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ORIGINAL ARTICLE

The tumor suppressor role of Src homology phosphotyrosine phosphatase 2 in hepatocellular carcinoma

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Abstract
Purpose The human gene PTPN11, which encodes the non-receptor protein tyrosine phosphatase of Src homology phosphotyrosine phosphatase 2 (Shp2), has been previously well interpreted as a proto-oncogene in a variety of malignancies. However, the tumor suppressor role of Shp2 has also been reported. The present study was conducted to investigate the role of Shp2 expression and its associated clinical manifestations in hepatocellular carcinoma (HCC).
Methods A tissue microarray of 333 pairs of HCC and self-matched adjacent non-tumor tissues was constructed, and the expression of Shp2 was determined by immunohistochemistry. The results were also conformed by Western blotting and quantitative PCR of 31 self-paired fresh HCC specimens. The associations of Shp2 expression with 25 clinicopathologic features were analyzed. Overall survival analysis and multivariate analysis were performed.
Results Significantly decreased Shp2 expression in tumor tissues (T) compared with adjacent non-tumor tissues (NT) could be detected, and the positive rate was 66.1 and 96.7%, respectively. We combined the T and NT Shp2 immunoreactivity by a variable of the decrease in Shp2 expression (ΔShp2) and divided cases into 2 groups: $T < NT$ and $T \geq NT$. Survival analysis showed both low Shp2 expression and $T < NT$ group were significantly associated with short overall survival. Multivariate analysis showed ΔShp2 was an independent prognostic marker ($P = 0.033$; HR: 0.527; 95% CI: 0.293–0.950).
Conclusion Shp2 is a tumor suppressor, and the decrease in Shp2 expression was a new prognostic marker in HCC. The oncogenic role of Shp2 was tissue specific, and the therapeutic target of human gene PTPN11 should be reconsidered.

Keywords Hepatocellular carcinoma · Src homology phosphotyrosine phosphatase 2 · PTPN11 · Tissue microarray · Immunohistochemistry

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Introduction

Hepatocellular carcinoma (HCC) is among the most common human cancers (Bard-Chapeau et al. 2011). The incidence and mortality of HCC may vary considerably among racial and ethnic groups. Asians, particularly the Chinese, have a high risk of HCC development (Xu et al. 2009). The poor prognosis of HCC could be mostly attributed to the multicentric development and metastatic nature of the disease, identification of oncogenes, and tumor suppressors have contributed greatly to the rapid advance in the oncogenesis of HCC (Bard-Chapeau et al. 2011; Xu et al. 2009).

Coordinately controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), the phosphorylation of tyrosine residues is a feature of many important signaling pathways that are involved in cell proliferation, adhesion, and migration (Kim et al. 2010). Naturally, PTPs function as tumor suppressors, and genetic data have implicated the inactivation or loss-of-function mutations of PTP genes in human cancer pathogenesis (Bard-Chapeau et al. 2011). Surprisingly, the human gene PTPN11, which encodes the non-receptor PTP Src homology phosphotyrosine phosphatase 2 (Shp2), has previously been reported as a proto-oncogene (Bard-Chapeau et al. 2011). Indeed, somatic missense gain-of-function mutations of PTPN11 gene have been reported in 50% of Noonan syndromes and certain types of leukemias, and the hyperactivation of the Ras/Erk pathway has been induced (Chan and Feng 2007; Kim et al. 2010; Keilhack et al. 2005). Although the frequency of PTPN11 mutations was much lower in human solid tumors, the oncogenic role of Shp2 was reported, such as in breast cancer (Zhou and Agazie 2008), gastric cancer (Matozaki et al. 2009; Kim et al. 2010), and lung cancer (Grinnell et al. 2010). However, the role of wild-type Shp2 in cancer progression might be more tissue specific as its tumor suppressor role was reported in liver cancer cell lines which due to the down-regulation of inflammatory signaling (Bard-Chapeau et al. 2011) as well as in colorectal cancer (Yu et al. 2011). Elucidation of the tissue-specific role of Shp2 may provide new insights into the human oncogenesis and provide novel therapeutic targets of human cancers (Chan et al. 2008).

