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Original Article

Over-expression of IMPDH2 is associated with tumor progression and poor prognosis in hepatocellular carcinoma

Ying He[1,2*], Zhousan Zheng[1*], Yi Xu[1,4*], Huiwen Weng[1], Ying Gao[1,2], Kai Qin[1,2], Jian Rong[3], Cui Chen[1], Miao Yun[2,5], Jiaxing Zhang[1,2], Sheng Ye[1]

[1]Department of Oncology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong Province, P. R. China; [2]Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, Guangdong Province, P. R. China; [3]Department of Extracorporeal Circulation, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong Province, P. R. China; [4]Department of Oncology, Zhongshan People's Hospital, Zhongshan 528400, Guangdong Province, P. R. China; [5]Department of Ultrasound, Cancer Center, Sun Yat-sen University, Guangzhou 510060, Guangdong Province, P. R. China. [*]Equal contributors.

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Abstract: Inosine monophosphate dehydrogenase type II (IMPDH2) has been found to play critical roles in the development and progression of several human cancers. However, the expression of IMPDH2 and its clinical significance in hepatocellular carcinoma (HCC) is little known. The expression of IMPDH2 in HCC cell lines and tissues were evaluated by Western blotting (WB), quantitative real-time PCR (q-PCR) and immunohistochemistry (IHC). We found that the expression of IMPDH2 was significantly up-regulated in HCC tissues than in adjacent non-tumorous tissues, and this was correlated with several clinicopathological features, including tumor multiplicity (P=0.001), TNM stage (P<0.001). Moreover, the Cox regression analysis suggested that the expression of IMPDH2 was an independent prognostic factor for overall survival (P<0.0001) and progression-free survival (P<0.0001). Further study showed that up-regulation of IMPDH2 expression increased the proliferation and tumorigenicity of HCC cells *in vitro*, by promoting cell growth rate, colony formation. Together, our results demonstrated that the over-expression of IMPDH2 was closely associated with poor survival outcome in patients with HCC and may present a novel prognostic and therapeutic target for this disease.

Keywords: IMPDH2, hepatocellular carcinoma, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide in men and one of the most common cancers in the world [1]. Despite curative hepatectomy along with other surgical and therapeutic approaches can improve short-term survival outcome, such as local ablation therapy and transarterial chemoembolization, the overall survival of HCC patients after curative hepatectomy remain unsatisfactory cause high recurrence and metastasis rates [2]. For several years, multiple molecular biomarkers for early diagnosis and additional prognostic information in HCC have been developed and investigated. For instance, serum a-fetoprotein (AFP) is the most widely accepted serum marker in HCC patients for its diagnostic and predictive value. Accumulating evidence indicates that altered expression of oncogenes and tumor suppressors is associated with the development and progression of HCC [3-7]. Therefore, it is an urgent need to investigate novel specific markers and new therapeutic targets to help in the management of HCC.

Inosine monophosphate dehydrogenase (IMPDH) is a rate-limiting enzyme which catalyzes a crucial step in the de novo biosynthesis of purine nucleotides [8], indicating that it is essential for DNA synthesis [9]. Previous researches have showed that IMPDH was involved with cell growth, malignant transformation and

differentiation [10-12]. In mammalian species, it has two ubiquitously expressed isoforms, IMPDH1 and IMPDH2, which are encoded by distinct genes with 85% of their amino acid sequence conserved [13]. IMPDH1 is constitutively expressed in normal cells, whereas expression of IMPDH2 is frequently elevated in malignant cells [14, 15]. Currently, the expression level of IMPDH2 was found to be remarkable up-regulated in prostate, kidney and bladder cancers and could be a potential biomarker for these diseases [2, 16]. Our previous study has found that high expression of IMPDH2 is associated with aggressive features and poor prognosis of human nasopharyngeal carcinoma (*Scientific Reports*, 2017) [17]. However, to the best of our knowledge, the expression pattern of IMPDH2 and its clinical significance in HCC remains inconclusive.

In this current study, we found that IMPDH2 was markedly overexpressed in HCC tissues and closely related with aggressive HCC progression, and that silencing IMPDH2 expression memorably suppressed the proliferation and tumorigenicity of HCC cells *in vitro*, whereas overexpression IMPDH2 had the opposite effect. The correlation between IMPDH2 expression and multiple clinicopathologic parameters was evaluated in order to investigate the prognostic impacts of IMPDH2 in HCC patients.

Materials and methods

Cell lines and cell culture

Seven HCC cell lines (97H, 97L, 7721, 7402, 7703, huh7 and 7701), and one immortalized primary hepatocellular epithelial cell line (Lo-2), were cultured routinely by our laboratory in DMEM medium (Gibco, Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum (FBS; Gibco). All cell lines were incubated at 37°C in a 5% CO_2 incubator.

