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The Role of *ZNF207* in Liver Hepatocellular Carcinoma: Expression Analysis and Prognostic Implications

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Abstract: *Objective:* To analyze the expression and clinical significance of the zinc finger protein *ZNF207* gene in liver hepatocellular carcinoma (LIHC) based on The Cancer Genome Atlas (TCGA) database. *Methods:* The mRNA sequencing data of 371 cases of primary liver cancer, 50 cases of normal tissues, and 3 cases of recurrent liver cancer were downloaded from the TCGA database. The corresponding clinical information of the 371 cases of hepatocellular carcinoma was subsequently analyzed. The difference in *ZNF207* expression between normal and tumor tissues was analyzed using the UALCAN online database. The impact of *ZNF207* expression on survival prognosis was assessed using the Kaplan-Meier method in R software. The GO and KEGG pathways of *ZNF207* were analyzed. The Cox proportional hazards model was used to evaluate the prognostic factors of patients with LIHC. RT-qPCR was employed to verify the expression of *ZNF207* in LIHC cells. *Results: ZNF207* was highly expressed in LIHC tissues and HepG2 cells, with a significant difference ($P < 0.05$). Multivariate Cox regression analysis revealed that patients with high *ZNF207* expression had a significantly shorter overall survival time compared to those with low *ZNF207* expression (HR = 1.466, 95% CI: 1.011–2.126, $P < 0.05$). GO enrichment analysis suggested that *ZNF207* may influence the onset and progression of hepatocellular carcinoma by regulating mRNA splicing and mRNA transcription processing through the spliceosome. KEGG pathway enrichment analysis indicated that *ZNF207* might affect the onset and progression of hepatocellular carcinoma through mitophagy, mRNA surveillance, homologous recombination, spliceosome, and nuclear-cytoplasmic transport. *Conclusion:* The expression of *ZNF207* may be an independent predictor of the prognosis of patients with LIHC and could influence the development of hepatocellular carcinoma through various gene functions and pathways. It has the potential to serve as a novel molecular marker for predicting the prognosis of hepatocellular carcinoma.

Keywords: *ZNF207*; Hepatocellular carcinoma; TCGA database

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1. Introduction

Liver cancer is one of the most common types of malignant tumors, with various pathogenic factors contributing to its occurrence and development. Hepatocellular carcinoma (LIHC) is the most prevalent form of primary liver cancer [1].

In countries with a high prevalence of hepatitis B virus, there is a significant progression from hepatitis to liver cirrhosis and eventually to liver cancer. The clinical symptoms and signs of liver cancer are often not obvious, with the disease typically being discovered at a late stage. Many patients with advanced liver cancer, who are less likely to undergo surgical resection, have limited treatment options and often resort to local or palliative treatments [2]. Early detection, diagnosis, and treatment are essential to reduce the mortality rate of liver cancer. Consequently, many researchers have conducted extensive studies to explore potential biomarkers of LIHC. Early diagnosis of hepatocellular carcinoma is crucial, and identifying these biomarkers provides a strong foundation for it.

ZNF207 is a protein-coding gene located on human chromosome 6p21.3 and is a member of the zinc finger protein family. *ZNF207* has been shown to regulate embryonic stem cell self-renewal and pluripotency [3]. Additionally, *ZNF207* can inhibit the immune microenvironment of hepatocellular carcinoma [4]. Zhou Chenghui *et al*. demonstrated that *ZNF207* is highly expressed in hepatocellular carcinoma [5]. However, due to the limited sample size and lack of ethnic diversity in studies of *ZNF207* expression, the clinical prognosis for LIHC patients remains uncertain. Furthermore, there is a lack of research on the function and pathway of *ZNF207* in hepatocellular carcinoma. This study aims to investigate the expression of *ZNF207* in LIHC, its clinical significance, and the function and pathways associated with *ZNF207*, using gene expression data from The Cancer Genome Atlas (TCGA) database and corresponding clinical information.

2. Materials and methods

2.1. Cell sample source and data download and collation

HepG-2 and Lo2 cells, obtained from the Shanghai Institute of Cell Biology, were used in this study at the Chinese Academy of Sciences. The RNA-Seq data of 50 normal tissues and 374 hepatocellular carcinoma tissues (including three patients with recurrent hepatocellular carcinoma) were retrieved and downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), along with clinical data of 371 patients with hepatocellular carcinoma.

