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Original Article

Decreased expression of schlafen5 (SLFN5) correlates with unfavorable survival in human hepatocellular carcinoma

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Abstract: As a type I IFN inducible-gene, schlafen5 (SLFN5) has been identified as a special tumor suppressor in the growth and invasion of human malignant melanoma and renal cell carcinoma. However, the association between SLFN5 expression and the clinicopathological characteristics of HCC is still unknown. We conducted this study to examine the expression of SLFN5 in HCC and investigate the association between SLFN5 expression and the clinicopathological manifestations of HCC patients. Real time-quantitative PCR, Western blotting and IHC analyses were used to evaluate the expression of SLFN5 in HCC cell lines and tissues. Kaplan-Meier survival and Cox regression analyses were applied to evaluate the prognosis of 90 HCC patients. The data showed significantly decreased SLFN5 expression in HCC tissues compared to corresponding non-cancerous cells and tissues. Moreover, we compared the HCC and self-paired non-cancerous tissue for SLFN5 expression by measuring the decrease in SLFN5 expression (denoted ΔSLFN5). Results showed that a higher ΔSLFN5 correlates with malignant behavior of HCC, including higher pathological grade (P = 0.003), higher TNM stage (P = 0.014) and bigger tumor diameter (P = 0.017). Cox regression analysis further revealed that ΔSLFN5 ≥ 150 is an independent prognostic factor for overall survival (HR: 4.101; 95% CI: 1.135-14.815; P = 0.031). These data are the first to indicate that decreased expression of SLFN5 in HCC, which is closely related to poor histological grade, advanced TNM and larger tumor size. The low-expression of SLFN5 suggests poor survival outcomes in HCC patients.

Keywords: SLFN5, IFN, hepatocellular carcinoma, biomarker

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer worldwide and China accounts for more than 50% of new cases, globally [1]. HCC represents more than 90% of primary liver cancer. The incidence of HCC increases progressively with age, reaching a peak at 70 years [2]. Men are more prone to this cancer than women, with a male to female ratio estimated to be 2.4:1 [1]. Many important risk factors for the development of HCC have been identified and thoroughly studied. These include alcohol intake, hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, hereditary hemochromatosis and cirrhosis of almost any cause [3]. Due to early metastasis and the rapid tumor progression of HCC, the prognosis of HCC is still poor. The CONCORD-2 study recently published in Lancet, reported that the global survival rate from liver cancer from 1995-2009 was generally low (10-20%) in most countries [4]. However, the mechanisms through which this cancer develops and progresses are still not fully understood.

The Schlafen (SLFN) gene family is divided into 3 categories by size of the molecular mass (Group I: range from 37 to 42 kDa; group II: range from 58 to 68 kDa, group III: range from 100 to 104 kDa). In 1998, Schwarts et al. identified 4 SLFN genes in mice and showed that they are involved in thymocyte maturation and T cell activation. At the moment, there are 10

known or predicted mouse SLFN genes and five human SLFN isoforms. Further studies revealed that they are involved in other important functions, such as the control of cell proliferation, induction of immune responses, and the regulation of viral replication. All SLFN isoforms contain one specific domain called the Slfn box, which is not found in other proteins and whose function is not yet fully understood. It is thought that it might be involved in GTP/ATP binding. Other domains, such as SWADL or domains which are involved in RNA metabolism and RNA structure-modeling activity are also described in the literature [5-7]. Recently, several studies focused on the negative regulatory effects of the SLFN gene family on tumorigenesis [8-10]. Among them, human SLFN5 was shown to inhibit malignant melanoma cell growth and invasion in vivo, and also inhibits motility and decreases invasiveness of renal cell carcinoma (RCC) cells [11, 12]. SLFN11 have recently been associated with some important biological functions. Interestingly, SLFN11 could block the production of the retrovirus, HIV-1, by blocking expression of viral proteins in a codon usage defined manner. Furthermore, other recent studies have demonstrated that SLFN11 has an important role in predicting OS of ovarian cancer patients treated with cisplatin-containing regimens and in sensitizing malignant cells to DNA damaging agents [13-16].

At the same time, SLFNs were deemed to be effectors of Type I interferon (IFNs) inducible responses. However, type I IFN-α has been used in HCC treatment for a long time. It activates the Janus kinase (JAK)/STAT signaling cascade, down-regulates vascular endothelial growth factor (VEGF) expression but is only effective in a subset of patients.

The role of human SFLN5 in HCC pathogenesis is still unclear. This study was conducted to investigate the relationship between SLFN5 expression and the clinicopathological course of HCC.