Patients and tissue specimens

We used 287 formalin-fixed paraffin-embedded (FFPE) tumorous and adjacent non-tumorous HCC tissues samples from 287 HCC patients, which from Sun Yat-sen University Cancer Center between February 1999 and June 2002. All the samples used in this study contained matched tumors (percentage of tumor cells ≥ 70%) and corresponding normal mucosal tissue (>5 cm laterally from the edge of the cancerous region); Before surgical therapy, none of the patients had received neoadjuvant chemotherapy, radiation therapy or immunotherapy. Related original clinical data, including gender, age, tumor size, AFP status, HBV infection, liver cirrhosis, pathological grade, portal vein invasion, lymph node metastasis, distant metastasis and TNM stage, were also collected simultaneously. The follow-up data were obtained by telephone or from the outpatient records. The patients who did not have the followed-up information were excluded from this study. The clinicopathologic characteristics of the patients in each cohort are summarized in **Table 1**. Clinical staging was performed according to the seventh edition of the American Joint Committee on Cancer Staging manual. The study was approved by the Ethics Committee of Sun Yat-sen University and written informed consent was obtained from all patients. All experimental methods were carried out in accordance with approved guidelines of Sun Yat-sen University.

Immunohistochemistry (IHC) analysis

The expression of IMPDH2 in the 287 paraffin-embedded HCC tissue specimens was examined by the immunohistochemical analysis. The paraffin-embedded HCC specimens were cut into 5 μm sections and placed in an oven at 65°C for 2 h. The sections were deparaffinized in xylene and hydrated through a series of graded ethanol. Subsequently, the sections were immersed in 3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. To retrieve the antigenicity, the slides were then boiled in citrate buffer solution (pH 6.5) for 20 min in a micro-wave oven. After washing three times for 5 min in phosphate buffered saline (PBS; PH=7.4), the sections were incubated with a primary antibody against IMPDH2 (1: 100 dilution; Abcam, Cambridge, MA, USA) at 4°C overnight. After rinsing three times for 5 min in PBS, the tissue sections were sequentially incubated with a secondary antibody for 1 hour at room temperature. After three further washes in PBS, the sections were stained with 3,3-diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's haematoxylin, dehydrated and mounted. PBS was used to replace anti-IMPDH2 antibody as a negative control.

Table 1. Correlation between IMPDH2 expression and clinical and pathological characters in HCC patients

Variable	All cases N=287 (%)	IMPDH2 protein		χ^2	*P* value
		Low expression N=123 (%)	High expression N=164 (%)		
Age (years)[a]				3.644	0.056
≤49	140 (48.8)	52 (37.1)	88 (62.9)		
>49	147 (51.2)	71 (48.3)	76 (51.7)		
Mean ± SD (49.5 ± 12.5)					
Gender				3.410	0.065
Female	24 (8.4)	6 (25.0)	18 (75.0)		
Male	263 (91.6)	117 (44.5)	146 (55.5)		
HBsAg				0.378	0.539
Yes	243 (84.7)	106 (43.6)	137 (56.4)		
No	44 (15.3)	17 (38.6)	27 (61.4)		
AFP (ng/ml)				0.465	0.495
<200	138 (48.1)	62 (44.9)	76 (55.1)		
≥200	149 (51.9)	61 (40.9)	88 (59.1)		
Liver cirrhosis				2.608	0.106
Yes	225 (78.4)	102 (45.3)	123 (54.7)		
No	62 (21.6)	21 (33.9)	41 (66.1)		
Tumor size (cm)				37.027	<0.001*
≤5	108 (37.6)	71 (65.7)	37 (34.3)		
>5	179 (62.4)	52 (29.1)	127 (70.9)		
Tumor multiplicity				11.676	0.001*
Single	230 (80.1)	110 (47.8)	120 (52.2)		
Multiple	57 (19.9)	13 (22.8)	44 (77.2)		
Pathological grade				1.096	0.778
Well (I)	25 (8.7)	11 (44.0)	14 (56.0)		
Moderate (II)	135 (47.0)	61 (45.2)	74 (54.8)		
Poor (III)	120 (41.8)	49 (40.8)	71 (59.2)		
Undifferentiated (IV)	7 (2.4)	2 (28.6)	5 (71.4)		
TNM stage				24.098	<0.001*
I	184 (64.1)	97 (52.7)	87 (47.3)		
II	24 (8.4)	9 (37.5)	15 (62.5)		
III	66 (23.0)	12 (18.2)	54 (82.8)		
IV	13 (4.5)	5 (38.5)	8 (61.5)		
Vascular invasion				2.261	0.133
Yes	30 (10.5)	9 (22.2)	21 (77.8)		
No	257 (89.5)	114 (41.2)	143 (58.8)		

a: patients were divided according to the median age; AFP: alpha-fetoprotein; HBsAg: hepatitis B surface antigen; *: P<0.05.

