Identification and validation of a five-necroptosis-related lncRNAs signature for prognostic prediction in hepatocellular carcinoma

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Abstract

Background

Hepatocellular carcinoma (HCC) is one of the most common digestive malignant tumors with poor prognosis. As a kind of death receptor-mediated regulated programmed death, necroptosis has the dual characteristics of necrosis and apoptosis. Long non-coding RNAs (lncRNAs) are reported to be key regulators in tumor necroptosis. This study aimed to identify the necroptosis-related IncRNAs (np-IncRNA) in HCC and investigate their relationships with prognosis.

Method

The RNA-sequencing data and clinicopathological and survival information of patients with HCC were retrieved from The Cancer Genome Atlas (TCGA) database. The np-IncRNAs were analyzed to predict the prognosis of patients with HCC. Stepwise multivariate Cox regression analysis was used to construct prognostic signatures related to necroptosis. Kaplan-Meier (KM) analysis was used to compare the prognosis of patients. Receiver operating characteristic (ROC) analysis and decision curve analysis (DCA) was used to assess the accuracy of the prognostic signature. Quantitative real-time polymerase chain reaction(qPCR) was used to validate the lncRNAs expression among samples from an independent cohort.

Results

The np-IncRNAs ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS were identified as potential prognostic biomarkers. The area under the curve (AUC) of the prognostic signature constructed by these np-IncRNAs reached 0.773. Patients were divided into two groups based on the risk score calculated by the signature, and poorer overall survival was shown in the high-risk group. Gene Set Enrichment Analysis (GSEA) suggested that tumor-related pathways (mTOR, MAPK and p53 signaling pathways) and immune-related functions (T cell receptor signaling pathway and natural killer cell mediated cytotoxicity) were significantly different between the low risk and high risk group. The increased expression of np-IncRNAs was confirmed in another independent HCC cohort.

Conclusions

This signature is a reliable tool for predicting the prognosis of HCC patients. Our findings provide a subset of np-IncRNA biomarkers for prognosis prediction and personalized treatment of HCC patients.

Introduction
Liver cancer is one of the most common digestive malignancies, and hepatocellular carcinoma (HCC) accounts for more than 90% of primary liver cancers[1], which seriously threatening people's health due to its high morbidity rate and fatality[2]. Although surgical resection is considered as the most effective treatment for HCC patients in the early stage, the high postoperative recurrence rate results in a poor overall prognosis [3; 4]. In recent years, with the advances in high-throughput sequencing technology, an increasing number of HCC-specific molecular biomarkers have been identified. Understanding the molecular mechanism of HCC will be of great significance for early diagnosis, prognostic monitoring, and the development of new molecular targeted drugs.

Necroptosis is a newly discovered regulating mode of programmed cell death that is morphologically characterized by necrosis [5]. Necroptosis plays a vital role in cancer development by promoting tumor cell death and regulating immune cells. Strategies for modulating necroptosis have brought new directions to cancer therapy [6; 7; 8]. It has been reported that necroptosis-inducing agents were more favorable for eliminating hepatoma cells than apoptosis-inducing agents [9].

Long non-coding RNA (lncRNA) is a non-coding transcript with a length of 200 nucleotides that regulates various biological behaviors in tumors [10]. Studies have shown that compared with normal liver tissues, some classic lncRNAs are significantly dysregulated in tumor tissues [11]. Besides, multiple lncRNAs related to liver cancer have been confirmed to be stably expressed in plasma, which is relatively easy to obtain. Therefore, they are expected to become novel biomarkers for HCC diagnosis and treatment [12]. Recent studies revealed that necroptosis-related lncRNA (np-lncRNA) signature has a good predictive effect on stomach adenocarcinoma, breast cancer, lung adenocarcinoma, and other cancers [13; 14; 15]. However, the application of np-lncRNAs in the prediction of HCC prognosis and the underlying mechanisms remain to be elucidated. In this study, we developed a promising prognostic signature for HCC based on differentially expressed np-lncRNAs.

Materials and methods

Datasets selection

To screen out the differentially expressed genes between HCC and adjacent normal tissue samples, we extracted RNA sequencing (RNA-seq) data of patients from The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) database. The corresponding clinicopathological information (age, gender, TNM, stage, grade, survival status and survival time) was also retrieved.

