Gene Risk Signature of m6A Regulators, KIAA1429, YTHDF1, YTHDF2, METTL3 and Its Relationship with Immune Infiltration in Hepatocellular Carcinoma

Jingjing Guo
The Second Hospital of Nanjing, Nanjing University of Chinese Medicine

Liwei Wang
The Affiliated Jiangning Hospital of Nanjing Medical University https://orcid.org/0000-0003-4796-4969

Hanfeng Xu
The second Hospital of Nanjing, Nanjing University of Chinese Medicine

Chuandong Zhu
The Second Hospital of Nanjing, Nanjing University of Chinese Medicine

Jianning Wang
The Affiliated Jiangning Hospital of Nanjing Medical University

Qin Zheng (njzq83626472@sina.com)

Research

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Abstract

**Background:** Hepatocellular carcinoma (HCC) is a tumor of the digestive system with high mortality. N6-methyladenosine (m6A) is one of the most common forms of mRNA modification. Tumor neoantigens play an important role in anti-tumor immune response and predict the clinical response of immunotherapy. There are few studies on the relationship between m6A regulators and immune infiltrating cells. The objective of this study was to determine the gene expression and prognostic value of m6A regulators in hepatocellular carcinoma. Further, we explored the relationship between m6A regulators and immune-infiltrating cells.

**Methods:** 13 m6A regulators expressions in 374 cancer tissues and 50 normal tissues were analyzed using RNA-seq data and clinical information in the TCGA database. Survival package was used to analyze the survival of patients in the two groups, and the corresponding clinical characteristics and gene expression were analyzed using univariate and multivariate Cox regression. We evaluated the expression of KIAA1429, YTHDF1, YTHDF2, and METTL3 in HCC and its correlation with TIICs using TIMER and CIBERSORT.

**Results:** Most m6A regulators had higher expression levels in 374 cancer tissues. We confirmed two groups of HCC patients using a consensus clustering method. The prognosis of the cluster 1 group was poor compared with that of the cluster 2 group. The m6A regulators, KIAA1429, YTHDF1, YTHDF2, and METTL3 are highly expressed in the high-risk group of HCC. Furthermore, it was found that four m6A regulators (KIAA1429, YTHDF1, YTHDF2, and METTL3) are closely related to the clinicopathological characteristics and poor prognostic marker of hepatocellular carcinoma. We analyzed the correlation between KIAA1429, YTHDF1, YTHDF2, and METTL3 expression and the level of immune invasion of HCC.

**Conclusion:** KIAA1429, YTHDF1, YTHDF2, and METTL3 as m6A regulators may regulate the tumor microenvironment through tumor immune infiltration cells to exert immune anti-tumor effects. KIAA1429, YTHDF1, YTHDF2, and METTL3 as molecular markers providing a new target for therapy of HCC in the further.

Introduction

Hepatocellular carcinoma (HCC) which the most common type of primary liver cancer is a tumor of the digestive system with high mortality. The latest cancer statistics indicate that hepatocellular carcinoma is the sixth most common cancer and the fourth leading cause of death from cancer worldwide. (1). Unfortunately, most liver cancer patients diagnosed at an advanced stage are not candidates for surgery treatment. Therefore, these patients display a dismal outcome. Novel drugs may improve the outcomes of patients with HCC, especially those in advanced stages. Exploring the molecular mechanism of liver cancer helps identify more potential therapeutic targets, providing more treatment options for HCC.

N6-methyl adenosine methylation (m6A), which occurs on mRNA and lncRNA (long chain noncoding RNA), is an epigenetic modification of RNA molecules (2-3). Geneticists discovered this methylation of
eukaryotic messenger RNA (mRNA) in the 1970s (3). The m6A methylation process involves methyltransferase complexes (writers), demethylases (erasers), and function managers (readers) (4). Writer genes, including WTAP, RBM15, KIAA1429(also called VIRMA), METTL3, METTL14, ZC3H13, mediate the methylation of RNA (5). Erasers mediate the process of RNA demethylation. FTO and ALKBH5 are two types of m6A demethylase genes (6). Readers are in charge of "reading" information about RNA modification and perform downstream RNA translation, degradation. Reader genes include YTHDFs (YTHDF1, YTHDF2, YTHDF3), YTHDC1, YTHDC2 and HNRNPC (7). m6A methylation is involved in the complex and delicate biological regulation of important functional genes in many cellular activities (8-10). Evidence also shows that m6A methylation is controlled by the biological clock (11) and by sperm production (12), embryo development (13), maintenance of pluripotency of embryonic stem cells (14), sex determination of fruit flies(15), T cell homeostasis (16), heat shock response (17), and regulation of cardiac contractile function (18). m6A modifications have been associated with multiple diseases, for instance obesity (19), type 2 diabetes (20), neurological diseases (21), and tumors (22-30).

