Original Paper

Identification of Potential Prognostic Long Non-Coding RNA Biomarkers for Predicting Survival in Patients with Hepatocellular Carcinoma

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Key Words

Lncrna • Hepatocellular carcinoma • Prognostic biomarker • TCGA • Bioinformatics analysis

Abstract

Background/Aims: The aim of the current study was to identify potential prognostic long non-coding RNA (lncRNA) biomarkers for predicting survival in patients with hepatocellular carcinoma (HCC) using The Cancer Genome Atlas (TCGA) dataset and bioinformatics analysis.

Methods: RNA sequencing and clinical data of HCC patients from TCGA were used for prognostic association assessment by univariate Cox analysis. A prognostic signature was built using stepwise multivariable Cox analysis, and a comprehensive analysis was performed to evaluate its prognostic value. The prognostic signature was further evaluated by functional assessment and bioinformatics analysis. **Results:** Thirteen differentially expressed lncRNAs (DELs) were identified and used to construct a single prognostic signature. Patients with high risk scores showed a significantly increased risk of death (adjusted $P < 0.0001$, adjusted hazard ratio = 3.522, 95% confidence interval = 2.307–5.376). In the time-dependent receiver operating characteristic analysis, the prognostic signature performed well for HCC survival prediction with an area under curve of 0.809, 0.782 and 0.79 for 1-, 3- and 5-year survival, respectively. Comprehensive survival analysis of the 13-DEL prognostic signature suggested that it serves as an independent factor in HCC, showing a better performance for prognosis prediction than

traditional clinical indicators. Functional assessment and bioinformatics analysis suggested that the prognostic signature was associated with the cell cycle and peroxisome proliferator-activated receptor signaling pathway. **Conclusions:** The novel lncRNA expression signature identified in the present study may be a potential biomarker for predicting the prognosis of HCC patients.

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Introduction

Liver cancer is prevalent in China and has become the fourth leading cause of male cancer-related death [1]. The global cancer statistics demonstrate that the number of new cases and deaths from liver cancer in China account for more than half the worldwide total [2]. Most liver cancers are diagnosed as hepatocellular carcinoma (HCC) [3]. Hepatocarcinogenesis is driven by the interaction of genetic and environmental factors [4, 5]. The main environmental factors associated with HCC are hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection and alcohol abuse [4]. Well-known genetic factors include the *TP53* mutation, *CTNNB1* mutation, methylation and dysregulation of cancer-relevant genes [4, 5]. The development of high-throughput technologies enabled researchers to investigate a variety of approaches for identifying the genetic factors associated with HCC through genome-wide screening, such as genome-wide association studies [6], genome-wide expression profiles [7] and comprehensive analysis of multiple genome-wide approaches [8].

Recent research advances support that the proportion of the genome termed non-coding RNAs (ncRNAs) reflects the complexity of the genome in higher eukaryotes [9, 10]. Among ncRNAs, long non-coding RNA (lncRNA), have been studied extensively and shown to play indispensable roles in many biological processes, and increasing evidence suggests that dysregulated lncRNAs are associated with HCC tumorigenesis, metastasis, and prognosis [11-13].

A growing number of studies demonstrate that aberrantly expressed lncRNAs serve as potential biomarkers for predicting HCC prognosis [14, 15]. Therefore, systematic efforts to investigate an expression-based lncRNA signature for prognosis prediction in patients with HCC are necessary. Genome-wide screening analysis is an effective method for the identification of the potential biomarkers for HCC diagnosis and prognosis prediction. The Cancer Genome Atlas (TCGA) is a project aimed at generating multi-dimensional maps of the key genomic changes in 33 types of cancer to discover potential genetic biomarkers for cancer prevention, diagnosis, and therapy based on comprehensive genomic data analysis [16]. The aim of the present study was to identify potential prognostic lncRNA biomarkers for predicting survival in patients with HCC using TCGA datasets and bioinformatics analysis.

Materials and Methods

Data source

An RNA sequencing (RNA-Seq) dataset of 371 HCC patients was obtained from TCGA data portal (<https://portal.gdc.cancer.gov/>, accessed November 5, 2017), including 374 HCC tumor tissues and 50 adjacent normal liver tissues [8]. The corresponding clinical information of TCGA HCC patients was download from the University of California Santa Cruz Xena browser (UCSC Xena: <http://xena.ucsc.edu/>, accessed November 5, 2017). Because the data were downloaded from TCGA, study approval by an ethics committee was not necessary. Data acquisition and application complied with TCGA publication guidelines and data access policies.

