Original Article

Bioinformatics analysis of the correlation between m6A RNA methylation regulators and the immune infiltration and prognosis of bladder cancer

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Background: To analyze the effect of N6-methyladenosine (m6A) RNA methylation regulators on the immune infiltration and prognosis of bladder cancer (BC). We explored the related signaling pathways and prognosis-related genes to provide candidate targets for the treatment and prognostic evaluation of BC.

Methods: After downloading BC data from The Cancer Genome Atlas (TCGA) database, the expressions of m6A-related genes were obtained. We then performed correlation and sample cluster analysis of the m6A methylation regulator genes as well as difference comparison and survival analysis for the clustered patients using R software. Gene set enrichment analysis (GSEA) was carried out on cluster-grouped samples. Finally, the prognosis-related genes of BC among the m6A methylation regulators were screened.

Results: Genomic alterations in the m6A regulators were linked to a poor BC prognosis. *HNRNPA2B1*, *HNRNPC*, *IGF2BP2*, *RBM15*, *YTHDF1*, and *YTHDF2* were found to be associated with advanced clinical stages of BC. Furthermore, the current study revealed that the levels of the m6A regulators were related to the expression levels and immune infiltration levels of immune regulators [immunosuppressive factors, immunostimulators, and major histocompatibility complex (MHC) molecules] in BC. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses suggested that in addition to the relevant immune responses, m6A regulators were involved in the poor prognosis of BC via their participation in blood vessels through regulatory RNA binding, telomeric DNA binding, microRNA (miRNA) binding, negative regulation of messenger RNA (mRNA) processing, negative regulation of DNA biosynthesis, branches of morphogenesis, positive regulation of the Notch receptor target gene transcription, etc.

Conclusions: The expression of m6A RNA methylation regulators is closely linked to immune infiltration and prognosis in BC. Thus, it can be utilized as a potential molecular target for the treatment and prognostic assessment of BC.

Keywords: Bioinformatics; bladder cancer (BC); N6-methyladenosine regulating factor (m6A regulating factor); immune infiltration; prognosis

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Introduction

Bladder cancer (BC) ranks among the most prevalent types of cancer, with approximately 546,000 new infections and 200,000 deaths annually. The incidence and mortality rates of men are higher than those of women, at 9.6/100,000 compared to 3.2/100,000, respectively (1,2). BC is categorized into two types based on its tumor stage: (I) muscle-invasive BC (MIBC) which is responsible for about 25% of early BC diagnoses; and (II) non-MIBC (NMIBC). The proportion of NMIBC progressing to MIBC is as high as 10–15% (3,4). Targeted therapy is a crucial component for the advancement of individualized treatments relating to BC, which is one of the most immune-infiltrating tumors (5). Signals in the immune microenvironment, including the accumulation of tumor metabolites or T-cell impairment, may substantially influence the response of patients to immune checkpoint therapy (ICT) (6,7). Recent advancements in monoclonal antibodies against programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) have demonstrated efficacy and clinical advantages in numerous malignancies, including BC (8,9). The cellular transcriptome is frequently modified by various chemical modification markers, which in turn have profound effects on its function. Among these modifications, N6-methyladenosine (m6A) RNA methylation has attracted significant attention in recent years and refers to the addition of a methyl group to the sixth nitrogenous base of adenine residues of RNA (10).

In cellular RNA, m6A RNA methylation is a common reversible internal modification. m6A methylation, like DNA methylation, can modulate post-transcriptional expression without causing any change in the base sequence (11,12). The reversibility of m6A marking differs from other previously known modifications (which are irreversible); it regulates genes and confers the additional flexibility required for gene expression and is dynamically modulated by methyltransferases including *KIAA1429*, *WTAP*, and *METTL3/14*. In addition to messenger RNA (mRNA), m6A is also present in other types of cellular RNAs viz. transfer RNA (tRNA), ribosomal RNA (rRNA), as well as non-coding RNAs such as circular RNA (circRNA), microRNA (miRNA), and long non-coding RNAs (lncRNAs) (13-16). As the most abundant internal RNA modification (17), m6A can be detected in about one-third of mammalian mRNAs, with an average of three to five m6A methylations per mRNA. Compared with humans, mice have evolved many m6A sites (18). The m6A modification site has the typical consensus sequence, DRACH (D = G, A or U; R = G or A; H = A, C or U), which contains more coding sequences and 3'-untranslated region (3'-UTR) and particularly high enrichment in the vicinity of the stop codon subregion (19).

A study has demonstrated a relationship between m6A alterations and numerous biological events, including metastasis, glycolysis, tumor proliferation, and apoptosis (20). In malignant tumors, m6A modification can play a tumor suppressor or oncogenic role. Numerous RNA-binding proteins, which regulate m6A progression, have been reported, such as *YTHDF1*, *YTHDC2*, *YTHDC1*, *YTHDF2*, *YTHDF3*, *ZC3H13*, *ZCCHC4*, etc. Zhao *et al.* showed that lung adenocarcinoma progression could be promoted by *KIAA1429*, which regulates the m6A level of *MUC3A* (21). Similarly, Pan *et al.* found that the progression of colorectal cancer could be promoted by *METTL3*, which regulates the m6A-*CRB3*-Hippo axis (22). Furthermore, another study showed that *YTHDF2* could promote colorectal cancer progression through the modulation of the m6A-*CRB3*-Hippo axis. Also, *METTL3*-mediated modification of m6A has been shown to activate nuclear factor kappa-B (NF-κB) and enhance glioma malignant progression, thereby promoting *UBXN1* mRNA decay (23). *ZC3H13* inhibits colorectal cancer invasion and proliferation by inactivating the Ras-extracellular signal-regulated kinase (Ras-ERK) signaling pathway (24).

Although tumorigenesis in various tumors is affected by m6A modification, the mechanism of m6A in BC remains unknown. Using data from The Cancer Genome Atlas (TCGA), the present study analyzed the expression of m6A

Highlight box

Key findings

- The expression of m6ARNA methylation regulator is closely related to the immune invasion and prognosis of BC, and can be used as a candidate molecular target for BC treatment and prognosis evaluation.

What is known and what is new?

- This article suggests that the imbalance of tumor related m6A regulatory factors is related to the prognosis of BC patients.
- At the same time, the expression of m6A regulatory factors is related to the level of immune invasion and the expression of immune regulatory factors in BC.

What is the implication, and what should change now?

- In the future, we will perform *in vivo* and *in vitro* cytological experiments to further study the mechanism of these regulators in BC.

regulators in BC; we also evaluated their association with clinicopathological features and predicted the potential functions of m6A regulators. We focus on the general direction of immune infiltration to screen candidate molecular targets for BC treatment and prognosis evaluation. This exploration can provide better reference for the development of *in vitro* and *in vivo* experiments in the future. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5993/rc).

Methods

Expression analysis of m6A regulators

The expression of m6A regulators was contrasted using Gene Expression Profiling Interactive Analysis 2 (GEPIA2), and the University of Alabama at Birmingham Cancer (UALCAN) was employed to evaluate the association between the m6A regulators and the clinical parameters of EC. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Prediction of immune responses

The correlation between m6A regulators' expression and the levels of infiltration of numerous BC immune cells [e.g., cancer-associated fibroblasts, bone marrow dendritic cells, T-cell regulators (Tregs, etc.), neutrophils, and $CD4^+$ T cells] and was explored using the Tumor Immune Estimation Resource (TIMER) database (proper citation). Meanwhile, the correlation between the expression of m6A modulators and immunomodulators [including immunosuppressants, immunostimulators, and major histocompatibility complex (MHC) molecules] was computed using the Tumor-Immune System Interactions Database (TISIDB).

