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ORIGINAL RESEARCH

Analysis of the Expression and Prognostic Value of SIRTs in Hepatocellular Carcinoma

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Purpose: This study contributes to the evolving understanding of the pivotal involvement of Sirtuins (SIRTs) in various human cancers, with a particular focus on elucidating their expression patterns and clinical relevance within the context of hepatocellular carcinoma (HCC). The investigation involves a comprehensive analysis of mRNA expression and prognostic implications associated with distinct SIRTs in HCC.

Patients and Methods: Initial data pertaining to SIRT expression in HCC patients were collated from publicly accessible databases. Subsequently, the expression levels of select members of the SIRT family were validated using clinicopathological specimens from HCC patients. Additionally, HCC tissue microarray was employed to scrutinize the correlation between SIRT7 expression and HCC prognosis.

Results: The findings indicated a substantial upregulation of SIRT2, SIRT3, SIRT4, SIRT6, and SIRT7 in HCC tissues. Survival analysis underscored a pronounced association between elevated mRNA levels of SIRT3, SIRT6, and SIRT7 and an adverse prognosis for HCC patients. Particularly, SIRT7 emerged as a potential independent risk factor for poor prognosis in HCC patients. Examination of the HCC tissue microarray revealed heightened expression of SIRT7 in 68 cases (54.8%) of HCC tissues. Multivariate analysis established high SIRT7 expression as an independent risk factor for diminished Disease-Free Survival (DFS) and Overall Survival (OS) in HCC patients.

Conclusion: The aberrant expression of SIRT7 presents itself may be as a novel biomarker for predicting the prognosis of HCC patients.

Keywords: HCC, microarray, prognosis, SIRT7, survivor

Introduction

The World Health Organization predicts that liver cancer will claim the lives of over one million individuals by 2030, with hepatocellular carcinoma (HCC) emerging as the predominant variant.[1] Despite notable strides in the prevention, detection, diagnosis, and treatment of HCC in recent years, patient prognosis remains suboptimal due to the intricate pathological mechanisms and the proclivity of HCC for invasion and metastasis. Currently, there exists a deficiency in effective biomarkers for early HCC diagnosis and prognosis prediction. Consequently, the identification of novel molecular biomarkers for the diagnosis, treatment, and prognosis of HCC holds significant clinical importance.

Silent mating type information regulation 2 homologs (sirtuins) represent NAD+-dependent histone deacetylases with regulatory roles in diverse cellular processes, including proliferation, apoptosis, and metabolism.[2–4] The expression patterns of sirtuin (SIRT) genes vary across different cancer types.[3] SIRT1 and SIRT3 may exhibit either upregulation or downregulation depending on the cancer type, functioning as oncogenes in colorectal and oral cancers or as tumor suppressor genes in bladder, breast, and anterior adenocarcinoma.[5–8] Conversely, SIRT2 and SIRT4 are deemed tumor suppressors, experiencing downregulation in glioma, HCC, bladder, stomach, and lung cancers.[9,10] While the role of SIRT5 in tumorigenesis remains poorly understood, its overexpression has been noted in colorectal and non-small cell lung cancers (NSCLC).[11,12] SIRT6 is downregulated in several cancers, including colorectal cancer, but is overexpressed

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in breast adenocarcinoma and NSCLC.[11,12] Current investigations reveal that SIRT7 is highly expressed in breast, ovarian, and kidney cancers, exerting cancer-promoting effects.[13–15] Conversely, it exhibits low expression levels in pancreatic cancer and head and neck squamous cell carcinoma, where it exerts tumor-suppressive effects.[6,16]

These studies suggest that the expression profiles of SIRT family members in different cancer types may be tissue-specific, rendering the exploration of SIRT family members in various tumors a subject of ongoing controversy. In this context, we conducted a review of studies related to SIRTs, revealing a paucity of research on SIRTs in HCC, particularly in relation to clinicopathological features and prognosis in HCC patients. Consequently, there is considerable clinical significance in investigating the expression of SIRT family members in HCC tissues, elucidating their association with clinicopathological features and patient prognosis, and identifying new prognostic biomarkers for HCC patients.

Materials and Methods

ONCOMINE Database Analysis: The ONCOMINE database (www.oncomine.org) serves as a bioinformatics tool meticulously crafted to aggregate, standardize, analyse, and disseminate cancer transcriptome data for the biomedical research community.[17] In this investigation, we harnessed the capabilities of the ONCOMINE database to scrutinise the expression levels of the SIRT gene family across prevalent cancer types. Differentially expressed mRNAs were selected using the cut-off criteria: p = 0.001 (Student's *t*-test), fold difference in expression 1.5, and differentially expressed gene rank ≤10%.

Gene Expression Profiling Interactive Analysis (GEPIA): GEPIA, a platform dedicated to the dynamic analysis of gene expression profile data,[18] seamlessly integrates The Cancer Genome Atlas (TCGA) cancer big data with GTEx normal tissue big data. Utilising advanced bioinformatics, GEPIA addresses pivotal questions in cancer biology, unraveling insights into cancer subtypes, driver genes, alleles, differential expression, and carcinogenic factors. In our current study, the SIRTs expression analysis was performed using Gene Expression Profiling Interactive Analysis (GEPIA), with $P < 0.05$ considered significantly different.

Download of Gene Expression and Clinical Data of Hepatocellular Carcinoma Patients

TCGA,[19] a collaborative effort of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) since 2006, offers comprehensive data across 36 cancer types. Gene expression data on SIRT mRNA levels for 424 HCC patients were acquired from the Firehose website. Due to the absence of follow-up data for certain patients, 50 of the 371 HCC patients were excluded, resulting in the enrolment of 321 HCC patients.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

The GO resource (http://geneontology.org) encapsulates gene participation in biological processes, cellular locations, and molecular functions, organising this information into directed acyclic graphs.[20] KEGG, a database amalgamating genomic, chemical, and system function information, aims to unveil the genetic and chemical blueprint of life phenomena.[21] Our study involved the analysis of SIRTs for enrichment of GO terms and KEGG pathways. The three enriched GO terms encompassed biological processes (BPs), cellular components (CCs), and molecular functions (MFs). Leveraging the LinkedOmics website[22] (http://www.linkedomics.org/), we conducted single-gene enrichment and visualisation for SIRTs and subsequently employed LinkedOmics for the enrichment analysis of gene set enrichment analysis (GSEA) for KEGG pathways, and the significant pathways were screened based on the criterion: $P < 0.05$ and FDR <0.05.

Metascape Analysis

Subsequently, we utilised Metascape[23] for the multi-gene enrichment and visualisation of SIRTs. Searched SIRTs interaction proteins in STRING database (https://string-db.org/). The functional proteins were studied by using protein-protein interaction network and genome-wide sequencing technology.

Quantitative Real-Time PCR

Total RNA extraction was performed using TRIzol reagent (15,596,026, Invitrogen, USA). RNA underwent reverse transcription with a TAKARA reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time quantitative PCR (RT-qPCR) was executed using the SYBR method with a LightCycler480 II real-time quantitative PCR instrument (CFX96 Bio-Rad, Hercules, CA, USA). The primers were synthesized by Shenggong Biotechnology Co., Ltd. (Shanghai) and belong to the SIRTs gene families of human (H) and mouse (M) sources, respectively. The primer sequence for SIRT3/6/7 is as follows: SIRT3 (5'-3'), Forward:AGAGATGCGGGACCTTGTGC, Reverse:TATTGTGTGCGGGCAGCCAT. SIRT6 (5'-3'), Forward:TTCCGGGAAGAAGCCACACC, Reverse:AGCCTCACCTCTGGACAACAC. SIRT7 (5'-3'), Forward: GGGTCCAGCCTGAAGGTTCT, Reverse:GGTCCACTGCAGGTTCACGA.

