The Expression of LIMK1 is Related to the Poor Prognosis and Immune Function of Hepatocellular Carcinoma

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Research

Keywords: hepatocellular carcinoma, LIMK1, biomarkers, prognosis, immune infiltration

Posted Date: October 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-910480/v1>

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Abstract

Background: According to reports, LIMK1 may have the effect of promoting tumor progression. However, the effect of the expression of LIMK1 on the healing of patients with hepatocellular carcinoma and its effect on the immune function are still not clear. Therefore, we analyzed the effect of LIMK1 on the healing of patients with hepatocellular carcinoma and its correlation with immunity through bioinformatics analysis.

Methods: Download the transcriptional expression profile of LIMK1 in hepatocellular carcinoma tissues and normal tissues in TCGA, and study its expression in hepatocellular carcinoma. Study the expression of LIMK1 in hepatocellular carcinoma through CPTAC and HPA database. The Kaplan-Meier method was used to evaluate the effect of LIMK1 expression on the survival of patients with hepatocellular carcinoma. Use the STRING database to construct a protein-protein interaction (PPI) network. Use the "ClusterProfiler" package for feature-rich analysis. Use TISIDB database and Xiantao platform to study the relationship between LIMK1 mRNA expression and immune infiltration.

Results: The expression of LIMK1 in hepatocellular carcinoma tissues was significantly up-regulated. Increased expression of LIMK1 mRNA is related to high TNM staging. In the ROC curve, when the cut-off level is 1.813, the sensitivity and specificity of LIMK1 to distinguish hepatocellular carcinoma from adjacent controls are 80.7% and 86%, respectively. The Kaplan-Meier curve shows that the higher the expression of LIMK1, the worse the survival of patients with hepatocellular carcinoma (42.2 months vs. 70 months, $P = 0.001$). Correlation analysis studies have shown that the expression of LIMK1 mRNA in hepatocellular carcinoma is related to immune cell infiltration.

Conclusion: Up-regulation of LIMK1 may affect the survival rate and immune invasion of hepatocellular carcinoma. Studies have shown that LIMK1 may be related to the poor prognosis of hepatocellular carcinoma, and has a certain relationship with the immune infiltration of hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) is still one of the malignant tumors with poor prognosis, and its effective treatment methods are very limited [1]. In addition, only a few HCC patients have the opportunity to undergo radical treatments such as liver transplantation, surgical resection, and radiofrequency ablation [2]. If some patients are found early, after surgical resection, their 5-year survival rate can reach more than 70% [3]. However, because early hepatocellular carcinoma has no obvious clinical symptoms, most patients with hepatocellular carcinoma have extensive metastasis when they appear, and the patients have missed the opportunity for radical surgery [4]. Lack of monitoring methods and insufficient accuracy of early diagnosis methods are the reasons for the poor prognosis and high mortality of HCC. Therefore, it is urgent to find new biomarkers to promote the prognosis of hepatocellular carcinoma.

LIMK1 is a molecule belonging to the serine/threonine kinase family and has the function of regulating actin polymerization [5]. Phosphorylated LIMK1 is related to a variety of cell functions, including cell

cycle, progression of cell metastasis, angiogenesis, and vascular proliferation [6, 7]. Previous studies have shown that the expression of LIMK1 may affect tumor progression and is related to the peritoneal metastasis of colorectal cancer, gastric cancer and other tumors [8–10]. In addition, the up-regulation of LIMK1 is associated with prostate cancer metastasis and lower survival rate [11]. In addition, in pancreatic tumors, knockdown of LIMK1 can inhibit tumor invasion and metastasis, as well as inhibit angiogenesis [12, 13]. In addition, some recent studies have shown that in hepatocellular carcinoma, the down-regulation of LIMK1 can inhibit tumor migration [14]. Therefore, we speculate that LIMK1 may be a biomarker for predicting the poor prognosis of hepatocellular carcinoma.

Through this study, we explored the prognostic value of LIMK1 in hepatocellular carcinoma and its correlation with immune infiltration. Through the Cancer Genome Atlas (TCGA) data, we found that the level of LIMK1 is related to the survival rate of hepatocellular carcinoma. The expression of LIMK1 is increased in hepatocellular carcinoma, and its up-regulation is related to the unfavorable clinical features of hepatocellular carcinoma. We further studied the relationship between LIMK1 in hepatocellular carcinoma, diagnosis and patient recovery. At the same time, we used TISIDB database and Xiantao platform to study the effect of LIMK1 on the immune function of hepatocellular carcinoma. We believe that LIMK1 may be a star target for diagnosing liver cancer and predicting the long-term recovery of patients.