Materials and methods

Tissue microarray and tissue specimens

For immunohistochemistry (IHC) analysis, 90 matched HCC tissues and adjacent non-cancerous tissues were arranged in a tissue microarray (TMA) by Outdo Biotech Co. Ltd (Shanghai, China). Patients, who had undergone curative hepatectomy at the Taizhou hospital (Zhejiang, China) from January 2010 to September 2011 were enrolled in this study, including 74 men and 16 women with median age of 54.5 years (range, 28-76 years). Among them, 33 patients were diagnosed with liver cirrhosis. Twenty-nine patients had primary tumors smaller than 5 cm, 39 patients had tumors between 5 to 10 cm, and the remaining 22 patients had tumors larger than 10 cm. Regarding the pathological stage, 17 patients were at grade 1, 63 were at grade 2, and 10 were at grade 3. In terms of TNM staging among all 90 HCC cases, 7 patients were in stage I, 45 were in stage II, while the remaining 31 patients were in advanced stage III. All patients were followed up, with the median duration of follow-up of 26 months (range, 0-44 months). A diagnosis of HCC was pathologically re-confirmed. The TNM stage of all HCC samples was confirmed according to the 2002 American Joint Committee on Cancer/International Union Against Cancer TNM staging system [17]. The grade of tumor differentiation was estimated by the Edmondson-Steiner grading system. The overall survival (OS) was defined as the survival time between surgery and death or between surgery and the last follow-up interview (Sep. 2013). We also collected 13 self-paired fresh HCC surgical samples used for subsequent RT-PCR and Western blot detection in the Second Affiliated Hospital of Chongqing Medical University from July 2013 to February 2014. The specimens were processed immediately after surgery and stored at -80°C before subsequent protein and total RNA extraction. None of the patients had received hepatic transplant, adjuvant chemotherapy, radiation therapy or immunotherapy before or after surgery. The study was approved by the Ethics Committee of Taizhou Hospital and Chongqing Medical University. Informed consent was obtained from all patients or their relatives.

Cell culture and reagents

Three HCC cell lines (SMMC-7721, BEL-7402 and Huh 7) were purchased from the Cell Bank of the Chinese Academy of Medical Science (Beijing, China). HepG2 and one human liver cell line (L02) was obtained from ATCC (Manassas, VA, USA). All cells were cultured in DMEM medium (HyClone, Waltham, MA) sup-

plemented with 10% fetal bovine serum (Gibco, San Diego, CA), penicillin (100 U/mL) and streptomycin (100 mg/mL).

Quantitative RT-PCR

Total RNA was extracted from frozen tissue using TriZol reagent (Invitrogen) following the manufacturer's instructions. Total RNA (500 ng) was mixed with oligo dT primers and reverse transcribed using PrimeScript™ RT Reagent Kit (TaKaRa Biotechnology). Real-time PCR was subsequently performed with SYBR® Priemix Ex Taq™ II (Takara Biotechnology). The primers of the PCR primers sets were as follows: schlafen5 (SLFN5), forward 5'-TTCTGCTGTGCGGTGTTTGCCA-3' and reverse 5'-CTGGAGAACCATCTCAGGACAC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5'-GTCTCCTCTGACTTCAACAGCG-3' and reverse 5'-ACCACCCTGTTGCTGTAGCCAA-3'. Expression levels were normalized against GAPDH, and the $2^{-\Delta\Delta Ct}$ method was used to evaluate the relative expression levels of SLFN5.

Western blotting assay

Frozen samples and harvesting cells were lysed using RIPA buffer in ice using homogenizers, then centrifuged (14,000×g; Eppendorf, Germany) for 30 min at 4°C. Protein concentration was determined by the BCA protein assay (Beyotime Institute of Biotechnology, Shanghai, China). Equal amounts of degenerated proteins were electrophoretically separated on 10% SDS/polyacrylamide gels (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skimmed milk in Tris-buffered saline with Tween-20 (TBS-T) for 1 h, and then incubated with Schlafen5 antibody (ab121537; diluted 1:1,000). Anti-human β-actin antibody (Santa Cruz, CW0266, diluted 1:2,000) was used as internal control. HRP-conjugated secondary antibodies were visualized using the ChemiDoc MP imaging system (Bio-Rad, Hercules, CA).

Immunohistochemistry (IHC)

SLFN5 expression was detected using immunohistochemistry through a standardized streptavidin-peroxidase (SP) method. The TMA slides were de-paraffinized and rehydrated, and epitope retrieval was performed in citrate buffer (pH 6.0) in a pressure cooker for 2 min after air jetting. Following peroxidase blocking, the slides were incubated with 10% normal goat serum for 30 min, and then incubated with the primary antibody Schlafen5 (ab121537; 1:200) overnight at 4°C. Slides were incubated with biotinylated goat anti-mouse IgG (Beijing Zhongshan Golden Bridge Biotechnology Co., Beijing, China; 1:100). Negative controls were conducted with Phosphate Buffered Saline (PBS) to substitute the primary antibody. Immunostaining scores were independently evaluated by two pathologists. Semi-quantification of SLFN5 protein expression was based on the percentage of positively stained cells (0-100%) and the staining intensity (scale 0-3). A final continuous IHC score of 0-300 was used.