Screening of differentially expressed lncRNAs

Genome-wide lncRNA expression profiles were obtained from the RNA-Seq dataset of TCGA, which consists of mRNA and lncRNA expression data. lncRNAs with mean values of >1 were included in the differentially expressed lncRNA (DEL) screening. The *edgeR* [17] and *DESeq* [18] packages of the R platform

were used for DEL screening. A false discovery rate (FDR) < 0.05 and $|\log_2 \text{fold change} (\log_2 \text{FC})| \geq 1$ were identified as DELs in the comparison of expression levels between HCC tumor and adjacent normal liver tissues. The overlapping DELs between *edgeR* and *DESeq* were used for further analysis.

Construction of the DEL expression-based prognostic signature

The survival information of one HCC patient from TCGA was unknown, therefore, 370 HCC patients were finally included in the survival analysis. A univariate Cox regression analysis was performed using the *survival* package to examine the association between the expression levels of DELs and the overall survival (OS) of patients with HCC. DELs with a *P*-value < 0.001 were considered prognostic DELs with expression levels significantly associated with OS. Prognostic DELs with a *P*-value of < 0.001 in the survival analysis were further assessed using the “*step*” function to select the optimal combination. The prognostic DELs selected by the “*step*” function were used for construction of the prognostic model. The relative contribution of these prognostic DELs in HCC survival prediction was evaluated by fitting them into a multivariate Cox regression analysis with OS as the dependent variable. A DEL expression-based prognostic risk score model was constructed using the linear combination of the expression levels of DELs with the multivariate Cox regression coefficient (β) as the weight. The risk score formula was as follows: risk score = expression of $\text{DEL}_1 \times \beta_1 \text{DEL}_1 + \text{expression of } \text{DEL}_2 \times \beta_2 \text{DEL}_2 + \dots \text{expression of } \text{DEL}_n \times \beta_n \text{DEL}_n$ [19-21]. This DEL prognostic model was used to divide HCC patients into high- and low- risk groups using the median score of the DEL expression-based risk scores. A time-dependent receiver operating characteristic (ROC) curve was generated using the *survivalROC* package in the R platform to evaluate the accuracy of the DEL expression-based prognostic signature for predicting OS in HCC [19].

Comprehensive analysis of the DEL expression-based prognostic signature

The prognostic model was evaluated by performing a comprehensive analysis of the DEL expression-based prognostic signature. The expression levels of the prognostic DELs were compared between the low- and high-risk groups. In addition, the predictive value of the risk score for clinical status was investigated. Stratified analysis and joint effect analysis were performed to investigate the association between the risk score and clinical status in HCC.

Functional assessment and bioinformatics analysis

Generally, lncRNAs do not encode proteins, and their function is associated with coexpressed protein-coding genes (PCGs) [22, 23]. The coexpression relationship between prognostic DELs and PCGs was evaluated using Pearson's correlation coefficient. Coexpressed PCGs were subjected to functional enrichment analysis to predict the biological function of the prognostic DELs using the Database for Annotation, Visualization, and Integrated Discovery version 6.7 (DAVID v6.7, <https://david.ncifcrf.gov/>, accessed November 5, 2017), which is a widely used bioinformatics resource [24-26]. GO terms and KEGG pathways with a *P*-value < 0.05 and enrichment score > 1.0 were considered as significantly enriched functional annotations.

The mRNA expression data of the PCGs were obtained from the same TCGA HCC patients and the same RNA-Seq dataset. Moreover, data processing of the PCG mRNA dataset was performed in the same manner as that of the lncRNA dataset, which was normalized by DESeq packages in the R platform. Prognostic DELs and PCGs with a Pearson correlation coefficient of > 0.4 were considered lncRNA-correlated PCGs, and used for coexpression network construction and prognostic DEL functional assessment. A coexpression network between prognostic DELs and the corresponding PCGs was constructed using Cytoscape software (version 3.4.0; <http://www.cytoscape.org/>, accessed November 5, 2017) [27].

Gene set enrichment analysis (GSEA v2-2.2.3: <http://software.broadinstitute.org/gsea/index.jsp>; accessed November 5, 2017) was performed to investigate the potential mechanisms underlying the effect of the risk score on HCC prognosis [28, 29]. The Molecular Signatures Database (MSigDB) of c2 (c2.cp.kegg.v6.1.symbols.gmt) and c5 (c5.all.v6.1.symbols.gmt) was used to assess the functional differences between the low- and high-risk groups [30]. The enrichment gene sets in the GSEA that reached a nominal *P*-value < 0.05 and FDR < 0.25 were considered significant.

Statistical analysis

FDRs in *edgeR*, *DESeq*, and GSEA were adjusted for multiple testing with the Benjamini–Hochberg procedure to control FDR [31–33]. Univariate analysis of clinical features and OS was performed using the log-rank test; those clinical features with $P < 0.05$ were included in the multivariate Cox proportional hazards regression model for adjustment. Coexpression relationships between prognostic DELs and PCGs were assessed using the Pearson correlation coefficient. Volcano plots and heat maps were drawn by the *ggplot2* package in the R platform. A P -value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) and R 3.3.0.