2. Material And Method

2.1 We download clinical information about LIMK1 from the official website of the Cancer Genome Atlas (TCGA) [15]. We analyzed the expression of LIMK1 in 33 tumor types.

2.2 We downloaded data about the expression of LIMK1 in liver cancer from TCGA, and extracted information about 374 hepatocellular carcinomas and 59 normal tissues adjacent to the cancer. We also extracted relevant clinical information, including gender, age, weight, BMI, AFP, T staging, N staging, M staging, TNM staging, tumor status, histological grade and OS events.

2.3 UALCAN is an open online network data resource used to analyze openly accessible cancer data [16]. In this study, we used UALCAN to analyze LIMK1 protein expression.

Human Protein Atlas (HPA) contains the expression information of human-related proteins[17]. Through the HPA database, we studied the protein expression of LIMK1 in hepatocellular carcinoma [17].

2.4 Using the STRING line database, a protein-protein interactions (PPI) Networks with was constructed [18]. Use the "ClusterProfiler" package and the "ggplot2" package to analyze and visualize the gene ontology (GO) enrichment of co-expressed genes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [19].

2.5 We use TISIDB7 online Web to study the effect of LIMK1 on the immune function of hepatocellular carcinoma [20].

We use the Xiantao platform (www.xiantao.love) to collect relevant gene expression profiles and relevant clinical information in hepatocellular carcinoma, and to study the correlation between LIMK1 and immune cells in hepatocellular carcinoma.

Kaplan-Meier plotter was used to study the effect of LIMK1 expression on the survival of hepatocellular carcinoma patients [21].

2.6 Statistical Analyses

Use R (V 3.6.3) to perform statistical analysis on all the data, and use the R package ggplot2 to visualize the difference in expression. The difference between hepatocellular carcinoma tissue and normal tissue was studied using paired t test and Mann-Whitney U test. The detection of the cut-off value of the ROC curve is performed using the pROC package [22]. In order to study the correlation between LIMK1 and the survival rate of hepatocellular carcinoma patients, we used the survminer package to perform Kaplan-Meier.

3. Results

3.1 The expression of LIMK1 in hepatocellular carcinoma and other tumors from the perspective of pan-cancer

We use the data of the TCGA platform to show that compared with adjacent normal tissues, the expression of LIMK1 in 19 types of tumors increased significantly, including: BLCA, BRCA, CESC, CHOL, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PCPG, PRAD, READ, STAD, THCA, UCEC. This study shows that in hepatocellular carcinoma, the expression of LIMK1 is up-regulated compared to normal tissues.

3.2 LIMK1 mRNA expression and protein expression are up-regulated in HCC

Using data from TCGA and HPA, the expression of LIMK1 in hepatocellular carcinoma was studied. Studies have shown that in matched hepatocellular carcinoma samples, the RNA expression level of LIMK1 in tumor samples ($n = 50$) was significantly increased (Fig. 2A, 1.637 ± 0.839 vs. 0.760 ± 0.322 , $P < 0.001$). In the unpaired samples, the results consistent with the matched samples were obtained, and the expression of LIMK1 in hepatocellular tumor specimens was significantly up-regulated (Fig. 2B, 2.687 ± 1.035 vs. 1.413 ± 0.501 , $P < 0.001$). The UALCAN database was used to analyze the expression of LIMK1, and the results showed that the mRNA expression of LIMK1 in hepatocellular carcinoma tissues was significantly increased (Fig. 2C). Using HPA to study the protein expression of LIMK1, the results showed that the protein expression of LIMK1 in cancer tissues was significantly up-regulated. In summary, the expression of LIMK1 is up-regulated in hepatocellular carcinoma tissues.

3.3 The expression level of LIMK1 mRNA in patients with HCC is correlated with clinical features

As shown in Table 1 and Fig. 3A-I, in patients with HCC, the high expression of LIMK1 is associated with higher T staging ($P = 0.009$), high TNM staging ($P = 0.003$), high AFP value ($P = 0.003$) and high histological grade ($P < 0.001$) are significantly correlated. However, our research showed that there was no significant correlation between the expression level of LIMK1 and age ($P = 0.151$) and gender ($P = 0.595$). In general, the above results show that the expression of LIMK1 is significantly correlated with higher T staging and higher TNM staging. This may suggest that LIMK1 may be a potential marker for predicting poor prognosis of HCC.

Table 1
Clinical characteristics of the HCC patients (TCGA).