2.2. UALCAN database analysis

The UALCAN database (UACAN.path.uab.edu/home) integrates clinical information and transcriptional data from the TCGA database of various tumors to enable visual online analysis [6]. The UALCAN database was used to analyze the difference in *ZNF207* expression between normal and tumor tissues.

2.3. Survival analysis

Using the “ggplot2,” “ggpubr,” “survminer,” and “survival” R packages in the R language, a graph illustrating the relationship between the expression of *ZNF207* and total survival time (K-M curve) was created.

2.4. Human Protein Atlas database analysis

The Human Protein Atlas (HPA) is a database (https://proteinatlas.org/) that integrates data from proteomics, transcriptomics, and systems biology to map tissues, cells, and organs. The protein expression data includes both tumor and normal tissues, and the survival curve of tumor patients can also be consulted [7]. The difference in *ZNF207* expression between normal tissues and LIHC tissues was investigated using the gene expression immunohistochemical staining map.

2.5. Relationship between ZNF207 expression level and clinical characteristics and survival status of LIHC

Clinical information and corresponding *ZNF207* expression data were removed for 2 patients with missing survival status. Using the "pheatmap" and "ggplot2" R packages in the R language, the elevation of *ZNF207* expression was plotted. This plot corresponds to the heatmap of clinical features and the dot plot of survival status.

2.6. Gene function enrichment analysis

Using Pearson correlation analysis, the most relevant gene set of *ZNF207* was selected (Pearson product-moment correlation coefficient cor > 0.5). The gene set was uploaded to The Database for Annotation, Visualization, and Integrated Discovery (DAVID, V2021). *Homo sapiens* was selected as the species, and the results of Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were obtained. The first five places with the largest *P*-values are shown as $P = -\text{Log}_{10}(P\text{Value})$.

2.7. The relationship between ZNF207 and clinical common immune checkpoints

Using the "Circlize" R package, chords representing the correlation degree between ZNF207 and common tumor immunosuppression targets such as "CD200R1," "CD47," "CTLA4," "Tim-3," "PD-1," "TIGIT," and "HVEM" were plotted. The correlation degree was determined using Pearson correlation analysis (COR > 0).

2.8. RT-qPCR verification of ZNF207 gene expression

Total RNA was isolated and extracted using the TRIZOL method, and the quality of the extracted RNA was assessed. Total RNA was reverse transcribed into cDNA using the PrimeScript RT Master Mix (Perfect Real Time) reverse transcription kit. RT-qPCR reaction assays were meticulously performed using the TB Green Premix Ex Taq II (TLI RNaseH Plus) fluorescent PCR kit, following specific instructions. Using β-actin as an internal reference, the relative expression of the ZNF207 gene was determined by the $2^{-\Delta\Delta CT}$ method. The primers used were Forward: GCCTCAACTTCATTT-CAGCCACAG and Reverse: CATTCCTGGTGCTCCTGGTACTG. The reaction conditions were as follows: 95°C for 30 seconds (one cycle), 95°C for 5 seconds, and 60°C for 30 seconds (39 cycles), followed by 95°C for 10 minutes, 65°C for 5 seconds, and 95°C for 5 minutes.

2.9. Statistical analysis

The Pearson correlation between *ZNF207* and other genes was calculated using R software. In IBM SPSS Statistics 29.0, a univariate Cox proportional hazards model was used to assess the association of ZNF207 expression and clinical characteristics with prognosis in patients with LIHC. To provide statistical evidence for the aforementioned studies, Prism 9 (version 9.4.1) was utilized to demonstrate the expression of ZNF207. Additionally, χ^2 was employed to assess the correlation between the expression of ZNF207 and the clinical features of 371 patients with LIHC after multiple imputations. The difference was statistically significant with $P < 0.05$.

3. Results

3.1. Expression of ZNF207 in LIHC

The UALCAN database showed that *ZNF207* was significantly upregulated in hepatocellular carcinoma compared to normal tissues, with a statistically significant difference ($P < 0.05$; **Figure 1A**). Immunohistochemical staining from the HPA database revealed a higher density of stained granules in LIHC

tissues than in normal tissues, indicating elevated *ZNF207* expression in LIHC (**Figure 1B**).

