

Development of N6-methyladenosine Related Signature as New Biomarker for Prognosis of Hepatocellular Carcinoma and Correlates with Sorafenib and anti-PD-1 Immunotherapy Treatment Response

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Abstract

Background: N6-methyladenosine (m6A) modification plays an essential role in diverse key biological processes and may take part in the development and progression of hepatocellular carcinoma (HCC). Here, we systematically analyzed expression profiles and prognostic values of 13 widely reported m6A modification related genes in HCC.

Methods: mRNA expression of 13 m6A modification related genes and clinical parameters of HCC patients were downloaded from TCGA, ICGC, GSE109211 and GSE78220. Univariate and LASSO analysis was used to develop risk signature. Time-dependent ROC was performed to assess the predictive accuracy and sensitivity of risk signature.

Results: FTO, YTHDC1, YTHDC2, ALKBH5, KIAA1429, HNRNPC, METTL3, RBM15, YTHDF2, YTHDF1 and WTAP were significantly overexpressed in HCC patients. YTHDF1, HNRNPC, RBM15, METTL3, YTHDF2 were independent prognostic factors for OS and DFS in HCC patients. Next, a risk signature was also developed and validated with five m6A modification related genes in TCGA and ICGC HCC cohort. It could effectively stratify HCC patients into high risk patients with shorter OS and DFS and low risk patients with longer OS and DFS and showed good predictive efficiency in predicting OS and DFS. Moreover, significantly higher proportions of macrophages M0 cells, neutrophils and Tregs were found to be enriched in HCC patients with high risk score, while significantly higher proportions of memory CD4 T cells, gamma delta T cells and naive B cells were found to be enriched in HCC patients with high low score. Finally, significantly lower risk scores were found at sorafenib treatment responders and anti-PD-1 immunotherapy responders compared to that in non-responders, and anti-PD-1 immunotherapy treated patients with lower risk score had better OS than patients with higher risk score.

Conclusion: A risk signature developed with the expression of 5 m6A related genes could improve the prediction of prognosis of HCC and correlate with sorafenib treatment and anti-PD-1 immunotherapy response.

Introduction

Hepatocellular carcinoma (HCC) is a common type of cancer and represents the leading cause of cancer-related death worldwide. HCC is still a serious burden to public health (1). There were about 841,000 patients developed HCC and 782,000 patients died from HCC alone in 2018 because of late diagnosis and limited treatment options (1, 2). Moreover, incidence of HCC is increasing rapidly with 50% recurrence rate after surgical treatment (3, 4). It is well recognized that development and progression of HCC is the result of multistep process, where interactions between genetics and epigenetics have played important roles (5–8). By understanding the pathogenesis of HCC is key to discover new diagnostic biomarkers and therapeutic targets.

RNA modification, discovered in the 1970s, has recently been recognized as a third layer of epigenetics that could modify a plethora of native cellular RNAs. (9–11). N6-methyladenosine (m6A) modification is

the most abundant form of internal mRNA methylation among the kinds of RNA modifications in eukaryotes (12). m6A modifications in mammalian cells are dynamic and reversible, and are commonly regulated by binding proteins ('readers'), methyl-transferases ('writers'), demethylases ('erasers') (13). Among m6A modification related genes, 13 genes, including *ZC3H13*, *WTAP*, *KIAA1429*, *METTL3*, *METTL14*, *RBM15*, *YTHDC1*, *YTHDC2*, *YTHDF1*, *YTHDF2*, *HNRNPC*, *ALKBH5* and *FTO*, are the most prominent m6A modification related genes (14–16). These m6A modification related genes are primarily involved in modulation of alternative mRNA splicing, precession of pre-miRNA, stability of mRNA and enhance of translation efficiency of mRNA [13]. Not only do these 13 m6A modification related genes play essential roles in many important biological processes, such as development of embryonic and neural cell, differentiation of stem cell, and stress responses (17–19), they also take part in the development, progression and radio resistance of various kinds of cancers (20–23). For example, overexpression of *YTHDF1* is found to be related with poorer survival of HCC patients, and *KIAA1429*, *METTL3* are found to regulate migration and invasion of HCC, indicating an important roles of m6A modifications related genes played in HCC (24–26).

Recently, Zhou et al explored expression pattern and prognostic values of m6A modification related genes of HCC patients, but they mainly focused on the role of *METTL3* and *YTHDF1* (27). In the present study, we comprehensively analyzed the expression pattern and prognosis of thirteen widely reported m6A modification related genes in TCGA HCC cohort. Besides, we also developed and validated a risk signature with expression of 5 selected m6A modification related genes, and analyzed its prognostic value for HCC patients and its relation with tumor-infiltrating immune cells in TCGA and ICGC HCC cohort. Moreover, the prediction values of risk signature in sorafenib treatment and anti-PD-1 immunotherapy response was also evaluated.

Materials And Methods

Ethics statement

All the data analyzed in the present study were received from TCGA, ICGC and GEO dataset, written consents were already obtained before our study.

Data collection

mRNA expression of TCGA HCC cohorts, which included 374 HCC cases and 50 normal controls, were got from GDC Data portal (<https://cancergenome.nih.gov/>). Meanwhile, corresponding clinical-pathological data, including gender, age, histologic grade, tumor T stage, N stage, M stage (M), TNM stage, overall survival (OS) time and disease-free survival (DFS) time were also downloaded. It was of note that 9 of 374 HCC patients were excluded because of absence of corresponding clinical-pathological data, and basic characteristics of 365 HCC patients were summarized in table 1. In addition, a total of 232 HCC patients with available OS information and mRNA expression were got from the ICGC portal

(<https://dcc.icgc.org/projects/LIRI-JP>). mRNA expression of 67 sorafenib-treated HCC patients of GSE109211 was downloaded from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) and there were 21 sorafenib treatment responders and 46 non-responder in GSE109211. Moreover, mRNA expression of 27 melanomas patients with anti-PD-1 checkpoint inhibition therapy of GSE78220 was also downloaded from GEO database. 4 patients achieved complete response, 10 patients achieved partial response and 13 patients achieved no response.

Table 1
Basic characteristics of 365 HCC patients from TCGA

Variables	HCC patients (N=365)
Gender (Male/female)	246/119
Age (years, ≤60/>60)	173/192
histologic grade(G1+G2/ G3+G4/NA)	230/130/5
T stage (T1+T2/ T3+T4/NA)	271/91/3
N stage (N0/N1/NA)	248/4/113
M stage (M0/N1/NA)	263/3/99
TNM stage(Stage I+II /Stage III+IV/NA)	254/87/24

Development and validation of risk signature

First, univariate analysis was carried out to select the genes related with survival. Then LASSO algorithm was used for selecting the most prognostic related genes (28). A risk signature was developed based on the coefficients weighted by LASSO analysis. With this signature, we calculated a risk score for HCC patients and divided HCC patients into high-risk group and low-risk group based on the median risk score.

CIBORSORT

CIBERSORT (<https://cibersort.stanford.edu>) is an online tool designed for estimating the abundances of 22 kinds of tumor-infiltrating immune cells with transcriptomic data(29), and we used it to calculate the tumor-infiltrating immune cells of HCC patients basing on the mRNA expression profiles of TCGA HCC cohort and ICGC HCC cohort, respectively.

Data analysis flow chart

To make the study to be better understood, a workflow of the study was depicted and was shown at figure 1.

