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ceRNA network development and tumor-infiltrating immune cell analysis in hepatocellular carcinoma

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Abstract
Hepatocellular carcinoma (HCC) is among the primary causes of cancer deaths globally. Despite efforts to understand liver cancer, its high morbidity and mortality remain high. Herein, we constructed two nomograms based on competing endogenous RNA (ceRNA) networks and invading immune cells to describe the molecular mechanisms along with the clinical prognosis of HCC patients. RNA maps of tumors and normal samples were downloaded from The Cancer Genome Atlas database. HTseq counts and fragments per megaparss per thousand bases were read from 421 samples, including 371 tumor samples and 50 normal samples. We established a ceRNA network based on differential gene expression in normal versus tumor subjects. CIBERSORT was employed to differentiate 22 immune cell types according to tumor transcriptomes. Kaplan–Meier along with Cox proportional hazard analyses were employed to determine the prognosis-linked factors. Nomograms were constructed based on prognostic immune cells and ceRNAs. We employed Receiver operating characteristic (ROC) and calibration curve analyses to estimate these nomogram. The difference analysis found 2028 messenger RNAs (mRNAs), 128 micro RNAs (miRNAs), and 136 long non-coding RNAs (lncRNAs) to be significantly differentially expressed in tumor samples relative to normal samples. We set up a ceRNA network containing 21 protein-coding mRNAs, 12 miRNAs, and 3 lncRNAs. In Kaplan–Meier analysis, 21 of the 36 ceRNAs were considered significant. Of the 22 cell types, resting dendritic cell levels were markedly different in tumor samples versus normal controls. Calibration and ROC curve analysis of the ceRNA network, as well as immune infiltration of tumor showed restful accuracy (3-year survival area under curve (AUC): 0.691, 5-year survival AUC: 0.700; 3-year survival AUC: 0.674, 5-year survival AUC: 0.694). Our data suggest that Tregs, CD4 T cells, mast cells, SNHG1, HMMR and hsa-miR-421 are associated with HCC based on ceRNA immune cells co-expression patterns. On the basis of ceRNA network modeling and immune cell infiltration analysis, our study offers an effective bioinformatics strategy for studying HCC molecular mechanisms and prognosis.

Keywords HCC · ceRNA network · Immune infiltration · Prognosis · Nomogram

Li Chen, Weijie Zou and Lei Zhang contributed equally to this study.

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Introduction

Hepatocellular carcinoma (HCC) is the 6th most frequent cancer globally and is linked to rising morbidity and mortality [1]. Despite advances in HCC treatment, including chemotherapy, targeted therapy, radiotherapy, Iodine125 seed implantation, transcatheter arterial chemoembolization (TACE), radiofrequency ablation, and immunotherapy, its overall 5-year survival has increased by 1–3% only and HCC relapse rate may reach 70% in the 5 years. Remarkably, a median survival of 7.1 months has been reported in advanced liver cancer patients without treatment [2]. Liver cancer is a major clinical challenge globally. Thus, effective personalized HCC biomarker and therapeutic strategies, as well as prognostic factors are urgently needed.

The hypothesis of competing endogenous RNA (ceRNA) refers to a brand-new gene expression regulation mode. CeRNAs, including long non-coding RNA (lncRNA) and circular RNA (circRNA), could competitively combine to micro RNA (miRNA) which will interfere miRNA binding to messenger RNA (mRNA) to regulate gene expression, thereby affecting cell function [3]. CeRNAs, correlations between mRNA, miRNA, and lncRNA have been identified in many diseases [4–7]. The tumor microenvironment (TME) is mainly composed of tumor cells and stromal components mixed with tumor-infiltrating immune cells. Understanding the immune infiltration is the key to improving the response rate and developing new strategies in tumor treatment [8, 9]. However, few previous studies have paid attention to ceRNA networks and tumor-infiltrating immune cells in HCC. Understanding the molecular mechanisms underlying HCC tumorigenesis is critical for early detection, diagnosis, successful treatment, and prognosis determination. Here, we established a HCC-associated ceRNA network on the basis of gene expression datasets from The Cancer Genome Atlas (TCGA). Using CIBERSORT, we evaluated HCC tumor sample infiltration by various immune cells. We then established 2 nomograms for predicting HCC prognosis on the basis of important immune cells and the ceRNA network. The association of HCC-linked ceRNA with immune cells networks was assessed to determine potential molecular mechanisms.