Identification of np-lncRNAs

A gene set including 67 necroptosis-related mRNAs were constructed based on previous study [16]. These genes were derived from the necroptosis gene set M24779.gmt (https://www.gsea-msigdb.org/gsea/msigdb/geneset_page.jsp?geneSetName=GOBP_NECROPTOTIC_SIGNALING_PATHWAY) which was previously reported to be involved in necroptosis. R software package named “LIMMA” was applied to identify differentially expressed
IncRNAs and mRNAs between HCC and normal tissues. The cut-off criteria were assigned as FDR < 0.01 and |logFC| > 1.5. Pearson correlation was used to confirm the relationship between the np-IncRNAs and necroptosis genes. The association was considered significant when the correlation coefficient |R| > 0.5 and P < 0.001 were satisfied at the same time. The cross-function of the Venn diagram was used to extract the intersection of these differentially expressed IncRNAs and mRNAs for subsequent analysis.

**Construction of the prognostic signature based on np-IncRNAs**

Univariate Cox regression and stepwise multivariate Cox regression analysis \[17\] were used to analyze the effects of np-IncRNAs on the patient's prognosis. Patient stratification was based on np-IncRNA risk score, which is determined by the following equation:

\[
\text{Risk score} = \sum (\text{Coefficient} \times \text{Expression})
\]

Each patient was designated with a corresponding risk score. We divided patients with risk score < median number into the low risk group, and those with risk score \(\geq\) median number into the high risk group.

**Gene set enrichment analysis (GSEA)**

GSEA 3.0 software was used to perform GSEA to determine the function or pathway of statistically significant and consistent differences between the two groups. The positive enrichment score (ES) and normalized enrichment score (NES) indicated that most genes in this gene set were positively correlated with our predefined group status. The condition of a normalized P-value (NOM P-value) < 0.05 was considered statistically significant.

**Immunity analysis and related gene expression**

To evaluate the cellular components and cellular immune response between the two groups of patients, we applied the TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCounter, XCELL and EPIC algorithms. The results were calculated and visualized with heatmaps \[18\]. Single-sample GSEA (ssGSEA) was used to assess differences in the number of tumor-infiltrating immune cells between groups, serving as an extension of the GSEA method at the single-sample level.

**Quantitative real-time polymerase chain reaction (qPCR)**

Total RNA was extracted using Trizol (Invitrogen, USA). The cDNA was prepared using HiScript III All-in-one RT SuperMix Perfect for qPCR (Vazyme, Nanjing, China). The qPCR was performed using ChamQ SYBR Color qPCR Master Mix (Vazyme, Nanjing, China) according to the manufacturer's instructions. U6 was used as endogenous control and primers used in this study were: ZFPM2-AS1 forward: 5'-GCAACTGTAGACAAGGAGGAAG-3', reverse: 5'-CAGAGAGCATCCATGGTCAATT A-3'; AC099850.3 forward: 5'-TCGCTATGTTTCCCAGGAGGAAG-3', reverse: 5'-CAGAGAGCATCCATGGTCAATT A-3'; BACE1-AS forward: 5'-GGCACCTCCTA AGTGTACCTGC-3', reverse: 5'-CTCTCTGCTGGGCACGATTC-3'; KDM4A-AS1
forward: 5′- TTGCCTGGATGGCTGAGAATC-3′, reverse: 5′- TTCCTTTCCACCCTCCTT CCTTC-3′ MKLN1-AS
forward: 5′- CTGGAGTAAGTCAGCAGGATTC-3′, reverse: 5′- CTGGTATTACGTCCACCTGATG-3′. The
expression of signature DE-FLs was normalized using the relative quantification method of $2^{-\Delta\Delta CT}$.

**Statistical analysis**

Statistical analysis was performed using R software and its appropriate packages (V 4.0.2). The
differences between the two groups were calculated by Student’s t test. Statistical significance was
indicated by *$P < 0.05$, **$P < 0.01$ or ***$P < 0.001$.**

**Results**

**Screening of differentially expressed np-IncRNAs in HCC**

To explore the necroptosis-related genes among HCC, we retrieved the RNA-seq results from the TCGA-
LIHC dataset. The expression data of mRNA and IncRNA were analyzed. A total of 3264 mRNAs and
2136 IncRNAs were found to be differentially expressed among HCC samples (N = 374) and normal liver
samples (N = 50; Fig. 1A). After overlapping the differentially expressed mRNAs and necroptosis-related
mRNAs[16], 10 differentially expressed necroptosis-related mRNAs were identified (Fig. 1B). Next, we
identified 103 np-IncRNAs by Person’s correlation analysis between necroptosis-related mRNAs and
IncRNAs (Fig. 1C). The intersection between the differentially expressed genes and the necroptosis-
related genes were extracted for stepwise multivariate Cox regression analysis (Fig. 1A and 1C). The 103
np-IncRNAs were used for further investigation.