Construction of the protein-protein interaction (PPI) network

PPI networks were constructed based on the differentially expressed genes (DEGs) using the STRING database and were visualized using Cytoscape software (Funding for continued development and maintenance of Cytoscape is provided by the U.S. National Institute of General Medical Sciences (NIGMS) under award number R01 GM070743. Cytoscape user support, education and new initiatives are supported by the National Resource for Network Biology (NRNB) under award number P41 GM103504 (25).

Genetic alterations of m6A regulators in BC

Genetic alterations of the regulators, from the perspective of multidimensional cancer genomics, were further analyzed through the cBioPortal website (https://www.cbioportal.org/).

Survival analysis and prognosis

The cBioPortal database was utilized to determine the correlations between m6A regulator aberrations and survival time in human cancers.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

Metascape (https://metascape.org), an online tool, was employed for functional analysis, in which differential genes were added to Metascape (proper citation).

Statistical analysis

Possible cellular mechanisms of action were examined by employing gene set enrichment analysis (GSEA). SPSS version 22.0 (IBM Corporation, USA) was used to perform statistical analysis. At $P<0.05$, the results of all data analyses were considered statistically significant.

Results

m6A regulator aberrations in the human genome are correlated with cancer prognosis

The correlation between m6A regulator aberrations and cancer patient survival times was analyzed by employing the cBioPortal database to assess the functional significance of these regulators in cancer patients. Our results showed that m6A regulators aberrations in the human genome were remarkably linked to a better prognosis in BC, pleural mesothelioma, and lung squamous cell carcinoma, and were significantly associated with adrenocortical tumors, breast cancer, skin melanoma, thyroid tumors, and lung adenocarcinoma. Poor prognosis was significantly associated with a statistically significant difference by Kaplan-Meier method ($P<0.05$, only the positive results are shown, see *Figure 1*).

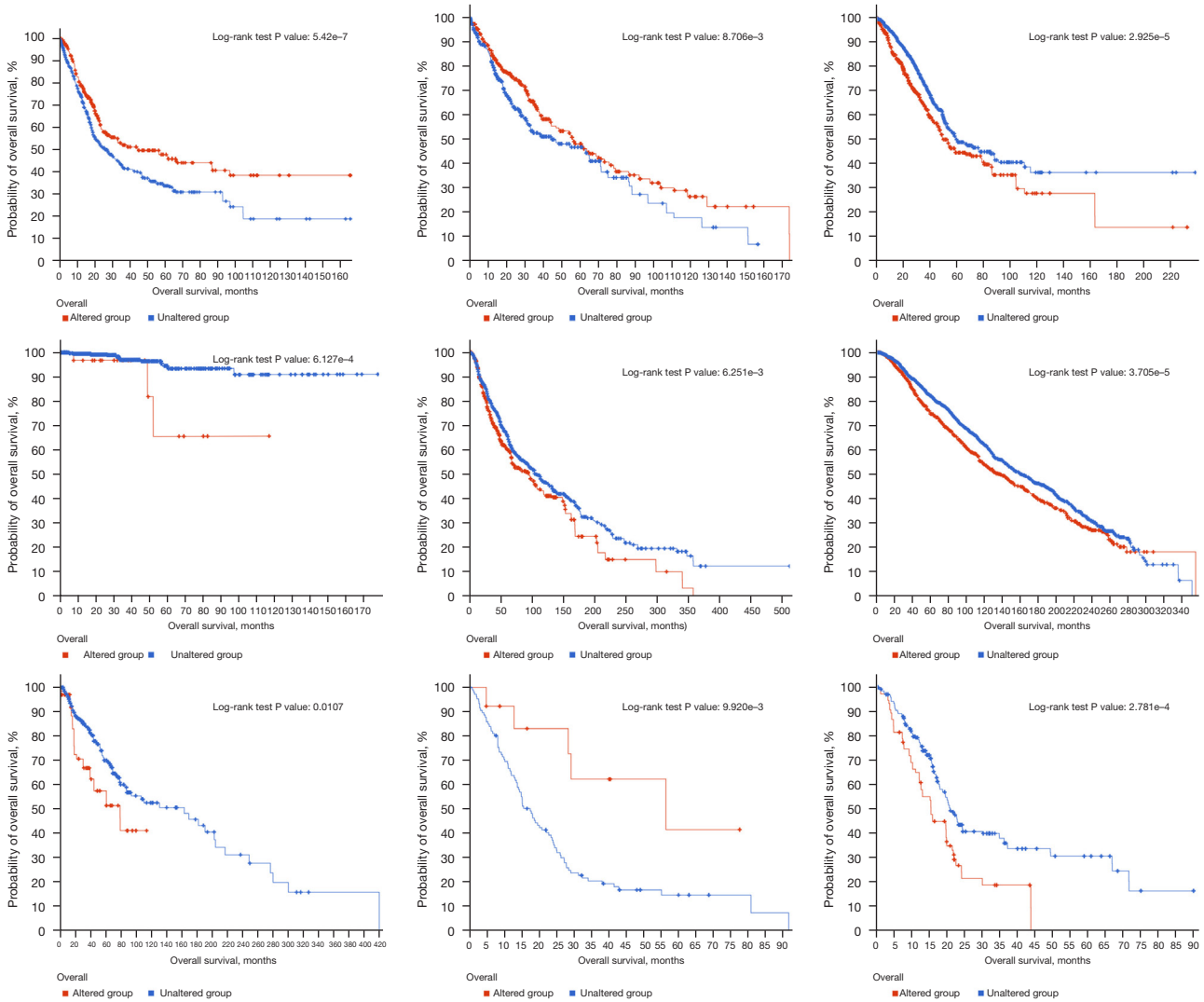


Figure 1 m6A regulator aberrations in the genome are related to the prognosis of various human cancers, including BC, pleural mesothelioma, lung squamous cell carcinoma, adrenocortical tumor, breast cancer, skin melanoma, thyroid tumor, and lung adenocarcinoma. m6A, N6-methyladenosine; BC, bladder cancer.

Amplification, mutation, and deletion of m6A regulators in BC

Studies investigating the role of m6A modulators in endometrial cancer (26), lung adenocarcinoma (27), and low-grade glioma (28) are available. The objective of the present study was to evaluate the functions of m6A modulators in BC. The genetic variations of m6A modulators in 2,410 BC patients were examined using the cBioPortal database. Our results revealed genetic variations of varying degrees in 23 m6A regulators, including *ZCCHC4*, *YTHDF2*, *YTHDF3*, *ZC3H13*, *YTHDF1*, *WTAP*, *YTHDC1*, *YTHDC2*, *VIRMA*, *RBM15*, *RBM15B*, *METTL14*, *METTL16*, *METTL3*, *VIRMA*, *IGF2BP3*, *ALKBH5*, *HNRNPA2B1*, *IGF2BP2*, *IGF2BP1*, *HNRNPC*, *FTO*, and *CBLL1*. The majority of these regulators were amplified, mutated, and deleted, with VIRMA displaying the highest incidence (11%) (see *Figure 2*).