IHC evaluation

Immunohistochemical staining was scored independently by three pathologists according to the intensity of staining and the proportion of positive tumor cells. In brief, the intensity of staining was scored as follows: 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The proportion of positively stained tumor cells was also scored according to the following standard: 0 (0%), 1 (1%-10%), 2 (11%-50%), 3 (51%-80%), and 4 (81%-100%). Then, the immunohistochemical scores (IHC scores) were generated by multiplying the intensity and the proportion scores. Based on this method, the final staining scores were classified as follow: "-" was given for IHC scores 0, "+" for IHC scores 1-4, "++" for IHC scores 5-8,

and "+++" for IHC scores 9-12. For statistical analysis, we defined cases with "-" or "+" as low IMPDH2 expression and cases with "++" or "+++" as high IMPDH2 expression.

Quantitative real-time polymerase chain reaction (q-PCR)

Total RNA was extracted from the HCC cell lines using Trizol regent (Invitrogen, Grand Island, NY, USA) and cDNA was synthesized by Superscript Reverse Transcriptase Kit (Promega, Madison, WI, USA) according to the manufacturer's instruction. The primer sequences used to amplify IMPDH2 were: 5'-GCT CCT GTG CCT GAT GGA AT-3' and 5'-CGG GCT CCT CCC CAA AAT AA-3'. GAPDH was used as an internal control for normalization.

Western blot

All of proteins were extracted using sample buffer (62.5 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 5% 2-β-mercaptoethanol), and the protein Protein concentration was quantified using the Pierce® BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Total protein (50 μg) was separated on 10% SDS-polyacrylamide gel electrophoresis (PAGE), and electrotransferred on a polyvinylidene difluoride (PVDF) membrane (Pall Corp., Port Washington, NY). After blocking non-specific binding sites for 1 h with 5% non-fat milk, the membranes were then incubated with primary mouse monoclonal antibodies against AZGP1 (Santa Cruz Biotechonlogy, CA, USA, at 1:1000 dilution), and GAPDH (Santa Cruz Biotechonlogy, CA, USA, at 1:4000 dilution) at 4°C overnight. After washing, the membrane was then incubated with the secondary anti-mouse antibody (Santa Cruz Biotechnology, CA, USA) for 1 h at room temperature. The immunoreactive signals were detected with enhanced chemiluminescence kit (Amersham Biosciences, Uppsala, Sweden). The procedures followed were conducted in accordance with the manufacturer's instructions.

Vector construction and retroviral infection

The coding sequences of IMPDH2 were amplified and cloned into pcDNA3.1 (+) to generate IMPDH2 expression vector. The sequences of two human short hairpin RNA (shRNA) sequences to repress IMPDH2 expression are listed as follows: IMPDH2 shRNA#1: 5'-CCAAGAGCTTGACCCAAGT-3'; IMPDH2 shRNA#2: 5'-GGCCAATGAAGTTCGGAAA-3'. Cell transfected with empty vector were used as controls. The vectors were packaged using the ViraPower Mix (Genecopoeia, Guangzhou, China) in 293FT cells. After culturing for 48 hours, the lentiviral particles in the supernatant were harvested and filtered by centrifugation at 500 g for 10 min, and then transfected into the HCC cells.

Colony formation assay

Cells were counted, plated in triplicate at 200 cells for the pooled population or 100 sorted cells per well in six-well plates, and cultured with DMEM medium (Gibco, Invitrogen, Carlsbad, California, USA) complete culture for 14 days. After most of the colonies had expanded to more than 50 cells, they were washed twice with PBS, fixed in methanol for 15 min, and dyed with crystal violet for 15 min at room temperature. After washing out the dye, the plates were photographed. To quantify the colonies objectively, the software Quantity One was used and colonies that lager than the averaging parameter of 3 or 1 and the minimum signal intensity of 1.0 were counted. At least three independent experiments were carried out for each assay.

CCK-8 assay

Cell viability was detected using Cell Counting kit-8 (CCK-8; Dojindo Labora-tories, Japan). Cells were plated at a density of 3 *10^3 cells/well in 96-well plates with four replicates. Then, 100 ml of serum-free cell culture medium containing 10 ml WST-8 reagent was added into each well every 24 h and the plates were incubated at standard conditions for 1 h. Optical absorbance of each well at 450 nm and 630 nm were measured with a microplate reader (Bio-Rad Laboratories, USA). Three independent experiments were performed for quantification.

