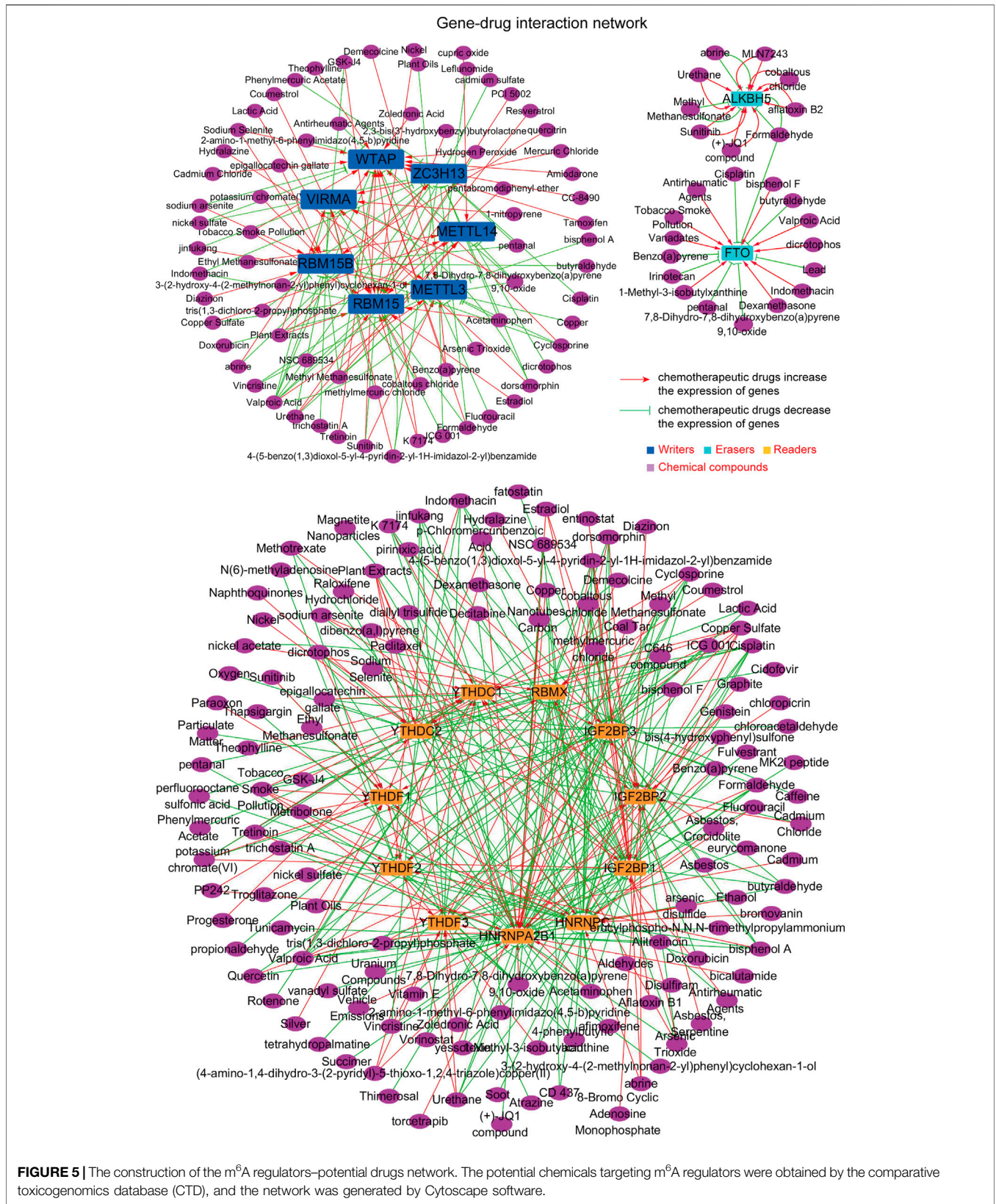
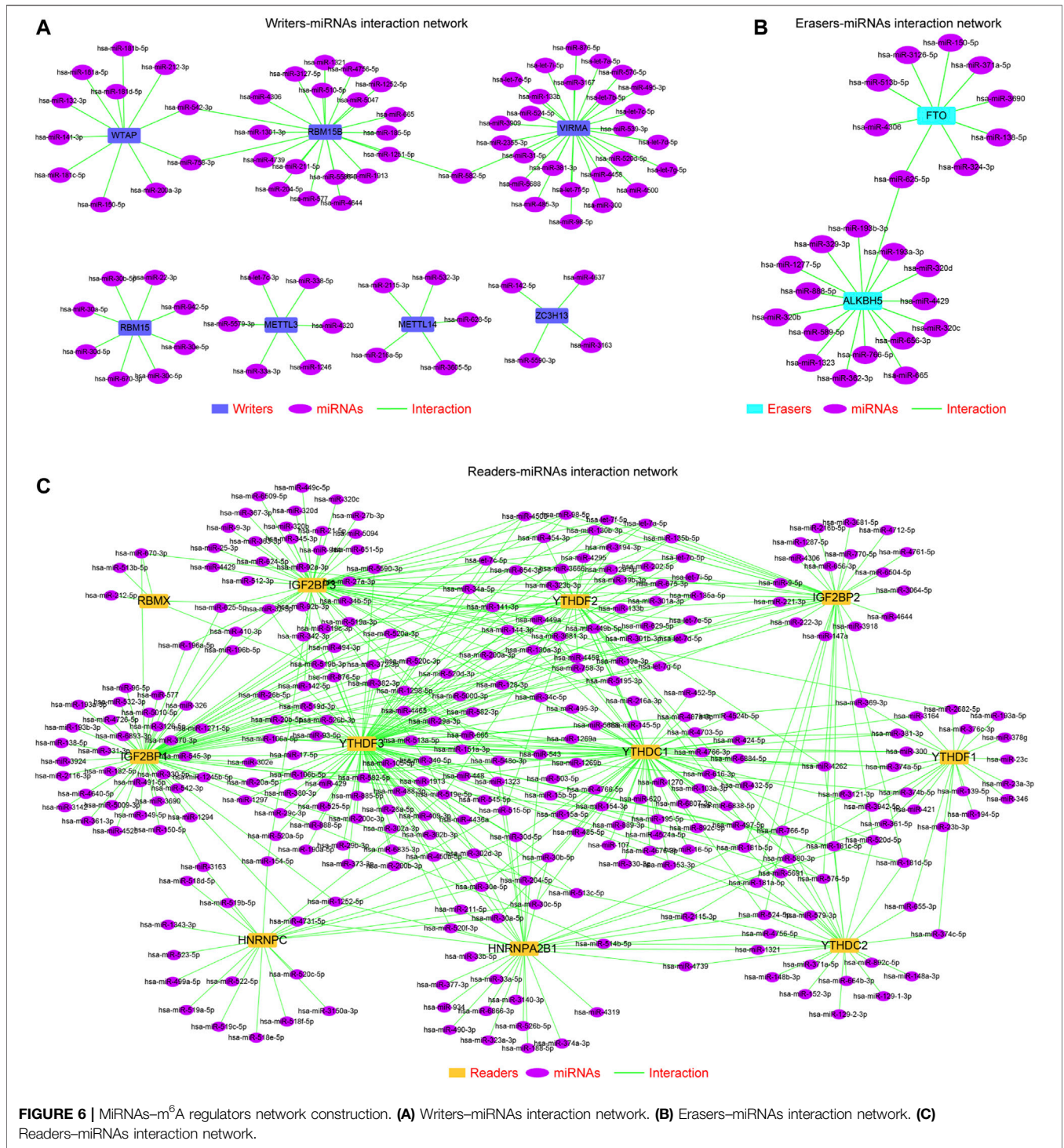


