N7-Methylguanosine Regulatory Genes Profoundly Affect the Prognosis, Progression, and Antitumor Immune Response of Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is a common abdominal cancer with poor survival outcomes. Although there is growing evidence that N7-methylguanosine (m7G) is closely associated with tumor prognosis, development, and immune response, few studies focus on this topic.

Methods: The novel m7G risk signature was constructed through the Lasso regression analysis. Its prognostic value was evaluated through a series of survival analyses and was tested in ICGC-LIRI, GSE14520, and GSE116174 cohorts. CIBERSORT, ssGSEA, and ESTIMATE methods were applied to explore the effects of the m7G risk score on tumor immune microenvironment (TIM). The GSEA method was used to evaluate the impacts of the m7G risk score on glycolysis, ferroptosis, and pyroptosis. The human protein atlas (HPA) database was used to clarify the histological expression levels of five m7G signature genes. The biofunctions of NCBP2 in hepatocellular cancer (HC) cells were confirmed through qPCR, CCK8, and transwell assays.

Results: Five m7G regulatory genes comprised the novel risk signature. The m7G risk score was identified as an independent prognostic factor of HCC and could increase the decision-making benefits of traditional prognostic models. Besides, we established a nomogram containing the clinical stage and m7G risk score to predict the survival rates of HCC patients. The prognostic value of the m7G model was successfully validated in ICGC and GSE116174 cohorts. Moreover, high m7G risk led to a decreased infiltration level of CD8+ T cells, whereas it increased the infiltration levels of CD8+ T cells.

Abbreviations: ACC, adrenocortical carcinoma; AJCC, American Joint Committee on Cancer; CBC, cap-binding protein complex; CC, intrahepatic cholangiocarcinoma; DCA, decision curve analysis; DEGs, differentially expressed genes; HCC, hepatocellular carcinoma; ICB, immune checkpoint blockade; ICC, intrahepatic cholangiocarcinoma; IFN, interferon; LADC, lung adenocarcinoma; m7G, N7-methylguanosine; m6A, N6-methyladenosine; MSigDB, Molecular Signatures Database; ORR, objective response rate; OS, overall survival; PAAD, pancreatic adenocarcinoma; RIG-I, retinoic acid inducible gene-I; ROC, receiver operating characteristic curve; TFH, T cells follicular helper; TIM, tumor immune microenvironment; Tregs, T cells regulator.
INTRODUCTION

Hepatocellular carcinoma (HCC) is a common digestive tumor, accounting for 6% of all cancer-related deaths in the United States (1). Despite considerable advances in its diagnosis and treatment, the overall survival (OS) is still unsatisfactory. Especially in China, the region having the largest number of HCC patients in the world, the median survival of patients is commonly less than 30 months (2). Regrettably, the effects of existing therapeutic approaches are unsatisfactory. Sorafenib is the first-line option for treating advanced HCC; the median OS of patients who receive sorafenib is only 18 months (3). Nivolumab, another commonly used immune checkpoint blockade (ICB) for HCC systemic treatment, only confers HCC patients with a median OS of 15 months (4). Meanwhile, its objective response rate (ORR) is less than 20% (5). Therefore, finding novel therapeutic strategies and establishing an accurate prognostic system are imperative.

RNA epigenetic modifications have been confirmed to be closely involved in the onset and progression of multiple cancers (6). One classic example is the N6-methyladenosine (m6A) modification. Ample evidence indicates that the m6A process profoundly affects the prognosis, tumor immune microenvironment (TIM), and therapeutic effects of various cancers, including adrenocortical carcinoma (ACC) (7), pancreatic adenocarcinoma (PAAD) (8, 9), lung adenocarcinoma (LADC) (10), HCC (11), etc. Unfortunately, extremely limiting research focuses on the N7-methylguanosine (m7G), which is another one of the most prevalent RNA modifications. m7G refers to the modification of the seventh N of RNA guanine with a methyl group (12). Mechanistically, this guanosine methylation at the 5′ cap of RNA relies on the catalyzation of METTL1 and its co-factor WD repeat domain 4 (WDR4) (13). Since m7G modification can improve the stability of mRNA, this process is speculated to mediate several biological functions through altering the expression levels of related genes (14, 15). Recently, Dai et al. found that m7G RNA modification could enhance oncogenic mRNA translation and promote intrahepatic cholangiocarcinoma (ICC) progression (16). Therefore, m7G, an unfamiliar epigenetic topic, has great potential to advance the paradigm of HCC treatment and clinical assessment.

