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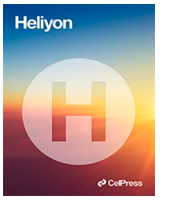

Research article

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 among the most prevalent digestive system malignancies and is associated with a poor prognosis. Necroptosis, a form of regulated death mediated by death receptors, exhibits characteristics of both necrosis and apoptosis. Long non-coding RNAs (lncRNAs) have been identified as crucial regulators in tumor necroptosis. This study aims to identify the necroptosis-related lncRNAs (np-lncRNA) in HCC and investigate their relationships with prognosis.

Method: The RNA-sequencing data, along with clinicopathological and survival information of HCC patients were sourced from The Cancer Genome Atlas (TCGA) database. The np-lncRNAs were analyzed to assess their potential in predicting HCC prognosis. Prognostic signatures related to necroptosis were constructed using stepwise multivariate Cox regression analysis. The prognosis of patients was compared using Kaplan-Meier (KM) analysis. The accuracy of the prognostic signature was evaluated using Receiver operating characteristic (ROC) analysis and decision curve analysis (DCA). Quantitative real-time polymerase chain reaction(qPCR) was employed to validate the lncRNAs expression levels of lncRNAs among samples from an independent cohort.

Results: The np-lncRNAs ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS were identified as potential prognostic biomarkers. The prognostic signature constructed from these np-lncRNAs achieved an Area Under the Curve (AUC) of 0.773. Based on the risk score derived from the signature, patients were divided into two groups, with the high-risk group exhibiting poorer overall survival. Gene Set Enrichment Analysis (GSEA) revealed significantly different between the low risk and high risk groups in tumor-related pathways (such as mTOR, MAPK and p53 signaling pathways) and immune-related functions (like T cell receptor signaling pathway and natural killer cell mediated cytotoxicity). The increased expression of np-lncRNAs was confirmed in another independent HCC cohort.

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Conclusions: This signature offers a dependable method for forecasting the prognosis of HCC patients. Our findings indicate a subset of np-lncRNA biomarkers that could be utilized for prognosis prediction and personalized treatment strategies of HCC patients.

1. Introduction

Liver cancer ranks among the most common digestive malignancies, with hepatocellular carcinoma (HCC) representing over 90 % of primary liver cancers [1]. It poses a significant threat to public health due to its high incidence and mortality rates [2]. Although surgical resection remains the most effective treatment option for early-stage HCC patients, the elevated rate of postoperative recurrence contributes to an overall poor prognosis. Although surgical resection remains the most effective treatment option for early-stage HCC patients, the elevated rate of postoperative recurrence contributes to an overall poor prognosis [3,4]. Recently, the rise of high-throughput sequencing technologies has facilitated the discovery of various HCC-specific molecular biomarkers. A more comprehensive understanding of the molecular mechanisms driving HCC is essential for improving early detection, prognostic assessment, and the creation of new molecular-targeted therapies.

Necroptosis, a recently discovered form of regulated programmed cell death, is morphologically characterized by necrosis [5]. This process is essential in cancer progression, as it promotes the death of tumor cells and modulates the activity of immune cells. Targeting necroptosis has opened new avenues for cancer therapy [6–8]. Research indicates that necroptosis-inducing agents may be more effective in eradicating hepatoma cells compared to apoptosis-inducing agents [9]. In the study by Xiang et al., HCC cells with high expression of connexin32 were found to resist streptonigrin-induced apoptosis [10]. However, when treated with the necroptosis inducer shikonin, a significant induction of necroptosis was observed in this subset of HCC cells [9].

Long non-coding RNAs (lncRNAs) are non-coding transcripts of at least 200 nucleotides that play key roles in regulating various tumor biological processes [11]. Research has demonstrated that, compared to normal liver tissues, several classic lncRNAs are notably dysregulated in tumor tissues [12]. For instance, lncSox4 pertains to the regulation of transcription factors, while lncRNA-PXN-AS1 is linked to post-transcriptional mRNA regulation [13]. Moreover, several liver cancer-related lncRNAs, such as lncRNA-H19 and lncRNA PCBP1-AS1, have been found to be stably expressed in plasma, making them easily accessible and promising as novel biomarkers for HCC diagnosis and treatment [14,15,16]. Recent studies have shown that necroptosis-related lncRNA (np-lncRNA) signatures have strong predictive potential in cancers such as stomach adenocarcinoma, breast cancer, and lung adenocarcinoma [17,18,19]. However, the potential of np-lncRNAs in predicting HCC prognosis and their underlying mechanisms remain unclear. In this study, we developed a novel prognostic signature for HCC using np-lncRNAs that are differentially expressed.

2. Materials and methods

2.1. Datasets selection

To identify differentially expressed genes between HCC and adjacent normal tissue samples, RNA sequencing (RNA-seq) data from patients were extracted from The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) database. The corresponding clinicopathological information, including age, gender, TNM stage, tumor stage, grade, survival status, and survival duration, was also obtained.

2.2. Identification of np-lncRNAs

A gene set containing 67 necroptosis-associated mRNAs was compiled based on prior research [20]. These genes were selected from the necroptosis gene set M24779.gmt (https://www.gsea-msigdb.org/gsea/msigdb/geneset_page.jsp? geneSet Na me = GOBP_NECROPTOTIC_SIGNALING_PATHWAY), previously recognized for their role in necroptosis. The "LIMMA" package in R software was employed to identify differentially expressed lncRNAs and mRNAs between HCC and normal tissues, with FDR <0.01 and $|\log FC| > 1.5$ as the cutoff criteria. Pearson correlation analysis was conducted to confirm the relationship between np-lncRNAs and necroptosis-related genes, with significance defined by a correlation coefficient $|R|>0.5$ and $P < 0.001$. Venn diagrams were utilized to identify the intersection of these differentially expressed lncRNAs and mRNAs for further analysis.

2.3. Construction of the prognostic signature based on np-lncRNAs

Univariate and stepwise multivariate Cox regression analyses [21] were applied to evaluate the impact of np-lncRNAs on patient prognosis. The np-lncRNA risk score for each patient was calculated using the formula: Risk score $= \sum$ (Coefficient $\times$ Expression)

Patients were categorized into low-risk and high-risk groups based on whether their risk scores were below or above the median value.

2.4. Gene set enrichment analysis (GSEA)

GSEA version 3.0 software was used to perform gene set enrichment analysis, identifying functions or pathways with statistically

significant and consistent differences between the two risk groups. A positive enrichment score (ES) and normalized enrichment score (NES) indicated that the majority of genes in a set were positively correlated with the predefined group status. Statistical significance was defined by a normalized P-value (NOM P-value) < 0.05.

2.5. Immunity analysis and related gene expression

To assess differences in cellular components and immune responses between the two patient groups, algorithms including TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL, and EPIC were employed. Results were visualized using heatmaps [21]. Additionally, single-sample GSEA (ssGSEA) was utilized to quantify differences in tumor-infiltrating immune cells between the groups, extending the GSEA methodology to the single-sample level.

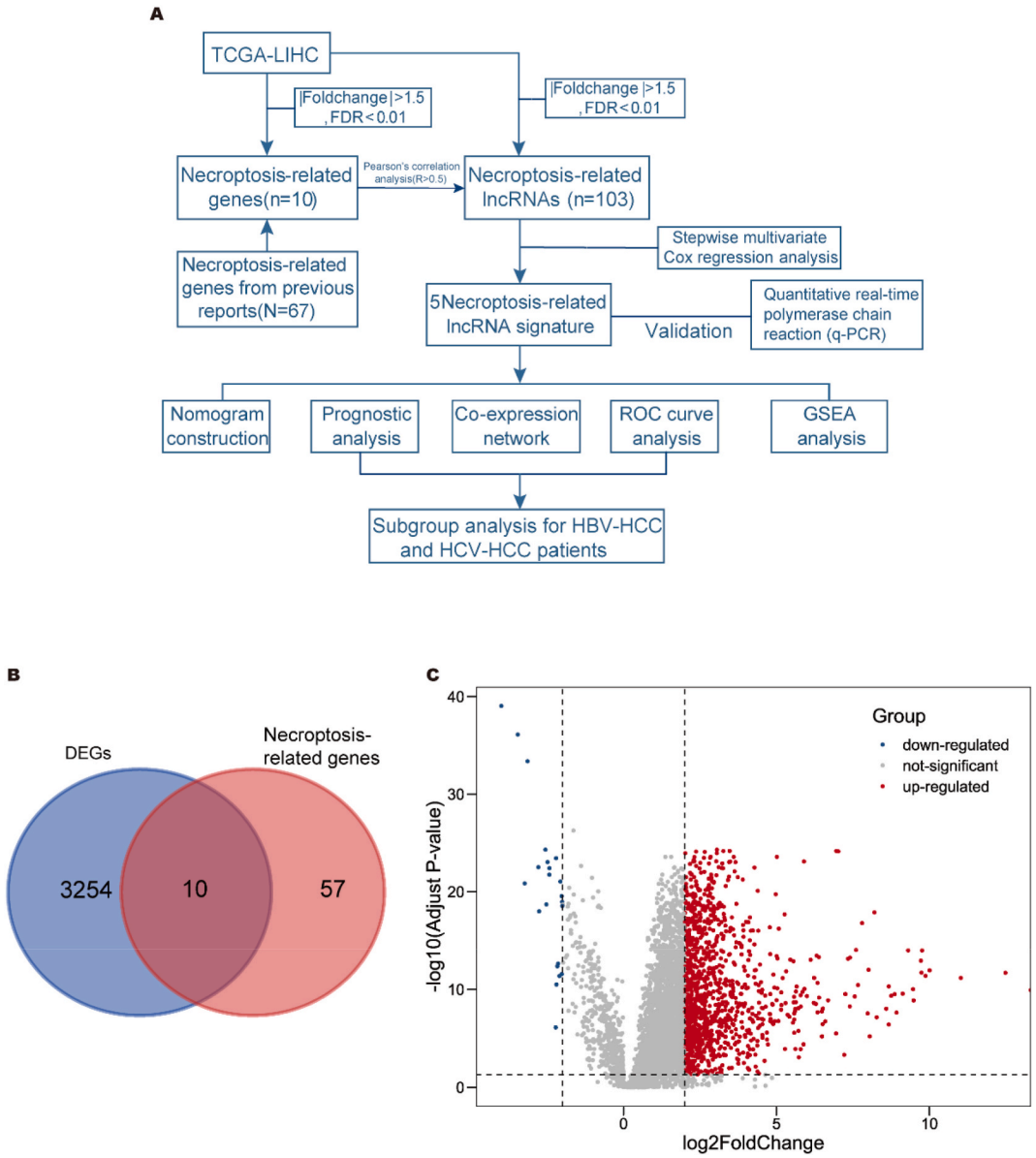

Fig. 1. Screening of differently expressed np-lncRNAs in HCC. (A) Schematic of the workflow used to establish the necroptosis-related signature in HCC. **(B)** Venn diagram illustrating the overlap of 40 differentially expressed genes associated with necroptosis. **(C)** Volcano plot displaying the differentially expressed lncRNAs in HCC patients.