Expression of m6A regulators in BC tissues in TCGA database

After discovering the amplification, mutation, and

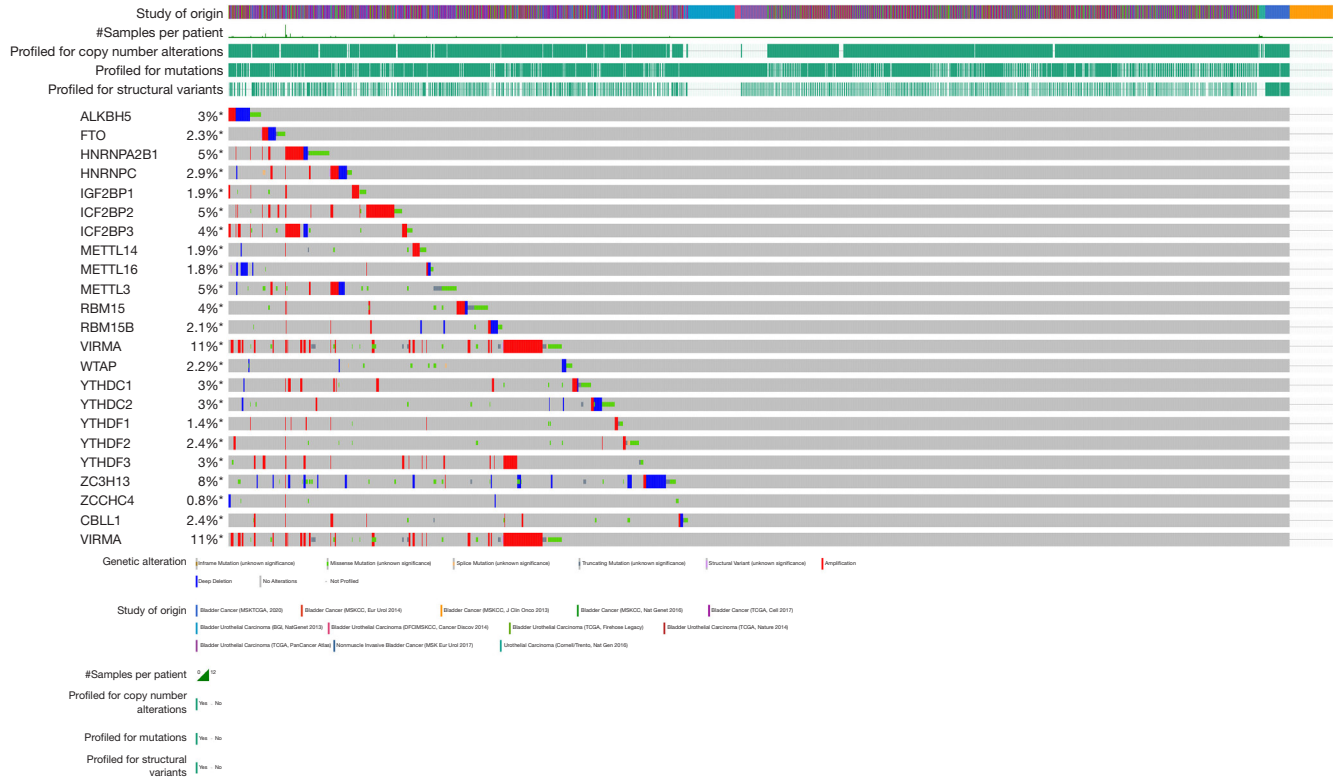


Figure 2 The amplification, mutation, and deletion of m6A regulators in BC. The gene variants of m6A regulators in 2,410 cases were examined using the cBioPortal database. #, prompt patient information; *, P<0.05. m6A, N6-methyladenosine; BC, bladder cancer.

deletion of the m6A regulators in BC, TCGA database was employed to assess the expression of regulators belonging to BC tissues. Our results showed that *FTO*, *ZC3H13*, *YTHDF3*, *YTHDC1*, *WTAP*, *METTL16*, and *METTL14* were down-regulated in the tumor tissues, while *HNRNPA2B1*, *IGF2BP1*, *IGF2BP3*, *METTL3*, *YTHDF2*, and *YTHDF1* were up-regulated. Meanwhile, *ALKBH5*, *CBLL1*, *HNRNPC*, *IGF2BP2*, *VIRMA*, *RBM15*, *RBM15B*, *VIRMA*, *YTHDC2*, and *ZCCHC4* exhibited no statistically noteworthy difference between the tumor and normal tissues (P>0.05) (*Figure 3*).

Correlation between m6A regulators and the prognosis of BC patients

The relationship between m6A regulators and the prognosis of BC patients was assessed by using a Kaplan-Meier plot, and the results showed that patients with a low expression of *FTO*, *ALKBH5*, *IGF2BP2*, *IGF2BP3*, *VIRMA*, *METTL16*, *YTHDF1*, and *ZC3H13* had better overall survival (OS) (*Figure 4A*). Similarly, patients exhibiting a low expression of *IGF2BP3*, *ALKBH5*, *VIRMA*, *FTO*, *IGF2BP2*, *YTHDC1*, *YTHDF1*, and *ZC3H13* had a better disease-specific survival (DSS) than those with high expression (*Figure 4B*). As for the progression-free interval (PFI) of patients, those with a low expression of *FTO*, *IGF2BP1*, *IGF2BP2*, *ZC3H13*, *YTHDF3*, *YTHDF1*, *VIRMA*, and *RBM15* had a better prognosis (*Figure 4C*).

Dysregulation of m6A regulators is associated with advanced clinical stages of BC

As shown in *Figure 4*, m6A regulators were linked with the prognosis of BC patients. Moreover, they were also correlated with the different clinical stages of BC patients. Our results revealed that, unlike normal tissues, the expressions of *HNRNPA2B1*, *HNRNPC*, *IGF2BP2*, *RBM15*, *YTHDF1*, and *YTHDF2* were higher in tumor tissues, which supports the above findings, and all clinical and N stages exhibited up-regulation of these factors. On the other hand, compared with the N1 stage, *YTHDF1* was up-regulated in the N2/3 stage; *HNRNPA2B1* and *YTHDF2* were down-

