Development of a prognostic signature based on six immune related lncRNAs for hepatocellular carcinoma.

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Primary research

Keywords: hepatocellular carcinoma, immune related lncRNAs, prognostic signature, competing endogenous RNA network

Posted Date: March 9th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-16348/v1>

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most common malignant tumor in the world which prognosis is poor. Therefore, a precise biomarker is needed to guide treatment and improve prognosis. More and more studies have shown that lncRNAs and immune response are closely related to the prognosis of hepatocellular carcinoma. The aim of this study was to establish a prognostic signature based on immune related lncRNAs for HCC.

Methods: Univariate cox regression analysis was performed to identify immune related lncRNAs, which had negative correlation with overall survival (OS) of 370 HCC patients from The Cancer Genome Atlas (TCGA). A prognostic signature based on OS related lncRNAs was identified by using multivariate cox regression analysis. Gene set enrichment analysis (GSEA) and a competing endogenous RNA (ceRNA) network were performed to clarify the potential mechanism of lncRNAs included in prognostic signature.

Results: A prognostic signature based on OS related lncRNAs (AC145207.5, AL365203.2, AC009779.2, ZFPM2-AS1, PCAT6, LINC00942) showed moderately in prognosis prediction, and related with pathologic stage (Stage I&II VS Stage III&IV), distant metastasis status (M0 VS M1) and tumor stage (T1-2 VS T3-4). CeRNA network constructed 15 axes among differentially expressed immune related genes, lncRNAs included in prognostic signature and differentially expressed miRNA. GSEA indicated that these lncRNAs were involved in cancer-related pathways.

Conclusion: We constructed a prognostic signature based on immune related lncRNAs which can predict prognosis and guide therapies for HCC.

Background

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the fourth leading cause of cancer death worldwide[1]. In recent years, although advances and developments in understanding molecular mechanism and therapeutic treatments for HCC[2–4], the overall survival rate for HCC remains low, the median survival rate is approximately 50% (17%-69%) after 5 years[5] and the median survival time of advanced HCC patients are only 1–2 years[6]. This poor prognosis is mainly due to its high recurrence rate (50%-70% at 5 years) of HCC [7–9].

Long non-coding RNAs (lncRNAs) are a class of poorly conserved non-coding RNA with transcripts longer than 200 nucleotides[10]. It has been revealed that various lncRNAs can function as signals, decoys, guides or scaffolds for other regulatory proteins[11–13]. For HCC, many studies have revealed that lncRNA can affect prognosis by targeting oncogenic or tumor suppressor gene mRNAs. For instance, lncRNA F11-AS1 suppresses HCC progression by competitively binding with miR-3146 to regulate PTEN expression[14], lncRNA ANCR promotes HCC metastasis through up-regulating HNRNPA1 expression[15].

Immune response and process are considered to be related with promoting tumorigenesis in many cancers. As we all know, the chronic inflammation induced by HBV infection can lead to liver damage. In China, more than 80% occurrence of HCC are attributed to cirrhosis and chronic inflammation, which is now considered as an important factor involved in cancer progression[16–18]. So, we consider that lncRNAs may affect HCC progression by targeting immune related genes, we called these lncRNAs as immune related lncRNAs.

The underlying molecular mechanisms that mediate recurrence and metastasis remain largely unclear. The construction of an appropriate survival prediction model will help improve the overall prognosis of HCC patients. In present study, we utilized the Cancer Genome Atlas (TCGA) database and other online database to identify a prognostic signature based on immune related lncRNAs for HCC patients.

Materials And Methods

Data acquisition

Transcriptome profiling RNA-seq data (HTSeq-fpkms) of 50 non-tumor liver specimens and 374 HCC specimens and corresponding clinical data were downloaded from the TCGA data portal (<https://portal.gdc.cancer.gov/>). Patients without complete follow-up data were excluded, 370 patients were enrolled in our study. We also downloaded the expression profile of miRNA (HTSeq-count) of 50

non-tumor liver specimens and 375 HCC specimens from TCGA data portal. There is no need to get ethical approval due to TCGA database is a public database from TCGA data portal. The present study complied with TCGA publication guidelines and data access policies.