2.6. Quantitative real-time polymerase chain reaction(qPCR)

Total RNA was isolated using Trizol (Invitrogen, USA), and cDNA synthesis was performed with the HiScript III All-in-one RT SuperMix Perfect for qPCR (Vazyme, Nanjing, China). qPCR was conducted using the ChamQ SYBR Color qPCR Master Mix (Vazyme, Nanjing, China) as per the manufacturer's instructions. U6 was used as an internal control. The primers used in this study were as follows: ZFPM2-AS1 (F: 5′- GCAACTGTAGACAAGGAGGAAG-3′, R: 5′-CAGAGAGCATCCATGGTCAATTA-3′); AC099850.3 (F: 5′-TCGCTATGTTTCCCAGGCTGTATT-3′, R: 5′-TGCCAAGGAATCTCTGAAGTCCAT-3′); BACE1-AS (F: 5′-GGCACCTCCTAA GTGTACCTGC-3′, R: 5′-CTCTCTGCTGGGCACGATTC-3′); KDM4A-AS1 (F: 5′-TTGCCTGGATGGCTGAGAATC-3′, R: 5′-TTCCTTTCACCCTCCTT CCTTC-3′); MKLN1-AS (F: 5′-CTGGAGTAAGTCAGCAGGATTC-3′, R: 5′-CTCGTATTACGTCCACCTGATG-3′). The relative expression levels were calculated using the $2^{-\Delta\Delta CT}$method.

2.7. Statistical analysis

Statistical analysis was performed using R software and its appropriate packages (V 4.0.2) and relevant packages. Differences between the two groups were calculated by Student's *t*-test. Statistical significance was denoted as $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$.

3. Results

3.1. Screening for differentially expressed np-lncRNAs in HCC

To investigate necroptosis-related genes in HCC, RNA-seq data from the TCGA-LIHC dataset was utilized. The expression profiles of mRNA and lncRNA were analyzed, revealing 3264 mRNAs and 2136 lncRNAs that were differentially expressed between HCC samples (N = 374) and normal liver samples (N = 50; Fig. 1A). Upon intersecting the differentially expressed mRNAs with necroptosis-related mRNAs [20], we identified 10 differentially expressed mRNAs linked to necroptosis (Fig. 1B). We further identified 103 np-lncRNAs through Pearson's correlation analysis between these necroptosis-related mRNAs and lncRNAs (Fig. 1C). Differentially expressed genes intersecting with necroptosis-related genes were subsequently subjected to stepwise multivariate Cox regression analysis (Fig. 1A and C), leading to the selection of 103 np-lncRNAs for deeper investigation.

3.2. Development of necroptosis-based lncRNAs prognostic signature and multivariate examination

Subsequently, univariate COX analysis identified 16 of the 103 np-lncRNAs as significant risk factors for HCC patient prognosis. Following incorporation into stepwise multivariate Cox regression, five differentially expressed np-lncRNAs were confirmed as independent prognostic indicators for HCC (Table 1). The total risk score for each patient was calculated using the following formula: Risk score(patients) = (0.0560*ZFPM2-AS1) + (0.0777*AC099850.3) + (0.0997*BACE1-AS) + (0.5056*KDM4A-AS1) + (0.8065*MKLN1-AS)

Based on these risk scores, patients were categorized into two groups (Table S1). A detailed analysis of the regulatory patterns and functional implications of these five np-lncRNAs in HCC and other cancers was provided (Table 2). Notably, the five np-lncRNAs were significantly upregulated in the high-risk group. Additionally, the histologic grade, pathologic stage, and TNM categories were elevated in the high-risk group, suggesting a positive correlation between these np-lncRNAs and the malignant phenotype of HCC (Fig. 2A). Kaplan-Meier (KM) analysis revealed that HCC patients with higher risk scores exhibited poorer survival outcomes compared to those with lower scores ($P < 0.01$, Fig. 2B). Consistently, a risk survival status plot indicated a higher mortality rate in the high-risk group (Fig. 2C). Interestingly, the area under the curve (AUC) for the np-lncRNA signature was 0.773, surpassing traditional clinicopathological characteristics in prognostic accuracy for HCC patients (Fig. 3A). Furthermore, decision curve analysis (DCA) validated that the np-lncRNA signature outperformed other variables (Fig. 3B). The AUC prediction values of the np-lncRNA signature for 1, 2, and 3-year survival rates of HCC patients were 0.773, 0.719, and 0.694, respectively (Fig. 3C).

Considering the high prevalence of HCC in Asia, particularly due to HBV, which accounts for 60 % of cases [42]. As HBV and HCV infection are well-established risk factors for HCC [43], we conducted stratification analysis for patients with HBV (HBV-HCC) and HCV (HCV-HCC) using the np-lncRNA signature. Kaplan-Meier analysis showed that HBV-HCC patients in the high-risk group had significantly shorter survival times than those in the low-risk group ($p < 0.001$, Fig. 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 (Fig. S1B). However, no

Table 1
Stepwise multivariate Cox regression analysis of np-lncRNAs.

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

Table 2
np-lncRNAs and their roles during cancer progression.

Np-lncRNAs	Biological roles in HCC	Cancer Impact in HCC	Regulatory mechanisms in HCC	Reference	The status in other tumors
ZFPM2-AS1	Promotion of cell proliferation, migration and invasion	Poor survival	miRNA sponge, regulation of GDF10 and GOLM1	[22,23]	Upregulated in breast cancer [24], colorectal cancer [25]and lung cancer (including both non-small cell lung cancer [26] and small cell lung cancer [27]
AC099850.3	Promotion of cell proliferation and invasion	Poor survival	Regulation of PRR11/PI3K/AKT axis	[28,29]	Upregulated in lung adenocarcinoma [30]
BACE1-AS	Enhances the invasive and metastatic capacity	Poor survival	miRNA sponge, regulation of APLN and CELF1	[31,32]	Upregulated in metastatic colorectal cancer [33]
KDM4A-AS1	Promotes cell proliferation, migration, and invasion; promotes epithelial-mesenchymal transition	Poor survival	miRNA sponge, regulation of STX6 and KPNA2; maintains the stability of AURKA mRNA	[34,35,36]	Weakens cancer cell viability and migratory capacity in esophageal squamous cell carcinoma [37]
MKLN1-AS	Promotes cell growth, angiogenesis, migration, and invasion; inhibits chemotherapeutic drugs	Poor survival	miRNA sponge, regulation of ETS1 and HDGF	[38–40]	Upregulated in pancreatic ductal adenocarcinoma [41]

significant difference was observed between high and low-risk groups for HCV-HCC patients based on Kaplan-Meier analysis (Fig. S1C). For patients without HBV and HCV, Kaplan-Meier analysis indicated that those in the low-risk group had longer survival times than those in the high-risk group ($p = 0.02$, Fig. S1D), with a 1-year survival AUC of 0.721 (Fig. S1E).

In summary, we developed a necroptosis-based lncRNA prognostic signature with robust predictive accuracy for assessing overall survival in HCC patients.

3.3. The prognostic signature as an independent prognostic factor in HCC

To further validate the reliability of the signature, we conducted univariate and multivariate COX analyses. The results confirmed that the risk score derived from the np-lncRNA signature was an independent prognostic factor (HR: 1.318, 95CI: 1.220–1.423) (Fig. 4A and B). Specifically, higher signature scores were associated with poorer prognoses. Subsequently, we developed a nomogram that integrates all prognostic variables to predict overall survival in HCC patients (Fig. 4C). The nomogram assigned specific points to each factor, including tumor grade, gender, TNM stage, age, and risk score. By summing the points of each variable, the total score indicated the survival probability of HCC patients. For example, a patient with a total score of 402 had probabilities of less than 1-year, 3-year, and 5-year survival of 0.235, 0.418, and 0.531, respectively (Fig. 4C). This nomogram may serve as a valuable tool for predicting HCC patient prognosis.

In summary, our findings suggest that the np-lncRNA risk score independently predicts prognosis in HCC patients.

3.4. Construction of co-expression network and gene set enrichment analysis

To elucidate the biological significance of these lncRNAs in HCC, we investigated co-expression networks between lncRNAs and their associated mRNAs. We identified correlations between lncRNAs KDM4A-AS and AC099850.3 with the DNA-binding protein gene TARDBP. Additionally, lncRNA AC099850.3 was associated with epigenetic regulators (DNMT1, HAT1), serine/threonine kinases (MAP3K7, PLK1), and mitochondrial-related genes (DIABLO). For lncRNA BACE1-AS, co-expression was observed with tumor suppressor TSC1 and E3 ubiquitin ligase TRIM11. Moreover, lncRNA MKLN1-AS and AFPM2-AS1 were correlated with protease CASP8 and scaffolding/adaptor protein SQSTM1, respectively (Fig. 5A).