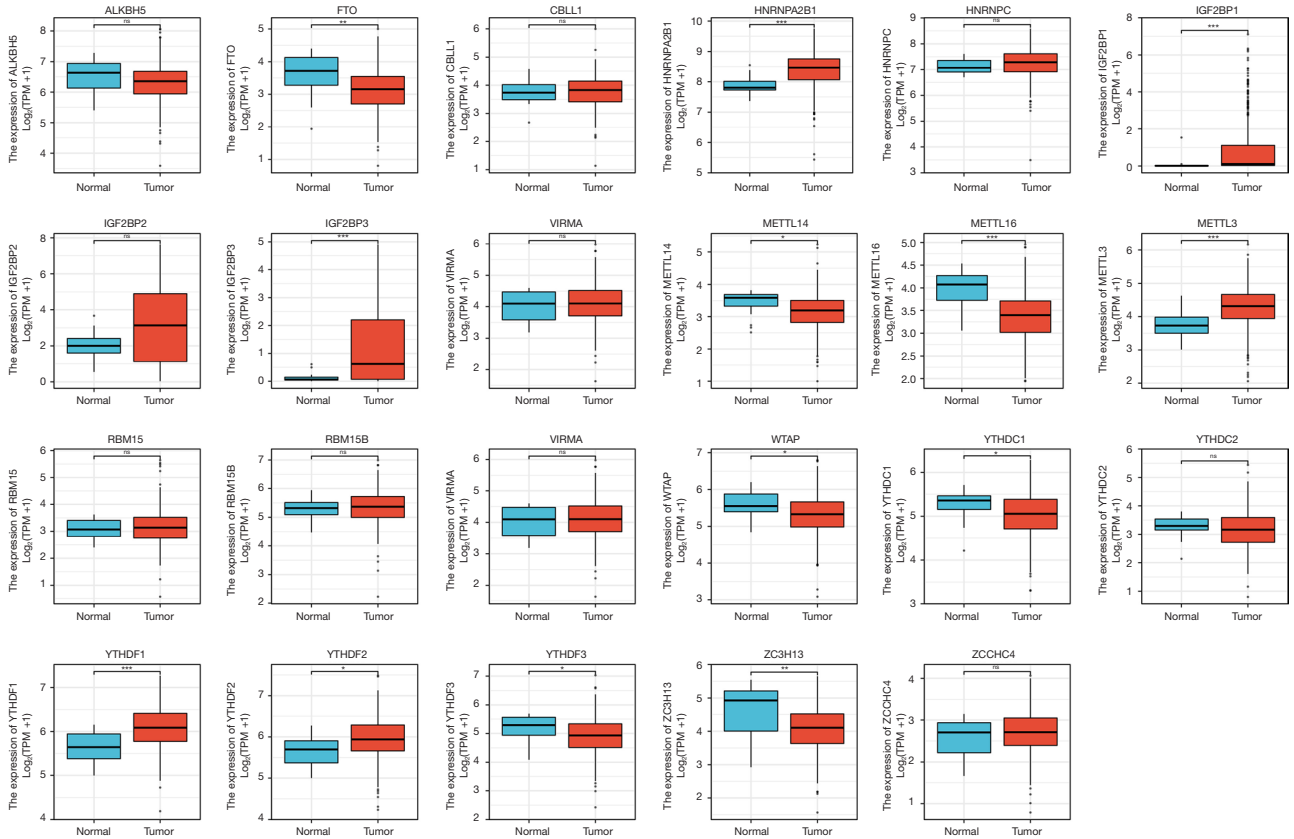


Figure 3 Expression of m6A regulators in BC tissues. "ns" means P≥0.05; *P<0.05; **P<0.01; ***P<0.001; TPM, transcripts per million; m6A, N6-methyladenosine; BC, bladder cancer.

regulated in the N2/3 stage; and *HNRNPC*, *IGF2BP2*, and *RBM15* were up-regulated in the N3 stage. Among these, it is important to note the increasing expression of *YTHDF1* with the increase of the N stage (*Figure 5A-5F*).

Correlation between the expression of m6A regulators and the immune infiltration levels in BC

The correlation between the expression of m6A regulators and the immune infiltration levels in BC was identified using the TIMER database. *HNRNPA2B1* was associated with $CD8^+$ T cells (r=0.267, P=2.14e-07), neutrophils (r=0.247, P=1.90e-06) and dendritic cells (r=0.226, P=1.26e-05). Similarly, *HNRNPC* was also associated with $CD8^+$ T cells (r=0.292, P=1.21e-08), neutrophils (r=0.254, P=9.14e-07), and dendritic cells (r=0.297, P=6.95e-09); *IGF2BP2* was associated with $CD8^+$ T cells (r=0.386, P=1.78e-14), $CD4^+$ T cells (r=0.223, P=1.64e-05), neutrophils (r=0.39, P=1.26e-14), and dendritic cells (r=0.559, P=1.97e-31); *RBM15* was associated with $CD8^+$ T cells (r=0.228, P=1.02e-05), neutrophils (r=0.288, P=2.34e-08), and dendritic cells (r=0.327, P=1.54e-10); *YTHDF1* was associated with B cells (r=0.135, P=9.96e-03) and macrophages (r=0.163, P=1.73e-03); and *YTHDF2* was significantly correlated with B cells (r=0.137, P=9.09e-03), $CD8^+$ T cells (r=0.141, P=7.00e-03), and neutrophils (r=0.165, P=1.57e-03) (*Figure 6A-6F*).

Correlation between m6A regulatory factor expression and immunomodulatory expression in BC

To analyze the effect of m6A regulators on the tumor immune response, the correlation between m6A regulatory factor expression and immunomodulatory expression in BC was evaluated in 30 different tumors using the TISIDB database. The analysis revealed that in BC tumors, *HNRNPA2B1*, *HNRNPC*, *YTHDF1*, and *YTHDF2* showed a negative correlation with immunosuppressive

