In recent years, there has been a growing recognition of the important role that long non-coding RNAs (lncRNAs) play in the immunological process of hepatocellular carcinoma (LIHC). An increasing number of studies have shown that certain lncRNAs hold great potential as viable options for diagnosis and treatment in clinical practice. The primary objective of our investigation was to devise an immune lncRNA profile to explore the significance of immune-associated lncRNAs in the accurate diagnosis and prognosis of LIHC. Gene expression profiles of LIHC samples obtained from TCGA database were screened for immune-related genes. The optimal immune-related lncRNA signature was built via correlational analysis, univariate and multivariate Cox analysis. Then, the Kaplan-Meier plot, ROC curve, clinical analysis, gene set enrichment analysis, and principal component analysis were performed to evaluate the capability of the immune lncRNA signature as a prognostic indicator. Six long non-coding RNAs were identified via correlation analysis and Cox regression analysis considering their interactions with immune genes. Subsequently, tumor samples were categorized into two distinct risk groups based on different clinical outcomes. Stratification analysis indicated that the prognostic ability of this signature acted as an independent factor. The Kaplan-Meier method was employed to conduct survival analysis, results showed a significant difference between the two risk groups. The predictive performance of this signature was validated by principal component analysis (PCA). Additionally, data obtained from gene set enrichment analysis (GSEA) revealed several potential biological processes in which these biomarkers may be involved. To summarize, this study demonstrated that this six-lncRNA signature could be identified as a potential factor that can independently predict the prognosis of LIHC patients.

Keywords: Long non-coding RNA, immune prognostic signature, hepatocellular carcinoma, overall survival

Introduction

Liver hepatocellular carcinoma, a prevalent malignancy globally, exhibits escalating rates of mortality and incidence [1, 2]. The primary approach utilized in LIHC administration is surgery, however, many patients are in the middle-advanced stage at first diagnosis and miss the chance of accepting surgery [3-5]. In a broad sense, liver is classified as a lymphoid organ [6]. It has been documented that during tumor progression, immunological tolerance is influenced by various factors including cytokines, hepatic nonparenchymal cells, dendritic cells, and lymphocytes, which actively modulate this process [7-10]. Meanwhile, immunology therapy comprising immune checkpoints, adoptive cellular immunotherapy (ACT) and vaccines has presented promising possibilities for the treatment of liver cancer (LIHC). These advancements have significantly broadened the horizons of LIHC treatment [11, 12]. Several studies have clarified that clinical application of immune checkpoint blockade programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA4) has enhanced the survival rate of some advanced patients [13-15]. Consequently, there is an urgent requirement for the study of immune biomarkers that exhibit both high sensitivity and specificity in terms of diagnosing and predicting the prognosis of hepatocellular carcinoma (LIHC). Long noncoding RNA is a type of poorly conserved RNA in length from 200 base pairs to 100 kilobase pairs. This particular RNA is capable of modulating gene expression at four primary levels: epigenetic regulation, epigenetic transcriptional regulation, posttranscriptional regulation and translational regulation [16-21]. According to their location with respect to protein-coding mRNAs, lncRNAs can be
categorized into four categories: antisense, pseudogene, long intergenic ncRNA and intronic IncRNA [22]. Recent research has brought to light the crucial significance of IncRNAs in the innate immune response and the development, differentiation, and activation of T cells [23, 24]. Additionally, certain investigations have examined the correlation between aberrant expression of IncRNAs and tumorsogenesis, metastasis, diagnosis or prognosis [25-27]. For example, HULC, a IncRNA that is specifically situated on cell plasma, exhibits a significant expression in hepatoma cells and promotes cell proliferation [28, 29]. In HBV-associated HCC, H19 has been documented to exhibit upregulated levels and represses the metastasis of tumors [30]. Through bioinformatic analysis, we developed a reliable immunological IncRNA model which serves as a valuable tool for facilitating the diagnosis and prognosis of liver hepatocellular carcinoma (LIHC). The long non-coding RNAs AC009005.1, AC099850.3, AL031985.3, AL117336.3, AL365203.2 and MSCP−AS1 were critical components of the whole model. The data suggested that a high expression level of these biomarkers was positively correlated with poor survival and malignant phenotypes in the TCGA dataset. Univariate Cox regression and multivariate Cox regression analyses further clarified that this signature had an independent influence on overall survival. The results derived from KM plot, ROC curve, and PCA further proved the sensitivity and reliability of this prognostic model.

