

# Effects Which M6A RNA Methylation Regulation Exerts on the Prognosis of Hepatocellular Carcinoma

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## Research article

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# Abstract

Background: Hepatocellular carcinoma (HCC), which has been known as the most common subtype in the range of primary liver cancer. Besides, it hails as one of China's common cancers, giving rise to the major cancer death cause in men. N6 methyladenosine (m6A) RNA methylation is under the regulation of m6A RNA methylation regulators in dynamic way (the proteins of "writer" "eraser"; "reader"). More and more evidences show that the m6A modification level is connected with self-renewal of tumor stem cells, the growth, proliferation, anti chemotherapy and radiosensitivity of tumor cells. The relationship between m6A RNA and human cancer types has been confirmed in a variety of cancers. This research aims to investigate the relationship between m6A RNA methylation regulators and liver cancer.

Methods: firstly, the comparison of the expression levels harbored by 13 major m6A RNA methylation regulators in liver cancer with normal tissues was conducted by means of the data of TCGA database. Secondly, we cluster the presentation data of m6A RNA methylated regulator uniformly and dissect HCC tissue into two subgroups (group 1 and 2) by comparing these subgroups according to the overall survival rate (OS), WHO phase and pathological level. Thirdly, based on the combination of least absolute contraction with selection operator (lasso) regression, the risk characteristics of m6A RNA methylation regulators was constructed, which affected OS in TCGA analysis.

Results: there were significant differences in the presentation grades held by 12 major m6A RNA methylation regulators in liver cancers and normal tissues. The primary liver cancer was divided into 1 and 2 groups. It was found that the OS of 1 subgroup was poor, the WHO stage was high and the pathological grade was high. In TCGA analysis, five m6A methylation regulators (YTHDF1, ZC3H13, YTHDF2, METTL3 and KIAA1429) were selected to affect OS, and a risk marker significantly related to who staging was constructed, which was also an independent prognostic marker of OS.

Conclusion: m6A RNA methylation regulator is a key player in the progression of HCC and has potential value in the prediction and treatment of HCC.

## Background

Hepatocellular carcinoma (HCC), which is known as the most common subtype under primary liver malignant tumors, ranks the world's third leading death cause related to cancer<sup>[1]</sup>. The disease has been proved to lead to the annual death of over 700000 people from liver cancer<sup>[2]</sup>. Therefore, it is touted as China's one of common malignant tumors. Besides, it occurs to men as the major cancer death cause<sup>[3]</sup>. Hepatitis B virus, hepatitis C virus and alcoholism constitute the major liver cancer cause<sup>[4]</sup>. High recurrence rate, together with high metastasis rate gives rise to poor prognosis of liver cancer patients<sup>[5]</sup>. Due to the limited effective treatment, patients often miss the best opportunity for clinical intervention, and HCC is usually diagnosed at a later stage<sup>[6]</sup>. Therefore, understanding the molecular system of hepatocellular carcinoma is of vital importance. In addition, it is essential to promote the invention of diagnosis and treatment in the future. It is exemplified that liver cancer occurrence performs a multi-

procedure phases involved in the complicated complex communications among genetics, epigenetics as well as transcriptional transformations. Recent genome-wide and exome sequencing analyses have described the mutations in liver cancer and found some new driving mutations. Strikingly, epigenetic regulation is one of the most common ways of variation, which may lead to profound changes in gene expression to promote the formation and development of liver cancer [7].

RNA modification, which was found first during the 1970s, is considered to be the third layer of epigenetics as well as new functions modulating RNA process and metabolism [8-14]. Recent years have witnessed the discoveries of a number of chemical modifications in different RNA patterns, which include tRNA, miRNA, mRNA, long noncoding RNA, etc. [12-17]. According to reports, these RNA modifications have more than one form, like N<sup>7</sup>-methyladenosine, 5-methylcytosine, N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) and 2'-O-methyl [12, 16, 18] respectively. N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), first reported in 1974 [19], is given identification first as the richest mRNA methylation form in eukaryotes [16, 20-21]. As we all know, RNA modification, which bears some similarity to DNA and protein modification, is put under the regulation of methyltransferase in a dynamical way (the "writer"), binding protein (the "reader") and demethylase (the "eraser") [20], respectively entitled "readers" for (YTHDC1, YTHDC2, YTHDF1, YTHDF2 and HNRNPC), "writers" for (METTL3, METTL4, WTAP, KIAA1429, RBM15 and ZC3H13); "eraser" for (FTO and ALKBH5) [18, 22-23]. The methylation of m<sup>6</sup>A RNA shows a reversing dynamic biological procedure via writer regulations, reader regulation and eraser regulation. In recent years, more and more evidences show that the degree of m<sup>6</sup>A modification embraces the relation with self-renewal of tumor stem cells, the growth and proliferation of tumor cells, the sensitivity of anti chemotherapy and radiotherapy [23-27]. The association between m<sup>6</sup>A RNA and human cancer types has been confirmed in a variety of cancers, which include cervical cancer, prostate cancer, breast cancer, pancreatic cancer, liver cancer as well as acute myeloid leukemia [28-30]. Up to now, although METTL4 has been reported to be a tumor suppressor by manipulating the pri Mir126 modified by m<sup>6</sup>A with the help of dgcr8 [31], in addition, METTL3 has also been reported to enhance the m<sup>6</sup>A modification of SOCS2 in a YTHDF2 dependent manner, thereby enhancing the progress of HCC [32]. It is rare to predict the prognosis of HCC through the construction of a prognostic risk assessment on the basis of m<sup>6</sup>A RNA methylation regulator.

In this study, we downloaded RNA sequencing data from the Cancer Genome Atlas (TCGA) data set. The expression of 13 widely reported m<sup>6</sup>ARNA regulatory factors in hepatocellular carcinoma and their relationship with clinicopathological features were analyzed systematically. Then, through bioinformatics analysis and statistical analyses, it aims to investigate the latent application value featured by M<sup>6</sup>A modification regulatory factors in hepatocellular carcinoma.

## Materials And Methods

### Data source

The RNA-seq transcriptome data of HCC samples, relevant clinical information and normal control samples were downloaded from nci-gdc (<https://portal.gdc.cancer.gov/>). Samples with incomplete data on age, gender, WHO stage and pathological grade were excluded from further evaluation. RNA SEQ data were collected in the form of fpkm<sup>[33]</sup>. A total of 374 HNSCC cases and 50 normal control samples were used for follow-up analysis.

## Screening of m6ARNA Methylation Regulators

In total, M6A associated genes exist in HCC and 13 of them are considered to be M6A methylation regulatory genes<sup>[13]</sup>. Then, based on TCGA data, the associated betwixt the presentation featured by these m6ARNA methylation regulators as well as distinct clinicopathological was under evaluation .