Results

DEL screening

Comprehensive gene annotation was downloaded from the Ensembl Genomes (Homo_sapiens.GRCh38.90.gtf: <http://www.ensembl.org/index.html>, accessed November 5, 2017) [34, 35]. Of the 14,447 lncRNAs obtained from the RNA-Seq dataset, 6,938 lncRNAs met the criterion of a mean value >1 and were included in the DEL screening. In the present study, 2626 lncRNAs were identified as DELs using *edgeR* (Fig. 1A, Table S1), whereas, 842 lncRNAs were identified as DELs using *DESeq* (Fig. 1B, Table S2). For all supplemental material see www.karger.com/doi/10.1159/000492507. The 827 DELs that overlapped in the *edgeR* and *DESeq* screening were used for further analysis (Fig. 1C). The heat map of the 827 DELs is shown in Fig. 2.

Construction of the
DEL-based prognostic
signature

A univariate Cox regression analysis was performed to examine the association between DELs and the OS of patients with HCC. The results are shown in Table S3. Forty-seven DELs with a P -value < 0.001 were regarded as prognostic DELs and selected using the “step” function to screen for the

Fig. 1. Analysis of differentially expressed lncRNAs (DELs). (A) DELs identified using the *edgeR* package; (B) DELs identified using the *DESeq* package; (C) Overlapping DELs.

Table 1. The detailed information of 13 prognostic DELs and prognosis analysis in patients with HCC. Notes: [‡]Derived from the univariate Cox proportional hazards regression analysis in 370 HCC patients cohort; [§]Derived from the multivariate Cox proportional hazards regression analysis in 370 HCC patients cohort. Abbreviations: DEL, differentially expressed lncRNAs; HCC, hepatocellular carcinoma.

Ensemble ID	Gene name	Hazard ratio [‡]	P-value [‡]	Coefficient [§]
ENSG00000264016	AC015908.3	0.778685	1.02E-06	-0.1935
ENSG00000237424	FOX2-AS1	1.264965	0.000723	0.1969
ENSG00000267751	AC009005.1	1.304236	3.79E-05	0.1459
ENSG00000251165	F11-AS1	0.854905	0.000398	0.1081
ENSG00000230587	AC093609.1	0.798914	0.000701	-0.1774
ENSG00000271270	TMCC1-AS1	1.561787	6.16E-08	0.3089
ENSG00000257732	AC089983.1	1.284594	0.000198	0.1706
ENSG00000227195	MIR663AHG	1.129568	0.000696	-0.0803
ENSG00000254290	AC124067.4	1.119317	0.000746	0.1108
ENSG00000276399	FLJ36000	1.229365	8.22E-05	0.1185
ENSG00000235356	AL592466.1	1.262443	0.000686	-0.1715
ENSG00000233571	AL592043.1	1.434065	1.17E-06	0.1593
ENSG00000248762	AC012339.1	1.342991	0.000219	0.1932

Fig. 2. Heat map of 827 DELs in HCC.

optimal combination. The following 13 prognostic DELs were used for the prognostic model construction (summarized in *Table 1*): AC015908.3, FOXD2-AS1, AC009005.1, F11-AS1, AC093609.1, TMCC1-AS1, AC089983.1, MIR663AHG, AC124067.4, FLJ36000, AL592466.1, AL592043.1, and AC012339.1. Kaplan-Meier curves and a scatter plot of the 13 prognostic DELs are shown in *Fig. 3A–M* and *Fig. 3N*, respectively. The relative contribution of these prognostic DELs to survival prediction was determined using multivariate Cox regression analysis. The risk score formula was as follows: risk score = expression of AC015908.3 $\times$ (−0.1935) + expression of FOXD2-AS1 $\times$ (0.1969) + expression of AC009005.1 $\times$ (0.1459) + expression of F11-AS1 $\times$ (0.1081) + expression of AC093609.1 $\times$ (−0.1774) + expression of TMCC1-AS1 $\times$ (0.3089) + expression of AC089983.1 $\times$ (0.1706) + expression of MIR663AHG $\times$ (−0.0803) + expression of AC124067.4 $\times$ (0.1108) + expression of FLJ36000 $\times$ (0.1185) + expression of AL592466.1 $\times$ (−0.1715) + expression of AL592043.1 $\times$ (0.1593) + expression of AC012339.1 $\times$ (0.1932). The results of the survival analysis of clinical features are summarized in *Table 2*. Tumor stage and radical resection that were significantly associated with HCC OS in the univariate analysis were included in the multivariate Cox proportional hazards regression model for adjustment. Survival analysis indicated that the 13-DEL prognostic signature could separate HCC patients into low-risk and high-risk groups, and HCC patients with high-risk scores had