Next, we conducted GSEA on samples with high lncRNA signature scores. Consistent with co-expression analysis, we found that genetic information in HCC samples with high lncRNA signature scores was enriched in the mTOR signaling pathway, critically regulated by TSC1, the MAPK signaling pathway induced by MAP3K7, and the well-known apoptosis-related p53 pathway. Notably, genes in HCC samples with high-risk scores were also enriched in immune-related pathways, such as FC gamma R-mediated phagocytosis, natural killer cell-mediated cytotoxicity, and the T cell receptor signaling pathway (Fig. 5B).

In conclusion, these findings suggest that the np-lncRNA signature, with its diverse genetic characteristics, may significantly influence various signaling pathways in HCC.

3.5. Immune analysis and related gene expression

Given that the np-lncRNA signature was correlated with multiple immune-related pathways, we further investigated these observations. We analyzed the expression of immune signature genes across different datasets. Analyses from TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL, and EPIC databases indicated that HCC samples with high-risk scores consistently exhibited increased expression of B cell signature genes and $CD4^+$ T cell signature genes, while expression of $CD8^+$ T cell signature genes and cancer-associated fibroblast signature genes was consistently decreased (Fig. 6A). Additionally, ssGSEA was used

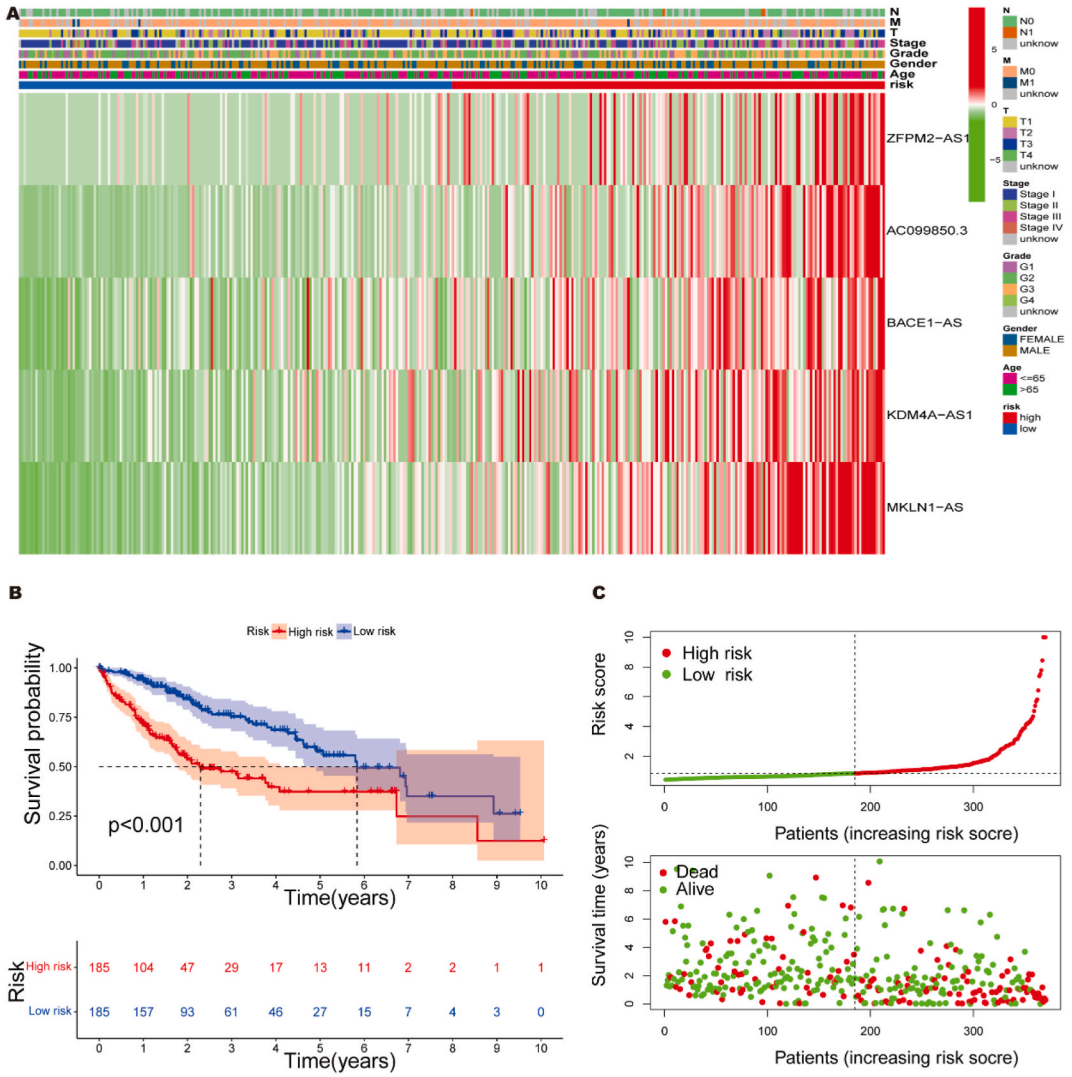

Fig. 2. Identification of np-lncRNAs signature. (A) Heatmap showing the expression levels of these lncRNAs alongside clinicopathological features.; **(B)** Kaplan-Meier curves result; **(C)** Risk survival status plot.

to explore immune status differences between the two groups, revealing that patients in the high-risk group exhibited more active class 1 MHC activity but lower cytolytic activity and type I and type II responses compared to the low-risk group (Fig. 6B). A recent study suggested that m6A methylation is significantly linked to necroptosis, as evidenced by the depletion of m6A writer METTL3, which enhanced oxaliplatin resistance in cancer patients through necroptotic cell death [44]. Therefore, we assessed the relevance of the np-lncRNA signature in m6A modification. As expected, ssGSEA analysis revealed upregulation of m6A-related factors, including METTL3, METTL14, and YTHDF1/2, in the high-risk group (Fig. 6C). These findings collectively indicate that HCC samples with high-risk and low-risk scores exhibit distinct differences in their immunological and epigenetic profiles.

3.6. Validation of the np-lncRNAs in clinical HCC tissues

We conducted a pan-cancer analysis of these lncRNAs using TCGA data, revealing that these lncRNAs were also highly expressed in various cancers, including UCEC, CHOL, and COAD. Prognostic analysis across different cancers indicated that these lncRNAs serve as independent risk factors in tumors such as PAAD, COAD, and STAD (Figs. S2 and S3). To validate these findings, we examined the expression levels of five lncRNAs in sixteen HCC samples through qPCR. The data demonstrated that all five np-lncRNAs (ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1, and MKLN1-AS) were significantly upregulated in tumor tissues ($p < 0.001$; Fig. 7A–E), aligning with the TCGA data (Fig. S2). The results of the cell experiments indicate that, in contrast to the non-tumor liver cell line L02, the expression of the remaining four np-lncRNAs, except for KDM4A-AS1, are significantly upregulated in the liver cancer cell lines Huh7 and HepG2 (Fig. 7F–J). These results substantiate the potential of the np-lncRNA signature for predicting the prognosis of HCC

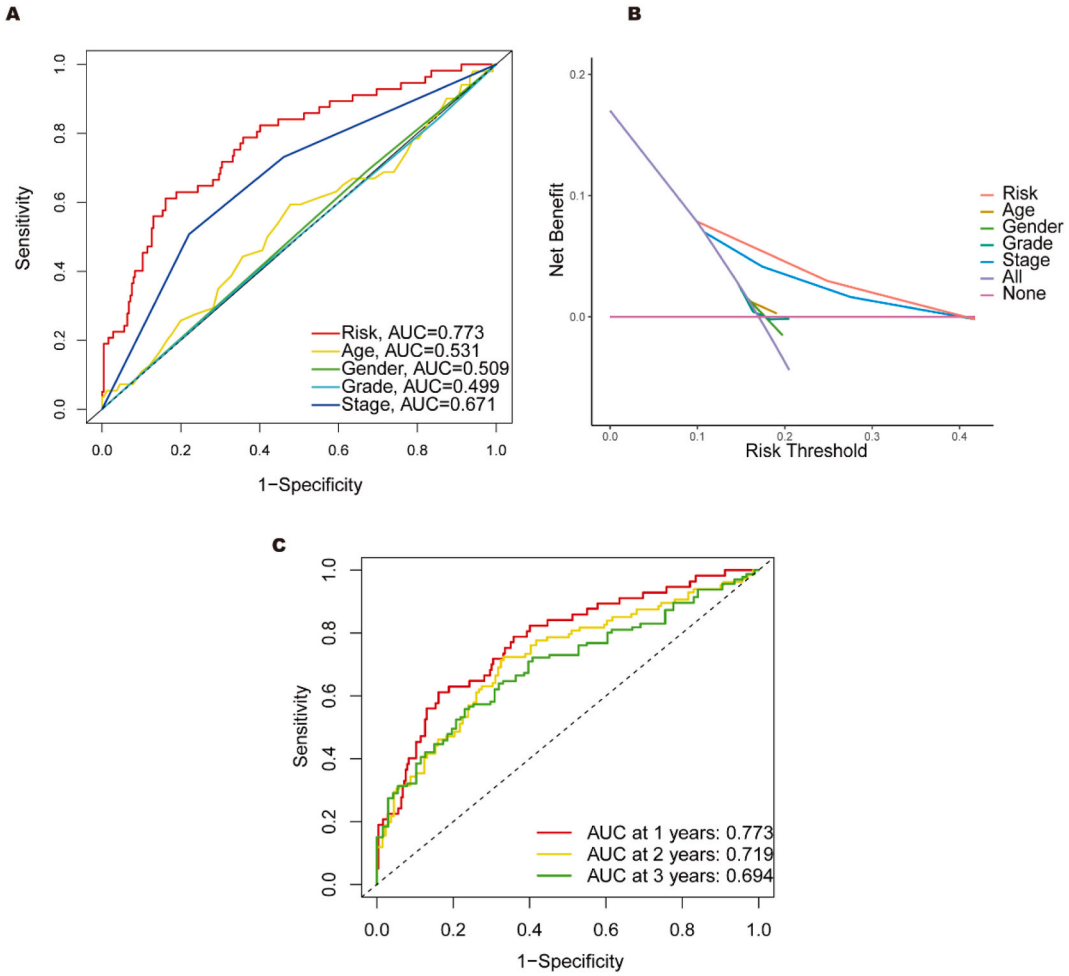

Fig. 3. Multivariate examination of the signature (A) AUC values of the risk factors; **(B)** The DCA of the risk factors; **(C)** The AUC values for the prediction of 1, 2, 3-years.

patients. Additionally, we found that compared to L02 cells, the expression level of RIPK3 was not significantly reduced in Huh7 cells, but was significantly reduced in HepG2 cells (Fig. 7K). This partially supports the hypothesis that the poor prognosis associated with high expression of these np-lncRNAs in patients may be related to their inhibition of necroptosis in HCC cells.