Statistical analysis

The association of Schlafen5 expression with clinical manifestations was analyzed using the t test, One-Way ANOVA, or Wilcoxon signed rank non-parametric test. Overall survival analysis and univariate analysis was performed using the Kaplan-Meier method with log rank tests. Multivariate analysis was performed using Cox proportional hazards regression models to identify important factors which are statistically associated with overall survival status (OS), 95% confidence intervals (CI), and hazard ratio (HR) are presented. All analyses were performed using SPSS for Windows Version 17.0 (SPSS Inc, Chicago, IL). $P < 0.05$ was designated as the level of significance.

Results

SLFN5 expression in HCC tissues

SLFN5 expression was measured in 13 fresh HCC tissues and matching non-tumor tissues using Real time-quantitative PCR. As shown in **Figure 1**, the SLFN5 expression in HCC tissues was significantly lower compared to the matching non-tumor tissue when normalized to GAPDH ($P = 0.027$). This was confirmed by Western blot analysis through which we also identified decreased expression of SLFN5 protein in fresh HCC tissue compared to non-tumor tissue.

Downregulation of SLFN5 mRNA and protein levels in HCC cell lines

SLFN5 expression was evaluated in four HCC cell lines (SMMC-7721, BEL-7402, Huh 7 and

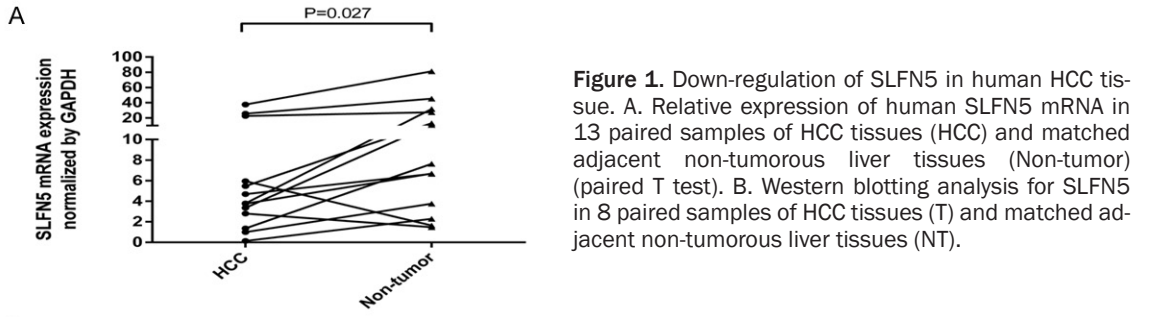

Figure 1. Down-regulation of SLFN5 in human HCC tissue. A. Relative expression of human SLFN5 mRNA in 13 paired samples of HCC tissues (HCC) and matched adjacent non-tumorous liver tissues (Non-tumor) (paired T test). B. Western blotting analysis for SLFN5 in 8 paired samples of HCC tissues (T) and matched adjacent non-tumorous liver tissues (NT).

