Research Article

LncRNA CARMN Affects Hepatocellular Carcinoma Prognosis by Regulating the miR-192-5p/LOXL2 Axis

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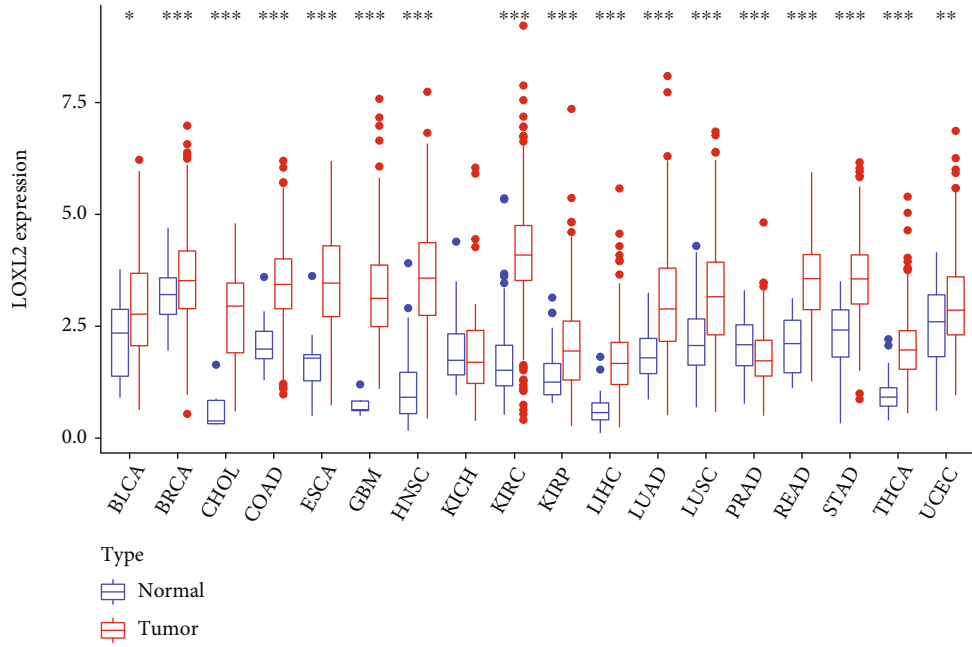
Background. Hepatocellular carcinoma (HCC) is aggressive cancer with a poor prognosis. It has been suggested that the aberrant expression of LOXL2 is associated with the development of HCC, but the exact mechanism remains unclear. This research is aimed at examining the expression level and prognostic value of LOXL2 in hepatocellular carcinoma and its relationship with immune infiltration and at predicting its upstream noncoding RNAs (ncRNAs). *Method*. The transcriptome data of HCC was first downloaded from The Cancer Genome Atlas (TCGA) database to investigate the expression and prognosis of LOXL2. Then, the starBase database was used to find the upstream ncRNAs of LOXL2, and correlation analysis and expression analysis were performed. Finally, the Tumor Immune Estimation Resource (TIMER) was used to explore the association between LOXL2 and immune cell infiltration. *Result*. CARMN was considered to be the potential upstream lncRNA for the hsa-miR-192-5p/LOXL2 axis in HCC. Furthermore, the level LOXL2 was markedly positively associated with tumor immune cell infiltration and immune checkpoint expression in HCC. *Conclusion*. Higher expression of LOXL2 mediated by microRNA (miRNA) and long noncoding RNAs (lncRNA) is associated with poor overall survival (OS), immune infiltration, and immune checkpoint expression in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the main type of liver cancer (LC). As the sixth most common cancer in the world, it is the second largest cause of cancer-related death and the most common primary liver cancer with poor prognosis [1, 2]. The morbidity and mortality rates of HCC are expected to significantly increase in the next few years [2]. Epidemiological data show that hepatitis virus infection [3], aflatoxin [4], type 2 diabetes [5], alcohol consumption [6], and smoking [7] are all predisposing factors for liver cancer. Despite remarkable improvements in the diagnosis and treatment of HCC, such as surgical resection [8] and sorafenib-regorafenib sequential therapy [9], patients with HCC often exhibit local invasion and metastasis resulting in a poor overall survival (OS) rate [10, 11]. Therefore, early screening and diagnosis of HCC are particularly crucial, and there is an urgent need to find specific and sensitive biomarkers.

Lysyl oxidase (LOX), an extracellular enzyme, plays a key role in the covalent cross-linking of collagen fibers by oxidizing deamino-specific lysine and hydroxylysine in the telopeptide structural domain of the collagen molecule to form allantoin [12]. In addition to LOX, there are four other members in the lox protein family, namely, LOX-like proteins (LOXL1, LOXL2, LOXL3, and LOXL4) [13, 14].
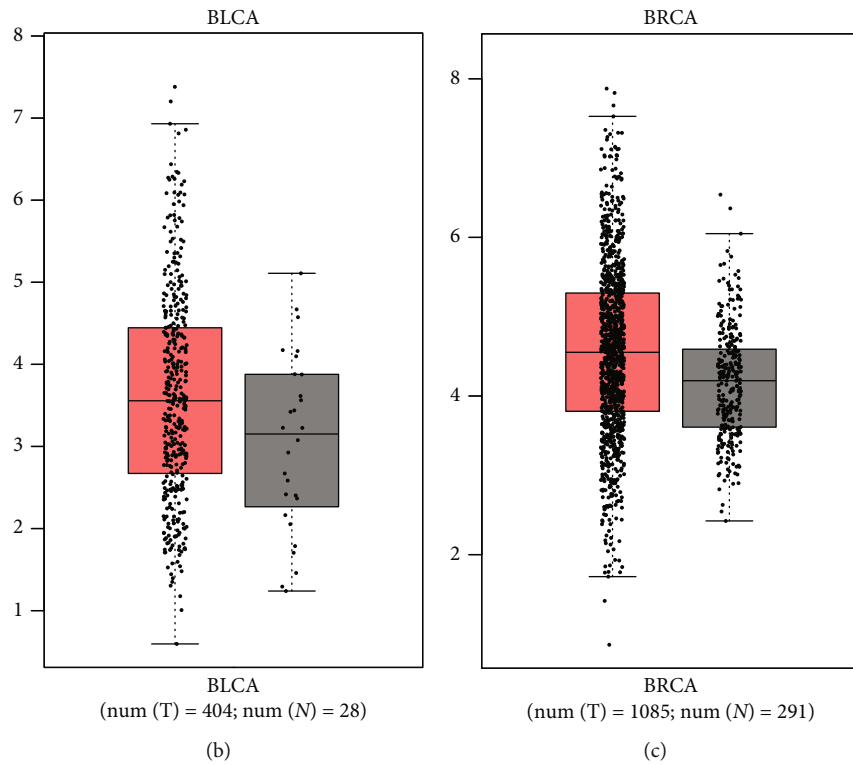
(a)

BLCA
(num (T) = 404; num (N) = 28)

(b)

BRCA
(num (T) = 1085; num (N) = 291)

(c)

Figure 1: Continued.

CHOL
(num (T) = 36; num (N) = 9)

(d)

COAD
(num (T) = 275; num (N) = 349)

(e)

ESCA
(num (T) = 182; num (N) = 286)

(f)

GBM
(num (T) = 163; num (N) = 207)

(g)

Figure 1: Continued.

HNSC
(num (T) = 519; num (N) = 44)

(h)

KICH
(num (T) = 66; num (N) = 53)

(i)

KIRC
(num (T) = 523; num (N) = 100)

(j)

KIRP
(num (T) = 286; num (N) = 60)

(k)

Figure 1: Continued.

LIHC
(num (T) = 369; num (N) = 160)

(l)

LUAD
(num (T) = 483; num (N) = 347)

(m)

LUSC
(num (T) = 486; num (N) = 338)

(n)

PRAD
(num (T) = 492; num (N) = 152)