Statistical analysis

Statistical analysis was performed with the SPSS statistical software package (standard version 19.0; SPSS, Chicago, IL). The relationship between IMPDH2 expression and the clinicapathologic features of the HCC patients were evaluated by a Pearson's chisquared test. Univariate and multivariate survival analysis was performed with the Cox proportional hazards

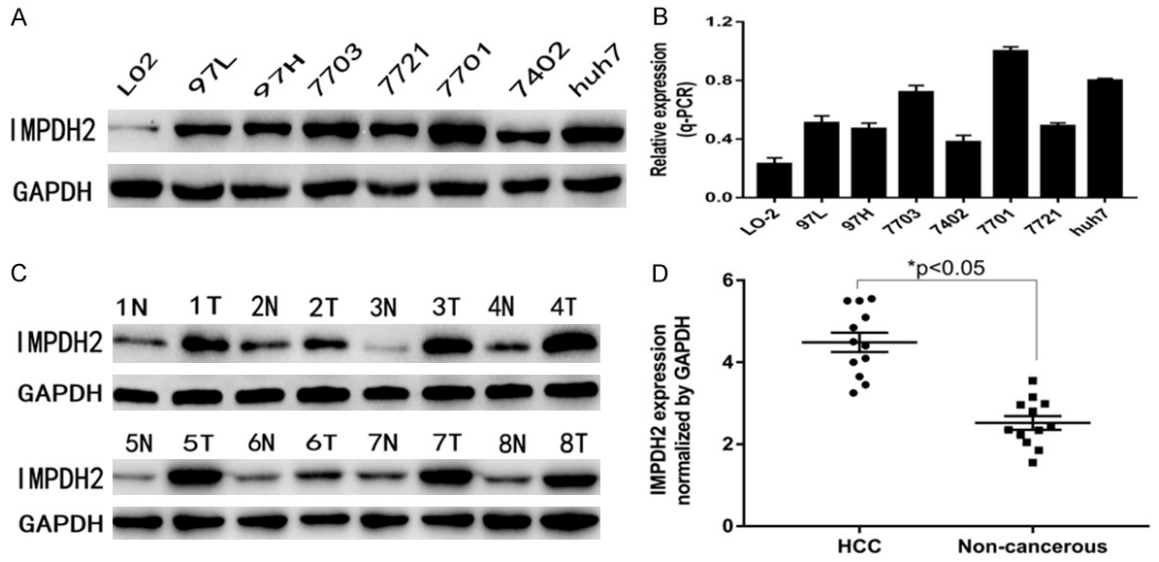

Figure 1. Expression and amplification of IMPDH2 in HCC cell lines and HCC tissues. A. The level of IMPDH2 protein examined by Western blotting in 8 human HCC cell lines (i.e., 97L, 97H, 7721, 7402, 7703, huh7 and 7701) and one immortalized primary hepatocellular epithelial cell line Lo-2. B. The expression level of IMPDH2 mRNA in 8 human HCC cell lines was evaluated by q-PCR. C. Among 12 HCC cases, increased expression of IMPDH2 was detected via western blotting in 10 pairs of HCC tissues compared with the matched non-cancerous liver tissues. The expression levels were normalized to those of GAPDH. D. One-step quantitative real-time polymerase chain reaction (q-PCR) demonstrated that the expression of IMPDH2 in HCC tissues was significantly higher than that in matched non-cancerous tissues, when normalized to the GAPDH internal control. *P<0.05.

regression model. The corresponding Hazard ratio (HR) and 95% CI were taken for from Cox regression models. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. Differences were considered significant if the *P*-value from a two-tailed test was <0.05.

Results

Detection of IMPDH2 expression in HCC based on western blotting and q-PCR

Western blotting and q-PCR was employed to examine the expression of IMPDH2 in seven HCC cell lines (7701, huh7, 7721, 7402, 97H, 97L and 7703) and one immortalized primary hepatocellular epithelial cell line (Lo-2), and ten fresh HCC tissues (T) with their paired adjacent normal-tissues (ANTs). We found that all seven HCC cell lines showed heightened IMPDH2 mRNA and protein expression compared with Lo-2 (**Figure 1A**, **1B**). Furthermore, both IMPDH2 protein and mRNA expression were higher in ten human HCC tissues than in the paired ANTs (**Figure 1C**, **1D**), indicating that IMPDH2 expression is up-regulated in HCC.

