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Original Article

Low MYH9 expression predicts a good prognosis for hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is currently one of the most common causes of cancer-related death and one of the most commonly diagnosed cancers. MYH9 is thought to play a critical role in cancer progression. However, the expression and prognostic value of MYH9 in HCC are still unknown. In this study, the expression and biological significance of MYH9 were evaluated in HCC, at mRNA and protein levels. We showed that both mRNA and protein levels of MYH9 were significantly upregulated in HCC relative to the levels in adjacent non-tumor tissues based on the TCGA database and immunohistochemical analysis, respectively ($P < 0.001$). Additionally, we found that high MYH9 protein levels correlated with poor patient prognosis (median survival 19 months *versus* 49 months) (Log-Rank, $P = 0.0292$). Meanwhile, our data suggested that MYH9 expression is an independent prognostic factor for HCC after surgical resection (HR = 0.675, 95% CI 0.452-1.008, $P = 0.054$) and can potentially serve as a biomarker for the clinical diagnosis and prognosis of HCC.

Keywords: MYH9, hepatocellular carcinoma, prognosis, bioinformatics analysis, immunohistochemistry

Introduction

Hepatocellular carcinoma (HCC) is currently the second most common cause of cancer-related deaths worldwide [1]. In China, HCC is one of the five most commonly diagnosed cancers among men, and also the third leading cause of cancer death among both men and women [2]. Although several approaches have been applied to the treatment of HCC, the unsatisfactory clinical outcomes highlight the need for novel indicators of survival, as well as for reliable therapeutic targets.

MYH9, also known as NMIIA, plays an important role in cytoskeleton reorganization, focal contact formation and lamellipodial retraction during cell spreading; moreover, MYH9 participates in the control of cell adhesion, cell migration and tissue architecture [3]. In recent years, multiple studies have reported the dysregulation of MYH9 in cancers. MYH9 functions as an oncogene in non-small cell lung cancer [4], gastric cancer [5], esophageal squamous cancer [6], breast cancer [7], and colorectal cancer [8, 9], contributing to progression and poor prognosis. Conversely, MYH9 was identified as a tumor suppressor in squamous cell carcinomas [10]. However, the role of MYH9 in HCC is unclear and worthy of further research.

In the present study, we investigated the expression and biological significance of MYH9 in HCC, at the mRNA and protein levels. Bioinformatics and immunohistochemical analysis suggested that both the mRNA and protein levels of MYH9 were upregulated in HCC relative to that in adjacent non-tumor tissues, and the MYH9 protein levels correlated with poor patient prognosis. Additionally, we demonstrated that MYH9 expression was an independent prognostic factor for HCC patients.

Materials and methods

Bioinformatics analysis

RNA-Seq data of hepatocellular carcinoma (LIHC) was downloaded from The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/). The data included 424 HCC patients, of which 50 patients were with paired non-tumor