To the best of our knowledge, no research has investigated the associations of m7G regulatory genes with the prognosis and immune response of cancers. Therefore, we constructed a novel m7G risk signature for the first time through the Lasso regression analysis. The roles of m7G risk score in prognosis, progression, TIM, and glycolysis metabolism of HCC were explored. Moreover, the biofunctions of NCBP2, a critical member of the m7G risk signature, were also confirmed through the experiments in vitro. It is conceivable that our findings could bring new insights into the treatment and prognosis assessment of HCC.

Keywords: hepatocellular carcinoma, N7-methylguanosine, risk signature, prognosis, tumor immune microenvironment, NCBP2

MATERIALS AND METHODS

Data Source

The clinical information and gene expression data were derived from TCGA (https://portal.gdc.cancer.gov/), ICGC (https://dcc.icgc.org/releases), and GEO (https://www.ncbi.nlm.nih.gov/geo/) databases. Given that the number of normal liver samples in TCGA (n = 50) did not well match that of HCC samples, we used 110 normal liver tissue samples from the GTEx database (https://xenabrowser.net/datapages/) to solve this shortage. The enrolled TCGA samples should provide both the gene expression matrix and the corresponding clinical information. Besides, 28 TCGA samples were excluded owing to their too short follow-up (less than 30 days). To minimize the batch effect between different datasets, we used the “ComBat” algorithm to correct it (17), which was implemented by the SVA package in R software. The normalized batch-corrected datasets were standardized by log2 (FPKM + 1) transformation. The clinical characteristics of TCGA, ICGC, and GEO cohorts are shown in Supplementary Table S1. Transcriptome data were provided by the Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org/gsea/msigdb/). As mentioned in some m7G-related reviews, this RNA modification refers to the methylation of the seventh guanosine at the 5′ end of RNA. In this context, we screened out three core m7G-related gene sets from the MSigDB database, including “GOMF RNA 7-Methylguanosine Cap Binding,” “GOMF m7G 5-PPPN Diphosphatase Activity,” and “GOMF RNA Cap Binding.” The detailed description of these gene sets is shown in Supplementary Table S2.

m7G-related Gene Set

In the present study, we established an m7G gene set using the Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org/gsea/msigdb/). As mentioned in some m7G-related reviews, this RNA modification refers to the methylation of the seventh guanosine at the 5′ end of RNA. In this context, we screened out three core m7G-related gene sets from the MSigDB database, including “GOMF RNA 7-Methylguanosine Cap Binding,” “GOMF m7G 5-PPPN Diphosphatase Activity,” and “GOMF RNA Cap Binding.” The detailed description of these gene sets is shown in Supplementary Table S3. Using these three MSigDB gene sets, we constructed a comprehensive m7G gene set, whose gene members are presented in Supplementary Table S4.
Construction of m7G Risk Signature

The m7G differentially expressed genes (DEGs) were screened out by the “Limma” package in R software (Ver. 3.6.3). The threshold for the screening criterion was set at adjusted P-value <0.05 and the absolute value of log2FC ≥ 0.58 (1.5-fold difference in gene expression). The m7G regulatory genes with prognostic values were identified by cox univariate regression analysis. The intersection part between DEGs and prognostic genes was selected by the Venn diagram. Finally, these intersection genes were applied to construct a novel m7G risk signature through the Lasso regression analysis.

Evaluation of the Prognostic Value

The m7G risk score of each HCC patient was calculated in terms of the novel risk signature. The Cutoff Finder online tool (http://molpath.charite.de/cutoff) was used to determine the optimal cutoff value of the m7G risk score (18). According to the value, patients were divided into high- and low-risk groups. Survival differences between different risk groups were compared based on the Kaplan–Meier method. Cox univariate and multivariate analyses were applied to identify the independent prognostic factors. The predictive accuracy of the m7G risk signature was evaluated by the receiver operating characteristic curve (ROC). Decision curve analysis (DCA) was used to determine whether the m7G risk score could improve the clinical decision-making benefit of two traditional prognostic models of HCC. The traditional model A was composed of age, histological grade, and TNM staging. The traditional model B was composed of age, histological grade, and clinical stage. Except for the M1 stage (n = 4), the clinical subgroup analyses were performed to assess the application scope of the m7G risk signature. A nomogram was constructed to predict the OSR of an individual at 1, 3, and 5 years. Besides, GSE14520 (n = 221), GSE116174 (n = 64) and ICGC-LIRI (n = 221) datasets as the validation cohorts were employed to further test the prognostic value of the m7G model.