4. Discussion

Given the critical roles that abnormal lncRNAs play in hepatocarcinogenesis, metastasis, angiogenesis, chemoresistance, and recurrence, these molecules are increasingly seen as potential targets for the diagnosis, treatment, and monitoring of HCC[45,46,47];. Necroptosis, a unique mode of cell death first identified by Degterev et al., is characterized by the RIPK1/RIPK3-mediated phosphorylation of MLKL/p-MLKL, sharing downstream pathways with apoptosis [48,49,50]. Intriguingly, liver cancer cells have been reported to be more sensitive to necroptotic inducers compared to apoptosis inducers [9]. Additionally, recent studies suggest that tumor cells resistant to apoptosis may be more susceptible to necroptosis [51,52], highlighting the significance of necroptosis in HCC-targeted therapy. In this study, we identified five np-lncRNAs and developed a prognostic signature for predicting mortality risk in HCC patients.

Using univariate and multivariate COX analyses, we screened five differentially expressed np-lncRNAs from the TCGA-LIHC dataset. After stratifying patients into high-risk and low-risk groups based on these molecules, we found that the survival rate in the high-risk group was significantly lower than in the low-risk group. Previous research has shown that lncRNAs such as BACE1-AS, MKLN1-AS, and KDM4A-AS1 are biomarkers for HCC diagnosis and prognosis, as they contribute to the progression of hepatocellular carcinoma through the ceRNA network [34,31,53]. LncRNA ZFPM2-AS1, highly expressed in HCC tissues, promotes HCC progression by competitively binding to miR-139 along with GDF10 mRNA [22]. The co-expression network in our study indicates that lncRNA AC099850.3 plays a central role. This lncRNA has been linked to patient prognosis and is involved in HCC proliferation and invasion through the PRR11/PI3K/AKT axis [28]. Interestingly, AC099850.3 was also included in a prognostic model for HCC based on

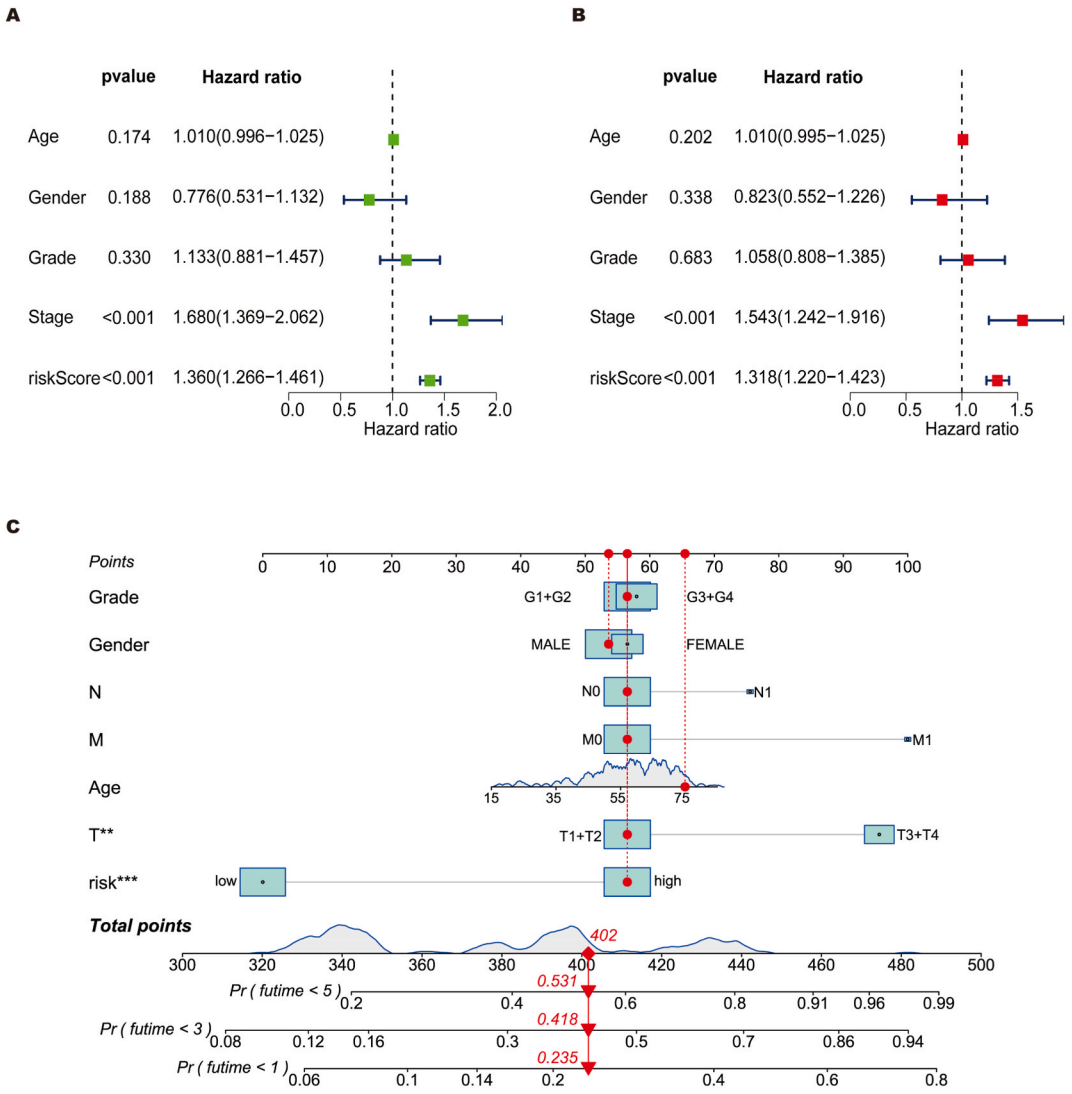

Fig. 4. COX analysis of np-lncRNAs signature and the construction of the nomogram and co-expression network. (A) Univariate COX analysis; **(B)** Multivariate COX analysis; **(C)**Nomogram developed to predict 1-, 3-, and 5-years OS.

immunoautophagy-related lncRNAs [54]. Similarly, Xu et al. constructed a prognostic model for HCC using lncRNAs like ZFPM2-AS1 and found correlations with immune responses [55]. Our findings, which show that immune-related functions like cytolytic activity and type I/II responses are downregulated in the high-risk group, are consistent with these studies. In *vitro,* vaccination with necrotic cancer cells has been shown to induce strong antitumor immunity by activating $CD8^+$ T cells through RIPK1 antigen cross-priming [56,57]. Collectively, these results support the idea that necroptosis enhances antitumor immunity.

We established a new prognostic signature based on these np-lncRNAs, achieving an AUC value of 0.773. This novel signature serves as an independent prognostic factor for HCC. The nomogram we constructed, incorporating this signature along with clinical features such as age, gender, and TNM stage, reliably predicts HCC patient prognosis. To our knowledge, previous studies have also developed prognostic signatures for HCC. For instance, Dai et al. created a prognostic signature based on necroptosis-related metabolic genes, with AUC values of 0.765 at 1 year, 0.684 at 3 years, and 0.642 at 5 years [58]. Bai et al. constructed a prognostic model using hypoxia-related genes, achieving an AUC of 0.621 at 1 year, 0.693 at 3 years, and 0.769 at 5 years [59]. Zhang also developed a prognostic signature based on RNA-binding protein genes for HCC, with an AUC value of 0.740 [60]. While other models have been developed recently [60,61], their AUC values are generally lower than our 0.773. Furthermore, our study goes beyond public database analysis by validating our findings through qPCR experiments conducted on clinical HCC patient samples, which may improve the precision of prognostic predictions for HCC patients.

In this study, GSEA revealed that the lncRNA signature is primarily involved in the mTOR, MAPK, p53, Wnt signaling pathways, and T-cell receptor signaling pathways. Investigating these pathways in conjunction with existing reports on lncRNAs may provide insights into the mechanisms we are focusing on. Hyperactivation of the mTOR signaling pathway is a key driver in HCC development.