(o)
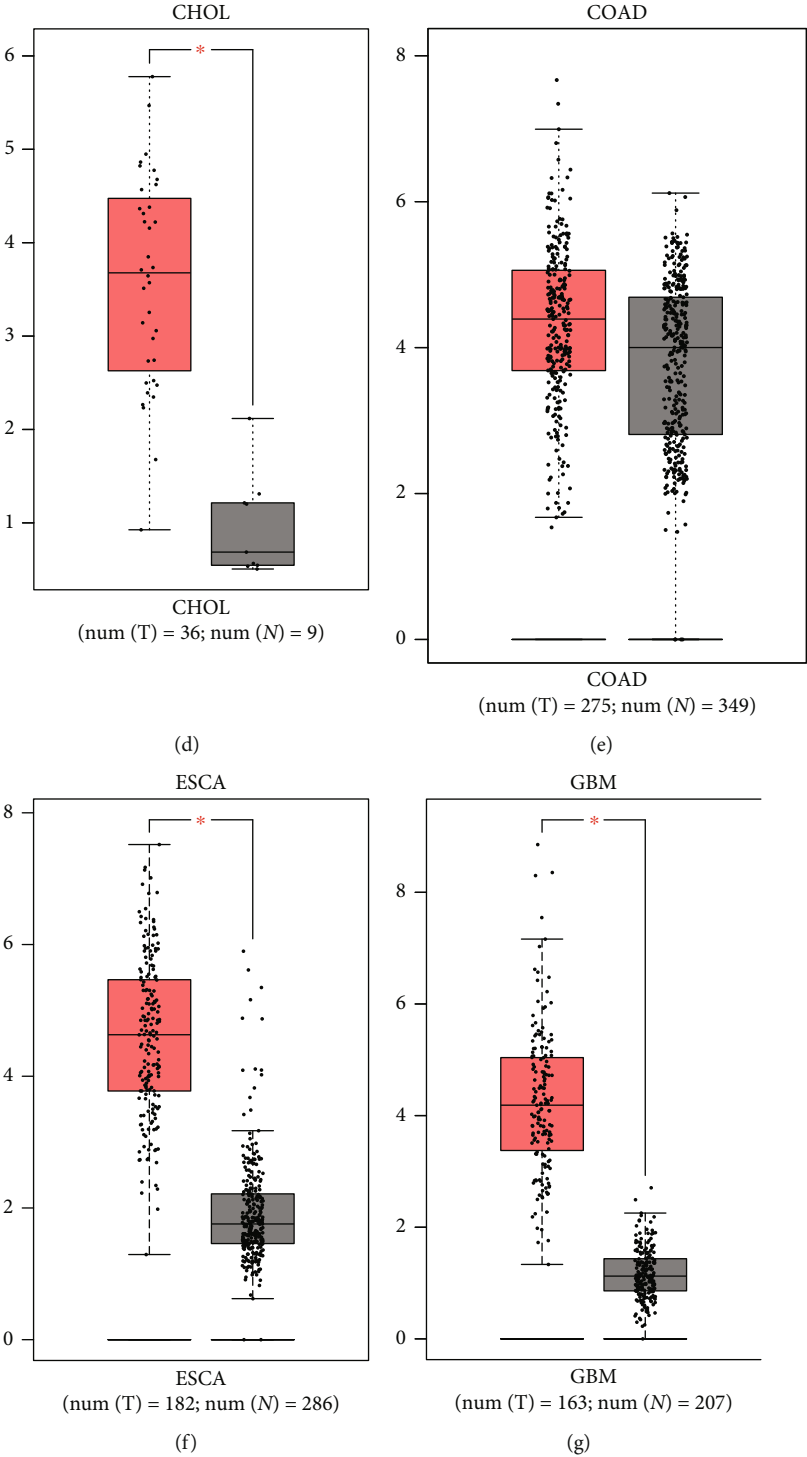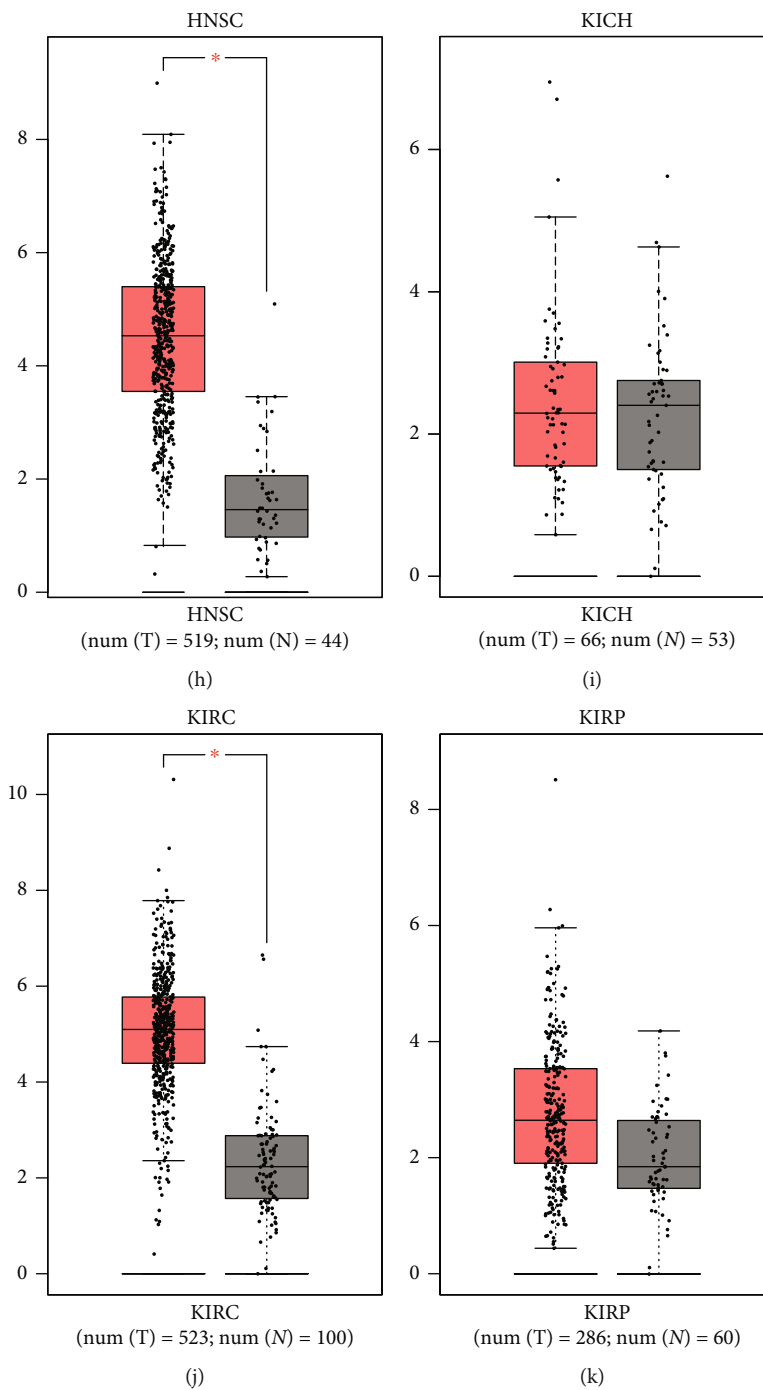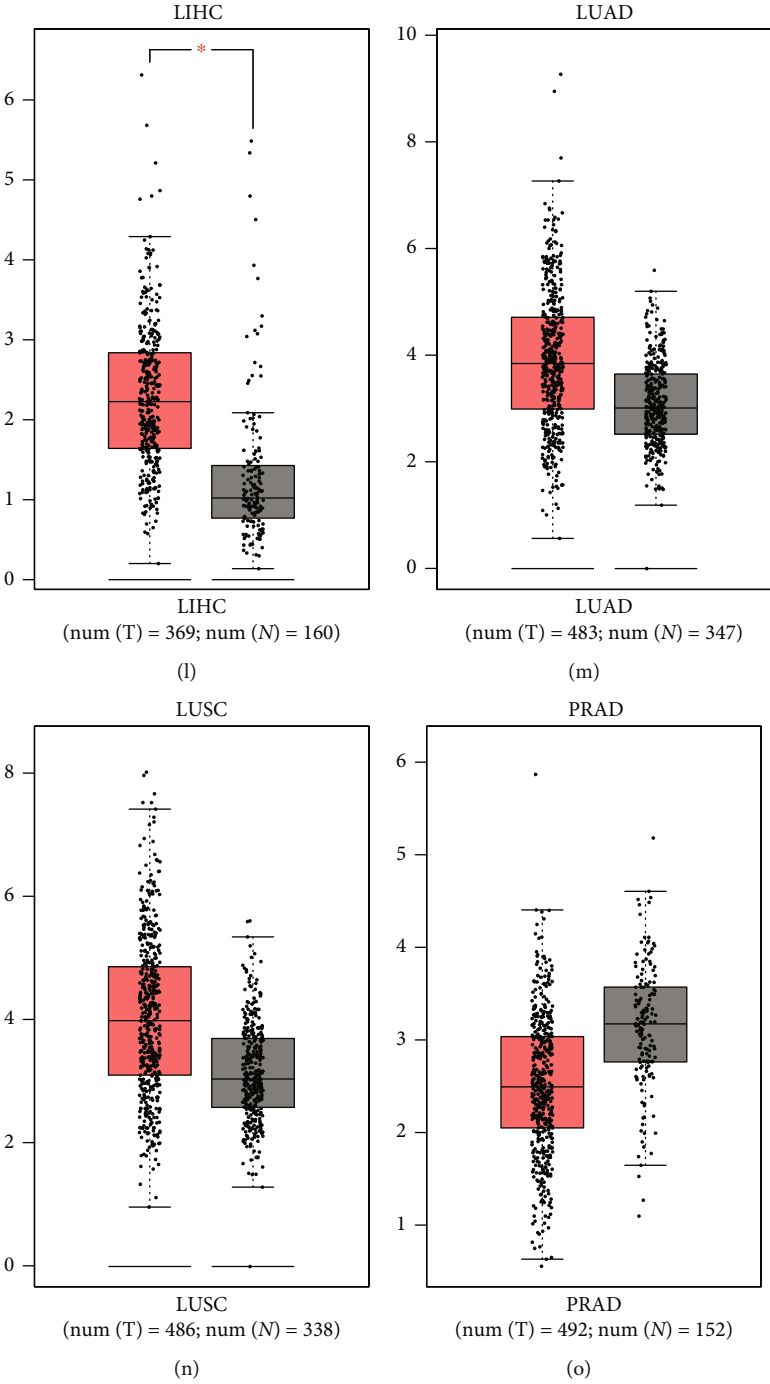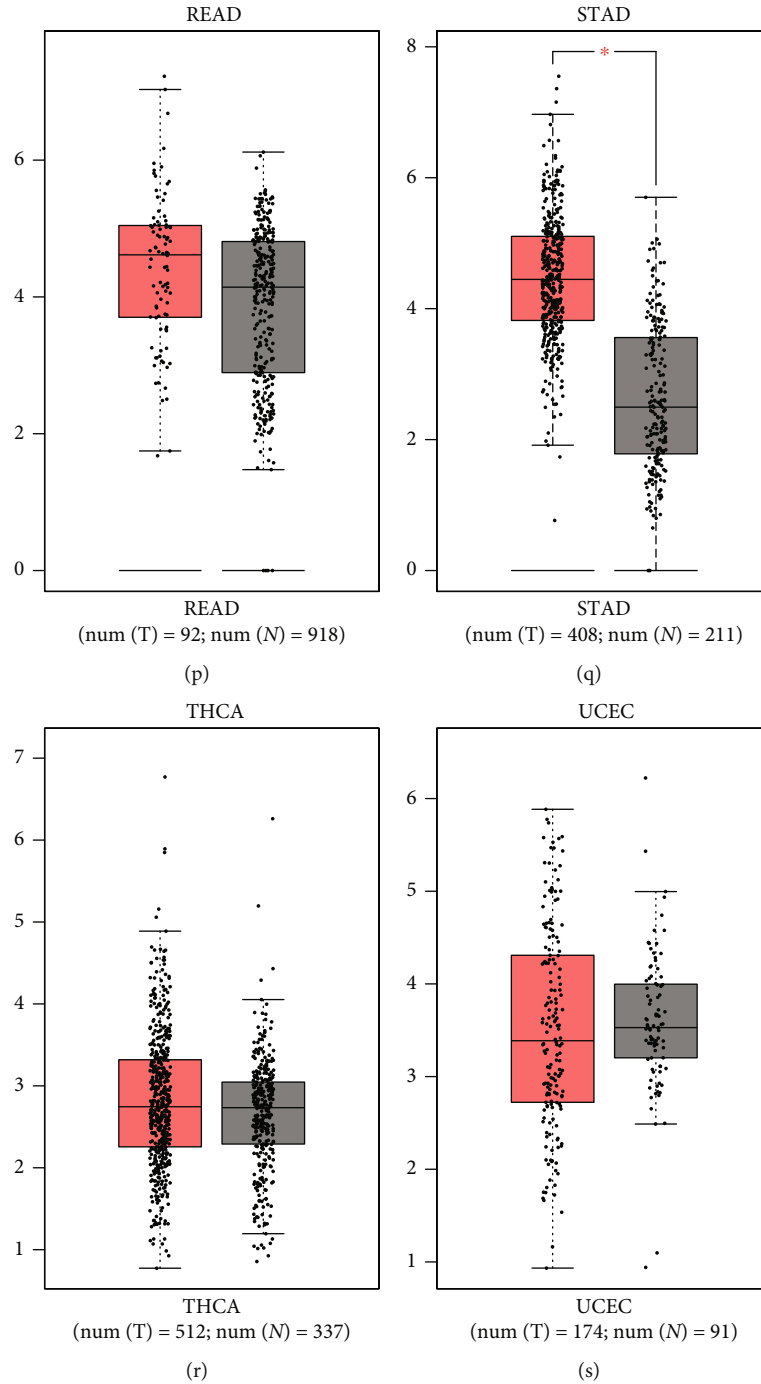
FIGURE 1: Continued.

FIGURE 1: Expression analysis for LOXL2 in multiple cancers. (a) The expression of LOXL2 in 18 types of human cancer based on TCGA cancer and normal data. (b)–(m) LOXL2 expression in GEPIA database. BLCA (b), BRCA (c), CHOL (d), COAD (e), ESCA (f), GBM (g), HNSC (h), KICH (i), KIRC (j), KIRP (k), LIHC (l), LUAD (m), LUSC (n), PRAD (o), READ (p), STAD (q), THCA (r), and UCEC (s) tissues compared with corresponding TCGA and GTEx normal tissues. $^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001.