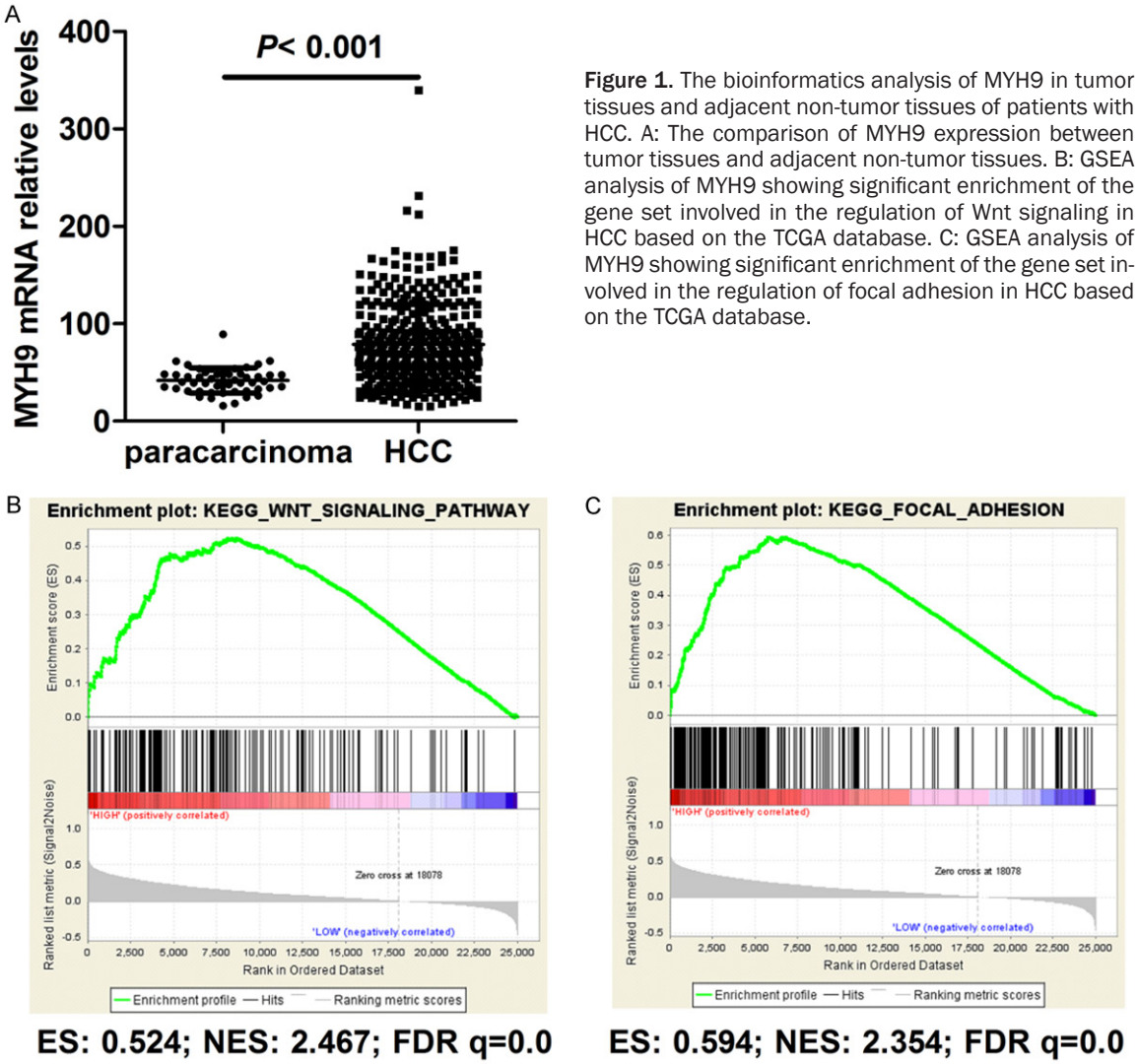

Figure 1. The bioinformatics analysis of MYH9 in tumor tissues and adjacent non-tumor tissues of patients with HCC. A: The comparison of MYH9 expression between tumor tissues and adjacent non-tumor tissues. B: GSEA analysis of MYH9 showing significant enrichment of the gene set involved in the regulation of Wnt signaling in HCC based on the TCGA database. C: GSEA analysis of MYH9 showing significant enrichment of the gene set involved in the regulation of focal adhesion in HCC based on the TCGA database.

tissues. The clinical information for only 377 of the patients was available on the website. For statistical analysis, patients with mRNA expression values greater than the median value were added to the high expression group, while the rest were classified into the low expression group.

Patients and tissues

Overall, 93 HCC and 87 non-tumor subjects with available clinical information and paraffin-embedded blocks were enrolled in this study. Tumors and adjacent non-tumor tissues were collected from the patients during surgery. Pathologic diagnosis was carried out by two pathologists, using the existing hematoxylin-eosin (H&E) slides. The follow-up ranged from 4 to 6.7 years. The protocols were approved by the Ethical Committee and Institutional Review Board of Traditional Chinese Medicine-Integrated Hospital of Southern Medical University, and written informed consent was obtained from each subject. All clinicopathological characteristics, including age, gender, tumor stage, presence of hepatic cirrhosis, Edmondson-Steiner grade, tumor number, tumor size and prognostic information, were retrospectively collected from the patients' medical records. Data collection, data analysis, and all other experiments were performed in accordance with the approved protocols.