Fig. 3. Kaplan-Meier curves of overall survival (OS) of 13 prognostic DELs in HCC and their expression in HCC tumor tissues and adjacent normal liver tissues. Notes: The order of Kaplan-Meier curves of 13 prognostic DELs were as follow: (A) AC015908.3, (B) FOXD2-AS1, (C) AC009005.1, (D) F11-AS1, (E) AC093609.1, (F) TMCC1-AS1, (G) AC089983.1, (H) MIR663AHG, (I) AC124067.4, (J) FLJ36000, (K) AL592466.1, (L) AL592043.1, (M) AC012339.1; (N) Scatter plot of 13 prognostic DELs in HCC tumor tissues and adjacent normal liver tissues; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Fig. 4. Prognostic risk score model analysis of 13 prognostic DELs in HCC patients and their expression level in low- and high-risk groups. Notes: (A) From top to bottom are the risk score, patients' survival status distribution, and the expression heat map of 13 prognostic DELs in the low- and high-risk groups. (B) Kaplan-Meier curves for the low- and high-risk groups. (C) ROC curve analysis for predicting survival in HCC patients according to the risk score. (D) Scatter plot of the expression of 13 prognostic DELs expression level in the low- and high-risk groups. *** $P < 0.0001$.

a significantly shorter median survival time (MST) than those with a low-risk score (802 vs. 2532 days for high-risk vs. low-risk, *Table 2*, *Fig. 4A, B*), and significantly increased risk of death (adjusted $P < 0.0001$, adjusted hazard ratio [HR] = 3.522, 95% confidence interval [CI] = 2.307–5.376 for OS), after adjusting for tumor stage and radical resection. Time-dependent ROC analysis showed that the risk score performed well for HCC OS prediction, as the AUC of the time-dependent ROC curve was 0.809, 0.782 and 0.79 for 1-, 3- and 5-year survival prediction (*Fig. 4C*), respectively. The expression level distribution of the 13 prognostic DELs in the low- and high-risk groups are presented in *Fig. 4D*. In addition, we investigated the relationship between the 13-DEL prognostic signature and various clinical features (*Fig. 5A–G*), and the risk score showed a potential predictive value for predicting the status of tumor stage ($P = 0.003$, AUC = 0.605, 95% CI = 0.535–0.676; *Fig. 5B*), histologic grade ($P < 0.0001$, AUC = 0.642, 95% CI = 0.585–0.700; *Fig. 5C*), AFP ($P = 0.002$, AUC = 0.628, 95% CI = 0.550–0.707; *Fig. 5D*), radical resection ($P = 0.007$, AUC = 0.630, 95% CI = 0.545–0.715; *Fig. 5E*) and micro vascular invasion ($P = 0.008$, AUC = 0.591, 95% CI = 0.524–0.657; *Fig. 5F*).

Stratified and joint effects analysis

To investigate the prognostic prediction value of the 13-DEL prognostic signature under different clinical conditions, stratified and joint effects analyses were performed after adjusting for tumor stage and radical resection. In the stratified analysis, high-risk scores predicted a significantly increased risk of death in patients with all favorable strata and all adverse strata except in patients without radical resection and serum AFP > 400 ng/mL (*Fig. 6A*), indicating that the risk score is independent from clinical information. To facilitate the use of the risk score, a survival nomogram was plotted (*Fig. 6B*) based on 155 patients with complete clinical information and considering the risk scores and the clinicopathological observations. Consistent with the survival analysis, the risk score contributed the greatest number of risk points (ranged 0–100), whereas the other clinical information had a considerably lower

Table 2. Distribution of HCC patients' characteristics and prognosis analysis. Notes: ^a Information of alcohol consumption was unavailable in 19 patients; ^b Information of Ishak fibrosis score was unavailable in 159 patients; ^c Information of tumor stage was unavailable in 24 patients; ^d Information of histologic grade was unavailable in 5 patients; ^e Information of serum AFP was unavailable in 93 patients; ^f Information of radical resection was unavailable in 7 patients; ^g Information of micro vascular invasion was unavailable in 56 patients; ^h Information of Child-Pugh score was unavailable in 132 patients; Abbreviations: HCC, hepatocellular carcinoma; MST, median survival time; HR, hazard ratio; CI, confidence interval; AFP, α -fetoprotein; MVI, micro vascular invasion.