## PPI Network Structure and the Correlation Analyses

The protein-protein interaction (PPI) between the methylation regulators of m6A RNA was analyzed, based on string<sup>[34]</sup> database (<http://string-db.org>). The "corrlot" software package in R software was used to analyze the correlation between M6A RNA methylation regulatory factors.

## Tumor classification

Using the consensusclusterplus package of R<sup>[35]</sup>, using the consistency clustering algorithm to evaluate the stability of clustering and determine the number of clustering samples. The classification principles are as follows: 1) the value of cumulative distribution function (CDF) grows slowly; 2) there is no small cluster in the cluster data; 3) the intra group correlation is high.

## Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) provided by R's limma package<sup>[36]</sup>, was used,; and the results were visualized by ggplot2 package<sup>[37]</sup>.

## Risk score and prognosis analysis

For the purpose of evaluating the prognostic significance of m6A RNA methylation regulators, lassocox regression algorithm was used to establish potential risk characteristics. Identify genes that are significantly related to survival. Further functional analysis of these genes was carried out, and the correlation coefficient was decided by the minimum standard, and the optimal penalty parameter  $\lambda$  related to the minimum 10 times cross validation was selected. The following formula was used for calculating the risk scores presented by the signature:

$$\text{Risk score} = \sum_{i=1}^n \text{coef}_i \times X_i$$

Where coef<sub>i</sub> refers to the coefficient; X<sub>i</sub> stands for the relative presentation of score transformation of per chosen gene. The formula is employed for calculating the risk scores for every patient on the data set of training and validation (TCGA) .

The presentation of RNA methylation regulators revealed that patients were dissected to be two subgroups, with lasso regression (according to risk characteristics) risk score as the boundary value. To classify the high-risk team and the low-risk team , and compare the two groups' overall survivals , the ROC curve<sup>[38]</sup> was used to test the predictive efficiency risk scores characterized by the survival model. Single factor and multi factor Cox regression analysis , which were applied to determining the prognostic value of the risk score, aimed to analyze the molecular pathological features held by each of subgroup according to the risk score.

## Statistical Analysis

Single factor analysis of variance was employed for the comparison on the presentation of m6A RNA methylation regulatory factor in TCGA tumor tissue and paracancerous tissue. Chi square test were conducted for comparing the clinical characteristics of various groups and the regulation of m6A RNA methylation. The Kaplan Meier approach was committed for the overall survival analysis. The tests, which, applied bilateral patterns and P < 0.05 was regarded to of statistical significance . All of the statistical analysis in this study is implemented in rv3.6.1 (<https://www.r-project.org/>).

## Results

### The different presentation of m6A RNA methylation regulatory factor affects tumor tissue and normal control tissues

Figures 1A and 1B illustrate the presentation 13 m6A RNA methylation regulators in the TCGA database. Red or green relatively represents a high or low expression, respectively (Figure 1A). The expression levels of FTO (P < 0.001), YTHDC2 (P < 0.001), YTHDC1 (P < 0.001), ALKBH5 (P = 0.001), KIAA1429 (P < 0.001), METTL3 (P < 0.001), HNRNPC (P < 0.001), YTHDF2 (P < 0.001), RBM15 (P < 0.001), YTHDF1 (P < 0.001) and WTAP (P < 0.001) in tumor tissues were significantly higher than those in normal control group. No dramatic difference can be seen betwixt METTL4 (P = 0.062) and ZC3H13 (P = 0.831) (Figure 1B).

### Interaction and Connection betwixt the Mthylation Regulators of m6A RNA

The relationship among 13 m6A RNA methylation genes was got back from string database for constructing PPI network (Figure 2A); the correlation between them was analyzed by "corplot" software package (Figure 2B) in R software. Consequently, a closely complex relationship were discovered among the six "writers" (Figure. 2A). Except for presentation of RBM25 and ZC3H13, ZC3H13 and KIAA1429 in HCC, the expressions of the other "writers" were all related (Figure. 2B). In PPI network, there was little interaction between the five "readers" (Figure. 2A). However, the observation was conducted on the correlation among HNRNPC, YTHDC2, YTHDC1, YTHDF1 and YTHDF2 in HCC (Figure. 2B), FTO, together ALKBH5 within PPI network were correlated (Figure. 2A). The presentation of FTO and ALKBH5 in HCC was of positive correlation (Figure. 2B). In addition, mettl13 had the strongest correlation with HNRNPC ( $r = 0.72$ ) among the interactions of all mRNA methylation regulators (Figure 2B).

## Consensus Clustering and Cluster Survival Rate of m6A RNA Methylation Regulators

The presentation similarity of m6A RNA methylation regulators were showing that  $k=2$  is considered to be most appropriate choice in dividing the HCC patient cohort to be two groups, namely group 1 and group 2 (Figure 3A-D). PCA analysis showed that RNA expression in the subgroup was specific, but in general, there were many overlapping regions between the two groups, indicating that there were some similarities betwixt the two teams (Figure.4). To evaluate the relationship between aggregation and clinicopathological features. There were dramatic differences at survival status, gender and grade between group 1 and group 2 ( $P < 0.05$ ), but no significant differences in other parameters such as age stage (Figure. 5A). The analysis on the total survival ration of the two teams was made by Kaplan Meier survival curve. The OS of HCC patients of Group 1 revealed a dramatical shorter value compared to that of Group 2 ( $P < 0.01$ ) (Figure 5B).

## Prognostic value of m6A RNA methylation regulatory factor and risk assessment based on m6A RNA methylation regulatory factor

It is convenient to study the prognostic value of 13 m6A RNA methylation regulators to HCC, and to conduct analysis on univariate Cox regression according to the expression level of TCGA regulators (Figure 6A). The results showed that 9 of the 13 regulatory factors expressed significant relevance to the total survival rate (OS) ( $P < 0.05$ ). Among the 9 regulatory factors, YTHDF1, YTHDC1, WTAP, YTHDF2, RBM15, METTL3, HNRNPC and KIAA1429 are deemed to be the risk genes of  $HR > 1$ , while ZC3H13 is considered as the protective gene of  $HR < 1$ .

In order for determining toppest effective prognostic factor exerted by m6A RNA methylation, the contraction and selection of 9 prognostic related genes were performed, and the lasso regression model

was established (Figure. 6B). Figure. 6B expressed the coefficients from every individual prognostic gene

The lasso findings displayed that 5 regulatory factors (YTHDF1, ZC3H13, YTHDF2, METTL3 and KIAA1429) were effective prognostic factors. The prognosis of the two regulatory factors was under the verification of Kaplan Meier plotter. The findings revealed that HCC patients who were undergoing high-level presentation of ZC3H13 were holding a good prognosis (Figure. 6C); comparatively, those with high-level presentation of YTHDF1, YTHDF2, METTL3 and KIAA1429 had a poor prognosis (Figure. 6C), which was consistent with the results of lasso regression model, which enhanced the reliability of the findings.