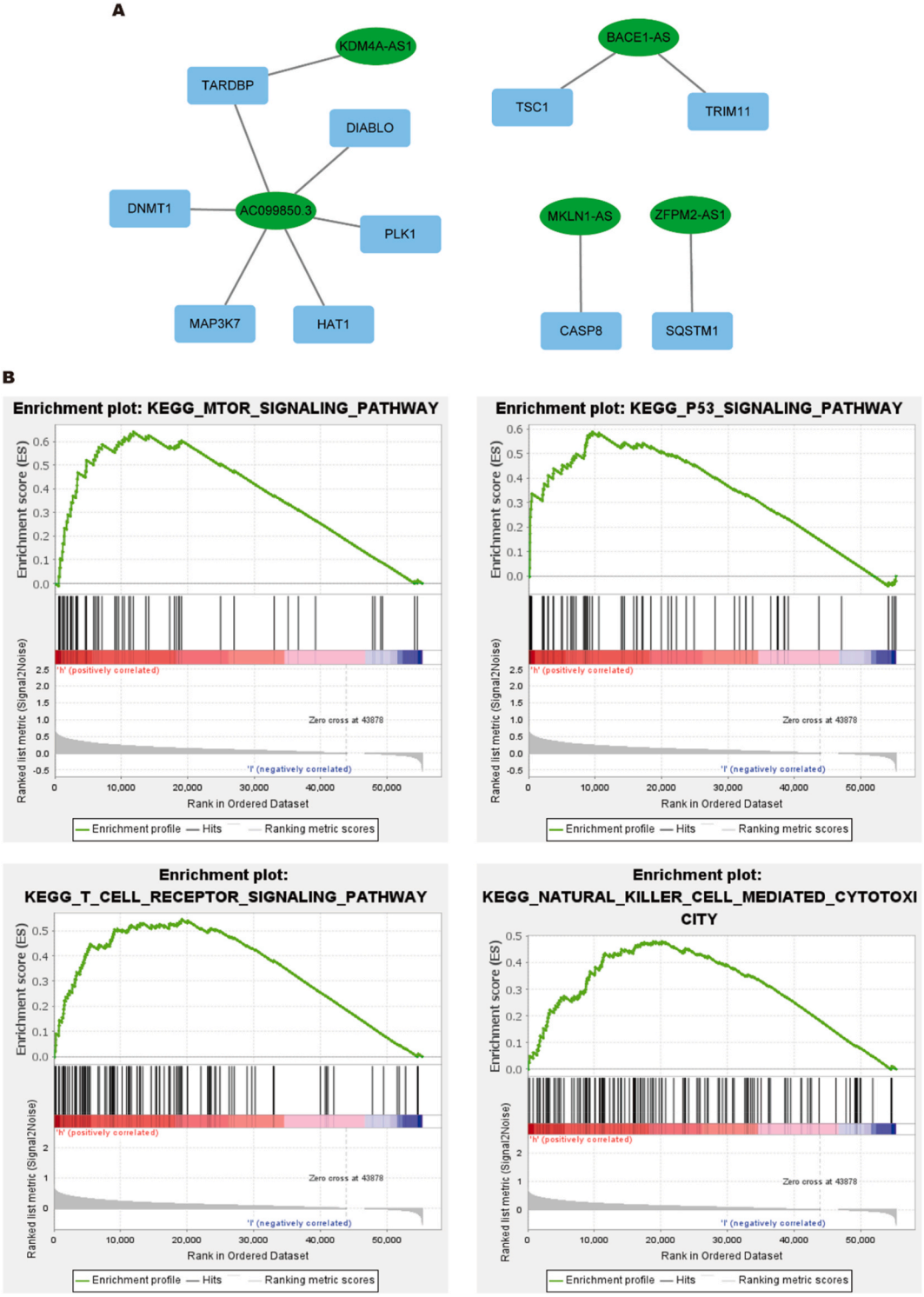

Fig. 5. Co-expression network construction and gene set enrichment analysis for np-lncRNAs. (A) Constructed a co-expression network of lncRNAs and necroptosis-related genes. DNA methyltransferase 1 (DNMT1), histone acetyltransferase 1 (HAT1), mitogen-activated protein kinase kinase kinase 7 (MAP3K7),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

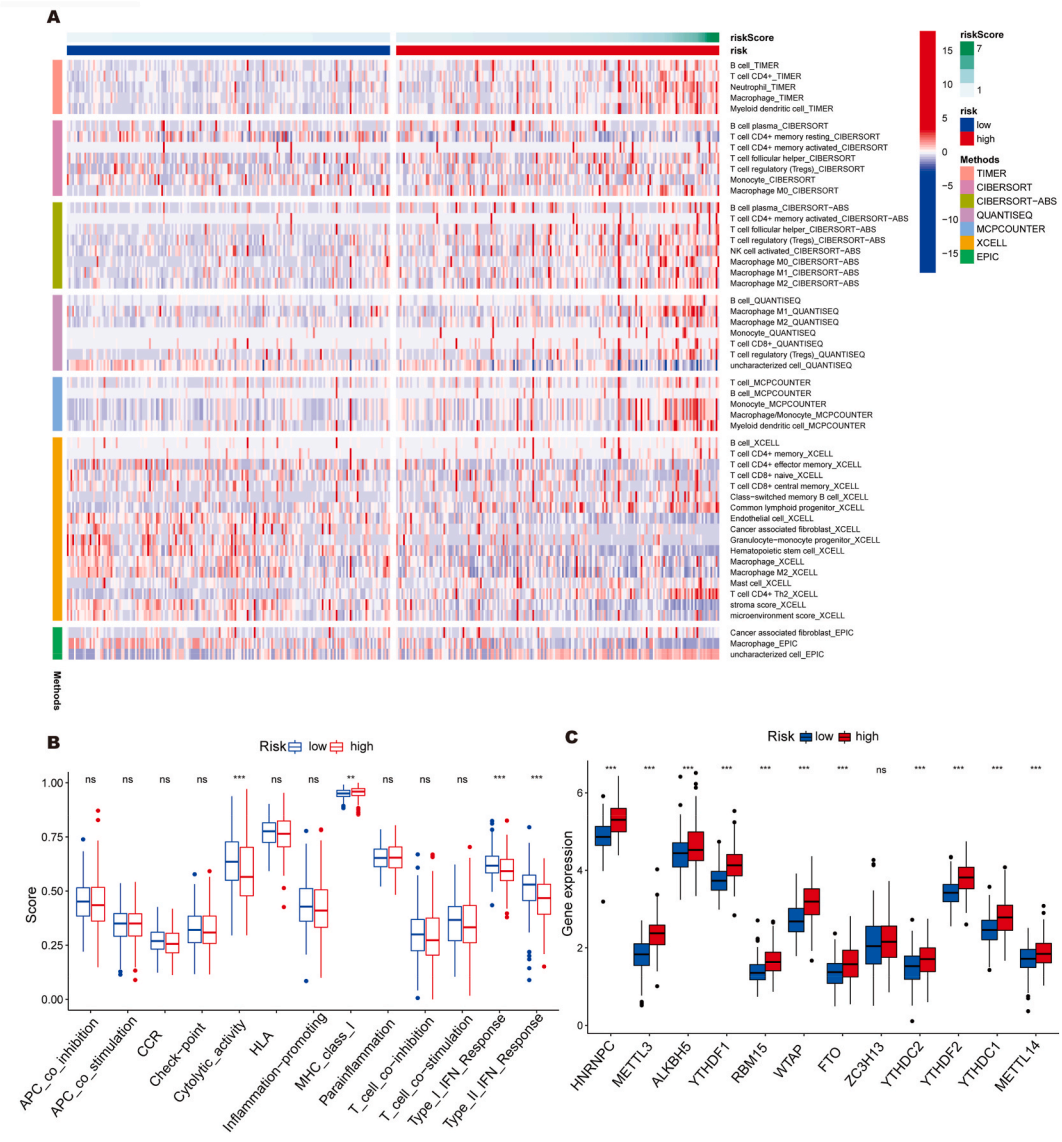

Fig. 6. Heatmap for immune responses and the expression of m6A-related genes between the two groups. (A) Heatmap for immune responses; **(B)** ssGSEA reveals the difference in immune cells and immune functions between the two groups; **(C)** The expression of m6A-related genes.

Inhibitors of this pathway effectively block aberrant signaling from various growth factors, thereby inhibiting HCC progression [62]. Some lncRNAs can enhance the mTOR signaling pathway, promoting HCC proliferation and migration by increasing the expression of MMPs and EMT [63]. Moreover, some lncRNAs modulate HCC cell apoptosis and autophagy by sponging microRNAs that regulate the mTOR signaling pathway [64]. Recently, third-generation mTOR inhibitors have been developed by scientists. The MAPK pathway plays a pivotal role in tumor progression and immunotherapy. Activation of the MAPK pathway has been reported to induce necroptosis of microvascular endothelial cells, promoting HCC metastasis [65]. Certain lncRNAs can enhance HCC radioresistance by modulating the MAPK pathway [66]. Additionally, some lncRNAs promote HCC progression by inducing mitochondrial fission and glycolysis in liver cancer cells through the activation of the MAPK pathway [67,68]. p53, the tumor suppressor gene most commonly associated with human cancers, loses its function in over a third of HCC cases. Recent studies have shown that combining p53 mRNA nanoparticles with immune checkpoint blockade (ICB) significantly enhances the antitumor immune response in liver cancer [69]. Some lncRNAs inhibit HCC cell growth and metastasis by activating the p53 signaling pathway, while others promote HCC tumorigenesis and progression by inactivating p53 signaling [70,71]. The development of potential drugs that inhibit the Wnt/β-catenin signaling pathway, such as small molecule inhibitors, traditional Chinese medicine extracts, and miRNAs, offers new possibilities for improving HCC therapy [72–74]. In neoplastic diseases, T-cell receptor signaling pathways play a critical role in T-cell activation and function. In HCC, persistent T-cell activation leads to T-cell exhaustion. Certain lncRNAs promote T-cell exhaustion, impairing anti-tumor immunity, such as lncRNA Lnc-Tim3 [75]. Additionally, some lncRNAs facilitate HCC immune evasion by promoting