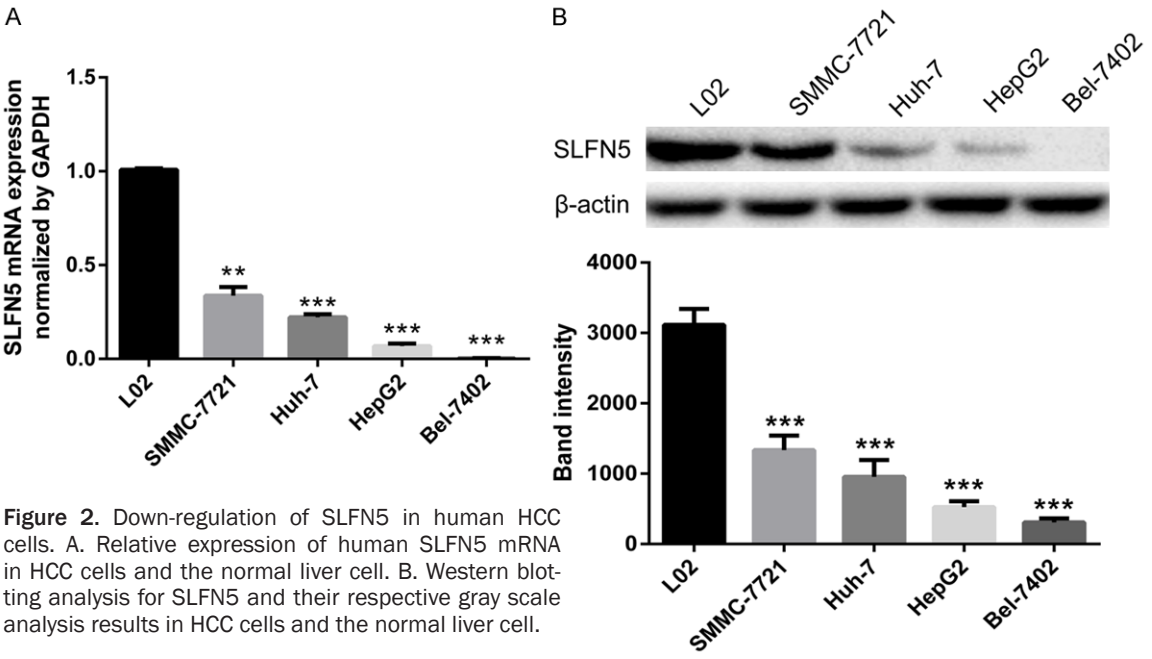

Figure 2. Down-regulation of SLFN5 in human HCC cells. A. Relative expression of human SLFN5 mRNA in HCC cells and the normal liver cell. B. Western blotting analysis for SLFN5 and their respective gray scale analysis results in HCC cells and the normal liver cell.

HepG2) and one normal liver cell line (L02) using RT-PCR and Western blotting analysis. As shown in **Figure 2**, RT-PCR and Western blotting analysis revealed that SLFN5 expression was decreased in the four HCC cell lines in comparison to the normal liver cell line.

Relationship of SLFN5 expression with clinicopathological features

IHC was conducted to evaluate SLFN5 protein expression. According to the H-score system, we detected significantly lower SLFN5 expression in HCC tissue samples compared to the matched non-tumor tissues (HCC: 95.33±60.82 & non-tumor tissue: 144.72±44.93, P < 0.001; 95% CI, -63.79 to -34.99). SLFN5 protein expression in HCC is shown in **Figure 3**. To further assess the clinical significance of SLFN5 expression, we calculated a new variable named ΔSLFN5 (the decrease in SLFN5 expression in HCC compared to self-paired non-tumor tissue). The relationship between ΔSLFN5 protein expression and clinicopathological parameters is demonstrated in **Table 1**. A larger decrease in SLFN5 expressionin HCC was associated with higher pathological grade (I:15.29±43.35 & II: 49.37±67.91 & III: 107.5±75.21; P = 0.003; 95% CI, 34.993-63.785), higher TNM stage (I: 17.14±41.91 & II: 36.28±56.89 & III: 76.53±78.32; P = 0.014; 95% CI,