Statistical analysis

R software (version 3.5.1) was used for statistical analysis. Wilcoxon test was performed to compare difference of m6A modification related genes between HCC and healthy controls. Correlation of the 13 m6A modification related genes with each other was compared by Spearman correlation analysis. One-way ANOVA was carried out to compare difference of m6A modification related genes among different histologic grades and TNM stages. Chi-square analysis was carried out to analyze distribution of clinical-pathologic parameters between high risk HCC patients and low risk HCC patients. Univariate and multivariate Cox regression analysis were carried out to analyze the prognostic value of m6A modification related genes and risk signature. Kaplan–Meier analysis with log-rank test was carried out to analyze difference of OS or DFS between patients of different clusters or with risk scores. Time-dependent ROC was carried out to analyze the predictive accuracy and sensitivity of risk signature. Additional statistical analyses were performed with STAMP(30). $P < 0.05$ was considered as statistically significant.

Results

Expression of m6A modification related genes of HCC patients and their associations with clinical-pathologic parameters

First, mRNA expression of 13 m6A modification related genes were download from *TCGA* and compared between HCC patients and normal controls. As was shown at figure 2A and 2B, significantly higher expression of *FTO*, *YTHDC1*, *YTHDC2*, *ALKBH5*, *KIAA1429*, *HNRNPC*, *METTL3*, *RBM15*, *YTHDF2*, *YTHDF1* and *WTAP* were found at HCC compared to normal tissues (all $p < 0.001$). Interestingly, we also found that expression of most of the 13 m6A modification related genes seemed to be lower than that of other 32 kinds of tumors. Besides, most of the 13 m6A modification related genes were positively correlated with each other (figure 2C). Moreover, genetic change, such as missense mutation, truncating mutation, amplification, deep deletion, diploid, gain were observed in near 80% of the HCC patients (figure 2D), Specifically, each HCC patient may have one or more kinds of genetic changes. The genetic rate of *WTAP*, *KIAA1429*, *RBM15*, *METTL3*, *METTL14*, *ALKBH5*, *YTHDC1*, *YTHDC2*, *HNRNPC*, *YTHDF1*, *YTHDF2*, *FTO* and *ZC3H13* were 7%, 4%, 17%, 40%, 5%, 5%, 7%, 8%, 18%, 11%, 9%, 13%, 17%, respectively, suggesting that higher expression of m6A modification related genes may be the result of genetic changes in related genes. Taken together, these results indicated that m6A modification related genes played important roles in HCC.

Prognostic value of m6A modification related genes in HCC cases

After discovering the expression of m6A modification related genes were associated with tumor histologic grade and TNM stage, we further analyzed their prognostic values. Univariate analysis showed that higher expression of *YTHDF1*, *WTAP*, *HNRNPC*, *RBM15*, *METTL3*, *KIAA1429*, *YTHDC1*, *YTHDF2* and lower expression of *ZC3H13* were statistically related to poorer OS of HCC patients (all $p < 0.05$, supplementary figure 1A); multivariate analysis showed that expression of *YTHDF1*, *WTAP*, *HNRNPC*, *RBM15*, *METTL3*, *KIAA1429* and *YTHDF2* still remained significantly related with OS after adjusting for gender, age, histologic grade, T stage, N stage, M stage and TNM stage (all $p < 0.05$, supplementary figure 1B-1J). Then, the prognostic values of m6A modification related genes for recurrence of HCC patients were also analyzed. Univariate analysis indicated that overexpression of *YTHDF1*, *WTAP*, *HNRNPC*, *RBM15*, *METTL3*, *YTHDC1* and *YTHDF2* were statistically related with shorter DFS (all $p < 0.05$, supplementary figure 2A); and, multivariate analysis showed that expression of *YTHDF1*, *HNRNPC*, *RBM15*, *METTL3* and *YTHDF2* were still statistically related with DFS after adjusting for gender, age, histologic grade, T stage, N stage, M stage and TNM stage (all $p < 0.05$, supplementary figure 2B-2H). These results strongly confirmed the important roles played by m6A modification related genes in HCC.

Development of risk signature with 5 m6A modification related genes and its association with clinical-pathologic parameters

To better explore the prognostic value of m6A modification related genes, a risk signature was developed. Based on the results of univariate analysis (figure 3A), *ZC3H13*, *YTHDF1*, *WTAP*, *HNRNPC*, *RBM15*, *METTL3*, *KIAA1429*, *YTHDC1* and *YTHDF2* were associated OS and were considered as prognostic-related genes. Then, LASSO analysis was used to further screen the prognostic-related genes. In the end, 5 genes, including *YTHDF2*, *YTHDF1*, *METTL3*, *KIAA1429* and *ZC3H13*, were used to develop the risk signature (figure 3A, 3B). The risk score was then constructed based on the coefficients weighted by LASSO analysis and calculated as follows: $\text{risk score} = (0.07 * YTHDF2) + (0.02 * YTHDF1) + (0.11 * METTL3) + (0.04 * KIAA1429) - (0.1 * ZC3H13)$. We calculated risk score for every HCC cases and assigned them into high risk group and low risk group on the basis of the median risk score. Expression of *YTHDF2*, *YTHDF1*, *METTL3*, *KIAA1429* tend to be higher in patients with high-risk score; and expression of *ZC3H13* seemed to be higher in patients with low-risk scores (figure 3C). Distribution of histologic grade, T stage, TNM stage were significantly different between high risk subgroup and low risk subgroup (all $p < 0.05$, figure 3C). High risk subgroup contained more patients with advanced histologic grade, T stage and TNM stage compared to patients of the low risk subgroup. Lastly, patients in the high risk subgroup had poorer OS (median OS time: 2.46 vs 5.79 years, HR=1.98, 95%CI: 1.39-2.83, $P < 0.001$, figure 3D) and shorter DFS (median DFS: 1.07 vs 2.97 years, HR=3.83, 95%CI: 2.56-5.90, $P < 0.001$, figure 3E) than that of patients of the low risk subgroup, which was consistent with previous result.

Prognostic value of risk signature for OS and DFS of HCC cases

The risk signature was found to be associated with clinical-pathologic parameters. We next performed univariate and multivariate analysis to analyze its prognostic value. Based on the univariate analysis, T stage, M stage, TNM stage and risk signature were statistically related with OS of HCC patients (all $p < 0.05$, figure 4A). The risk signature still remained statistically related with OS after adjusting for T stage, M stage and TNM stage by multivariate analysis. In multivariate analysis, after adjusting for TNM stage, the risk signature was still significantly related with OS ($p < 0.01$, figure 4B). Similarly, univariate analysis also showed that T stage, TNM stage and risk signature were statistically related with DFS of HCC patients. In univariate analysis, T stage, TNM stage and the risk signature were also significantly associated with DFS in HCC patients (all $p < 0.001$, figure 4C). By incorporating these factors into multivariate analysis, the result suggested only the risk signature was statistically related with DFS ($p < 0.001$, figure 4D). To conclude, these results indicated that risk signature was independent prognostic factor for OS and DFS of HCC patients.

Next, we used time-dependent ROC curve analysis to analyze the predictive value of risk signature for HCC patients. As were shown at figure 5, the AUC of risk signature for predicting 1-, 3- and 5-year OS were 0.765, 0.73 and 0.678, respectively, which exhibited better predictive efficiency compared to TNM stage, *YTHDF2*, *YTHDF1*, *METTL3*, *KIAA1429* and *ZC3H13* (figure 5A, C, E). Likewise, the AUC of risk signature for predicting 1-, 3- and 5-year DFS were 0.695, 0.643 and 0.68, respectively, which also showed better predictive accuracy than TNM stage, *YTHDF2*, *YTHDF1*, *METTL3*, *KIAA1429* and *ZC3H13* (figure 5B, D, F).