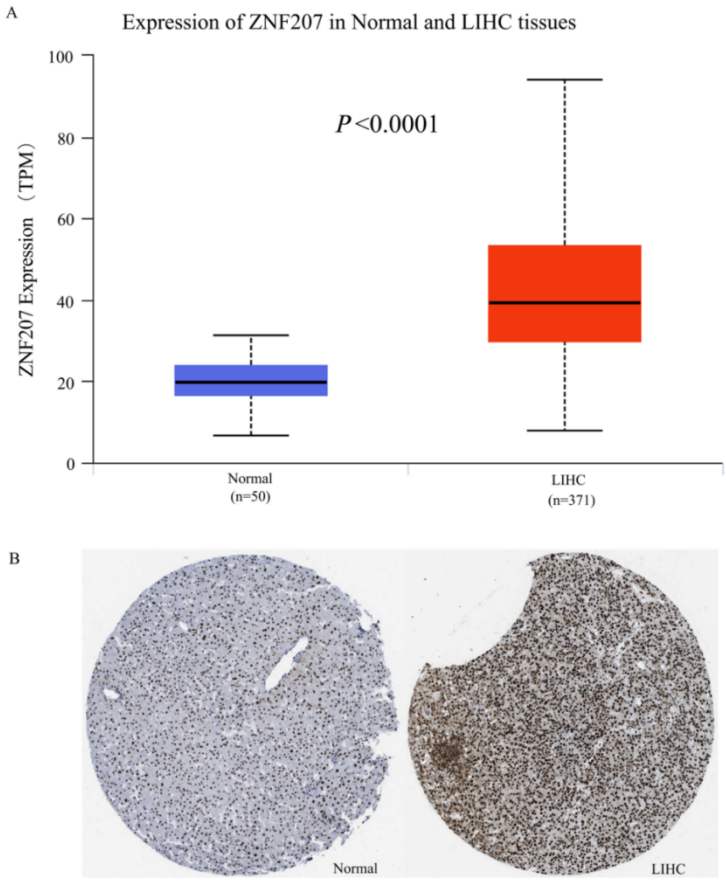

Figure 1. Expression of *ZNF207* in normal and LIHC tissues. **(A)** Comparison of *ZNF207* expression levels in normal tissues and LIHC tissues using the UALCAN database (ualcan.path.uab.edu/home); **(B)** *ZNF207* immunohistochemical staining in normal and LIHC tissue as seen on the HPA online database (The Human Protein Atlas, https://proteinatlas.org/).

3.2. Influence of different expressions of ZNF207 on survival prognosis

The Kaplan-Meier curve illustrating the impact of varying *ZNF207* expression on survival prognosis was generated using R software. As depicted in **Figure 2**, the overall survival rate of the high-expression *ZNF207* group was significantly lower than that of the low-expression group, with a statistically significant difference ($P < 0.05$).

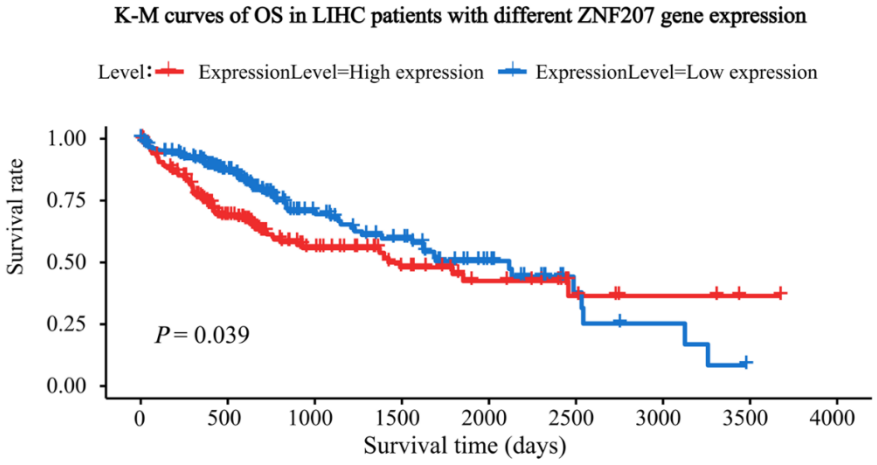

Figure 2. Relationship between *ZNF207* expression and survival prognosis

3.3. Relationship between ZNF207 expression and clinical characteristics and survival status in LIHC

As *ZNF207* expression increased, the rate of cell death also rose, suggesting a synchronous relationship between *ZNF207* and LIHC, as shown in **Figure 3A**. Additionally, changes in the T stage of the TNM classification in LIHC were synchronized with changes in *ZNF207* expression. However, there were no significant changes in age, sex, and race in the clinical characteristics of LIHC with increased *ZNF207* expression, as shown in **Figure 3B**.