**Methods**

**Data Source and Processing**

RNA-sequencing data of LIHC samples were retrieved from the TCGA repository [31]. The data was generated using the Illumina HiSeq RNA-Seq platform. Additionally, we collected the corresponding clinical data, including survival time, TNM classification information, and risk factors. The dataset consisted of a total of 424 samples, with 50 being normal and 374 being primary hepatocellular carcinoma. The utilization and acquisition of this data were conducted in accordance with TCGA data access policies and publication guidelines. In our study, we excluded clinical samples that did not have precise outcomes or had follow-up times of less than 30 days. To match the names of mRNAs and long non-coding RNAs in the ensemble, we utilized the human general transfer format (HUGTF) to map them. To ensure the reliability of the data, we excluded clinical samples that did not have precise outcomes or had follow-up times of less than 30 days. To match the names of mRNAs and long non-coding RNAs in the ensemble, we utilized the human general transfer format (HUGTF) to map them.

**Selection of Immune-Related Long Non-Coding RNAs**

**Construction of the Prognostic IncRNA Signature and Statistical Analysis**

The gene sets for ‘immune response’ and ‘immune system process’ were obtained from the Molecular Signatures Database [33]. These gene sets were then used to identify immune-related genes in LIHC samples. Correlation analysis was performed on these genes using the ‘limma’ packages and the ‘cor function’ in R. The filter criteria were set as absolute correlation coefficient (corresponding coefficients) > 0.4 and adjusted P value < 0.001 to identify the correlated IncRNAs. The network was visually represented using the Cytoscape software [34]. To ensure data reliability, specific criteria were applied to filter out IncRNAs that did not meet the following conditions: (1) IncRNAs with expression levels (FPKM ≥ 1) in at least 50% of the samples from patients with LIHC; and (2) IncRNAs that demonstrated consistent expression levels across all samples with no significant fluctuations [35]. We conducted a Univariate Cox regression analysis to identify a subset of candidate IncRNAs that exhibited a significant correlation (P < 0.001) with patient overall survival (OS). Subsequently, we performed multivariate Cox regression analysis to develop a prognostic IncRNA model. To validate the predictive significance of the 5- IncRNA signature in patients with HCC, we calculated risk scores for the test group of patients. These scores were determined using a formulated equation that incorporates the expression levels of the 6 IncRNAs. The formulas for calculating the risk scores are provided below. The tumor samples were categorized into a high-risk cohort and a low-risk cohort by applying the risk score threshold. To determine the survival rate, we performed Kaplan-Meier survival analysis. Additionally, we evaluated the performance of this long non-coding RNA (IncRNA) model using receiver operating characteristic (ROC) curve analysis. The optimization process involved the use of the Akaike Information Criterion (AIC). For all these statistical analyses, we utilized the R platform and the following packages: ‘survival,’ ‘survminer,’ ‘survival ROC,’ and ‘pheatmap’.

\[
\text{IncRNA risk score} = [|\text{coef}| \times \text{expression value}]
\]

\[
\text{risk score of samples} = \sum (\text{IncRNA risk score})
\]

**Analysis of the Clinical Features of the IncRNA Signature**

Following the preceding analysis, we obtained the expression matrix of IncRNA biomarkers in individuals diagnosed with LIHC. We subsequently merged the clinical information with the expression data. We then examined the relationship between the expression of IncRNA biomarkers (significance level: P < 0.05) and different clinical features, including tumor stage (T stage), histologic grade (G stage), and pathological stage (S stage). This analysis was performed using the ‘ggpubr’ package in the R programming language.

**Gene Set Enrichment Analysis**

The utilization of Gene Set Enrichment Analysis (GSEA) allowed for the investigation of the underlying relationship between risk scores obtained from co-expression analysis. For reference purposes, two sets of immune genes, namely ‘immune system process’ (M13664 genes annotated by GO term GO:0002376) and ‘immune response’ (M19817 genes annotated by GO term GO:0006955), were obtained from the Molecular Signatures Database (MSigDB). Enrichment outcomes were considered statistically significant if they met the criterion of FDR < 0.25.
Principal Component Analysis

Principal component analysis (PCA) was conducted to visualize the separation of samples with different risk scores based on the six-lncRNA signature, immune lncRNAs, immune-related genes, and all genes. Prior to PCA, the expression matrices were preprocessed by deduplicating the values through averaging and excluding any data with no change in expression level. The resulting graphs exhibited the three major components (PC1, PC2, PC3) in a three-dimensional space. The analysis was performed using the ‘limma’ and ‘scatterplot3d’ packages.

Statistics

Data expression was performed using the mean ± SD. Two-group comparisons were analyzed using the Student’s t-test, while multigroup comparisons were analyzed using one-way ANOVA. Spearman’s correlation analysis was used to evaluate expression correlation. Kaplan Meier analysis was conducted to analyze overall survival. A P value < 0.05 was considered statistically significant.