Patient Samples

Tissue specimens from both cancerous and paracancerous regions were gathered from 30 HCC patients (22 males and 8 females) undergoing their first HCC resection at Liuzhou People's Hospital affiliated with Guangxi Medical University from November 2018 to December 2020. The inclusion criterion was limited to patients with HCC who had undergone postoperative pathological biopsy. Ethical approval for this study protocol was obtained from the Ethics Committee of Liuzhou People's Affiliated with Guangxi Medical University for Human Study, and the study adhered to the principles of the Declaration of Helsinki. Prior to surgery, written informed consent was obtained from each patient.

Tissue Microarray

The Hepatocellular Carcinoma (HCC) tissue microarray (TMA), sourced from Liao Ding Biotechnology Co., Ltd. (Product Catalog Number: LD-LVC1603), encompassed patients exclusively diagnosed with HCC, who had not undergone radiotherapy, chemotherapy, or any other antitumor adjuvant therapy pre-surgery. Surgical procedures were conducted between December 2008 and September 2012 on individuals aged 18–74, with subsequent follow-up until November 2016. Based on immunohistochemical SIRT7 results, data were categorised into the SIRT7 high expression group and the SIRT7 low expression group. Post exclusion of cases with tissue defects and incomplete follow-up data, 124 HCC patients with comprehensive clinicopathological information were included. Primary endpoints encompassed disease-free survival (DFS) and overall survival (OS), while secondary endpoints comprised clinicopathological features such as tumour stage and pathological grade. Pathologists meticulously evaluated all samples and subsequently provided histological diagnoses.

Histopathology and Immunohistochemistry

Liver tissues, following resection, underwent fixation in 10% formalin for 12 hours. Subsequently, 5-μm thick paraffin-embedded sections were prepared for immunohistochemical and hematoxylin and eosin (H&E) staining. The sections were subjected to overnight incubation at 4°C with primary antibodies against SIRT3 (Ab9755, Abcam, UK), SIRT6 (Ab191385, Abcam, UK), or SIRT7 (Ab259968, Abcam, UK). Following this, the sections were labelled using an anti-rabbit universal two-step detection kit (PV-9001, Zhongshan Jinqiao Biotechnology Co. Ltd., China). The signal was then detected through the 3.3'-diaminobenzidine (DAB) method (ZLI-9018, Zhongshan Jinqiao Biotechnology Co. Ltd., China), and images were captured using the Nano-Zoomer S60 C13210 series (S60 C13210, Hamamatsu Photonics K.K., Japan). TMA staining intensity was assessed according to a predefined protocol,[24] where a staining score equal to or less than 4 was considered negative, and a score greater than 4 was regarded as positive. The immunohistochemical score was determined collaboratively by two pathologists.

Statistical Analysis

Baseline data were presented as mean ± standard deviation, median (P25, P75), or percentage. Statistical analysis employed the independent *t*-test (for non-normally distributed data or Mann–Whitney *U*-test), square difference analysis (for non-normally distributed data or Kruskal–Wallis test), and Chi-square test, contingent on the specific circumstances. Kaplan-Meier analysis and the Log rank test were employed to assess DFS and OS differences between groups.

Following univariate analysis, the Cox proportional risk model facilitated multivariate survival analysis. SPSS 23.0 software (SPSS Inc, IL, USA) was utilised for all analyses, with $P < 0.05$ considered significantly different.

Results

Analysis of SIRT Expression in Various Tumors

The ONCOMINE database served as the foundation for evaluating the expression patterns of SIRTs across diverse cancer types. To ascertain the expression characteristics of the SIRT family in Hepatocellular Carcinoma (HCC), we initially utilised the ONCOMINE database to scrutinise the expression trends of SIRTs across distinct tumour tissues. The results, depicted in Figure 1, underscored discernible differences in the expression trends of SIRTs in various cancer types.

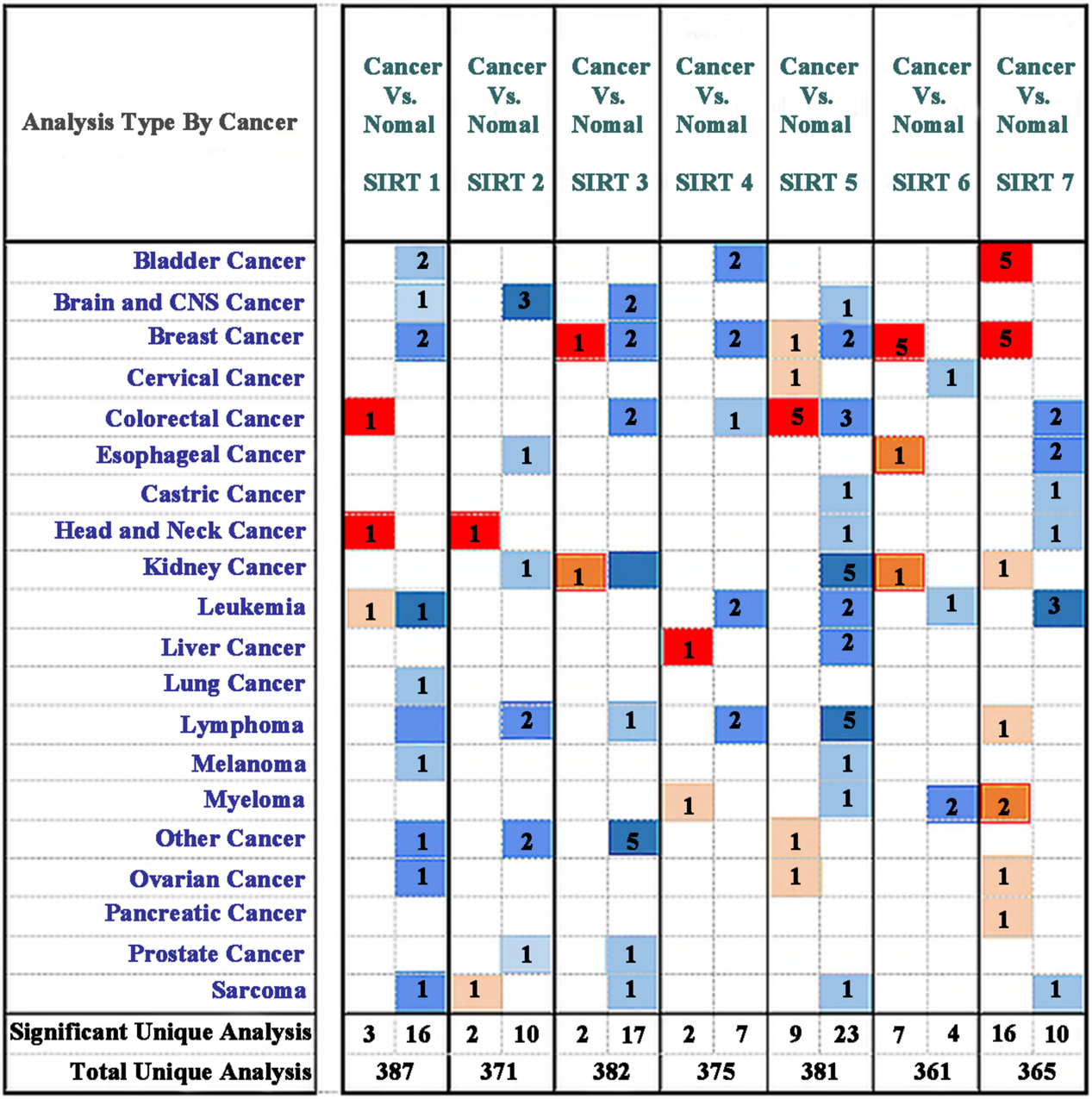

Figure 1 mRNA expression of SIRTs across various cancer types (Oncomine database). Retrieval conditions: $P < 0.001$, fold change: 1.5, gene rank: 10%, data type: mRNA. SIRTs, silent information regulators.