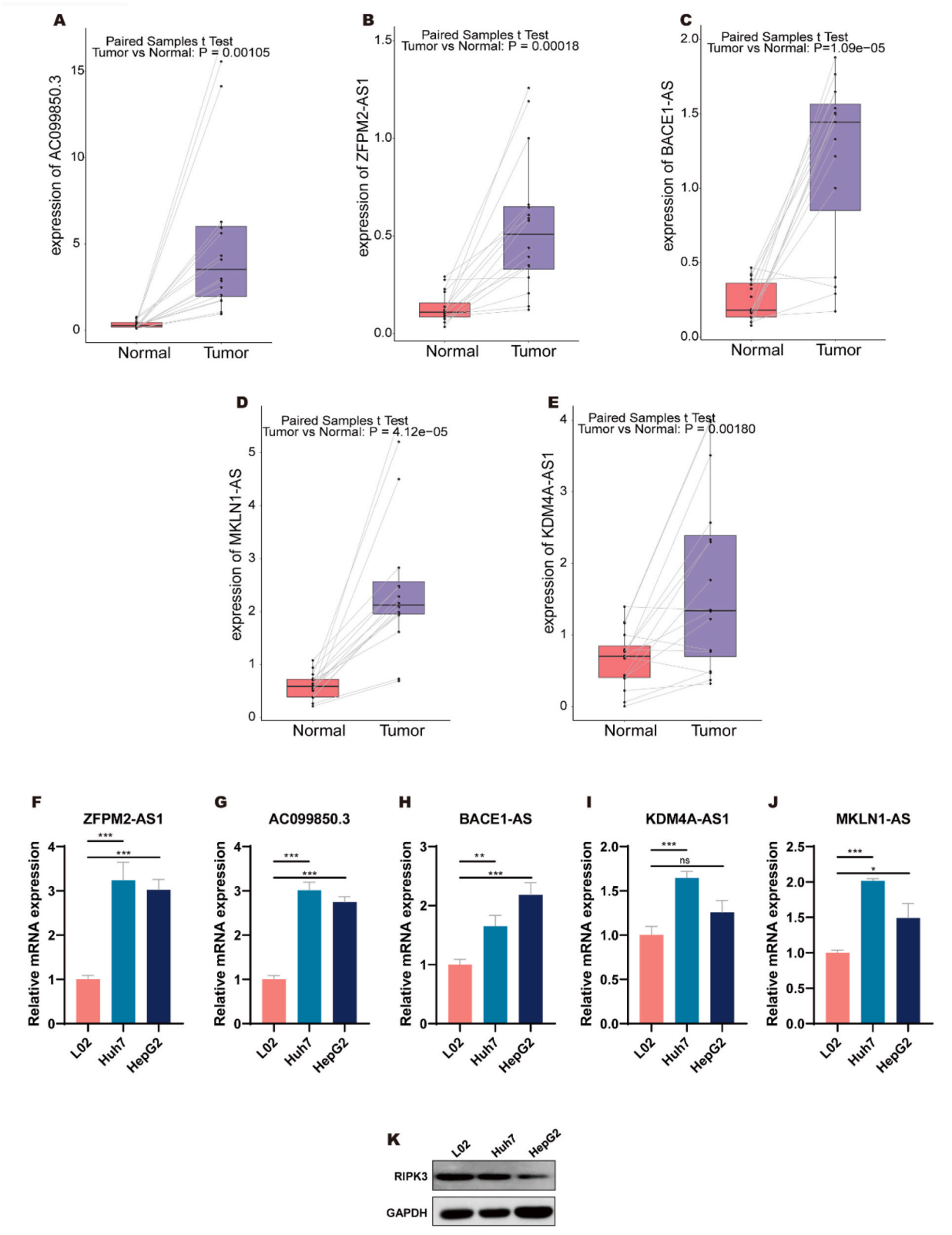

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Fig. 7. Validation of the np-lncRNAs in clinical HCC tissues. (A–E) qRT-PCR result of expression level of ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS in HCC tissues compared with paired paratumor tissues. **(F–J)** qRT-PCR result of expression level of ZFPM2-AS1, AC099850.3, BACE1-AS, KDM4A-AS1 and MKLN1-AS in L02, Huh7 and HepG2. (**K**) Western blot of expression level of necroptosis-related protein RIPK3 in L02, Huh7 and HepG2.

regulatory T cell differentiation, such as lnc-EGFR [76].This study illustrated that mTOR, MAPK, p53, Wnt signaling pathways, and T-cell receptor signaling pathways were all enriched in the high risk group, indicating that our new signature is supported by previous findings. Furthermore, given the growing interest in RNA m6A modification in HCC, we assessed the relevance of the np-lncRNA signature in m6A modification. As anticipated, m6A-related factors like METTL3, METTL14, and YTHDF1/2 were upregulated in the high-risk group, suggesting a significant correlation between m6A methylation and necroptosis. The specific relationship between necroptosis and m6A in HCC warrants further exploration.

5. Conclusion

This study identified five lncRNAs associated with necroptosis and developed a novel np-lncRNA signature with significant predictive value for assessing the prognosis of HCC patients.

Ethical approval statement

Sixteen pairs of HCC tumor and adjacent non-tumor tissues were obtained from West China Hospital, Sichuan University. The study protocols were approved by the Biomedical Ethics Review Committee of West China Hospital, Sichuan University (Ethics Approval Number: 2022-221, Approval date: 2022.02.25). All patients provided written informed consent prior to participation.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Hao Chen: Writing – original draft. **Guimin Hou:** Resources, Methodology. **Tian Lan:** Methodology, Conceptualization. **Shuai Xue:** Visualization. **Lin Xu:** Methodology. **Qingbo Feng:** Software. **Yong Zeng:** Funding acquisition, Conceptualization. **Haichuan Wang:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37403.