Detection of IMPDH2 expression in HCC by immunohistochemistry (IHC)

The expression of IMPDH2 protein was detected by IHC in 287 HCC tissues and adjacent normal hepatic tissues. Compared with adjacent non-tumorous hepatic tissues, HCC tissues exhibited higher expression levels of IMPDH2 protein (**Figure 2A**, **2B**). The four categories of the intensity of IMPDH2 immunostaining were described in **Figure 2C-F**.

The association between IMPDH2 expression and cliniopatheological parameters

Pearson's chi-square (χ^2) test/Fisher's exact test was performed to investigate the relationship between IMPDH2 expression and clinicopathological characteristics in 287 patients with HCC. Our results showed significant correlations between IMPDH2 expression and three characteristics including tumor multiplicity (P= 0.001), TNM stage (P<0.001) and tumor size (P<0.001). However, we found no statistically significant correlations between IMPDH2 expression and the rest of clinicopathological features, such as patient age, gender, AFP, HBsAg,

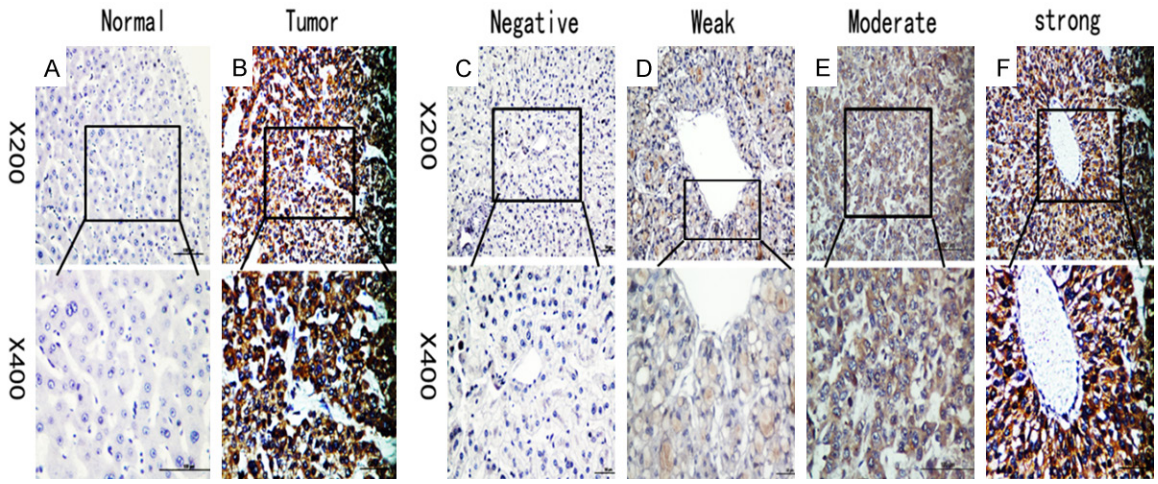

Figure 2. The Representative images of IMPDH2 expression in HCC tissues via IHC. IMPDH2 was absent from or only weakly detected in adjacent normal liver cells (A), whereas its up-regulation was mainly detected in HCC tissues (B) (original magnification, ×200 and ×400). The representative images show different staining intensities of IMPDH2: (C) negative staining, (D) weak staining, (E) moderate staining, and (F) strong staining (original magnification, ×200 and ×400).

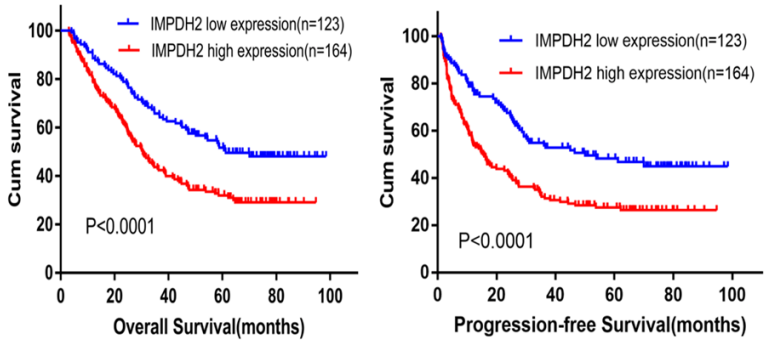

Figure 3. Kaplan-Meier analysis indicating the correlation of IMPDH2 overexpression with poorer overall survival and progression-free survival rates of 287 HCC patients (log-rank test).

Liver cirrhosis, tumor size, pathological grades, and vascular invasion (P>0.05, **Table 1**).