Materials and methods

Data abstraction and differential gene expression analysis

HCC RNA profiles along with the matching patient clinical data were abstracted from TCGA data resource (https://tcga-data.nci.nih.gov/tcga/). HTseq counts and fragment mapping per million exons per thousand bases for 421 samples (371 tumors and 50 normal samples) were assembled. edgeR was employed to uncover mRNA, lncRNA, and miRNA that were differentially expressed. False discovery rate (FDR), log(fold change)> 1.0 coupled with \( P < 0.05 \) were set as cutoff thresholds for differentially expressed genes.

Construction of the ceRNA network

LncRNA-miRNA-mRNA ceRNA networks are hinged on the theory that lncRNAs can directly or indirectly serve as miRNA sponges to modulate mRNA activity. The ceRNA network was built using the package “GDCRNATools” R (http://bioconductor.org/packages/devel/bioc/html/GDCRNATools.html) [10]. Cytoscape web resource (http://www.cytoscape.org/) was employed to visualize the expression locations of the ceRNA network [11].

Survival analyses and nomograms of essential members in the ceRNA network

Kaplan–Meier (K–M) survival assessment was done to evaluate correlation between Biomarker expression and HCC survival [12]. Next, important variables in the initial Cox model were analyzed to identify those with prognostic value and important biomarkers integrated into the reduced Cox proportional hazard model. LASSO (minimal equivalent contraction and recovery of selective operators) utilizes contraction to lower the data value to a specific point to verify the validity of the constructed multifactorial model. Finally, a nomogram was created on the basis of the multivariate model to predict HCC prognosis. The nomogram may enable clinicians determine a prognostic score for each biomarker based on its expression. The sum of individual scores may indicate prognosis and overall three- and five-year survival. Calibration and recipient behavioral characteristic (ROC) curve analyses were used to assess the nomograms accuracy.

CIBERSORT estimation

CIBERSORT uses gene expression data to determine the abundance, as well as the proportion of various types of immune cells in a mixed cell population [13, 14]. Here, we employed CIBERSORT to evaluate the fractions of 22 immune cell types in HCC samples. Only cases with CIBERSORT outputs of \( P < 0.05 \) were eligible for subsequent analysis. Wilcoxon rank-sum was used identify important immune cells in tumor vs non-malignant samples. Next,
K–M survival assessment was employed to assess the association of the proportion of specific immune cells with HCC overall survival. Upon LASSO regression analysis, distinct immune cells were integrated into a Cox proportional hazard model and a nomogram developed for HCC prognosis prediction. The concordance index of the Cox model was employed to assess the nomogram’s bias and precision. Pearson’s correlation explored correlation between HCC biomarkers and immune cells.

**Explanation and use of the nomograms**

A nomogram is a graphical representation of an equation that predicts medical outcomes. Nomograms use a points-based system in which patients accumulate point based on the level of risk factors. The risk factors of two nomograms we built in the study were based on the fold change of gene expression between tumor tissues and normal tissues. Thus, comparing the target genes’ expression of patients’ sample and a normal sample, we could get the corresponding log (fold change) and scores in the nomograms. Then, we could predict the 1-, 3- or 5-year survival rate of patients.

**Statistical analysis**

Statistical analyses were done on R version 4.0.2 software using edgeR [15], ggplot2 [16], GDCRNATools [10], rms [17], glmnet [18], preprocessCore [19], survminer [20], and timeROC [21] packages. Two-sided $P < 0.05$ indicates statistical significance.

**Results**

**Criterion and expression analysis of genes with essential differences**

A schematic of our analysis process is shown in Fig. 1. Genes with FDR = $< 0.05$ as well as a log fold change $> 1.0$ or $< −1.0$ were regarded as differentially expressed. Differential expression of mRNAs, miRNAs, and lncRNAs in tumor vs normal samples was calculated using DESeq2 (Fig. 2a-c). Our analysis identified 104 lncRNAs, 107 miRNAs, and 1222 mRNAs as upregulated and 32 lncRNAs, 21 miRNAs, and 806 mRNAs as downregulated (Fig. 2d).