In this study, the 13 m6A regulators expression in 374 patients with HCC was systematically analyzed using the Cancer Genome Atlas (TCGA) data set. Using bioinformatics and statistical analysis, this study explored the potential application value of m6A regulators and its correlation with immune infiltration in hepatocellular carcinoma.

**Materials & Methods**

**Datasets**

We downloaded the RNA-seq transcriptome data and clinical information of HCC patients using the TCGA database (https://cancergenome.nih.gov/).

**Immune infiltrates analysis**

TIMER is a tool for systematic analysis of immune infiltration of different cancer types (https://cistroshinyapps.io/timer/) (31). We evaluated the expression of four m6A regulators in HCC and its correlation with TIICs including B cells, CD4+ T cells, CD8+ T cells, neutrophils, dendritic cells, and macrophages. We used CIBERSORT (https://cibersortx.stanford.edu/) to investigate the relationship between high and low levels of the four m6A regulators in the tumor immune microenvironment of HCC.

**Bioinformatic Analyses**

We analyzed the 13 genes expression in 374 tumor tissues and 50 normal liver tissues using Limma software package. Then, we used the vioplot software package to visualize 13 genes in 374 tumor tissues and 50 normal liver tissues. Next, in this study, 50 normal tissue samples were extracted, 374 cancer tissues were grouped using the Consensus ClusterPlus package, and the grouping results were
verified using Principal Component Analysis (PCA). Finally, we analyzed the survival of the two clusters using the survival package. We used univariate and multivariate Cox regression to analyze the corresponding clinical features and gene expression in the TCGA data set.

**Statistical Analyses**

The 13 m6A regulators expression in 374 HCC tissues and 50 normal liver tissues in the TCGA dataset were contrasted using one-way ANOVA. The clinical characteristics of the primary liver cancer samples in the TCGA dataset were compared using a t-test. Kaplan-Meier method was used for the bilateral logarithmic rank test in overall survival analysis. All the statistical analyses in this study were implemented by R v3.6.3, and P < 0.05 means the difference is statistically significant.

**Results**

**Identification of m6A regulators in hepatocellular carcinoma.**

The 13 m6A regulators expression in the TCGA database is shown in Figure 1A and 1B. Except for METTL14 and ZC3H13, the expression of the other 11 genes was strikingly different among tumor group and normal group. The expression of ALKBH5 (p=0.001), WTAP (p<0.05), YTHDF1, YTHDF2, FTO, HNRNPC, YTHDC1, YTHDC2, RBM15, KIAA1429, and METTL3 (P<0.001) in HCC tissues was upregulated compared with normal liver tissues (Figure 1A, 1B). 13 m6A regulators expression were positively correlated with each other (Figure 1C).

**Consistent clustering of m6A regulators determined two clusters**

In this study, we used the Consensus Cluster Plus package to extract 50 normal liver tissues and 374 liver cancer tissues. On the basis of the expression similarity of m6A regulators in the TCGA dataset, k = 2 seemed to have the smaller Cumulative distribution function (CDF) value (Figure 2 B, C). We divided the patients into two groups (Figure 2A). Next, we used PCA to analyze the two clusters. The results showed that cluster 1 and cluster 2 were distinct. (Figure 2D)

**Correlation between consensus cluster classification and clinical characteristics**

We analyzed the OS curves of two groups of patients. Kaplan-Meier survival curve was used to analyze OS rate of HCC patients between two clusters. In this study, we found that the OS of cluster 1 was striking
shorter than cluster 2 (Figure 3A). Most m6A regulators were highly expressed in the cluster 2. Patients of HCC in the cluster 1 had higher scores compared with the cluster 2. In this study, we investigated the positive relationship of m6A regulator and the pathological characteristics such as age, gender, grade, stage, TMN status of HCC. (Figure 3B)