Results

Construction of the Six-LncRNA Signature

In the present research, the transcriptome data of both LIHC tissues (n = 374) and normal tissues (n = 50) were obtained from the TCGA database. To classify the immune genes based on the patients’ gene expression patterns, immune gene sets such as "immune response" and "immune system process" were utilized from the GSEA. Further correlational analysis was conducted on a total of 331 immune-related genes that were retained. Using R, lncRNAs meeting the criteria of |cor| > 0.4 and P < 0.001 were identified as potential candidates. Fig. 1A illustrates the visual representation of connections between immune genes and linked lncRNAs using Cytoscape software. Table 1 provides information on the coefficient and attributes of positive or negative regulation. Additionally, the candidate lncRNAs underwent both univariate and multivariate Cox regression analyses. Among them, 16 lncRNAs exhibited a strong association with survival outcomes (P < 0.0001). From these, 6 high-risk prognostic lncRNAs were identified (Fig. 1B). The prognostic optimization model yielded an AIC value of 1156.35. For detailed information on the six-lncRNA signature, please refer to Table 2. Based on the risk score, LIHC samples were divided into two groups: a high-risk group (n = 171) and a low-risk group (n = 172). Subsequently, the relative RNA expression levels of the six-lncRNA signature were assessed across different groups. Interestingly, the results indicated a significant upregulation of all six lncRNAs in LIHC tissues (Fig. 1C-1H).

Prognostic Value of the LncRNA Signature for Assessing Clinical Outcome

In Fig. 2A, we present the final survival state and expression profiles of the six-lncRNA signature for each sample, aiming to assess its potential in predicting the prognosis of LIHC patients. The scatter graph demonstrates a clear correlation between increasing risk score and worsened survival estimate. Furthermore, our survival analysis reveals significantly lower death rates in the low-risk group compared to the high-risk group (P = 6.75E−11). In

Table 1. Correlation of immune genes and associated lncRNAs.

| Immune Gene | lncRNA | Cor  | Pvalue | Regulation |
|-------------|--------|------|--------|------------|
| KMT2A       | AP001318.2 | 0.460 | 5.45E-21 | Positive   |
| NCOA6       | AC012510.1 | 0.462 | 3.35E-21 | Positive   |
| TRAF2       | SREBF2-AS1 | 0.451 | 3.66E-20 | Positive   |
| RPS19       | AC132192.2 | 0.614 | 4.03E-40 | Positive   |
| TRAF2       | AL035446.1 | 0.463 | 3.12E-21 | Positive   |
| HELS        | AL360181.2 | 0.455 | 1.53E-20 | Positive   |
| PRKRA       | AP002884.1 | 0.416 | 4.67E-17 | Positive   |
| RPS19       | AL109615.3 | -0.401 | 7.64E-16 | Negative  |
| APOA2       | FLJ42351  | -0.460 | 6.18E-21 | Negative  |
| NCK2        | MSC-AS1   | 0.408 | 2.10E-16 | Positive   |
| KMT2A       | AC009005.1 | 0.483 | 2.82E-23 | Positive   |
| ITGB2       | AL031985.3 | 0.679 | 7.50E-52 | Positive   |
| DPP4        | AL117336.3 | 0.512 | 2.18E-26 | Positive   |
| HDAC7       | AL365203.2 | 0.462 | 3.49E-21 | Positive   |
| CKLF        | AC099850.3 | 0.431 | 2.26E-18 | Positive   |

Table 2. Detailed information of the six-lncRNA signature.

| Gene symbol | Gene_ID       | Location  | coef  |
|-------------|---------------|-----------|-------|
| MSC-AS1     | ENSG00000235531.8 | chr8:71828167-72002405 | 0.3293 |
| AC009005.1  | ENSG00000267751.4 | chr19:567212-571745 | 0.3111 |
| AL117336.3  | ENSG00000271335.4 | chr10:3531452-35320998 | 0.3428 |
| AL031985.3  | ENSG00000260920.2 | chr1:4046419-40466767 | 0.4886 |
| AL365203.2  | ENSG00000273038.2 | chr10:32887255-32889311 | 0.2210 |
| AC099850.3  | ENSG00000265415.1 | chr17:59202677-59203829 | 0.1741 |
**Fig. 1.** Construction of an immune lncRNA signature for liver hepatocellular carcinoma. (A) The network of partial immune genes and associated lncRNAs. B. Candidate lncRNA for the prognostic model with information about hazard ratio. (C) Relative gene expression of MSC-AS1 among the low-risk group, high-risk group, and non-tumor samples. (D) Relative gene expression of AC009005.1 among the low-risk group, high-risk group, and non-tumor samples. (E) Relative gene expression of AL365203.2 among the low-risk group, high-risk group, and non-tumor samples. (F) Relative gene expression of AC099850.3 among the low-risk group, high-risk group, and non-tumor samples. (G) Relative gene expression of AL031985.3 among the low-risk group, high-risk group, and non-tumor samples. (H) Relative gene expression of AL117336.3 among the low-risk group, high-risk group, and non-tumor samples.
fact, the five-year survival rate was 62.2% in the low-risk group and only 34.1% in the high-risk group (Fig. 2B). We further employed an ROC curve to evaluate the predictive accuracy of the combined six-lncRNA signature. Interestingly, the results indicate that this signature outperforms other clinical parameters, such as age, sex, grade, and TNM staging, in terms of prediction. The risk score system achieved an AUC of 0.779 (Fig. 2C).