Variables	Events/total(n=370)	MST (days)	HR (95% CI)	Log-rank P
Age (years)				0.194
≤65	73/232	2465	1	
>65	57/138	1423	1.265(0.893-1.791)	
Sex				0.261
female	51/121	1490	1	
male	79/249	2486	0.817(0.573-1.164)	
Alcohol consumption ^a				0.896
NO	84/234	1694	1	
YES	40/117	1624	1.026(0.703-1.496)	
Ishak fibrosis score ^b				0.872
0 - No Fibrosis	30/74	2131	1	
1,2 - Portal Fibrosis	9/31	1372	0.917(0.429-1.962)	
3,4 - Fibrous Speta	6/28	NA	0.682(0.281-1.654)	
5 - Nodular Formation and Incomplete Cirrhosis	2/9	1386	0.750(0.177-3.167)	
6 - Established Cirrhosis	17/69	NA	0.766(0.418-1.403)	
Tumor stage ^c				<0.0001
I	42/171	2532	1	
II	26/85	1852	1.427(0.874-2.330)	
III+IV	48/90	770	2.764(1.823-4.190)	
Histologic grade ^d				0.748
G1	18/55	2116	1	
G2	60/177	1685	1.181(0.697-2.000)	
G3	43/121	1622	1.233(0.711-2.140)	
G4	5/12	NA	1.693(0.626-4.584)	
Serum AFP (ng/mL) ^e				0.832
≤400	62/213	2456	1	
>400	22/64	2486	1.055(0.645-1.724)	
Radical resection ^f				0.006
R0	110/323	1852	1	
R1+R2+RX	17/40	837	2.030(1.213-3.395)	
MVI ^g				0.154
NO	60/206	2131	1	
YES	36/108	2486	1.351(0.892-2.047)	
Child-Pugh score ^h				0.18
A	59/216	2542	1	
B+C	9/22	1005	1.614(0.796-3.270)	
Risk score				<0.0001
Low	39/185	2532	1	
High	81/185	802	3.929(2.678-5.765)	

Table 3. Joint effects survival analysis of clinical factors and the DELs signature risk score with OS in HCC patients. Notes: ^bInformation of Ishak fibrosis score was unavailable in 159 patients; ^cInformation of tumor stage was unavailable in 24 patients; ^dInformation of histologic grade was unavailable in 5 patients; ^eInformation of serum AFP was unavailable in 93 patients; ^fInformation of radical resection was unavailable in 7 patients; ^gInformation of micro vascular invasion was unavailable in 56 patients; ^hInformation of Child-Pugh score was unavailable in 132 patients; [£]Adjusted for tumor stage and radical resection. Abbreviations: DEL, differentially expressed lncRNA; OS, overall survival; HCC, hepatocellular carcinoma; MST, median survival time; HR, hazard ratio; CI, confidence interval; AFP, α -fetoprotein; MVI, micro vascular invasion.

Group	Risk Score	Variables	Events/total(n=370)	MST (days)	Crude HR (95% CI)	Crude P	Adjusted HR (95% CI)	Adjusted P E
Ishak fibrosis score ^b								
1	Low risk	0	13/47	3125	1		1	
2	Low risk	1/2/3/4/5/6	13/77	NA	0.842(0.374-1.895)	0.678	0.935(0.398-2.196)	0.877
3	High risk	0	17/27	660	4.037(1.871-8.711)	0.0004	3.584(1.600-8.030)	0.002
4	High risk	1/2/3/4/5/6	21/60	1372	2.870(1.348-6.112)	0.006	3.235(1.465-7.144)	0.004
Tumor Stage ^c								
A	Low risk	Stage I	14/100	NA	1		1	
B	Low risk	Stage II	6/38	3258	1.176(0.451-3.0650)	0.74	1.174(0.450-3.062)	0.743
C	Low risk	Stage III+IV	13/34	1622	3.463(1.625-7.381)	0.001	3.431(1.609-7.316)	0.001
D	High risk	Stage I	28/71	1372	4.701(2.451-9.017)	<0.0001	4.643(2.416-8.923)	<0.0001
E	High risk	Stage II	20/47	848	5.724(2.868-11.427)	<0.0001	5.661(2.833-11.310)	<0.0001
F	High risk	Stage III+IV	35/56	419	7.954(4.254-14.872)	<0.0001	7.300(3.852-13.832)	<0.0001
Histologic Grade ^d								
a	Low risk	G1	8/37	2131	1		1	
b	Low risk	G2	18/95	3125	0.900(0.391-2.073)	0.804	1.189(0.437-3.237)	0.735
c	Low risk	G3+G4	10/49	NA	0.885(0.348-2.250)	0.798	1.256(0.428-3.686)	0.678
d	High risk	G1	10/18	581	3.514(1.372-9.005)	0.009	3.764(1.198-11.833)	0.023
e	High risk	G2	42/82	802	4.087(1.912-8.737)	0.0003	4.459(1.734-11.467)	0.002
f	High risk	G3+G4	38/84	899	3.461(1.606-7.458)	0.002	3.920(1.523-10.088)	0.005
Serum AFP (ng/mL) ^e								
I	Low risk	≤400	20/121	2532	1		1	
II	Low risk	>400	6/26	NA	1.112(0.443-2.790)	0.821	1.206(0.478-3.040)	0.691
III	High risk	≤400	42/92	1149	4.113(2.397-7.056)	<0.0001	3.978(2.269-6.976)	<0.0001
IV	High risk	>400	16/38	2486	3.217(1.659-6.237)	0.001	2.671(1.301-5.481)	0.007
Radical resection ^f								
11	Low risk	R0	33/169	3125	1		1	
22	Low risk	R1+R2+RX	6/14	837	4.207(1.752-10.103)	0.001	3.739(1.300-10.753)	0.014
33	High risk	R0	77/154	802	4.337(2.860-6.579)	<0.0001	3.991(2.557-6.230)	<0.0001
44	High risk	R1+R2+RX	11/26	837	4.820(2.418-9.607)	<0.0001	3.692(1.719-7.929)	0.01
MVI ^g								
i	Low risk	NO	23/119	3125	1		1	
ii	Low risk	YES	11/50	3258	1.364(0.663-2.806)	0.399	1.104(0.470-2.595)	0.82
iii	High risk	NO	37/87	1372	3.853(2.248-6.603)	<0.0001	3.608(2.019-6.447)	<0.0001
iv	High risk	YES	25/58	1149	4.144(2.317-7.411)	<0.0001	3.399(1.697-6.807)	0.001
Child-Pugh score ^h								
①	Low risk	A	21/116	3258	1		1	
②	Low risk	B+C	3/13	NA	1.188(0.351-4.024)	0.782	1.240(0.366-4.198)	0.729
③	High risk	A	38/100	1386	3.272(1.883-5.6860)	<0.0001	3.098(1.771-5.420)	<0.0001
④	High risk	B+C	6/9	535	9.017(3.530-23.034)	<0.0001	7.111(2.747-18.408)	<0.0001