Immune Analysis

The immune abundance of 21 lymphocyte subtypes in each HCC sample was calculated using the CIBERSORT algorithm (19). The activities of 10 immune-related pathways were calculated based on the ssGSEA (single-sample gene set enrichment analysis) method (20). The ESTIMATE algorithm is an approach to quantify the immune cell admixture in tumor parenchyma and stroma, by which the tumor immune microenvironment (TIM) could be depicted to some extent (21). The scores of stromal, immune, and ESTIMATE in different risk groups were calculated.

GSEA

GSEA (Gene Set Enrichment Analysis) was utilized to analyze the impacts of m7G risk levels on the glycolysis process, ferroptosis, and pyroptosis. The used gene sets were obtained from the MsigDB database, including “GO Glycolytic Process,” “Hallmark Glycolysis,” “Reactome Pyroptosis,” and “WP Ferroptosis.” Phenotype labels were set as high-m7G risk samples versus low-m7G risk ones. The number of permutations was set as 1,000. There was no collapse in gene symbol. The descriptions of these gene sets are given in Supplementary Table S5.

HPA Database

The human protein atlas (HPA) database exhibits the landscape of cancer proteomes in 32 different tissues and organs (https://www.proteinatlas.org/) (22). Using the HPA database, we confirmed the histological expression levels of m7G signature genes between normal liver and HCC tissues.

Cell Culture and Transfection

Two human hepatocellular cancer cell lines (HepG2 and Huh-7) were purchased from Procell Life Science&Technology Company (Wuhan, China). One normal liver cell line (THLE-3) was purchased from Otwo Biotechnology (Guangzhou, China). HepG2 and THLE-3 cells were both cultured in MEM (minimum essential medium) containing 10% FBS (fetal bovine serum) and 1% P/S (penicillin/streptomycin) (Procell, Wuhan, China). Huh-7 cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium) containing 10% FBS and 1% P/S (Procell, Wuhan, China). Specific siRNA and amplification plasmids were designed by HanHeng Biotechnology (Shanghai, China). Lentiviruses (HanHeng Biotechnology, Shanghai, China) are used to transfect liver cancer (LC) cells.

Clinical Samples and RT-qPCR

We collected 15 pairs of HCC and adjacent normal tissues from the Third Affiliated Hospital of Chongqing Medical University. RT-qPCR and western blot tests were employed to confirm the differential expression of NCBP2 in HCC specimens. We received informed consent from all patients. The study protocol was approved by the Ethics Committees of the Third Affiliated Hospital of Chongqing Medical University.

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription (RT) was performed using the PrimeScript RT reagent kit with gDNA eraser (Takara, Japan). Transcript levels were measured using the SYBR-Green PCR reagent (Takara, Japan). Then, RT-qPCR was performed on an ABI Prism 7900 sequence detection system. GAPDH was used as an internal reference. mRNA expression was calculated based on the 2−ΔΔCt method. The primer list is presented in Supplementary Table S6.

Western Blot

The detailed manipulations were similar to those previously described (23). After washing with PBS twice, transfected cells were lysed on ice by RIPA buffer (Beyotime, China). Centrifugation was performed at 12,000 rpm for 4 min. Then, the supernatant was collected and moved into an EP tube. The protein concentration was measured by the BCA kit (Phygene Life Sciences Company, Fuzhou, China). Sample absorbance at 562 nm was measured after 30 min incubation with the BCA working reagent. The protein concentration of each sample was calculated according to the standard curve.
Sample proteins were separated by 10% SDS-PAGE. After electrophoresis, protein samples were transferred to PVDF membranes (BestBio, Shanghai, China). The PVDF membranes were blocked by 5% skim milk at 37°C for 2 h and then were washed with TBST buffer (BIOSIC, Nanjing, China) three times. The treated membranes were incubated with the primary antibody overnight at 4°C. After washing again with TBST three times, the membranes were incubated with the secondary antibody. The primary and secondary antibodies were both purchased from Abcam Scientific (Shanghai, China). The primary antibodies were as follows: rabbit polyclonal anti-NCBP1 (anti-CBP80) (1:1000, ab154532) and rabbit polyclonal anti-GAPDH antibody (1:1,000, ab22555). The secondary antibody was the goat-anti rabbit IgG-HRP secondary antibody (1:2000, ab205718).