Correlation between LncRNA Signature and Clinical Characteristics

To assess the correlation between patients’ clinical indicators and outcomes based on their risk score, we conducted a stratified analysis on a total of 373 samples obtained from the TCGA cohort (Table 3). Moreover, a univariate analysis highlighted a significant association between TNM staging, as well as the six-lncRNA signature, and OS.

Table 3. Relationship between the risk score of the lncRNA signature for OS and clinical features.

| Clinical Feature | Low risk/high risk | Pearson χ² | P   |
|-----------------|--------------------|------------|-----|
| Age             |                    |            |     |
| ≥ 55            | 119/116            | 0.072      | 0.788 |
| < 55            | 53/55              |            |     |
| Gender          |                    |            |     |
| Female          | 47/63              | 3.565      | 0.059 |
| Male            | 125/108            |            |     |
| TNM stage       |                    |            |     |
| I/II            | 130/108            | 8.404      | 0.004 |
| III/IV          | 30/53              |            |     |
| G               |                    |            |     |
| G1/G2           | 123/91             | 12.032     | 0.001 |
| G3/G4           | 47/77              |            |     |
| AFP (ng/ml)     |                    |            |     |
| ≥ 20            | 58/94              | 5.917      | 0.015 |
| < 20            | 64/57              |            |     |
| BMI             |                    |            |     |
| ≥ 25            | 80/73              | 0.213      | 0.645 |
| < 25            | 81/82              |            |     |
| Race            |                    |            |     |
| White           | 83/86              | 0.268      | 0.605 |
| Asian           | 77/71              |            |     |
with overall survival (OS). Furthermore, through multivariate analysis, T staging along with the six-lncRNA signature emerged as potential independent prognostic factors (Fig. 3A and 3B). Additionally, we investigated the relationship between the expression of the six lncRNAs and tumor stage as well as tumor grade. Our findings indicated a strong connection between the expression of AC009005.1, AC099850.3, AL031985.3, and MSC−AS1 with clinical grade. Additionally, AC009005.1 and AC099850.3 displayed associations with TNM staging, while AC009005.1, AC099850.3, and AL031985.3 levels were found to be correlated with T staging (Fig. 3C-3E).

**Gene Set Enrichment Analysis**

The utilization of GSEA was employed in order to investigate the potential connection between biomarkers and biological processes based on the risk score. As depicted in Fig. 4A-4B, GSEA data exhibited that protein–coding genes coexpressed within the high-risk group demonstrated significant enrichment in the reference gene sets "immune response" and "immune system process" (with FDR q-value < 0.25). Additionally, Fig. 4C-4L showcases the top ten biological processes that were highly enriched, indicating that the coexpressed genes associated with the six-lncRNA signature potentially participate in various cellular activities such as cell cycle phase transition, RNA splicing, regulation of chromosome organization, etc.

**Principal Component Component Analysis**

Principal component analysis (PCA) was conducted to aggregate samples based on gene expression patterns in two groups at risk. By reducing multiple indices and extracting main parameters, tumor samples were examined at four levels: lncRNA signature, immune lncRNAs, immune genes, and all genes. Sample data from the high-risk group is depicted by red points on the graph, while green points represent the low-risk group. Our analysis
Fig. 4. Highly enriched biological pathways for corresponding immune genes of six-lncRNA signature in TCGA. (A) Regulation of cell cycle phase transition (FDR < 0.007), (B) RNA splicing (FDR < 0.004), (C) mRNA CIS splicing via spliceosome (FDR < 0.002) (D) Positive regulation of mRNA processing (FDR < 0.002) (E) Regulation of cell cycle G1 S phase transition (FDR < 0.002) (F) ATP dependent chromatin remodeling (FDR < 0.002) (H) Regulation of response to DNA damage stimulus (FDR < 0.002) (I) Regulation of chromosome organization (FDR < 0.002) (J) Recombinational repair (FDR < 0.001) (K) Immune response (FDR < 0.203) (L) Immune system process (FDR < 0.087).