References

[1] S. Tohme, H.O. Yazdani, A. Rahman, S. Handu, S. Khan, T. Wilson, D.A. Geller, R.L. Simmons, M. Molinari, C. Kaltenmeier, The use of machine learning to create a risk score to predict survival in patients with hepatocellular carcinoma: a TCGA cohort analysis, Can J Gastroenterol Hepatol 2021 (2021) 5212953.
[2] M. Sayiner, P. Golabi, Z.M. Younossi, Disease burden of hepatocellular carcinoma: a global perspective, Dig. Dis. Sci. 64 (2019) 910–917.
[3] N. Fujiwara, S.L. Friedman, N. Goossens, Y. Hoshida, Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine, J. Hepatol. 68 (2018) 526–549.
[4] S. Famularo, S. Di Sandro, A. Giani, A. Lauterio, M. Sandini, R. De Carlis, V. Buscemi, F. Uggeri, F. Romano, L. Gianotti, L. De Carlis, Recurrence patterns after anatomic or parenchyma-sparing liver resection for hepatocarcinoma in a western population of cirrhotic patients, Ann. Surg Oncol. 25 (2018) 3974–3981.
[5] A.G. Snyder, N.W. Hubbard, M.N. Messmer, S.B. Kofman, C.E. Hagan, S.L. Orozco, K. Chiang, B.P. Daniels, D. Baker, A. Oberst, Intratumoral activation of the necroptotic pathway components RIPK1 and RIPK3 potentiates antitumor immunity, Sci Immunol 4 (2019).
[6] T. Tenev, K. Bianchi, M. Darding, M. Broemer, C. Langlais, F. Wallberg, A. Zachariou, J. Lopez, M. MacFarlane, K. Cain, P. Meier, The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs, Mol Cell 43 (2011) 432–448.
[7] M.F. Brown, B.J. Leibowitz, D. Chen, K. He, F. Zou, R.W. Sobol, D. Beer-Stolz, L. Zhang, J. Yu, Loss of caspase-3 sensitizes colon cancer cells to genotoxic stress via RIP1-dependent necrosis, Cell Death Dis. 6 (2015) e1729.
[8] Y. Xu, H.B. Ma, Y.L. Fang, Z.R. Zhang, J. Shao, M. Hong, C.J. Huang, J. Liu, R.Q. Chen, Cisplatin-induced necroptosis in TNFalpha dependent and independent pathways, Cell. Signal. 31 (2017) 112–123.
[9] Y.K. Xiang, F.H. Peng, Y.Q. Guo, H. Ge, S.Y. Cai, L.X. Fan, Y.X. Peng, H. Wen, Q. Wang, L. Tao, Connexin32 activates necroptosis through Src-mediated inhibition of caspase 8 in hepatocellular carcinoma, Cancer Sci. 112 (2021) 3507–3519.
[10] Y. Xiang, Q. Wang, Y. Guo, H. Ge, Y. Fu, X. Wang, L. Tao, Cx32 exerts anti-apoptotic and pro-tumor effects via the epidermal growth factor receptor pathway in hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 38 (2019) 145.
[11] H. Bao, H. Su, Long noncoding RNAs act as novel biomarkers for hepatocellular carcinoma: progress and prospects, BioMed Res. Int. 2017 (2017) 6049480.
[12] H. Mai, B. Zhou, L. Liu, F. Yang, C. Conran, Y. Ji, J. Hou, D. Jiang, Molecular pattern of lncRNAs in hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 38 (2019) 198.
[13] J.H. Yuan, X.N. Liu, T.T. Wang, W. Pan, Q.F. Tao, W.P. Zhou, F. Wang, S.H. Sun, The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of lncRNA-PXN-AS1, Nat. Cell Biol. 19 (2017) 820–832.
[14] A. Rojas, A. Gil-Gomez, P. de la Cruz-Ojeda, R. Munoz-Hernandez, Y. Sanchez-Torrijos, R. Gallego-Duran, R. Millan, M.C. Rico, R. Montero-Vallejo, S. Gato-Zambrano, D. Maya-Miles, M.T. Ferrer, J. Muntane, M.J. Robles-Frias, J. Ampuero, F.J. Padillo, M. Romero-Gomez, Long non-coding RNA H19 as a biomarker for hepatocellular carcinoma, Liver Int. 42 (2022) 1410–1422.
[15] T. Luo, Y. Gao, G. Zhangyuan, X. Xu, C. Xue, L. Jin, W. Zhang, C. Zhu, B. Sun, X. Qin, lncRNA PCBP1-AS1 aggravates the progression of hepatocellular carcinoma via regulating PCBP1/PRL-3/AKT pathway, Cancer Manag. Res. 12 (2020) 5395–5408.
[16] C. Tan, J. Cao, L. Chen, X. Xi, S. Wang, Y. Zhu, L. Yang, L. Ma, D. Wang, J. Yin, T. Zhang, Z. John Lu, Noncoding RNAs serve as diagnosis and prognosis biomarkers for hepatocellular carcinoma, Clin. Chem. 65 (2019) 905–915.
[17] N. Wang, D. Liu, Identification and validation a necroptosisrelated prognostic signature and associated regulatory Axis in stomach adenocarcinoma, OncoTargets Ther. 14 (2021) 5373–5383.
[18] F. Chen, J. Yang, M. Fang, Y. Wu, D. Su, Y. Sheng, Necroptosis-related lncRNA to establish novel prognostic signature and predict the immunotherapy response in breast cancer, J. Clin. Lab. Anal. 36 (2022) e24302.
[19] Y. Lu, X. Luo, Q. Wang, J. Chen, X. Zhang, Y. Li, Y. Chen, X. Li, S. Han, A novel necroptosis-related lncRNA signature predicts the prognosis of lung adenocarcinoma, Front. Genet. 13 (2022) 862741.
[20] Z. Zhao, H. Liu, X. Zhou, D. Fang, X. Ou, J. Ye, J. Peng, J. Xu, Necroptosis-related lncRNAs: predicting prognosis and the distinction between the cold and hot tumors in gastric cancer, J Oncol 2021 (2021) 6718443.
[21] A.M. Newman, C.L. Liu, M.R. Green, A.J. Gentles, W. Feng, Y. Xu, C.D. Hoang, M. Diehn, A.A. Alizadeh, Robust enumeration of cell subsets from tissue expression profiles, Nat. Methods 12 (2015) 453–457.
[22] H. He, Y. Wang, P. Ye, D. Yi, Y. Cheng, H. Tang, Z. Zhu, X. Wang, S. Jin, Long noncoding RNA ZFPM2-AS1 acts as a miRNA sponge and promotes cell invasion through regulation of miR-139/GDF10 in hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 39 (2020) 159.
[23] X.W. Zhang, Q.H. Li, Z.D. Xu, J.J. Dou, STAT1-induced regulation of lncRNA ZFPM2-AS1 predicts poor prognosis and contributes to hepatocellular carcinoma progression via the miR-653/GOLM1 axis, Cell Death Dis. 12 (2021) 31.
[24] Y.F. Zhao, L. Li, H.J. Li, F.R. Yang, Z.K. Liu, X.W. Hu, Q. Wang, LncRNA ZFPM2-AS1 aggravates the malignant development of breast cancer via upregulating JMJD6, Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 11139–11147.
[25] M. Xiao, Z. Liang, Z. Yin, Long non-coding RNA ZFPM2-AS1 promotes colorectal cancer progression by sponging miR-137 to regulate TRIM24, Mol. Med. Rep. 23 (2021).
[26] X. Wang, J. Tang, J. Zhao, B. Lou, L. Li, ZFPM2-AS1 promotes the proliferation, migration, and invasion of human non-small cell lung cancer cells involving the JAK-STAT and AKT pathways, PeerJ 8 (2020) e10225.
[27] Z. Yan, Q. Yang, M. Xue, S. Wang, W. Hong, X. Gao, YY1-induced lncRNA ZFPM2-AS1 facilitates cell proliferation and invasion in small cell lung cancer via upregulating of TRAF4, Cancer Cell Int. 20 (2020) 108.
[28] F. Zhong, S. Liu, D. Hu, L. Chen, LncRNA AC099850.3 promotes hepatocellular carcinoma proliferation and invasion through PRR11/PI3K/AKT axis and is associated with patients prognosis, J. Cancer 13 (2022) 1048–1060.
[29] Q. Wang, Q. Fang, Y. Huang, J. Zhou, M. Liu, Identification of a novel prognostic signature for HCC and analysis of costimulatory molecule-related lncRNA AC099850.3, Sci. Rep. 12 (2022) 9954.
[30] X. Chen, J. Guo, F. Zhou, W. Ren, J. Pu, L. Mutti, X. Niu, X. Jiang, Over-expression of long non-coding RNA-ac099850.3 correlates with tumor progression and poor prognosis in lung adenocarcinoma, Front. Oncol. 12 (2022) 895708.
[31] C. Liu, H. Wang, L. Tang, H. Huang, M. Xu, Y. Lin, L. Zhou, L. Ho, J. Lu, X. Ai, LncRNA BACE1-AS enhances the invasive and metastatic capacity of hepatocellular carcinoma cells through mediating miR-377-3p/CELF1 axis, Life Sci. 275 (2021) 119288.
[32] Q. Tian, X. Yan, L. Yang, Z. Liu, Z. Yuan, Y. Zhang, Long non-coding RNA BACE1-AS plays an oncogenic role in hepatocellular carcinoma cells through miR-214-3p/APLN axis, Acta Biochim. Biophys. Sin. 53 (2021) 1538–1546.
[33] X. Wang, Y. Liu, M. Zhou, L. Yu, Z. Si, m6A modified BACE1-AS contributes to liver metastasis and stemness-like properties in colorectal cancer through TUFT1 dependent activation of Wnt signaling, J. Exp. Clin. Cancer Res. 42 (2023) 306.
[34] T. Chen, R. Liu, Y. Niu, H. Mo, H. Wang, Y. Lu, L. Wang, L. Sun, Y. Wang, K. Tu, Q. Liu, HIF-1alpha-activated long non-coding RNA KDM4A-AS1 promotes hepatocellular carcinoma progression via the miR-411-5p/KPNA2/AKT pathway, Cell Death Dis. 12 (2021) 1152.
[35] H.M. Shen, D. Zhang, P. Xiao, B. Qu, Y.F. Sun, E2F1-mediated KDM4A-AS1 up-regulation promotes EMT of hepatocellular carcinoma cells by recruiting ILF3 to stabilize AURKA mRNA, Cancer Gene Ther. 30 (2023) 1007–1017.
[36] W. Cao, Y. Ren, Y. Liu, G. Cao, Z. Chen, F. Wang, KDM4A-AS1 promotes cell proliferation, migration, and invasion via the miR-4306/STX6 Axis in hepatocellular carcinoma, Crit. Rev. Eukaryot. Gene Expr. 34 (2024) 55–68.
[37] B. Zhou, Y. Wu, P. Cheng, C. Wu, Long noncoding RNAs with peptide-encoding potential identified in esophageal squamous cell carcinoma: KDM4A-AS1-encoded peptide weakens cancer cell viability and migratory capacity, Mol. Oncol. 17 (2023) 1419–1436.
[38] X. Chen, Q. Ye, Z. Chen, Q. Lin, W. Chen, C. Xie, X. Wang, Long non-coding RNA muskelin 1 antisense RNA as a potential therapeutic target in hepatocellular carcinoma treatment, Bioengineered 13 (2022) 12237–12247.
[39] G. Pan, J. Zhang, F. You, T. Cui, P. Luo, S. Wang, X. Li, Q. Yuan, ETS Proto-Oncogene 1-activated muskelin 1 antisense RNA drives the malignant progression of hepatocellular carcinoma by targeting miR-22-3p to upregulate ETS Proto-Oncogene 1, Bioengineered 13 (2022) 1346–1358.