**CCK8 Assay**

Cell counting kit-8 assays (CCK-8, Dojindo, Tokyo, Japan) were employed to evaluate the viability of HepG2 and HuH-7 cells. Transfected cells were plated into 96-well plates with a density of 5 × 10^3 cells per well and incubated overnight at 37°C. At each observed time point, the cells were incubated with 10 μL of CCK-8 reagent for 2 h at 37°C. The absorbance of each well was detected at 450 nm by a spectrophotometer.

**Transwell Migration and Invasion Assays**

Transwell migration and invasion assays were carried out to assess the malignant behaviors of LC cells. The experiment procedure was as described in a previous study (7). Transfected cells (2 × 10^4 per well) were placed into 24-well Transwell chambers (Corning, NY, USA). DMEM or MEM medium containing 0.1% FBS was added to upper chambers, while that with 10% FBS was added to the lower ones. After 48 h incubation, the migratory cells were fixed by paraformaldehyde and stained with 0.1% crystal violet. Cell counting was performed under a high-magnification microscope (100-fold) from five random visual fields. For invasion assays, the upper chambers were precoated with Matrigel.

**RESULTS**

**Novel m7G Risk Signature Is Constructed Through the Lasso Regression Analysis**

The flowchart of this study is presented in Figure 1. Most of the m7G regulatory genes (73.5%, 25/34) are differentially expressed in HCC samples. Among them, NUDT4B, EIF4A1, NUDT10, EIF4E, NCBP3, and EIF4E3 were downregulated, while others were upregulated (Figure 2A). Meanwhile, 15 m7G genes were

![Flowchart of the present study](https://example.com/flowchart.png)

**FIGURE 1** | Flow chart of the present study. m7G, N7-methylguanosine; DEGs, differentially expressed genes; ROC, receiver operating characteristic curve; DCA, decision curve analysis; GSEA, gene set enrichment analysis; HPA, Human Protein Atlas.
found to have abilities to affect the prognosis of HCC patients (Figure 2B). Interestingly, all these prognostic genes were risk factors for the poor prognosis. Finally, a total of 13 m7G genes were screened out to construct the risk signature (Figure 2C). Through the Lasso regression analysis (Figure 2DE), we established a novel m7G risk signature that comprised five members. The m7G risk score of each HCC patient was calculated according to the following formula (Figure 2F): m7G risk score = 0.249 * (WDR4 relative expression) + 0.334 * (EIF4E relative expression) + 0.065 * (NCBP1 relative expression) + 0.275 * (NCBP2 relative expression) + 0.011 * (NUDT1 relative expression). According to the optimal cutoff value of risk score (1.912), 442 HCC patients were divided into high- and low-risk groups. The m7G risk plots are exhibited in Supplementary Figure S2. Moreover, the m7G risk score could explain the 80.9% of the prognostic variation (Figure 1G), suggesting that the m7G risk signature had outstanding abilities to discriminate the HCC patients with different survival outcomes.

**m7G Risk Signature Provides Crucial Prognostic Information**

High m7G risk resulted in a poor prognosis (HR = 2.78, \(P < 0.01\)) (Figure 3A). In contrast to the clinicopathological features of HCC, the m7G risk score possessed an excellent
predicted accuracy (AUC = 0.733) (Figure 3B). Besides, it had the highest accuracy for predicting the five-year survival rate (AUC = 0.747) (Figure 3C). Through the cox regression analyses, only the m7G risk score was identified as the independent prognostic factor of HCC (Figures 3D,E). More importantly, DCA plots revealed that the m7G risk score greatly increased the clinical-decision benefit of two traditional prognostic models (Figure 3F). Meanwhile, the combination of the m7G risk score with either TNM staging or AJCC stage could significantly improve the predicted accuracy of the previous prognostic system (Figures 3G,H vs. 3B).