The relationship of high IMPDH2 expression with poor survival in HCC patient

The relationship between IMPDH2 expression in HCC patients and the survival time of these patients was analyzed by Kaplan-Meier analysis and the log-rank test (**Figure 3**). The log-rank test showed that the survival time was different between high and low IMPDH2 expression groups. The median overall survival (OS) time was 44.4 months for the patients with high IMPDH2 expression groups, significantly shorter than that for the patients exhibiting low IMPDH2 expression (62.9 months) (log-rank test, P<0.001, **Figure 3A**). Furthermore, high IMPDH2 expression group had poorer progression-free survival (PFS) time than low IMPDH2 expression group (log-rank test, P<0.001, **Figure 3B**). Our results showed that the high expression of IMPDH2 could be a prognostic factor for HCC patients with pathological grade.

In addition, the prognostic value of IMPDH2 expression was analyzed using univariate analysis model and multivariate Cox regression against OS and PFS (**Tables 2** and **3**). The univariate analysis results confirmed the impact of IMPDH2 expression and pathological grade on survival. The Cox regression further suggested IMPDH2 expression and pathological grade stages as independent prognostic factors for OS and PFS in HCC patients. Therefore, the results demonstrated that the IMPDH2 expression level was closely associated with the prognosis of HCC.

IMPDH2 promotes the proliferation of HCC cells

To further demonstrate the role of IMPDH2 in HCC progression, IMPDH2 was stably transfect-

Table 2. Univariate and multivariate Cox regression analysis of prognostic factors in 287 HCC patients for overall survival

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Overall survival						
Age (years)						
>49 vs. ≤49	1.053	0.782-1.418	0.733			
Sex						
Male vs. Female	1.245	0.707-2.190	0.448			
AFP (ng/ml)						
≥200 vs. <200	1.188	0.882-1.600	0.257			
Liver cirrhosis						
Positive vs. Negative	1.261	0.867-1.835	0.225			
HBsAg						
Positive vs. Negative	1.152	0.749-1.773	0.519			
Tumor size (cm)						
>5 vs. ≤5	1.819	1.318-2.510	<0.001*	1.450	1.022-2.056	0.037*
Tumor multiplicity						
Multiple vs. Single	1.898	1.350-2.668	<0.001*	1.524	0.990-2.347	0.056
Vascular invasion						
Positive vs. Negative	1.492	0.936-2.380	0.093			
Pathological grade						
III-IV vs. I-II	1.560	1.159-2.101	0.003*	1.538	1.127-2.099	0.007*
TNM stage						
III-IV vs. I-II	1.781	1.298-2.443	<0.001*	1.061	0.697-1.615	0.782
IMPDH2 expression						
High vs. Low	1.816	1.329-2.482	<0.001*	1.513	1.079-2.123	0.016*

*: P<0.05.

ed into HCC cell lines 7402, which showed endogenous low IMPDH2 expression (**Figure 1**). CCK8 and colony formation assays showed that the proliferation rate of IMPDH2-overexpressing cells was significantly higher than in the vector-control cells (**Figure 4D**). To confirm this result, we knocked down endogenous IMPDH2 in huh7 and 7701 HCC cells by expressing short hairpin RNAs (shRNA). Consistently, CCK8 and colony formation assay showed that the proliferation rates were remarkably compromised (**Figure 4A-C**, **4E**). These result showed that IMPDH2 has important role in the proliferation of HCC cells in vitro.

Discussion

Currently, tumor clinical/pathological stage is the most commonly and widely used predictive factor for the prognosis of HCC patients. However, patients with the same clinical/pathological stage of HCC sometimes show considerable variability in tumor recurrence and metastasis. Thus, it will be meaningful to search for novel biomarkers that can provide additional staging information to assist the choice of therapy and optimize treatment outcomes.

Accumulating evidence has shown that expression of IMPDH2 was significantly increased in various types of malignancies [18, 19]. These findings reveal a potential carcinogenic role of IMPDH2 in multiple human malignancies. To date, however, the expression status of IMPDH2 in HCC and its relationship with the cliniclpathological parameters have not been elucidated. In the current study, we first performed immunohistochemistry to evaluate the expression status of IMPDH2 in 287 HCC patients. IMPDH2 was significantly up-regulated in HCC paraffin-embedded tissues compared with that in adjacent normal tissues. In particular, overexpression of IMPDH2 protein was detected in 59.2% of HCC tissues. By contrast, the expres-

Table 3. Univariate and multivariate Cox regression analysis of prognostic factors in 287 HCC patients for progression-free survival