[40] W. Gao, X. Chen, W. Chi, M. Xue, Long non-coding RNA MKLN1-AS aggravates hepatocellular carcinoma progression by functioning as a molecular sponge for miR-654-3p, thereby promoting hepatoma-derived growth factor expression, Int. J. Mol. Med. 46 (2020) 1743–1754.
[41] J. Chen, L. Li, Y. Feng, Y. Zhao, F. Sun, X. Zhou, D. Yiqi, Z. Li, F. Kong, X. Kong, MKLN1-AS promotes pancreatic cancer progression as a crucial downstream mediator of HIF-1alpha through miR-185-5p/TEAD1 pathway, Cell Biol. Toxicol. 40 (2024) 30.
[42] M. Omata, A.L. Cheng, N. Kokudo, M. Kudo, J.M. Lee, J. Jia, R. Tateishi, K.H. Han, Y.K. Chawla, S. Shiina, W. Jafri, D.A. Payawal, T. Ohki, S. Ogasawara, P. J. Chen, C.R.A. Lesmana, L.A. Lesmana, R.A. Gani, S. Obi, A.K. Dokmeci, S.K. Sarin, Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update, Hepatol Int 11 (2017) 317–370.
[43] M.A. El-Maksoud, M.R. Habeeb, H.F. Ghazy, M.M. Nomir, H. Elalfy, S. Abed, M.E.S. Zaki, Clinicopathological study of occult hepatitis B virus infection in hepatitis C virus-associated hepatocellular carcinoma, Eur. J. Gastroenterol. Hepatol. 31 (2019) 716–722.
[44] H. Lan, Y. Liu, J. Liu, X. Wang, Z. Guan, J. Du, K. Jin, Tumor-associated macrophages promote oxaliplatin resistance via METTL3-mediated m(6)A of TRAF5 and necroptosis in colorectal cancer, Mol. Pharm. 18 (2021) 1026–1037.
[45] C. Yan, S. Wei, D. Han, L. Wu, L. Tan, H. Wang, Y. Dong, J. Hua, W. Yang, LncRNA HULC shRNA disinhibits miR-377-5p to suppress the growth and invasion of hepatocellular carcinoma in vitro and hepatocarcinogenesis in vivo, Ann. Transl. Med. 8 (2020) 1294.
[46] C.N. Ma, L.L. Wo, D.F. Wang, C.X. Zhou, J.C. Li, X. Zhang, X.F. Gong, C.L. Wang, M. He, Q. Zhao, Hypoxia activated long non-coding RNA HABON regulates the growth and proliferation of hepatocarcinoma cells by binding to and antagonizing HIF-1 alpha, RNA Biol. 18 (2021) 1791–1806.
[47] X. Tang, W. Zhang, Y. Ye, H. Li, L. Cheng, M. Zhang, S. Zheng, J. Yu, LncRNA HOTAIR contributes to sorafenib resistance through suppressing miR-217 in hepatic carcinoma, BioMed Res. Int. 2020 (2020) 9515071.
[48] K.D. Marshall, C.P. Baines, Necroptosis: is there a role for mitochondria? Front. Physiol. 5 (2014) 323.
[49] W. Declercq, T. Vanden Berghe, P. Vandenabeele, RIP kinases at the crossroads of cell death and survival, Cell 138 (2009) 229–232.
[50] A. Degterev, Z. Huang, M. Boyce, Y. Li, P. Jagtap, N. Mizushima, G.D. Cuny, T.J. Mitchison, M.A. Moskowitz, J. Yuan, Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury, Nat. Chem. Biol. 1 (2005) 112–119.
[51] Z. Su, Z. Yang, L. Xie, J.P. DeWitt, Y. Chen, Cancer therapy in the necroptosis era, Cell Death Differ. 23 (2016) 748–756.
[52] G.W. He, C. Gunther, V. Thonn, Y.Q. Yu, E. Martini, B. Buchen, M.F. Neurath, M. Sturzl, C. Becker, Regression of apoptosis-resistant colorectal tumors by induction of necroptosis in mice, J. Exp. Med. 214 (2017) 1655–1662.
[53] C. Guo, S. Zhou, W. Yi, P. Yang, O. Li, J. Liu, C. Peng, Long non-coding RNA muskelin 1 antisense RNA (MKLN1-AS) is a potential diagnostic and prognostic biomarker and therapeutic target for hepatocellular carcinoma, Exp. Mol. Pathol. 120 (2021) 104638.
[54] Y. Wang, F. Ge, A. Sharma, O. Rudan, M.F. Setiawan, M.A. Gonzalez-Carmona, M.T. Kornek, C.P. Strassburg, M. Schmid, I.G.H. Schmidt-Wolf, Immunoautophagy-related long noncoding RNA (IAR-lncRNA) signature predicts survival in hepatocellular carcinoma, Biology 10 (2021).
[55] Z. Xu, B. Peng, Q. Liang, X. Chen, Y. Cai, S. Zeng, K. Gao, X. Wang, Q. Yi, Z. Gong, Y. Yan, Construction of a ferroptosis-related nine-lncRNA signature for predicting prognosis and immune response in hepatocellular carcinoma, Front. Immunol. 12 (2021) 719175.
[56] N. Yatim, H. Jusforgues-Saklani, S. Orozco, O. Schulz, R. Barreira da Silva, C. Reis e Sousa, D.R. Green, A. Oberst, M.L. Albert, RIPK1 and NF-kappaB signaling in dying cells determines cross-priming of CD8(+) T cells, Science 350 (2015) 328–334.
[57] T.L. Aaes, A. Kaczmarek, T. Delvaeye, B. De Craene, S. De Koker, L. Heyndrickx, I. Delrue, J. Taminau, B. Wiernicki, P. De Groote, A.D. Garg, L. Leybaert, J. Grooten, M.J. Bertrand, P. Agostinis, G. Berx, W. Declercq, P. Vandenabeele, D.V. Krysko, Vaccination with necroptotic cancer cells induces efficient anti-tumor immunity, Cell Rep. 15 (2016) 274–287.
[58] T. Dai, J. Li, X. Lu, L. Ye, H. Yu, L. Zhang, M. Deng, S. Zhu, W. Liu, G. Wang, Y. Yang, Prognostic role and potential mechanisms of the ferroptosis-related metabolic gene signature in hepatocellular carcinoma, Pharmgenomics Pers Med 14 (2021) 927–945.
[59] Y. Bai, W. Qi, L. Liu, J. Zhang, L. Pang, T. Gan, P. Wang, C. Wang, H. Chen, Identification of seven-gene hypoxia signature for predicting overall survival of hepatocellular carcinoma, Front. Genet. 12 (2021) 637418.
[60] H. Zhang, P. Xia, W. Ma, Y. Yuan, Development and validation of an RNA binding protein-associated prognostic model for hepatocellular carcinoma, J Clin Transl Hepatol 9 (2021) 635–646.
[61] D. Tian, Y. Yu, L. Zhang, J. Sun, W. Jiang, A five-gene-based prognostic signature for hepatocellular carcinoma, Front. Med. 8 (2021) 681388.
[62] Y. Chen, X. Zhou, Research progress of mTOR inhibitors, Eur. J. Med. Chem. 208 (2020) 112820.
[63] X. Wu, S. Wang, X. Wu, Q. Chen, J. Cheng, Z. Qi, Analysis of m(6)A-related lncRNAs for prognostic and immunotherapeutic response in hepatocellular carcinoma, J. Cancer 15 (2024) 2045–2065.
[64] N. Peng, J. He, J. Li, H. Huang, W. Huang, Y. Liao, S. Zhu, Long noncoding RNA MALAT1 inhibits the apoptosis and autophagy of hepatocellular carcinoma cell by targeting the microRNA-146a/PI3K/Akt/mTOR axis, Cancer Cell Int. 20 (2020) 165.
[65] X. Chen, B. Cheng, D. Dai, Y. Wu, Z. Feng, C. Tong, X. Wang, J. Zhao, Heparanase induces necroptosis of microvascular endothelial cells to promote the metastasis of hepatocellular carcinoma, Cell Death Discov 7 (2021) 33.
[66] Z. Wang, X. Wang, Z. Rong, L. Dai, C. Qin, S. Wang, W. Geng, LncRNA LINC01134 contributes to radioresistance in hepatocellular carcinoma by regulating DNA damage response via MAPK signaling pathway, Front. Pharmacol. 12 (2021) 791889.
[67] T. Yi, H. Luo, F. Qin, Q. Jiang, S. He, T. Wang, J. Su, S. Song, X. Qin, Y. Qin, X. Zhou, Z. Huang, LncRNA LL22NC03-N14H11.1 promoted hepatocellular carcinoma progression through activating MAPK pathway to induce mitochondrial fission, Cell Death Dis. 11 (2020) 832.
[68] H. He, T. Chen, H. Mo, S. Chen, Q. Liu, C. Guo, Hypoxia-inducible long noncoding RNA NPSR1-AS1 promotes the proliferation and glycolysis of hepatocellular carcinoma cells by regulating the MAPK/ERK pathway, Biochem. Biophys. Res. Commun. 533 (2020) 886–892.
[69] Y. Xiao, J. Chen, H. Zhou, X. Zeng, Z. Ruan, Z. Pu, X. Jiang, A. Matsui, L. Zhu, Z. Amoozgar, D.S. Chen, X. Han, D.G. Duda, J. Shi, Combining p53 mRNA nanotherapy with immune checkpoint blockade reprograms the immune microenvironment for effective cancer therapy, Nat. Commun. 13 (2022) 758.
[70] P. Liu, Q. Zhong, Y. Song, D. Guo, D. Ma, B. Chen, J. Lan, Q. Liu, Long noncoding RNA Linc01612 represses hepatocellular carcinoma progression by regulating miR-494/ATF3/p53 axis and promoting ubiquitination of YBX1, Int. J. Biol. Sci. 18 (2022) 2932–2948.
[71] G. Tang, H. Zhao, Z. Xie, S. Wei, G. Chen, Long non-coding RNA HAGLROS facilitates tumorigenesis and progression in hepatocellular carcinoma by sponging miR-26b-5p to up-regulate karyopherin alpha2 (KPNA2) and inactivate p53 signaling, Bioengineered 13 (2022) 7829–7846.
[72] R. Galuppo, E. Maynard, M. Shah, M.F. Daily, C. Chen, B.T. Spear, R. Gedaly, Synergistic inhibition of HCC and liver cancer stem cell proliferation by targeting RAS/RAF/MAPK and WNT/beta-catenin pathways, Anticancer Res. 34 (2014) 1709–1713.
[73] Z. Guo, Y. Zhou, J. Yang, X. Shao, Dendrobium candidum extract inhibits proliferation and induces apoptosis of liver cancer cells by inactivating Wnt/beta-catenin signaling pathway, Biomed. Pharmacother. 110 (2019) 371–379.
[74] K. Jin, H. Chen, Q. Zuo, C. Huang, R. Zhao, X. Yu, Y. Wang, Y. Zhang, Z. Chang, B. Li, CREPT and p15RS regulate cell proliferation and cycling in chicken DF-1 cells through the Wnt/beta-catenin pathway, J. Cell. Biochem. 119 (2018) 1083–1092.
[75] J. Ji, Y. Yin, H. Ju, X. Xu, W. Liu, Q. Fu, J. Hu, X. Zhang, B. Sun, Long non-coding RNA Lnc-Tim3 exacerbates CD8 T cell exhaustion via binding to Tim-3 and inducing nuclear translocation of Bat3 in HCC, Cell Death Dis. 9 (2018) 478.
[76] R. Jiang, J. Tang, Y. Chen, L. Deng, J. Ji, Y. Xie, K. Wang, W. Jia, W.M. Chu, B. Sun, The long noncoding RNA lnc-EGFR stimulates T-regulatory cells differentiation thus promoting hepatocellular carcinoma immune evasion, Nat. Commun. 8 (2017) 15129.