Extracting expression profile of immune related lncRNAs

Firstly, we distinguish the expression profile of genes and lncRNAs. We extracted the expression profile of immune related genes with the help of immune response (M19817), immune system process (M13664) gene set and transcriptome expression profile of TCGA. With the help of co-expression analysis between immune related genes and lncRNAs, we extracted the expression profile of immune related lncRNAs, a threshold of correlation scores more than 0.4 and $P < 0.001$ were considered significant.

Differentially expressed analysis of immune related lncRNAs, immune related genes and miRNAs

Differentially expressed immune related lncRNAs and immune related genes between the HCC specimens and non-tumor liver specimens were identified with the help of limma package and differentially expressed miRNAs were identified by edgeR package. A threshold of $|\log_2 \text{fold change} (\log_2 \text{FC})| > 1$ and false discovery rate (FDR) < 0.05 were considered significant.

Identification of OS related immune related lncRNA

Due to the low overall survival (OS) rate of HCC, we chose OS as the primary endpoint. We performed univariate cox proportional hazards regression analysis to identify OS related immune related lncRNAs, $P < 0.01$ was selected as the cut off value.

Construction and evaluation of an immune related lncRNAs prognostic signature

We performed LASSO analysis based on the results of univariate cox analysis to avoid over-fitting the prognostic signature. According to the results of LASSO analysis, we performed multivariable cox proportional hazards regression analysis to construct prognostic signature based on immune related lncRNAs. The risk-score is calculated by multiplying the cox regression coefficient by the gene expression data. Patients were categorized as high- and low-risk groups according to the median risk score. Kaplan-Meier curves were plotted to compare the OS of high-risk and low-risk groups, we also performed KM analysis of the lncRNAs included in prognostic signature. The receiver operating characteristic (ROC) curve was plotted as well and the area under the ROC curve (AUC) was calculated to evaluate the predicting efficacy of prognostic signature. Univariate Cox analysis and multivariate Cox analysis were performed among clinicopathologic characteristics and risk score to identify the independent prognostic factors for HCC. The relevance between clinicopathologic characteristics and risk score were performed as well.

Exploration of the molecular mechanisms of lncRNAs included in prognostic signature.

In order to explore potential molecular mechanisms of the lncRNAs included in prognostic signature, we performed a competing endogenous RNA (ceRNA) network among differentially expressed immune related genes, lncRNAs included in prognostic signature and differentially expressed miRNA. We predicted the target miRNAs of lncRNAs through lncbasev3 online tool (<http://diana.e-ce.uth.gr/lncbasev3/interaction>). Target genes of miRNAs were predicted via miRDB (<http://mirdb.org>) [19], miRTarbase (<http://mirtarbase.mbc.nctu.edu.tw/php/download.php>) [20] and targetScan database (http://www.targetscan.org/faqs.Release_7.html) [21] and differentially expressed immune related genes, only genes included in all four gene sets were considered target genes of miRNAs. Gene set enrichment analysis (GSEA) of lncRNAs included in prognostic signature were performed to explore the potential pathways affecting the prognosis of HCC.

Statistical analysis

All the statistical analysis was performed by R software (version 3.6.1) and perl software (version 5.30). Univariate and multivariable cox proportional hazards regression analysis were calculated by survival R software package. Survival ROC R software package was used to calculate AUC of the survival ROC curve. Cluster heatmaps and volcano maps were generated using gplots and heatmap packages. ceRNA network was constructed by Cytoscape software (version 3.6.1). GSEA was performed using GSEA software (version 4.0.1).

Results

Identification of differentially expressed immune related lncRNAs, immune related genes and miRNAs

In present study, the expression profile of 331 immune related genes, 544 immune-related lncRNAs and 1881 miRNAs were extracted. We identified 273 differentially expressed lncRNAs, including 6 down-regulated and 267 up-regulated (Figure 1A and 1B), 86 differentially expressed genes, including 16 down-regulated and 70 up-regulated (Figure 1C and 1D), 251 differentially expressed miRNAs, including 22 down-regulated and 229 up-regulated (Figure 1E and 1F).