SIRT Expression in HCC Based on TCGA Database

The TCGA database facilitated an in-depth analysis of SIRT expression in HCC. Through the GEPIA website, we conducted a verification analysis of SIRT expression in HCC and adjacent normal tissues. The outcomes revealed significant overexpression of SIRT2/3/4/6/7, except for SIRT1 and SIRT5, in 369 HCC tissues compared to 160 normal tissues ($P < 0.05$). Figure 2 provides a visual representation of these findings.

Metabolic Pathways and Functional Enrichment Analysis of SIRTs in HCC

Utilising the GO and KEGG databases, we explored the metabolic pathways and functional enrichment of SIRTs in HCC, considering $P < 0.05$ as the significance threshold. Figure 3 illustrates that SIRTs exhibit consistent changes in single-gene work energy enrichment across BPs, CCs, and MFs. In BPs, enrichment primarily pertains to biological regulation, metabolic processes, and response to stress. CCs show concentration in the membrane, nucleus, and membrane-enclosed lumen. Additionally, significant enrichment in protein binding, ion binding, and nucleic acid binding is evident in MFs.

KEGG Pathway Analysis of SIRTs in HCC

The KEGG pathway analysis reveals specific enrichment patterns for SIRT1, SIRT2, SIRT3, SIRT5, SIRT4/6/7 in various pathways, as demonstrated in Figure 4A–H. Notable pathways include ribosomes, proteasomes, Huntington's disease, oxidative phosphorylation, and niacin and nicotinamide metabolism. This comprehensive analysis provides valuable insights into the diverse metabolic functions associated with SIRTs in HCC.

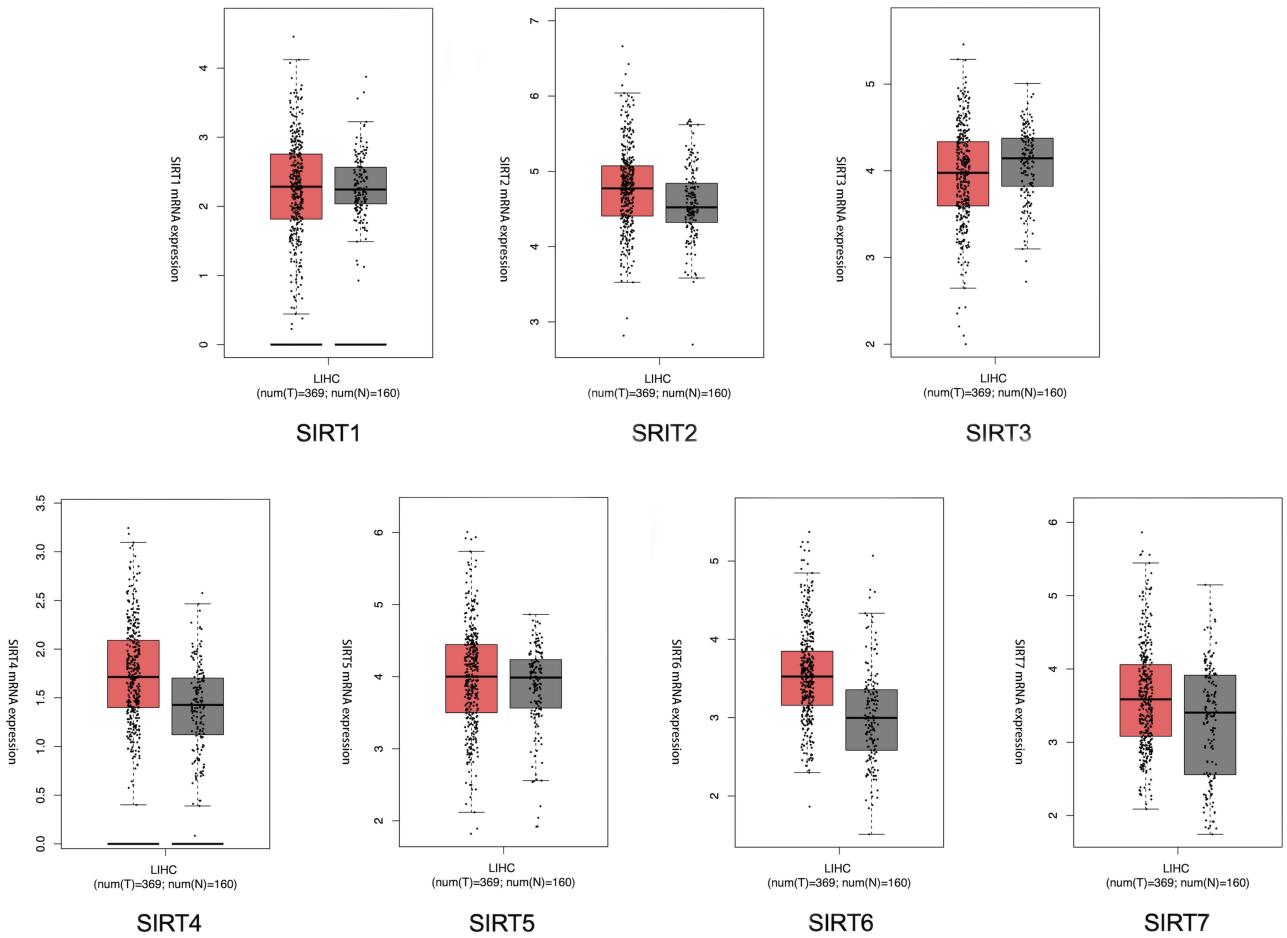

Figure 2 mRNA expression of distinct SIRT family members in HCC and adjacent normal liver tissues (GEPIA). *P < 0.05. GEPIA, gene expression profiling interactive analysis.