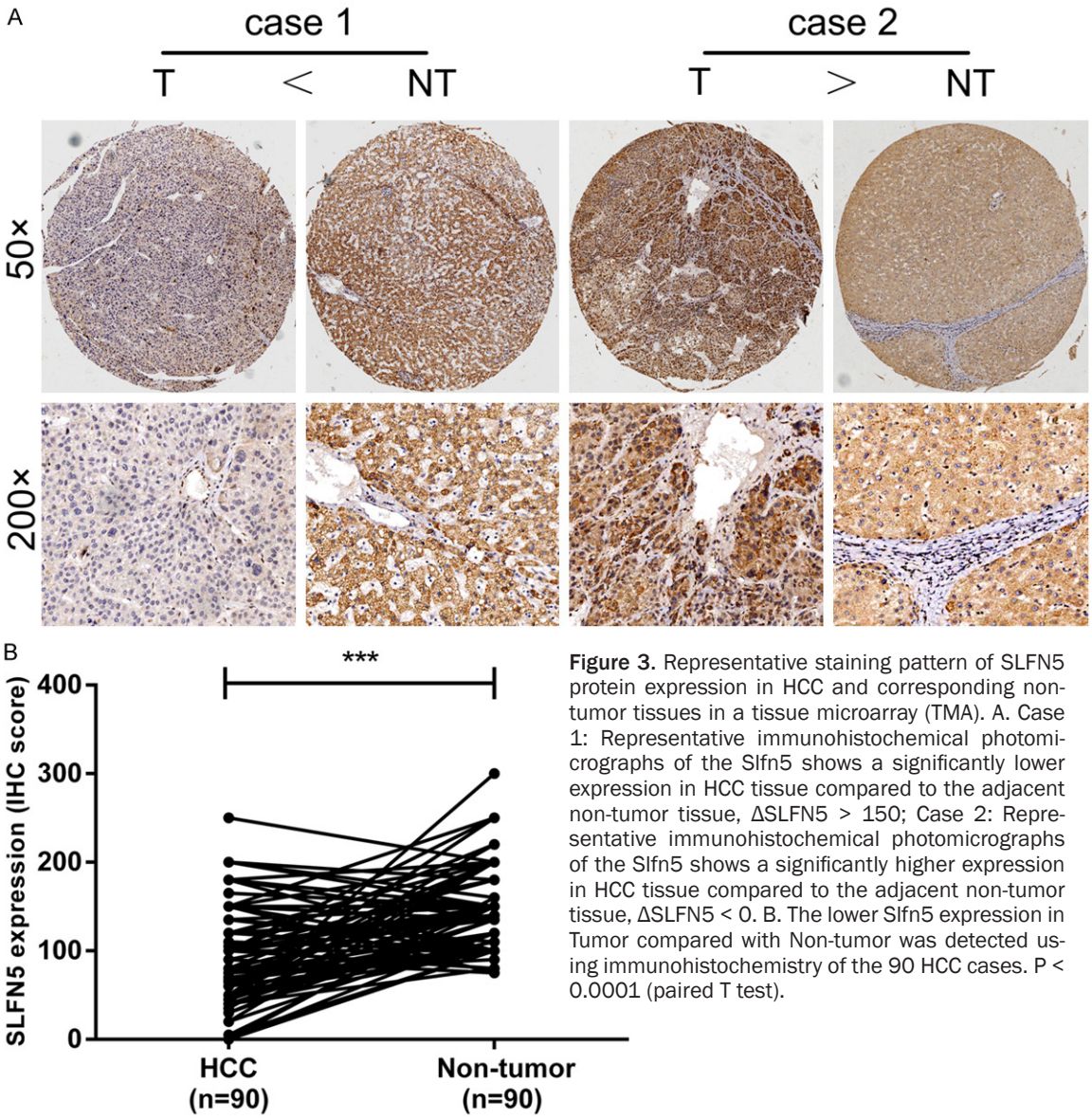

Figure 3. Representative staining pattern of SLFN5 protein expression in HCC and corresponding non-tumor tissues in a tissue microarray (TMA). A. Case 1: Representative immunohistochemical photomicrographs of the Slfn5 shows a significantly lower expression in HCC tissue compared to the adjacent non-tumor tissue, ΔSLFN5 > 150; Case 2: Representative immunohistochemical photomicrographs of the Slfn5 shows a significantly higher expression in HCC tissue compared to the adjacent non-tumor tissue, ΔSLFN5 < 0. B. The lower Slfn5 expression in Tumor compared with Non-tumor was detected using immunohistochemistry of the 90 HCC cases. P < 0.0001 (paired T test).

34.936-64.461) and larger tumor diameter (< 5 cm: 31.72±55.94 & 5~10 cm: 42.88±61.71 & ≥ 10 cm: 84.20±84.73; P = 0.017; 95% CI, 34.993-63.785) (**Table 1**). In comparison, no significant correlation was discovered between ΔSLFN5 expression and other clinical variables, including gender, age, liver cirrhosis and tumor node.

Relationship of SLFN5 expression with clinical outcome

The overall survival (OS) analysis using the Kaplan-Meier method revealed that the prognosis of HCC patients with a higher decrease of SLFN5 expression (ΔSLFN5 ≥ 100 or ΔSLFN5 ≥ 150) was significantly poorer compared to patients with low decrease of SLFN5 expression (ΔSLFN5 < 100 or ΔSLFN5 < 150) (**Figure 4**; P = 0.021 & P < 0.001). Univariate analysis demonstrated that tumor size, histological grade, tumor stage, and ΔSLFN5 expression were significantly associated with overall survival of HCC patients (all P < 0.05, **Table 2**). No significant associations were found for age, gender, tumor node and Ki67 expression. To determine the independent prognostic marker of HCC, all variables of P < 0.10 in univariate survival analysis were included in the Cox proportional hazards model. As shown in **Table 2**, out of 7 included variables (TNM stage, patho-

Table 1. Clinical correlation of ΔSLFN5[a] expression in HCC (IHC score)

Parameter	Total patients (N)	ΔSLFN5 expression	
		Mean ± SD	*P* value[b]
Age (years)			
< 60	62	55.24±64.95	0.231
≥ 60	28	36.43±76.10	
Gender			
Male	74	49.26±68.25	0.969
Female	16	50±73.21	
Tumor diameter (cm)[c]			
< 5	29	31.72±55.94	0.017*
5~10	39	42.88±61.71	
≥ 10	22	84.20±84.73	
Tumor nodules			
Single	78	50.48±68.48	0.703
Multi	12	42.29±73.03	
Pathological grade			
I	17	15.29±43.35	0.003*
II	63	49.37±67.91	
III	10	107.5±75.21	
AJCC tumor stage			
I	7	17.14±41.91	0.014*
II	45	36.28±56.89	
III	31	76.53±78.32	
Adjacent non-cancerous tissue			
Cirrhotic	33	39.32±66.65	0.208
Non-cirrhotic	51	58.87±70.43	
HCC & Non-cancerous	90	95.33±60.82	< 0.001*
	90	144.72±44.93	

*Statistically significant; a: the decrease in SLFN5 expression in T compared with self-paired NT; b: the association of ΔSLFN5 expression with clinical manifestations were determined by the one-way analysis of variance (ANOVA) and t test; c: Tumor size was measured by the diameter of the largest tumor nodule.

logical grade, liver cirrhosis, tumor node, gender and ΔSLFN5 ≥ 100 or ΔSLFN5 ≥ 150), two of these were identified as independent prognostic markers for OS. These were ΔSLFN5 ≥ 150 (P = 0.031; HR: 4.101; 95% CI: 1.135-14.815) and gender (P = 0.014).