Construction of prognostic signature

According to the results of univariate cox regression analysis ($P < 0.05$), 31 lncRNAs were identified that were related to OS (Figure 2A). LASSO analysis revealed that 11 lncRNAs were properly considered to construct a prognostic signature (Figure 3). According to the results of multivariable cox regression analysis, six OS related immune related lncRNAs were included in the prognostic signature (Figure 2B). We constructed the prognostic signature according to the expression level of these six lncRNAs and their coefficients. The formula was as follows: risk score = $(0.3342 * \text{the expression level of AC145207.5}) + (0.0865 * \text{the expression level of AL365203.2}) + (0.092 * \text{the expression level of AC009779.2}) + (0.0441 * \text{the expression level of ZFPM2-AS1}) + (0.0801 * \text{the expression level of PCAT6}) + (0.0266 * \text{the expression level of LINC00942})$. HCC patients were categorized as high-risk group ($n=185$) and low-risk group ($n=185$) according to the median risk score.

Evaluation of prognostic signature based on OS related immune related lncRNA

Based on the results of Kaplan-Meier analysis, we found that high-risk group had a significantly poorer OS than low-risk group ($P=2.094e-06$, Figure 4A), the 1-, 3- and 5-year survival rates of high-risk group were 72.3%, 50.1% and 36.2%, respectively. However, in the low-risk group, the corresponding survival rates were 92.6%, 73% and 58.5%, respectively. All lncRNAs included in prognostic signature were found to be negative correlation with OS of HCC patients (Figure 5A-F).

Compared with other Clinicopathological parameters, the AUC for the prognostic signature was the highest (0.778), suggesting moderate predicting efficacy in OS monitoring (Figure 4B). A heat map about the expression profile of the six lncRNAs show that all lncRNAs were negative with OS (Figure 6A). A dot plot of survival status revealed that patients in high risk group had much higher mortality rate than those in low-risk group (Figure 6B). Figure 6C showed the rank of prognostic index and distribution of groups.

Principal component analysis (PCA) revealed that risk-score can better distinguish high-risk and low risk patients compared with all lncRNAs, immune related lncRNAs, differentially expressed immune related lncRNAs (Figure 7)

Univariate cox regression analysis revealed that pathologic stage, tumor stage, distant metastasis status and risk-score were related to OS (Figure 8A). Multivariate cox regression analysis suggested that risk-score could become an independent predictor after other parameters were adjusted, including age (≥ 65 years old), gender, tumor grade, pathologic stage, tumor stage, lymph node metastasis status and distant metastasis status (Figure 8B).

Analysis the clinical relevance of the prognostic signature

In order to apply the prognostic signature to the clinical, we analyzed the relevance between risk score and clinicopathologic characteristics, including age (≥ 65 years old), gender, tumor grade (Grade I&II VS Grade III&IV), pathologic stage (Stage I&II VS Stage III& IV), tumor stage (T1-2 VS T3-4), lymph node metastasis status (N0 VS N1) and distant metastasis status (M0 VS M1). The result showed that risk-score were relevant with distant metastasis status, pathologic stage and tumor stage ($P < 0.05$, Figure 9). We also analyzed the relevance between these clinicopathologic characteristics and the six lncRNAs. The result showed that AC145207.5 was relevant with age, tumor grade, pathologic stage and tumor stage (Supplementary figure 1), LINC00942 was relevant with gender, distant metastasis status and lymph node metastasis status (Supplementary figure 2), AL365203.2 were relevant with age, pathologic stage and tumor stage (Supplementary figure 3A-C), ZFPM2-AS1 was relevant with age and distant metastasis status (Supplementary figure 3D).

Potential molecular mechanisms of lncRNAs included in prognostic signature

According to the results of the ceRNA network (Figure 10A), five of the lncRNAs included in prognostic signature, six differentially expressed immune related genes and 32 differentially expressed miRNAs were included in the network. Finally, 15 immune-related

ceRNA axes were constructed (Table 1).

The results of GSEA (c2.cp.kegg.v7.symbol.gmt) showed that mainly function of these six lncRNAs were related with cancer, including nine high expressed pathways (cell cycle, DNA replication, NOTCH signaling pathway, tight junction, ERBB signaling pathway, bladder cancer, pathways in cancer, rig i like receptor signaling pathway, nod like receptor signaling pathway) and three low expressed pathways (Complement and coagulation cascades, PPAR signaling pathway, Drug metabolism cytochrome P450). (Table 2 and Figure 10B). High expressed pathways were considered to promote cancer formation, invasion and metastasis, while low expressed pathways were considered to inhibit cancer formation, invasion and metastasis.