Validation of risk signature

To independently test the applicability of the signature, 232 HCC patients with available OS information from the ICGC portal (<https://dcc.icgc.org/projects/LIRI-JP>) was further used to examine the applicability of the signature. Risk score for every patient was computed. Similarly, the signature could effectively stratify high risk HCC patients with poorer OS and low risk patients with better OS (HR=2.309, 95%CI: 1.302-4.369, $P=0.006$, figure 6A). Moreover, the AUC of risk signature for predicting 1-, 3- and 5-year OS were 0.7, 0.74 and 0.714 (figure 6B), respectively, which convincingly suggested the good discrimination and prediction of our signature.

Correlation of risk signature with tumor-infiltrating immune cells in TCGA and ICGC HCC cohort

CIBERSOR was used to calculate 22 kinds of infiltrating immune cells in patients with different risk scores. In TCGA HCC cohort, significantly higher proportions of macrophages M0 cells, memory B cells, follicular helper T cells and neutrophils were found to be enriched in HCC patients with high risk score,

while significantly higher proportions of resting memory CD4 T cells and monocytes were found to be enriched in HCC patients with low risk score (all $p < 0.05$, figure 7A). In ICGC HCC cohort, significantly higher proportions of macrophages M0 cells and Treg cells were found to be enriched in HCC patients with high risk score, while significantly higher proportions of naive B cells and gamma delta T cells were found to be enriched in HCC patients with low risk score (all $p < 0.05$, figure 7B). These results suggested that the risk signature was significantly associated with tumor-infiltrating immune cells, and different kinds of infiltrating immune cells in patients with different risk score may contribute to their different prognosis.

Risk signature as indicator in sorafenib treatment response for HCC patients

To investigate the association between risk signature and sorafenib treatment response, we calculated risk score for each HCC patients treated with sorafenib of GSE109211, which contained 21 sorafenib treatment responders and 46 non-responders. Significantly lower risk scores were found at sorafenib treatment responders compared to that in non-responders ($P < 0.001$, figure 8A). Moreover, the AUC for predicting sorafenib treatment response was 0.794 (figure 8B). Taken together, the risk signature may be served as an indicator for sorafenib treatment response in HCC patients.

Correlation of risk signature with anti-PD-1 immunotherapy

As a major breakthrough in cancer therapy, immunotherapies represented by immunological checkpoint blockade (PD-1/L1 and CTLA-4) has proved promising clinical efficacy and previous study have proved that combination treatment with anti-PD-1 antibodies and Sorafenib exhibited a more potent antitumor effect, but only a small number of patients could achieved durable responses (31, 32), so in the present study, we also explored whether the risk signature could predict patients' response to immune checkpoint blockade therapy in an anti-PD-1 cohort of GSE78220. Encouragingly, patients with lower risk score had better OS than patients with higher risk score (HR=3.81, 95%CI: 1.13-11.08, $p=0.03$, figure 9A). Besides, despite there was no statistical difference, lower risk score was found at patients with complete immunotherapeutic response compared to that in patients with partial response and patients with no response, and lower risk score was also found in alive patients treated with anti-PD-1 than that in patients of death, which may due to the limitation number of patients in the cohort (figure 9B-9C). Moreover, the AUC of the risk signature for predicting 1 year-, 1.5-year and 2-year OS of patients with anti-PD-1 immunotherapies was 0.669, 0.725 and 0.639 (figure 9D). In a word, the aboved results strongly indicated that risk signature was significantly correlated with response to anti-PD-1 immunotherapy, which may be used as a new biomarker for predicting the response to anti-PD-1/L1 immunotherapy.

Discussion

m6A modifications are mainly controlled by methyl-transferases, binding proteins and (13). Studies have reported conservative role and mechanism of m6A modification related genes in regulating RNA modification, but only a few literatures have studied the role of m6A modification related genes in HCC patients. Zhou et al found that *YTHDF1* was significantly up-regulated in HCC and positively correlated with pathology stage (24). Cheng et al also reported that expression of *KIAA1429* was higher in HCC and HCC cell lines, and *KIAA1429* could regulate the progression of HCC by regulating ID2 m6A modification (26). Chen et al discovered that *METTL3* was significantly up-regulated in HCC. Knockdown of *METTL3* was also found to suppress tumorigenicity and progression of HCC through *YTHDF2*-dependent post transcriptional silencing of *SOCS2* (25). Moreover, Yang et al found *YTHDF2* was significantly related to malignancy of HCC, and miR-145 could inhibit tumorigenicity of HCC by decreasing *YTHDF2* (33). Collectively, these results indicated that m6A modification related genes promoted the tumorigenesis of HCC.

Whether expressions of m6A modification related genes could be considered as prognostic biomarker is one of the trending research topics in m6A modification research (20). Up-regulation of *YTHDF1* and *METTL3* expression were found to be related to poorer OS of HCC patients (24, 25, 27). Similarly, in our study, *THDF1*, *HNRNPC*, *RBM15*, *METTL3*, *YTHDF2* are independent prognostic factors for OS and DFS in HCC patients. Next, a risk signature based on the expression five genes could differentiate HCC patients into high risk patients with poorer OS and DFS and low risk patients with better OS and DFS. Interestingly, this risk signature together showed better predictive efficiency in predicting OS and DFS than TNM stage, or any single gene estimation *alone*. Therefore, this risk signature may be an advantageous method for individualized therapeutic strategies in HCC patients. In addition, we also found that the risk signature was significantly associated with tumor-infiltrating immune cells, which may influence prognosis of patients with different risk scores. Significantly higher proportions of macrophages M0 cells, neutrophils and Tregs cells were found to be enriched in HCC patients with high risk score. Previous studies have showed that macrophages could be recruited to tumor tissues and become pro-angiogenic cells, which was significantly associated with micro-vessel density and poor OS and DFS of HCC(34, 35); Zhou et al also found that tumor-associated neutrophils could promote the progression of HCC and resistance to sorafenib by recruiting macrophages and Tregs cells (36). These results may partly explain the reason for poorer OS and DFS in HCC patients with high risk score. Moreover, significantly higher proportions of memory CD4 T cells, gamma delta T cells and naive B cells were found to be enriched in HCC patients with low risk score, suggesting higher proportions infiltrated T cells and B cells. Garnelo et al. found that the degree of infiltrated T cells and B cells of tumor tissues significantly related with improved prognosis of HCC patients(37), which may also partly explain the reason for longer OS and DFS in HCC patients with low risk score.

As an oral multi kinase inhibitor, sorafenib is one of the standard care therapy for advanced stage HCC patients approved by FDA. It can prolong the survival time of HCC patients by inhibiting cell proliferation and angiogenesis, and promoting cell apoptosis through inhibiting a variety of intracellular and cell surface kinases (such as c-raf, BRAF, RET) and vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) (38, 39). However, some studies have also found that

HCC rapidly become sorafenib-resistant and only about 30% of patients can benefit from sorafenib treatment, which may greatly limit the wide clinical application of sorafenib(40, 41). Besides, as a major breakthrough in cancer therapy, immunotherapies represented by immunological checkpoint blockade (PD-1/L1 and CTLA-4) has proved promising clinical efficacy and previous study have proved that combination treatment with anti-PD-1 antibodies and Sorafenib exhibited a more potent antitumor effect, but only a small number of patients could achieved durable responses(31, 32), so identifying the HCC patients suitable for sorafenib treatment or anti-PD-1 immunotherapy or their combination therapy may be urgent and clinical significance. Encouragingly, in the present study, we found the m6A-related risk signature was significantly correlated with response to sorafenib treatment and anti-PD-1 immunotherapy. Significantly lower risk scores were found at sorafenib treatment responders or anti-PD-1 immunotherapy responders and anti-PD-1 immunotherapy treated patients with lower risk score had better OS than patients with higher risk score, which strongly indicated that the risk signature may be used as a new biomarker for predicting the response to sorafenib treatment and anti-PD-1 immunotherapy, and even the combination of them, but independent prospective studies with a larger sample size are still needed to confirm our findings.