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Disease-free survival						
Age (years)						
>49 vs. ≤49	1.144	0.874-1.497	0.328			
Sex						
Male vs. Female	1.242	0.735-2.100	0.419			
AFP (ng/ml)						
≥200 vs. <200	1.166	0.891-1.526	0.264			
Liver cirrhosis						
Positive vs. Negative	1.552	1.087-2.214	0.015*	1.866	1.293-2.692	0.001*
HBsAg						
Positive vs. Negative	1.258	0.850-1.862	0.251			
Tumor size (cm)						
>5 cm vs. ≤5 cm	1.415	1.069-1.874	0.015*	1.148	0.837-1.573	0.393
Tumor multiplicity						
Multiple vs. Single	2.057	1.499-2.823	<0.001*	1.495	0.619-3.612	0.371
Vascular invasion						
Positive vs. Negative	1.705	1.122-2.591	0.013*	1.517	0.995-2.311	0.053
Pathological grade						
III-IV vs. I-II	1.449	1.107-1.898	0.007*	1.601	1.200-2.135	0.001*
TNM stage						
III-IV vs. I-II	1.649	1.232-2.208	0.001*	0.949	0.642-1.405	0.795
IMPDH2 expression						
High vs. Low	1.662	1.260-2.194	<0.001*	1.567	1.149-2.136	0.005*

*: P<0.05.

sion level of IMPDH2 in the adjacent HCC tissues was low or absent. We also observed that IMPDH2 was highly expressed at both the protein and mRNA levels in HCC cell lines compared with LO-2. These findings suggest that high expression of IMPDH2 may provide a selective advantage in the HCC tumorigenic processes.

In previous studies, the expression of IMP-DH2 was discovered to be remarkable elevated and closely related with tumor progression and unfavorable prognosis in diversified types of malignancies [20]. IMPDH2 was found to be overexpressed in the subgroup of patients with poor response to chemotherapy, rendering it an independent prognostic factor for chemotherapy response and event-free survival [21]. He et al. showed that high IMPDH2 expression was frequently observed in colorectal cancer tissues and might play a crucial role in colorectal carcinogenesis [22]. A study by Toubiana et al. demonstrated that IMPDHII plays an important role in the negative regulation of TLR2 signaling by modulating PI3K activity [23]. In the present study, we observed that increased expression of IMPDH2 was significantly associated with proliferation characteristics of HCC. Importantly, the Kaplan-Meier curve and multivariate Cox regression analysis were performed to show that high IMPDH2 expression was identified as an independent predictor for shorter OS and PFS in HCC patients: tumor size >5 cm, TNM stage III/IV. Therefore, high IMPDH2 expression seems to have the potential to predict poor OS and PFS outcomes in patients with HCC. The detection of IMPDH2 expression status might be served as an integrated approach for identifying HCC patient at high risk of cancer progression. Thus, HCC patients with high IMPDH2 expression should be paid much more attention to and/or should be more closely followed up after surgical resection.

As we known, the majority of cancer deaths are a result of tumor metastasis rather than prima-