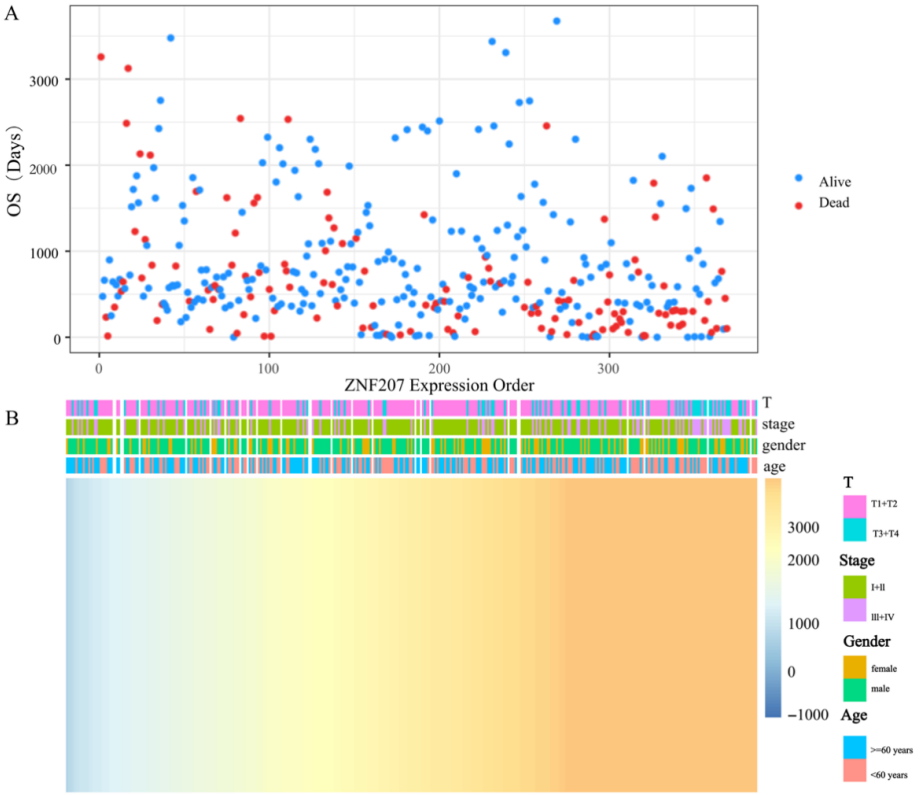

Figure 3. *ZNF207* elevated expression correlates with changes in patient survival status and clinical characteristics. **(A)** Scatter plot showing patient survival in relation to the increase in *ZNF207* expression; **(B)** Heatmaps illustrating patients' clinical features as *ZNF207* expression increases.

3.4. ZNF207 expression correlated with clinical features

As shown in **Table 1**, *ZNF207* expression was not correlated with sex or age 60 or older in patients with LIHC ($P > 0.05$). However, it was associated with the clinical stage (I + II vs. III + IV) and TNM stage (T1 + T2 vs. T3 + T4) in patients with LIHC ($P < 0.05$).

3.5. Gene enrichment analysis results

The biological processes involved in the enrichment analysis of the ZNF207 gene ontology (GO) mainly include the negative regulation of mRNA splicing by the spliceosome, regulation of RNA splicing, RNA splicing, mRNA processing, and splicing of mRNA by the spliceosome (**Figure 4A**). The cellular components mainly include catalytic Step II spliceosomes, nuclear speckles, ribonucleoprotein complexes, nuclei, and cytoplasm (**Figure 4B**). Molecular functions mainly include nucleic acid binding, transcription cofactor activity, protein binding, mRNA binding, and RNA binding (**Figure 4C**). In the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of ZNF207, the main pathways identified were mitophagy-animal, mRNA surveillance, homologous recombination, spliceosome, and nucleoplasmic transport (**Figure 4D**).