Though the risk signature exhibited good performance for the prognosis of HCC, several limitations should be addressed. First of all, although the prognostic value of the risk signature has been validated in external cohort, independent cohorts consist of more HCC patients are required to further verify the model. Secondly, we did not explore the potential biological functions and pathways of risk signature. In vitro and in vivo experiment should be carried out to uncover the relevant mechanisms. Finally, previously, Huang et al. suggested significant expression of m6A modification related genes were found in circulating tumor cells (CTCs) (42). Further studies are needed to examine whether these m6A modification related genes could be detected in peripheral blood in HCC patients and whether the risk signature in blood could still have good prognostic value.

In conclusion, *THDF1*, *HNRNPC*, *RBM15*, *METTL3*, *YTHDF2* are independent prognostic factors for OS and DFS in HCC patients. A risk signature developed with the expression of *YTHDF2*, *YTHDF1*, *METTL3*, *KIAA1429* and *ZC3H1* could improve the prediction of prognosis and correlate with sorafenib treatment and anti-PD-1 immunotherapy response.

Abbreviations

HCC: hepatocellular carcinoma, m6A: N6-methyladenosine, OS: overall survival, DFS : disease free status, LASSO : least absolute shrinkage and selection operator, GSC: glioblastoma stem cell, VEGFR: vascular endothelial growth factor receptor, PDGFR: platelet-derived growth factor receptor, CTCs: circulating tumor cells.

Declarations

Ethics approval

All the data analyzed in the present study were got from TCGA and ICGC. Informed consents had already been obtained from the patients before the present study.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data of the study are available from the corresponding web page link, including GDC Data portal (<https://cancergenome.nih.gov/>), ICGC portal (<https://dcc.icgc.org/projects/LIRI-JP>) and GEO database (<https://www.ncbi.nlm.nih.gov/geo/>).

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Code availability

Not applicable.

Authors' contributions

Hong-ye Jiang and Gang Ning analyzed the data and wrote the paper; Yen-sheng Wang downloaded the data and wrote the R codes to process the data. Wei-biao Lv got the idea of this study and designed the experiment. Wei-biao Lv discussed with all of the authors and provided the suggestions about the experiments. All authors read and approved the final manuscript.

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Figures

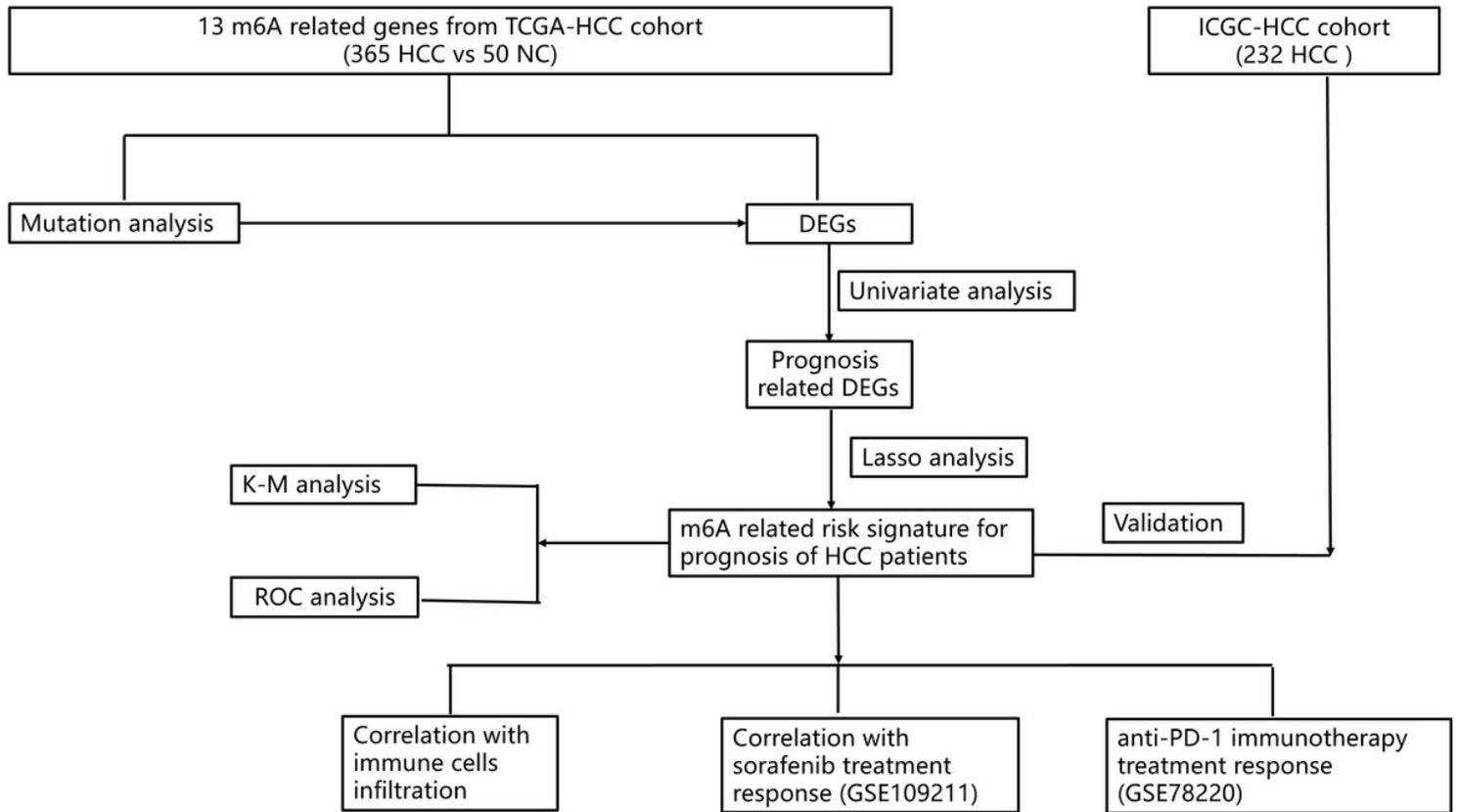


Figure 1

The workflow chart of the present study.