contribution.

Joint effects analysis of the 13-DEL prognostic signature and clinical features indicated that the prognostic signature performed well in clinical outcome prediction, and in combination with clinical features, it showed a strong interaction and better predictive value for HCC prognosis (Fig. 7A–G and Table 3).

Functional assessment and bioinformatics analysis

A total of 17, 682 PCGs were extracted from the TCGA HCC patients' dataset and used for coexpression network construction. The Pearson correlation coefficients of 686

Fig. 5. The predictive value of the risk score for HCC clinical parameters. Notes: ROC curve of risk score for clinical parameters: (A) Ishak fibrosis score, (B) tumor stage, (C) histologic grade, (D) serum AFP, (E) radical resection, (F) micro vascular invasion, and (G) Child-Pugh score.

Fig. 6. The relationship between risk score and clinical information. Notes: (A) Stratified analysis of the association between risk score and OS in HCC. (B) Nomogram for predicting the 1-, 3- and 5-year event (death) with risk score and clinical information.

Fig. 7. Joint effects analysis of OS stratified by risk score and HCC clinical parameters. Notes: Joint effects analysis stratified by risk score and following clinical parameters: (A) Ishak fibrosis score, (B) tumor stage, (C) histologic grade, (D) serum AFP, (E) radical resection, (F) micro vascular invasion; (G) Child-Pugh score.

Fig. 8. Co-expression network of 13 prognostic DELs and corresponding lncRNA-correlated PCGs.

Fig. 9. Functional assessment of lncRNA-correlated PCGs of 13 prognostic DELs. Notes: (A) GO term enrichment results. (B) KEGG enrichment results.

lncRNA-correlated PCGs with 738 edges greater than 0.4 were included in the coexpression network (Fig. 8 and Table S4). To investigate the potential functional roles of these 13 prognostic lncRNAs in HCC, we performed functional enrichment analysis of lncRNA-correlated PCGs using DAVID v6.7. GO term enrichment analysis suggested that these lncRNA-correlated PCGs were significantly enriched in 273 GO terms (Table S5), of which cell cycle-related biological processes were frequently enriched (Fig. 9A). Consistent with the GO enrichment analysis, KEGG enrichment analysis confirmed that these lncRNA-correlated PCGs were significantly enriched in the cell cycle pathway. Additionally, the peroxisome proliferator-activated receptor (PPAR) signaling pathway and multiple metabolic pathways were also enriched, including glyoxylate and dicarboxylate metabolism, metabolism of xenobiotics by cytochrome P450, beta-alanine metabolism, and propanoate metabolism (Fig. 9B).

GSEA analysis of the low- and high-risk groups supported the results of functional enrichment analysis for lncRNA-correlated PCGs. Cell cycle-related biological processes and pathways, and the PPAR signaling pathway were significantly enriched (Table S6 and S7; Fig. 10A–F), indicating that the 13-DEL prognostic signature may have a marked influence on these biological processes and pathways in HCC.