**Construction of necroptosis-based IncRNAs prognostic
signature and multivariate examination**

Next, 16 of the 103 np-IncRNAs were identified to be the statistical risk factors of the prognosis for
patients with HCC by using univariate COX analysis. Incorporating them into the stepwise multivariate
Cox regression analysis, we confirmed 5 differentially expressed np-IncRNAs (ZFPM2-AS1, AC099850.3,
BACE1-AS, KDM4A-AS1, MKLN1-AS) as the independent prognostic predictors of HCC (Table 1). The total
risk score is determined by the following equation:
Table 1

<table>
<thead>
<tr>
<th>ID</th>
<th>Coef</th>
<th>HR</th>
<th>HR.95L</th>
<th>HR.95H</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZFPM2-AS1</td>
<td>0.0560</td>
<td>1.0576</td>
<td>1.0107</td>
<td>1.1066</td>
<td>0.0155</td>
</tr>
<tr>
<td>AC099850.3</td>
<td>0.0777</td>
<td>1.0808</td>
<td>1.0163</td>
<td>1.1494</td>
<td>0.0133</td>
</tr>
<tr>
<td>BACE1-AS</td>
<td>0.0997</td>
<td>1.1048</td>
<td>0.9782</td>
<td>1.2479</td>
<td>0.1085</td>
</tr>
<tr>
<td>KDM4A-AS1</td>
<td>0.5056</td>
<td>1.6581</td>
<td>1.0241</td>
<td>2.6845</td>
<td>0.0397</td>
</tr>
<tr>
<td>MKLN1-AS</td>
<td>0.8065</td>
<td>2.2400</td>
<td>1.4151</td>
<td>3.5459</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Risk score (patients) = (0.0560*ZFPM2-AS1) + (0.0777*AC099850.3) + (0.0997*BACE1-AS) + (0.5056*KDM4A-AS1) + (0.8065*MKLN1-AS)

We divided patients into two groups based on risk scores (Table S1). Of interest, we found that these 5 np-lncRNAs were significantly up-regulated in the high risk group. In addition, the neoplasm histologic grade, pathologic stage and TNM categories were graded higher in the high-risk group, suggesting that the 5 np-lncRNAs were positively correlated with the malignant phenotype of HCC (Fig. 2A). Kaplan-Meier (KM) analysis showed that HCC patients with high risk scores had poorer survival rates compared to HCC patients with low risk scores ($P < 0.01$, Fig. 2B). Consistently, the risk survival status plot also showed that the high risk group was associated with larger number of death (Fig. 2C). Intriguingly, the area under the curve (AUC) of the np-lncRNA signature was 0.773, which was higher than the conventional clinicopathological characteristics for predicting prognosis for HCC patients (Fig. 3A). Besides, the decision curve analysis (DCA) confirmed that the np-lncRNA signature was superior to the other variates (Fig. 3B). The np-lncRNA signature’s AUC prediction values for 1, 2, and 3-year survival rates of HCC patients reached 0.773, 0.719, and 0.694, respectively (Fig. 3C).

HCC is particularly prevalent in Asia, where HBV is hyperendemic, and 60% of HCC is associated with HBV [19]. As HBV and HCV infection are well-established risk factors for HCC [20], we performed stratification analysis for the patients with HBV (HBV-HCC) subgroup and patients with HCV (HCV-HCC) subgroup using the self-generated np-lncRNA signature. According to the Kaplan-Meier survival analysis, for HBV-HCC patients, a significantly shorter survival time was observed in the high risk group than patients in low risk group ($p = 0.001$, Figure S1A). The np-lncRNA signature’s AUC prediction values for 1, 2, and 3-year survival rates for HBV-HCC patients were 0.755, 0.785, and 0.792, respectively (Figure S1B). In contrast, there was no significant difference between high risk and low risk group of HCV-HCC patients according to Kaplan-Meier survival analysis (Figure S1C). Moreover, for patients without HBV and HCV, Kaplan-Meier analysis suggested that patients in the low risk group have a longer survival time than patients in high risk group ($p = 0.02$, Figure S1D). The AUC was 0.721 for the 1-year survival time (Figure S1E).