We also observed that the m7G risk score is equipped with a wide range of applicability. The m7G risk signature could distinguish the survival differences of HCC patients in most of the clinical subgroups (Figures 4A–I). For advancing clinical practice, we constructed a nomogram that consisted of clinical stage and m7G risk level (Figure 4J). The calibration plot showed that the predicted probabilities well-matched with actual survival rates (Figure 4K).
m7G risk Signature Is Also Applicable to Validation Cohorts

Using three validation cohorts, we tested the prognostic value of the m7G risk signature. In the GSE14520 cohort, although the overall survival rates between different risk groups presented a certain difference, it was not statistically significant (HR = 1.48, $P = 0.082$) (Figure 5A). The predicted accuracy of the m7G risk score in GSE14520 was around 0.5 but was still higher than most HCC clinical indicators (Figure 5B). Besides, the m7G risk score in the patients with high AFP levels and advanced clinical stages was much higher than that in the patients with low AFP levels and local cases.
However, the m7G risk score was not associated with the clinical history of cirrhosis and ALT level (Figures 5E, F). Different findings were observed in GSE116174 and ICGC-LIRI cohorts. In these two cohorts, high risk conferred unfavorable survival outcomes (Figures 5G, I). Moreover, the m7G risk score was superior in predicting prognosis compared with the age and clinical stage (Figures 5H, J). Overall, the m7G risk signature was successfully validated in two external cohorts.

**High m7G Risk Is Detrimental to the Anticancer Immune Process**

The infiltrating abundances of 21 immune cells in each HCC sample are presented in Supplementary Figure S3. High m7G risk significantly reduced the infiltrating levels of CD8 T cells, resting NK cells, gamma delta T cells, monocytes, M1 macrophages, M2 macrophages, and resting mast cells; inversely, it increased that of follicular helper T cells.
regulatory T cells (Tregs), and macrophages M0 (Figure 6A). As shown in Table 1, most of the alterations of immune abundances suppressed the antitumor process and promoted the formation of the immunotolerant microenvironment. As for immune-related pathways, high m7G risk was accompanied by the low activities of cytolytic activity and type-II IFN (interferon) response (Figure 6B). ESTIMATE analyses showed that stromal and ESTIMATE scores in the high-risk group were all significantly lower than those in the low-risk group (Figure 6C), which indicated that high m7G risk was difficult to activate the antitumor immunity.

m7G Risk Score Is Associated With the Glycolysis and Pyroptosis

Glycolysis is the main hallmark of cancer metabolism. In view of this, we investigated the effects of the m7G risk score on the enrichments of the glycolytic process. As expected, glycolysis was significantly enriched in the HCC samples with high m7G risk (Figures 6D,E). Interestingly, m7G risk levels failed to affect the enrichment of ferroptosis (Figure 6F), whereas high m7G risk could stimulate pyroptosis (Figure 6G), which revealed that different patterns of programmed cell death (PCD) acted different roles in m7G-mediated HCC progression.

FIGURE 6 | Effects of the m7G risk score on TIM. (A) Differences in infiltrating levels of 21 immune cells between high- and low-m7G-risk groups based on the CIBERSORT algorithm. High m7G risk is red, and low m7G risk is blue. (B) Differences in activities of 10 immune-related pathways between different m7G risk groups based on the ssGSEA algorithm. (C) Differences in immune scores between different m7G risk groups based on the ESTIMATE method. (D–G) Effects of m7G risk levels on the enrichments of glycolysis, ferroptosis, and pyroptosis based on GSEA analyses. TIM, tumor immune microenvironment; GSEA, gene set enrichment analysis; *P < 0.05, **P < 0.01, ***P < 0.001.
m7G Signature Genes Differentially Express in Hepatocellular Carcinoma Tissues

The mRNA expression levels of five m7G signature genes were all significantly upregulated in HCC tissues compared to normal liver tissues (Figures 7A–E). Using the HPA database, we investigated the histological expression levels of these genes (Figures 7F–J). All these signature genes presented low or nondetectable expression in normal tissues. In tumor tissues, EIF4E and NCBP2 were highly expressed, while WDR4 and NCBP1 were moderately expressed. Despite low expression of NUDT1 in tumor tissues, NUDT1 was not detectable in normal ones.

m7G, N7-methylguanosine; TFH, follicular helper T cells; Tregs, regulatory T cells.