The construction of a risk score was based on the predicted risk factors (YTHDF1, ZC3H13, YTHDF2, METTL3 and KIAA1429). The evaluation of risk score is based on the coefficient of regression analysis of lasso. In accordance with the median risk score, the patients undergoing TCCA (n = 377) got divided to be the member of high-risk team and low-risk team. A drastic difference existed in the OS ration betwixt the two teams. The OS ratio in the high-risk group expressed a lower value compared to the one from the low-risk team (Figure. 6D, P = 1.969e-04). 40.0% stood for the five-year survival ratio of high-risk team; 56% expressed in low-risk team. The prediction efficiency of the constructed survival curve is further analyzed, as shown in Figure.6E. AUC = 0.619 indicates that the prediction efficiency of ROC curve is good.

## The influence of risk scores and clinicopathological variables for HCC patients' prognosis

The thermogram showed the presentation of five selected m6A RNA methylation regulators as well as clinicopathological variables in the groups of high-risk as well as low-risk. There were significant differences in tumor classification, T phase (P < 0.05) and stage (P < 0.01) between the two groups (Figure 7A). We use Cox univariate and multivariate analysis to determine whether risk characteristics are an independent predictor. As is shown in single factor analysis, stage, T stage, and risk score revealed significant relevance to OS (P < 0.001, Figure.7B). Multivariate analysis exhibited a dramatic association betwixt risk score and OS (P < 0.001, Figure 7C). On the basis of these findings, m6A RNA methylation regulator can take the risk score as an independently defined prognostic factor for HCC.

## Discuss

Considering that the high rate of recurrence and metastasis rate, HCC boasts a toppest common malignant tumors for adults., patients with liver cancer are usually diagnosed with poor prognosis, especially in the late stage<sup>[39]</sup>. The development of HCC is of complication complicated. As multi factor, it is composed of multi-step complex process, in connection with exterior environmental influence, diets, living routines, tissue and cell differentiation, genetic transformation cell cycle changes, gene expression, metabolism, molecular interaction, signal transduction pathway variations, as well as host immune

conditions, dynamic balance and some other influential aspects<sup>[40]</sup>. Despite that the treatment of liver cancer has made great progress in recent decades, from interventional therapy, radical resection or liver transplantation to targeted therapy or immunotherapy, the prognosis of liver cancer is still not ideal<sup>[5]</sup>. Therefore, it is still a challenging problem to explore the molecular mechanism and new therapeutic targets of HCC.

More and more evidences show that RNA modification is a common feature of mammalian cells, which may play a role in development and disease<sup>[41]</sup>. Among them, m6A modification mainly plays a role by regulating the splicing, output and stability of mRNA<sup>[42-43]</sup>. Studies have shown that cancer occurrence ratio harbors some association with the interaction of multiple genes<sup>[44]</sup>, and m6A RNA methylation regulators exert a crucial part during cancer growth<sup>[45]</sup>. With the membership of RNA epigenetic modification family, m6A has no "good" or "bad" distinction according to the existing comprehension to m6A and tumors. Also, it is able of enhance or prohibit tumor cells in virtue of adjusting the mRNA presentation of the association oncogenes and tumor suppressor genes. Under the action of "writers", m6A methylation site presented its appearance within nuclear RNA. With of the reaction from "eraser", the elimination of m6A methylation site of RNA within the area of the nucleus could be realized. Later, further process of nuclear RNA allow the reader in the nucleus to be bonded with the methylation site of m6A. As the mature RNA arises from the nucleus, several "readers" exterior to the nucleus have binded to the m6A site. What is mentioning is that various "reader" combination and m6A are going to create various biological influences<sup>[46]</sup>. The methylation level of m6A is closely related to the expression level of "writers" and "eraser", while the protein molecule expressed by "readers" gene combines with the methylation site of m6A and plays a series of biological functions<sup>[47]</sup>. Thereby, the expression level of m6A related combination of genes with proteins, which might grow to be a latent tumor molecular diagnostic marker, is also supply a novel target for developing clinical molecular targeted therapeutic drugs.

The research aimed to investigate the function exerted by m6A-rna methylation regulatory factor on HCC. It was discovered that m6A-rna methylation regulatory factor is closely related to the pathological characteristics of HCC. Applied uniform were applied to gathering m6A RNA methylation regulators to identify two subgroups of HCC, and the prognosis of subgroup 1 was poor. More than that, a risk signal was attained in virtue of five m6A RNA methylation regulators. Risk score worked as both independent prognostic indicator and a predictor of the clinicopathological characteristics of HCC. In addition, YTHDF1, YTHDF2, METTL3 and KIAA1429 took on high expression within HCC's high-risk group, while ZC3H13 possesses high expression in HCC's low-risk group.

Many studies have indicated the relation between liver cancer occurrence and abnormal presentation m6A RNA methylation regulatory factor<sup>[30-32,48-51]</sup>. ZC3H13 is a classical CCCH zinc finger protein, which locates on human chromosome 13q14.1<sup>[52]</sup>. Kim, Y.R., et al. Found that ZC3H13 gene has somatic cell migration mutation in colon cancer, suggesting that ZC3H13 may be a tumor suppressor gene<sup>[53]</sup>. However, Gewurz et al. found that ZC3H13 may be the upstream key factor of NF- $\kappa$ B, responsible for its activation<sup>[54]</sup>. It is exemplified that activation of NF- $\kappa$ B signaling can accelerate tumor proliferation and

invasion, suggesting that ZC3H13 may be a carcinogenic protein [55]. Luo et al. showed that ZC3H13 may combine with K-ras, and K-ras often mutates in various cancers like non-small cell lung cancer [56]. Dehuazhu et al. found that ZC3H13 an indispensable part in inhibiting the invasion and proliferation of colorectal cancer cells by regulating K-ras and ERK signals' expression [57]. Our group put hands on ZC3H13 presentation hold no difference in HCC as well as the normal tissues; however, it embraced some significance grading. It was highly expressed in low-risk group, suggesting that ZC3H13 might also be an anti-cancer gene in HCC. Zhao et al. studied that YTHDF1 growth is of association with the poor prognosis of liver cancer patients. YTHDF1 exerts a key role in modulating the metabolism and cell cycle of liver cancer cells [50].