To sum up, we generated a necroptosis-based lncRNAs prognostic signature with accurate predictive ability for evaluating the overall survival of HCC patients.
The prognostic signature is an independent prognostic factor in HCC

To further verify the reliability of the signature, we performed univariate and multivariate COX analysis. The results showed that the risk score defined by the np-IncRNA signature was an independent prognostic factor (HR: 1.318, 95CI: 1.220–1.423) (Fig. 4A, 4B). Specifically, a high signature score was associated with poor prognosis. Next, a nomogram was developed to predict the overall survival of HCC patients using all of the prognostic variables (Fig. 4C). Taking the tumor grade, gender, TNM stage, age and risk score into consideration, the nomogram assigned each factor a specific point. By adding up the points of each variable, the total points indicated the survival probability of the HCC patients. For example, for a patient with total points of 402, the probability of less than 1-year, 3-year, and 5-year survival was 0.235, 0.418 and 0.531, respectively (Fig. 4C). Therefore, this nomogram might serve as a useful tool for predicting prognosis of HCC patients.

In summary, our results indicated that the np-IncRNA risk score independently predicted the prognosis in HCC patients.

Construction of co-expression network and gene set enrichment analysis

To understand the biological implications of these lncRNAs in HCC patients, we explored the co-expression networks among the lncRNAs and their associated mRNAs. We found that IncRNA KDM4A-AS and AC099850.3 correlated with a DNA-binding protein encoding gene TARDBP. In addition, the IncRNA AC099850.3 also correlated with epigenetic regulating genes (DNMT1, HAT1), serine/threonine protein kinase (MAP3K7, PLK1) and mitochondrial related genes (DIABLO). For IncRNA BACE1-AS, it generally co-expressed with tumor suppressor TSC1 and E3 ubiquitin ligase TRIM11. Additionally, the IncRNA MKLN1-AS and AFPM2-AS1 correlated with protease CASP8 and scaffolding/adaptor protein SQSTM1, respectively (Fig. 5A).

Next, we performed GSEA of the sample with high score of IncRNA signature. Consistent with co-expression analysis, we found that the genetic information of HCC samples with high IncRNA signature score were enriched in mTOR signaling pathway critically modulated by TSC1, MAPK signaling pathway induced by protein kinase MAP3K7, and a renowned apoptosis-related pathway p53. Of great interest, we also found that genes in HCC samples with high risk score were also enriched in immune-related pathways, such as FC gamma R mediated phagocytosis, natural killer cell mediated cytotoxicity and T cell receptor signaling pathway (Fig. 5B).

Altogether, the above data indicated that the np-IncRNA signature, with diverse genetic features, might exert great impacts on various signaling pathways of HCC.

Immune analysis and related gene expression
Since the previous results indicated that the np-lncRNA signature was correlated with multiple immune-related pathways, we performed further analysis to substantiate the observations. First, we analyzed the expression of immune signature genes based on different datasets. According to the TIMER database, the results demonstrated that HCC samples with high risk scores had higher expressions of various immune-related genes, including B cell signature genes, T cell signature genes, neutrophil signature genes, macrophage signature genes and myeloid dendritic cell signature genes. Similar results were further verified in CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL and EPIC databases (Fig. 6A).

Next, we applied ssGSEA to explore the differences in immune status between the two groups. The results revealed that patients in the high-risk group were more active in class 1 MHC activity, but had lower cytolytic activity, type I and type II response than patients in the low-risk group (Fig. 6B). Recent study showed that the depletion of m6A writer METTL3 improved anticarcinogen oxaliplatin resistance in cancer patients by contributing to necroptotic cell death, suggesting the significant correlation between m6A methylation and necroptosis [21]. Therefore, we evaluated the importance of the np-lncRNA signature in m6A modification. Expectedly, the ssGSEA analysis found that m6A-related factors such as METTL3, METTL14 and YTHDF1/2 were upregulated in the high-risk group (Fig. 6C). Taken together, the results suggested that HCC samples with high/low risk scores had distinct immunological and epigenetic signatures.