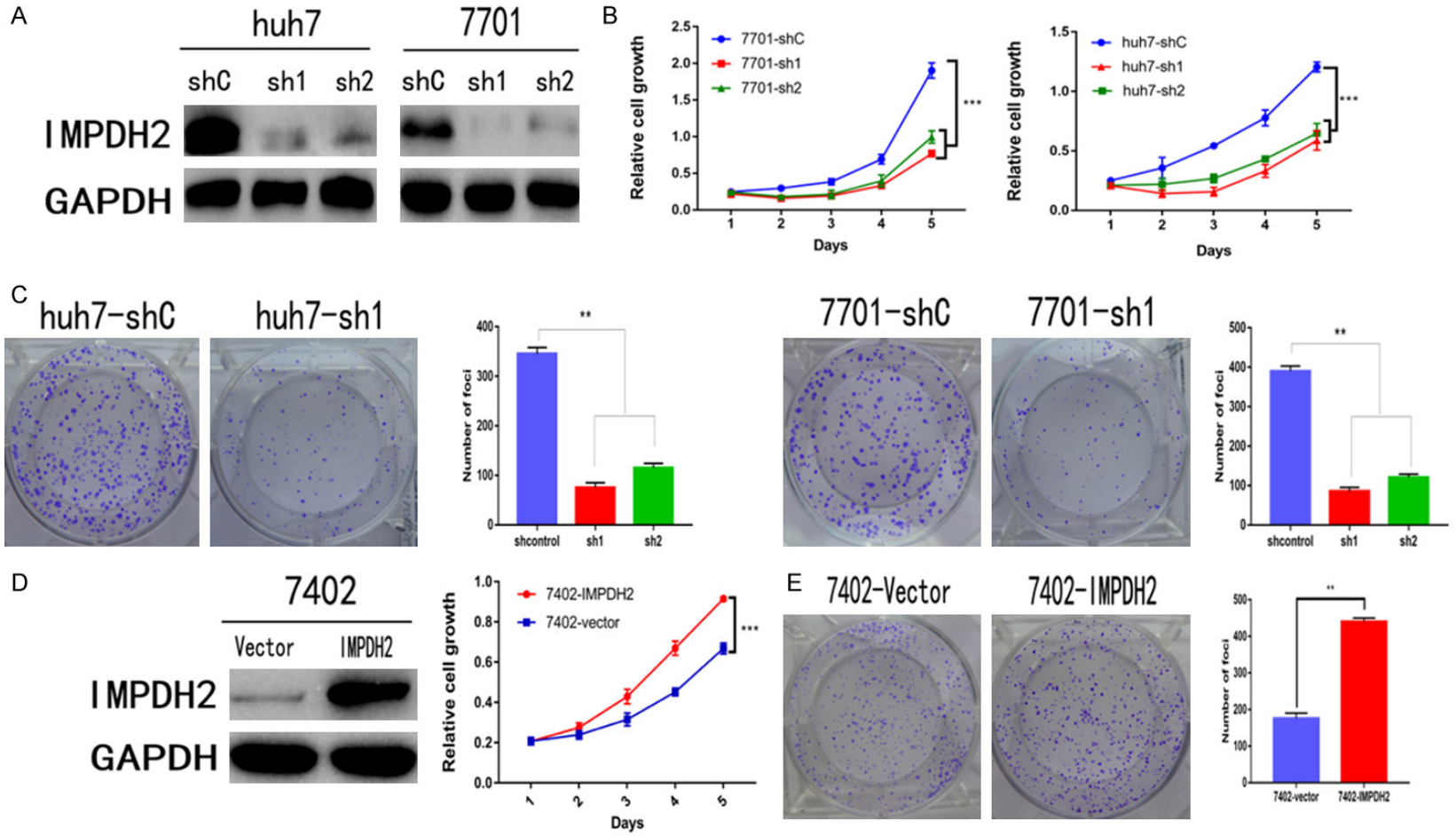

Figure 4. IMPDH2 has strong oncogenic function in HCC cells. A. Western blotting reveals that IMPDH2 was efficiently knocked down by the treatment of IMPDH2-shRNA-1 or IMPDH2-shRNA-2 in huh7 and 7701 HCC cells. B. Rate of cell growth between shIMPDH2 and shControl HCC cells by CCK-8 kit. *P<0.05, **P<0.01 by Student's t-test. C. Representative images of decreased foci formation in monolayer culture induced by IMPDH2 silenced in HCC cells. Data are the means ± s.d. of three independent experiments. **P<0.01 by Student's t-test. D. The levels of IMPDH2 were substantially increased in 7402-IMPDH2 cells compared with that in control 7402-vector cells by western blotting (left). Overexpression of IMPDH2 promoted 7402 cells growth rate (right). **P<0.01 by Student's t-test. E. Representative images of increased foci formation in monolayer culture induced by IMPDH2 overexpression in 7402 cells. Data are the mean ± s.d. of three independent experiments. **P<0.01 by Student's t-test.

ry tumors, and the vascular invasion and metastasis are important steps in cancer progression [24]. The potential function of IMPDH2 in cancer progression has been explored in recently investigations. A study by Takebe N et al. demonstrated that inhibition of IMPDH had been able to induce caspase-dependent apoptosis and cell-cycle arrest in multiple myeloma cells [25]. Furthermore, IMPDH inhibitors have been found to induce terminal differentiation in several types of human cancer cells [26, 27], and have been successfully applied in clinical trials [27, 28]. All these study illustrated that the function of IMPDH2 in human cancer might be tissue-specific. Although in present study, we identified the correlation of IMPDH2 expression in cancer and HCC patients' survival, more works are needed in the future study to elucidate the mechanisms by which IMPDH2 is involved in the development and progression of HCC.

Conclusion

In summary, our study demonstrated for the first time that IMPDH2 was markedly overexpressed in HCC tissues and closely related with aggressive HCC progression, and that silencing IMPDH2 expression memorably suppressed the proliferation and tumorigenicity of HCC cells in vitro, whereas overexpression IMPDH2 had the opposite effect. These date indicated the expression levels of IMPDH2, as detected by IHC, could be a potential biomarker for poor differentiation and a useful predictor for unfavorable prognosis of HCC patients after curative hepatectomy.

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Disclosure of conflict of interest

None.

Address correspondence to: Sheng Ye and Jiaxing Zhang, Department of Oncology, The First Affiliated Hospital, Sun Yat-sen University, No 58, Zhongshan Road II, Guangzhou 510080, Guangdong Province, P. R. China. Tel: 86-20-87755766; Fax: 86-20-87-755766; E-mail: yesheng2@mail.sysu.edu.cn (SY); zjxlundy@hotmail.com (JXZ)

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