Fig. 5. Principal component analysis of samples for TCGA. (A) PCA shows samples divisibility from the low risk- and high-risk group based on all gene expression. (B) PCA shows samples divisibility from the low risk- and high-risk group based on immune gene expression. (C) PCA shows samples divisibility from the low risk- and high-risk group based on immune lncRNA expression. (D) PCA shows samples divisibility from the low risk- and high-risk group based on lncRNA signature expression.
revealed that the six-lncRNA signature exhibited the highest degree of separation among the four levels (Fig. 5A-5D). These findings partially support the prognostic accuracy of the immune lncRNA model. Additionally, we assessed the expression profiles of these six lncRNAs in pan-cancers using the Lnc2Catlas database, and the resulting boxplots are displayed in Fig. 6.

**Discussion**

Long noncoding RNA has drawn widespread attention as a potential molecular target for cancer diagnosis and treatment in recent years. Multomics has developed rapidly following the application of high-throughput screening to cancer diagnosis and therapy [36-38]. Several studies have proposed that long noncoding RNAs are involved in tumor genesis and progression and that they contribute to the immune system [39-41]. For example, Jiang et al. demonstrated that a high level of lnc-EGFR in Tregs could enhance immunosuppression in hepatoma cells via activating AP-1/NF-AT1signaling [42]. Moreover, immunotherapy has advanced dramatically, the administration of anti-PD-1 antibodies has shown definite efficacy in virus-associated hepatocellular carcinoma [43, 44]. In this study, the potential role of immune lncRNAs as prognostic or therapeutic biomarkers was examined, and this finding may provide some insights for further research. From the TCGA dataset, we discovered a predictive immune pattern consisting of six lncRNAs: MSC−AS1, AC009005.1, AL117336.3, AL365203.2, and AC099850.3. We subsequently confirmed its predictive significance for patients with HCC. The current investigation categorized patients into low-risk and high-risk groups according to the risk score of the previously mentioned lncRNAs. Univariate Cox analysis and multivariate Cox analysis were conducted to assess the suitability of these biomarkers. HCC was classified based on the TNM classification of the American Joint Society.
Committee on Cancer (AJCC) [45]. In addition, the AUC of the ROC system was higher than that of TNM classification and other indices, proving the validity of the proposed risk system as a predictor of overall survival independently. Several studies have indicated that MSC-AS1 is associated with the tumor microenvironment (TME) and can be utilized to evaluate the impact of immunotherapy and prognosticate hepatocellular carcinoma patients [46]. Moreover, the overexpressions of IncRNA AC0099850.3 and AL365203.2 in HCC tumor tissue is correlated with an increased presence of a proinflammatory senescence-associated secretory phenotype (SASP), enhanced infiltration of regulatory T (Treg) cells, and reduced infiltration of naïve B cells [47]. Xing et al. demonstrated that patients with cervical squamous cell carcinoma and endocervical adenocarcinoma patients who exhibited down-regulated expression levels of lncRNAs AL117336 showed increased infiltration of immune cells and expression of various immune checkpoints. These findings suggest that these patients may derive greater benefits from immune checkpoint blockade therapy [48]. Additionally, IncRNA AC0099850.3 was found to promote malignant behavior in HCC cells, and analysis of immune cell infiltration revealed a positively correlation between AC099850.3 and T follicular helper cells, M0 macrophages, CD4+ memory T cells, and memory B cells [49]. Alpha-fetoprotein (AFP) is extensively employed in clinical practice for the diagnosis of liver cancer and assessment of treatment effectiveness [50-52]. In a physically normal human body, AFP expression remains minimal due to its synthesis by the fetal liver, which is subsequently downregulated after birth [53]. Notably, significant alternations in AFP serological levels are observed in approximately half of liver malignancies [54]. A concentration exceeding 400 ng/ml may suggest the presence of liver cancer [55]. Nevertheless, elevated AFP concentrations have also been documented in various other conditions, including cerebellar ataxia and testicular cancer [56, 57]. In the current stratified analysis, samples with AFP levels higher than 20 ng/ml (pathological threshold) were roughly concentrated in the high-risk group. These results inspired us that the expression of this lncRNA signature can be used together with serum AFP as an indicator for early diagnosis, efficacy assessment, and prognostic evaluation. In recent years, significant efforts have been made to examine IncRNAs in cancer immunology. Extensive research has been conducted to uncover the mechanism by which IncRNAs regulate the immune response [58]. For instance, IncRNA NKILA plays a crucial role in boosting immune evasion by associating STAT1 and NF-κB signaling in various tumors [59, 60]. LncRNA ID2-AS1 inhibits HCC metastasis by activating the HDAC8/ID2 pathway [61]. In this study, six prognostic biomarkers showed high expression levels in tumor samples. Analysis using GSEA revealed the enrichment of corresponding genes in a range of GO biological process pathways, such as the cell cycle, RNA splicing, DNA repair, and chromosome regulation. However, further investigation is required to determine the specific roles of these lncRNAs in regulating biological pathways.