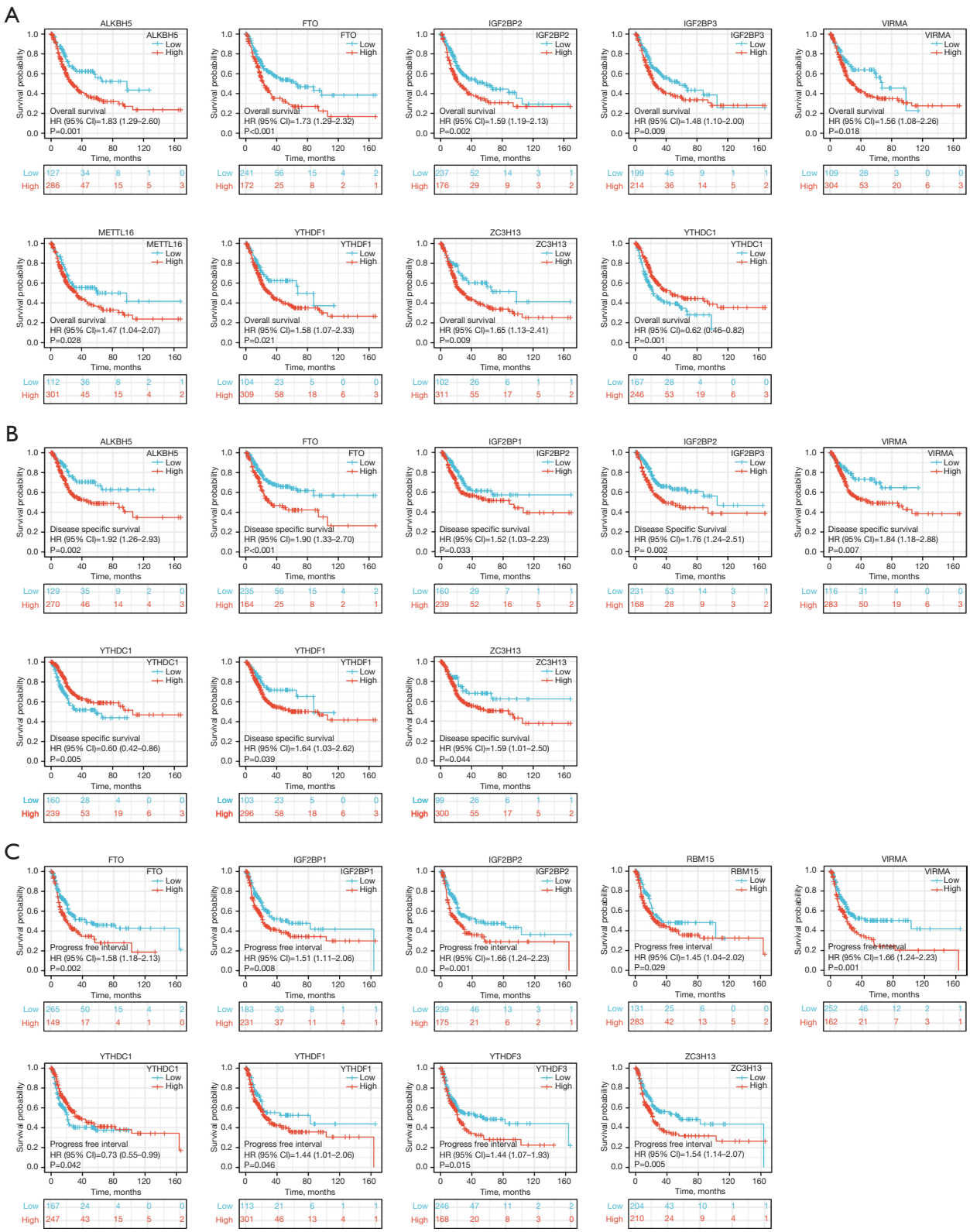
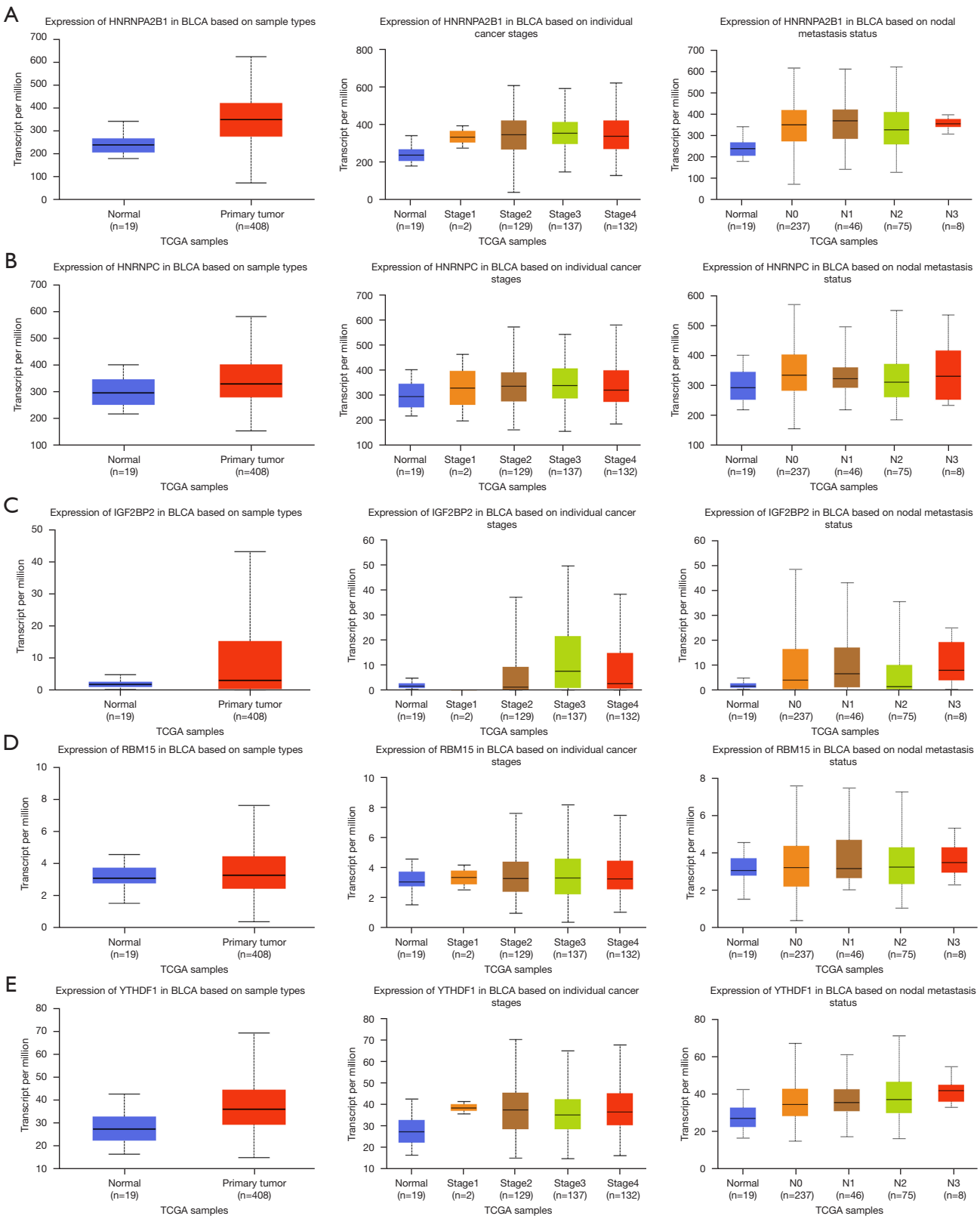


Figure 4 Correlation between prognosis and m6A regulators in BC patients. (A) OS. (B) DSS. (C) PFI. m6A, N6-methyladenosine; BC, bladder cancer; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.



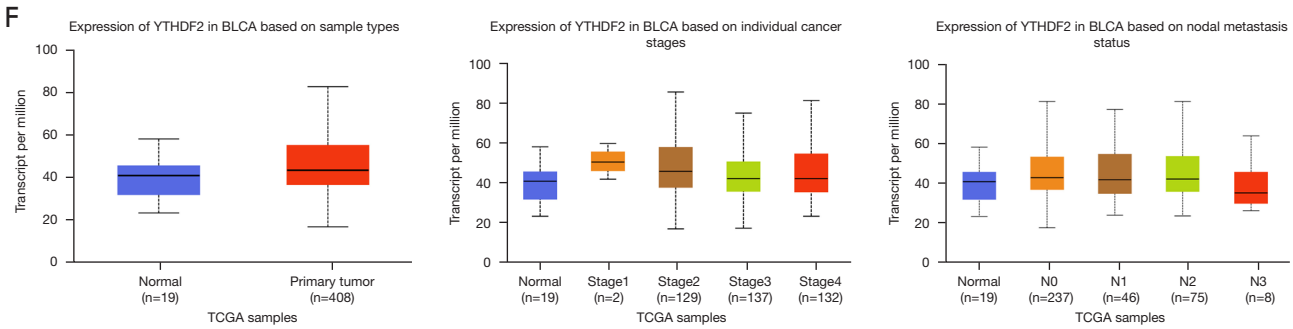


Figure 5 Dysregulation of m6A regulators [(A) *HNRNPA2B1*, (B) *HNRNPC*, (C) *IGF2BP2*, (D) *RBM15*, (E) *YTHDF1*, and (F) *YTHDF2*] was associated with advanced clinical stages of BC. BLCA, bladder urothelial carcinoma; TCGA, The Cancer Genome Atlas; m6A, N6-methyladenosine; BC, bladder cancer.

agents, and *IGF2BP2* showed a positive correlation with immunosuppressive agents (*Figure 7A*). Moreover, *HNRNPA2B1*, *HNRNPC*, *YTHDF1*, and *YTHDF2* were negatively correlated with immunostimulants, while *IGF2BP2*, *RBM15* were positively correlated with immunostimulants (*Figure 7B*). Likewise, *HNRNPA2B1*, *HNRNPC*, *YTHDF1*, and *YTHDF2* exhibited a negative correlation with MHC molecules, while *IGF2BP2* showed a positive correlation with these molecules (*Figure 7C*).

GO and KEGG enrichment analyses

GO and KEGG enrichment analyses were performed on the co-expressed genes of six m6A regulators to evaluate their downstream pathways in BC. Among all of the regulators, only *HNRNPA2B1*, *HNRNPC*, *RBM15*, *YTHDF1*, and *YTHDF2* exhibited positive results. We found that *HNRNPA2B1* binds to regulatory RNA, telomeric DNA, miRNA, sequence-specific single-stranded DNA, single-stranded telomeric DNA, and G-strand-rich telomeric DNA, enabling telomere elongation. Furthermore, it is a positive regulator of telomere maintenance and granzyme activity, and negative regulator of mRNA processing and RNA splicing through the spliceosome (*Figure 8A*). *HNRNPC* binds to polypyrimidine tracts, poly(U)RNA, and telomerase RNA to form a telomerase-holoenzyme complex, which leads to negative regulation of the DNA biosynthesis process, a negative response to telomere maintenance, a negative effect of telomere elongation on telomere maintenance, and negative regulation of the 3'-UTR-mediated mRNA stabilization of telomere maintenance by telomerase (*Figure 8B*). *RBM15* was associated with a branch involved in vascular morphogenesis, up-regulation of the transcription of target gene of the Notch receptor (*Figure 8C*). *YTHDF1* was implicated in oocyte maturation as well as the regulation of cell maturation, hematopoietic stem cell proliferation, oogenesis, and oocyte development (*Figure 8D*). *YTHDF2* was implicated in the regulation of antigen processing and presentation (*Figure 8E*).