Discussion

HCC is one of the most common malignant cancer around the world with poor prognosis. It is necessary to construct an effective survival prediction model to help improve the overall prognosis of HCC patients. In present study, with the help of TCGA data portal, we constructed a prognostic signature based on immune related lncRNAs. Six lncRNAs (AC145207.5, AL365203.2, AC009779.2, ZFPM2-AS1, PCAT6, LINC00942) related to OS were included in the prognostic signature.

We then evaluated the prognostic signature using various kinds of analysis. In present study, HCC patients with high risk score based on the prognostic signature had a poorer OS compared with those with low risk score. The results of the multivariate cox regression analysis showed that the risk score was an independent predict factor for predicting the OS of HCC patients. The area under the ROC curve (AUC) for the prognostic signature also suggested moderate predicting efficacy in OS monitoring. In our study, risk score was relevant with distant metastasis status, pathologic stage and tumor stage. PCA revealed that risk score can better distinguish high-risk and lower risk patients. The results of these analysis suggest that the prognostic value of the prognostic signature is robust and reliable for predicting OS in HCC patients.

With respect to lncRNAs included in prognostic signature, previous studies has revealed that ZFPM2-AS1 can promote proliferation and tumorigenesis of lung adenocarcinoma, renal cell cancer and gastric cancer[22–24]. Jiang H et al reported that dysregulated PCAT6 were significantly associated with HCC patients' poor outcomes[25]. Although there are no studies on the effect of AC145207.5, AL365203.2, AC009779.2, ZFPM2-AS1 and LINC00942 on the prognosis of HCC, KM analysis of these lncRNAs were significant difference in our present study. Our study provided novel evidence that AC145207.5, AL365203.2, AC009779.2, ZFPM2-AS1 and LINC00942 might be potential predictors of HCC prognosis, and further studies are needed to validate these results and investigate its molecular mechanisms.

Previous studies provided limited information about the mechanisms of these six lncRNAs in HCC patients survival, we then performed ceRNA network to expose potential molecular mechanisms of lncRNAs in HCC prognosis. According to the results of the network, 15 ceRNA axes were showed. Four (CCL20, IL12A, TPD52, CDK6) of the six immune related genes included in the ceRNA network have been reported to be related with the migration and invasion of HCC cells. He H et al revealed that CCL20/CCR6 chemokine/receptor axis can contribute to the initiation and progression of HCC through the recruitment of CCR6-positive leukocytes to the tumor micro-environment[26]. Liu L et al and Elsayed HM et al reported that IL12A rs568408 (G > A) polymorphism may contribute to the risk of HCC[27, 28]. Gong Y et al and Zhu H et al showed that up-regulated CDK6 can promote the proliferation and metastasis of HCC[29, 30]. In the work of Wang Y et al, they revealed that Decreased TPD52 expression is associated with poor prognosis in HCC[31]. However, TPD52 was up-regulating in HCC tissue in our study, which means the expression level of TPD52 may negative correlation with the prognosis of HCC patients. In the work of Zhao P et al, the suppression of TPD52 resulted in significant decrease of migration and invasion capabilities of 16HBE-C cells[32], which may in accord with us. However, the molecular mechanisms of immune related lncRNAs in HCC have not be fully explored, the ceRNA network axes we constructed may be the underlying molecular mechanisms of immune related lncRNAs on prognosis of HCC, our present study could provide a perspective to explore the molecular mechanisms, further research is needed in the future.

According to the results of GSEA, mainly function of immune related lncRNAs included in prognostic signature were involved in pathways related with cancer, including cell cycle, DNA replication and NOTCH signaling pathway. So, we hypothesize that these pathways may play an important role in HCC prognosis. Further studies are needed to validate the role of these pathways in HCC.

There are some limitations of our present study. First, the present study lacks independent validation cohort. Second, in vitro or in vivo experiments are needed to validate the molecular mechanisms and pathways.

Conclusion

We constructed a prognostic signature based on immune related lncRNAs which can predict prognosis and guide therapies of HCC.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are openly available in TCGA data portal (<https://portal.gdc.cancer.gov/>), Incbasev3 online tool (<http://diana.e-ce.uth.gr/Incbasev3/interaction>), miRDB (<http://mirdb.org>), miRTarbase (<http://mirtarbase.mbc.nctu.edu.tw/php/download.php>) and targetScan database (http://www.targetscan.org/faqs.Release_7.html).