NCBP2 Has Cancer-Promoting Capacities in Hepatoma Cells

As shown in Table 2, there have been several studies that probed into the roles of m7G signature genes in multiple cancers.
However, the biofunctions of NCBP2 in HCC remain elusive. In this context, NCBP2 was selected for further investigation. We first detected the NCBP2 expression in clinical specimens through RT-qPCR and western blot tests. RT-qPCR results showed that the mRNA expression of NCBP2 in HCC tissues was significantly higher than that in adjacent normal tissues (Figure 8A). The western blot detection on four pairs of clinical specimens also confirmed this expressive tendency. The protein expression levels of NCBP2 in tumor tissues were markedly upregulated compared to those in normal tissues (Figure 8B).

NCBP2 was also significantly overexpressed in hepatoma cells (HepG2 and HuH-7) compared to that in normal liver epithelial cells (THLE-3) (Figure 8C). Meanwhile, we observed that si-NCBP2 and OE-NCBP2 could effectively manipulate the mRNA and protein expression levels of NCBP2 (Figures 8D–G). CCK8 assays revealed that overexpression of NCBP2 promoted the proliferation of hepatoma cells (Figures 8H,I). Inversely, silencing NCBP2 led to a decreased tendency (Figures 8H,I). Next, we applied transwell assays to evaluate the migrative and invasive abilities of hepatoma cells. Overexpression of NCBP2 was enhanced, whereas blocking NCBP2 expression suppressed the migration of hepatoma cells (Figures 9A–D). Similar alterations were observed in cellular invasive ability. Overexpression of NCBP2 increased the number of cells that penetrated Matrigel (Figures 9E–H). However, silencing NCBP2 retarded the invasive process (Figures 9E–H).

**Comparison Between Five Existing HCC Signatures and Our m7G Risk Signature**

Given that there have been some studies constructing signatures for HCC clinical assessments, we analyzed the similarities and differences between five existing signatures and ours (24–28) (Table 3). It was not difficult to perceive that our novel m7G model exhibited several strengths. First, we applied three validation cohorts to test the prognostic value of the m7G risk signature, which effectively assured its applicable scope due to the maximum sample size. Second, our risk signature (AUC = 0.737) (27), the gene number of our signature is much smaller than their ones (5 vs. 19 and 10, respectively). Considering this fact, the calculation of the m7G risk score based on our model was more accessible, which was more beneficial for clinical practice. Third, other studies did not investigate the biofunctions of core signature genes in HCC through an experimental approach, which attenuated the credibility and value of the models. Nevertheless, in the present study, we ascertained the pro-oncogenic capacities of NCBP2 in HCC for the first time, providing new insights into HCC treatment. Briefly, our m7G signature was reliable and had great potential in the clinical assessment of HCC.

**DISCUSSION**

HCC is a common abdominal tumor with a poor prognosis, leading to more than 30,000 deaths across the United States in 2021 (1). Owing to the easy metastasis and the limited efficacy of current therapeutic approaches, finding breakthrough treatment and establishing an accurate prognostic assessment system are extremely meaningful. m7G is a pivotal RNA modification that widely exists in various types of RNA, especially in tRNA (29). Due to its stimulative effects on the stability of RNA, it has been demonstrated that the m7G process could promote cancer progression by upregulating the expressions of oncogenic genes (16, 30). In the present study, we preliminarily probed the functions of m7G regulatory genes in HCC.

Accurate prognostic evaluation has pivotal instructive significance for making therapeutic strategies. Regrettably, the current prognostic model has some defects. Beumer’s team has reported that the American Joint Committee on Cancer (AJCC) system failed to operate in an external cohort (31). Meanwhile, its predictive C-index was less than 0.7 (31). Hence, utilizing the TNM or AJCC prognostic system could not completely meet the demand of accurately predicting the survival outcomes. In this work, the novel m7G risk score not only increased the clinical making-decision benefit of TNM and clinical stage models but also elevated their predicted...
accuracy. All of these findings highlighted that the m7G risk score was a critical supplement for the prognostic assessment of HCC.