**Risk Signature and Poor prognosis of m6A Regulators**

Univariate Cox regression analysis was used to the expression of the TCGA dataset. The results showed that HNRNPC (HR = 1.024, 95% CI = 1.009–1.039), RBM15 (HR = 1.264, 95% CI = 1.031–1.548), WTAP (HR = 1.086, 95% CI = 1.029–1.147), KIAA1429 (HR = 1.158, 95% CI = 1.070–1.253), YTHDF2 (HR = 1.117, 95% CI = 1.070–1.165), METTL3 (HR = 1.252, 95% CI = 1.128–1.390), YTHDC1 (HR = 1.091, 95% CI = 1.015–1.173) and YTHDF1 (HR = 1.080, 95% CI = 1.045–1.115) corresponded to lower survival rate in patients. Instead, increased expression of ZC3H13 (HR = 0.907, 95% CI = 0.833–0.988) was associated with a higher survival rate in patients (Figure 4A). The 13 genes in the TCGA dataset were analyzed using the least absolute shrinkage and selection operator (LASSO) Cox regression algorithm. (Figure 4B, C). We separated the patients into a low-risk group and a high-risk group. The results indicated that the high-risk group of patients had poor survival (Figure 4D).

**Correlation between prognostic risk score and clinicopathological characteristics**

We investigated the role of the five genes in the high and low-risk patients, and the pathological characteristics including age, stage, TMN status of liver cancer (Figure 5A). Higher expression of KIAA1429, YTHDF1, YTHDF2, and METTL3 and lower expression of ZC3H13 in hepatocellular carcinoma (Figure 5A). Using the ROC curve, we predicted prognostic risk scores and 3-year survival rates in HCC patients. The consequences showed that risk score predicted 3-year survival in patients (AUC = 0.619) (Figure 5B). Univariate and multivariate Cox regression analysis confirmed that the risk score, stage, T status were all related to OS (Figure 5C,D). The clinicopathological characteristics of primary liver cancer, and KIAA1429, YTHDF1, YTHDF2, and METTL3 were risk factors for poor prognosis of liver cancer patients.

**Relationship between KIAA1429, YTHDF1, YTHDF2, and METTL3 expression and tumor-infiltrating immune cells**

Independent tumor-infiltrating lymphocytes play a pivotal role in predicting prognosis (32). This study used TIMER to analyze the correlation between KIAA1429, YTHDF1, YTHDF2, and METTL3 expression and the level of immune invasion of liver cancer. KIAA1429 expression was associated with B cells (p-value = 1.39 × 10^{-7}), CD^4^+ T cells (p-value = 4.20 × 10^{-7}), Neutrophil (p-value = 2.46 × 10^{-7}), and
Dendritic cells ($p$-value = $1.85 \times 10^{-7}$). YTHDF1 expression was associated with B cells ($p$-value = $2.70 \times 10^{-11}$), CD$^4^+$ T cells ($p$-value = $5.11 \times 10^{-16}$), Macrophage ($p$-value = $5.23 \times 10^{-21}$), Neutrophil ($p$-value = $2.25 \times 10^{-18}$), and Dendritic cells ($p$-value = $1.36 \times 10^{-14}$). YTHDF2 expression was associated with CD$^4^+$ T cells ($p$-value = $8.97 \times 10^{-13}$), Macrophage ($p$-value = $7.23 \times 10^{-16}$), Neutrophil ($p$-value = $3.02 \times 10^{-21}$), and Dendritic cells ($p$-value = $6.71 \times 10^{-11}$). METTL3 expression was associated with B cells ($p$-value = $9.51 \times 10^{-7}$), CD$^4^+$ T cells ($p$-value = $5.73 \times 10^{-14}$), Macrophage ($p$-value = $1.25 \times 10^{-11}$), Neutrophil ($p$-value = $8.70 \times 10^{-9}$), and Dendritic cells ($p$-value = $6.21 \times 10^{-9}$) (Figure 6A-6D). We further investigated whether there is a difference in the tumor immune microenvironment between high and low levels of the four m6A regulators in HCC. The CIBERSORT algorithm was applied to 22 immune cell subtypes to evaluate the difference in their expression levels between the high expression group and the low expression group (Figure 7A-7D). There was no significant difference in immune infiltrating cells between the KIAA1429 high expression group and the low expression group. Compared with the low expression group, the neutrophils in the YTHDF1 high expression group increased ($p$-value = 0.039), the neutrophils in the YTHDF2 high expression group increased ($p$-value = 0.023), and the METTL3 memory B cells increased ($p$-value = 0.006). The results indicate that KIAA1429, YTHDF1, YTHDF2, and METTL3 may play a crucial part in immune invasion of liver cancer.