Immunohistochemistry (IHC)

Tissue sections (4-μm thick) were de-paraffinized and rehydrated in a xylene and alcohol bath solution. Antigen retrieval was then conducted by incubating the slides in 0.01 M citrate buffer (pH 6.0), and the slides were

Table 1. Correlations between MYH9 expression and the clinicopathological features of hepatocellular carcinoma patients

Characteristics	Total	MYH9 expression		*P* value
		Low, n (%)	High, n (%)	
Age (years)				
≤ Median	49	23 (46.9%)	26 (53.1%)	0.686
> Median	43	22 (51.2%)	21 (48.8%)	
Gender				
Male	83	42 (50.6%)	41 (49.4%)	0.384
Female	10	4 (40.0%)	6 (60.0%)	
AJCC stage				
I-II	43	25 (58.1%)	18 (41.9%)	0.159
III-IV	42	18 (42.9%)	24 (57.1%)	
T classification				
T1-T2	43	25 (58.1%)	18 (41.9%)	0.159
T3-T4	42	18 (42.9%)	24 (57.1%)	
N classification				
N0	83	42 (50.6%)	41 (49.4%)	0.512
N1	1	1 (100.0%)	0 (0.0%)	
Distant metastasis				
No	84	44 (52.4%)	40 (47.6%)	0.482
Yes	1	0 (0.0%)	1 (100.0%)	
Hepatic cirrhosis				
No	52	24 (46.2%)	28 (53.8%)	0.472
Yes	41	22 (53.7%)	19 (46.3%)	
Edmondson-Steiner grade				
I-II	61	32 (52.5%)	29 (47.5%)	0.425
III-IV	32	14 (46.7%)	18 (56.2%)	
Tumor number				
Single	24	10 (41.7%)	14 (58.3%)	0.691
Multiple	45	21 (46.7%)	24 (53.3%)	
Tumor size (cm)				
≤ 5	42	23 (54.8%)	19 (45.2%)	0.304
> 5	50	22 (44.0%)	28 (56.0%)	

blocked in 3% hydrogen peroxide for 10 minutes, to eliminate endogenous peroxidase activity. Then, the slides were incubated for 60 minutes, at room temperature, with primary antibodies for either MYH9 (1:100, Proteintech, USA, 14844-1-AP). Finally, the slides were incubated with a horseradish peroxidase-labeled secondary antibody, and counterstained using 3, 3'-diaminobenzidine and hematoxylin.

Evaluation and scoring

The IHC sections were scored by two blinded pathologists, and any disagreement was resolved by reaching consensus. A third pathologist was then invited to review the scores judged by the two pathologists. The staining was scored according to the intensity and the percentage of positively-stained cells. Staining intensity was scored as 0 (no staining), 1 (weakly positive staining), 2 (moderately positive staining), and 3 (strong staining). The percentages were estimated on a scale of 0 to 4, as follows: 0 (0%), 1 (≤ 25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%). The final staining scores were calculated through multiplying the intensity score by the percentage score. For statistical analysis, a score ≤ 8 was regarded as low expression, and > 8 as high expression.

Statistical analysis

All data analyses were performed using the SPSS software, version 21.0 (SPSS Inc., USA). For comparison, a nonparametric test was performed to investigate the differential expression of each protein between the two groups of patients. The relationships between clinicopathological factors and gene expression were investigated using the Pearson X^2 test. Log-rank tests were performed on Kaplan-Meier survival curves to elucidate any significant relationship between gene expression and overall patient survival. Univariate and multivariate survival analysis were performed using the Cox proportional hazards regression model. Univariate regression models were fitted to identify independent factors associated with overall survival, and only variables with $P < 0.1$ were included in the multivariate analysis. The hazard ratio (HR) and corresponding 95% confidence intervals (95% CI) were calculated for each factor. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

MYH9 mRNA levels are upregulated in HCC

To explore the role of MYH9 in HCC, we initially assessed its mRNA expression in HCC based