The YTH521-B homologous (YTH) domain protein family (including YTHDF1, YTHDF2, YTHDC1 and YTHDC2), acting as a straightforward m6A reader, holds a preserved m6A binding site [58-59]. Despite that YTHDF1, YTHDF2, ythdf3, YTHDC1 and YTHDC2 is defined as the membership of the YTH domain family, they still exerts different function. For instance, YTHDC1-2 occupies the location of the nuclear chamber and YTHDF1-2 holds the location of the cytoplasmic chamber [60-61]. The nuclear reader YTHDC1 performs the modulation of alternative splicing via direct bond of m6A and the recruitment of splicing factors [62]; the cytoplasmic YTHDF1, as the "writers" for m6A modified mRNA performs the interaction with the binding sites upon m6A modified mRNA by promoting translation initiation [58]. Han et al. announced that m6A RNA modification involved in YTHDF1 is able to modulate antitumor immune reaction [62]. According to the research findings, made by Zhao et al. And Zhou et al, YTHDF1, which harbored high expression in HCC took on significant association with poor prognosis [50,63]. YTHDF2 exerts the major function, regulation the degeneration of m6A modified mRNA [64]. According to the researches in recent years, YTHDF2 presents close association with of HCC's malignant. The positive rate of YTHDF2 in HCC is 35.6% (67 / 188) [49]. In addition, reports have been published that YTHDF2 displayed high expression on pancreatic cancer, and it can promote pancreatic cancer cells' proliferation by inhibiting pancreatic cancer cells from migrating and invading [65]. This research reveals that YTHDF1 and YTHDF2 offered high expression on cancer tissues, and also gave high expression on high-risk groups, as well as correlation with poor prognosis, indicating that YTHDF1 and YTHDF2 were oncogenes promoting tumor progression. METTL3 is an S-adenosylmethionine binding protein with methyltransferase ability. Also, it is an indispensable elements of human methyltransferase complex which regulates those abundance and distribution of m6A modification at transcriptome level [66]. It has exemplified that METTL3 mediated m6A modification are associated with the occurrence and development of different cancer patterns. Besides, the role of METTL3 in cancer seems to depend on the type of tumor. For example, it has been reported that METTL3 can promote the development of ovarian cancer and epithelial to mesenchymal transformation of pancreatic cancer cells, and promote chemotherapy and radiotherapy resistance [29,67]; on the contrary, METTL3 may play an anti-cancer role in renal cancer or endometrial cancer patients [68,69]. Recent studies have found that METTL3 can regulate mRNA structure independently of its m6A catalytic activity, thus promoting oncogene translation [70]. KIAA1429 also given the name :virus, just similar to m6A methyltransferase related gene. It is

reported that KIAA1429 promotes the multiplication, shift and invasion of liver cancer via modulating the mRNA methylation level where cell line was situated Id2<sup>[30]</sup>. Also, a study expressed that KIAA1429 presented significant up-regulation in seminoma and down-regulation in the non-tumours<sup>[71]</sup>. KIAA1429, which features high expression on breast cancer, is able to enhance the multiplication and metastasis of breast cancer not only in vivo but also in vitro<sup>[72]</sup>. In our study, METTL3 and KIAA1429 present high expression on cancer tissues and high expression on high-risk groups. They are associated with poor prognosis, indicating that METTL3 and KIAA1429 are oncogenes promoting tumor progression in HCC. The carcinogenic effect of m6A regulatory gene seems questioned. That might be a result of tumor heterogeneity, giving rise to the different presentation of m6A regulatory genes on different tumors. The identified gene features various parts of various types of cancers.

In this study, the prognostic markers obtained by using five m6A RNA methylation regulators (ZC3H13, YTHDF1, YTHDF2, METTL3 and KIAA1429) are of great value in HCC. The ROC of the determined risk assessment shows good prediction performance in HCC. In addition, the prognostic value of risk assessment on HCC in groups of low risk and high risk. The risk assessment in this study may be helpful to estimate individual survival prediction more accurately.

## Conclusions

There are several limitations to this study. First of all, the quantities of normal samples (50) constitutes smaller tumor sample numbers (374), which might influence on the result dependability. Secondly, this research is based on pure computation. It is required that the future, experimental and clinical data should verify the findings we have made. Thirdly, a number of crucial clinical parameters cannot be obtained from TCGA. To sum up, the achieved research demonstrated that 13 m6A RNA methylation regulators feature close relevance to the clinicopathological HCC feature as well as the biological process and pathway HCC' malignant development. The achieved results might be of vitally crucial proof for the part exerted by m6A RNA methylation in HCC. It is necessary that future experimental and clinical researches should confirm our results.

## Abbreviations

HCC: Hepatocellular carcinoma; m6A, N6-methyladenosine; HR: Hazard ratio; LASSO: Least absolute shrinkage and selection operator; miRNA: microRNA; mRNA: Messenger RNA; OS: Overall survival; ROC: Receiver operating characteristic; TCGA: The Cancer Genome Atlas; WHO: World Health Organization;

## Declaration

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### Authors' contribution

Xiaojian Pei conceived and designed the idea to this paper; Baoxue Jia ,Yue Sun and Qiong wang collected and analyzed the data, and drafted the paper; Yaming Xing,Yukun Wang and Jinna Hu analyzed the data and revised the final paper. All authors read and approved the final manuscript.

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## **Availability of data and materials**

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

## **Ethics approval and consent to participate**

Not applicable.

## **Consent for publication**

Not applicable.

## **Competing interests**

All authors certify that we have no conflict of interest in this study.