Characteristic	Low expression of LIMK1	High expression of LIMK1	p
n	187	187	
T stage, n (%)			0.003**
T1	108 (29.1%)	75 (20.2%)	
T2	42 (11.3%)	53 (14.3%)	
T3	30 (8.1%)	50 (13.5%)	
T4	4 (1.1%)	9 (2.4%)	
N stage, n (%)			0.123
N0	125 (48.4%)	129 (50%)	
N1	0 (0%)	4 (1.6%)	
M stage, n (%)			0.623
M0	132 (48.5%)	136 (50%)	
M1	1 (0.4%)	3 (1.1%)	
Age, median (IQR)	63 (52, 69.5)	60 (51, 68)	0.151
** $p < 0.01$			

3.4 The expression level of LIMK1 RNA-Seq may be a potential marker for the identification of hepatocellular carcinoma

We use ROC curve to study the potential role of LIMK1 in the identification of hepatocellular carcinoma. ROC curve shows that the AUC value of LIMK1 predicting hepatocellular carcinoma is 0.885 (95% CI: 0.842–0.929) (Fig. 4A). When the cutoff value is 1.813, the sensitivity and specificity of LIMK1 are 80.7% and 86%, respectively. The positive predictive value was 97.7%, and the negative predictive value was 37.4%. According to these studies, LIMK1 may be a biomarker that distinguishes hepatocellular carcinoma tissues from normal tissues.

3.5 The expression of LIMK1 mRNA is related to the shortening of overall survival in patients with HCC

We used Kaplan-Meier curve database and XIANTAO platform to study the correlation between LIMK1 mRNA expression level and (OS) of patients with HCC. In patients with hepatocellular carcinoma, the higher the expression of LIMK1, the shorter the patient's OS, and the difference was statistically significant (42.2 months vs. 71 months, $P = 0.001$) (Fig. 4B).

Subgroup K-M analysis showed that OS in patients with hepatocellular carcinoma was associated with high LIMK1 and high TNM staging, further indicating that LIMK1 may predict the poor outcome of patients with hepatocellular carcinoma (Fig. 4C, D, E). The results of the Kaplan-Meier curve database also indicate that the high expression of LIMK1 is related to the poor OS of hepatocellular carcinoma (Fig. 4F). The above research results indicate that LIMK1 may indicate that patients with hepatocellular carcinoma have poor recovery.

3.6 In HCC, construct PPI network and functional annotations

In HCC, we found that LIMK1 and 10 genes form a network of co-expressed genes through string database (Fig. 5A). At the same time, GO and KEGG analysis showed that the changes in the biological process of LIMK1 are related to the regulation of actin cytoskeleton, axon guidance, regulation of actin cytoskeleton organization Related, regulation of actin filament-based processes, and actin filament organization (Fig. 5B). Functional annotations indicate that these genes are involved in Rho GTPase binding. Correlation analysis showed that there is a correlation between LIMK1 and HCC overexpressed genes (Fig. 5C-I).

3.7 There is a correlation between LIMK1 and immune infiltration in HCC

We used the TISIDB database to evaluate the correlation between LIMK1 expression and 28 TILs. Studies have shown that there is a certain relationship between the expression of LIMK1 in human tumors and 28 TILs (Fig. 6A). The expression of LIMK1 is related to Tcm-CD8 + T cells ($r = 0.109$, $P = 0.0351$), Tcm-CD4 + T cells ($r = 0.42$, $P < 2.2e-16$), and Treg cells ($r = 0.314$, $P = 7.29e-10$), Act-CD4 cells ($r = 0.41$, $P < 2.2e-16$), CD56bright cells ($r = 0.191$, $P = 0.000205$), MDSC cells ($r = 0.332$, $P = 6.64e-11$), B Cells ($r = 0.327$, $P =$

1.25e-10), Th1 cells ($r = 0.205$, $P = 6.79\text{e-}5$), Th2 cells ($r = 0.211$, $P = 4.11\text{e-}5$), Th17 cells ($r = 0.19$, $P = 0.000233$) and NKT cells ($r = 0.285$, $P = 2.5\text{e-}8$) are correlated (Fig. 6B). We use the XIANTAO platform to analyze the correlation between LIMK1 and immune cells (Fig. 6C). As shown in Fig. 6D, in HCC, the expression of LIMK1 is related to the infiltration of immune cells such as T cells, B cells, NK cells, Th1 cells, Th2 cells, and CD56bright cells. LIMK1 expression and CD56 bright, T cells, NK cells, B cells, Th17 cells, TReg cells, Th1 cells, Th2 cells (Fig. 7E). These data indicate that LIMK1 may have a certain relationship with the immune infiltration of hepatocellular carcinoma and play a specific role.