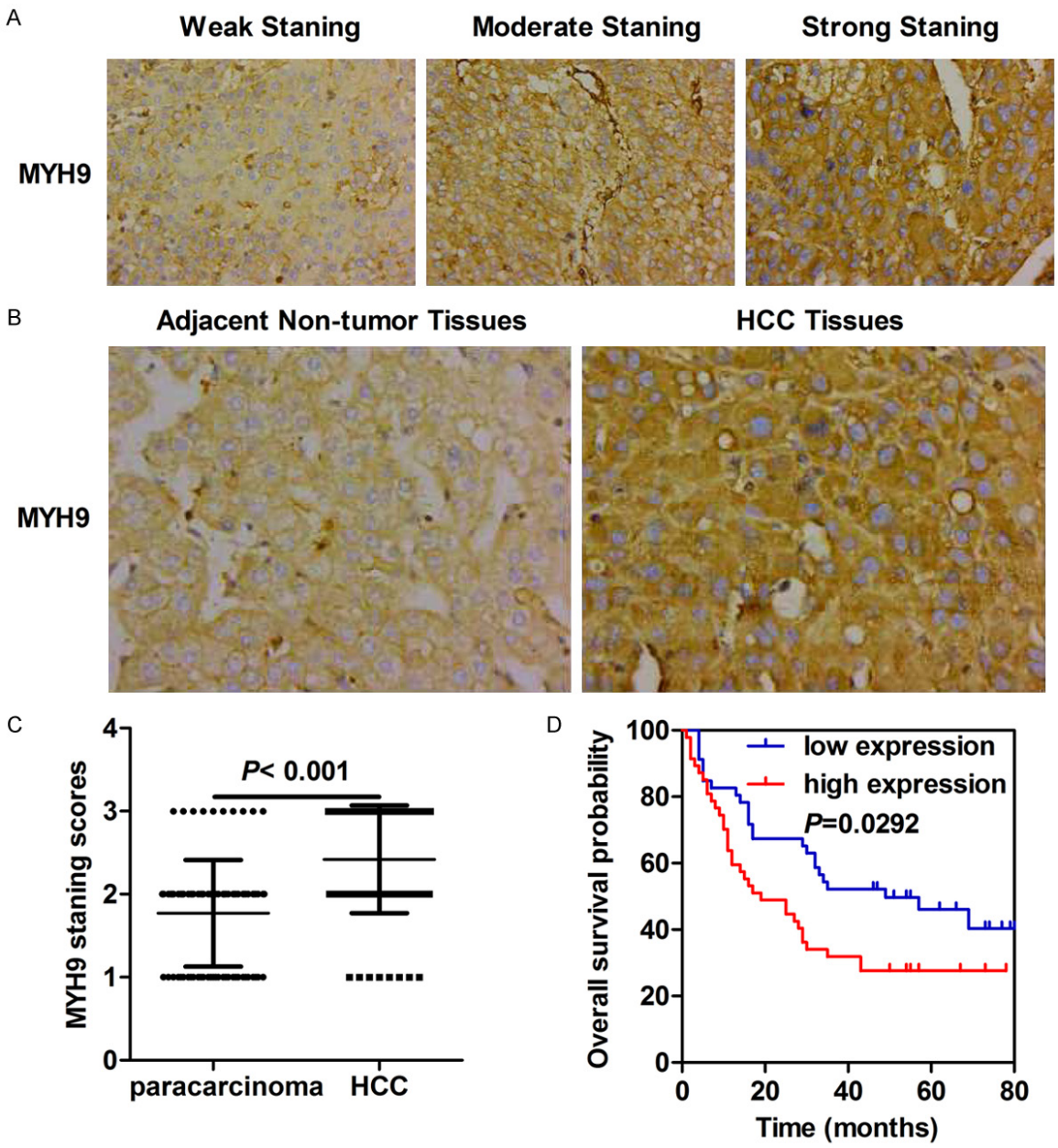

Figure 2. MYH9 protein levels are upregulated in HCC tissues and confer poor prognosis for HCC patients. A: Representative images of MYH9 staining in HCC (original magnification ×400). B: Representative images of MYH9 staining in tumor tissues and adjacent non-tumor tissues (original magnification ×400). C: Differential expression levels of MYH9 in tumor tissues and adjacent non-tumor tissues. D: Kaplan-Meier survival analysis based on MYH9 expression.