Despite the molecular mechanisms, to the best of our knowledge the clinical significance of Shp2 in human HCC has not been explored. The present study was performed to investigate the Shp2 expression in a tissue microarray (TMA) of 333 HCC specimens by immunohistochemistry and analyze its associated clinical manifestations. Here, we found that Shp2 was a tumor suppressor and that the decrease in Shp2 expression was a new prognostic marker in HCC, which is in stark contrast to previous studies, indicating the role of promoting carcinogenesis in other cancers.

Patients and methods

Patients and clinical manifestations

A retrospective cohort of HCC cases who underwent initial curative hepatectomy at the Chinese PLA General Hospital (Beijing, China) between 2000 and 2007 were retrieved. The curative hepatectomy was defined as a surgery in which all tumors, peripheral non-tumor (NT) tissues within 2 cm, and probable metastatic lymph nodes were macroscopically resected. The formalin-fixed and paraffin-embedded tumor specimens were obtained from the Pathology Department, and all the diagnoses of HCC were pathologically reconfirmed. The exclusion criteria were preoperative chemotherapy or radiotherapy, hepatic transplantation before or after surgery, no tumor tissue or matched adjacent NT tissue available, cases lost to follow-up or died from other causes, and individuals unwilling to be involved. Eventually 333 cases were included, including 297 men and 36 women with a mean age of 51.8 ± 10.5 years (range: 17–77 years). The median follow-up period was 56.0 months (mean 63.0 months, 38–134 months), and 180 (54.1%) patients had died during the follow-up. We also collected 31 self-paired fresh HCC surgical specimens (adjacent NT tissues were collected at least 2 cm from the tumor edge). The specimens were processed immediately after surgery and stored below −80°C before the following protein and total RNA extraction. The study was approved by the local Institutional Review Board of the PLA General Hospital. The informed consent was obtained from all patients or their relatives.

Clinicopathological information for all cases was retrieved from the Clinical Database of our hospital (summarized in Table 1). We defined the tumor size as the maximum tumor diameter measured on surgical specimens. The number of tumor nodules, lymph nodes metastasis, peplos infiltration, satellite tumor nodules, and venous infiltration was assessed by the combination of radiological examinations, intraoperative findings, and histopathological findings. Peplos infiltration was defined as the tumor peplos that was infiltrated by more than 2/3 in depth. Tumor differentiation grades were determined according to the World Health Organization International Histological Classification of Tumors. Tumor stage was graded according to the American Joint Committee on Cancer (AJCC) (Fleming et al. 1997). The preoperative local infiltration and metastasis were defined as the presence at least one of the following events: intra-hepatic metastasis, local lymphatic metastasis, and adjacent tissue infiltration. The overall survival time was measured from the hepatectomy to death.

TMA construction and immunohistochemistry staining

Immunohistochemistry staining of Shp2 was performed on the same paraffin-embedded tissue blocks used for clinical diagnosis of the expression of Ki-67, P53, DNA topoisomerase II (Top II), vascular endothelial growth factor (VEGF), P16, P170, and human epidermal growth factor receptor 1 and 2 (HER1 and HER2). The representative area was carefully selected according to the hematoxylin-eosin-stained slide (both tumor and adjacent NT areas; NT area was defined as at least 1 cm from the tumor edge).