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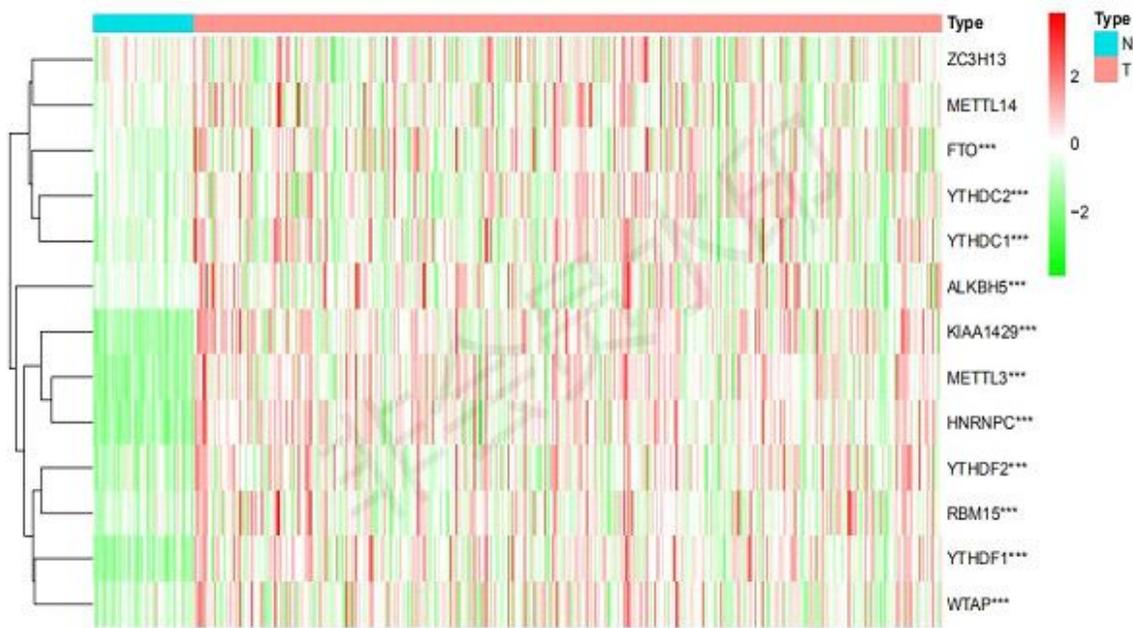
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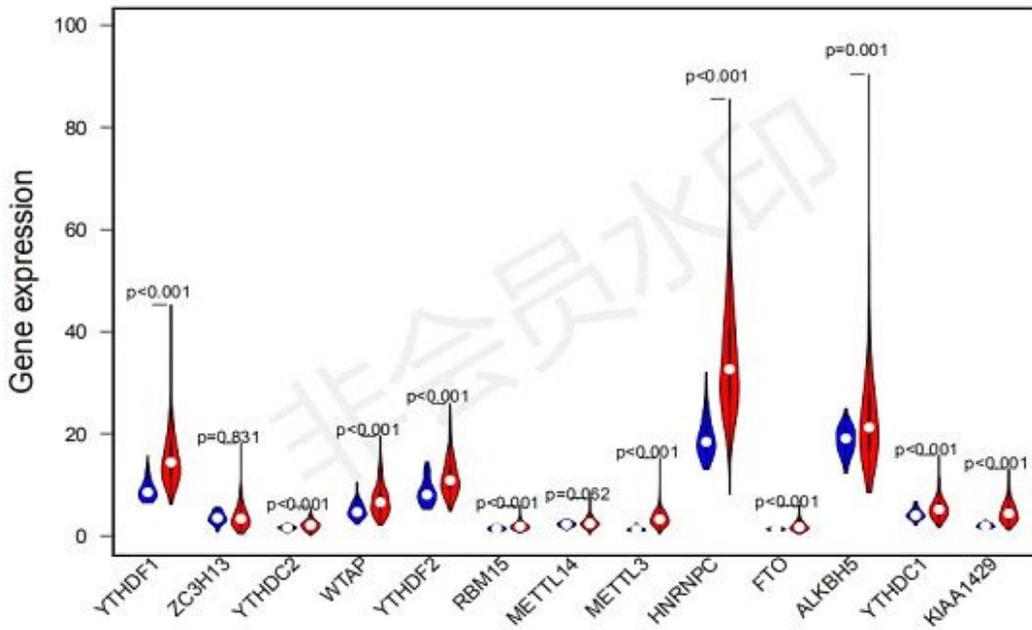
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## Figures

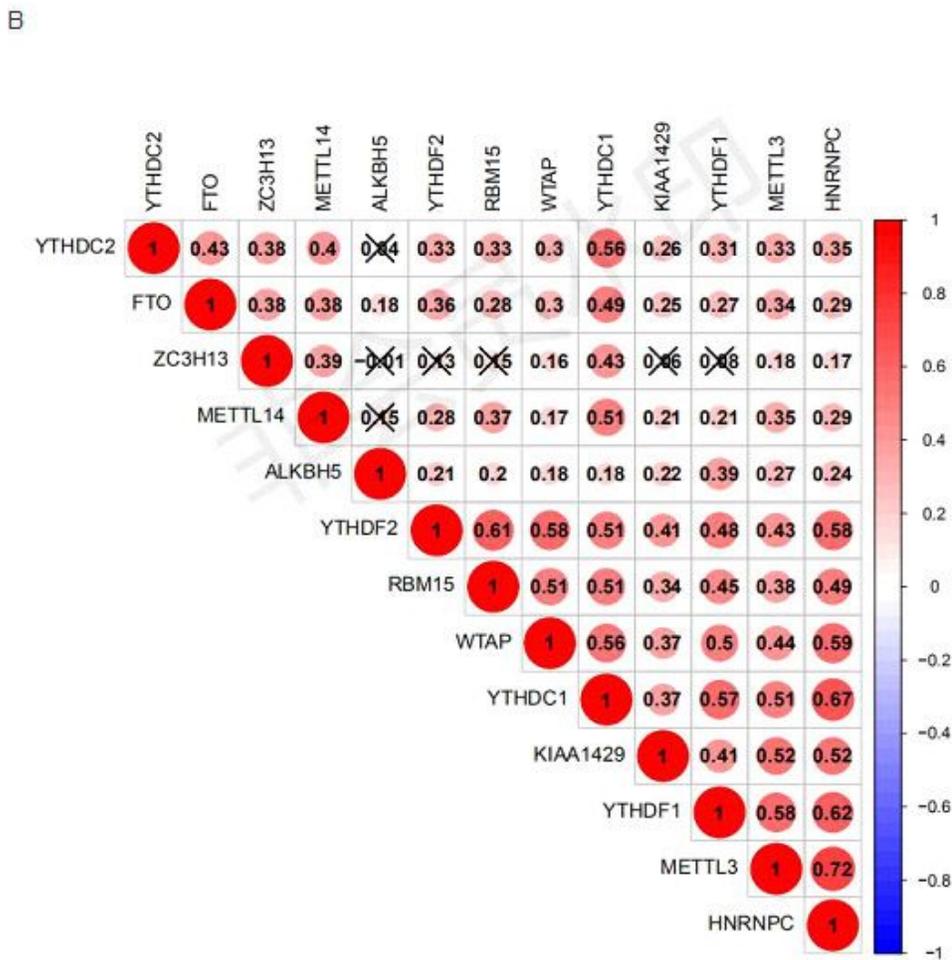
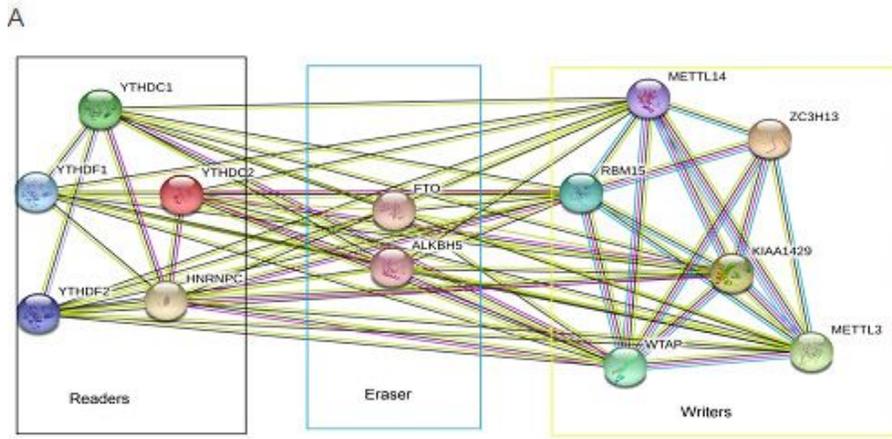