Among these proteins, LOXL2 is considered to be an important regulator of tumor progression, and previous studies reported that LOXL2 is significantly overexpressed in human HCC tissues compared to nontumor tissues [15]. Studies demonstrated that elevated levels of LOXL2 might contribute to tumor progression and metastasis by promoting tumor cell invasion and remodeling of the tumor microenvironment [16, 17]. Considering the major role of LOXL2,

we further investigated the role of LOXL2 in the development of HCC progression based on previous studies.

In this study, the expression level of the LOXL2 and its relationship with prognosis were first analyzed in various common cancers. Next, we found some noncoding RNAs (microRNAs (miRNAs)) and long noncoding RNAs (lncRNAs) as regulatory molecules of LOXL2 by bioinformatics analysis, so as to establish the LncRNA-miRNA-
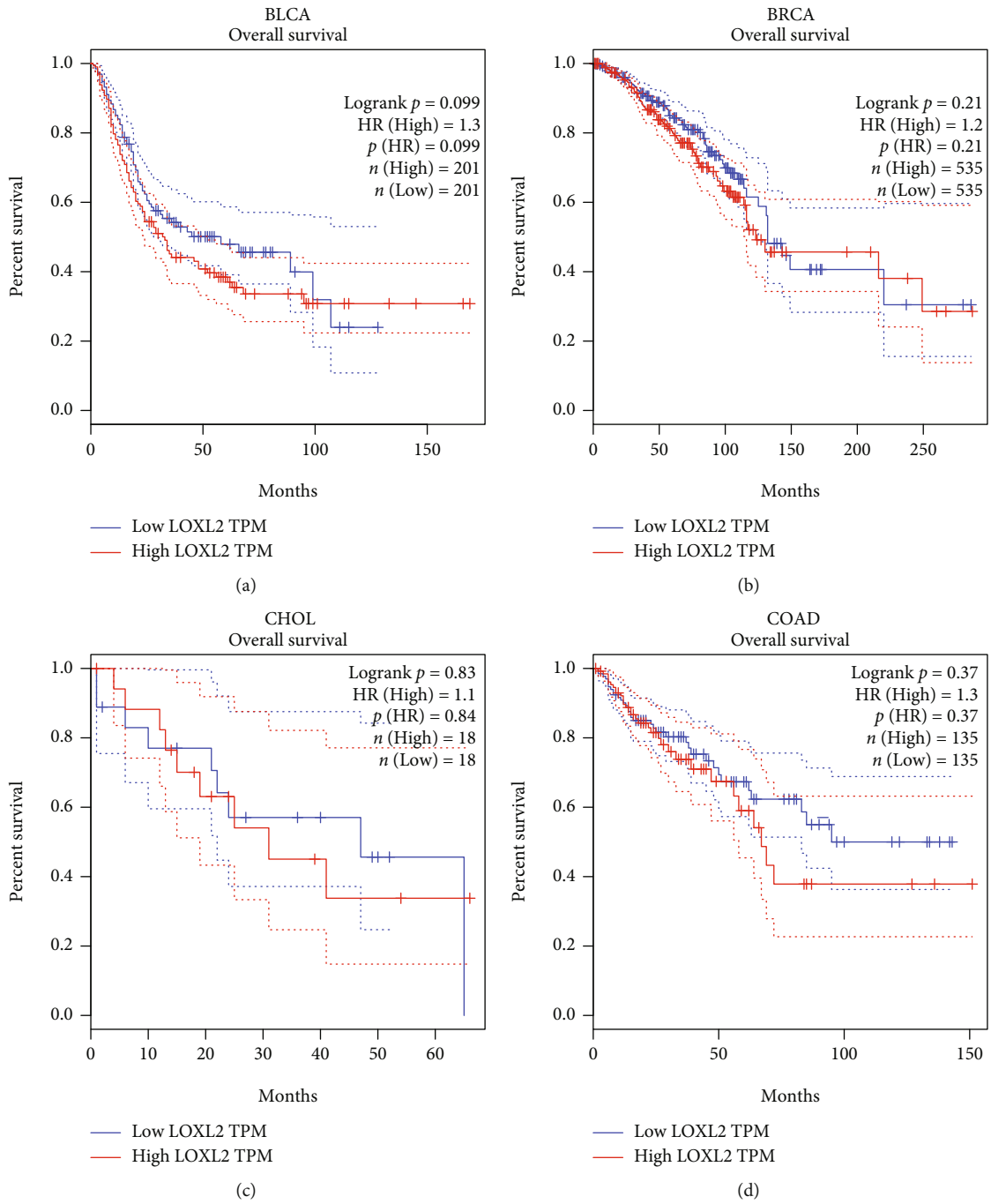
BLCA
Overall survival
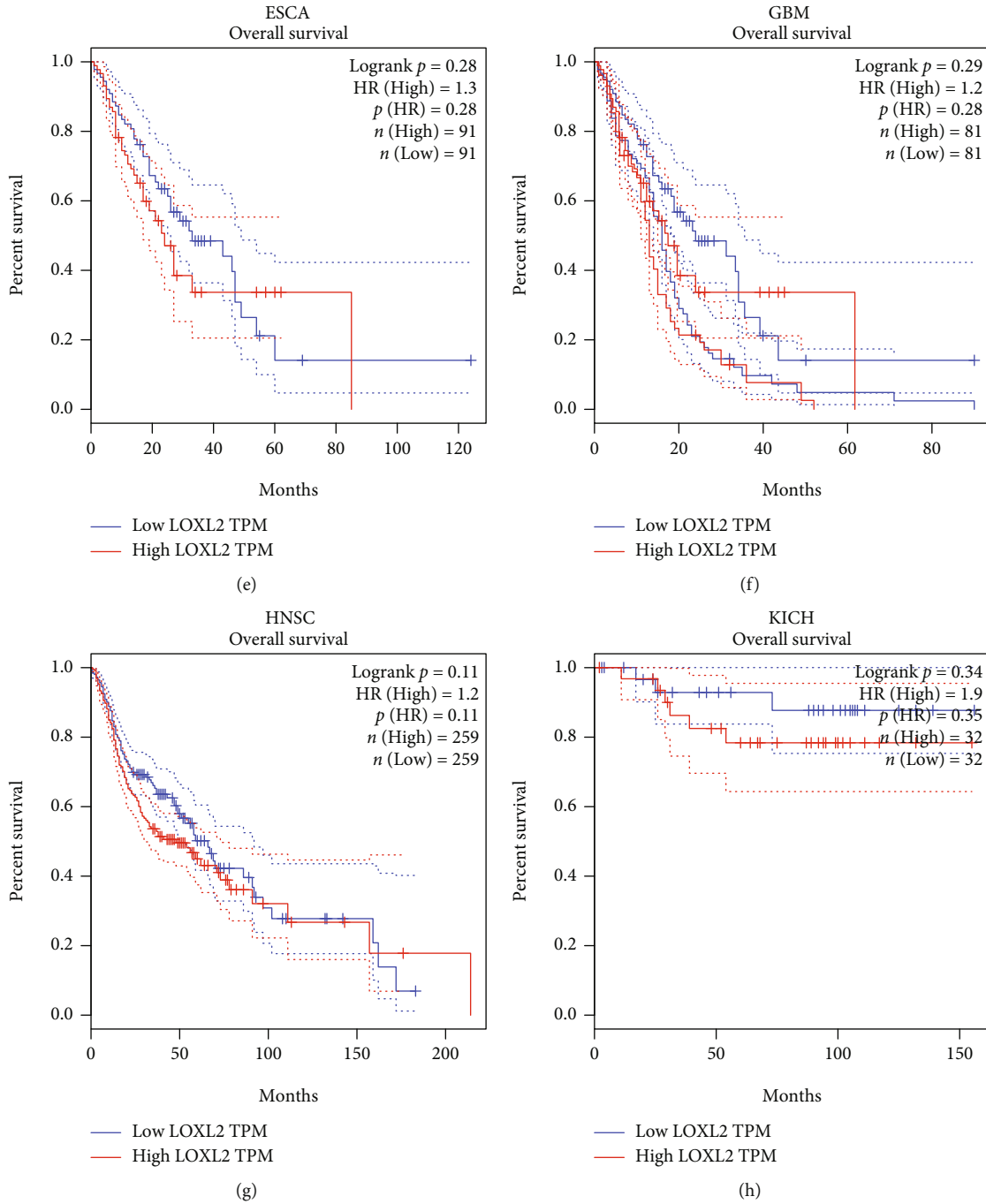
Logrank $p = 0.099$
HR (High) = 1.3
p (HR) = 0.099
n (High) = 201
n (Low) = 201

— Low LOXL2 TPM
— High LOXL2 TPM

(a)

BRCA
Overall survival

Logrank $p = 0.21$
HR (High) = 1.2
p (HR) = 0.21
n (High) = 535
n (Low) = 535

— Low LOXL2 TPM
— High LOXL2 TPM

(b)

CHOL
Overall survival

Logrank $p = 0.83$
HR (High) = 1.1
p (HR) = 0.84
n (High) = 18
n (Low) = 18

— Low LOXL2 TPM
— High LOXL2 TPM

(c)

COAD
Overall survival

Logrank $p = 0.37$
HR (High) = 1.3
p (HR) = 0.37
n (High) = 135
n (Low) = 135

— Low LOXL2 TPM
— High LOXL2 TPM

(d)

Figure 2: Continued.

(e)

(f)

(g)

(h)

FIGURE 2: Continued.

FIGURE 2: Continued.

LUSC
Overall survival

Logrank $p = 0.0045$
HR (High) = 1.5
p (HR) = 0.0047
n (High) = 241
n (Low) = 241

— Low LOXL2 TPM
— High LOXL2 TPM

(m)

PRAD
Overall survival

Logrank $p = 0.27$
HR (High) = 2.1
p (HR) = 0.28
n (High) = 246
n (Low) = 246

— Low LOXL2 TPM
— High LOXL2 TPM

(n)

READ
Overall survival

Logrank $p = 0.46$
HR (High) = 0.69
p (HR) = 0.46
n (High) = 46
n (Low) = 46

— Low LOXL2 TPM
— High LOXL2 TPM

(o)