Table 1. Relationship between *ZNF207* expression and clinical characteristics of patients

Clinical features	Cases	Expression level of *ZNF207*/case		*P*
		Low (*n* = 185)	High (*n* = 186)	
Gender				
Male	250	128	122	0.460
Female	121	57	64	
Age (years)				
< 60	170	81	89	0.432
≥ 60	201	104	97	
Clinical stage				
I + II	273	145	128	0.037*
III + IV	98	40	58	
TNM stage				
T1 + T2	278	147	131	0.045*
T3 + T4	93	38	55	

*$P < 0.05$

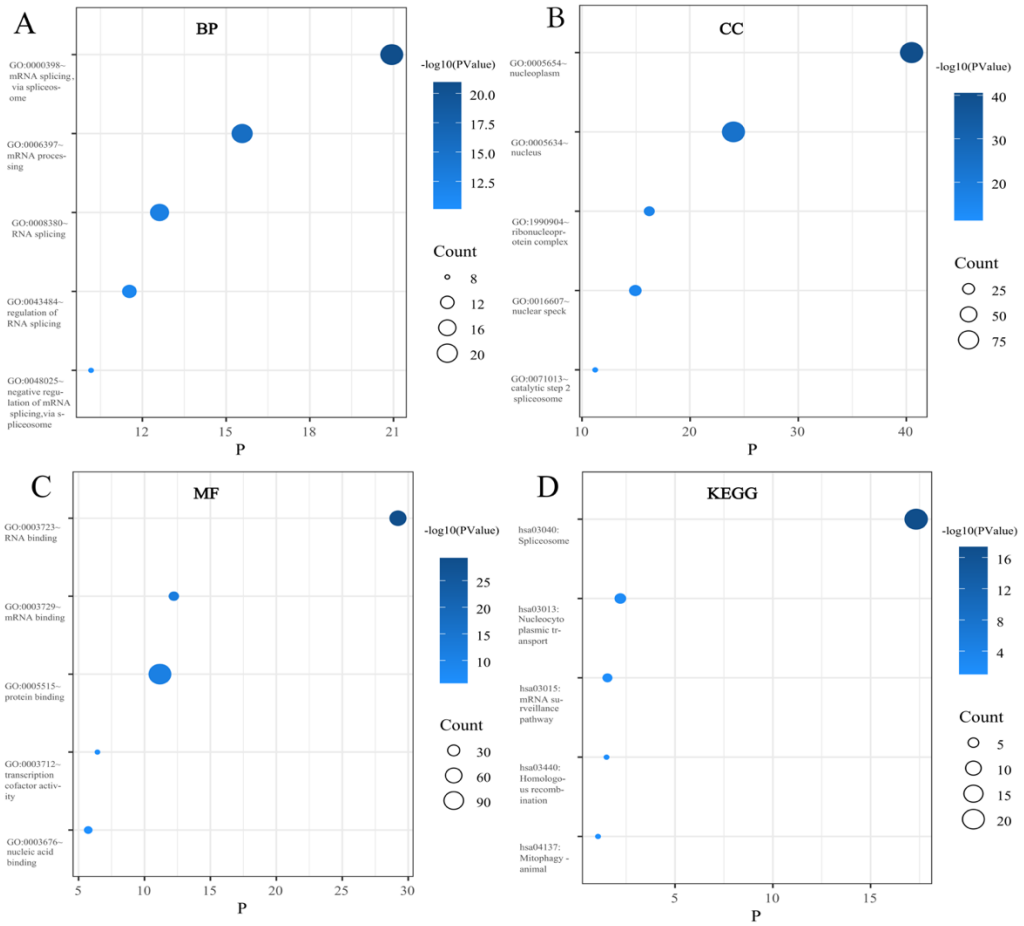

Figure 4. Results of gene enrichment analysis. **(A)** Results of enriched biological processes in Gene Ontology (GO) analysis; **(B)** Results of enriched cellular components in GO analysis; **(C)** Molecular function results enriched in GO analysis; **(D)** Pathway results from KEGG enrichment analysis.

3.6. ZNF207 and common clinical immune checkpoints

ZNF207 showed a positive correlation with CD200R1, CD47, CTLA4, Tim-3, PD-1, TIGIT, and HEVM (correlation coefficient > 0), with the strongest correlation observed with CD47. See **Figure 5**.