**CeRNA network construction and survival assessment**

The ceRNA network was developed on the basis of interactions between 14 pairs of lncRNA–miRNA and 42 pairs of miRNA–mRNA (Fig. 3a, Table 1). K–M survival assessment evaluated the relationship of the prognosis with biomarkers in the HCC-linked ceRNA network. These analysis revealed that hsa-miR-421 ($P < 0.001$) and hyaluronan mediated motility receptor (HMMR) ($P < 0.001$) signified significance. These analysis revealed that hsa-miR-421...
Fig. 2  Heat maps of differentially expressed a RNAs, c miRNAs, e lncRNAs between the tumor and normal tissues of HCC. b Bar plot showing differentially expressed protein-coding genes, long non-coding genes, pseudogenes, and other RNAs. Red and blue represent upregulated and downregulated RNAs, respectively. It shows that 806 of 2028 differentially expressed protein-coding genes are downregulated and 1222 are upregulated. Besides, among 136 differentially expressed lncRNAs, 32 lncRNAs are downregulated, and 104 are upregulated. Volcano plots of differentially expressed miRNAs (d) and lncRNAs (f). We defined the log (fold change) > 1.0 or < −1.0 and FDR < 0.05 as the critical point. Thus, the red and green dots in the plots represent high and low expression RNAs with statistical significance, respectively. Meanwhile, black dots represent mRNAs and lncRNAs without statistical significance.
Fig. 3  

(a) Overview of the lncRNA–miRNA–mRNA ceRNA network of HCC with 14 pairs of lncRNA–miRNA and 42 pairs of miRNA–mRNA. Kaplan–Meier survival curves based on the expression of biomarkers involved in ceRNA network related to the bone metastasis in melanoma shows that (b) HMMR (P < 0.001), (c) hsa-miR-421 (P < 0.001), (d) E2F8 (P < 0.001) and (e) FANCE (P < 0.001) had significantly prognostic values. The flowchart of the analysis process.
and hyaluronan mediated motility receptor (HMMR) \((P < 0.001)\) signified significance (Fig. 3b, c).

**Establishment of the prediction model on the basis of the ceRNA network**

LASSO regression analysis found HMMR, ARL2, RNF24, has-miR-326, RAP2A, S100A10, and has-miR-421 as essential for modeling, which were then integrated into Cox regression analysis and a nomogram built for prognosis prediction. Area under curve (AUC) values for three- and five-year survival were 0.691 and 0.700, respectively, indicating good efficiency and accuracy. Additionally, the identification of the nomogram can be seen through the calibration curves (Fig. 4a-g). Immune cells related to the HCC, Histograms and heat maps which illustrated the composition of immune cells in HCC were evaluated by the CIBERSORT algorithm (Fig. 5a, b). The results of Wilcoxon rank-sum test showed that there were relatively high levels of T CD4 naive cells \((P = 0.004)\), T gamma delta cells \((P = 0.005)\), T regulatory (Tregs) cells \((P = 0.016)\), mast resting cells \((P = 0.005)\) and dendritic activated cells \((P = 0.031)\) were relatively in liver cancer (Fig. 5c).

**Development of the prediction model on the basis of the immune cells**

We integrated five of 22 immune cells with remarkable prognostic potential in the Cox regression into the final multivariable model with satisfactory estimation power (concordance index = 0.660) and used to establish the

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**Table 1 Hypergeometric testing and correlation analysis results of ceRNAs network**