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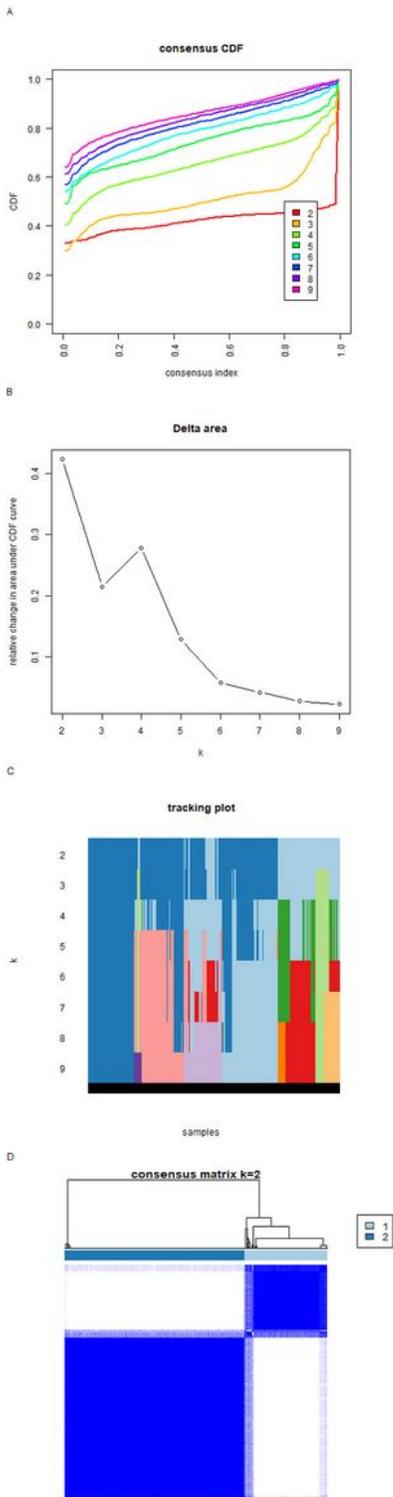
**Figure 1**

The expression of m6aarna methylation regulatory factors in HCC A. the expression of 13 m6aarna methylation regulatory factors in different HCC showed a high expression level in the red depth of thermogram, and a low expression level in the green depth ( $P < 0.001$ ,  $** P < 0.01$  and  $* P < 0.05$ ). B.The violin diagram shows the median expression of 13 m6A RNA methylation regulators in HCC, and the position of the middle white dot represents the median expression.



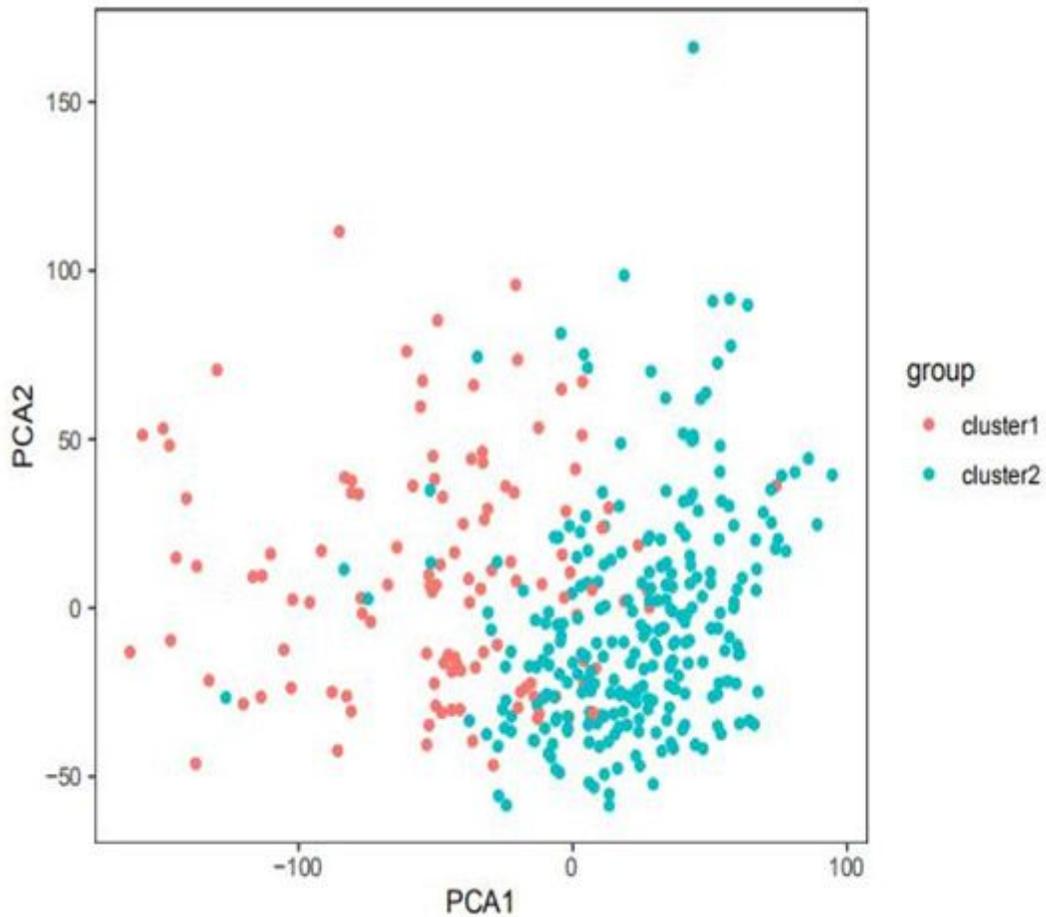
**Figure 2**

The interaction and correlation between m6A RNA methylation regulators. A. PPI networks were constructed to evaluate the interaction between the m6A RNA methylation regulators. B. Spearman correlation analysis of 13 m6A methylation regulators