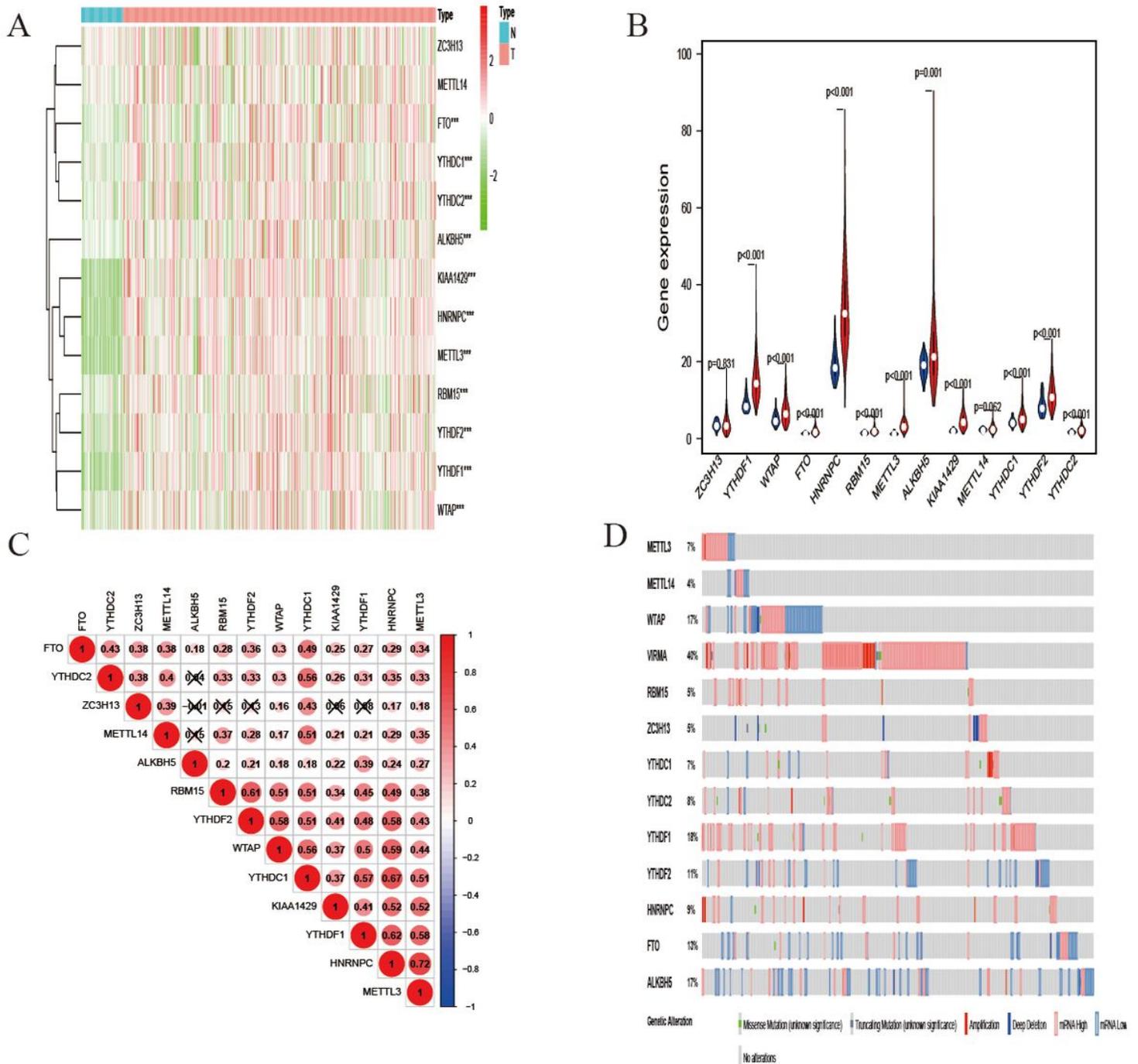


Figure 2

Expression of 13 m6A modification related genes in HCC and their associations with clinical-pathologic parameters. Heatmap of log₂ transformed expression of 13 m6A modification related genes between HCC patients and normal controls (A). Violin plot of expression of 13 m6A modification related genes between HCC patients and normal controls (B). Correlation of the 13 m6A modification related genes with each other (C). Genetic changes of the 13 m6A modification related genes (D). Associations of m6A modification related genes with histologic grade (E) and TNM stage (F). * P < 0.05, ** P < 0.01, *** P < 0.001.

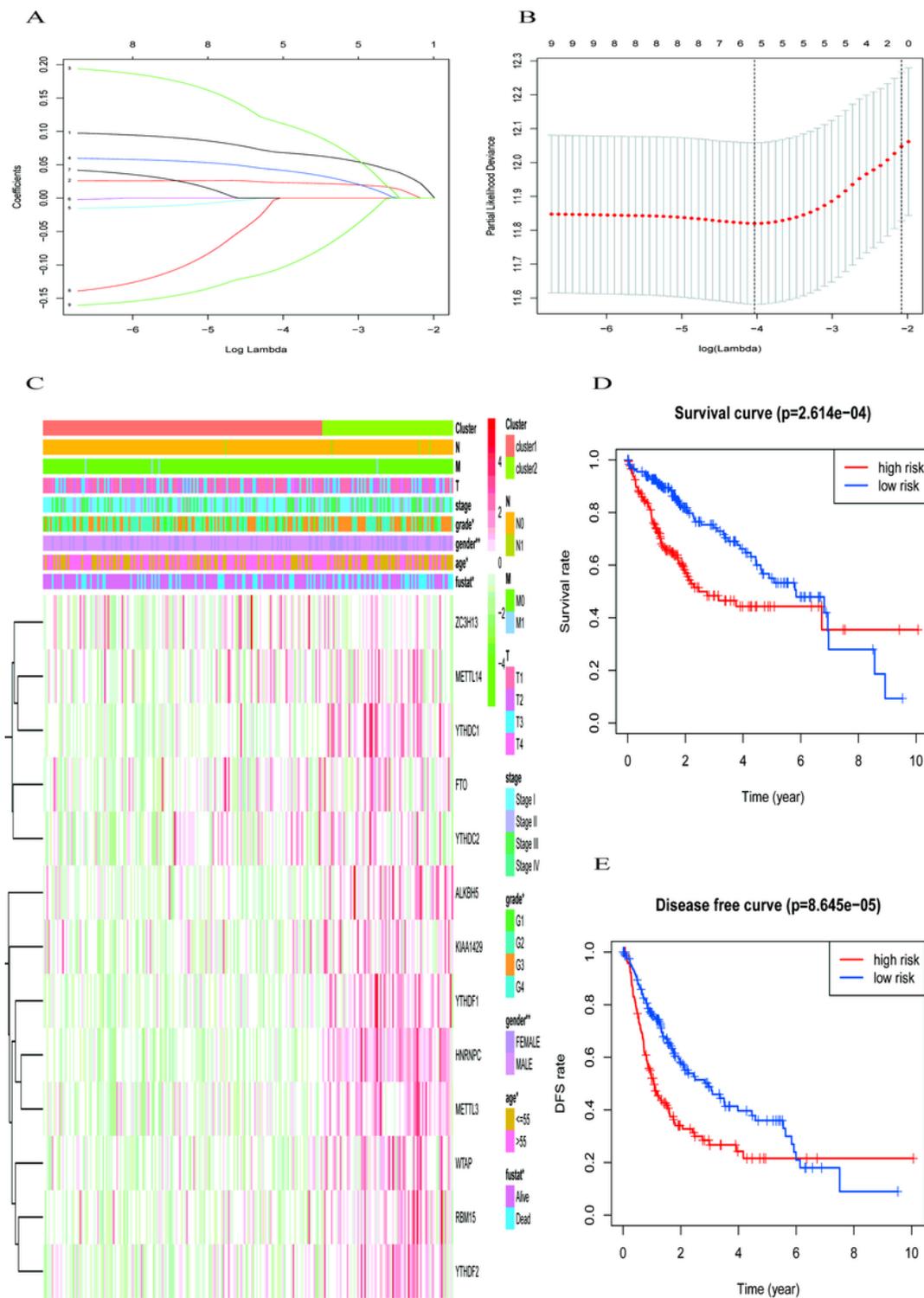


Figure 3

Construction of risk signature with 5 m6A modification related genes and its association with clinical parameters. 5 m6A modification related genes identified by LASSO analysis (A-B). Heatmap of the association of risk score with clinical-pathologic parameters (C). Kaplan-Meier analysis of OS of patients of high-risk subgroup and low-risk subgroup (D). Kaplan-Meier analysis of DFS of patients of high-risk

subgroup and low-risk subgroup (E). T: tumor stage; N: lymph node stage; M: metastasis stage; stage: TNM stage; *: P < 0.05; **: P < 0.01; ***: P < 0.001.