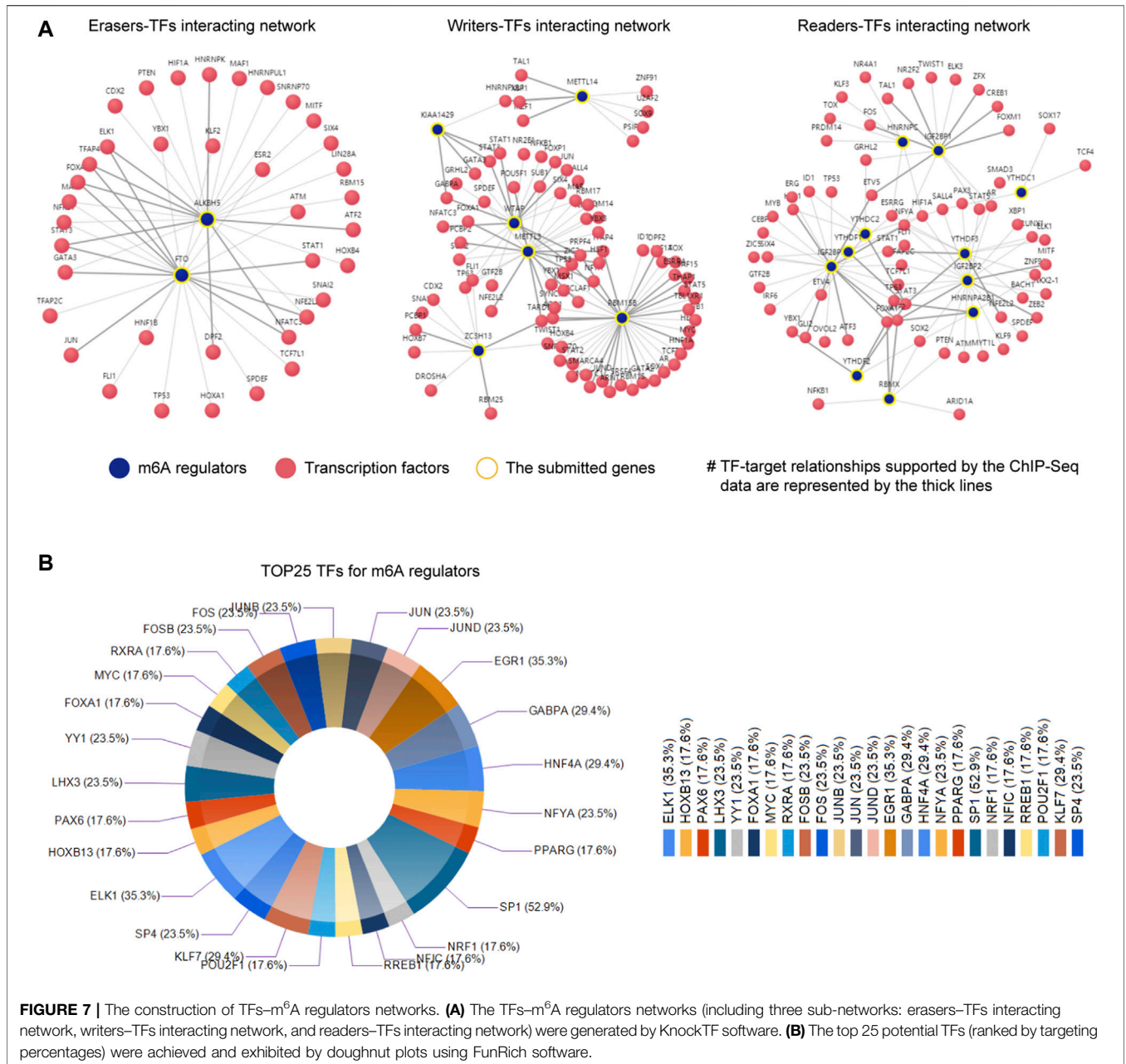
FIGURE 5 | The construction of the m⁶A regulators–potential drugs network. The potential chemicals targeting m⁶A regulators were obtained by the comparative toxicogenomics database (CTD), and the network was generated by Cytoscape software.