4. Discussion

Research shows that the expression of LIMK1 is increased in hepatocellular carcinoma. At the same time, when the TNM stage of hepatocellular carcinoma patients is higher, the mRNA expression value of LIMK1 is also higher. According to the ROC curve results, LIMK1 may be a potential marker for differentiating tumor tissues from normal tissues in hepatocellular carcinoma. According to Kaplan-Meier curve and subgroup analysis, when LIMK1 is highly expressed, the OS of hepatocellular carcinoma patients is shortened. In addition, the expression of LIMK1 in HCC may be related to tumor immune infiltration.

In recent years, there have been many studies on the carcinogenic effects of LIMK1 in human tumors such as pancreatic tumors, stomach tumors and HCC [23–25]. In addition, the increased expression of LIMK1 in various cancers has a certain correlation with the poor prognosis of the tumor [11, 26]. However, the expression of LIMK1 in patients with HCC and its correlation with the prognosis have not yet been fully studied. In this study, the pan-cancer analysis of the TCGA database was first used, and the results obtained were consistent with the reported results of over-expression of LIMK1 mRNA in various tumors such as lung tumor and gastric tumor. At the same time, based on paired data analysis, we also confirmed that the expression of LIMK1 is significantly increased in hepatocellular carcinoma. Our research results are consistent with those previously obtained by Guo et al. [14]. Correlation analysis shows that there is a certain correlation between the expression of LIMK1 and high TNM staging, and the results show a positive correlation [27]. These findings show that LIMK1 may be related to the prognosis of hepatocellular carcinoma and can be used to predict the adverse prognosis of HCC.

At present, the role of LIMK1 in HCC has not been fully reported. According to previous research results, miRNA-27-3p and miRNA-520-3p are the targets of LIMK1, and they can inhibit tumor development [14, 27, 28]. At the same time, studies of potential mechanisms have shown that LIMK1 has a certain influence in tumor progression [23, 29]. Through previous research, we speculate that LIMK1 may be a potential substance or new target that affects HCC [30]. Since the expression of LIMK1 mRNA is significantly increased in HCC, we speculate that LIMK1 may have a role in predicting the prognosis of HCC. We use the ROC curve to study the role of LIMK1 in the diagnosis of HCC. ROC studies have shown that LIMK1 has a high AUC value in the diagnosis of hepatocellular carcinoma, and the ROC curve shows that its sensitivity is 80.7% and specificity is 86%. Based on our findings, we believe that LIMK1 can be used to distinguish HCC tissues from normal tissues. Given that the up-regulation of LIMK1 is positively correlated with high TNM, we speculate that LIMK1 may promote the progression of hepatocellular

carcinoma. In addition, when the TNM stage is higher, the patient's survival rate is lower. We speculate that the up-regulation of LIMK1 is a potential marker of a poorer prognosis for patients. In addition, the results of K-M curve and log-rank test showed that compared with patients with low LIMK1 expression, patients with hepatocellular carcinoma with high LIMK1 mRNA expression had a lower survival rate. Based on our results, we speculate that LIMK1 can be used to predict the prognosis of patients with HCC.

LIMK1 is an important part of the Rac1/PAK1/LIMK1 signaling pathway and is related to a variety of cancers [31]. For example, in esophageal tumors, miR-384 can regulate this pathway to promote cell apoptosis in esophageal tumors and increase the sensitivity of patients to chemotherapy [32]. Studies have shown that inhibiting Rho GDP dissociation inhibitor 2 in gastric cancer can inhibit tumor progression by regulating the LIMK1 signaling pathway [30, 33]. Research by Lu et al. showed that the expression of LIMK1 has an effect on the expression of Rac1 and PAK1 [34]. Based on the research results, we believe that the overexpression of LIMK1 will have influence of the expression of the entire pathway of Rac1/PAK1/LIMK1. However, this result should be verified in other experiments.

Through the TISIDB database, we found that the expression of LIMK1 in HCC is correlated with some tumor infiltrating immune cells (Tcm-CD8 + T cells, Tcm-CD4 + T cells, Treg cells, Act-CD4 cells, CD56bright cells). At the same time, through the Xiantao platform, we also observed that the expression of LIMK1 has an effect on T, B, NK, Th1, Th2 and CD56bright cells. These findings indicate that LIMK1 may affect the immune infiltration function of patients with hepatocellular carcinoma to play a role. In the future, we will design further studies to verify this relationship.

However, this study has several flaws. First of all, this study only used online public databases such as TCGA and TISIDB to study the effect of LIMK1 expression on the prognosis of hepatocellular carcinoma. However, these results need to be verified by further clinical studies. Second, there is a lack of relevant experiments to verify the mechanism of LIMK1's effect on hepatocellular carcinoma immune infiltration.