Due to participating in the maturation and response function of immune cells, RNA methylation profoundly affects the TIM (12). For instance, m7G recognition is responsible for driving the retinoic acid-inducible gene-I (RIG-I)-mediated innate immune (32). In the current study, we confirmed that the m7G risk score was tightly associated with the TIM of HCC. Especially, high m7G risk obviously decreased the immune abundance of CD8+ T cells, whereas it increased that of Tregs and macrophages. As is well known, CD8+ T cells have potent abilities to eradicate tumor cells through the Fas/FasL pathway (33). However, Tregs and macrophages could make...
FIGURE 9  |  Overexpression of NCBP2 promotes the migration and invasion of LC cells. (A,B) Results of transwell migration assays in HepG2 and HuH-7 cells. (C,D) Migrative cell numbers in different experimental groups. (E-G) Results of transwell invasion assays in HepG2 and HuH-7 cells. (F,H) Invasive cell numbers in different experimental groups. *P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 3  |  Comparison between five related research studies and our study.

| Study       | PMID      | Model type     | Number of signature genes | Validation cohort          | Predictive accuracy (Mean value) | Experimental validation |
|-------------|-----------|----------------|---------------------------|----------------------------|---------------------------------|-------------------------|
| Wu et al.   | 34869364  | Pyroptosis     | 2                         | ICGC (n = 231)             | 0.698                           | NA                      |
| Deng et al. | 33686959  | Glycolysis     | 19                        | ICGC (n = 231)             | 0.757                           | NA                      |
| Gao et al.  | 33789609  | Immune-related | 7                         | Internal validation       | 0.614                           | NA                      |
| He et al.   | 34257161  | FA metabolism  | 6                         | ICGC (n = 231)             | 0.705                           | NA                      |
| Shen et al. | 34820373  | Autophagy      | 10                        | TCGA (n = 342)             | 0.737                           | NA                      |
| Our         | NA        | m7G            | 5                         | GSE14520 (n = 221)         | 0.733                           | Biofunctions of NCBP2   |
|             |           |                |                           | GSE116174 (n = 64)         |                                 |                         |

FA, fatty acid; m7G, N7-methylguanosine; NA, not available.
immunologic barriers against CD8+ T cell-mediated antitumor immune response (34). Therefore, high m7G risk may herald that the antitumor immune process is arrested.

Cancer progression is commonly accompanied by metabolic reprogramming. Glycolysis, also named the “Warburg effect”, acts as a core hallmark of cancer metabolism. It is now established that glycolysis contributes to tumor growth, drug resistance, and immune escape (35–37). For example, the glycolysis process was found to be remarkably upregulated in RCC samples, and its core regulatory genes PLOD1, PLOD2 and CD44 could promote the proliferation of renal cancer cells (38). Through GESA analyses, we observed that high m7G risk resulted in a similar metabolic alteration; for example, the glycolysis process was significantly enriched in the HCC samples with high m7G risk. We speculated the m7G signature gene, EIF4E, may act as the intrinsic driving force. Evidence has emerged that the mTORC1/EIF4E/HIF-1α pathway is capable of mediating glycolysis (39). Moreover, EIF4E-mediated glycolysis has been proven to be a critical link in PAAD development (40).

There has been some research to unravel the roles of some m7G signature genes in cancer progression. For example, Zeng et al. revealed that WDR4, the core subunit of the m7G functional complex, extensively affected the cancer immunity in pan cancer (41). Silencing METTL1 or WDR4 inhibits the malignant behavior of HCC cells (42). NCBP1 promotes the development of LUAD through upregulating CUL4B (43). Herein, we focused on the lesser-studied NCBP2. NCBP2 encodes a product that is a component of the nuclear cap-binding protein complex (CBC), which binds to the monomethylated 5′ cap of nascent pre-mRNA in the nucleoplasm (44). In the present study, we ascertained its tumorigenicity in HCC cells for the first time. Blocking NCBP2 markedly inhibited the proliferation, migration, and invasion of HCC cells, which indicated that NCBP2 is a potential therapeutic target for HCC.