Validation of the np-lncRNAs in clinical HCC tissues

We performed a pan-cancer analysis of these lncRNAs using data from TCGA, the results showed that these lncRNAs were also highly expressed in other cancers such as UCEC, CHOL and COAD. The pan-cancer prognosis analysis indicated these lncRNAs as independent risk factors for PAAD, COAD, STAD and other tumors (Figure S2 and S3). To further confirm the observations, we investigated the expressions of the five lncRNAs in sixteen HCC samples by qPCR. The data revealed that all the five np-lncRNAs (ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS) were upregulated in the cancerous tissues (p < 0.001; Fig. 7), which were consistent with the results from TCGA (Figure S2). The results confirmed that the np-lncRNA signature could be applied to predict the prognosis of HCC patients.

Discussion

Given that abnormal IncRNAs play crucial roles in the hepatocarcinogenesis, metastasis, angiogenesis, chemoresistance and recurrence, IncRNAs are expected to be the targets for the diagnosis, treatment and surveillance of HCC [22; 23; 24]. As a unique cell death mode first reported by Degterev et al., necroptosis is characterized by serine/threonine protein kinase 1/3 (RIPK1/RIPK3)-mediated phosphorylation of activating mixed lineage kinase domain-like proteins (MLKL/p-MLKL), sharing the same subsequent pathway with apoptosis [25; 26; 27]. Intriguingly, it is reported that liver cancer cells are more sensitive to necroptotic inducers than apoptosis inducers[9]. Moreover, recent studies show that tumor cells with high resistance to apoptosis are more likely to be induced into necroptosis[28; 29], suggesting the significance of necroptosis in HCC targeted treatment. Thus, using np-IncRNAs for the diagnosis and treatment of
HCC patients would be a promising strategy. In this study, we identified 5 np-lncRNAs and constructed a signature for predicting mortality risk in HCC patients.

We screened five differentially expressed np-lncRNAs in the TCGA-LIHC dataset by using univariate and multivariate COX analysis. After dividing patients into high risk and low risk groups based on these molecules, we were excited to find that the survival rate of patients in the high risk group was significantly lower than that in the low risk group. Previous studies confirmed that the IncRNAs BACE1-AS, MKLN1-AS, and KDM4A-AS1 become biomarkers for the diagnosis and prognosis of HCC, as they can promote hepatocellular carcinoma progression by mediating the competing endogenous RNA (ceRNA) network [30; 31; 32]. LncRNA ZFPM2-AS1, highly expressed in HCC tissues, facilitates the progression of HCC by competitively binding to miR-139 with GDF10 mRNA [33]. The co-expression network of this study shows that IncRNA AC099850.3 plays a central role in the network. Studies demonstrated that IncRNA AC099850.3, associated with patient prognosis, contributes to HCC proliferation and invasion through the PRR11/PI3K/AKT axis [34]. Interestingly, AC099850.3 was also included in the HCC prognostic model constructed by using immunoautophagy-related IncRNAs [35]. Similarly, Xu et al. constructed an HCC prognostic model through IncRNAs such as ZFPM2-AS1 and found the model was correlated with the immune response [36]. These studies are highly consistent with our findings that immune-related functions such as cytolytic activity, type I and type II responses were downregulated in the high risk group. In vitro, vaccination with necrotic cancer cells induces potent antitumor immunity by activating CD8+ T cells via RIPK1 antigen cross-priming [37; 38]. Taken together, the above results support the concept that necroptosis promotes antitumor immunity.

Based on these np-lncRNAs, we established a new prognostic signature with an AUC value of 0.773. The novel prognostic signature is an independent prognostic factor for HCC. The nomogram constructed by this signature and clinical features (such as age, gender, TNM stage) can faithfully predict the prognosis of HCC patients. As far as we know, some previous studies have also constructed new signatures to predict the prognosis of HCC. In the study by Dai et al., a prognostic signature was constructed based on the necroptosis-related metabolic genes to predict the prognosis of HCC. In their study, the AUC values were 0.765 at 1 year, 0.684 at 3 years, and 0.642 at 5 years [39]. Using hypoxia-related genes, the work of Bai et al. built a prognostic model based on seven genes, and the AUC of this model was 0.621 at 1 year, 0.693 at 3 years, and 0.769 at 5 years [40]. Similarly, Zhang built a prognostic signature based on the RNA binding protein gene for HCC, and the AUC value was 0.740[41]. In addition, some other prognostic models have been established in recent years [41; 42], but their AUC values are lower than our 0.773. Moreover, we not only conduct the mining and exploration of public databases but also conducted the qPCR experiment to verify our findings in clinical HCC patient samples. Therefore, our research may faithfully improve the prognostic prediction of patients with HCC.