Discussion

Human SLFN5 has mainly been investigated because of its anti-tumor properties. Antonella et al. reported that SLFN5 could downregulate expression of matrix metalloproteinases (MMP)-1 and -13 and several other genes, which are involved in the control of malignant cell motility [11]. SLFN5 also exhibits negative regulatory effects on anchorage-independent malignant melanoma cell growth and invasion of malignant cells in collagen according to the study conducted by Katsoulidis et al. Researchers recently described that SLFN5 and SLFN11 could bind NOTCH in the nucleus [18]. Since it is well known that the NOTCH signaling pathway is activated in human HCC, we can hypothesize that SLFN5 could affect biological function through interacting with NOTCH [19, 20].

In this study, we first investigated differential expression of SLFN5 in fresh HCC tissues. The results suggested a significantly lower SLFN5 expression at both the mRNA and protein levels in HCC tissues at different levels compared to matched non-cancerous tissues, indicating that the expression decrease potentially occurs at the transcriptional level. The expression of SLFN5 in cell lines shows the same trend corresponding to the normal liver cell line.

To further characterize the SLFN5 expression in HCC, an IHC analysis in a HCC cohort of 90 cases was conducted. Our results showed significantly lower SLFN5 expression in HCC tissue compared with matched non-cancerous tissues (P < 0.001). The IHC scores was 95.33±60.82 and 144.72±44.93, respectively. These data are consistent with our results and support our findings. Taking into account a different degree of decline in SLFN5 expression in every case of HCC, we evaluated the relationship of ΔSLFN5 (SLFN5 expression in self-paired non-cancerous tissue minus that of HCC tissue) with certain clinical parameters. We found that highly decreased expression of SLFN5 (ΔSLFN5 ≥ 100 or 150) was associated with higher pathological grade (P = 0.003), advanced TNM stage (P = 0.014) and larger