regulators networks, including the writers-miRNAs interaction network (Figure 6A), erasers-miRNAs interaction network (Figure 6B), and readers-miRNAs interaction network (Figure 6C). These miRNAs-m⁶A regulators networks provided new supplementary knowledge about the modulation of the m⁶A regulators' dysregulation across cancer types.

The Upstream TFs-m⁶A Regulators Networks

Besides, the transcription factors (TFs) could also contribute to the dysregulation of m⁶A regulators. Hence, we next sought to uncover the potential TFs that were capable to modulate the expression of the m⁶A regulators. To achieve that, we first utilized an online database,

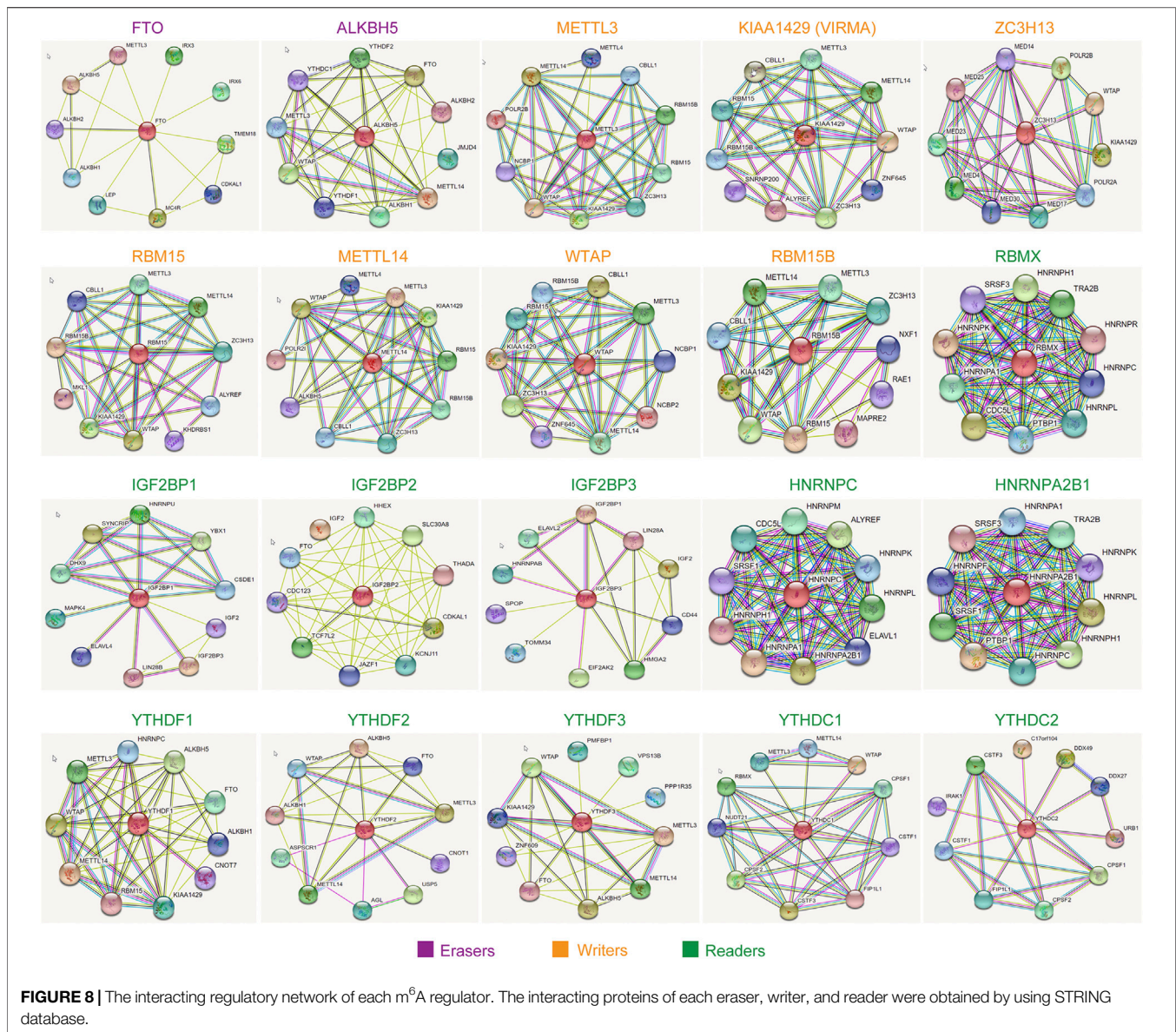


KnockTF, to analyze the possible TFs of the writers, erases, and readers, respectively. We generated the TFs–m⁶A regulators networks (including three sub-networks: erasers–TFs interacting network, writers–TFs interacting network, and readers–TFs interacting network), and they are presented in **Figure 7A**. The TFs–m⁶A regulators networks indicated that a plethora of TFs might regulate the m⁶A regulators. For example, there were more than 20 TFs that were possibly able to target the promoter of RBM15B. In addition, another software, FunRich, was also utilized for calculating the potential TFs targeting the m⁶A regulators. The top 25 potential TFs (ranked by targeting percentages) were achieved and exhibited by doughnut plots (**Figure 7B**). The data demonstrated that SP1 were possibly able to modulate more than half of the 20 m⁶A regulators

(52.9%), ELK1 regulated 35.3% of the 20 m⁶A regulators, and EGR1 also orchestrated 35.3% of the 20 m⁶A regulators. Indeed, analyses from the UALCAN database revealed that the expressions of SP1, ELK1, and EGR1 were remarkably aberrant in many TCGA cancer types (**Supplementary Figure S2**). Collectively, these data provided novel insights into the possible molecular mechanisms of the m⁶A regulators’ dysregulation in TCGA cancer types.