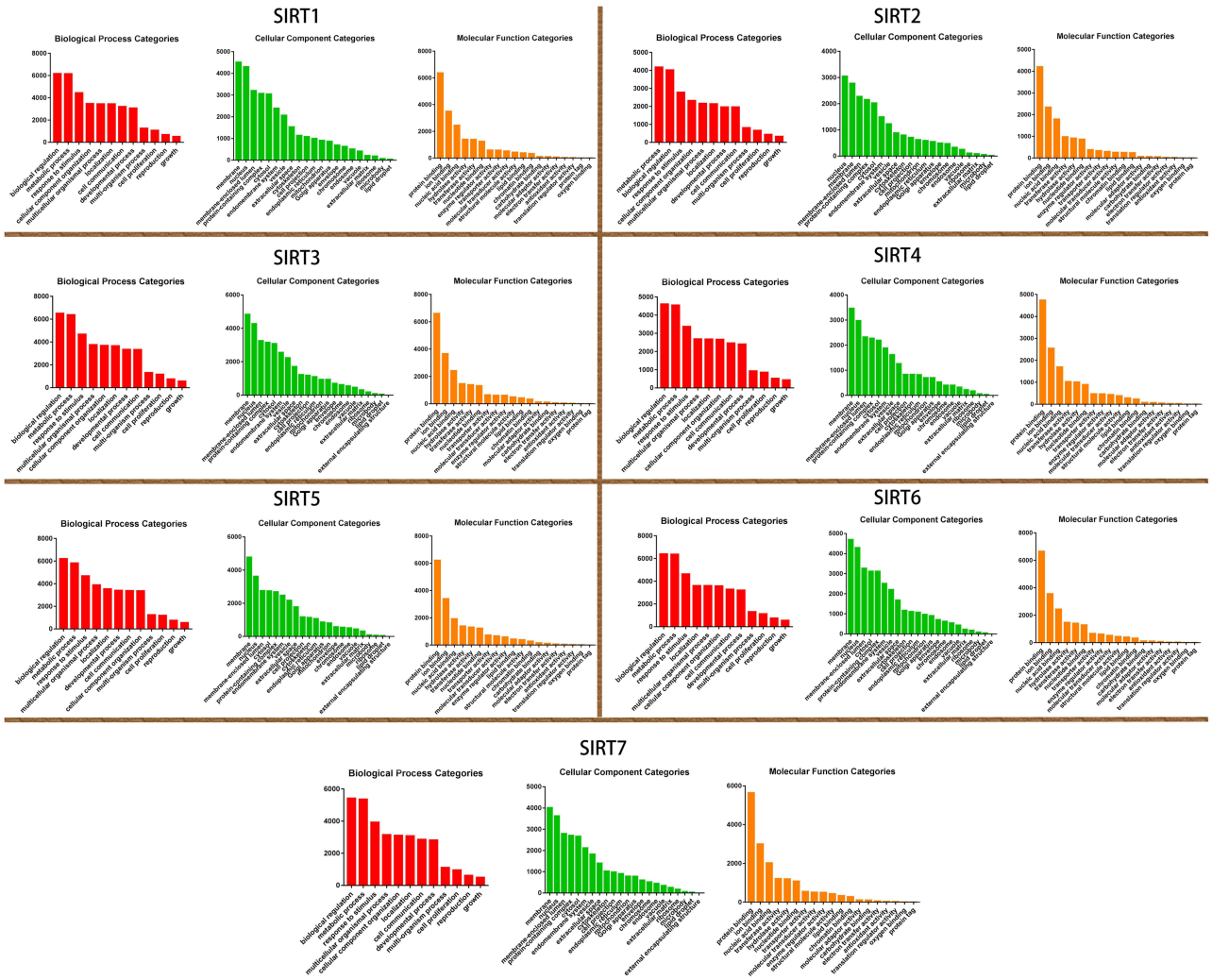

Figure 3 Functional enrichment analysis of SIRTs by single genes. Enriched biological processes, cellular components, and molecular functions of SIRT1-7 (20). GO, gene ontology.

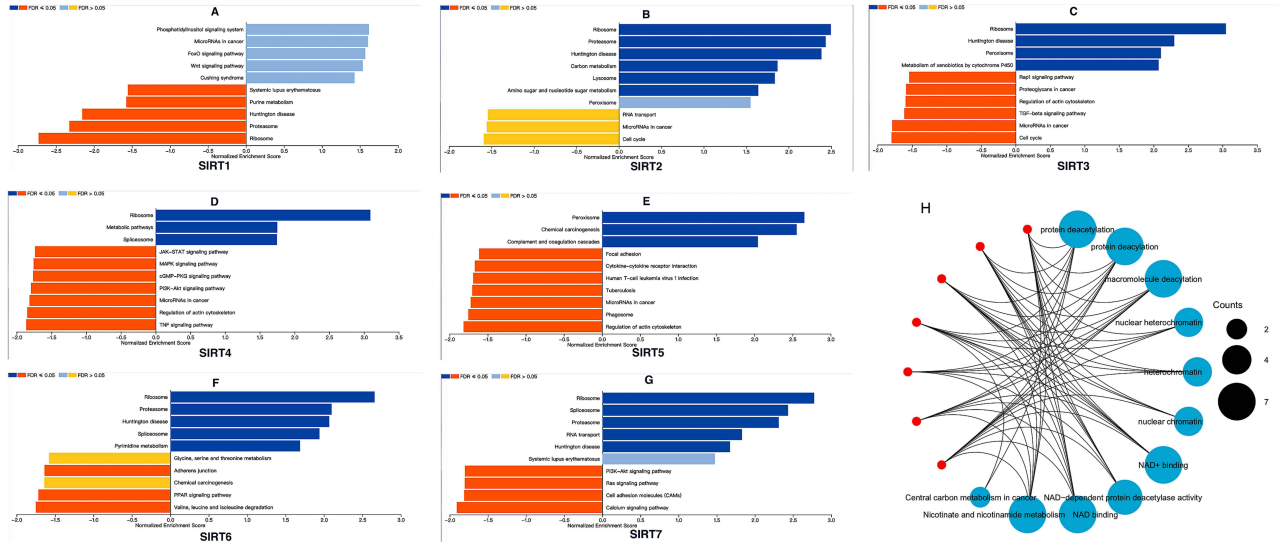

Figure 4 KEGG pathway enrichment analysis of SIRT1-7 (KEGG) (**A**–**G**). KEGG, Kyoto encyclopedia of genes and genomes (**H**).

Protein-Protein Interaction Network Analysis

Differential genes identified in this study were input into the STRING website to construct a Protein-Protein Interaction (PPI) network. The analysis of neighbouring genes in SIRTs revealed significant correlations with SIRT functions, as depicted in Figure 5. Liver disease-related genes such as Foxo3, Foxo1, TP53, EP300, and HDAC1 exhibited substantial associations with SIRT functions, indicating their close relevance to tumour formation. These findings underscore the potential significant role of SIRTs in Hepatocellular Carcinoma.

Analysis of the Prognostic Value of SIRTs in HCC Patients

The correlation between differential expression of SIRTs and the prognosis of Hepatocellular Carcinoma (HCC) patients was examined utilising the TCGA database. Data from the TCGA database were employed to assess the prognostic significance of differential SIRT expression in HCC patients. Furthermore, we scrutinised the association between the expression levels of SIRT3/6/7 and the tumour stage as well as the pathological grade in HCC patients. Results indicated that mRNA overexpression of SIRT3 ($P = 0.003$), SIRT6 ($P = 0.001$), and SIRT7 ($P < 0.001$) significantly correlated with a shorter Overall Survival (OS) in HCC patients. Conversely, SIRT1/2/4/5 demonstrated no significant correlation with the prognosis of HCC patients (refer to Figure 6A–G). Similarly, the mRNA expression levels of SIRT3/6/7 exhibited significant correlation with both tumour stage and pathological grade, as depicted in Figure 6H–M. These findings propose that SIRT3/6/7 may serve as potential biomarkers for predicting the survival and prognosis of HCC patients, although further validation is imperative.

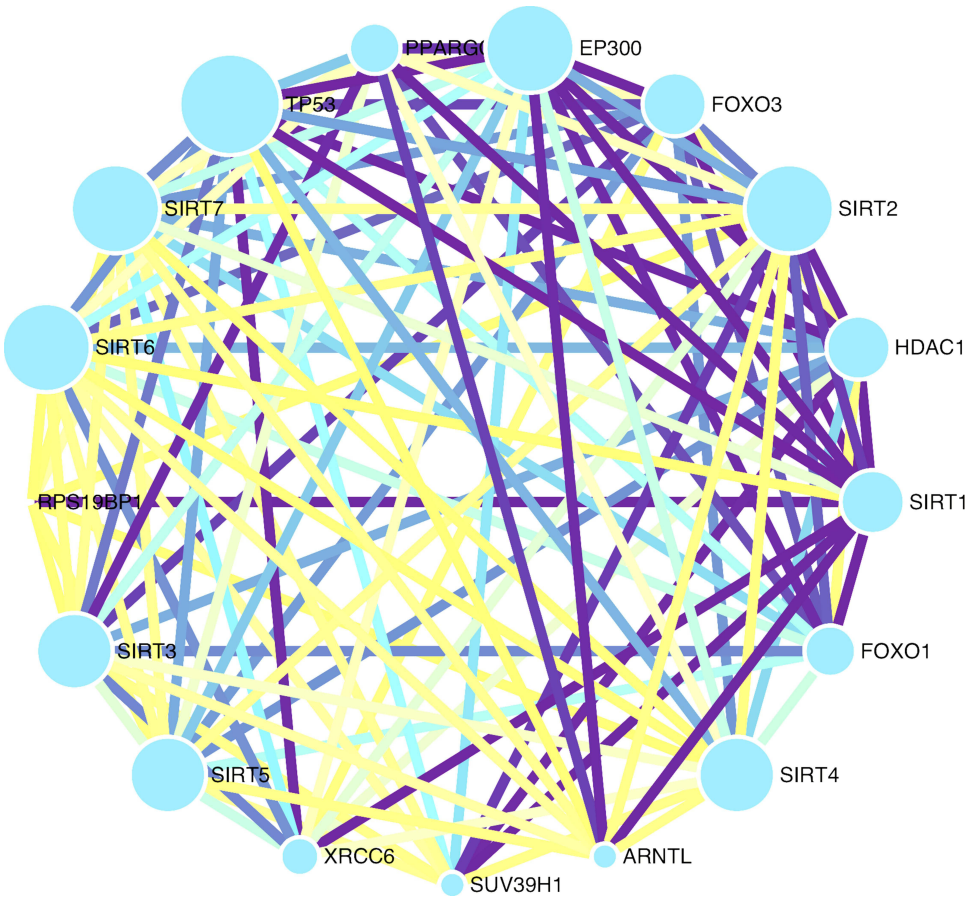

Figure 5 Construction of protein-protein interaction (PPI) networks (PPI networks of SIRT1-7).