**Discussion**

Globally, liver cancer is the sixth most frequent cancer and the fourth leading cause of cancer death. (1) Surgery is the preferred treatment for patients with liver cancer at an early stage (33). More evidence indicated that primary liver cancer is a multistep process including the complex interactions between genetics, epigenetics, and transcriptional changes (34).

The m6A modification usually first causes the m6A methylation group to be written into the RNA through coding genes called writers (35) and then eraser genes remove the m6A methylation group on the RNA, thereby affecting the tumor biological process (30). Finally, the reader gene binds to the m6A site on the RNA in the nucleus or cytoplasm to play a specific biological role (36,37). The expression level of m6A gene and protein may a new target of molecular targeted therapy for primary liver cancer.

In this study, we investigated that there is a close relationship between the m6A regulators and pathological characteristics of prime liver cancer. KIAA1429, YTHDF1, YTHDF2, and METTL3 were higher expressed in a high-risk group of liver cancer. The risk score is an independent prognostic indicator, which is related to the clinicopathological features of primary liver cancer.

As a downstream target of METTL3, cytokine signaling inhibitor 2 (SOCS2) is also a tumor suppressor. Through the m6A-YTHDF2-dependent signal way, METTL3 reduced the stability of SOCS2 and promotes the occurrence and development of HCC (38). METTL3, YTHDF2, and ZC3H13 were found to be independent prognostic factors. In HCC, the expression of METTL3 is up-regulated through CNV and DNA methylation mechanisms. METTL3 may be a biomarker for the prognosis of liver cancer (39). YTHDF1 expression is related to the pathological stage of patients with HCC. Patients with low expression of
YTHDF1 related to higher survival rates (40). The decreased expression of METTL14 enhanced the ability of metastasis in liver cancer cells, and the OS rate of liver cancer patients was lower. Increased METTL14 expression inhibited the ability of migration and invasion in vitro tumor cell (41). METTL14 may play a role in the progression of HCC by regulating the m6A levels of CSAD, GOT2, and SOCS2(42). In this study, we also found that KIAA1429 protein expression was not detected in normal liver tissues and HCC tissues. Protein expression data of YTHDF1 and METTL3 were missing from the HPA data. The expression of YTHDF2 was not detected in normal tissues or moderately expressed in liver tissues, and the expression of the four genes in normal tissues and liver cancer tissues was not consistent with their mRNA expression levels. This conclusion is only based on the HPA database and needs to be confirmed by further experimental data. The study demonstrated the expression and poor prognosis of m6A regulators in HCC. The expression of m6A regulator are positively correlated with the clinicopathological features of HCC.

The composition of immune cells in the tumor microenvironment is closely related to prognosis. M2 macrophages and pro-inflammatory M1 macrophages are associated with the growth and spread of tumors and the formation of immunosuppressive microenvironments (43,44). High concentrations of CD3+ T cells, CD8+ cytotoxic T cells, and CD45RO+ memory T cells suggest a better prognosis. (45,46) Compared with normal livers, there were fewer mast cells, monocytes, and plasma cells activated in HCC, and more resting mast cells, total B cells, and naive B cells, CD4+ memory resting T cells, and CD8+ T cells (47). Tumor neoantigens play an important role in anti-tumor immune response and predict the clinical response of immunotherapy (48,49). The potential role of m6A modification in the host's anti-tumor immune response is unclear. Studies have shown that long-lasting new antigen-specific immunity can be controlled by m6A modification of m6A binding protein YTHDF1 to achieve anti-tumor therapy, suggesting that YTHDF1 may be a potential therapeutic target for anti-cancer immunotherapy (50). We found that KIAA1429, YTHDF1, YTHDF2, and METTL3 are positively correlated with various immune cells, suggesting that they may participate in the tumor microenvironment through the composition of immune cells and play a role in the occurrence and development of tumors.