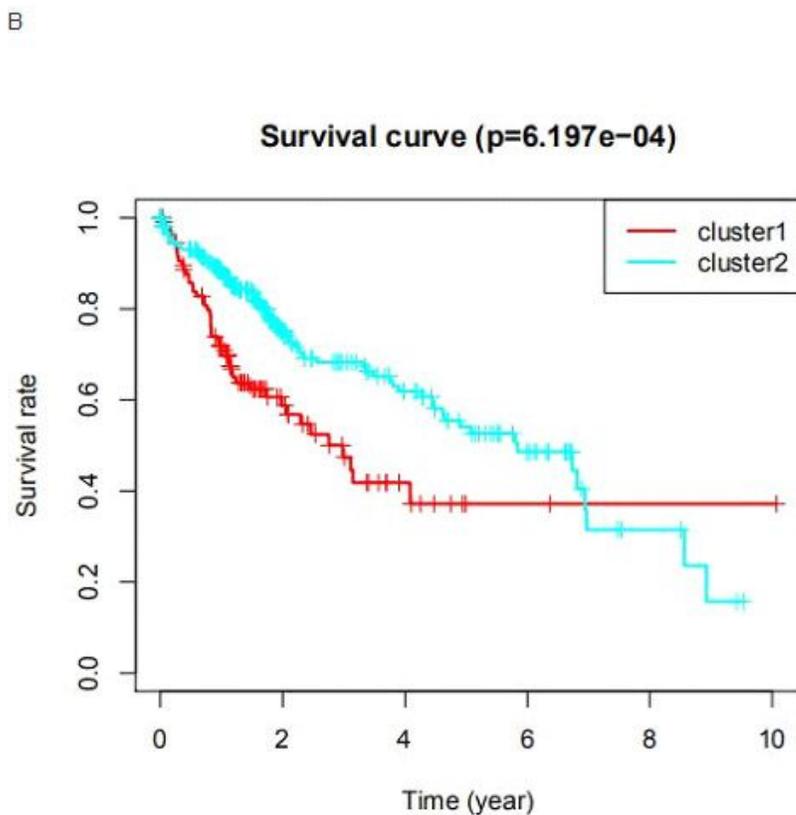
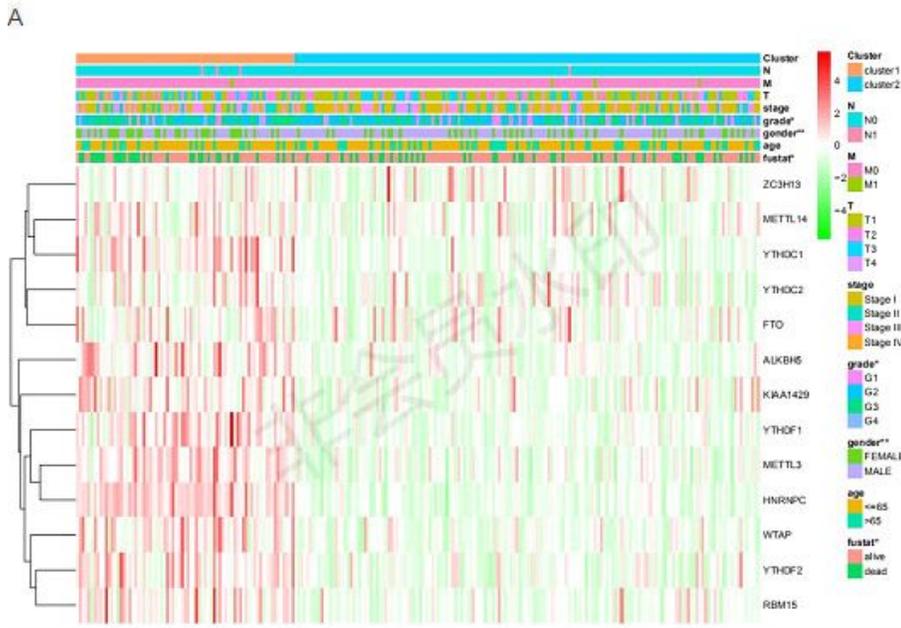
**Figure 3**

Cluster analysis of HCC A.The relative change of area under CDF curve from 2 to 10. B.When k is between 2 and 10, the consistency clustering cumulative distribution function (CDF). C.When k is between 2 and 10, the distribution of the sample. D.When k = 2, the correlation between groups