Conclusion

We have successfully devised an immune model comprising of six lncRNAs to forecast the outcome of LIHC samples. Our study extensively examined the bioinformatics analysis to evaluate the functionality and efficacy of this lncRNA signature. The results derived from the observations suggest that this signature holds the potential to introduce a novel method for precise diagnosis and prognosis. Nevertheless, it is crucial to conduct clinical trials and functional tests in order to comprehend the underlying mechanism before considering its applicability in a clinical setting.

Abbreviations

ACT: adoptive cellular immunotherapy, AFP: alpha-fetoprotein, AIC: Akaike information criterion, AJCC: American Joint Committee on Cancer, AUC: area under the curve, BMI: body mass index, CTLA4: cytotoxic T-lymphocyte antigen-4, FPKM: fragments per kilobase of exon model per million mapped fragments, GO: gene ontology, GSEA: gene set enrichment analysis, LIHC: liver hepatocellular carcinoma, lncRNA: long non-coding RNA, OS: overall survival, PD-1: programmed cell death-1, ROC: receiver operating characteristic, PCA: principal component analysis, TCGA: The Cancer Genome Atlas, TME: tumor microenvironment, Treg cells: regulatory T cells.

Acknowledgments

This study was supported by: (1) "Special Project for Scientific Research and Cultivation of Young Physician" of Gusu School, Nanjing Medical University (GSKY20230503) to Rui Kong. (2) Shanghai "Rising Stars of Medical Talent" Youth Development Program-Outstanding Youth Medical Talents (No.SHWJRS2021-99) to Jie Lu. (3) Shanghai Pudong New Area Science and Technology Commission (No.PKJ2021-Y10) to Jie Lu. (4) Specialty Feature Construction Project of Pudong Health and Family Planning Commission of Shanghai (PWZzb2022-14) to Jie Lu.

Authors Contributions

Rui Kong and Nan Wang performed the data analyses. Rui Kong and Jie Lu wrote and revised the manuscript. Jie Lu and Chunli Zhou designed the study. All the authors have read and approved the final manuscript.

Conflict of Interest

The authors have no financial conflicts of interest to declare.
References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71: 209-249.

2. Takayama T, Sekine T, Makuuchi M, Yamatsuka S, Kosuge T, Tamamoto J, et al. 2000. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet 355: 151-156.

3. Dimitroulis D, Damaskos C, Valsami S, Davakos S, Garmpas N, Spartalis E, et al. 2017. From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J. Gastroenterol. 23: 5282-5294.

4. Brux J, Sherman M. 2011. Management of hepatocellular carcinoma: an update. Hepatology 53: 1020-1022.

5. Forner A, Reig M, Bruix J. 2018. Liver cancer. Lancet 391: 1301-1314.

6. Sangro B, Sarobe E, Hervas-Stubs S, Meler O. 2021. Advances in immunotherapy for hepatocellular carcinoma. Nat. Rev. Gastroenterol. Hepatol. 18: 525-543.

7. Kairaluoma V, Kemi N, Huhta H, Pohjanen VM, Helminen O. 2021. Toll-like receptor 5 and 8 in hepatocellular carcinoma. J. Immunol. 199: 450-456.

8. Ungerleider N, Song K, Zhang J, Han C, Wang Y, Unfried JP, Marin-Baquero M, et al. 2022. Landscape of transcription in human cells. Nat. Cell Biol. 24: 101-108.

9. Rosenberg SA, Restifo NP. 2015. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348: 62-68.

10. Forner A, Reig M, Bruix J. 2018. Hepatocellular carcinoma. Lancet 392: 123-134.

11. Rosenberg SA, Restifo NP. 2015. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348: 62-68.

12. Wang YL, Liu JY, Yang JE, Yu XM, Chen ZL, Chen YJ. 2019. Lnc-UCID promotes G1/S transition and hepatoma growth by inhibiting CUL4A-mediated ubiquitination of LATS1. J. Hematol. Oncol. 12: 91-108.

13. Pieter D, Nunez NG, Pinyol R, Govaere O, Pinter M, Saydowska M, et al. 2021. NASH limits tumour surveillance in immunotherapy-treated HCC. Nature 599: 450-456.