STAD
Overall survival

Logrank $p = 0.073$
HR (High) = 1.3
p (HR) = 0.073
n (High) = 192
n (Low) = 192

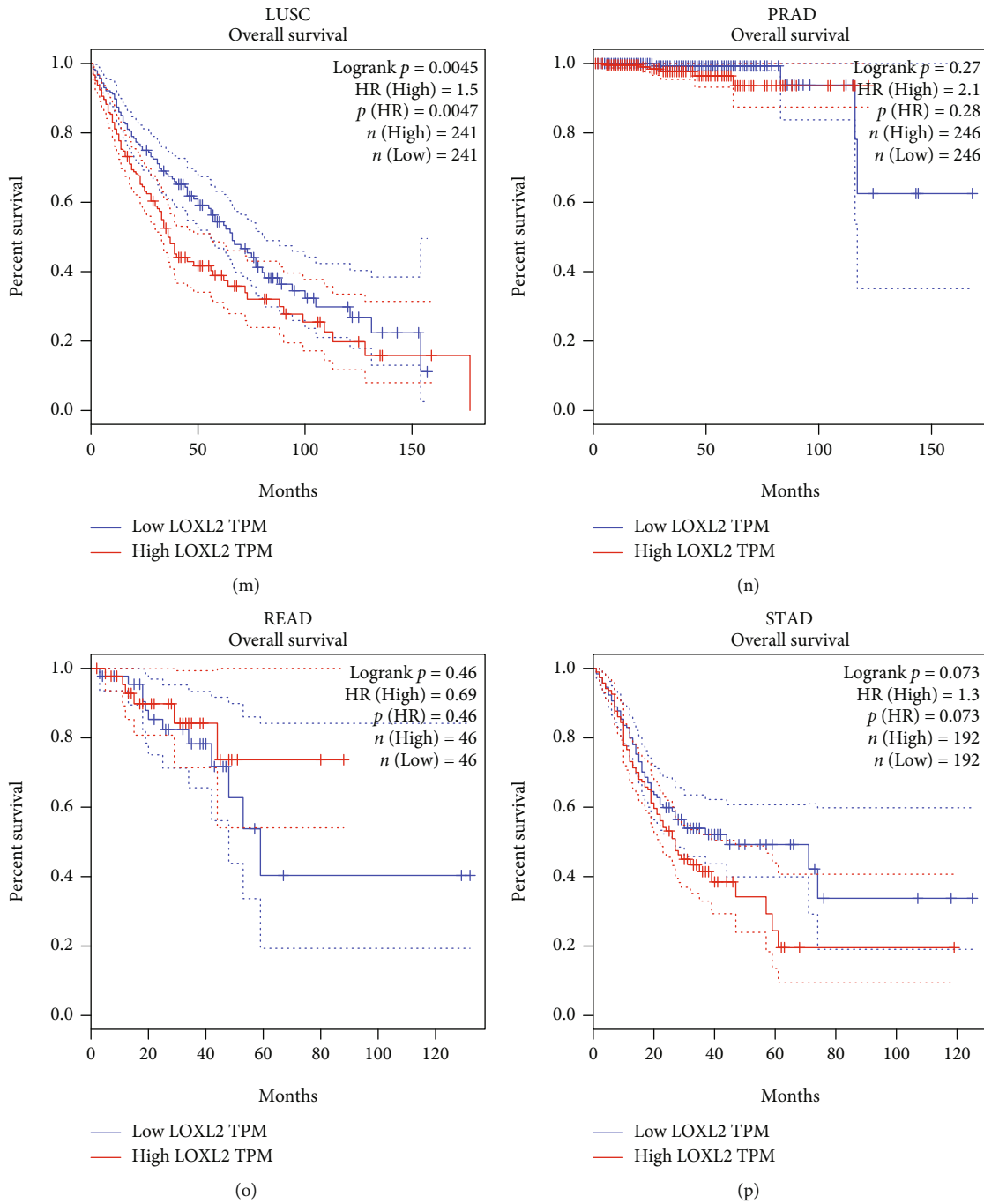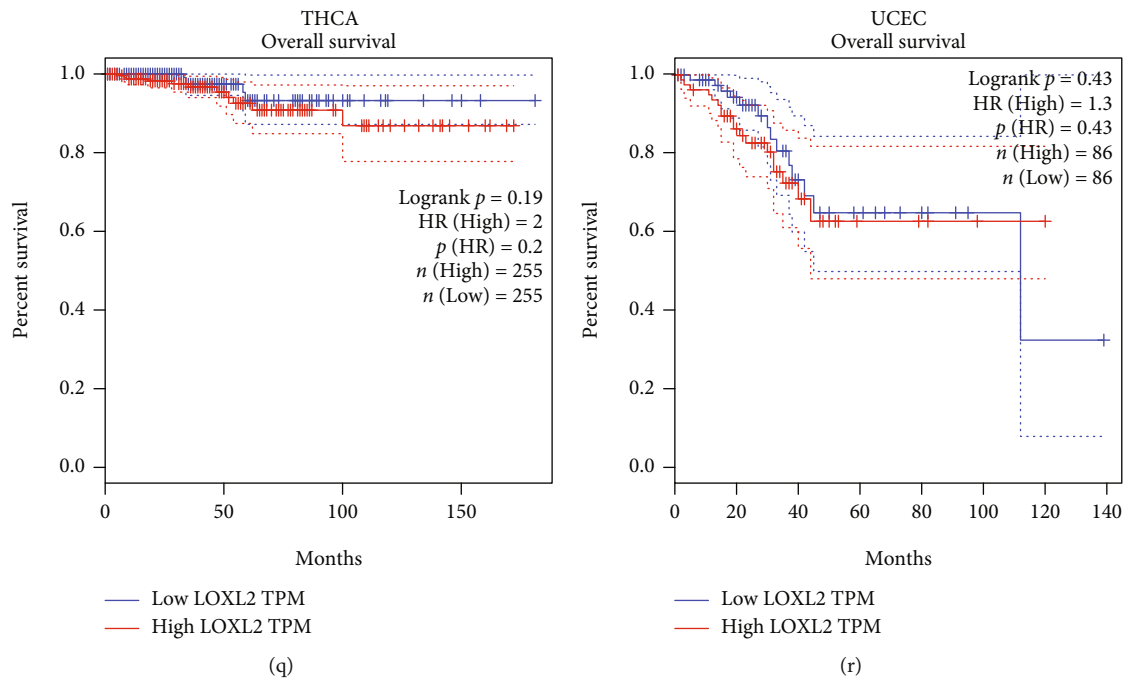— Low LOXL2 TPM
— High LOXL2 TPM

(p)

Figure 2: Continued.

FIGURE 2: The overall survival (OS) analysis for LOXL2 in various human cancers determined by the GEPIA database. (a)–(l) The OS plot of LOXL2 in BLCA (a), BRCA (b), CHOL (c), COAD (d), ESCA (e), GBM (f), HNSC (g), KICH (h), KIRC (i), KIRP (j), LIHC (k), LUAD (l), LUSC (m), PRAD (n), READ (o), STAD (p), THCA (q), and UCEC (r).

mRNA regulatory network and explore the mechanism of HCC at a deeper level. Moreover, the correlation of LOXL2 expression with immune cell infiltration, biomarkers of immune cells and immune checkpoints was finally discussed.

2. Methods

2.1. Download, Process, and Analysis of The Cancer Genome Atlas (TCGA) Data. The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer. gov/) is a collaboration between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) for cancer research. It provides a large and free reference for cancer research by collecting and organizing various cancer-related histological data. A total of 33 cancer types are currently included. Data in this research was obtained from the Liver Hepatocellular Carcinoma (LIHC) cohort, and then Log2 transformed. The expression levels of LOXL2 in tumor tissues were compared with normal tissues using Wilcoxon rank sum test in the eighteen cancers (BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC), and then visualized by box plots. The analysis was performed using the R software "limma" and "ggplot2" packages [18].

2.2. Analysis of LOXL2 Expression and Prognosis in Pan-Cancer by GEPIA Database. The GEPIA database (http://gepia.cancer-pku.cn/) integrates TCGA cancer data with GTEx normal tissue data, exploiting bioinformatics techniques to drill down into novel cancer targets and markers.

In this research, we analyzed the expression LOXL2 in tumor and normal samples and prognosis in pan-cancer by the GEPIA database [19]. The boxplots and Kaplan–Meier plots were downloaded for visualizing the results of differential expression analysis and survival analysis.

2.3. Prediction and Analysis of Upstream miRNAs of LOXL2. The starBase database (https://starbase.sysu.edu.cn/) is used to analyze data related to multiple cancers integrated from the TCGA project. It provides a platform for predicting miRNA targets by searching for miRNA targets through high-throughput CLIP-Seq experimental data and degradome experimental data which include lncRNAs, miRNAs, snoR-NAs, and mRNAs [20]. We use this platform to detect the upstream miRNA of LOXL2 in this research. After searching with the keyword "LOXL2" in "Target Gene" module and selecting the "miRNA-mRNA" option, the upstream miRNAs that LOXL2 may bind to would be presented. The filtering condition for screening is that the miRNAs would be predicted in two or more programs. The regulatory network between these predicted miRNAs and LOXL2 was demonstrated with Cytoscape software [21]. Among them, miRNAs with correlation coefficients greater than 0.2 were included in the subsequent analysis. In addition, expression analysis for the selected miRNA was also performed.

2.4. Prediction and Analysis of Upstream lncRNAs of miRNA. The upstream lncRNA of the miRNAs selected in the prior step was also identified in starBase database [20]. It has been known that miRNAs can bind to target mRNAs and inhibit their translation or cause mRNA degradation to achieve the function of posttranscriptional regulation of gene expression.
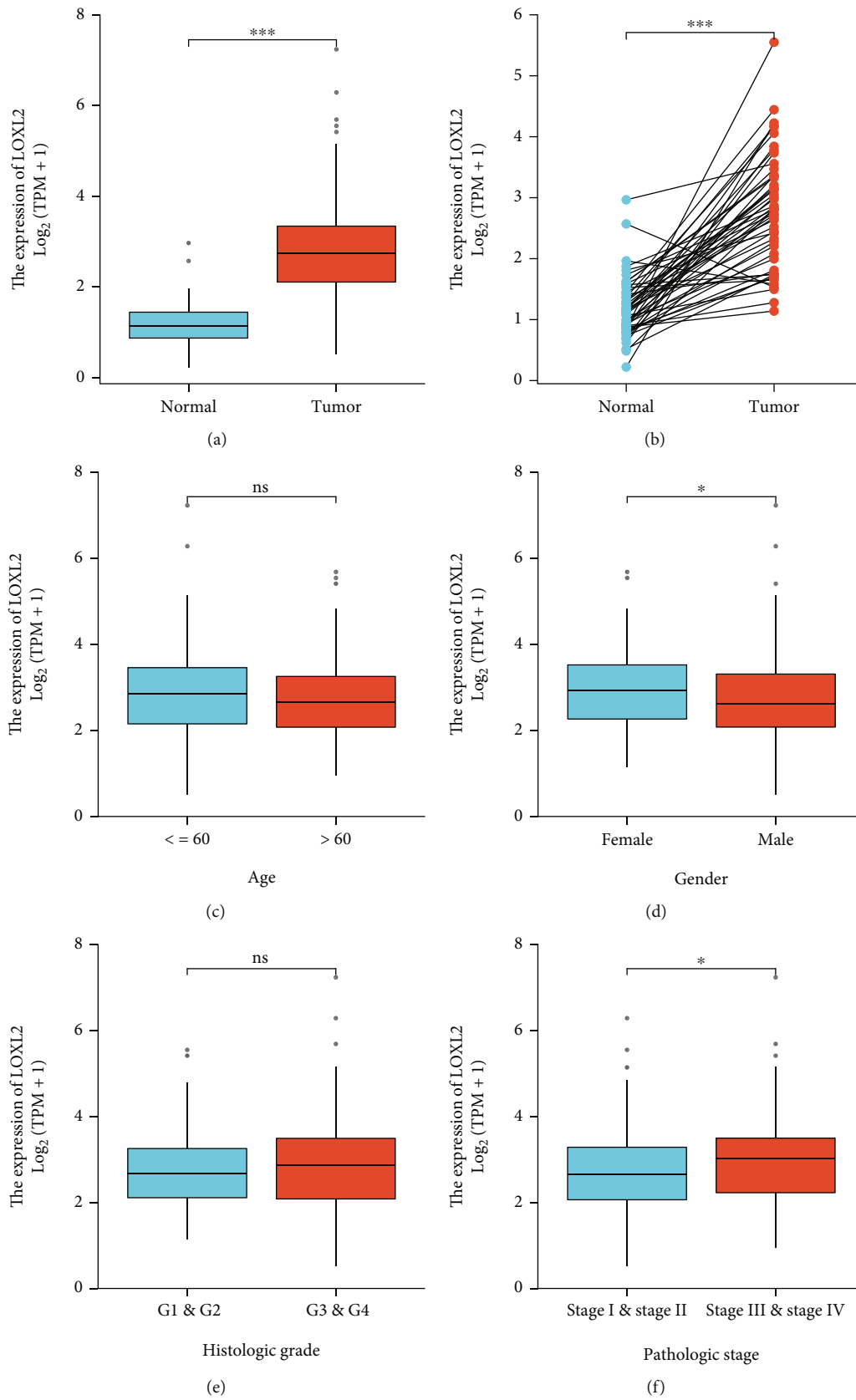
FIGURE 3: (a) Expression analysis for LOXL2 in unpaired sample. (b) Expression analysis for LOXL2 in paired sample. (c)–(f) Expression of LOXL2 in different clinical subgroups.