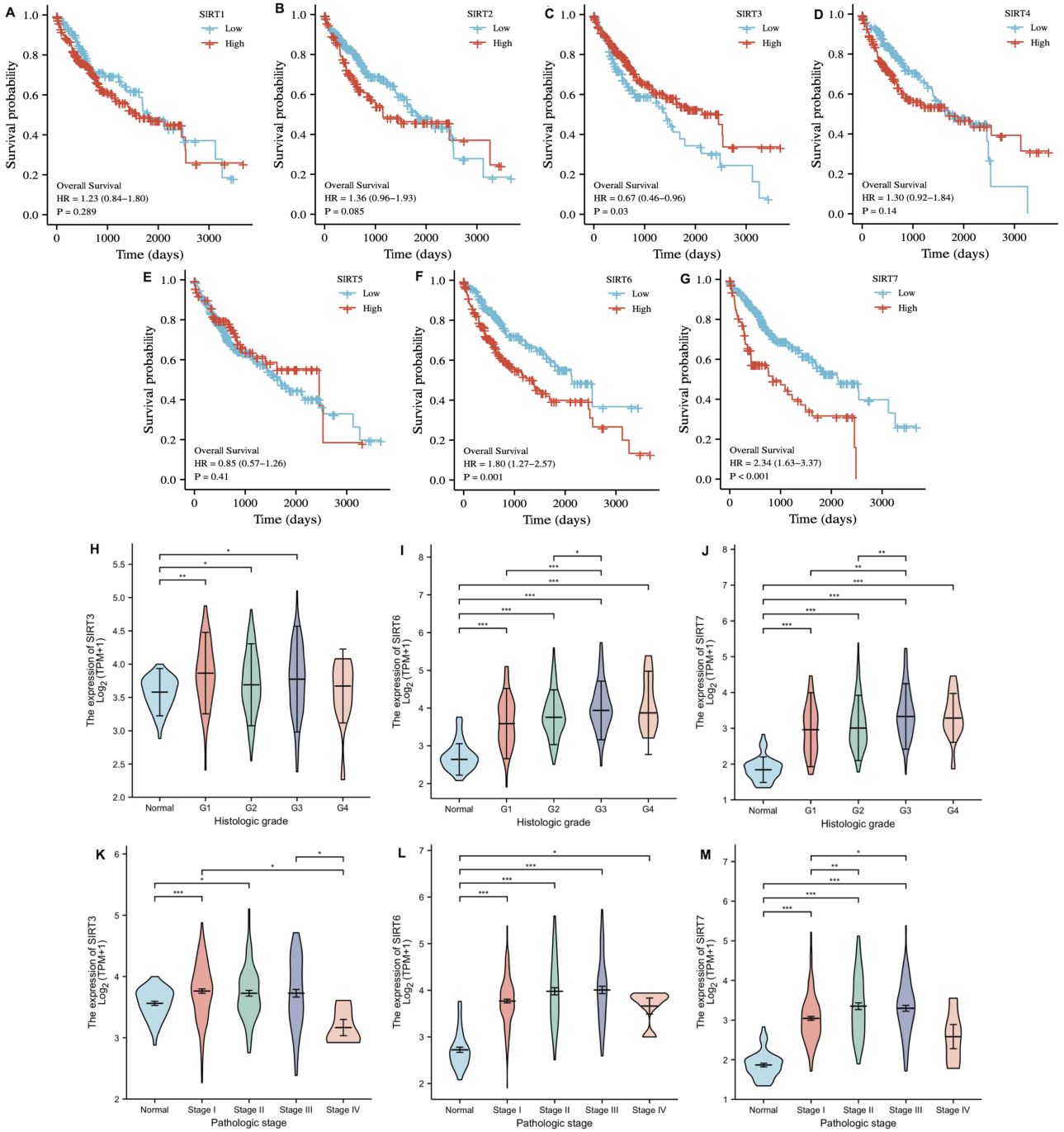

Figure 6 Prognostic evaluation of mRNA expression levels of SIRT family members in HCC patients (TCGA) (**A**–**G**). Association between SIRT3/6/7 mRNA expression and the pathological stages and histological grade of HCC patients (**H**–**M**). *p<0.05, **p<0.01, ***p<0.001.

Validation of SIRT 3/6/7 Expression in HCC Patients

To further verify the expression of SIRT3/6/7 in HCC, tissue specimens from 30 patients meeting the inclusion criteria were examined. Results demonstrated that the gene expression levels of SIRT3/6/7 in HCC tissues were significantly elevated compared to adjacent normal tissues (Figure 7E–G). Subsequent Hematoxylin and Eosin staining, along with IHC detection on 30 HCC tissues and adjacent normal tissues, aimed to delineate the cellular position and expression levels of SIRT3/6/7 protein. HE staining revealed a lack of lobular structures in cancerous tissues, replaced by disordered