14. DiStefano JK. 2017. Long noncoding RNAs in the initiation, progression, and metastasis of hepatocellular carcinoma. J. Hepatol. 67: 101-108.

15. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Libbrecht L, et al. 2012. Landscape of transcription in human cells. Nat. Genet. 44: 573-581.

16. Wang YL, Liu JY, Yang JE, Yu XM, Chen ZL, Chen YJ. 2019. Lnc-UCID promotes G1/S transition and hepatoma growth by inhibiting CUL4A-mediated ubiquitination of LATS1. J. Hematol. Oncol. 12: 91-108.

17. Ge Set Enrichment Analysis. http://software.broadinstitute.org/gsea/msigdb/index.jsp. Accessed 02, November, 2022.

18. Ensemble website. http://asia.ensembl.org/index.html. Accessed 26 October, 2022.

19. The Cancer genome atlas. https://portal.gdc.cancer.gov/. Accessed 26 October, 2022.

20. Zhang J, Han C, Song K, Chen W, Unferleider N, Yao L, 2020. The long-noncoding RNA MALAT1 regulates TGF-beta/Smad signaling through formation of an IncRNA-protein complex with Smads, SETD2 and PPM1A in hepatic cells. PLoS One 15: e228160.

21. Lin C, Xiang Y, Sheng J, Liu S, Cui M, Zhang X. 2020. Long non-coding RNA CRNDE promotes malignant progression of hepatocellular carcinoma. J. Mol. Med. 98: 527-536.

22. Wang BG, Lv Z, Ding HX, Fang XX, Wen J, Xu Q, 2018. The association of lncRNA-HULC polymorphisms with hepatocellular carcinoma. J. Natl. Cancer Inst. 110: 1129-136.

23. Zhang J, Han C, Song K, Chen W, Unferleider N, Yao L, 2020. The long-noncoding RNA MALAT1 regulates TGF-beta/Smad signaling through formation of an IncRNA-protein complex with Smads, SETD2 and PPM1A in hepatic cells. PLoS One 15: e228160.

24. Jieblai S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. 2012. Landscape of transcription in human cells. Nature 489: 101-108.

25. Forner A, Reig M, Bruix J. 2018. Hepatocellular carcinoma. Lancet 392: 123-134.

26. Sangro B, Sarobe E, Hervas-Stubbs S, Meler O. 2021. Advances in immunotherapy for hepatocellular carcinoma. Nat. Rev. Gastroenterol. Hepatol. 18: 525-543.

27. Kairaluoma V, Kemi N, Huhta H, Pohjanen VM, Helminen O. 2021. Toll-like receptor 5 and 8 in hepatocellular carcinoma. J. Immunol. 199: 450-456.

28. Ungerleider N, Song K, Zhang J, Han C, Wang Y, Unfried JP, Marin-Baquero M, et al. 2022. Landscape of transcription in human cells. Nat. Cell Biol. 24: 101-108.

29. Rosenberg SA, Restifo NP. 2015. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348: 62-68.

30. Forner A, Reig M, Bruix J. 2018. Liver cancer. Lancet 391: 1301-1314.

31. The Cancer genome atlas. https://portal.gdc.cancer.gov/. Accessed 26 October, 2022.

32. Ensemble website. http://asia.ensembl.org/index.html. Accessed 26 October, 2022.

33. Gene Set Enrichment Analysis. http://software.broadinstitute.org/gsea/msigdb/index.jsp. Accessed 02, November, 2022.

34. Ungerleider N, Song K, Zhang J, Han C, Wang Y, Unfried JP, Marin-Baquero M, et al. 2022. Landscape of transcription in human cells. Nat. Cell Biol. 24: 101-108.

35. Forner A, Reig M, Bruix J. 2018. Liver cancer. Lancet 391: 1301-1314.

36. Sangro B, Sarobe E, Hervas-Stubs S, Meler O. 2021. Advances in immunotherapy for hepatocellular carcinoma. Nat. Rev. Gastroenterol. Hepatol. 18: 525-543.

37. Kairaluoma V, Kemi N, Huhta H, Pohjanen VM, Helminen O. 2021. Toll-like receptor 5 and 8 in hepatocellular carcinoma. J. Immunol. 199: 450-456.

38. Ungerleider N, Song K, Zhang J, Han C, Wang Y, Unfried JP, Marin-Baquero M, et al. 2022. Landscape of transcription in human cells. Nat. Cell Biol. 24: 101-108.