| LncRNA | Protein-coding RNA | MiRNAs | Hypergeometric test P | Correlation P |
|-------|--------------------|--------|-----------------------|---------------|
| SNHG1 | E2F8               | has-miR-421 | 0.00784745          | 8.58865E−31   |
| SNHG1 | HMMR               | has-miR-421 | 0.042928879       | 2.93371E−27   |
| SNHG1 | NMB                | has-miR-421 | 0.042928879       | 1.9713E−25    |
| SNHG1 | MSH2               | has-miR-21-5p| 0.042928879       | 1.54818E−24   |
| SNHG1 | CRYBG2             | has-miR-21-5p| 0.042928879       | 9.00723E−19   |
| SNHG1 | NETO2              | has-miR-21-5p| 0.041029046       | 1.71971E−08   |
| SNHG1 | S100A10            | has-miR-21-5p| 0.042928879       | 5.98165E−05   |
| SNHG1 | LMNB2              | has-miR-326,hsa-miR-330-5p| 0.002308944      | 1.56875E−38   |
| SNHG1 | FANCE              | has-miR-326,hsa-miR-330-5p| 0.000392403      | 6.37741E−30   |
| SNHG1 | ANKRD13B           | has-miR-326,hsa-miR-330-5p| 0.041029046      | 3.34609E−20   |
| PVT1  | ABC5               | hsa-miR-17-5p,hsa-miR-93-5p| 0.026876318      | 1.97873E−13   |
| PVT1  | RNF24              | hsa-miR-17-5p,hsa-miR-20a-5p,hsa-miR-93-5p,hsa-miR-20b-5p| 0.000133481      | 3.03984E−09   |
| PVT1  | ANKRD52            | hsa-miR-17-5p,hsa-miR-20a-5p,hsa-miR-93-5p,hsa-miR-106b-5p,hsa-miR-20b-5p| 0.007044169      | 1.04891E−08   |
| PVT1  | PLAG1              | hsa-miR-17-5p,hsa-miR-20a-5p,hsa-miR-93-5p,hsa-miR-106b-5p,hsa-miR-20b-5p| 0.000138113      | 0.000120269   |
| KCNQ1OT1 | NDOR1            | hsa-miR-214-3p | 0.006618532      | 8.40638E−11   |
| KCNQ1OT1 | CDC7            | hsa-miR-335-5p | 0.008147588      | 1.13552E−09   |
| KCNQ1OT1 | KLC2            | hsa-miR-214-3p | 0.000490319      | 8.95697E−05   |
| KCNQ1OT1 | ARL2            | hsa-miR-214-3p | 0.040952018      | 0.000207007   |
| KCNQ1OT1 | CDC25B         | hsa-miR-214-3p | 0.020800997      | 0.001761624   |
| KCNQ1OT1 | LMNB2          | hsa-miR-326,hsa-miR-330-5p | 0.001910986      | 6.64665E−15   |
| KCNQ1OT1 | ANKRD13B       | hsa-miR-326,hsa-miR-330-5p | 0.00598274      | 4.41844E−06   |
| KCNQ1OT1 | FANCE         | hsa-miR-326,hsa-miR-330-5p | 0.006618532      | 6.25071E−06   |
| KCNQ1OT1 | RAP2A         | hsa-miR-326,hsa-miR-330-5p | 0.020888159     | 6.77642E−06   |
| KCNQ1OT1 | MECOM         | hsa-miR-326,hsa-miR-338-3p | 0.015398832     | 0.034334548   |
| KCNQ1OT1 | ANKRD52       | hsa-miR-326,hsa-miR-326,hsa-miR-330-5p | 0.038275504     | 2.38934E−13   |

**CeRNAs** competing endogenous RNAs, **lncRNA** long non-coding RNA, **miRNA** microRNA
nomogram. LASSO regression analysis revealed that all 6 genes were important for modeling. ROC and calibration curve analysis revealed acceptable accuracy (three-year survival AUC: 0.674; 5-year survival AUC: 0.694), indicating good concordance (Fig. 6a–g).

**Comprehensive assessment of genes and immune cells**

Pearson’s correlation analyses of co-expression patterns between various immune cells and the correlation between biomarkers and immune cells (Fig. 7a, b) found that HMMR and M0 macrophages ($P=0.00046$, $R=0.18$), RAP2A and M0 macrophages ($P=0.000017$, $R=−0.22$), RNF24 and M0 macrophages ($P=0.000057$, $R=0.21$) and RNF24 and resting mast cells ($P=0.00000009$, $R=−0.3$) had good correlation (Fig. 7c–f).

**Discussion**

Mounting evidence shows that genetic alterations in signaling pathways drive HCC tumorigenesis, highlighting the importance of molecular biomarkers in liver cancer detection. Although the mechanisms underlying HCC tumorigenesis are unclear, cellular and molecular changes are influential factors. Here, we first evaluated differentially expressed ceRNA and immune cells infiltration in normal vs tumor HCC tissues. We then developed nomograms for predicting HCC prognosis. The excellent concordance index and AUC value in the 2 nomograms may guide HCC survival prediction. K–M survival along with correlation analysis indicated that the ceRNA modulatory network of SNHG1 (lncRNA), HMMR (mRNA), hsa-miR-421 (miRNA), and Tregs may influence HCC progression. It is estimated < 2% of the human genome is protein-coding, suggesting that most human transcripts are non-coding [22]. mRNAs, miRNAs, and lncRNAs are linked in an intricate crosstalk network by competing endogenous RNA [23]. Interaction between miRNA, IncRNA, and mRNA, in ceRNA networks is extensively studied in various disorders, including lung, gastric, and gallbladder cancers [24, 25]. However, the mechanisms of ceRNA networks in HCC oncogenesis are largely unclear. Here, we find that in a ceRNA network, SNHG1 (a lncRNA), may downregulate hsa-miR-421 and upregulate HMMR to promote HCC tumorigenesis. Hsa-miR-421 is reported to play a core role in malignant transformation, which is consistent with our findings [26, 27]. HMMR is an important component of polo-like kinase 1 (PLK1)-dependent mitotic spindle localization cascade, which is essential for neurodevelopment, neonatal survival, and tumorigenesis [28]. HMMR is upregulated in many cancers, such as lung cancer, glioblastoma, prostatic adenocarcinoma, and leukemia [29–32]. HMMR has been documented to enhance EMT (epithelial-mesenchymal transition) and carcinogenesis by activating TGF-β/Smad2 signaling in gastric cancer [33]. Assessment of 1420 colorectal cancer tissues indicated that HMMR may be a better prognostic factor relative to tumor grade as well as vascular infiltration [34]. How HMMR modulates cell cycle-linked gene expression has not been studied. HMMR antisense IncRNA HMMR-AS1 stabilizes HMMR mRNA and enhances the progress of lung adenocarcinoma, epithelial ovarian cancer, as well as glioblastoma [35–37]. HMMR negatively correlates with poor HCC prognosis, highlighting it as a potential indicator of HCC prognosis [38].