Notably, there are several limitations to this study. First, the m7G risk signature has not been validated in a real clinical cohort. Second, we did not compare the differences in the m7G modification intensity between high- and low-m7G-risk groups. Third, the oncogenic capacities of NCBP2 have not been verified in a xenograft tumor model of nude mice. Fourth, the immune effects of NCBP2 in HCC were not confirmed by experiments in vitro. These issues warrant further research.

CONCLUSIONS

HCC is a common digestive tumor bringing a great health burden on patients. As one of the most common RNA modifications, m7G shows promising potential to improve cancer treatment. In view of this, we constructed a novel m7G risk signature through lasso regression analysis. The m7G risk score exhibited pivotal functions in the prognostic assessment of HCC. Meanwhile, it extremely improved the predictive performance of the TNM staging or AJCC system. Moreover, high m7G risk was detrimental to antitumor immune but increased the enrichments of glycolysis metabolism. Of note, we ascertained the cancer-promoting abilities of NCBP2 in HCC for the first time. In conclusion, these findings provide new clues for prognostic assessment and treatment of HCC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XQZ conceived and designed the study. KXZ, JQY, and XYL analyzed and interpreted the data. KXZ, JQY, WX, and PBZ wrote the manuscript. KXZ, JQY, XYL, WX, and PBZ conducted the in vitro experiments. KXZ, JQY, and XYL made a revised version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2022.893977/full#supplementary-material.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. (2021) 71(1):7–33. doi: 10.3322/caac.21654

2. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol. (2019) 16(10):589–604. doi: 10.1038/s41575-019-0186-y
3. Gordan JD, Kennedy EB, Abou-Alfa GK, Beg MS, Brower ST, Gade TP, et al. Systemic therapy for advanced hepatocellular carcinoma: ASCO guideline. J Clin Oncol. (2020) 38(36):e3417-45. doi: 10.1200/JCO.20.02572

4. Liu JKH, Irvine AF, Jones RL, Samson A. Immunotherapies for hepatocellular carcinoma. JNM, et al. Systematic review and meta-analysis of validation of a glycolysis-associated multiomics prognostic model for hepatocellular carcinoma. Aging (Albany NY). (2021) 13(14):7847-98. doi: 10.18632/aging.202613

5. Zhang LS, Liu C, Ma H, Dai Q, Sun HL, Luo G, et al. Transcriptome-wide RNA methylation modulators pattern was a potential biomarker for prognosis and associated with tumor immune microenvironment of pancreatic adenocarcinoma. Cancer Invest. (2021) 39 (1):39-54. doi: 10.1080/07357907.2020.1834576

6. Wiener D, Schwartz S. The epitranscriptome beyond m(6)A. Nucleic Acids Res. (2016) 44(16):7511-58. doi: 10.1093/nar/gkw551

7. Xu F, Zhang Z, Yuan M, Zhao Y, Zhou Y, Pei H, et al. MeA regulatory genes play an important role in the prognosis, progression and immune microenvironment of pancreatic adenocarcinoma. Cancer Invest. (2021) 39 (1):54-68. doi: 10.1080/07357907.2020.1834576

8. Weng Q, Jia L, Wang X, Liu S, Li C, Yu G, et al. Comprehensive analysis of immune-related prognostic genes in the tumor microenvironment of hepatocellular carcinoma. BMC Cancer. (2021) 21(1):331. doi: 10.1186/s12885-021-08052-8

9. He D, Cai I, Huang W, Weng Q, Lin X, You M, et al. Prognostic value of fatty acid metabolism-related genes in patients with hepatocellular carcinoma. Aging (Albany NY). (2021) 13(13):17847-63. doi: 10.18632/aging.203288

10. Shen S, Wang R, Qiu H, Li C, Wang J, Xue J, et al. Development of an autophagy-based and stemness-correlated prognostic model for hepatocellular carcinoma using bulk and single-cell RNA-sequencing. Front Cell Dev Biol. (2021) 9:743910. doi: 10.3389/fcell.2021.743910

11. Zhou et al. m7G in HCC
44. Dou Y, Barbosa I, Jiang H, Iasillo C, Molloy KR, Schulze WM, et al. NCBP3 positively impacts mRNA biogenesis. *Nucleic Acids Res*. (2020) 48 (18):10413–27. doi: 10.1093/nar/gkaa744

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