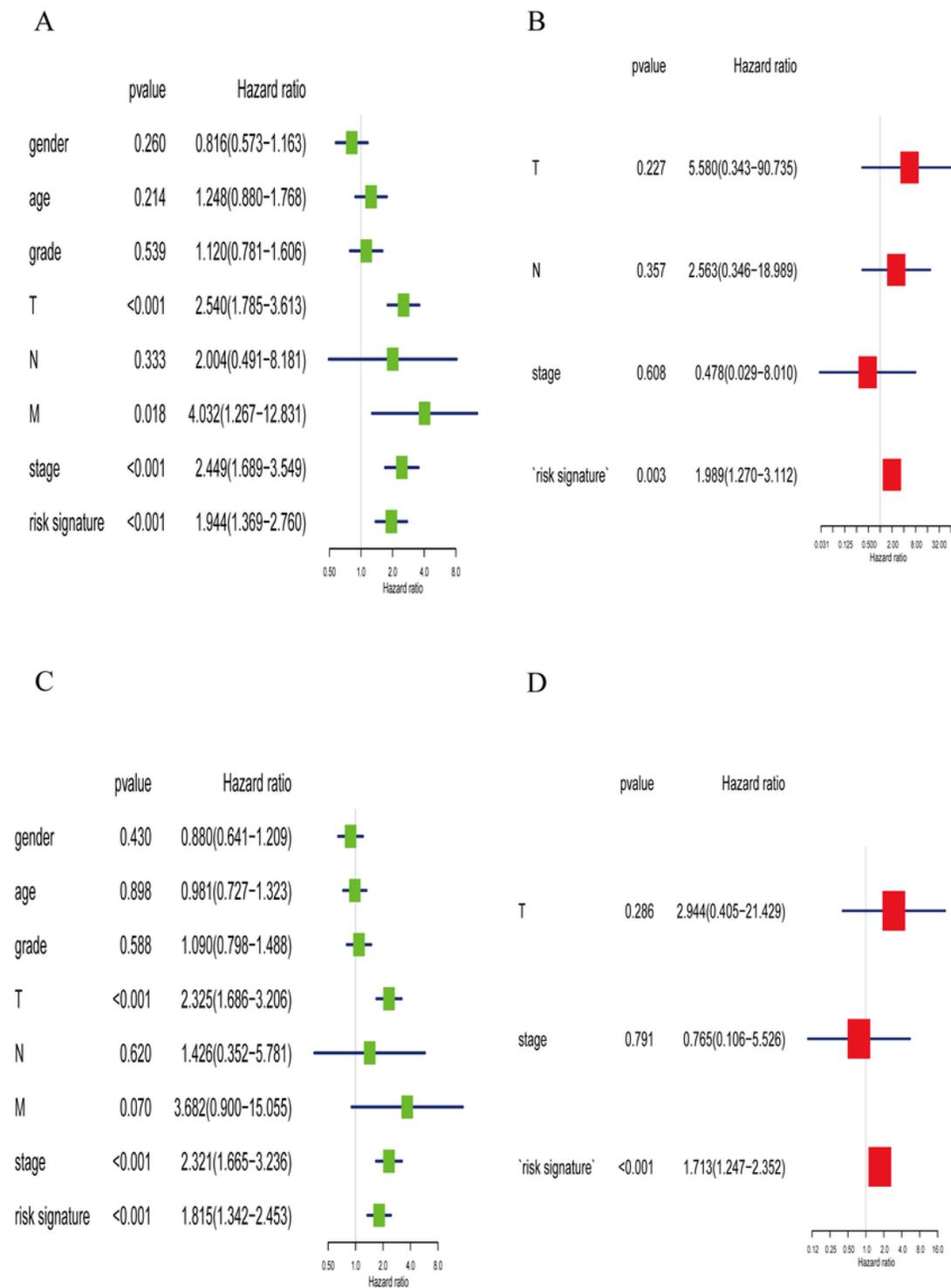


Figure 4

Prognostic value of risk signature for OS and DFS of HCC patients. Univariate analysis of risk signature with OS of HCC patients (A). Multivariate analysis of risk signature with OS of HCC patients (B). Univariate analysis of risk signature with DFS of HCC patients (C). Multivariate analysis of risk signature

with DFS of HCC patients (D) Gender: male vs female; age: >60 vs ≤60; grade: G3+G4 vs G1+G2; T: T1 vs T0; N: N1 vs N0; M: M1 vs M0; TNM stage: stage III+IV vs stage I+II.