Table 2. MYH9 expression in HCC tissues and adjacent non-tumor tissues

Group	Cases (n)	MYH9 expression		*P* value
		Low	High	
Hepatocellular carcinoma	93	46 (52.4%)	47 (47.6%)	< 0.001
Para-carcinoma tissue	87	77 (72.6%)	10 (27.4%)	

on the TCGA LIHC dataset. RNA-seq data from HCC and adjacent non-tumor tissues showed that MYH9 expression was significantly elevated in HCC tissues compared with adjacent non-tumor tissues ($P < 0.001$, **Figure 1A**). Furthermore, the gene set enrichment analysis (GSEA) based on the TCGA LIHC dataset indicated that high

MYH9 expression positively regulates Wnt signaling (**Figure 1B**) and focal adhesions (**Figure 1C**).

Clinicopathological characteristics

The clinicopathological characteristics of 93 HCC patients are summarized in **Table 1**. Patient age ranged from 25 to 73 years, and the median age was 54 years. Moreover, 43 out of 93 patients (46.7%) were older than 54 years, most patients (89.2%) were male, and all patients had undergone surgery as initial treatment. Based on the criteria set by the AJCC 8th edition, 12 HCC patients were in stage I, 31 in stage II, 40 in stage III, and only 2 in stage IV. According to the available medical records, only one patient had lymph node metastasis, and one other case had distant metastasis.

Correlations between the expression of MYH9 and the clinicopathological characteristics of HCC patients

To confirm the results obtained *via* bioinformatics analysis, we then detected the protein levels of MYH9 by immunohistochemistry, in 93 HCC cases. The analysis showed that patients exhibited different MYH9 protein levels, indicated by the strength of the staining (weak to strong; **Figure 2A**). There were significant differences in MYH9 protein levels between the HCC specimens and adjacent non-tumor tissues ($P < 0.001$, **Table 2**; **Figure 2B**, **2C**). However, we detected no correlation between MYH9 protein levels and the clinicopathological features, such as age, gender, TNM classification, hepatic cirrhosis, Edmondson-Steiner grade, number of tumors and tumor size in HCC (**Table 1**).

MYH9 expression is indicative of poor prognosis in HCC patients

The relationship between MYH9 protein levels and the overall survival of HCC patients was explored by survival analysis. Obtained results suggeste that high MYH9 expression correlates with poor prognosis in HCC patients (median survival 19 months *versus* 49 months) (Log-Rank, $P = 0.0292$, **Figure 2D**). Following the above findings, a univariate Cox proportional hazard analysis was conducted to correlate the MYH9 expression with the clinicopathological parameters and the overall survival of the 93 HCC patients (as shown in **Table 3**). Low MYH9 expression, AJCC stages I-II, without distant metastasis, and small tumor size (≤ 5 cm in diameter) contributed to longer overall survival time of HCC patients. Subsequently, a multivariate Cox proportional hazard analysis was performed to evaluate the meaningful parameters identified by the univariate Cox analysis. Since T classification was consistent with the AJCC stage classification, and only one patient had lymph node metastasis and one case had distant metastasis, we included MYH9 expression, AJCC stage, and tumor size in the multivariate Cox analysis for HCC patients. MYH9 expression (HR = 0.675, 95% CI 0.452-1.008, $P = 0.054$), and AJCC stage (HR = 0.405, 95% CI 0.243-0.675, $P = 0.001$), served as independent prognostic factors for HCC patients (**Table 3**).

Discussion

MYH9 is involved in the regulation of cancer progression and may function as a "tumor promoter" or "tumor suppressor" for certain types of tumors. In the present work, MYH9 expression and its biological significance in HCC were studied. Both mRNA and protein levels of MYH9 were upregulated in HCC, and low MYH9 protein levels were indicative of good prognosis for HCC patients. In addition, MYH9 expression served as an independent prognostic factor for HCC patients.