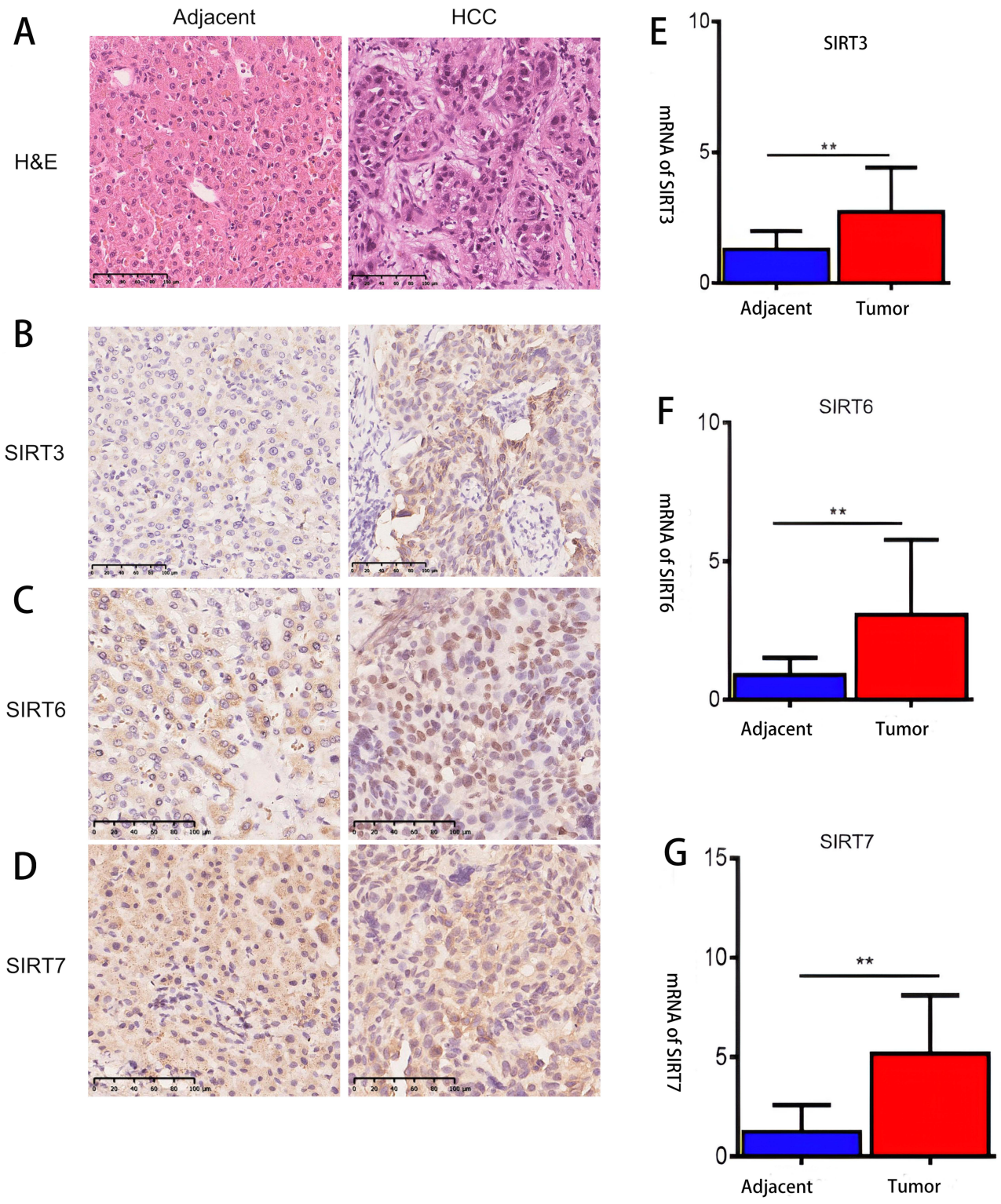

Figure 7 H&E and immunohistochemical staining of liver tissues from HCC patients. (**A**) (**H** and **E**) staining of normal tissues and tumor tissues. (**B**–**D**) Expression of SIRT3/6/7 in normal tissues and tumor tissues. SIRT 3/6/7 expression levels are upregulated in tumor tissues. (**E**–**G**) Gene expression of SIRT 3/6/7 in 30 paired tumor and normal tissues was plotted. **P < 0.05.

cancer cells with nuclear atypia (Figure 7A). Immunohistochemical results indicated higher SIRT3/6/7 protein expression levels in HCC tissues compared to normal tissues (Figure 7B–D). Notably, SIRT3 was predominantly expressed in mitochondria, while SIRT6/7 exhibited nuclear localisation, aligning with previous research findings.[2]

Association Between SIRT3/6/7 mRNA Expression Levels and HCC Patient Prognosis

Following the confirmation from the TCGA database that the expression levels of SIRT3/6/7 mRNA significantly correlated with the prognosis of HCC patients, we conducted further analyses to ascertain the relationship between SIRT3/6/7 expression levels and OS in HCC patients. Clinical data from 321 HCC patients were obtained from the Firehose website (http://gdac.broadinstitute.org). Univariate and multivariate Cox survival analyses were then performed for SIRT3/6/7.

Univariate analysis revealed a positive correlation between poor pathological differentiation and shorter OS in HCC patients (HR = 1.649, 95% CI: 1.315–2.067). Notably, high SIRT6 expression (HR = 1.001, 95% CI: 1.000–1.001, P = 0.021) and high SIRT7 expression (HR = 1.001, 95% CI: 1.000–1.001, P < 0.001) were also positively correlated with shorter OS in HCC patients (refer to Figure S1).

For SIRT3 multivariate Cox survival analysis, higher tumour stage (HR = 1.726, 95% CI: 1.365–2.182, P < 0.001) and poor tumour histological grade (HR = 1.413, 95% CI: 1.050–1.903, P = 0.023) emerged as independent risk factors for shorter OS in HCC patients. No significant correlation was found between age, gender, and SIRT3 high expression and OS in HCC patients (see Figure S2).

Similarly, SIRT6 multivariate Cox survival analysis revealed that higher tumour grade (HR = 1.691, 95% CI: 1.336–2.140, P < 0.001) and poor tumour histological grade (HR = 1.375, 95% CI: 1.091–1.853, P = 0.037) were independent risk factors for shorter OS in HCC patients. Age, gender, and SIRT6 high expression demonstrated no significant correlation with OS in HCC patients (refer to Figure S3).

Importantly, high SIRT7 expression (HR = 1.001, 95% CI: 1.000–1.001, P = 0.015) emerged as an independent risk factor for shorter OS in HCC patients. Furthermore, higher tumour stage (HR = 1.673, 95% CI: 1.321–2.118, P < 0.001) and poorer tumour histological grade (HR = 1.344, 95% CI: 0.994–1.818, P = 0.055) were associated with shorter OS in HCC patients. Age and gender demonstrated no significant correlation with OS in HCC patients (see Figure S4). These findings underscore that elevated SIRT7 expression serves as an independent risk factor for a poor prognosis in HCC patients and may serve as a biomarker for predicting HCC patient prognosis.

Expression of SIRT7 in HCC Tissues and Adjacent Normal Tissues

To delve into the correlation between SIRT7 protein expression and the clinical characteristics of HCC, immunohistochemical analysis was conducted on tissue chip slides. SIRT7 protein expression primarily localizes to the nucleus, exhibiting negative staining, weak positivity with light yellow staining, moderate positivity with brownish-yellow staining, and strong positivity with tan staining. Five high-power fields (400×) were randomly selected from each tissue core under the microscope, with 100 cancer cells counted in each field, and the average was computed. Based on the percentage of positive cells, scoring was assigned as 0 for ≤5%, 1 for 6%–25%, 2 for 26%–50%, 3 for 51%–75%, and 4 for ≥76%. Considering the cell staining intensity, a score of 0 represented negative, 1 for weakly positive, 2 for moderately positive, and 3 for strongly positive. The total score, obtained by multiplying the scores of the two criteria, ranged from 0 to 3 denoting low expression and 4 to 7 denoting high expression. The findings indicated low or no expression of SIRT7 in normal adjacent tissues, with only 12 cases (17.8%) exhibiting high expression, as illustrated in Figure 8A and B. In cancerous tissues, SIRT7 manifested high expression in 68 cases (54.8%), predominantly in the nucleus, as depicted in Figure 8C and D. The expression rate of SIRT7 in HCC tissues was significantly higher than that in normal adjacent tissues, as indicated in Figure 8D and B.

Correlation Between Abnormal Expression of SIRT7 and Clinicopathological Features in HCC

Complete clinical follow-up data were available for 124 of the 135 HCC patients, and the comparative analysis of baseline data for these patients is detailed in Table S1. The correlation between SIRT7 expression in cancer and the clinicopathological characteristics of HCC patients was investigated. The results revealed that high expression of SIRT7 significantly correlated with TNM stage (P < 0.001), pathological differentiation (P = 0.037), and vascular tumour thrombolus (P = 0.015), but not with tumour size, tumour number, or tumour envelope, as outlined in Table S1.