TABLE 1: Correlation between LOXL2 and different clinicopathological features.

Characteristic	Low-expression of LOXL2	High-expression of LOXL2	p
n	187	187	
Age, n (%)			0.107
≤ 60	80 (21.4%)	97 (26%)	
> 60	106 (28.4%)	90 (24.1%)	
Gender, n (%)			0.027
Female	50 (13.4%)	71 (19%)	
Male	137 (36.6%)	116 (31%)	
Race, n (%)			0.188
Asian	81 (22.4%)	79 (21.8%)	
Black or African American	12 (3.3%)	5 (1.4%)	
White	88 (24.3%)	97 (26.8%)	
T stage, n (%)			0.013
T1	101 (27.2%)	82 (22.1%)	
T2	49 (13.2%)	46 (12.4%)	
T3	33 (8.9%)	47 (12.7%)	
T4	2 (0.5%)	11 (3%)	
N stage, n (%)			0.125
N0	121 (46.9%)	133 (51.6%)	
N1	0 (0%)	4 (1.6%)	
M stage, n (%)			1.000
M0	130 (47.8%)	138 (50.7%)	
M1	2 (0.7%)	2 (0.7%)	
Pathologic stage, n (%)			0.121
Stage I	96 (27.4%)	77 (22%)	
Stage II	44 (12.6%)	43 (12.3%)	
Stage III	34 (9.7%)	51 (14.6%)	
Stage IV	2 (0.6%)	3 (0.9%)	
Histologic grade, n (%)			0.029
G1	37 (10%)	18 (4.9%)	
G2	87 (23.6%)	91 (24.7%)	
G3	54 (14.6%)	70 (19%)	
G4	7 (1.9%)	5 (1.4%)	
Tumor status, n (%)			0.305
Tumor free	106 (29.9%)	96 (27%)	
With tumor	71 (20%)	82 (23.1%)	

The ceRNA theory represents a new model of gene expression regulation. ceRNA molecules (lncRNA, circRNA, etc.) can compete to bind the same miRNA through miRNA Response Element (MRE) to regulate each other's expression levels [22–24]. Therefore, the eligible lncRNA should be negatively correlated with miRNA and positively correlated with mRNA.

2.5. Analysis of Immune Infiltration in HCC. The TIMER database (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for the systematic analysis of immune infiltrates in diverse cancer types [25]. TIMER database is used to detect six types of immune cell (including dendritic cells, macrophages, neutrophils, CD4+ T cells, CD8+ T cells, and B cells) infiltration in tumor tissues with RNA-Seq expression profiling data. In this study, the TIMER database was

used to estimate the correlation between LOXL2 and the extent of infiltration of specific immune cell subpopulations. Additionally, considering the potential oncogenic role of LOXL2 in HCC, the relationship of LOXL2 with immune checkpoints (involving CTLA4/PDCD1/CD274) was assessed as well.

2.6. Statistical Analysis. The statistical analysis was automatically calculated by the online database or statistical software. p value less than 0.05 was considered statistically significant.

3. Result

3.1. The Expression Level of LOXL2 in Pan-Cancers. To explore the role of LOXL2 in the development of HCC
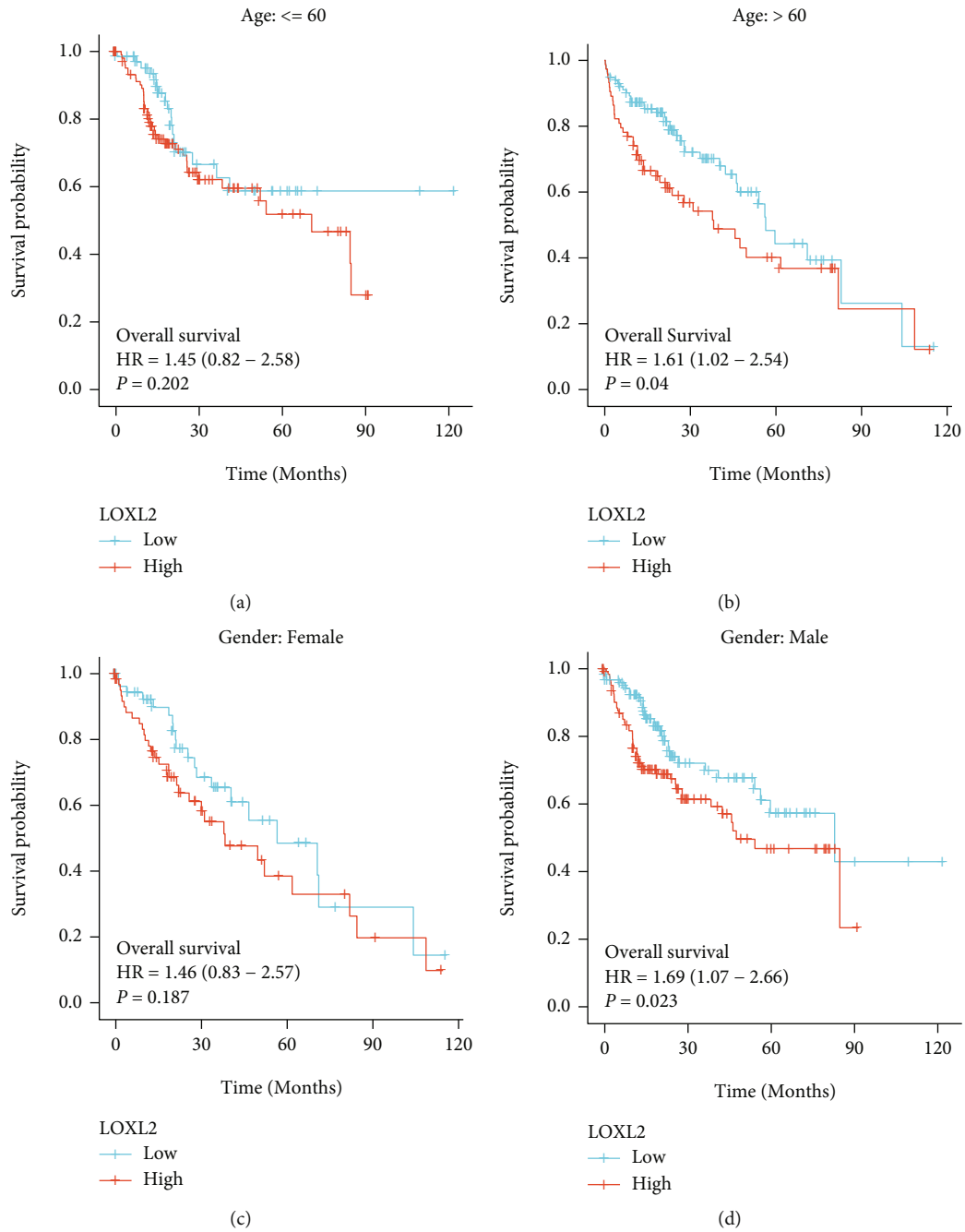
(a)

(b)