**Conclusions**

KIAA1429, YTHDF1, YTHDF2, and METTL3 can not only be used for tumors by m6A modification mechanism, but also may regulate the tumor microenvironment through tumor immune infiltration cells to exert immune anti-tumor effects. KIAA1429, YTHDF1, YTHDF2, and METTL3 as molecular markers providing a new target for therapy of HCC in the further.

**Declarations**

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Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. (2018) 68(6):394–424. https://doi.org/10.3322/caac.21492 PMID:30207593.
2. Dubin DT, Taylor RH. The methylation state of poly A-containing messenger RNA from cultured hamster cells. Nucleic Acids Res. (1975)2(10):1653–68. doi: 10.1093/nar/2.10.1653.
3. Boccaletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, et al. MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res. (2018)46(D1): D303–D307. doi: 10.1093/nar/gkx1030.
4. Yang Y, Hsu PJ, Chen YS, Yang YG. Dynamic transcriptomic m6A decoration: writers, erasers, readers and functions in RNA metabolism. Cell Res. (2018)28(6):616–624. doi: 10.1038/s41422-018-0040-8.
5. Schöller E, Weichmann F, Treiber T, Ringle S, Treiber N, FlatleyA, et al. Interactions, localization, and phosphorylation of the m6A generating METTL3-METTL14-WTAP complex. RNA. (2018)24(4):499–512. doi: 10.1261/rna.064063.117.
6. Tang C, Klukovich R, Peng H, Wang Z, Yu T, Zhang Y, et al. ALKBH5 dependent m6A demethylation controls splicing and stability of long 3'-UTR mRNAs in male germ cells. Proc Natl Acad Sci USA.
7. Ding C, Zou Q, Ding J, Ling M, Wang W, Li H, et al. Increased N6-methyladenosine causes infertility is associated with FTO expression. J Cell Physiol. (2018)233(9):7055–7066. doi: 10.1002/jcp.26507.

8. Wojtas MN, Pandey RR, Mendel M, Homolka D, Sachidanandam R, Pillai RS. Regulation of m6A transcripts by the 3′→5′ RNA helicase YTHDC2 is essential for a successful meiotic program in the mammalian germline. MolCell. (2017)68(2):374–387.e12.doi: 10.1016/j.molcel.2017.09.021.

9. Meyer KD, Saleitore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell. (2012) 149(7):1635–1646. doi: 10.1016/j.cell.2012.05.003.

10. Bodi Z, Bottley A, Archer N, May ST, Fray RG. Yeast m6A methylated mRNAs are enriched on translating ribosomes during meiosis, and under rapamycin treatment. PLoS ONE. (2015)10(7):e0132090. doi: 10.1371/journal.pone.0132090.

11. Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, et al. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell. (2013)155(4):793-806. doi: 10.1016/j.cell.2013.10.026.

12. Lin Z, Hsu PJ, Xing X, Fang J, Lu Z, Zou Q, et al. Mettl3-/Mettl14-mediated mRNA N6-methyladenosine modulates murine spermatogenesis. Cell Res. (2017)27(10):1216-1230. doi: 10.1038/cr.2017.117.

13. Mendel M, Chen KM, Homolka D, Gos P, Pandey RR, McCarthy AA, et al. Methylation of structured RNA by the m6A writer METTL16 is essential for mouse embryonic development. Mol Cell. (2018)71(6):986-1000.e11. doi: 10.1016/j.molcel.2018.08.004.

14. Aguilo F, Zhang F, Sancho A, Fidalgo M, Di Cecilia S, Vashisht A, et al. Coordination of m6 a mRNA methylation and gene transcription by ZFP217 regulates pluripotency and reprogramming. Cell Stem Cell. (2015)17(6):689-704. doi: 10.1016/j.stem.2015.09.005.

15. Haussmann IU, Bodi Z, Sanchez-Moran E, Mongan NP, Archer N, Fray RG, et al. m6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination. Nature. (2016) 540(7632):301-304. doi: 10.1038/nature20577.

16. Li HB, Tong J, Zhu S, Batista PJ, Duffy EE, Zhao J, et al. m6A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOC5 pathways. Nature. (2017)548(7667):338-342. doi: 10.1038/nature23450.

17. Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. Nature. (2015) 526(7574):591-594. doi: 10.1038/nature15377.