Discussion

TCGA is a comprehensive expression database of multiple types of cancer. Several recent studies used the lncRNA dataset from TCGA to investigate the potential use of lncRNAs for cancer diagnosis and prognosis prediction. He et al., Tang et al. and Zang et al. used TCGA lncRNA dataset to identify a lncRNA expression-based prognostic signature for assessing the survival of patients with soft-tissue sarcoma, lung cancer, and colorectal adenocarcinoma [36–38]. TCGA lncRNA datasets of cutaneous melanoma [39], bladder urothelial carcinoma [40], breast cancer [41], clear cell renal cell carcinoma [42], gastric cancer [43, 44], head and neck squamous cell carcinoma [45], and HCC [46] have been used in lncRNA expression-based prognostic signature investigations by other researchers. These studies used a genome-wide screening method to identify potential prognostic lncRNAs and calculated a risk score based on lncRNA expression. This is an effective method to screen for prognostic lncRNAs and investigate their optimal combination for prognosis prediction.

Numerous studies by multiple researchers, who systematically and comprehensively reviewed the existing literature on the role of lncRNAs in HCC indicate that lncRNAs can be dysregulated in HCC and play a critical role in hepatocarcinogenesis, metastasis, as therapeutic targets, and in prognosis or diagnosis [14, 47, 48]. Yang et al. identified numerous recurrently deregulated tumorigenesis- and metastasis-associated lncRNAs, and most of these were experimentally validated and mechanistically linked to cancer development and progression [49]. Yuan et al. reported that a lncRNA associated with microvascular invasion in HCC (lncRNA MVIH) was frequently overexpressed in HCC patients with microvascular

Fig. 10. GSEA results of c2 and c5 MSigDB between low- and high-risk groups. Notes: GSEA results of c2 MSigDB (A–C) and c5 MSigDB (D–F).

invasion, and deregulation of lncRNA MVIH serves as a predictor for poor OS and recurrence-free survival after hepatectomy [12]. Because lncRNAs play important roles in HCC, it is necessary to identify potential lncRNA biomarkers in HCC, and the use of TCGA HCC lncRNA dataset is a reliable method.

In the present study, we identified 827 DELs *via* the *edgeR* and *DESeq* packages, and identified 13 DELs that were suitable for the construction of a prognostic signature through screening with the “*step*” function. The DEL-based prognostic signature performed well in HCC prognosis prediction, and the time-dependent ROC analysis showed that it performed well in 1-, 3-, and 5-year survival prediction. In addition, the DEL-based prognostic signature identified in the present study performed well compared with the previous prognostic signature reported by Wang et al., and the prediction accuracy was significantly improved (AUC in 5-year survival prediction: 0.79 vs. 0.701, respectively) [46]. Consistent with previous findings, AC093609.1 and AC124067.4 were also identified as HCC prognostic DELs in the present analysis [46]. In addition, AC124067.4, also known as RP11-150012.3, was previously identified as a prognostic DEL in a TCGA lncRNA-based prognostic signature investigation in colorectal adenocarcinoma and gastric cancer [38, 44]. A previous study showed that the expression levels of TMCC1-AS1, which was included in the 13-DEL prognostic signature, are associated with OS in patients with HCC [50]. Yu et al. reported that another prognostic DEL, FOXD2-AS1, was significantly differentially expressed in association with the progression of gastric cancer, and high FOXD2-AS1 expression was associated with a significantly increased risk of death [51]. Similar results regarding FOXD2-AS1 were reported in non-small cell lung cancer. Rong et al. showed that high expression of FOXD2-AS1 promotes non-small cell lung cancer progression via Wnt/ β -catenin signaling and is associated with poor OS [52]. A study in colorectal cancer (CRC) also suggested that FOXD2-AS1 was significantly upregulated in tumor tissues and acts as a tumor promoter in CRC, inducing CRC progression by regulating epithelial–mesenchymal transition and the Notch signaling pathway [53]. Transcriptional fingerprint analysis in pancreatic ductal adenocarcinoma (PDAC) demonstrated that F11-AS1 is downregulated in PDAC tumor tissues and cell lines. These results suggest that dysregulated lncRNAs play important roles in PDAC development and progression [54]. A literature review revealed that the remaining prognostic DELs identified in the present study were not reported previously, and their function in HCC and other cancers needs to be further investigated and verified. The present findings have limitations because they were obtained from a single cohort; therefore, external validation cohorts are necessary to confirm the present results.