Table 1 Clinical correlation of Shp2 expression in HCC

	Shp2 expression in tumor tissues				ΔShp2 expression[a]			
	T < 0.8 N = 271 (81.4%)	T ≥ 0.8 N = 62 (18.6%)	Total N = 333 (100%)	P valua[b]	T < NT N = 235 (70.6%)	T ≥ NT N = 98 (29.4%)	Total N = 333 (100%)	P value[b]
Age (mean ± SD), years								
	51.9 ± 10.7	51.3 ± 9.6	51.8 ± 10.5	0.679	51.5 ± 10.9	52.6 ± 9.5	51.8 ± 10.5	0.366
Gender								
Male	236 (79.5%)	61 (20.5%)	297	0.010*	208 (70%)	89 (30%)	297	0.537
Female	35 (97.2%)	1 (2.8%)	36		27 (75%)	9 (25%)	36	
Hepatitis B								
Absent	49 (87.5%)	7 (12.5%)	56	0.193	39 (69.6%)	17 (30.4%)	56	0.88
Present	221 (80.1%)	55 (19.9%)	276		195 (70.7%)	81 (29.3%)	276	
Hepatitis C								
Absent	261 (81.6%)	59 (18.4%)	320	0.567	230 (71.9%)	90 (28.1%)	320	0.004*
Present	9 (75%)	3 (25%)	12		4 (33.3%)	8 (66.7%)	12	
Serum AFP level, ng/mL								
<400	163 (78%)	46 (22%)	209	0.06	142 (67.9%)	67 (32.1%)	209	0.227
≥400	92 (86.8%)	14 (13.2%)	106		79 (74.5%)	27 (25.5%)	106	
Median tumor size (25–75%), cm[c]								
	5.5 (3.5–8.0)	3.5 (2.5–7.0)	5.0 (3.2–8.0)	0.001*	6.0 (3.5–8.5)	4.0 (2.5–6.1)	5.0 (3.2–8.0)	<0.001*
Tumor nodules								
Single	219 (79.6%)	56 (20.4%)	275	0.075	187 (68%)	88 (32%)	275	0.025*
Multi	52 (89.7%)	6 (10.3%)	58		48 (82.8%)	10 (17.2%)	58	
Venous infiltration[d]								
Absent	131 (77.5%)	38 (22.5%)	169	0.066	105 (62.1%)	64 (37.9%)	169	0.001*
Present	140 (85.4%)	24 (14.6%)	164		130 (79.3%)	34 (20.7%)	164	
Preoperative local infiltration and metastasis[e]								
Absent	180 (78.9%)	48 (21.1%)	228	0.093	145 (63.6%)	83 (36.4%)	228	<0.001*
Present	91 (86.7%)	14 (13.3%)	105		90 (85.7%)	15 (14.3%)	105	
Cellular differentiation[f]								
Well	25 (75.8%)	8 (24.2%)	33	0.006*	16 (48.5%)	17 (51.5%)	33	<0.001*
Moderate	199 (79%)	53 (21%)	252		176 (69.8%)	76 (30.2%)	252	
Poor	47 (97.9%)	1 (2.1%)	48		43 (89.6%)	5 (10.4%)	48	
AJCC tumor stage								
I	9 (69.2%)	4 (30.8%)	13	0.004*	4 (30.8%)	9 (69.2%)	13	<0.001*
II	96 (73.8%)	34 (26.2%)	130		76 (58.5%)	54 (41.5%)	130	
III	83 (83%)	17 (17%)	100		70 (70%)	30 (30%)	100	
IV	83 (92.2%)	7 (7.8%)	90		85 (94.4%)	5 (5.6%)	90	
Satellite tumor nodules								
Absent	204 (82.3%)	44 (17.7%)	248	0.483	169 (68.1%)	79 (31.9%)	248	0.097
Present	67 (78.8%)	18 (21.2%)	85		66 (77.6%)	19 (22.4%)	85	
Peplos infiltration								
No infiltration	80 (73.4%)	29 (26.6%)	109	0.025*	68 (62.4%)	41 (37.6%)	109	0.031*
Infiltration	116 (85.9%)	19 (14.1%)	135		105 (77.8%)	30 (22.2%)	135	
No peplos fromation	75 (85.2%)	13 (14.8%)	88		61 (69.3%)	27 (30.7%)	88	
Adjacent non-tumor liver status								
Noncirrhotic	7 (100%)	0	7	0.389	6 (85.7%)	1 (14.3%)	7	0.334
Chronic hepatitis	48 (78.7%)	13 (21.3%)	61		39 (63.9%)	22 (36.1%)	61	
Cirrhotic	215 (81.4%)	49 (18.6%)	264		189 (71.6%)	75 (28.4%)	264	