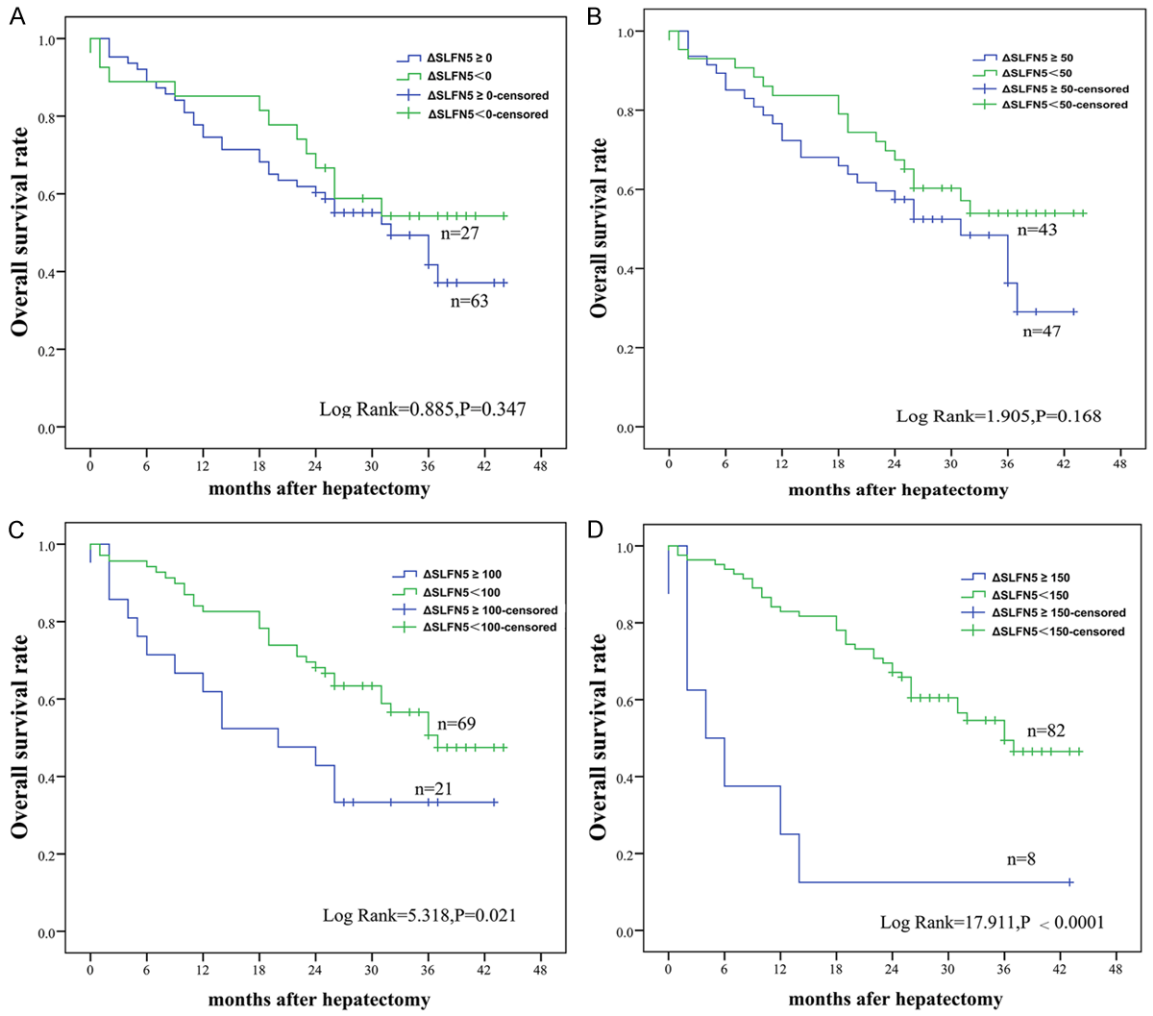

Figure 4. Kaplan-Meier overall survival analysis of the 90 HCC patients who underwent curative surgery according to different cut-off value of ΔSLFN5 (the vertical bar represented the cases censored). A. ΔSLFN5 ≥ 0; B. ΔSLFN5 ≥ 50; C. ΔSLFN5 ≥ 100; D. ΔSLFN5 ≥ 150.

tumor diameter (P = 0.017), strongly suggesting that SLFN5 might be involved in the carcinogenesis and progression of HCC.

Furthermore, we showed that in our group, a decrease in SLFN5 expression (ΔSLFN5 ≥ 100 or 150) was significantly associated with OS of patients with HCC. To support this, Kaplan-Meier analysis of OS was conducted showing that patients whose tumors had higher ΔSLFN5 (≥ 100 or 150) tend to have a significantly poorer OS, indicating that a high ΔSLFN5 level could be a marker of poor prognosis for patients with HCC. Moreover, the Cox proportional hazards model showed that ΔSLFN5 ≥ 150 was a marker of poor OS independent of the known clinical prognostic indicators such as tumor size, histological grade, and tumor stage. Therefore, SLFN5 could be a candidate for a molecular prognostic marker in these patients, which could contribute to the identification of patients with poor prognosis and those who need to be treated more aggressively.

However, this study has several limitations. Firstly, some clinical information was missing such as presence of HBV/HCV infection, alcohol consumption. Secondly, the follow-up time was not long enough.

In summary, our findings showed for the first time that SLFN5 is down-regulated both in mRNA and protein expression level in HCC. The low-expression of SLFN5 is closely related to poor histological grade, advanced TNM and larger tumor size. Although further studies are

Table 2. Cox regression analysis of the overall survival in 90 HCC cases

Variables	Univariate analysis[a]	Multivariate analysis	
	P value	*P* value	Hazards ratio (95% CI)
ΔSLFN5[b]			
NT-T ≥ 100 & NT-T < 100	0.021*	0.579	
NT-T ≥ 150 & NT-T < 150	< 0.001*	0.031*	4.101 (1.135-14.815)
AJCC tumor stage			
I & II & III	0.006*	0.208	
Pathological grade			
I & II & III	0.002*	0.083	
Adjacent non-tumor liver			
With cirrhosis & Without cirrhosis	0.004*	0.259	
Tumor size (cm)			
< 5 & 5~10 & ≥ 10	0.006*	0.617	
Age (years)			
< 60 & ≥ 60	0.944	0.63	
Gender			
Male & Female	0.077	0.014*	4.347 (1.392-13.575)
Tumor node			
Single & Multi	0.081	0.274	
Ki67			
Low & High	0.235	0.784	

*Statistically significant; a: Univariate Analysis was performed using Kaplan-Meier analysis; b: ΔSLFN5: the decrease in SLFN5 expression in T compared with self-paired NT.

needed, low-expression of SLFN5 may be used as a novel biomarker for HCC prognosis. Future studies are needed to search for the molecular mechanism of SLFN5 in tumorigenesis and metastasis of HCC. With regard to the special relationship between SLFN5 and interferon, more studies are necessary to identify whether SLFN5 could predict the effect of interferon treatment.

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Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] El-Serag HB and Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999; 340: 745-750.

[3] Tsochatzis E, Meyer T, O'Beirne J and Burroughs AK. Transarterial chemoembolisation is not superior to embolisation alone: the recent European Association for the Study of the Liver (EASL)-European Organisation for Research and Treatment of Cancer (EORTC) guidelines. Eur J Cancer 2013; 49: 1509-1510.