Discussion

Numerous studies evaluating the function of m6A regulators in BC have been published, such as those showing, for example, the inhibition of cell proliferation by *ALKBH5* through m6A-CK2α-mediated glycolysis, making BC cells resistant to cisplatin (29). Also, by interacting with *IGF2BP1*, circPTPRA inhibits BC progression by preventing the recognition of the m6A of RNA (30). Circ0008399 interacts with *WTAP* to promote m6A-methyltransferase complex assembly and activity and encourage cisplatin resistance in BC (31). By degrading *KLF4* and *SETD7* mRNA in BC, the *METTL3/YTHDF2* m6A axis promotes tumorigenesis (32). *FTO* alters the *MALAT* m6A levels and promotes BC progression (33). Moreover, by controlling the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway in human BC, *IGF2BP3* promotes both tumorigenesis and cell proliferation (34). However, given its high malignancy and recurrence, further in-depth study into BC is still needed to improve patient prognosis. Therefore, a comprehensive study on the correlation between BC progression and m6A regulators is still required. A significant prognostic value of m6A regulators in BC was observed in this study by using multiple datasets. Moreover, the relationship between antitumor immune responses and m6A regulators was also investigated. Our

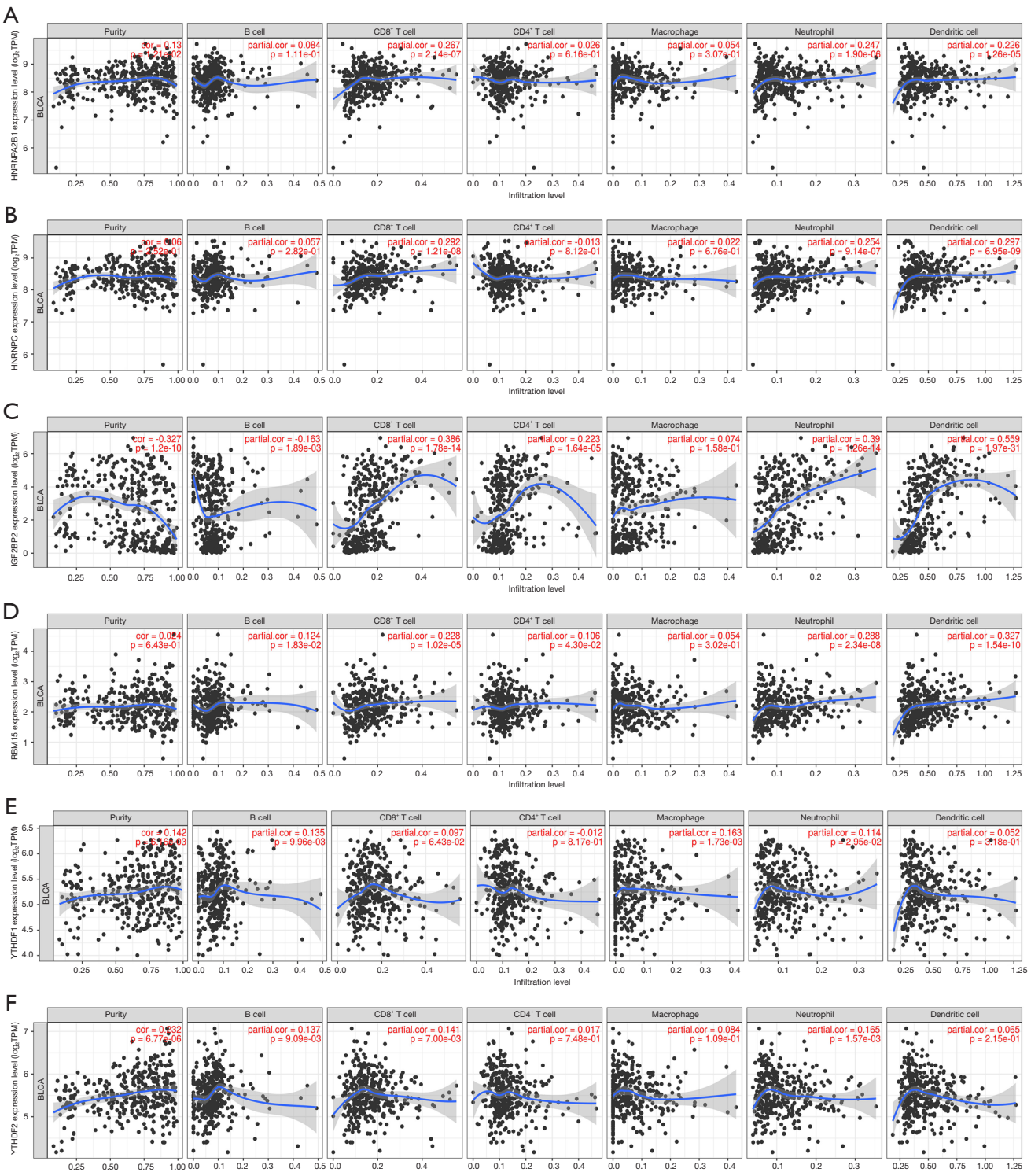
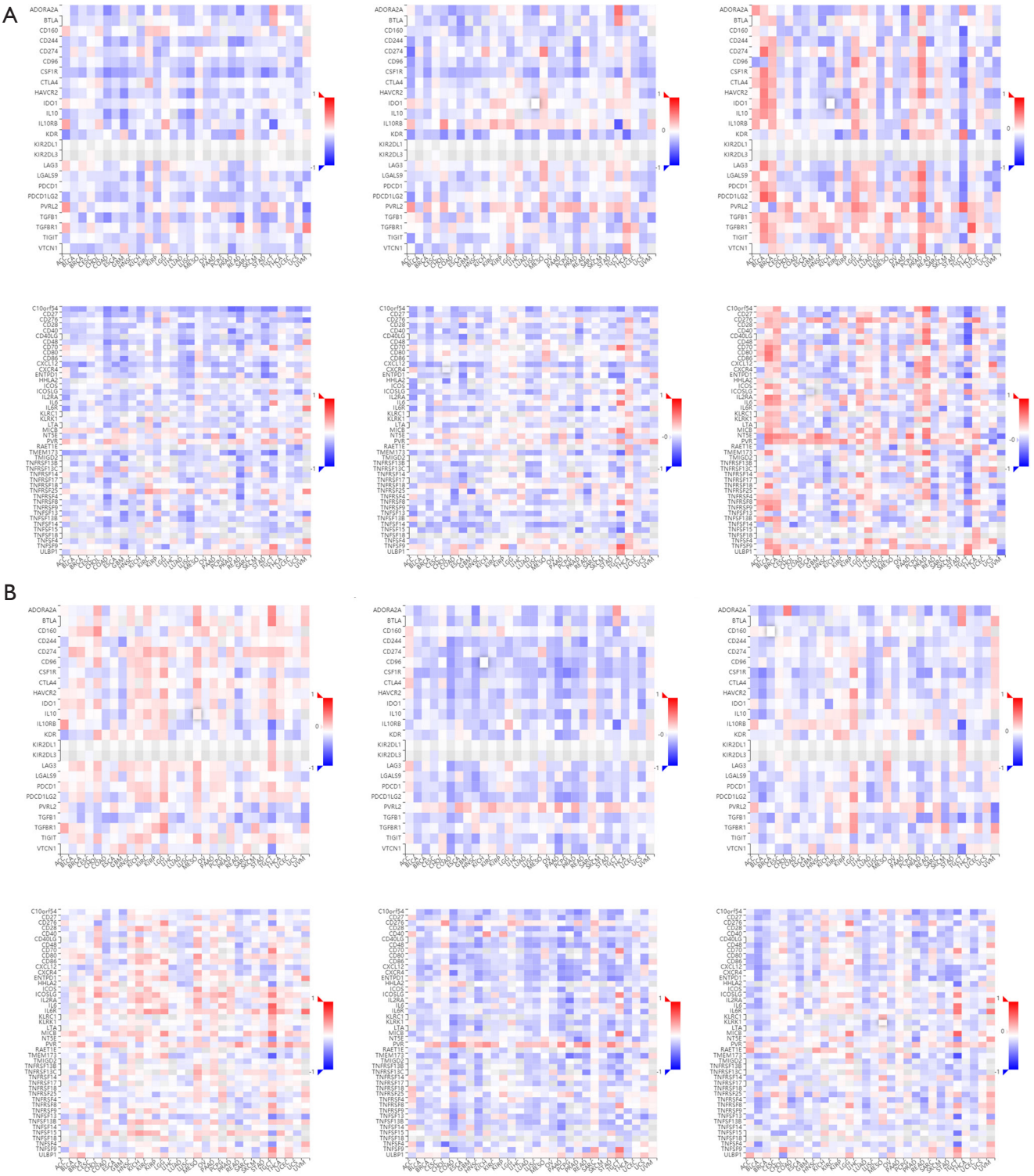