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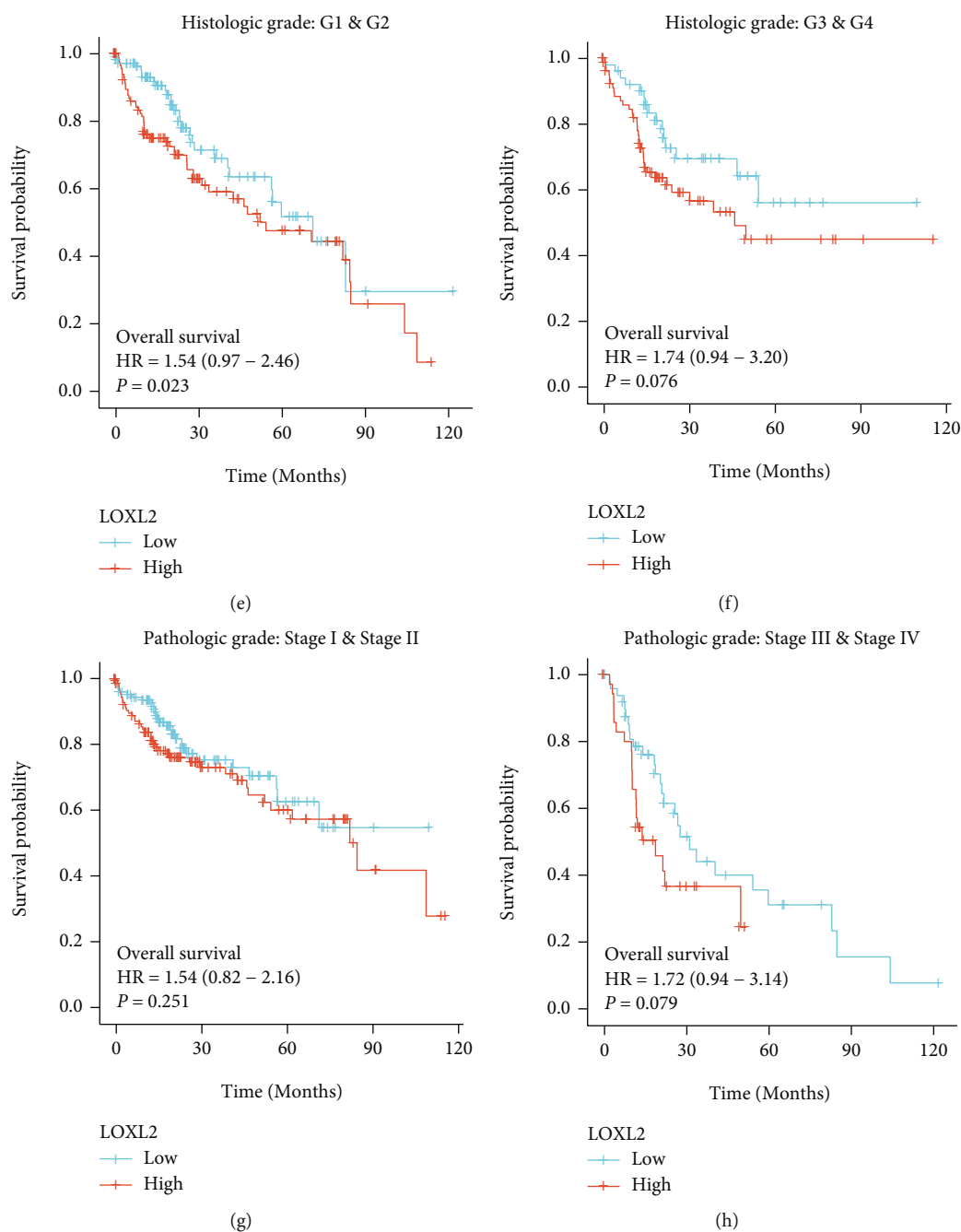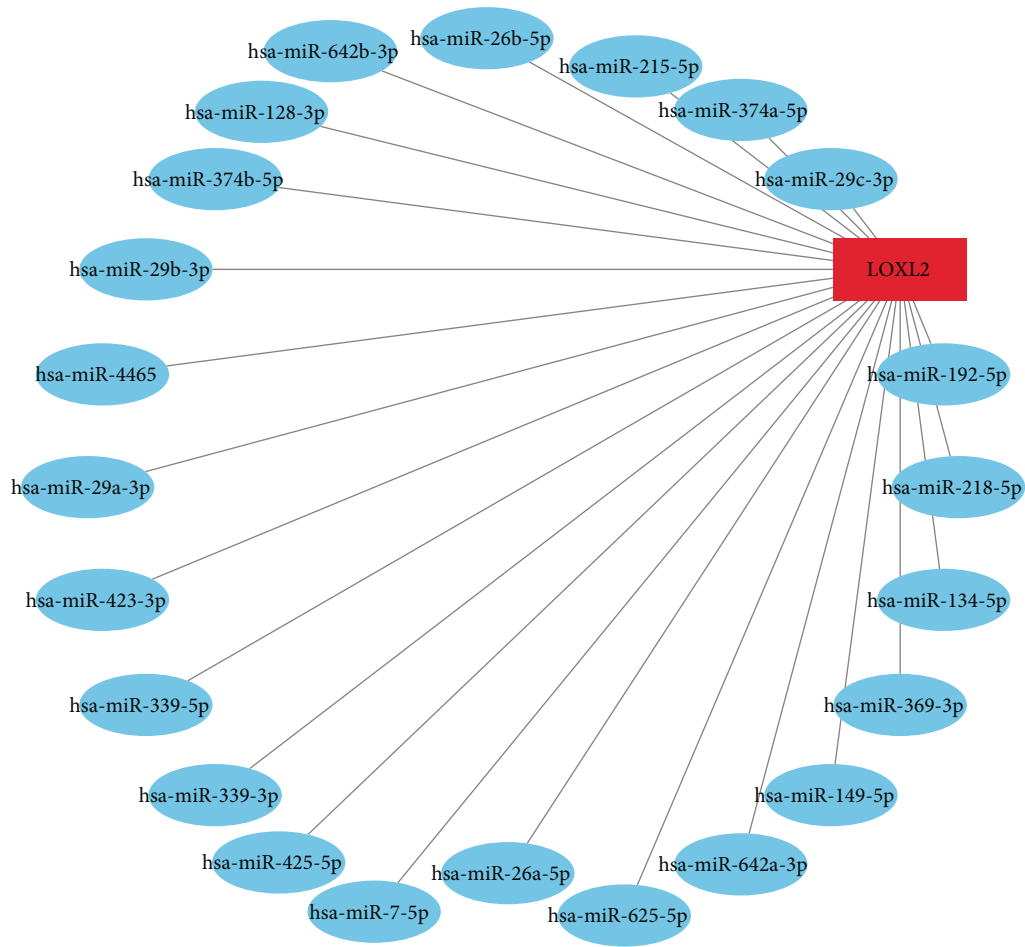
FIGURE 4: Continued.

(e)

(f)

(g)

(h)

Figure 4: Subgroup Kaplan–Meier curve analysis for different clinicopathological factors.

progression, we first analyzed the expression level of LOXL2 in 18 types of cancer based on the TCGA database, which found that LOXL2 was markedly upregulated in 16 cancer types, including BLCA, BRCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, READ, STAD, THCA, and UCEC and was significantly downregulated in PRAD. However, there was no significant difference between KICH and normal tissues (Figure 1(a)). Next, to further confirm this result, the GEPIA database was also used to evaluate the LOXL2 expression levels in these cancers. As presented in Figure 1(b), LOXL2 was significantly increased in CHOL, ESCA, GBM, HNSC, KIRC, LIHC,

and STAD, whereas it was decreased in PRAD, compared with normal tissues. Also, no statistically significant difference was observed in BLCA, BRCA, COAD, KICH, KIRP, LUAD, LUSC, READ, THCA, and UCEC (Figures 1(c)– 1(s)). Taken together, LOXL2 was upregulated in CHOL, ESCA, GBM, HNSC, KIRC, LIHC, and STAD and downregulated in PRAD, which indicated that LOXL2 was in connection with the development of the above cancer types.
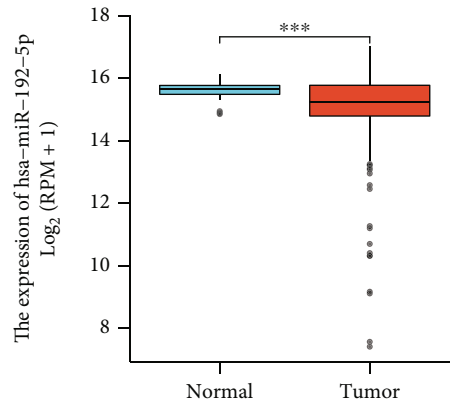
3.2. The Prognostic Value of LOXL2 in Pan-Cancers. GEPIA platform was used to analyze the prognostic value of LOXL2 in pan-cancers, and OS is selected to be the outcome indicator. As
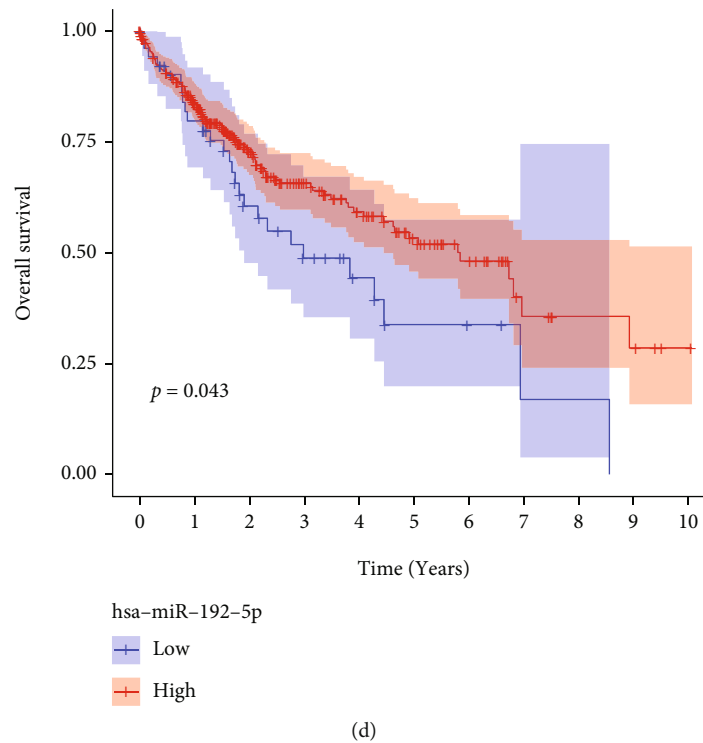
(a)
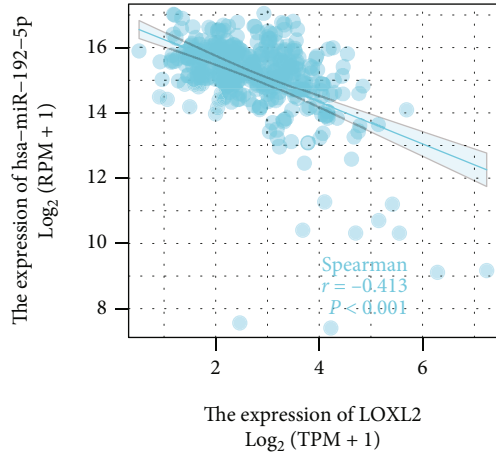
(b)

(c)

Figure 5: Continued.

(d)

FIGURE 5: Identification of hsa−miR−192−5p as a potential upstream miRNA of LOXL2 in HCC. (a) The miRNA-LOXL2 regulatory network. (b) The correlation between hsa−miR−192−5p and LOXL2 in HCC. (c) The expression of hsa−miR−192−5p in HCC and control normal samples. (d) The prognostic value of hsa−miR−192−5p in HCC.

shown in Figure 2, higher expression of LOXL2 was associated with worse OS in LIHC, LUAD, and LUSC. Consequently, combining the expression of LOXL2 between tumor and normal tissues and its prognostic value, LOXL2 may be utilized as an unfavorable prognostic biomarker in patients with LIHC.

3.3. Further Exploration of LOXL2 Expression Levels and Survival Conditions in HCC.
The expression analyses of LOXL2 in unpaired samples, paired samples, and different clinical subgroups are shown in Figure 3. With the increased expression level of LOXL2, the pathologic stage of HCC increases accordingly. Also, we found that in female, the expression level of LOXL2 was higher than that in male. Table 1 listed the LOXL2 expression data and clinical data for 374 HCC patients. We observed a significant association between LOXL2 expression and clinicopathological features, such as gender, T stage, and histologic grade ($p < 0.05$).

As for survival, we further performed subgroup analyses to assess the impact of LOXL2 expression on OS of patients with HCC according to age, gender, pathological stage, and differentiation grade. We found that high-expression of LOXL2 resulted in poor survival in patients older than 60 years old and in male patients (Figure 4).

3.4. Prediction and Analysis of Upstream miRNA of LOXL2.
MiRNAs represent a class of noncoding single-stranded RNA molecules of approximately 22 nucleotides in length encoded by endogenous genes, which are involved in the posttranscriptional regulation of gene expression. Twenty-two possible miRNAs were found in the starBase database

according to the rules, and the regulatory network was presented in Figure 5(a). Notably, the miRNA that regulates the mRNA must be negatively correlated with that mRNA. Therefore, the screening criteria were set: the value of the correlation coefficient was less than -2 and the p value was less than 0.05. Finally, only hsa−miR−192−5p met the conditions (Figure 5(b)). Furthermore, hsa−miR−192−5p was found to be lowly expressed in HCC tissues and patients with lower hsa−miR−192−5p expression had a better prognosis. (Figures 5(c) and 5(d)).

3.5. Prediction and Analysis of Upstream lncRNAs of Hsa−miR−192−5p.
Also, the upstream lncRNAs of miRNAs can be searched in the starBase database. After downloading the twelve relevant lncRNAs from the database, the regulatory network was visualized using Cytoscape software (Figure 6(a)). However, among these lncRNAs, only CARMN was negatively correlated with hsa−miR−192−5p ($R < −2$, $P < 0.05$) and positively correlated with LOXL2 ($R > 2$, $P < 0.05$) (Figures 6(b) and 6(c)). Besides, expression analysis showed that CARMN was highly expressed in the tumor samples (Figure 6(d)). Thus, CARMN was selected as a promising upstream lncRNA for the miR-192-5p/LOXL2 axis in HCC.