Table 1 continued

	Shp2 expression in tumor tissues				ΔShp2 expression[a]			
	T < 0.8 N = 271 (81.4%)	T ≥ 0.8 N = 62 (18.6%)	Total N = 333 (100%)	P valua[b]	T < NT N = 235 (70.6%)	T ≥ NT N = 98 (29.4%)	Total N = 333 (100%)	P value[b]
Peripheral inflammation								
Absent	7 (100%)	0	7	0.073	6 (85.7%)	1 (14.3%)	7	0.099
Mild	111 (86%)	18 (14%)	129		98 (76%)	31 (24%)	129	
Moderate	117 (79.6%)	30 (20.4%)	147		94 (63.9%)	53 (36.1%)	147	
Severe	35 (71.4%)	14 (28.6%)	49		37 (75.5%)	12 (24.5%)	49	
Peripheral fatty degeneration								
Absent	141 (83.4%)	28 (16.6%)	169	0.705	128 (75.7%)	41 (24.3%)	169	0.024*
Mild	70 (77.8%)	20 (22.2%)	90		65 (72.2%)	25 (27.8%)	90	
Moderate	30 (78.9%)	8 (21.1%)	38		20 (52.6%)	18 (47.4%)	38	
Severe	28 (82.4%)	6 (17.6%)	34		21 (61.8%)	13 (38.2%)	34	
Ki67 index								
<0.4	98 (77.8%)	28 (22.2%)	126	0.059	84 (66.7%)	42 (33.3%)	126	0.608
≥0.4	68 (88.3%)	9 (11.7%)	77		54 (70.1%)	23 (29.9%)	77	
P53								
Negative	173 (82.4%)	37 (17.6%)	210	0.540	139 (66.2%)	71 (33.8%)	210	0.022*
Positive	98 (79.7%)	25 (20.3%)	123		96 (78%)	27 (22%)	123	
Mean survival time (95% CI), months[g]								
	59.6 (52.6–66.6)	98.9 (85.2–112.7)	67.1 (60.5–73.7)	<0.001*	59.0 (51.6–66.4)	86.6 (74.0–99.2)	67.1 (60.5–73.7)	<0.001*

Shp2 Src homology phosphotyrosine phosphatase 2, *HCC* hepatocellular carcinoma, *T* tumor tissue, *NT* adjacent non-tumor tissue, *AJCC* American Joint Committee on Cancer, *SD* standard deviation, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *AFP* α-fetoprotein, *CI* confidence interval

* Statistically significant

[a] ΔShp2: the decrease in Shp2 expression in T compared with self-paired NT. The two groups were divided according to whether they were decreased or not

[b] P value of age was calculated by the Fisher's exact test, of tumor size was by Kruskai–Wallis test, of survival time by log rank test, others by Pearson's chi-square test

[c] Tumor size was measured by the diameter of the largest tumor nodule

[d] Venous infiltration was defined based on the final histological examination

[e] Preoperative local infiltration and metastasis indicate preoperative intra-hepatic metastasis, local lymphatic metastasis, and adjacent tissue infiltration

[f] Cellular differentiation was based on World Health Organization International Histological Classification of Tumors

[g] Mean survival time was calculated instead of median survival time for the follow-up period was not long enough for T ≥ 0.8 and T ≥ NT groups

The TMA was constructed, and 4-μm sections were cut by regular histology procedures. One TMA was stained with hematoxylin and eosin for pathological reassessment. Immunohistochemistry of Shp2 was performed using the PV9000 method. After the TMA, slides were de-paraffinized and rehydrated, and epitope retrieval was performed in citrate buffer (pH 6.0) for 2.5 min at 120°C. After peroxidase blocking, the slides were incubated with 10% normal goat serum for 30 min, and then incubated with the primary antibody (Bioworld Technology, BS1705, diluted 1:75) overnight at 4°C. Slides were incubated with signal enhancer for 30 min and then incubated with horseradish-peroxidase (HRP)-conjugated secondary antibody (Zhongshan Technology, China) for 30 min. All above incubations were performed at room temperature, and slides were washed with phosphate-buffered saline. Sections were then incubated within 3,3′-diaminobenzidine substrate (Zhongshan Technology, China) and counterstained with hematoxylin. Negative control (the primary antibody was replaced by 10% normal goat serum) and positive control (breast carcinoma in which Shp2 was known to be highly expressed) were included.

The immunohistochemical staining of Shp2 was independently evaluated by two pathologists who were blinded to the clinical and follow-up information. The widely accepted *H*-score system (McClelland et al. 1990) that

considered both the staining intensity and the percentage of cells stained at the specific range of intensity was adapted. The *H*-score was calculated following the equation: *H*-score = $\sum(Pi \cdot i)$, where i is the intensity of the stained tumor cells (0 to 3+, no staining = 0, weak staining = 1+, moderate staining = 2+ and strong staining = 3+), and Pi is the percentage of stained tumor cells for each intensity varying from 0 to 100%. The *H*-score ranged from 0 (the minimum score) to 3 (the maximum score), and we interpreted tumors ≤ 0.3 as negative (−).