Competing interests

The authors have declared no conflict of interests

Funding

This work was supported by no funding.

Authors' contributions

Conceptualization: Shao-qiang Li.

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Final approval of manuscript: All authors.

Acknowledgements

The authors would like to thank the TCGA database, Incbasev3 online tool, miRDB, miRTarbase and targetScan for the availability of the data.

Abbreviations

HCC: Hepatocellular carcinoma

lncRNAs: Long non-code RNAs

TCGA: The Cancer Genome Atlas

GSEA: Gene set enrichment analysis

ceRNA: Competing endogenous RNA

log2FC: log2 fold change

ROC: The receiver operating characteristic curve

AUC: the area under the ROC

PCA: Principal component analysis

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA: a cancer journal for clinicians* 2018, **68**(6):394-424.
2. Zheng G, Zhao R, Xu A, Shen Z, Chen X, Shao J: **Co-delivery of sorafenib and siVEGF based on mesoporous silica nanoparticles for ASGPR mediated targeted HCC therapy.** *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 2018, **111**:492-502.
3. Wu L, Nguyen LH, Zhou K, de Soysa TY, Li L, Miller JB, Tian J, Locker J, Zhang S, Shinoda G *et al*: **Precise let-7 expression levels balance organ regeneration against tumor suppression.** *eLife* 2015, **4**:e09431.
4. Shen Z, Li B, Liu Y, Zheng G, Guo Y, Zhao R, Jiang K, Fan L, Shao J: **A self-assembly nanodrug delivery system based on amphiphilic low generations of PAMAM dendrimers-ursolic acid conjugate modified by lactobionic acid for HCC targeting therapy.** *Nanomedicine : nanotechnology, biology, and medicine* 2018, **14**(2):227-236.
5. Takayama T: **Surgical treatment for hepatocellular carcinoma.** *Japanese journal of clinical oncology* 2011, **41**(4):447-454.
6. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK: **Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases.** *Hepatology (Baltimore, Md)* 2018, **68**(2):723-750.
7. Joliat GR, Allemann P, Labgaa I, Demartines N, Halkic N: **Treatment and outcomes of recurrent hepatocellular carcinomas.** *Langenbeck's archives of surgery* 2017, **402**(5):737-744.
8. Bruix J, Gores GJ, Mazzaferro V: **Hepatocellular carcinoma: clinical frontiers and perspectives.** *Gut* 2014, **63**(5):844-855.
9. Zheng J, Kuk D, Gonen M, Balachandran VP, Kingham TP, Allen PJ, D'Angelica MI, Jarnagin WR, DeMatteo RP: **Actual 10-Year Survivors After Resection of Hepatocellular Carcinoma.** *Annals of surgical oncology* 2017, **24**(5):1358-1366.
10. Yao J, Wu L, Meng X, Yang H, Ni S, Wang Q, Zhou J, Zhang Q, Su K, Shao L *et al*: **Profiling, clinicopathological correlation and functional validation of specific long non-coding RNAs for hepatocellular carcinoma.** *Molecular cancer* 2017, **16**(1):164.
11. Thai P, Statt S, Chen CH, Liang E, Campbell C, Wu R: **Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines.** *American journal of respiratory cell and molecular biology* 2013, **49**(2):204-211.
12. Chen Y, Yu X, Xu Y, Shen H: **Identification of dysregulated lncRNAs profiling and metastasis-associated lncRNAs in colorectal cancer by genome-wide analysis.** *Cancer medicine* 2017, **6**(10):2321-2330.
13. Gao YS, Liu XZ, Zhang YG, Liu XJ, Li LZ: **Knockdown of Long Noncoding RNA LUCAT1 Inhibits Cell Viability and Invasion by Regulating miR-375 in Glioma.** *Oncology research* 2018, **26**(2):307-313.
14. Du J, Chen M, Liu J, Hu P, Guan H, Jiao X: **LncRNA F11-AS1 suppresses liver hepatocellular carcinoma progression by competitively binding with miR-3146 to regulate PTEN expression.** *J Cell Biochem* 2019, **120**(10):18457-18464.
15. Wen Z, Lian L, Ding H, Hu Y, Xiao Z, Xiong K, Yang Q: **LncRNA ANCR promotes hepatocellular carcinoma metastasis through upregulating HNRNPA1 expression.** *RNA biology* 2019.
16. de Martel C, Maucourt-Boulch D, Plummer M, Franceschi S: **World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma.** *Hepatology (Baltimore, Md)* 2015, **62**(4):1190-1200.