We also uncovered different proportions of immune cells in liver cancer. Naïve CD4 T cells, resting mast cells, gamma delta T cells, Tregs, and activated dendritic cells have been shown to be associated with HCC. A nomogram based on 5 immune cell types, built for overall survival prediction (consistency index = 0.66) may have high clinical value.

Tregs, which are CD25+, account for 5–10% of CD4+ T cells and are one of the most widely studied immune cell types due to their inhibitory effects on tumor pathogenesis. Tregs repress the activation as well as the proliferation of CD4+ and CD8+ T cells in vitro and in vivo using various mechanisms consisting of cell–cell contact along with secretion of immunosuppressive cytokines like TGF-β, IL-10, and IL-35 [39]. Tregs are believed to be the most important determinant of hepatitis B prognosis [40]. Infection with hepatitis B virus (HBV) is linked to > 60% of liver cancer cases in developing countries and < 25% in developed countries. Our data show that Tregs are upregulated in HCC. It is suggested that Tregs depletion may slow HCC progression [41].

Levels of tumor-infiltrating dendritic cells (DCs) correlate with clinical outcomes. CD14+CTLA4+ regulatory DCs have the same inhibitory effect as Tregs in HCC. These DCs inhibit T-cell response and upregulate PD-1 via IL-10 and CTLA4 [42].

High CD4+ T-cell levels also hinder liver cancer development. Anti-programmed death 1 antibody on the surface of CD4+ cells can demonstrate the clinical outcomes of HCC patients.
### a) Hazard ratio

| Gene             | Hazard Ratio (95% CI) | p-value |
|------------------|-----------------------|---------|
| HMMR             | (1.16 - 1.6)          | <0.001 ** |
| RNF24            | (0.94 - 1.5)          | 0.146   |
| RAP2A            | (1.04 - 1.6)          | 0.019 * |
| S100A10          | (1.02 - 1.4)          | 0.029 * |
| ARL2             | (1.07 - 1.5)          | 0.085   |
| hsa-miR-326      | (1.06 - 1.4)          | 0.003 **|
| hsa-miR-421      | (1.06 - 1.4)          | 0.121   |

# Events: 127, Global p-value (Log-Rank): 2.682e-10
AIC: 1251.63; Concordance Index: 0.71

### b) Coefficients

![Coefficient plot](image)

### c) Partial Survival Curves

![Survival curve](image)

### d) Sensitivity

![Sensitivity plot](image)

### e) Point Scores

| Gene            | Point Score |
|-----------------|-------------|
| HMMR            | -1.5        |
| RNF24           | -1          |
| RAP2A           | 0           |
| S100A10         | 1           |
| ARL2            | 2           |
| hsa-miR-326     | 4           |
| hsa-miR-421     | 8           |
| Total Points    | 3           |

1-year survival: 0.99, 0.9
2-year survival: 0.89, 0.8
3-year survival: 0.8, 0.7

### f) Nomogram Predicted Probability of 3-Year OS

![Nomogram plot](image)

### g) Nomogram Predicted Probability of 5-Year OS

![Nomogram plot](image)
In the innate immune cells, total mast cells, monocytes, DCs, as well as neutrophils are considerably changed. Mast cells have key immune regulation functions, and the mechanism of cell inactivation in HCC remains unclear. IgE, a mast cell activator has been reported in HBV-linked HCC [43]. Our data show a higher proportion of active mast cells in healthy livers and a higher proportion of inactive subtypes in advanced HCC. COX evaluation also established that the proportion of resting mast cells remarkably correlated with prognosis. Mast cells constitute the fastest immune cell responders that are particularly enriched in barrier sites. Their rapid release is mediated by factors like vasoactive amines, proteases, cytokines, and proteoglycans, from intracellular storage upon FCER1-bound IgE crosslinking [44]. However, mast cell function in abnormal livers is still unclear and warrants further investigation.