3.6. LOXL2 Correlates with Immune Cell Infiltration in HCC.
The relationship between LOXL2 and immune cell infiltration was investigated using the timer database because the level of immune cells is associated with the proliferation and development of tumor cells.
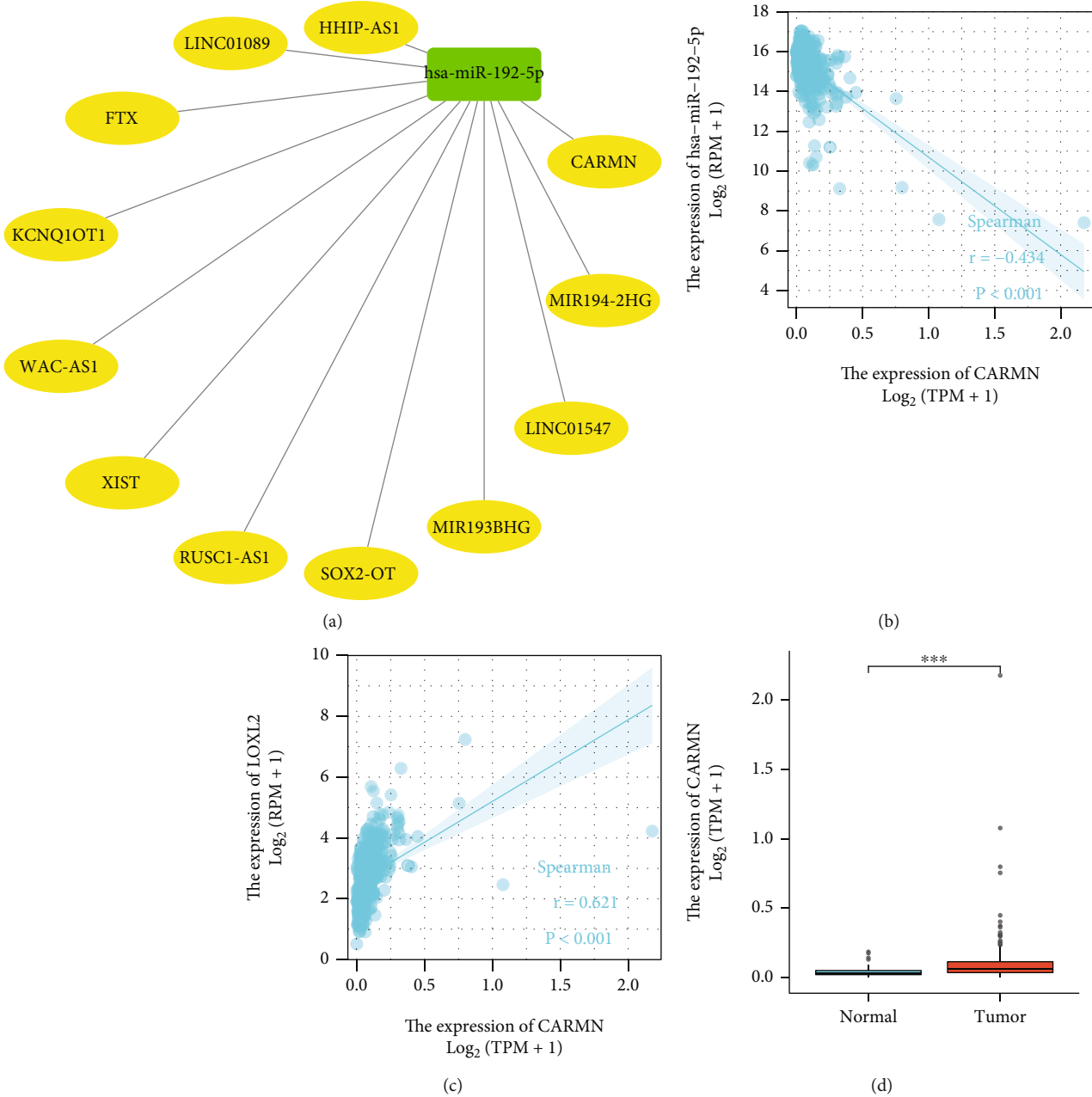
(a)

(b)

(c)

(d)

FIGURE 6: Identification of CARMN as a potential upstream LncRNA of hsa−miR−192−5p in HCC. (a) The LncRNA- hsa−miR−192−5p regulatory network. (b) The expression correlation between hsa−miR−192−5p and CARMN in HCC. (c) The correlation between LOXL2 and CARMN in HCC. (d) The expression of CARMN in HCC and control normal samples.
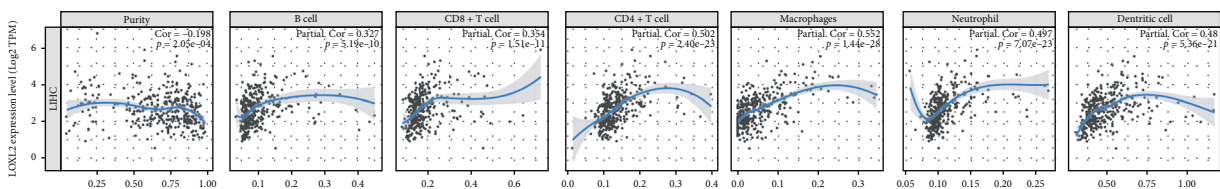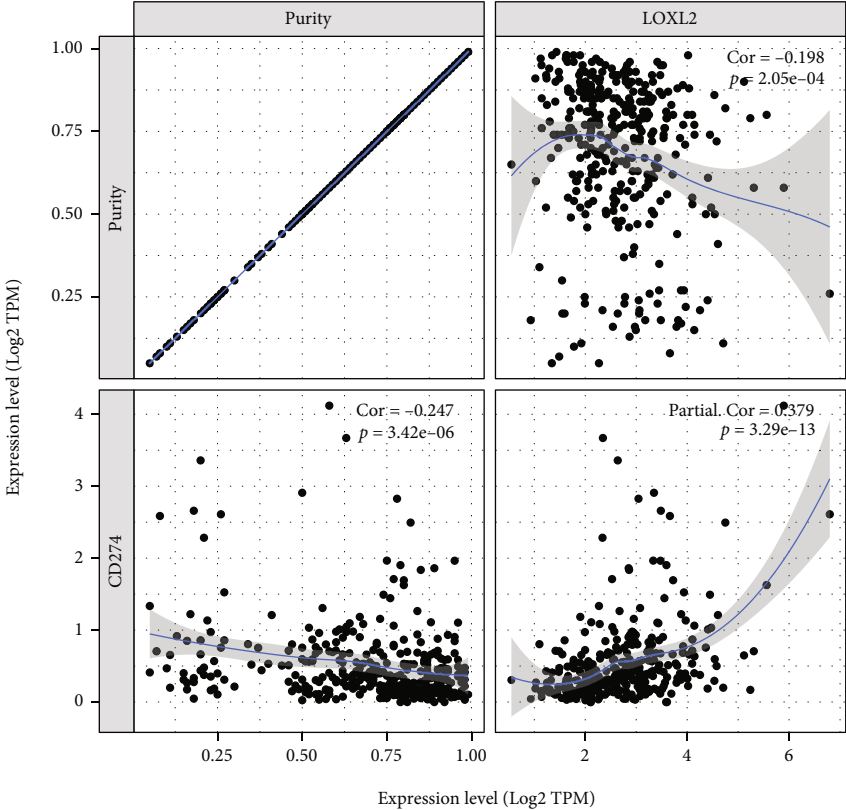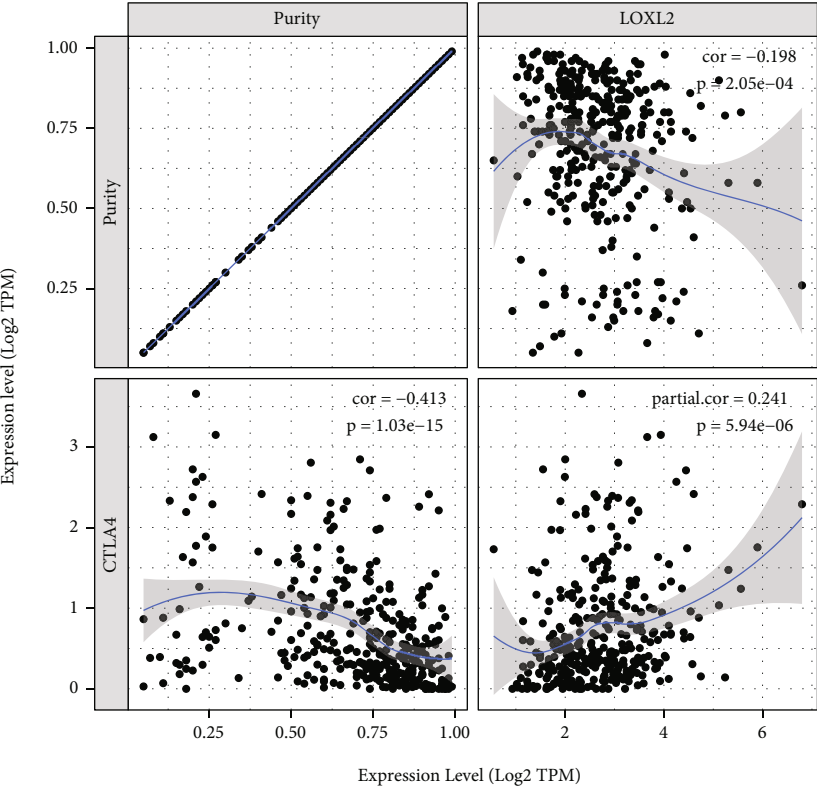