The MYH9 gene encodes a conventional nonmuscle myosin, which is involved in many vital functions, including cell motility, cytokinesis and maintenance of cell shape. Defects in MYH9 have been associated with Fechtner syndrome [11], Epstein syndrome [12], Sebastian syndrome [13], Alport syndrome with macrothrombocytopenia [14], macrothrombocytopenia with progressive sensorineural deafness [15], and non-syndromic sensorineural autosomal dominant deafness type 17 [16]. Recently, many researchers have reported the dysregulation of MYH9 in cancers and its value in the prediction of cancer prognosis. Here, we suggest that MYH9 plays an oncogenic role in HCC. Both bioinformatics and immunohistochemical analysis revealed that mRNA and protein levels of MYH9 were elevated in HCC relative to the levels in adjacent normal tissues, and MYH9 protein levels were positively associated with poor prognosis of HCC patients. Previous studies showed that MYH9 expression not only correlated with poor prognosis but also associated with several clinicopathological parameters in cancers [4-6]. Although no correlation between MYH9 protein levels and the clinicopathological

Table 3. Univariate and multivariate survival analysis of clinicopathological variables of hepatocellular carcinoma patients

	Overall survival					
	Univariate analysis			Multivariate analysis		
Clinical parameters	HR	95% CI	*P* value	HR	95% CI	*P* value
MYH9 expression	0.548	(0.324-0.926)	0.029	0.675	(0.452-1.008)	0.054
Low						
High						
Age (years)	1.260	(0.751-2.113)	0.382			
≤ Median						
> Median						
Gender	1.393	(0.626-3.097)	0.416			
Male						
Female						
AJCC stage	0.340	(0.192-0.602)	< 0.001	0.405	(0.243-0.675)	0.001
I-II						
III-IV						
T classification	0.340	(0.192-0.602)	< 0.001			
T1-T2						
T3-T4						
N classification	0.121	(0.004-3.930)	0.235			
N0						
N1						
Distant metastasis	0.000	(0.000-0.000)	< 0.001			
No						
Yes						
Hepatic cirrhosis	0.858	(0.508-1.450)	0.568			
No						
Yes						
Edmondson-Steiner grade	0.784	(0.451-1.363)	0.388			
I-II						
III-IV						
Tumor number	1.796	(0.912-3.540)	0.101			
Single						
Multiple						
Tumor size (cm)	0.490	(0.289-0.831)	0.008	0.912	(0.549-1.518)	0.724
≤ 5						
> 5						

features were detected in this study, we considered that the small sample volume might explain the obtained results. Additionally, MYH9 participates in miRNA-mediated invasion and metastasis in gastric cancer [17, 18] and colorectal cancer [8], interacts with CXCR4 to promote migration and invasion in renal cell carcinoma [19], and regulates posttranscriptional p53 stabilization in squamous cell carcinomas [10]. In this work, the GSEA analysis hinted that MYH9 might promote the stemness of HCC and accelerate cancer progression through Wnt signaling and focal adhesion. It is well known that the Wnt signaling pathway plays important roles in the regulation of cancer stemness in various tumor types, including HCC [20, 21]. Together with this, focal adhesion is also reported to be involved in regulating cell stemness [22, 23]. However, whether MYH9 regulates cell stemness to modulate HCC progression and the specific mechanism involved is yet to be explored.

In addition, a previous study suggested that the phosphorylation of MYH9 also plays a regulatory role in HCC [24]. It was the phosphorylation but not the basic expression of MYH9 that mattered for the migration of HCC cells, and MYH9 wasn't prominent in the mRNA overexpression analysis of HCC cell lines and clinical specimens [25]. By contrast, we demonstrat in the present study that MYH9 mRNA levels were indeed upregulated in HCC based on the TCGA database. By immunohistochemistry, we found that it was the basic MYH9 protein levels that conferred a poor prognosis of HCC patients.

Taken together, our results aid in unravelling the oncogenic roles of MYH9 in HCC. We also show that MYH9 can potentially serve as a biomarker for the clinical diagnosis and prognosis evaluation of HCC. Lastly, the targeted inhibition of MYH9 might be an alternative strategy for the management of HCC.

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Disclosure of conflict of interest

None.

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