18. Mathiyalagan P, Adamiak M, Mayourian J, Sassi Y, Liang Y, Agarwal N, et al. FTO-dependent m6 a regulates cardiac function during remodeling and repair. Circulation. (2019)139(4):518-532. doi: 10.1161/CIRCULATIONAHA.118.033794.

19. Ben-Haim MS, Moshitch-Moshkovitz S, Rechavi G. FTO: linking m6A demethylation to adipogenesis. Cell Res. (2015)25(1):3-4. doi: 10.1038/cr.2014.162.
20. Shen F, Huang W, Huang JT, Xiong J, Yang Y, Wu K, et al. Decreased N(6)-methyladenosine in peripheral blood RNA from diabetic patients is associated with FTO expression rather than ALKBH5. J Clin Endocrinol Metab. (2015)100(1): E148-54. doi: 10.1210/jc.2014-1893.

21. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell. (2013) 49(1):18-29. doi: 10.1016/j.molcel.2012.10.015.

22. Angelova MT, Dimitrova DG, Dinges N, Lence T, Worpenberg L, Carré C, et al. The emerging field of epitranscriptomics in neurodevelopmental and neuronal disorders. Front Bioeng Biotechnol, (2018), 6:46. doi: 10.3389/fbioe.2018.00046.

23. Wang S, Sun C, Li J, Zhang E, Ma Z, Xu W, et al. Roles of RNA methylation by means of N(6)-methyladenosine (m6A) in human cancers. Cancer Lett. (2017) 408:112-120. doi: 10.1016/j.canlet.2017.08.030.

24. Chen XY, Zhang J, Zhu JS. The role of m6A RNA methylation in human cancer. Mol Cancer. (2019) 18(1):103. doi: 10.1186/s12943-019-1033-z.

25. Chen B, Li Y, Song R, Xue C, Xu F. Functions of RNA N6-methyladenosine modification in cancer progression. Mol Biol Rep. (2019) 46(2):2567–2575. doi: 10.1007/s11033-019-04655-4.

26. Zhou S, Bai ZL, Xia D, Zhao ZJ, Zhao R, Wang YY, et al. FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting b-catenin through mRNA demethylation. Mol Carcinog. (2018) 57(5): 590–597. doi: 10.1002/mc.22782.

27. Taketo K, Konno M, Asai A, Koseki J, Toratani M, Satoh T, et al. The epitranscriptome m6A writer METTL3 promotes chemo-and radioresistance in pancreatic cancer cells. Int J Oncol. (2018); 52(2): 621–629. doi: 10.3892/ijo.2017.4219.

28. Cheng X, Li M, Rao X, Zhang W, Li X, Wang L, et al. KIAA1429 regulates the migration and invasion of hepatocellular carcinoma by altering m6A modification of ID2 mRNA. Onco Targets Ther. (2019)12: 3421–3428. doi: 10.2147/OTT.S180954.

29. Dai D, Wang H, Zhu L, Jin H, Wang X. N6-methyladenosine links RNA metabolism to cancer progression. Cell Death Dis. (2018) 9(2): 124. doi: 10.1038/s41419-017-0129-x.

30. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, et al. m6A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell Rep. (2017) 18(11): 2622–2634. doi: 10.1016/j.celrep.2017.02.059.

31. T. Li, J. Fan, B. Wang, et al., TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells, Cancer Res. 77 (21) (2017) e108–e110.

32. H. Ohtani, Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer, Cancer Immunity. 7 (2007) 4.

33. Orcutt ST, Anaya DA. Liver Resection and Surgical Strategies for Management of Primary Liver Cancer. Cancer Control. (2018)25(1):1073274817744621. doi: 10.1177/1073274817744621.

34. Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets.
35. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. (2012)485(7397): 201–206. doi: 10.1038/nature11112.

36. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, et al. Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. Nucleic Acids Res. (2015)43(1):373-384. doi: 10.1093/nar/gku1276.

37. Meyer KD, Jaffrey SR. Rethinking m6A Readers, Writers, and Erasers. Annu Rev Cell Dev Biol. (2017)33:319-342. doi: 10.1146/annurev-cellbio-100616-060758.

38. Chen M, Wei L, Law CT, Tsang FH, Shen J, Cheng CL, et al. RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2. Hepatology. (2018) 67(6):2254-2270. doi: 10.1002/hep.29683.