17. Papatheodoridis GV, Chan HL, Hansen BE, Janssen HL, Lampertico P: **Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy.** *Journal of hepatology* 2015, **62**(4):956-967.
18. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY: **The global burden of liver disease: the major impact of China.** *Hepatology (Baltimore, Md)* 2014, **60**(6):2099-2108.
19. Wong N, Wang X: **miRDB: an online resource for microRNA target prediction and functional annotations.** *Nucleic acids research* 2015, **43**(Database issue):D146-152.
20. Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, Huang WC, Sun TH, Tu SJ, Lee WH *et al*: **miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions.** *Nucleic acids research* 2018, **46**(D1):D296-d302.
21. Agarwal V, Bell GW, Nam JW, Bartel DP: **Predicting effective microRNA target sites in mammalian mRNAs.** *eLife* 2015, **4**.
22. Li J, Ge J, Yang Y, Liu B, Zheng M, Shi R: **Long noncoding RNA ZFPM2-AS1 is involved in lung adenocarcinoma via miR-511-3p/AFF4 pathway.** *J Cell Biochem* 2019.
23. Liu JG, Wang HB, Wan G, Yang MZ, Jiang XJ, Yang JY: **Long noncoding RNA ZFPM2-AS1 promotes the tumorigenesis of renal cell cancer via targeting miR-137.** *European review for medical and pharmacological sciences* 2019, **23**(13):5675-5681.
24. Kong F, Deng X, Kong X, Du Y, Li L, Zhu H, Wang Y, Xie D, Guha S, Li Z *et al*: **ZFPM2-AS1, a novel lncRNA, attenuates the p53 pathway and promotes gastric carcinogenesis by stabilizing MIF.** *Oncogene* 2018, **37**(45):5982-5996.
25. Jiang H, Shi X, Ye G, Xu Y, Xu J, Lu J, Lu W: **Up-regulated long non-coding RNA DUXAP8 promotes cell growth through repressing Kruppel-like factor 2 expression in human hepatocellular carcinoma.** *OncoTargets and therapy* 2019, **12**:7429-7436.
26. He H, Wu J, Zang M, Wang W, Chang X, Chen X, Wang R, Wu Z, Wang L, Wang D *et al*: **CCR6(+) B lymphocytes responding to tumor cell-derived CCL20 support hepatocellular carcinoma progression via enhancing angiogenesis.** *American journal of cancer research* 2017, **7**(5):1151-1163.
27. Elsayed HM, Nabel Y, Sheta T: **IL12 Gene Polymorphism in Association with Hepatocellular Carcinoma in HCV-infected Egyptian Patients.** *Immunological investigations* 2017, **46**(2):123-133.
28. Liu L, Xu Y, Liu Z, Chen J, Zhang Y, Zhu J, Liu J, Liu S, Ji G, Shi H *et al*: **IL12 polymorphisms, HBV infection and risk of hepatocellular carcinoma in a high-risk Chinese population.** *International journal of cancer* 2011, **128**(7):1692-1696.
29. Gong Y, Mao J, Wu D, Wang X, Li L, Zhu L, Song R: **Circ-ZEB1.33 promotes the proliferation of human HCC by sponging miR-200a-3p and upregulating CDK6.** *Cancer cell international* 2018, **18**:116.
30. Zhu H, Wang G, Zhou X, Song X, Gao H, Ma C, Chang H, Li H, Liu FF, Lu J *et al*: **miR-1299 suppresses cell proliferation of hepatocellular carcinoma (HCC) by targeting CDK6.** *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2016, **83**:792-797.
31. Wang Y, Chen CL, Pan QZ, Wu YY, Zhao JJ, Jiang SS, Chao J, Zhang XF, Zhang HX, Zhou ZQ *et al*: **Decreased TPD52 expression is associated with poor prognosis in primary hepatocellular carcinoma.** *Oncotarget* 2016, **7**(5):6323-6334.
32. Zhao P, Zhong W, Ying X, Yao B, Yuan Z, Fu J, Zhou Z: **Comparative proteomic analysis of anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide-transformed and normal human bronchial epithelial G0/G1 cells.** *Chemico-biological interactions* 2010, **186**(2):166-173.