39. DiStefano JK. 2017. Long noncoding RNAs in the initiation, progression, and metastasis of hepatocellular carcinoma. J. Hepatol. 67: 101-108.
43. Liu X, Li M, Wang X, Dang Z, Jiang Y, Wang X. 2019. PD-1+ TIGIT+ CD8+ T cells are associated with pathogenesis and progression of patients with hepatitis B virus-related hepatocellular carcinoma. *Cancer Immunol. Immunother.* 68: 2041-2054.

44. McLane LM, Abdel-Hakeem MS, Wherry EJ. 2019. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu. Rev. Immunol.* 37: 457-495.

45. Matos LL, Dedivitis RA, Kulcsar M, de Mello ES, Alves V, Cernea CR. 2017. External validation of the AJCC Cancer Staging Manual, 8th edition, in an independent cohort of oral cancer patients. *Oral Oncol.* 71: 47-53.

46. Zhou P, Lu Y, Zhang Y, Wang L. 2021. Construction of an immune-related six-lncRNA signature to predict the outcomes, immune cell infiltration, and immunotherapy response in patients with hepatocellular carcinoma. *Front. Oncol.* 11: 661758.

47. Gao C, Zhou G, Cheng M, Feng J, Cao P, Zhou G. 2022. Identification of senescence-associated long non-coding RNAs to predict prognosis and immune microenvironment in patients with hepatocellular carcinoma. *Front. Genet.* 13: 956094.

48. Zhou J, Xu L, Zhou H, Wang J, Xing X. 2023. Prediction of prognosis and chemotherapeutic sensitivity based on cuproptosis-associated lncRNAs in cervical squamous cell carcinoma and endocervical adenocarcinoma. *Front. Genet.* 13: 956094.

49. Zhong F, Liu S, Hu D, Chen L. 2022. LncRNA AC099850.3 promotes hepatocellular carcinoma proliferation and invasion through PRR11/P3K/PI3K/AKT axis and is associated with patients prognosis. *J. Cancer* 13: 1048-1060.

50. Bird TG, Dimitropoulou P, Turner RM, Jenks SJ, Cusack P, Hey S, et al. 2016. Alpha-fetoprotein detection of hepatocellular carcinoma leads to a standardized analysis of dynamic AFP to improve screening based detection. *PLoS One* 11: e0156801.

51. Johnson P, Zhou Q, Dao DY, Lo Y. 2022. Circulating biomarkers in the diagnosis and management of hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* 19: 670-681.

52. Tzartzeva K, Obr J, Rich NE, Parikh ND, Marrerro JA, Yopp A. 2018. Surveillance imaging and alpha-fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 154: 1706-1718.

53. Galle PR, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, et al. 2019. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. *World J. Gastroenterol.* 15: 7068-7072.

54. Tzartzeva K, Obr J, Rich NE, Parikh ND, Marrerro JA, Yopp A. 2018. Surveillance imaging and alpha-fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 154: 1706-1718.

55. Sterling RK, Wright EC, Morgan TR, Seef L, Hoefs JC, Di Bisceglie AM, et al. 2012. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am. J. Gastroenterol.* 107: 64-74.

56. Bennett CL, Dastidar SG, Ling SC, Malik B, Asher T, Wadlova M, et al. 2018. Senataxin mutations elicit motor neuron degeneration phenotypes and yield TDP-43 mislocalization in ALS4 mice and human patients. *Acta Neuropathol.* 136: 425-443.

57. Wymers KM, Daneshmand S, Pierroz PM, Pearce SM, Harris KT, Eggener SE. 2017. Mildly elevated serum alpha-fetoprotein (AFP) among patients with testicular cancer may not be associated with residual cancer or need for treatment. *Ann. Oncol.* 28: 899-902.

58. Sun W, Yang Y, Xu C, Guo J. 2017. Regulatory mechanisms of long noncoding RNAs on gene expression in cancers. *Cancer Genet.* 261-267: 105-110.

59. Huang D, Chen J, Yang L, Ouyang Q, Li J, Liao L, et al. 2018. NKILA lncRNA promotes tumor immune evasion by sensitizing T cells to activation-induced cell death. *Nat. Immunol.* 19: 1112-1125.

60. Wu W, Chen F, Cui X, Yang L, Chen J, Zhao L, et al. 2018. LncRNA NKILA suppresses TGF-beta-induced epithelial–mesenchymal transition by blocking NF-κB signaling in breast cancer. *Int. J. Cancer* 143: 2213-2224.

61. Zhou Y, Huan L, Wu Y, Bao C, Chen B, Wang L. 2019. LncRNA ID2-AS1 suppresses tumor metastasis by activating the HDAC8/ID2 pathway in hepatocellular carcinoma. *Cancer Lett.* 469: 399-409.