The Interacting Regulatory Network of Each m⁶A Regulators

Although we had generated the PPI network of the 20 m⁶A regulators in the above findings, which demonstrated that



these 20 m⁶A regulators interacted with each other with very high frequency, the m⁶A regulators exerted their functions and might also collaborate with other factors. Therefore, we next attempted to construct the interacting regulatory network of each m⁶A regulator alone. The interacting proteins of each eraser, writer, and reader were obtained by using the STRING database, and their corresponding interacting networks are presented in **Figure 8**. The results indicated that every m⁶A regulator had a complex interacting regulatory network, and these interacting networks might provide new insights into how the m⁶A regulators exerted their modulatory functions.

Furthermore, a more complex interacting regulatory network including m⁶A regulators and their corresponding interacting factors was constructed by applying FunRich software. This network not only validated that m⁶A regulators interacted

with other relevant factors, but also revealed that the erasers, writers, and readers interacted with each other frequently (**Figure 9A**). In addition, we then attempted to explore the expressions of the m⁶A regulators-related genes in that network using GSCALite database, because these genes might be the downstream targets of the m⁶A regulators or even the modulators of the m⁶A regulators, and their dysregulation should be critical in m⁶A regulation. According to the data, numerous genes (such as H2AFX, CDKN2A, TTF2, IKBKE, and UBE2I) were markedly upregulated in many cancer types, while some genes (such as ARRB1, LMO3, KHDRBS2, CIRBP, and RALYL) were remarkably downregulated in multiple cancer types (**Figure 9B**). Therefore, these data suggested that the majority of the m⁶A regulators-related genes were dysregulated across many cancer types.