[4] Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, Bannon F, Ahn JV, Johnson CJ, Bonaventure A, Marcos-Gragera R, Stiller C, Azevedo e Silva G, Chen WQ, Ogunbiyi OJ, Rachet B, Soeberg MJ, You H, Matsuda T, Bielska-Lasota M, Storm H, Tucker TC, Coleman MP; CONCORD Working Group. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). Lancet 2015; 385: 977-1010.

[5] Mavrommatis E, Fish EN and Platanias LC. The schlafen family of proteins and their regulation by interferons. J Interferon Cytokine Res 2013; 33: 206-210.

[6] Geserick P, Kaiser F, Klemm U, Kaufmann SH and Zerrahn J. Modulation of T cell development and activation by novel members of the Schlafen (slfn) gene family harbouring an RNA

helicase-like motif. Int Immunol 2004; 16: 1535-1548.
[7] Neumann B, Zhao L, Murphy K and Gonda TJ. Subcellular localization of the Schlafen protein family. Biochem Biophys Res Commun 2008; 370: 62-66.
[8] Kovalenko PL and Basson MD. Schlafen 12 expression modulates prostate cancer cell differentiation. J Surg Res 2014; 190: 177-184.
[9] Mavrommatis E, Arslan AD, Sassano A, Hua Y, Kroczynska B and Platanias LC. Expression and regulatory effects of murine Schlafen (Slfn) genes in malignant melanoma and renal cell carcinoma. J Biol Chem 2013; 288: 33006-33015.
[10] Oh PS, Patel VB, Sanders MA, Kanwar SS, Yu Y, Nautiyal J, Patel BB and Majumdar AP. Schlafen-3 decreases cancer stem cell marker expression and autocrine/juxtacrine signaling in FOLFOX-resistant colon cancer cells. Am J Physiol Gastrointest Liver Physiol 2011; 301: G347-355.
[11] Sassano A, Mavrommatis E, Arslan AD, Kroczynska B, Beauchamp EM, Khuon S, Chew TL, Green KJ, Munshi HG, Verma AK and Platanias LC. Human Schlafen 5 (SLFN5) Is a Regulator of Motility and Invasiveness of Renal Cell Carcinoma Cells. Mol Cell Biol 2015; 35: 2684-2698.
[12] Katsoulidis E, Mavrommatis E, Woodard J, Shields MA, Sassano A, Carayol N, Sawicki KT, Munshi HG and Platanias LC. Role of interferon {alpha} (IFN{alpha})-inducible Schlafen-5 in regulation of anchorage-independent growth and invasion of malignant melanoma cells. J Biol Chem 2010; 285: 40333-40341.
[13] Zoppoli G, Regairaz M, Leo E, Reinhold WC, Varma S, Ballestrero A, Doroshow JH and Pommier Y. Putative DNA/RNA helicase Schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents. Proc Natl Acad Sci U S A 2012; 109: 15030-15035.
[14] Tian L, Song S, Liu X, Wang Y, Xu X, Hu Y and Xu J. Schlafen-11 sensitizes colorectal carcinoma cells to irinotecan. Anticancer Drugs 2014; 25: 1175-1181.
[15] Kang MH, Wang J, Makena MR, Lee JS, Paz N, Hall CP, Song MM, Calderon RI, Cruz RE, Hindle A, Ko W, Fitzgerald JB, Drummond DC, Triche TJ and Reynolds CP. Activity of MM-398, nanoliposomal irinotecan (nal-IRI), in Ewing's family tumor xenografts is associated with high exposure of tumor to drug and high SLFN11 expression. Clin Cancer Res 2015; 21: 1139-1150.
[16] Tang SW, Bilke S, Cao L, Murai J, Sousa FG, Yamade M, Rajapakse V, Varma S, Helman LJ, Khan J, Meltzer PS and Pommier Y. SLFN11 is a transcriptional target of EWS-FLI1 and a determinant of drug response in Ewing's sarcoma. Clin Cancer Res 2015; 21: 4184-93.
[17] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.
[18] Yatim A, Benne C, Sobhian B, Laurent-Chabalier S, Deas O, Judde JG, Lelievre JD, Levy Y and Benkirane M. NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function. Mol Cell 2012; 48: 445-458.
[19] Huntzicker EG, Hotzel K, Choy L, Che L, Ross J, Pau G, Sharma N, Siebel CW, Chen X and French DM. Differential effects of targeting Notch receptors in a mouse model of liver cancer. Hepatology 2015; 61: 942-952.
[20] Villanueva A, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, Rodriguez-Carunchio L, Sole M, Thung S, Stanger BZ and Llovet JM. Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. Gastroenterology 2012; 143: 1660-1669, e1667.