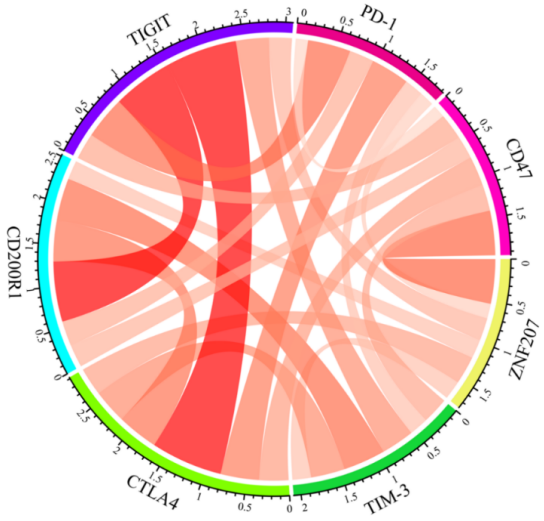

Figure 5. *ZNF207* correlates with common clinical immune checkpoints

3.7. Results of univariate and multivariate Cox proportional hazards models

Univariate Cox proportional hazards model assessment suggested that T stage in TNM and *ZNF207* expression levels were significantly associated with the prognosis of patients with LIHC, as depicted in **Figure 6A**. The multivariate Cox proportional hazards model suggested that high *ZNF207* expression was an independent prognostic factor for LIHC, as depicted in **Figure 6B**.

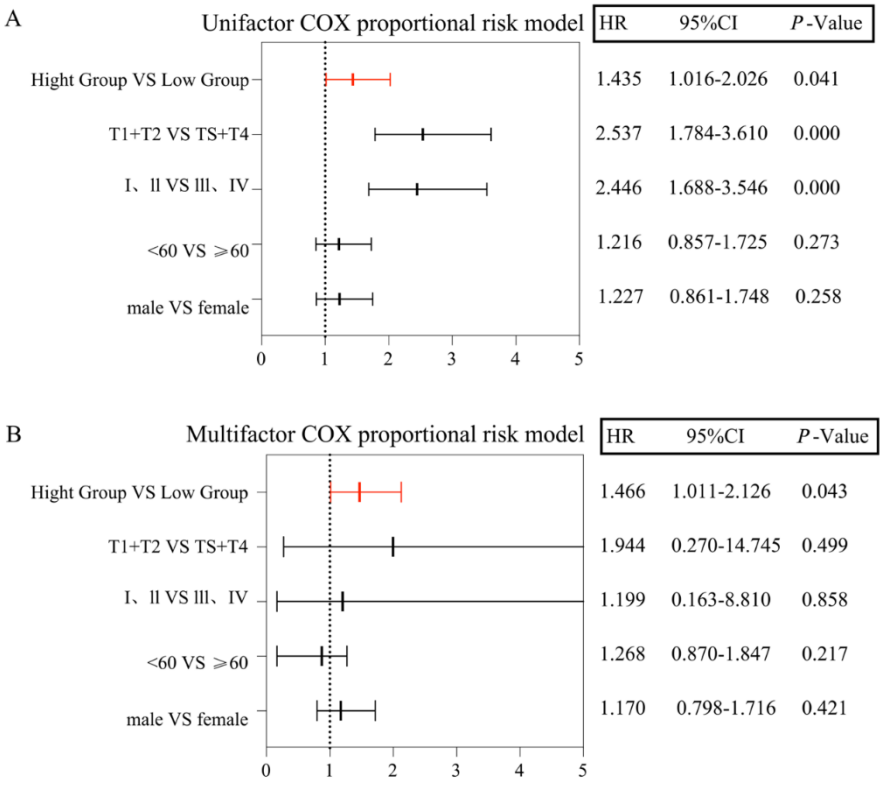

Figure 6. Results of univariate and multivariate Cox proportional hazards models

3.8. RT-qPCR verification of ZNF207 gene expression

The results showed that *ZNF207* expression was significantly higher in HepG-2 cells compared to Lo2 cells ($P < 0.05$), as depicted in **Figure 7**.