Correlation analysis found that CD4 T cells were linked to HMMR (R = −0.164, p = 0.0016). CeRNA network correlation analysis and hypergeometry tests found that SNHG1 (a lncRNA), HMMR (a protein-coding RNA), and hsa-miR-421 (a miRNA) were remarkably associated (p = 0.007847), suggesting that the interaction between hsa-miR-421, SNHG1, HMMR, and CD4 T cells is associated with HCC development.

This study has several limitations. Firstly, multivariable survival assessment included only basic prognostic factors from TCGA and could not suggest other potential clinical factors like metastatic lesions status and patients’ performance status. Secondly, preponderant evidence suggests that HCC is a very heterogeneous tumor at genetic and molecular levels. Due to study limitations, gene expression data from Gene Expression Omnibus (GEO), Sun Yat-sen Memorial Hospital (SYMH), and International Cancer Genome Consortium (ICGC) cohorts was determined from one piece of HCC tissue from a single patient. Future studies should evaluate gene expression in several HCC specimens from a patient via single-cell whole-genome sequencing or Quantitative Real-time PCR (RT-qPCR) analysis to determine if the line map is a reliable and viable predictor of HCC overall survival markers. In addition, cooperation with other hospitals should be considered to recruit more patients and samples for gene model validation.
Fig. 5  

a Bar plot showing cell types and relative percent in HCC. Different colors represent different cell types, which are listed in the right as y axis, while x axis represents different samples.  
b Heat map of tumor-infiltrating cells in tumor tissues in HCC patients. Annotations on top show clustering of samples. While the blue represents the normal, the red symbolizes the HCC.  
c Violin plot for comparing cells’ proportion between the tumor and normal. It illustrates that the proportion of the T cells CD4 naïve in tumor tissues was relatively less than that in the normal tissues (P = 0.004), and Tregs, T cells gamma delta dendritic cell resting and mast cell activator were relatively greater in the tumor than the normal tissues (P = 0.016, 0.005, 0.031 and 0.005)
Fig. 6  

(a) Cox proportional hazards model integrated by 4 different types of immune cells. *P < 0.05; **P < 0.001  

(b) The results of the Lasso regression.  

c) ROC curve analysis for predicting the 1-, 3-, and 5-year survival.  

d) Nomogram for predicting patients’ outcome based on 4 cells in (a).  

e) Calibration curve for 3-, and 5-year survival of the nomogram.
Fig. 7  a Correlation analysis (Pearson analysis) of different tumor-infiltrating cells and b the relationships between different tumor-infiltrating cells and differentially expressed genes in HCC. Scatterplots further illustrate the exact relationship between HMMR and Macrophages M0 \((P=0.00046, R=0.18)\) (c), RAP2A and Macrophages M0 \((P=0.00017, R=-0.22)\) (d), RNF24 and Macrophages M0 \((P=0.000057, R=0.21)\) (e) and RNF24 represented good correlation with Mast cells resting \((P=0.0000000009, R=-0.3)\) (f). Besides, gray-shaded areas in two graphs represent the standard errors of the blue regression lines. \(R\) correlation coefficient.
Conclusions

Based on a ceRNA network along with tumor-invading immune cells, we established 2 nomograms with high concordance index as well as AUC values for predicting HCC prognosis. On the basis of comprehensive clinical data from predictive nomograms, individualized management of HCC patients can be significantly enhanced and has huge potential to be a candidate biomarker for the diagnosis, prognosis as well as therapeutic targets of HCC patients.

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Author contributions

L.C. and C.N. conceived and designed the experiments; W.Z. performed the experiments; L.C., H.S. and L.Z. analyzed the data; Z.L. and L.C. contributed reagents and materials.

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Data availability

The data were downloaded from The Cancer Genome Atlas (TCGA) database.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The studies involving human participants were reviewed and approved by all data are from public database on the internet.

Consent to participate

All authors agree to submit articles for publication.

Consent for publication

All authors agree with publication.

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