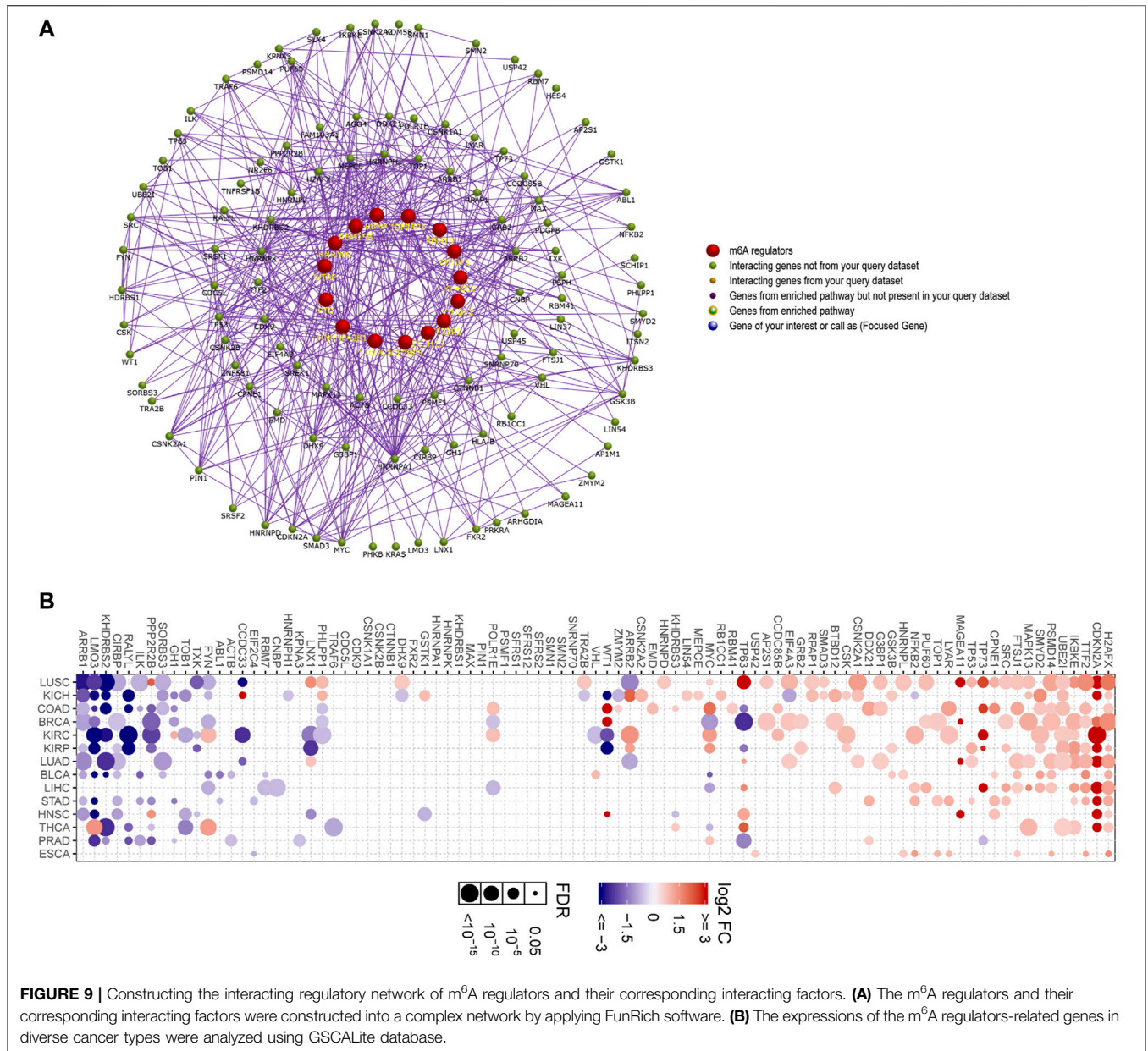


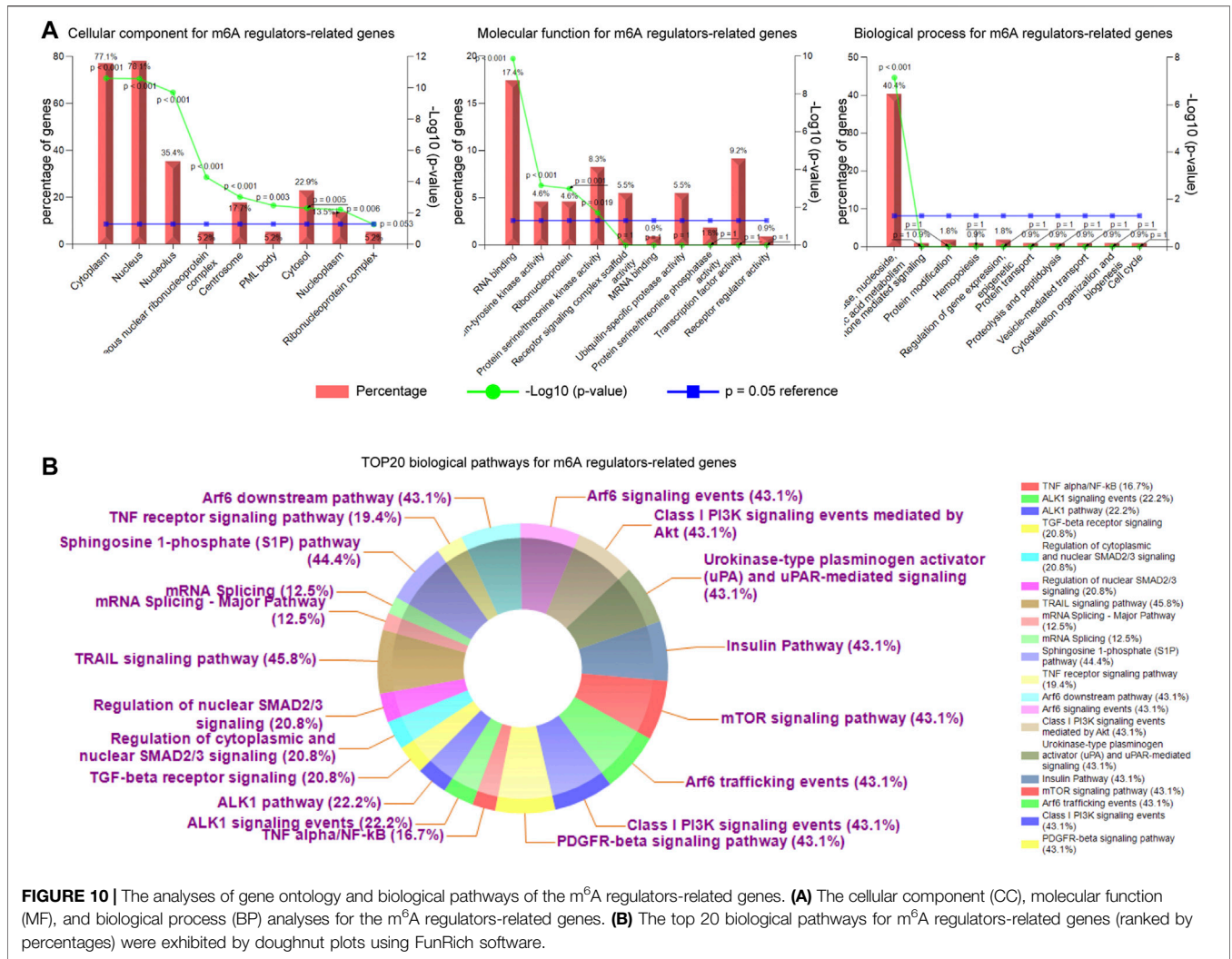
FIGURE 9 | Constructing the interacting regulatory network of m⁶A regulators and their corresponding interacting factors. **(A)** The m⁶A regulators and their corresponding interacting factors were constructed into a complex network by applying FunRich software. **(B)** The expressions of the m⁶A regulators-related genes in diverse cancer types were analyzed using GSCALite database.