Figure 6 Correlation between the expression of m6A regulators and the level of immune infiltration in BC (A-F) based on the TIMER database. Correlation between (A) *HNRNPA2B1*, (B) *HNRNPC*, (C) *IGF2BP2*, (D) *RBM15*, (E) *YTHDF1*, and (F) *YTHDF2* and neutrophils, macrophages, dendritic, $CD4^+$ T, $CD8^+$ T cells, and B cells. TPM, transcripts per million; m6A, N6-methyladenosine; BC, bladder cancer; TIMER, Tumor Immune Estimation Resource.



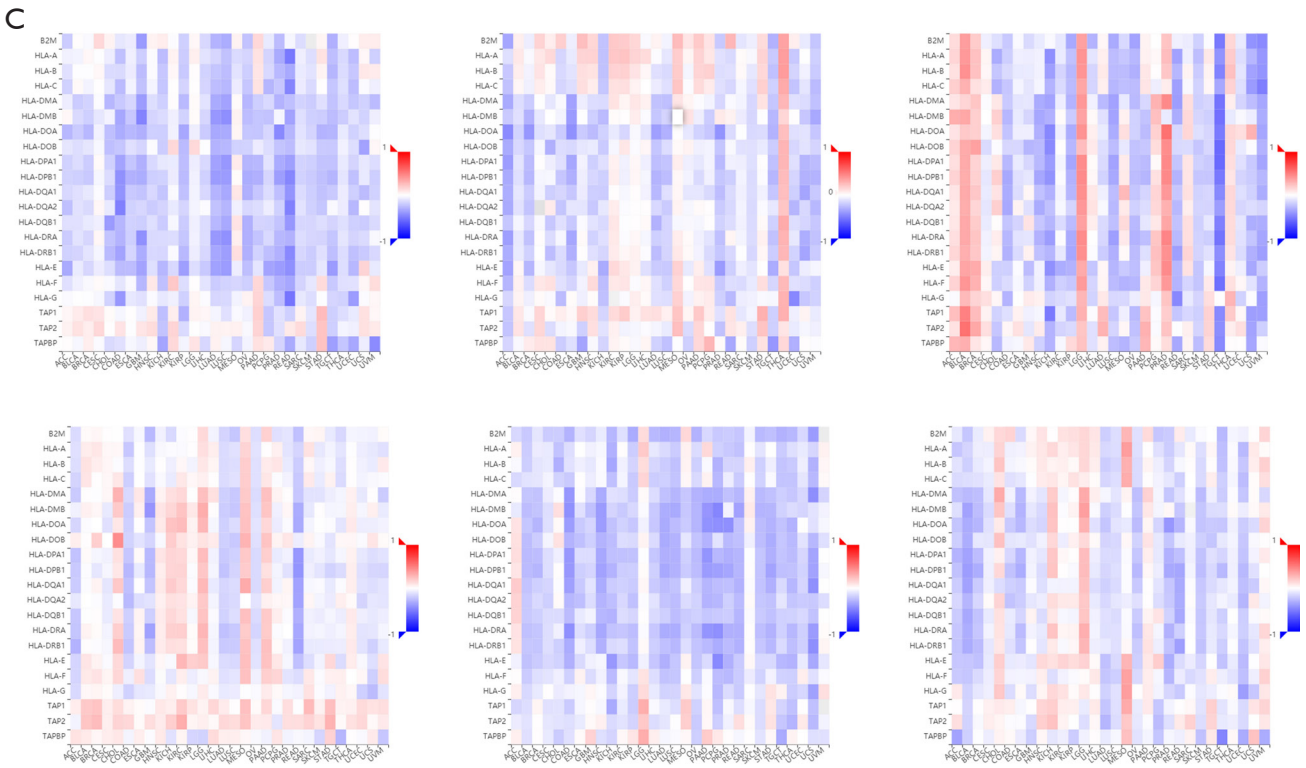


Figure 7 Correlation between the expression of immune regulators and that of m6A regulators in all tumors, especially BC. The correlations between *HNRNPA2B1*, *HNRNPC*, *IGF2BP2*, *RBM15*, *YTHDF1*, and *YTHDF2* and the expression of (A) immunosuppressive factors, (B) immunostimulants, and (C) MHC molecules were analyzed using the TISIDB database. m6A, N6-methyladenosine; BC, bladder cancer; MHC, major histocompatibility complex; TISIDB, Tumor-Immune System Interactions Database; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; 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; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

results demonstrated that m6A regulators could be utilized as novel effective biomarkers for immunotherapy and prognostic evaluation of BC. We also showed that m6A regulator aberrations in the genome are associated with the prognosis of human BC. Furthermore, numerous m6A regulators were found to be mutated, deleted, and amplified in BC, with *VIRMA* exhibiting the highest incidence (11%).