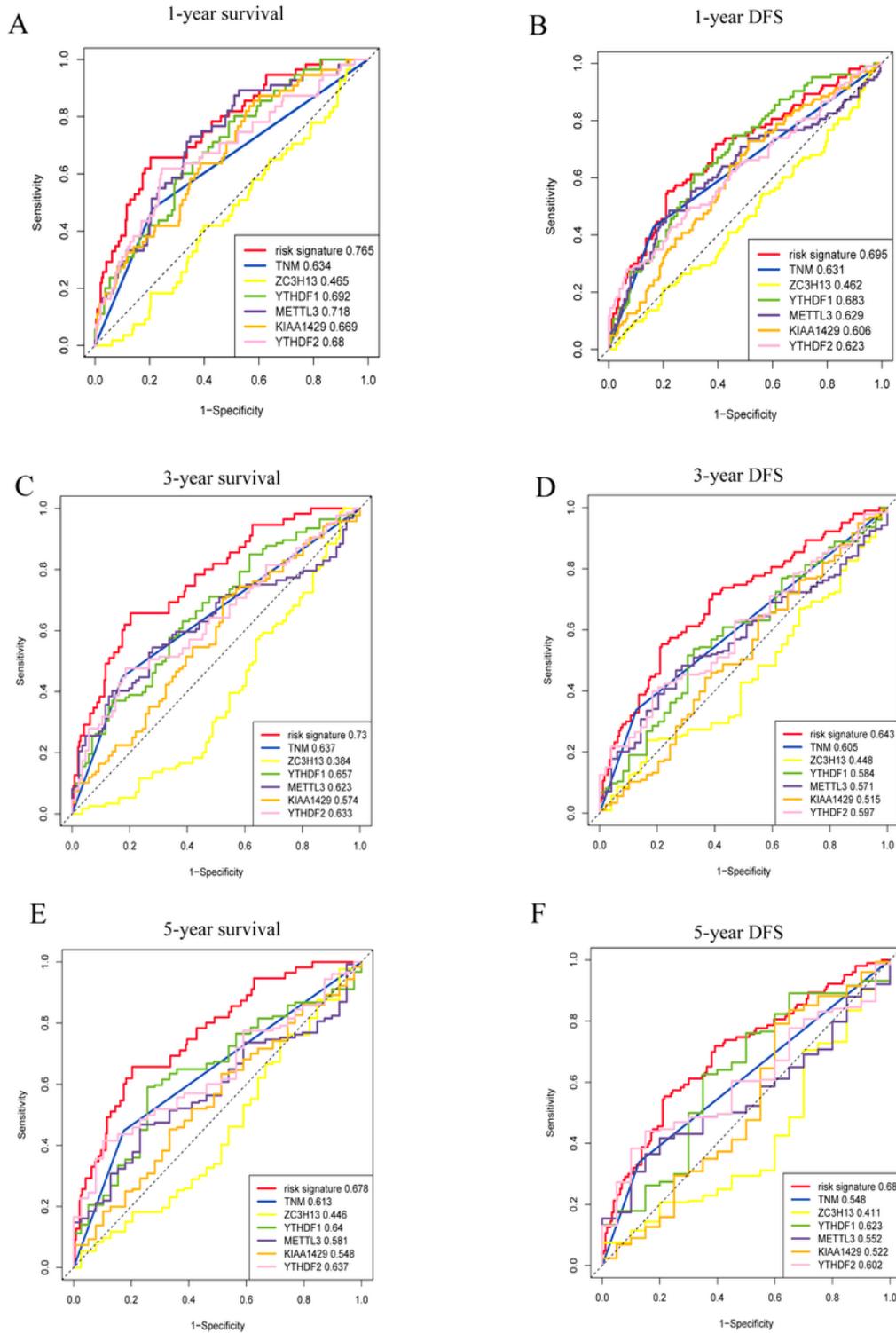


Figure 5

Predictive value of risk signature, TNM stage, YTHDF2, YTHDF1, METTL3, KIAA1429 and ZC3H13. Time-dependent ROC analysis was used to evaluate the predictive value in predicting 1-year (A), 3-year (C) and 5-year (E) OS and predicting 1-year (B), 3-year (D) and 5-year (F) DFS in HCC patients.

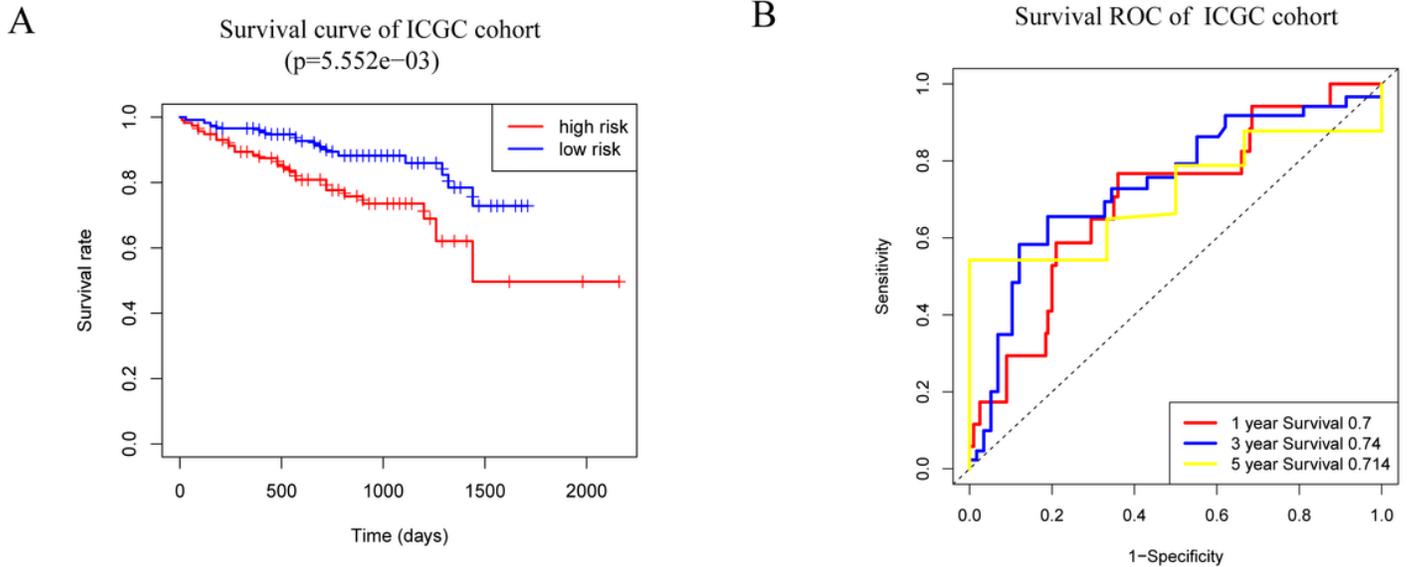


Figure 6

External validation of the applicability of the signature in ICGC HCC cohort. Kaplan-Meier analysis of OS of patients of high-risk subgroup and low-risk subgroup in ICGC cohort (A); AUC of risk signature in predicting 1-year, 3-year and 5-year OS in HCC patients (B).

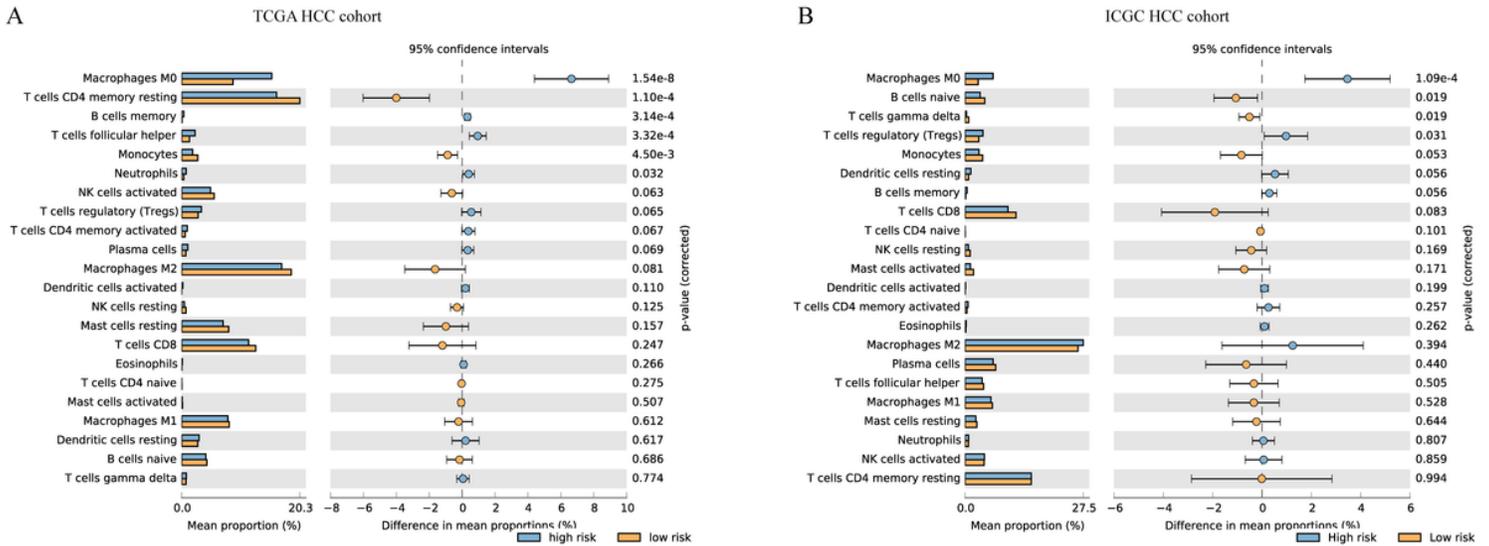


Figure 7

Correlation of risk signature with tumor-infiltrating immune cells in TCGA and ICGC HCC cohort. Difference of 22 kinds of infiltrating immune cells between patients with different risk scores of TCGA HCC cohort (A); Difference of 22 kinds of infiltrating immune cells between patients with different risk scores of ICGC HCC cohort (B).