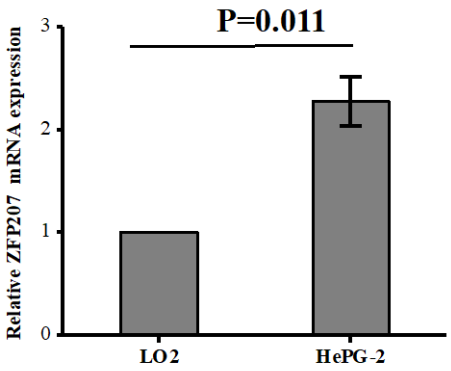

Figure 7. *ZNF207* expression in normal cells and LIHC (verified by RT-qPCR)

4. Discussion

Currently, approximately 383,000 people die from liver cancer in China each year, representing 51% of the global liver cancer deaths. This dire situation imposes a significant burden on our society and healthcare system [8]. The traditional methods for diagnosing and screening LIHC primarily include a combination of ultrasound imaging (US), computed tomography imaging (CT), magnetic resonance imaging (MRI), and serum alpha-fetoprotein (AFP) levels. Serum AFP levels ≥ 400 μg/L strongly indicate liver cancer, provided that pregnancy, chronic or active liver disease, germ cell embryonal tumors, and digestive tract tumors have been ruled out [9]. However, biomarkers are needed to supplement ultrasound in the early detection of liver cancer, as AFP is not the optimal choice [10]. Even the American Association for the Study of Liver Diseases' liver cancer screening guidelines have removed AFP from screening recommendations due to its overall underperformance [11]. Thus, discovering tumor diagnostic markers that can diagnose early hepatocellular carcinoma with high sensitivity and specificity has become a key focus and challenge in basic and clinical research.

Zinc finger protein (ZNF) was first discovered in *Xenopus* oocyte transcription factor IIIA [12]. ZNF is the largest transcription factor family in the human genome, with various combinations and functions of zinc finger motifs providing these proteins with a broad range of applications in biological processes such as development, differentiation, metabolism, and autophagy [13]. *ZNF207*, a member of the zinc finger protein family, was first discovered in human vascular smooth muscle cells (VSMC) [14].

The authors believe that the results of this study warrant further exploration of the specific mechanisms of *ZNF207* in hepatocellular carcinoma. According to the results, *ZNF207* expression in LIHC tissues was significantly higher than in normal tissues. High *ZNF207* expression significantly decreased the overall survival of patients. The immunohistochemistry results from the HPA database showed higher *ZNF207* expression in LIHC than in normal tissues, consistent with Zhou Chenghui's findings [5]. The main biological processes involved in gene enrichment include negative regulation of mRNA splicing by the spliceosome, regulation of RNA splicing, RNA splicing, mRNA processing, and mRNA splicing by the spliceosome. The cellular components involved are the catalytic step II spliceosome, nuclear speckles, ribonucleoprotein complex, nucleus, and cytoplasm. The molecular functions include nucleic acid binding, transcription cofactor activity, protein binding, mRNA binding, and RNA binding. The enriched pathways included mitophagy-animal, mRNA surveillance, homologous recombination, spliceosome, and nuclear-cytoplasmic transport. *ZNF207* has

been shown to inhibit the immune microenvironment of LIHC. The authors further analyzed the correlation between *ZNF207* and common clinical immune checkpoints (CD200R1, CD47, CTLA4, Tim-3, PD-1, TIGIT, HVEM) in hepatocellular carcinoma. The related mechanisms warrant further exploration. The univariate and multivariate proportional hazards models suggested that high *ZNF207* expression could be an independent prognostic factor in patients with LIHC. These results align with previous studies, indicating that the prognosis of hepatocellular carcinoma patients is not influenced by their sex or age. The high expression of *ZNF207* in LIHC patients was confirmed through Western blot assay, RT-qPCR assay in HepG-2 cells, TCGA database, UALCAN database, and HPA database. These findings provide a theoretical foundation for investigating the role of *ZNF207* in hepatocellular carcinoma and lay the groundwork for early screening and diagnosis, clinical gene therapy, and the development of anti-tumor drugs for LIHC patients.

In conclusion, the role of *ZNF207* in embryonic stem cell self-renewal, pluripotency, and tumor immunity in the development of hepatocellular carcinoma warrants further investigation. Based on the data presented, *ZNF207* was found to be highly expressed in LIHC and correlated with prognosis. This finding could potentially broaden the sample study and provide reliable tumor diagnostic markers for the early detection of LIHC.

Disclosure statement

The authors declare no conflict of interest.

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