Next, we aimed to investigate the gene ontology analyses (GO analyses; including three sub-analyses: CC: cellular component; MF: molecular function; BP: biological process) and biological pathways of the m⁶A regulators-related genes. To achieve that, the FunRich software was utilized. The gene ontology analyses revealed that the m⁶A regulators-related genes were significantly associated with cytoplasm (CC), nucleus (CC), RNA binding (MF), regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism (BP) (Figure 10A). Moreover, the top 20 biological pathways for m⁶A regulators-related genes (ranked by percentages) were exhibited by doughnut plots and the data showed that these genes were dramatically correlated with many tumorigenesis-relevant pathways, such as TRAIL signaling pathway, S1P pathway, and mTOR signaling (Figure 10B).

Therefore, these results indicated that m⁶A regulators as well as their relevant genes were potentially associated with cancers.

DISCUSSION

Cancers account for the major public health problems, and it leads to the second cause of death, ranking behind cardiovascular diseases, in most countries (Siegel et al., 2019). Therefore, seeking novel approaches for cancer therapy is urgent. Based on deeply understanding the molecular mechanisms, several effectively new methods for treating cancers such as cellular immuno-therapy and PD1/PDL1 antibodies therapy had emerged currently (Sharpe and Pauken, 2018). Moreover, epigenetics including



m⁶A methylation, as a popular field of cancer research, might also emerge as a new approach for cancer treatment if its detailed molecular mechanisms in tumorigenesis were deeply unraveled (Zhao et al., 2020). Hence, it is necessary to investigate the effects of m⁶A RNA methylation regulators on multiple cancer types. In the present study, we applied bioinformatics analyses to explore the expression, genetic alterations, prognosis significance, the networks between m⁶A regulators, and potential chemical drugs, miRNAs, or upstream transcriptional factors in multiple cancer types, which deeply uncovered the critical roles and molecular mechanisms of m⁶A regulators in cancers.

Emerging lines of evidence had indicated that the m⁶A regulators served as critical roles in regulating numerous biological processes, diseases, and especially tumor development. For example, m⁶A regulator YTHDF1 was recently identified as a novel prognostic marker and potential target for HCC (Bian et al., 2020). Moreover, m⁶A regulator HNRNPA2B1 was found to function as an oncogenic factor to accelerate esophageal cancer (ESCA) progression, and it might be a promising prognostic biomarker for ESCA (Guo et al., 2020). In addition, the low expression of METTL3, an important m⁶A

writer, was found to be correlated with the poor prognosis of triple-negative breast cancer (TNBC), and METTL3 might serve as a novel therapeutic target in TNBC metastasis (Shi et al., 2020). In the present study, our bioinformatics analysis also revealed that YTHDF1, HNRNPA2B1, and METTL3 were highly expressed in colon adenocarcinoma (COAD), lung squamous cell carcinoma (LUSC), and liver hepatocellular carcinoma (LIHC), respectively, and the expression of many other m⁶A regulators such as IGF2BP1, IGF2BP2, and IGF2BP3 was remarkably dysregulated across multiple cancer types.