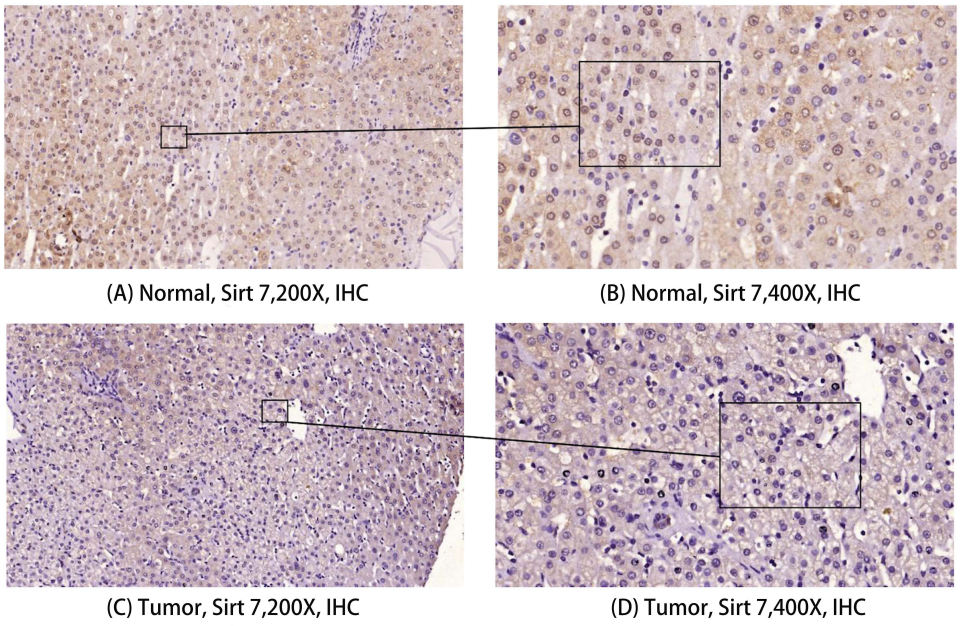

Figure 8 The expression of SIRT7 protein in HCC is higher than in normal tissue. (**A**) Immunohistochemical staining with the SIRT7 antibody of normal tissues (200X, IHC), (**B**) Immunohistochemical staining with the SIRT7 antibody of normal tissues (400X, IHC), (**C**) Immunohistochemical staining with the SIRT7 antibody of tumor tissues (200X, IHC), (**D**) Immunohistochemical staining with the SIRT7 antibody of tumor tissues (400X, IHC).

The Association Between Abnormal Expression of SIRT7 in HCC and Patient Prognosis

The relationship between SIRT7 expression and the prognosis of HCC patients was further elucidated through Kaplan-Meier survival analysis. The HCC tissue chips we acquired encompassed data from a total of 135 patients, aged 18–74 years, who underwent HCC resection from December 2008 to September 2012, with HCC confirmed by postoperative pathology. The median follow-up time was 48.0 months, with the follow-up deadline set at November 2016. Owing to chip defects and incomplete follow-up data for nine patients in this group, these cases were excluded, and the clinical data for 124 patients were ultimately included for Kaplan-Meier survival analysis. The analysis results are outlined below:

(1) The DFS of the SIRT7 high-expression group was significantly lower than that of the SIRT7 low-expression group ($P < 0.001$, Figure 9). The 1-year, 2-year, and 3-year DFS rates for the SIRT7 high-expression group were 58.8%, 29.4%,

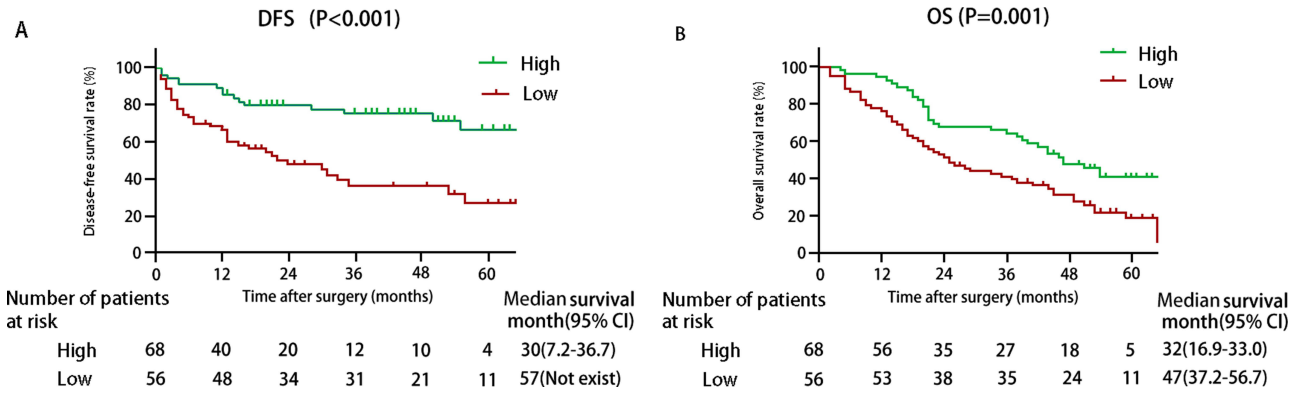

Figure 9 Kaplan-Meier survival curves for DFS and OS in patients (n=124). (**A**) The DFS rate of patients with low SIRT7 expression was significantly higher than that of patients with high SIRT7 expression (P<0.001), (**B**) The OS rate of patients with low SIRT7 expression was significantly higher than that of patients with high SIRT7 expression (P = 0.001).

and 17.6%, respectively, while the 1-year, 2-year, and 3-year DFS rates for the SIRT7 low-expression group were 85.7%, 60.7%, and 55.3%, respectively. Univariate analysis revealed that multiple tumors, larger tumor diameter, and high SIRT7 expression were risk factors for postoperative recurrence of HCC ($P < 0.05$), as indicated in Table S2. Multivariate analysis suggested that high expression of SIRT7 (HR = 3.104, 95% CI: 1.697–5.678, $P < 0.001$) was an independent risk factor for a shorter postoperative DFS rate in HCC patients, as shown in Table S3.

(2) Kaplan-Meier survival analysis demonstrated that the Overall Survival (OS) rate of patients with low SIRT7 expression was significantly higher than that of patients with high SIRT7 expression ($P = 0.001$, Figure 9). The median survival time of HCC patients in the SIRT7 high-expression group and the low-expression group was 32 months and 47 months, respectively. The OS rates of the SIRT7 low-expression group at 1, 2, and 3 years were 94.6%, 67.8%, and 62.5%, respectively, while the OS rates of the SIRT7 high-expression group at 1, 2, and 3 years were 82.3%, 51.4%, and 39.7%, respectively.

The findings from univariate analysis revealed that ascites, larger tumor diameter, elevated glutamyl transferase (GGT) levels, TNM VI stage, multiple tumors, absence of a tumor capsule, and high SIRT7 expression constituted risk factors for a shortened Overall Survival (OS) rate in HCC patients ($P < 0.05$), as indicated in Table S3. The outcomes of multivariate analysis further indicated that a larger tumor diameter (HR = 1.066, 95% CI: 1.009–1.127, $P = 0.022$), a greater number of tumors (HR = 2.088, 95% CI: 1.301–3.352, $P = 0.002$), the presence of ascites (HR = 2.981, 95% CI: 1.794–4.952, $P < 0.001$), and high SIRT7 expression (HR = 1.949, 95% CI: 1.239–3.065, $P = 0.004$) were independent risk factors contributing to a shorter OS rate in HCC patients, as displayed in Table S3.

To further corroborate the association between abnormal expression of SIRT7 and the prognosis of HCC patients, data from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) were utilized, specifically the dataset GSE54236. This dataset comprised 161 samples, including 81 HCC samples. The results also affirmed that a high expression of SIRT7 was a risk factor for a shorter OS rate in HCC patients (HR = 1.91, 95% CI: 1.19–3.06, $P = 0.007$), as illustrated in Figure S5.

Discussion

SIRT genes intricately participate in regulating various physiological and biochemical processes in humans, encompassing cellular stress response, metabolism, aging, chromatin remodeling, and autophagy, as well as apoptosis. Their aberrant expression is intimately associated with diverse human diseases, including malignant tumors, cardiovascular disease, type II diabetes mellitus, skeletal disorders, neurological conditions, retinopathy, chronic obstructive pulmonary disease, and liver ailments.[4,25–27] Previous studies have highlighted the involvement of SIRTs in the onset and progression of various cancers, including HCC.[3,4] Nevertheless, comprehensive bioinformatics analyses and research covering the entire SIRT family in the context of HCC are seldom conducted. This study aims to explore the mRNA expression and prognostic significance of different SIRTs in HCC, with the anticipation that our findings will contribute to identifying new biomarkers for predicting the prognosis of HCC patients.