VIRMA, an essential component and the largest methyltransferase, promotes cancer progression and is related to poor survival in various cancers. To achieve its carcinogenic effects, *VIRMA* can regulate cancer cell proliferation, migration, invasion, metastasis, tumor growth, and apoptosis resistance by affecting stem factors (including *CDK1*, *GATA3*, *CCAT1/2*, and *ID2*) and other pathways in an m6A-dependent (or independent) manner (35). For instance, VIRMA promotes the progression of non-small cell lung cancer via m6A-dependent post-transcriptional alteration of *DAPK3* (36). It also activates the cell cycle, RNA polymerase, RNA degradation, DNA replication, nucleoside acid excision repair and basal transcription factors, as well as other related signaling pathways, which contribute to the poor

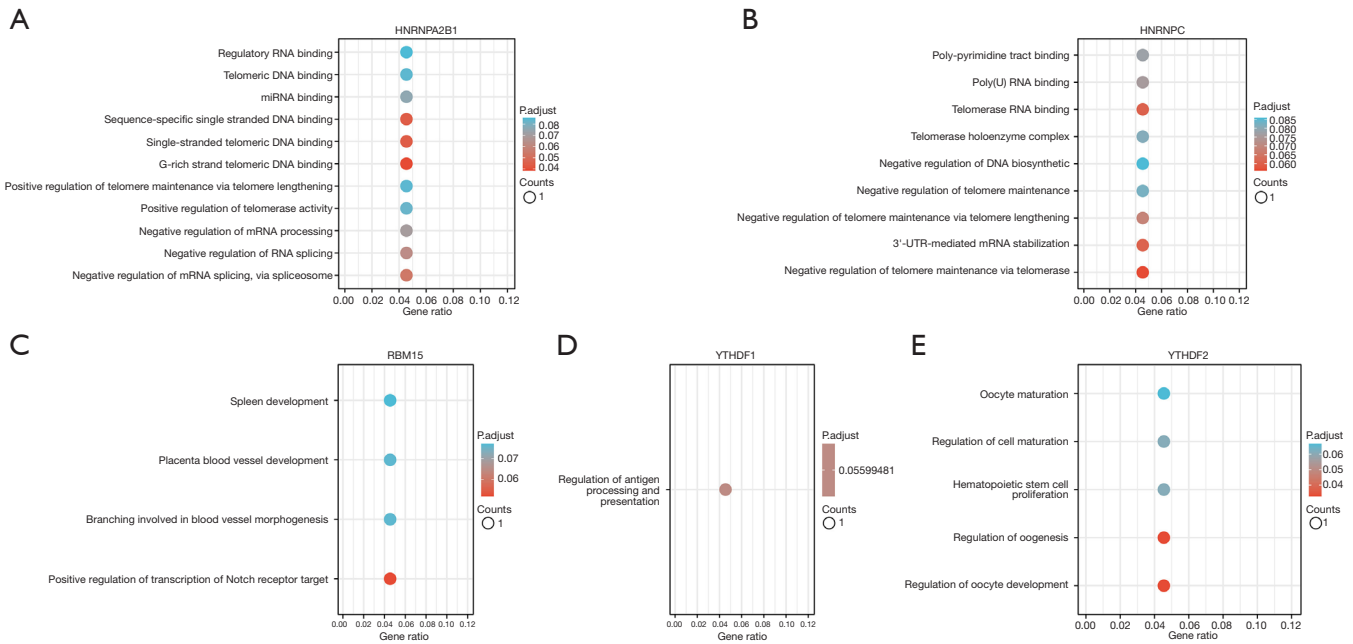


Figure 8 GO and KEGG enrichment analyses. (A-E) Bioinformatics analysis of *HNRNPA2B1*, *HNRNPC*, *RBM15*, *YTHDF1*, and *YTHDF2* in BC. MiRNA, microRNA; mRNA, messenger RNA; 3'-UTR, 3'-untranslated region; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BC, bladder cancer.

prognosis of lung adenocarcinoma (37).

Meanwhile, we also found that *HNRNPA2B1*, *IGF2BP1*, *IGF2BP3*, *METTL3*, *YTHDF1*, and *YTHDF2* were up-regulated in tumor tissues, and *HNRNPA2B1*, *HNRNPC*, *IGF2BP2*, *RBM15*, *YTHDF1*, and *YTHDF2* were positively regulated in all clinical and N stages of BC and were associated with BC. Poorer cancer outcomes were remarkably associated with advanced stages, suggesting that m6A regulators play a significant role in BC and patient prognosis.

HNRNPA2B1 is a crucial subunit of the m6A-methyltransferase complex and plays a crucial role in cancer among such genes (*HNRNPA2B1*, *IGF2BP1*, *IGF2BP3*, *METTL3*, *YTHDF1*, and *YTHDF2*). It stimulates multiple myeloma progression through m6A-dependent stabilization of *ILF3* mRNA by increasing *AKT3* expression (38). *H19*, a lncRNA, stimulates colorectal cancer metastasis by binding to *HNRNPA2B1* (39). Also, m6A-induced lncRNA *RP11* promotes the transmission of colorectal tumor cells by up-regulating *Zeb1* (40). *YTHDF1* has been identified as an m6A reader. *YTHDF1* enhances translation of the key Wnt receptor *FZD7* in an m6A-dependent way. Mutant *YTHDF1* increases FZD7 expression, leading to the Wnt/β-catenin pathway and the promotion of gastric cancer development (41). MiR-6125 binds to the 3'-UTR of *YTHDF2* and down-regulates the YTHDF2 protein, thereby enhancing the stability of m6A-modified *GSK3β* mRNA. Elevated *GSK3β* protein levels suppress the expression of proteins related to the Wnt/β-catenin/cyclin D1 pathway, resulting in G0–G1 phase arrest and subsequently preventing the propagation of colorectal cancer cells (42).

Current immunotherapy targeting anti-PD-L1 (and anti-PD-1) has completely transformed the treatment of many different advanced cancers (43). Although immune checkpoint inhibitors can greatly enhance the prognosis of BC patients, there are still significant numbers of BC patients with low sensitivity or direct resistance to this treatment. Previous research has suggested that intrinsic tumor cell factors [such as PD-L1 expression, microsatellite instability-high (MSI-H), and tumor mutation burden (TMB)] are related to the efficacy of immune checkpoint inhibitors, while extrinsic factors, such as lymphocytes infiltrating the tumor, can also play a role in immunotherapy resistance (44). The present study aimed to examine the relationship between m6A regulators and the levels of cancer immune cell infiltration using the TISIDB database. *HNRNPA2B1*, *YTHDF1*, and *YTHDF2* are all correlated with neutrophils, B cells, $CD4^+$ T, $CD8^+$ T, and dendritic cells. $CD8^+$ T cells, which play

an important role in anti-tumor immunity, are capable of proliferating and differentiating into potent cytotoxic cells with specific tumoricidal ability upon stimulation by specific cytokines (45).

Bioinformatics analysis regarding m6A in BC was also performed. Our results showed that, in addition to the relevant immune responses, *HNRNPA2B1* was also associated with regulatory RNA binding, telomeric DNA binding, miRNA binding, negative regulation of mRNA processing, etc. Furthermore, HNRNPC was found to be related to the negative regulation of DNA biosynthesis, 3'-UTR-mediated mRNA stabilization, and so on. *RBM15* was implicated in the up-regulation of the transcription of Notch receptor target genes, which is a branch involved in vascular morphogenesis. *YTHDF1* was related to hematopoietic stem cell proliferation, etc. Thus, we observed that these m6A regulators play a significant role in modulating biological events such as cancer cell proliferation, which is consistent with previous reports.

This project is mainly based on database research, which has certain limitations, including: (I) the relevant basic experiments of these single effective genes in BC have not been carried out to clarify the correlation between m6A changes and biological events such as tumor metastasis, glycolysis, tumor proliferation and apoptosis; (II) there is no detailed and comprehensive study on the role of lymphocytes in immune infiltration. Therefore, in the future further research, we will carry out *in vitro* and *in vivo* experiments of relevant target genes, and carry out relevant multicenter clinical trial validation to further evaluate the actual value of m6A regulatory factor as a clinical biomarker at the mRNA and protein levels.

Conclusions

Based on bioinformatics analysis, the present study demonstrated that the dysregulation of tumor-associated m6A regulators is correlated with the prognosis of BC and may therefore be used as biomarkers for prognostic evaluation. Moreover, the relationship between the expression of m6A regulators and the levels of immune infiltration and the expression of immune regulators in BC was also evaluated. The current research suggests that m6A regulators may be used as potential drug targets in BC.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5993/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5993/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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