Hyperactivation of the mTOR signaling pathway is an important mechanism to facilitate the development of HCC. Inhibitors of the mTOR signaling pathway effectively block the abnormal signal transduction of various growth factors, thereby blocking the occurrence and development of HCC [43]. Recently, third-generation mTOR inhibitors have been developed by scientists. The p53 is a tumor suppressor gene with
the highest correlation with human tumors, which is the most studied gene in tumors so far. However, in more than one-third of HCC cases, the p53 gene loses its function. Recent studies revealed that p53 mRNA nanoparticles combined with immune checkpoint blockade (ICB) significantly improve the anti-tumor immune response of liver cancer\[44\]. The emergence of potential drugs that inhibit the Wnt/β-catenin signaling pathway, such as small molecule inhibitors, traditional Chinese medicine extracts, and miRNAs, provides more possibilities for improving the therapeutic effect of HCC \[45; 46; 47\]. This study illustrated that mTOR, p53, and Wnt signaling pathways were all enriched in the high risk group, indicating that our new signature is supported by previous findings. Furthermore, as the role of RNA m6A modification in HCC has attracted increasing attention, we evaluated the importance of the np-IncRNA signature in m6A modification. Expectedly, m6A-related factors such as METTL3, METTL14 and YTHDF1/2 were upregulated in the high-risk group, suggesting the significant correlation between m6A methylation and necroptosis. The specific relationship between necroptosis and m6A in HCC deserves further investigation.

Conclusion

The current study identified 5 IncRNAs that were related to necroptosis and constructed a novel np-IncRNA signature with high predictive value for predicting the prognosis of HCC.

Declarations

Ethical approval statement Sixteen pairs of HCC tumor and para-tumor tissues were collected from West China Hospital, Sichuan University. The protocols used in this study were approved by the Ethical Review Committees of Sichuan University, and written informed consent was provided from all the patients.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors’ contributions All authors participated in the design, interpretation of the studies and analysis of the data and review of the article. HCW and YZ designed the research; HC, LX, and QBF performed the statistical analysis; GMH and SX analyzed and interpreted the data; HC and TL drafted the article; HCW and TL revised the article for important intellectual content.

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Declaration of conflicting interests The author(s) declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

References


Figures
Figure 1

Screening of differently expressed np-IncRNAs in HCC. (A) Workflow for the construction of the necroptosis-related signature in HCC; (B) Venn diagram showing the intersection of 40 differentially expressed necroptosis-related genes; (C) Volcano plot of the differently expressed IncRNAs in HCC patients.
Figure 2

Identification of np-IncRNAs signature. **(A)** Heatmap for these IncRNAs and clinicopathological manifestations; **(B)** Kaplan-Meier curves result; **(C)** Risk survival status plot.
Figure 3

Multivariate examination of the signature (A) The AUC values of the risk factors; (B) The DCA of the risk factors; (C) The AUC values for the prediction of 1, 2, 3-years.
Figure 4

COX analysis of np-lncRNAs signature and the construction of the nomogram and co-expression network. 
(A) Univariate COX analysis; (B) Multivariate COX analysis; (C) Constructed a nomogram to predict 1-, 3-, and 5-years OS.
Figure 5

Co-expression network construction and gene set enrichment analysis for np-lncRNAs. (A) Constructed a co-expression network of lncRNAs and necroptosis-related genes. DNA methyltransferase 1 (DNMT1), histone acetyltransferase 1 (HAT1), mitogen-activated protein kinase kinase kinase 7 (MAP3K7), polo-like kinase 1 (PLK1), Diablo IAP-binding mitochondrial protein (DIABLO); (B) Gene set enrichment analysis for the 5 np-lncRNAs.
Figure 6

Heatmap for immune responses and the expression of m6A-related genes between the two groups. (A) Heatmap for immune responses; (B) ssGSEA reveals the difference in immune cells and immune functions between the two groups; (C) The expression of m6A-related genes.
Figure 7

Validation of the np-lncRNAs in clinical HCC tissues. (A-E) qRT-PCR result of expression level of ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS in HCC tissues compared with paired paratumor tissues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
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