In conclusion, through this study, we found that the expression of LIMK1 mRNA in HCC was up-regulated, and it was positively correlated with high TNM staging. At the same time, our research shows that LIMK1 may be a potential target for predicting the poor outcome of hepatocellular carcinoma patients, and may play a certain role in immune infiltration.

Declarations

Acknowledgements

Not applicable

AUTHOR CONTRIBUTIONS

Bo Li and Xiaoli Yang conceived and designed the study; Yisheng Peng and Jun Fan performed data analysis and wrote the manuscript; Gang Zhu, Sunde Tan and Su Song contributed analysis tools; Jianfei

Chen, Xuewen Wang and Haoxian Gou contributed to photo editing and data analysis; all authors reviewed the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81802778), Southwest Medical University Foundation (2018-ZRZD-010), Doctoral Startup Fund of Affiliated Hospital of Southwest Medical University.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets analyzed during the current study are available in the TCGA Database, <https://xenabrowser.net/datapages/>

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Figures

Figure 1

The expression of LIMK1 in normal and tumor tissues (data from TCGA). Compared with normal tissues, LIMK1 mRNA expression is up-regulated in 19 types of tumors. (***) $P < 0.001$, (**) $P < 0.01$. ns, meaningless

Figure 2

The expression of LIMK1 in hepatocellular carcinoma. (A) In matched samples, LIMK1 mRNA expression levels in cancer tissues and normal tissues. (B) In unpaired samples, LIMK1 mRNA expression levels in

cancer and normal tissues. (C) LIMK1 mRNA expression in the UALCAN database. (D) The protein level of LIMK1 in hepatocellular carcinoma tissue. Normal tissue, <https://www.proteinatlas.org/ENSG00000106683-LIMK1/tissue/liver#img>; Tumor tissue, <https://www.proteinatlas.org/ENSG00000106683-LIMK1/pathology/liver+cancer#img> (** $P < 0.001$).

Figure 3

The clinical correlation between LIMK1 mRNA level and hepatocellular carcinoma. LIMK1 mRNA expression and high T stage (A), high N stage (B), high M stage (C), high TNM stage (D), tumor status (E), high AFP (J) and high histological grade (K), there is a significant correlation. However, no obvious connection was found between LIMK1 and gender (F) and age (G). (** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns, no significance).

Figure 4

ROC and Kaplan-Meier curve in hepatocellular carcinoma. (A) ROC curve shows that LIMK1 distinguishes hepatocellular carcinoma tissue from normal tissue with an AUC value of 0.885. (B) Kaplan-Meier survival curve shows that in hepatocellular carcinoma, the higher the expression of LIMK1, the shorter the patient's OS. (42.2 vs. 71 months, $P = 0.001$). (C) The Kaplan-Meier survival curve of the subgroup showed that in patients without lymph node metastasis, the higher the expression of LIMK1 mRNA, the shorter the OS ($P = 0.03$). (D) and (E) The subgroup Kaplan-Meier survival curve shows that TNM has a significant impact on the patient's OS. (F) The results of the Kaplan-Meier curve database show that the expression of LIMK1 is related to the survival of the patient.

Figure 5

PPI network and feature-rich analysis results. (A) Network of co-expressed genes. (B) Functional enrichment analysis. (C-L) Correlation analysis shows that there is a correlation between LIMK1 and hepatocellular carcinoma co-expressed genes.

Figure 6

In hepatocellular carcinoma, there is a correlation between the expression of LIMK1 and immune infiltration. (A) In human tumors, the expression of LIMK1 is correlated with 28 TILs. (B) In hepatocellular carcinoma, the expression of LIMK1 is related to Tcm-CD8⁺ T cells, Tcm-CD4⁺ T cells and Treg cells, Act-CD4 cells, CD56bright cells, MDSC cells, B cells, Th1 cells, Th2 cells, Th17 cells and NKT cells. (C) The expression of LIMK1 is associated with 24 tumor-infiltrating immune cells in hepatocellular carcinoma.

(D) The expression of LIMK1 is related to T cells, B cells, NK cells, Th1 cells, Th2 cells and CD56bright cells. (E) LIMK1 is related to the abundance of CD56 bright ($r=0.41$, $P<0.001$), T cells ($r=0.17$, $P=0.001$), NK cells ($r=0.11$, $P=0.035$), B cells ($r=0.21$, $P<0.001$), Th17 cells ($r=-0.25$, $P<0.001$), TReg cells (-0.11 , $P=0.032$), Th1 cells ($r=0.32$, $P<0.001$) and Th2 cells ($r = 0.42$, $P < 0.001$).