FIGURE 7: The correlation of LOXL2 expression level with B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil, or dendritic cell infiltration level in HCC.
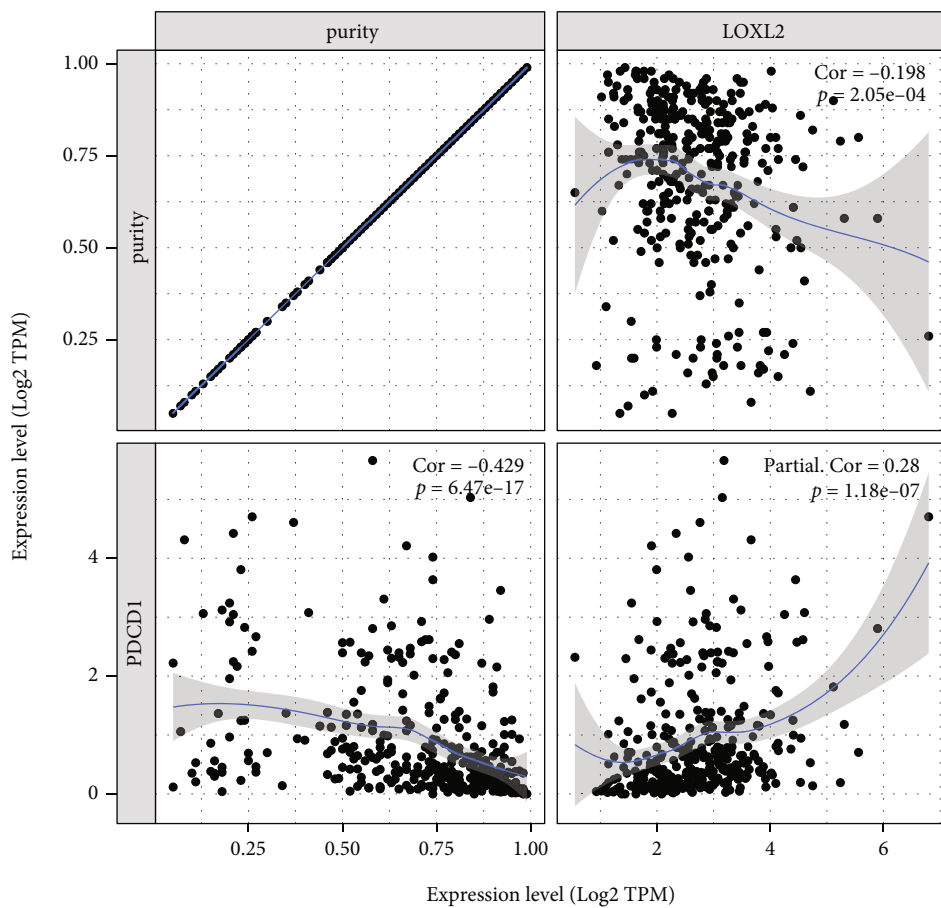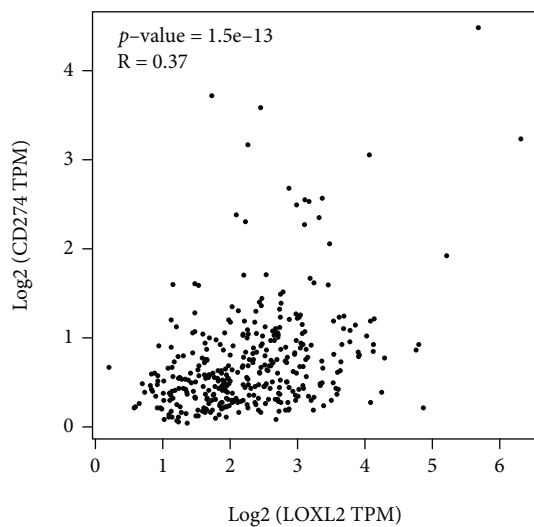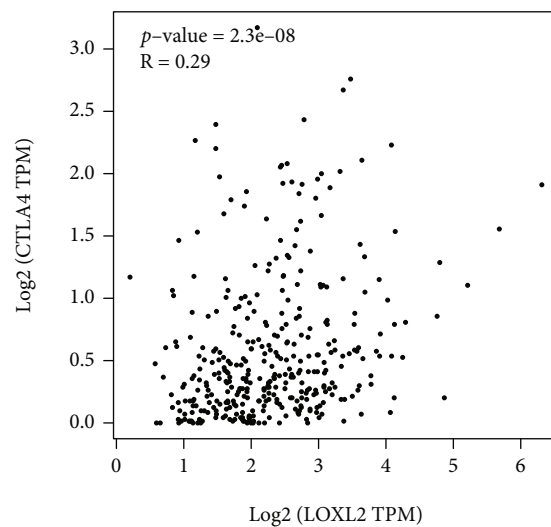
(a)

(b)

FIGURE 8: Continued.

(c)
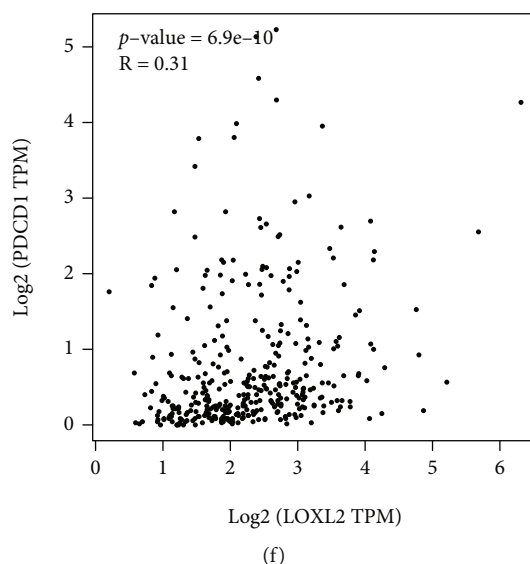

(d)

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Figure 8: Continued.

(f)

Figure 8: Correlation of LOXL2 expression with CD274, PDCD1, and CTLA-4 expression in HCC (a) Spearman correlation of LOXL2 with expression of CD274 in HCC adjusted by purity using TIMER. (b) Spearman correlation of LOXL2 with expression of PDCD1 in HCC adjusted by purity using TIMER. (c) Spearman correlation of LOXL2 with expression of CTLA-4 in HCC adjusted by purity using TIMER. (d) The expression correlation of LOXL2 with CD274 in HCC determined by GEPIA database. (e) The expression correlation of LOXL2 with PDCD1 in HCC determined by GEPIA database. (f) The expression correlation of LOXL2 with CTLA-4 in HCC determined by GEPIA database.

The expression of LOXL2 was positively correlated with the infiltration of dendritic cells, neutrophils, macrophages, CD8+ T cells, CD4+ T cells, and B cells (Figure 7). Among them, CD4+ T Cells, macrophages, and neutrophils showed the strongest positive correlation.

3.7. The Relationship between LOXL2 and Immune Checkpoints in HCC.

CD274, PDCD1, and CTLA-4 were known as critical immune checkpoints that are associated with immune escape in cancers. So, the relationship between LOXL2 and these checkpoints was analyzed via online tools. Both TIMER data analysis and GEPIA data analysis found a significant positive correlation of LOXL2 with CD274, PDCD1, and CTLA-4 (Figures 8(a)–8(f)).

4. Discussion

HCC is one of the most common malignant tumors in the world, and with the continuous development of medical technology and research on the molecular biology of tumors, targeted therapy for hepatocellular carcinoma is developing rapidly [1, 2]. TERT, MLL4, CCNE1, TP53, and CTNNB1 were identified as commonly mutated genes in HCC [26–30]. Despite the availability of many potential therapeutic targets, the incidence and mortality rates of patients with HCC are still increasing currently [8–11]. The overall survival rate remains suboptimal, so it is crucial to explore the underlying molecular mechanisms and oncogenes to provide new ideas for the diagnosis and treatment of HCC. Previous studies had uncovered the value of LOXL2 in different tumors, but the exact mechanism remains unclear [15, 31–33]. In this study, we discussed the role of LOXL2 in

HCC through bioinformatics analysis to further understand the potential value of LOXL2.

In this present study, the expression of LOXL2 in various cancers was analyzed using different databases (TCGA database and GEPIA database), and it was concluded that LOXL2 was significantly differentially expressed in HCC. The next survival analysis using GEPIA online tool revealed that LOXL2 overexpression leads to a poorer prognosis in patients with HCC. Wu et al. constructed LOXL2-small interfering RNA using a lentiviral vector and investigated the effect of LOXL2 on the proliferation of HCC cell lines by reverse transcription-quantitative polymerase chain reaction and other experimental methods [34]. The results showed that LOXL2 was highly expressed in HCC tissues and that LOXL2 silencing reduced cell number, proliferation, colony formation, and cell growth, induced cell cycle arrest, and increased apoptosis [34]. This study and our results both demonstrated the oncogenic role of LOXL2 in HCC.

The starBase database contains seven programs that can be used to predict miRNAs, including TargetScan, miRmap, miRanda, PicTar, RNA22, PITA, and microT. Through these programs, twenty-two potential miRNAs were found for LOXL2. Among all these 22 possible miRNAs, only hsa-miR-192-5p showed a significant negative correlation with LOXL2. Subsequently, the data of miRNAs were downloaded from the TCGA database, and the differential analysis showed that hsa-miR-192-5p was lowly expressed in the tumor samples, and its low-expression was related to the poor prognosis of HCC. During the past decade, more and more studies are focusing on the role of miR-192-5p in cancers. For example, in Wang et al.'s experiment, they found that miR-192-5p-modified tumor-associated macrophages-

derived exosome suppressed endometrial cancer progression by targeting IRAK1/NF-κB signaling [35]. The role of miR-192-5p in HCC is also noteworthy. Previous studies revealed that miR-192-5p loss enhanced glycolysis and over produced lactate might further increase HCC malignant features via interacting with environmental nontumor cells [36]. Previous findings are consistent with our predictions. To summarize, hsa-mir-192-5p was found to be a vital regulatory molecule of LOXL2 in HCC.

According to the ceRNA theory, it is known that ceRNA inhibits the inhibitory effect of miRNA on mRNA by binding to it. Therefore, it is essential to find the upstream lncRNA of hsa-mir-192-5p because it plays a role in cancer development. StarBase bioinformatics software predictions showed that CARMN may be the upstream lncRNA of miR-192-5p. The expression analysis also showed that CARMN was highly expressed in the tumor samples. There is a paucity of research on CARMN in cancers. Sheng et al. found that overexpression of CARMN can promote the prognosis and chemosensitivity in breast cancer [37]. Other reports on the role of lncRNA CARMN in cancers are currently scarce. However, the results of this paper suggested that CARMN might influence the progression of HCC by regulating the miR-192-5p/LOXL2 axis.

The tumor microenvironment is a hot topic of research in recent years. The immune microenvironment, consisting of tumor-infiltrating lymphocytes (B cells, T cells) and other immune cells (such as dendritic cells, macrophages, and neutrophils), is an important part of the tumor microenvironment and is considered as the "seventh hallmark feature" of tumors, so more research is urgently needed to focus on the link between immune cell infiltration and tumors [38–40]. In our research, a positive correlation between LOXL2 expression and dendritic cells, neutrophils, macrophages, CD8+ T cells, CD4+ T cells, and B cell in HCC was observed. In addition, the correlation between LOXL2 expression and immune checkpoint markers (CD274, PDCD1, and CTLA-4) suggested a role for LOXL2 in immune regulation of tumor immunity. Consequently, it is hypothesized that tumor immune infiltration exerts an influential role in LOXL2-mediated HCC development.

The limitation of this study should be mentioned. First, this is a bioinformatics study based on an online database that lacks experimental validation. Second, in identifying CARMN as a potential upstream LncRNA for hsa-miR-192-5p in HCC, serval extreme values may leverage the true correlation and may lead to overestimation of the correlation of CARMN with hsa-miR-192-5p. Third, the results need to be interpreted carefully because many of the data do not have high-correlation values.

In short, CARMN was identified as a possible upstream lncRNA for miR-192-5p, which affects LOXL2 expression and promotes the progression of HCC. Our study suggests that LOXL2 may exert its tumorigenic effects by potentiating tumor immune cell infiltration and immune checkpoint expression. However, in the future, more relevant studies are still needed to verify these predictions by bioinformatics.

Data Availability

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

Conception and design are contributed by Xiaokang Wang; administrative support is contributed by Shulong Wu and Yi Yang; data analysis and interpretation, manuscript writing, and final approval of the manuscript are contributed by all the authors.

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