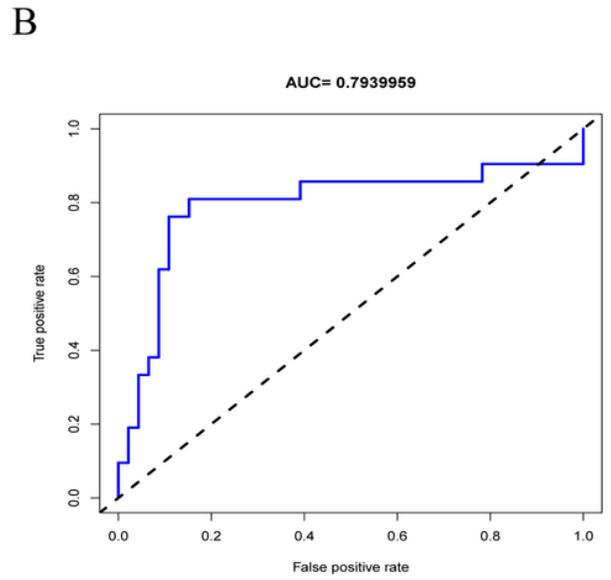
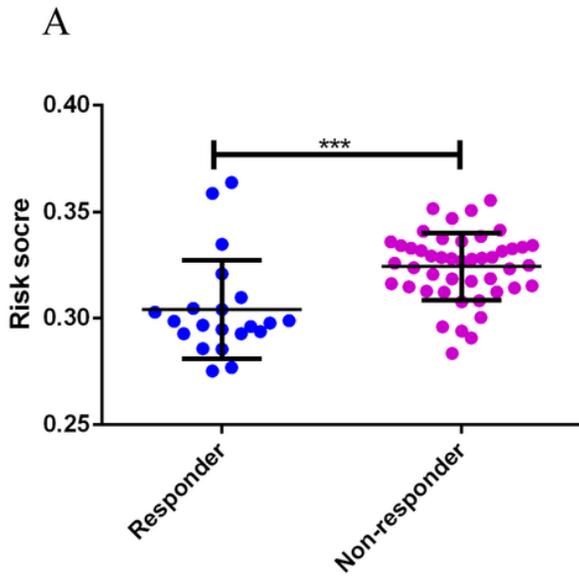


Figure 8

Association of risk signature with sorafenib treatment response of GSE109211 cohort. Difference of risk score between sorafenib treatment responders and non-responders (A); AUC of risk signature in predicting in sorafenib treatment response (B).

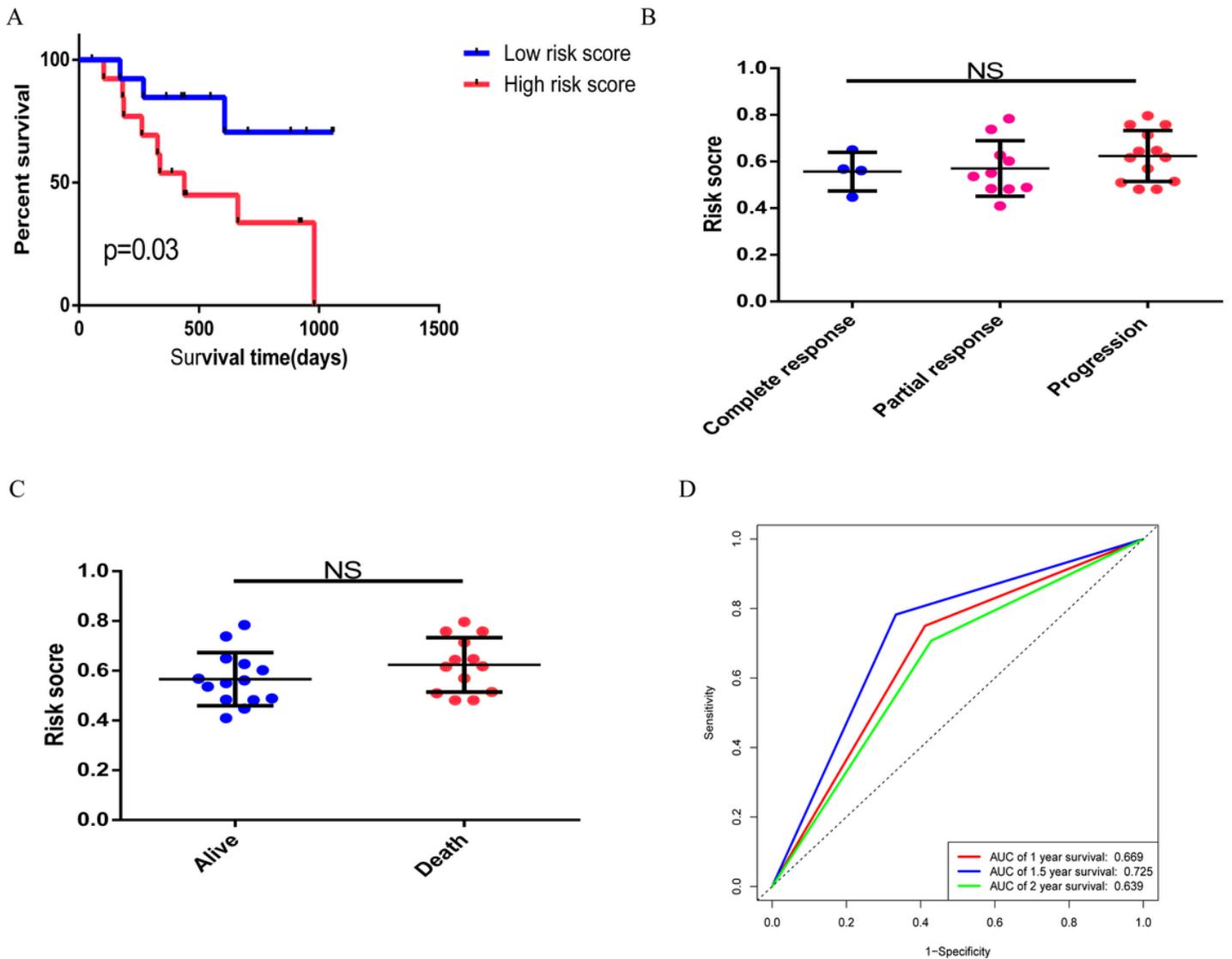


Figure 9

Association of risk signature with anti PD-1 immunotherapy treatment response of GSE78220 cohort. Kaplan-Meier analysis of OS of anti PD-1 immunotherapy treated patients with different risk score (A); Difference of risk score among complete anti PD-1 immunotherapy response, partial anti PD-1 immunotherapy response and no anti PD-1 immunotherapy response (B); Difference of risk score between alive patient with anti PD-1 immunotherapy and dead patients with anti PD-1 immunotherapy (C); AUC of risk signature in predicting 1-year, 1.5-year and 2-year OS in patients 1-year, 3-year and 5-year OS in patients with anti PD-1 immunotherapy response (D).

Supplementary Files

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