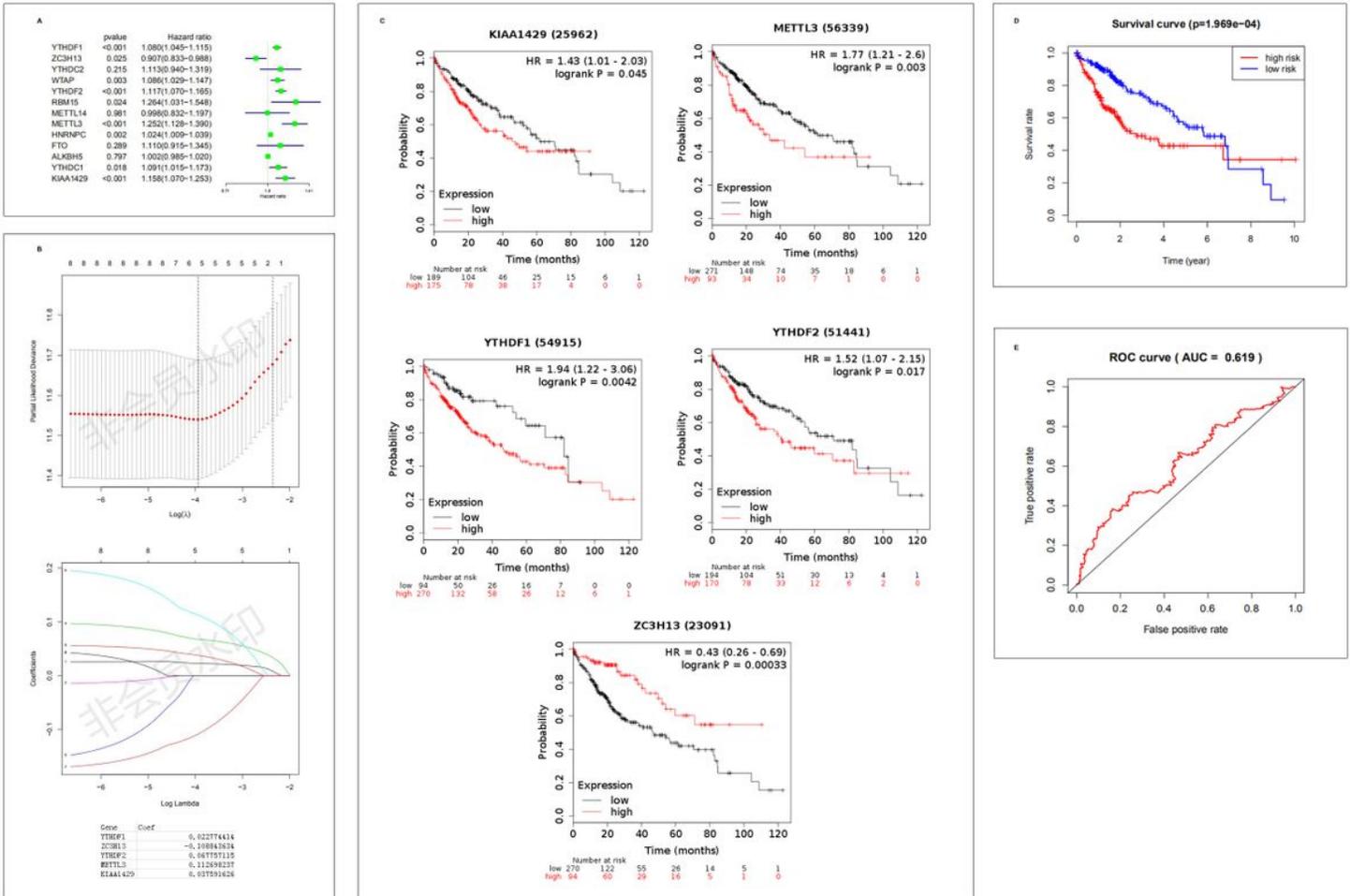
**Figure 4**

After the consistency analysis of HCC in TCGA data set, principal component analysis was carried out for the total RNA expression profiles of the two clusters. Cluster I subgroup was marked in red, cluster II subgroup in blue. Scatter plots show the distribution of RNA expression in two subgroups.



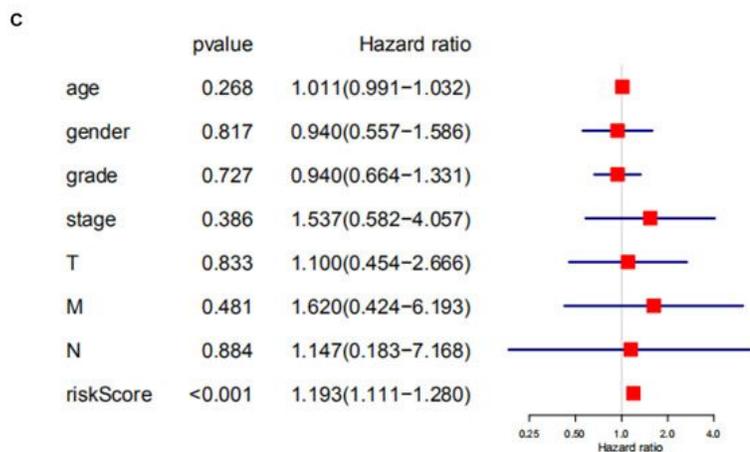
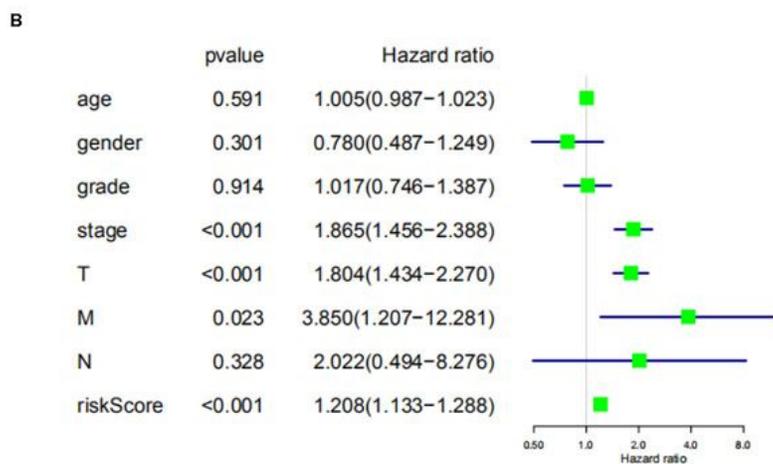
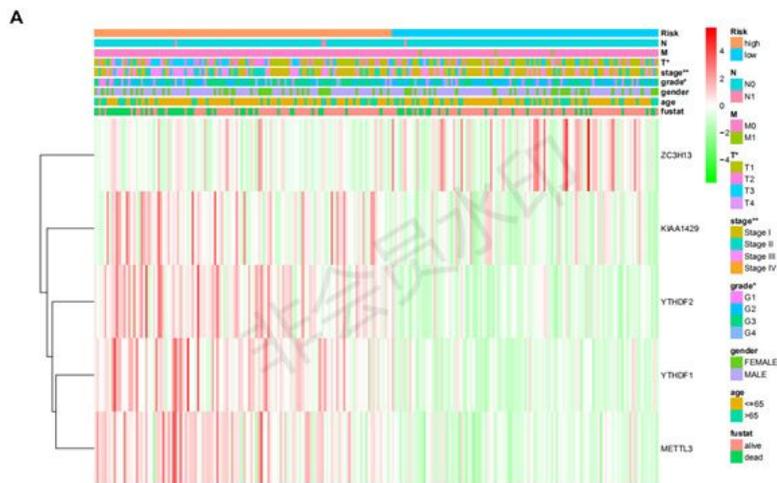
**Figure 5**

To compare the clinical characteristics and the expression of m6A RNA methylated regulator between the two groups by cluster analysis. A m6A RNA methylation regulators consistently expressed the thermogram and clinicopathological characteristics of two defined clusters (RM1 / 2). \*\*\*P < 0.001, \*\* P < 0.01 and \* P < 0.05. B. Comparison of Kaplan meiros rate of total survival (OS) curve in patients with HCC.



**Figure 6**

Risk profile of 5 m6A RNA methylation regulators. (A, B) Based on the construction process of two groups of m6A RNA methylation regulators. The hazard ratio (HR), 95% confidence interval (CI) and multivariate Cox regression coefficients (A, B) of single variable Cox regression (a) were given. (C) The survival curves of five adjustment factors estimated by Kaplan Meier plotter. The log rank  $P < 0.05$  was statistically significant.  $HR > 1$ , gene expression was negatively correlated with OS,  $HR < 1$ , gene expression was positively correlated with OS. (D) Kaplan Meier overall survival (OS) curve was divided into high-risk group and low-risk group according to risk score. (E) ROC analysis and AUC value of ROC curve indicate the sensitivity and specificity of risk characteristics.



**Figure 7**

A. Thermogram showed the expression of 5 m6A RNA methylation regulators in high-risk and low-risk HCC. The clinicopathological characteristics of high-risk group and low-risk group were compared. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$ . B. Cox univariate analysis of clinicopathological variables (including risk score) and overall survival rate. C. Cox multivariate analysis of clinicopathological variables (including risk score) and OS.

## Supplementary Files

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