Tables

Table 1. The ceRNA network among differentially expressed immune related genes, lncRNAs included in prognostic signature and differentially expressed miRNA.

lncRNA	miRNA	gene
ZFPM2-AS1	hsa-mir-33b	SEMA7A
ZFPM2-AS1	hsa-mir-21	CCL20/IL12A
AC145207.5	hsa-mir-93/hsa-mir-33b/hsa-mir-20a/hsa-mir-17	SEMA7A
AC145207.5	hsa-mir-10b	NCOA6
AC145207.5	hsa-mir-183/hsa-mir-34a	CDK6
AC145207.5	hsa-mir-34a	TPD52
AC009779.2	hsa-mir-93	SEMA7A
AL365203.2	hsa-mir-17	SEMA7A
AL365203.2	hsa-mir-34a	CDK6/TPD52

Table 2. GSEA of the six lncRNAs included in prognostic signature

NAME	ES	NES	NOM p-val	FDR q-val
KEGG_PPAR_SIGNALING_PATHWAY	-0.56233007	-1.8340175	0	0.010671181
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	-0.80476934	-2.2798862	0	0
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	-0.6372146	-1.9294395	0.002016129	0.003940492
KEGG_CELL_CYCLE	0.6979802	1.9863397	0	0.027493307
KEGG_NOTCH_SIGNALING_PATHWAY	0.6204506	1.8016059	0.001972387	0.036784
KEGG_DNA_REPLICATION	0.76476115	1.7826561	0.001964637	0.039617956
KEGG_TIGHT_JUNCTION	0.50900465	1.752197	0.007476636	0.043791786
KEGG_RIG_I LIKE RECEPTOR SIGNALING_PATHWAY	0.5359764	1.7333766	0.017110266	0.043962717
KEGG_ERBB_SIGNALING_PATHWAY	0.5460123	1.730117	0.009615385	0.044393964
KEGG_NOD LIKE RECEPTOR SIGNALING_PATHWAY	0.5838806	1.7236259	0.02008032	0.04588009
KEGG_PATHWAYS_IN_CANCER	0.49706513	1.7110907	0.011363637	0.048517283
KEGG_BLADDER_CANCER	0.57523596	1.7126355	0.01171875	0.04939836

Figures

Figure 1

Differentially expressed immune-related lncRNAs, immune-related genes and miRNAs. Differentially expressed immune-related lncRNAs are shown in heatmap (A) and volcano plot (B). Differentially expressed immune-related genes are shown in heatmap (C) and volcano plot (D). Differentially expressed miRNAs are shown in heatmap (E) and volcano plot (F). Blue dots represent down expressed genes/lncRNAs/miRNAs, red dots represent up expressed genes/lncRNAs/miRNAs, and black dot represents no differentially expressed genes/lncRNAs/miRNAs.

Figure 2

Univariate and multivariable Cox regression analysis of immune related lncRNAs. OS related lncRNAs are shown in forest plot (A). lncRNAs included in prognostic signature are shown in forest plot (B).

Figure 3

LASSO analysis of OS related lncRNAs.

Figure 4

Kaplan-Meier analysis of high-risk group and low-risk group and ROC curve validation of prognostic value of the risk score. A: the results of KM analysis. B: the results of ROC curve.

Figure 5

Kaplan-Meier analysis of lncRNAs included in prognostic signature.

Figure 6

Development of the prognostic index based on OS related lncRNAs. A: Heatmap of expression profiles of included lncRNAs. B: Survival status of patients in high-risk groups and low-risk group. C: Rank of prognostic index and distribution of groups.

Figure 7

Principal component analysis of all lncRNAs, immune related lncRNAs, differentially expressed immune related lncRNAs and risk score. Figure A represents all lncRNAs. Figure B represents immune related lncRNAs. Figure C represents differentially expressed immune related lncRNAs. Figure D represents risk score. Blue dots represent low-risk group, red dots represent high risk group.

Figure 8

Univariate and multivariate Cox regression analysis of clinicopathologic characteristics and risk score. Figure A represents the results of univariate cox regression analysis. Figure B represents the results of multivariate cox regression analysis.