Survival analysis of the DEL-based prognostic signature in the present study showed a significantly different clinical outcome after stratification by risk score. Stratification according to risk score and clinical features suggest that the prognostic signature constructed in the current study may serve as an independent risk factor for HCC. Joint effects analysis of risk scores and clinical features suggested that the prognostic signature may have a strong interaction with clinical features and serve as an independent factor for HCC prognosis. The nomogram substantiated that the risk score contributed most to HCC prognosis prediction. However, because of the lack of validation cohorts, further studies are needed to confirm our results. Functional analysis of these prognostic DELs have rarely been reported in previous studies, therefore, the mechanism underlying the effect of these prognostic DELs on HCC prognosis still needs in-depth exploration.

Functional assessment of the HCC prognostic DELs in PCGs showed that cell cycle biological processes and pathways were significantly enriched. Previous genome-wide screening studies using lncRNA datasets from GEO and TCGA in lung cancer [55, 56], gastric cancer [44] and breast cancer [57, 58] also identified cell cycle-related lncRNAs. Several studies reported that dysregulation of specific lncRNAs in HCC cell lines caused cell cycle arrest and induced apoptosis, and affected the prognosis of HCC [59, 60]. KEGG enrichment analysis revealed a significant enrichment in the PPAR signaling pathway. Zhang et al. reported that the lncRNA HOTTIP is upregulated and has a high diagnostic value in HCC, and the genes coexpressed with HOTTIP are related to the PPAR signaling pathway [61]. In a genome-wide

analysis of intrahepatic cholangiocarcinoma (iCCA), coexpressed mRNAs of DELs in iCCA tissues were significantly enriched in the PPAR signaling pathway, and KEGG enrichment of DEL target genes was demonstrated in papillary thyroid carcinoma [62, 63]. The most enriched functional terms of the KEGG analysis in coexpressed PCGs were metabolism-related pathways, such as glyoxylate and dicarboxylate metabolism, and metabolism of xenobiotics by cytochrome P450. These metabolism-related pathways are associated with liver function and indicating that dysregulated DELs are involved the function of the liver and may play a role in hepatocarcinogenesis. GSEA of low- and high-risk groups demonstrated that cell cycle and PPAR signaling pathways were significantly enriched, and this was confirmed by the PCG enrichment results. We speculate that the prognostic signature identified in the present study may play a vital role in cell cycle and PPAR signaling pathway regulation in HCC. However, these findings need further experimental verification.

Although TCGA database was previously used to analyze lncRNA-related signatures for HCC prognosis, the present study was different in that it investigated the optimum combination of these prognostic lncRNAs using the “step” function in the R platform [46]. Therefore, the prognostic signature constructed in the present study is more accurate than that identified in a previous study [46]. In addition, comprehensive survival analysis of the present 13-DEL prognostic signature was performed by developing a nomogram, and stratified and joint effects survival analysis was performed to investigate its potential application. The stratified and joint effects analysis of the risk score and clinical features suggested that the 13-DEL prognostic signature was an independent risk factor for HCC, and the associated risk score could serve as a prognostic indicator for HCC patients. The nomogram consisting of the risk score and other clinical information indicated that the contribution of the risk score to HCC prognosis prediction was greater than that of other traditional clinical indicators.

The present study had several limitations. First, the results generated in the present study were derived from a single cohort, and the demographics of the patients from TCGA may not be representative of all patient populations; therefore, the genetic alterations may be biased, and further validation in other HCC cohorts is necessary. Second, the clinical information from TCGA database was incomplete; therefore, we were unable to perform a comprehensive stratification analysis in all strata of HCC, as well as adjust all the features that may affect HCC OS in the Cox proportional hazard regression model.

Despite these limitations, the current study has identified numerous DELs with potential diagnostic and prognostic value for HCC. In addition, we constructed an effective prognostic signature to predict HCC prognosis that may be valuable for clinical application.

Conclusion

Here, we performed a genome-wide screening analysis using an HCC dataset and identified 827 DELs. We then constructed a prognostic signature based on the expression of 13 prognostic DELs. Comprehensive survival analysis of the 13-DEL prognostic signature suggested that the risk score of the prognostic signature serves as an independent factor in HCC and performs better for prognosis prediction than other traditional clinical indicators. The results suggest that the 13-DEL prognostic signature is a potential biomarker for the prognosis of HCC patients, and could be a clinically useful tool that can be easily incorporated into an RNA-Seq clinical sequencing program to individualize HCC therapy. Functional assessment and bioinformatics analysis showed that cell cycle-related processes and pathways and the PPAR signaling pathway were significantly associated with the risk score, indicating that the 13-DEL prognostic signature may be involved in the regulation of the status of HCC cells. However, further studies are necessary to elucidate the mechanism underlying the roles of these DELs in HCC, and additional cohorts are needed to validate our findings. Additional findings of the present study are also worthy of further investigation.

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Disclosure Statement

The authors declare to have no conflict of interests.

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