Deeply understanding cancer hallmarks requires the detailed information of molecular alterations at multiple dimensions such as gene expression, genetic alteration, epigenomics, clinical information, and metabolome. Therefore, the multi-omics analysis approaches were particularly valuable to deeply discover the molecular alterations in pan-cancer. For example, a multi-omics approach was applied to characterize brain metastasis, and the findings revealed that two molecular subtypes showed notably differential prognosis irrespective of brain tumor subtype (Su et al., 2020). Besides, single-cell sequencing was also an important aspect of multi-omics

analysis. Recently, several single-cell datasets, including CancerSEA and scLM, were developed to facilitate the mechanism discovery and understanding of complex biosystems such as in cancers (Yuan et al., 2019; Song et al., 2020). In the present study, although lacking single-cell sequencing data, we also applied the multi-omics analysis to uncover the molecular mechanisms of m⁶A regulators in pan-cancer at levels of gene expression, genetic alteration, epigenomics, and clinical information, which might help to facilitate the deep understanding of the modulating mechanisms of m⁶A regulators in pan-cancer.

Up to now, the great majority of the studies focused on researching one or several m⁶A RNA methylation regulators in one or several cancer types (Barbieri et al., 2017; Huang et al., 2019). However, the m⁶A regulators exerted their functions in tumor development and might also collaborate with each other or other factors, and accumulating lines of evidence had indicated that m⁶A regulators might play a dual role as tumor promoters or tumor suppressors in variously different cancer types, implying that the levels or functions of m⁶A RNA methylation were determined by the collaboration of m⁶A regulators in certain conditions (Roundtree et al., 2017; Panneerdoss et al., 2018). Therefore, the comprehensive analyses of all the m⁶A regulators but not several of them across all the cancer types might help supply unique insights into the molecular mechanisms of m⁶A RNA methylation in many cancer types. In the present study, the landscapes of the gene expression, genetic alterations, the prognosis significance, and interacting networks of the 20 m⁶A regulators across dozens of cancer types were revealed by integrative bioinformatics analyses. These results provided new supplementary knowledge about the modulation of the m⁶A regulators' dysregulation across cancer types and novel insights into the possible molecular mechanisms of the m⁶A regulators' dysregulation in TCGA cancer types.

The expression alteration of m⁶A regulators in various cancer types might provide novel insight into the molecular mechanisms of tumorigenesis and new therapy approaches (Chen and Wong, 2020). In addition, many aspects such as genetic alternations, epigenetics, and transcriptional factors could contribute to the dysregulation of the m⁶A regulators in cancers (Li et al., 2019). For example, the m⁶A levels were increased through miR-145 targeting YTHDF2, which caused the suppression of cancer cell proliferation in HCC (Yang et al., 2017). Another study demonstrated that SPI1, as a transcriptional factor in hematopoietic cancer cells, could directly suppress the expression of METTL14 (Weng et al., 2018). Therefore, in this study, we not only explored that the methylation and genetic alterations were capable of affecting the expression of the m⁶A regulators across TCGA cancer types, but also investigated the potential upstream miRNAs and transcriptional factors that were able to target these m⁶A regulators. Our results including miRNAs or TF-m⁶A regulators networks provided new supplementary knowledge about the modulation of the m⁶A regulators' dysregulation across cancer types.

The dysregulation of m⁶A regulators was involved in the procedures of cancer development (Huang et al., 2020). Hence, discovering novel drugs targeting these m⁶A regulators

was critical for cancer therapy. For example, a chemical compound, MA2, as an inhibitor of FTO, could effectively suppress the tumor progression of glioblastoma (Cui et al., 2017). Besides, FB23-2 was also capable of inhibiting FTO expression to suppress the proliferation of AML cells (Huang et al., 2019). In the present study, we thereby investigated whether there were some potential chemicals that could increase or decrease the expression of the m⁶A regulators. By analyzing the chemical database, the m⁶A regulators-potential drugs network was constructed and it might provide benefits for potential drugs discovery to target specific m⁶A regulators.

CONCLUSION

In summary, our results not only systematically analyze the expression, genetic alterations, oncogenic pathways, and prognosis significance of m⁶A regulators across multiple cancer types, but also constructed the networks between m⁶A regulators and potential chemical drugs, miRNAs, or upstream transcriptional factors. These comprehensive analyses might provide novel understanding of these m⁶A regulators' roles and shed light on their potential molecular mechanisms in cancers as well as help develop new therapy approaches for cancers.

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 author.

AUTHOR CONTRIBUTIONS

Conceptualization, XS and JL; methodology, XS and JZ; software, XS; validation, CZ, XL, and JW; formal analysis, YJ; investigation, XS; resources, JZ; data curation, CZ; writing—original draft preparation, XS; writing—review and editing, XS and JZ; visualization, XS; supervision, YJ; project administration, XS; funding acquisition, JL. All authors have 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/fgene.2021.771853/full#supplementary-material>

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