Numerous researchers have documented the prevalent overexpression of SIRT1 in cancerous cells, suggesting its potential to promote tumorigenesis. SIRT1 is implicated in cancer progression by targeting cell growth signaling pathways like Wnt-β and Akt/PI3K.[2,28–30] However, the specific role of SIRT1 in the Wnt/β-catenin signaling pathway within HCC remains unclear. Previous studies have underscored significant overexpression of SIRT1 in tumor tissues and HCC cell lines.[31,32] In parallel, investigations into the correlation of SIRT2 with HCC are scarce. Existing reports on SIRT2 attribute either tumor suppressor or oncogenic functions to this gene. Recent studies indicate that inhibiting SIRT2 yields broad anticancer activity.[33,34] Owing to tumor heterogeneity, the expression of SIRT2 varies across different malignancies. SIRT2 expression shows an increase in neuroblastoma, uveal melanoma, renal cell carcinoma, and acute myeloid leukemia, while it decreases in glioma, cervical squamous cell carcinoma, as well as breast, prostate, and liver cancers.[35,36] In the present study, our analysis of the TCGA database and GEPIA website revealed that SIRT1 and SIRT2 were not overexpressed in HCC.

The role of SIRT3 in cancer has been extensively documented, revealing its dual impact within tumours.[37,38] Some studies have indicated a low expression of SIRT3 in patients with HCC, associating it with an independent risk factor for poor prognosis in HCC.[39,40] Research by Song[41] has proposed that SIRT3 inhibits HCC growth through the glycogen

synthase kinase-3β/BCL2-associated X protein-dependent apoptotic pathway. However, a recent study reported downregulated protein expression of SIRT3 despite elevated mRNA levels in the human hepatoma cell line.[42] In the present study, both mRNA and protein levels of SIRT3 were found to be elevated in tumour tissues. The expression of SIRT3 significantly correlated with tumour stage and grade, aligning with previously published results.[27,43] Elevated SIRT3 mRNA expression emerges as a prognostic factor associated with poor overall prognosis in HCC patients. However, these findings are solely based on bioinformatics analysis and necessitate further verification.

The mitochondrial SIRTs, SIRT4 and SIRT5, have received limited attention in HCC studies to date. SIRT4 exhibits a tumour-suppressive role in patients with HCC,[24,44] with significantly downregulated expression in HCC cell lines and tissues.[45] Conversely, the role of SIRT5 in HCC remains contentious. While Guo, Song, Guo, Gu, Chang, Su, Yang, Liang, and Huang[46] reported low SIRT5 expression in HCC tissues, two other studies argued for high expression in HCC cell lines and tissues.[47,48] Our analysis of publicly accessible databases revealed low expression of SIRT4/5 in HCC patients.

SIRT6 has emerged as a potential candidate target in tumour treatment.[49,50] However, recent reports on SIRT6 in HCC tumorigenesis present conflicting results. While one study noted low expression of SIRT6 in HCC cells compared to primary human hepatocytes,[51] another study demonstrated its overexpression in an HCC cell line, regulating proliferation and apoptosis via the ERK1/2 signalling pathway.[52] Some studies indicated SIRT6 overexpression in HCC patients, associating it with an independent risk factor for poor prognosis in HCC.[53–55] Although we found that high expression of SIRT6 may be a factor in poor prognosis in HCC patients by using the TCGA database, subsequent multivariate Cox survival analysis found that high expression of SIRT6 was not related to the prognosis of HCC patients. Therefore, further research is needed to confirm the relationship between the expression of SIRT6 and the prognosis of HCC patients.

Several studies have substantiated the high expression of SIRT7 in HCC.[56–58] In our current investigation, we confirmed the overexpression of SIRT7 in HCC tissues compared to normal tissues. Moreover, our study identified a correlation between elevated SIRT7 expression and the pathological grade, vascular tumour thrombin, and TNM stage in HCC patients. Yanai, Kurata, Muto, Iha, Kanao, Tatsuzawa, Ishibashi, Ikeda, Kitagawa, and Yamamoto[57] demonstrated the overexpression of SIRT7 in cancer tissues, associating it with vascular infiltration and poor differentiation of HCC.

Our findings also indicated a significantly higher proportion of TNM stage III and IV in the SIRT7 high-expression group compared to the SIRT7 low-expression group. TNM staging serves as an indicator of the depth of tumour invasion to a certain extent. Li, Sun, Chen, Wang, Geng, and Tao[59] showcased SIRT7 overexpression in the tissues and cell lines of cholangiocarcinoma patients, linking high SIRT7 expression to tumour size and TNM stage. While few reports exist on the relationship between SIRT7 and HCC tumour stage, our study suggests a potential role for SIRT7 in the invasion and metastasis of HCC.

Numerous studies have underscored the association between abnormal SIRT7 expression and poor prognosis in various tumours.[13,14,16,60] Abnormal SIRT7 expression is considered a novel biomarker for predicting cancer patients' prognosis. Univariate analysis in our study revealed that patients with high SIRT7 expression experienced poorer DFS and OS compared to those with low SIRT7 expression. Multifactorial analysis further indicated that high SIRT7 expression was linked to an adverse prognosis for HCC patients, encompassing DFS and OS, thus corroborating the outcomes of our bioinformatics analysis. Additionally, our study identified tumour size, multiple tumours, and ascites as independent risk factors for shorter OS in HCC patients, aligning with prior research.[61–63] While Lee, Jung, Lee, Chang, Choi, Kim, Kim, and Kim[64] associated SIRT7 with poor prognosis in HCC patients, they could not establish SIRT7 as an independent risk factor. Another study indicated that high SIRT7 expression was not significantly correlated with poor prognosis in HCC patients.[41] Therefore, further investigations are warranted to validate the relationship between SIRT7 and the prognosis of HCC patients.

In the examination of SIRT expression and its correlation with the prognosis of HCC, Liu, Yu, Jin, Wang, Ding, Xing, He, and Zeng[55] utilised TCGA database analysis, revealing that only the mRNA expression level of SIRT1 was not elevated in HCC. Nevertheless, our study has certain limitations. Firstly, there is a scarcity of research on the potential mechanisms of SIRT3/6/7 in HCC, necessitating further investigations to elucidate their specific modes of action in this

context. Secondly, the prognosis of HCC patients is influenced not only by tumour characteristics but also by the state of liver disease.

In our current investigation, we were unable to access data on the degree of cirrhosis, preoperative alpha-fetoprotein levels, surgical margins, microvascular invasion, liver function grading, antiviral status, and other pertinent patient information, all of which hold significant implications for patient prognosis. Additionally, information on patients' underlying conditions such as coronary heart disease, diabetes, or hypertension, which can also impact long-term survival, was not available. Due to funding and other reasons, we have not been able to further elaborate the mechanism of SIRT7 and its important role in targeted therapy, which lead to the limitations and potential biases of the study, and we will further study its mechanism and role in the future. Consequently, a prospective, large-sample, multicentre, randomised controlled study is imperative to further validate the association between high SIRT expression and the prognosis of HCC patients.

Conclusions

This study illustrates that SIRT3/6/7 exhibit high expression levels in HCC tissues, with elevated SIRT7 expression identified as a potential independent risk factor for poor prognosis in HCC patients. Consequently, the aberrant expression of SIRT7 could be regarded as a novel biomarker for predicting the prognosis of HCC patients.

Data Sharing Statement

The public database data used have been described in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. This study protocol was approved by the Ethics Committee of Liuzhou People's Hospital Affiliated with Guangxi Medical University for Human Study (Ethics approval no KY2022-027-01, May 18th, 2022).

Patient Consent for Publication

Written informed consent has been obtained from the patients before surgery.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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