39. Gao-Min Liu, Hua-Dong Zeng, Cai-Yun Zhang, Ji-Wei Xu. Identification of METTL3 as an Adverse Prognostic Biomarker in Hepatocellular Carcinoma. Dig Dis Sci. 2020 Apr 24. doi: 10.1007/s10620-020-06260-z.

40. Zhao X, Chen Y, Mao Q, Jiang X, Jiang W, Chen J, et al. Overexpression of YTHDF1 is associated with poor prognosis in patients with hepatocellular carcinoma. Cancer Biomark, (2018) 21(4):859-868. doi: 10.3233/CBM-170791.

41. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N6-methyladenosine-dependent primary MicroRNA processing. Hepatology. (2017) 65(2):529-543. doi: 10.1002/hep.28

42. Zedong Li, Fazhan Li, Yu Peng, Jianyu Fang, Jun Zhou. Identification of Three m6A-related mRNAs Signature and Risk Score for the Prognostication of Hepatocellular Carcinoma. Cancer Med. 2020 Mar; 9(5):1877-1889. doi: 10.1002/cam4.2833. Epub 2020 Jan 13.

43. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. (2008) Jul 24; 454 (7203):436-44. doi: 10.1038/nature07205

44. Bronte V, Brandau S, Chen S H, Mario P Colombo, Alan B Frey, Tim F Greten. et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun. (2016) Jul 6(7): 12150. doi: 10.1038/ncomms12150.

45. Fridman W H, Zitvogel L, Sautes-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol.(2017)14(12):717-734.

46. Mlecnik B,Bindea G,Angell H K. Pauline Maby, Mihaela Angelova, David Tougeron. et al. Integrative analyses of colorectal cancer show immunoresponse is a stronger predictor of patient survival than microsatellite instability. Immunity. (2016)44(3):698-711

47. Rohr-Udilova N, Klinglmuller F, Schulte-Hermann R, Judith Stift, Merima Herac, Martina Salzmann et al. Deviations of the immune cell landscape between healthy liver and hepatocellular carcinoma. Sci Rep. (2018)8(1):6220. doi: 10.1038/s41598-018-24437-5
48. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. Science. 2015 Apr 3;348(6230):69-74. doi: 10.1126/science.aaa4971.

49. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature.2017 Jul 13;547(7662):217-221. doi: 10.1038/nature22991.

50. Han D, Liu J, Chen C, Dong L, Liu Y, Chang R.et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. Nature.2019 Feb; 566 (7743):270-274. doi: 10.1038/s41586-019-0916-x.

Figures
Figure 1

Gene expression and role of m6A regulators in HCC. (A) m6A RNA regulators expression in normal tissues and tumor tissues. High expression in red and low expression in green. (B) Vioplot visualization of m6A regulators expression in tumor tissues and normal tissues (tumor tissues in red and normal tissues in blue). (C) Spearman correlation analysis of 13 m6A regulators.
Figure 2

Description of consistent clustering. (A) Matrix $k = 2$; (B) Cumulative distribution function (CDF) when $k = 2-9$; (C) The area under the CDF curve when $k = 2-9$; (D) Principal component analysis (PCA) of m6A regulators expression. The group of cluster 1 are marked red, and the group of cluster 2 are marked blue.
Overall survival rate and clinicopathologic features in both groups. (A) Overall survival (OS) curve using Kaplan-Meier. Cluster 1 in red and cluster 2 in blue. (B) Heatmap of the 13 m6A regulators expression in patients of different pathological characteristics of two clusters in HCC.
Figure 4

Risk characteristics of m6A regulators. (A) Univariate Cox regression was used to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs). (B, C) Multivariate Cox regression was performed with LASSO. (D)
Figure 5

The relationship between risk score, clinicopathological characteristics. (A) Expression of the three m6A regulators in low and high-risk groups. (B) ROC curve verifies the validity of risk signatures. (C) Univariate Cox regression analysis for OS and clinicopathological factors in patients (D) Multivariate Cox regression analysis for clinicopathological factors and OS in patients.
Figure 6
Relationship between KIAA1429(A), YTHDF1(B), YTHDF2(C), and METTL3(D) expression and tumor-infiltrating immune cells.
The immune cells in high and low expression groups of KIAA1429(A), YTHDF1(B), YTHDF2(C), and METTL3(D).