Western blotting

Frozen tissue samples were lysed in RIPA buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS). Shp2 antibody (Bioworld Technology, BS1705, diluted 1:1,000) was used, and anti-human GAPDH antibody (CoWin Biotech, CW0266, diluted 1:1,000) was used as internal control. The Western procedure and semiquantitative analysis methods are detailed elsewhere (Xu et al. 2009).

Quantitative RT-PCR

Total RNA was extracted from frozen tissue using TriZol reagent (Invitrogen) following the manufacturer's instructions. RNA quality was assessed by electrophoresis on a denaturing agarose gel (MOPS). There were 19 self-paired specimens that could be considered of intact total RNA (18S and 28S ribosomal RNA bands are clearly visible), and quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed. 500 ng of total RNA was subjected to reverse transcription to synthesize cDNA using the ProtoScript M-MuLV Taq RT-PCR Kit (New England Biolabs), according to the manufacture's instruction, followed by real-time PCR using the TransStart Green qPCR SuperMix (TransGen Biotech). The primers of Shp2 was detailed in PrimerBank (ID: 33356176b1).The transcription of GAPDH was used as an internal control for normalization (Li et al. 2008).

Statistical analysis

The association of Shp2 expression with clinical manifestations was analyzed by the Pearson's chi-square test, Kruskai–Wallis test, Mann–Whitney test, or Fisher's exact test. Overall survival analysis was performed using the Kaplan–Meier method with log rank tests. All variables of $P < 0.10$ in the univariate survival analysis were included in multivariate analysis using the Cox proportional hazards model (Back Wald method with $P < 0.05$ included and $P > 0.10$ excluded). Overall survival times, 95% confidence interval (CI), and hazard ratio (HR) are presented. All analyses were performed with SPSS for Windows Version 17.0 (SPSS Inc, Chicago, Ill), and two-sided P values of <0.05 were considered of statistically significance.

Results

Decreased Shp2 expression in HCC and the associated clinical manifestations

To evaluate the prevalence and clinical manifestations of Shp2 in HCC, immunohistochemistry was performed on a TMA of 333 self-paired HCC specimens. Presented in Fig. 1a and b, the immunostaining of Shp2 was cytoplasmic, and negative control showed no signal (data not shown). According to the *H*-score system, the positive rate of Shp2 expression (score > 0.3) in HCC tumor tissues was 66.1% (220/333), and in adjacent, NT was 96.7% (322/333). Presented in Fig. 1c, significantly low Shp2 expression in T could be detected (T: 0.52 ± 0.37; NT: 0.97 ± 0.40, $P < 0.001$; 95% CI: −0.49 to −0.39, Paired-*t* test). We graded the immunoreactivity of Shp2 expression into two groups: <0.8 as low expression (271 in T and 121 in NT) and ≥0.8 as high expression (62 in T and 212 in NT). To further detect the clinical significance of Shp2 expression, we combined the self-paired T and NT immunoreactivity by a calculated variable of ΔShp2 (the decrease in Shp2 expression in T compared with self-paired NT). According to whether there were decreased Shp2 expression, cases were divided into 2 groups: T < NT (T-NT < −0.20, 235 cases, 70.6%) and T ≥ NT (T-NT ≥ −0.20, 98 cases, 29.4%). The representative immunohistochemical photomicrographs of the 2 ΔShp2 groups were presented in Fig. 1a and b. The decreased expression of Shp2 in T compared with NT was also confirmed by Western blotting of 31 self-paired fresh HCC specimens (Fig. 2a, b), as well as adjacent significantly decreased mRNA level by real-time PCR considering that there were only 19 pairs of specimen performed (Fig. 2c).

Twenty-five clinicopathological variables were evaluated of the association with Shp2 expression. The results of 19 variables are presented in Table 1, data not shown for the remaining 6 immunohistochemical variables that were of no significant associations, including the expression of Top II, VEGF, HER1, HER2, P16, and P170. Analyzed by Pearson chi-square test, the low Shp2 expression in HCC tumor tissues was significantly associated with female gender ($P = 0.010$), large tumor size ($P = 0.001$), poor cellular differentiation ($P = 0.006$), high AJCC tumor stage ($P = 0.004$), and peplos infiltration ($P = 0.025$). ΔShp2 was associated with hepatitis C infection ($P = 0.004$), tumor size ($P < 0.001$), number of tumor nodules ($P = 0.025$), venous infiltration ($P = 0.001$), preoperative local infiltration and

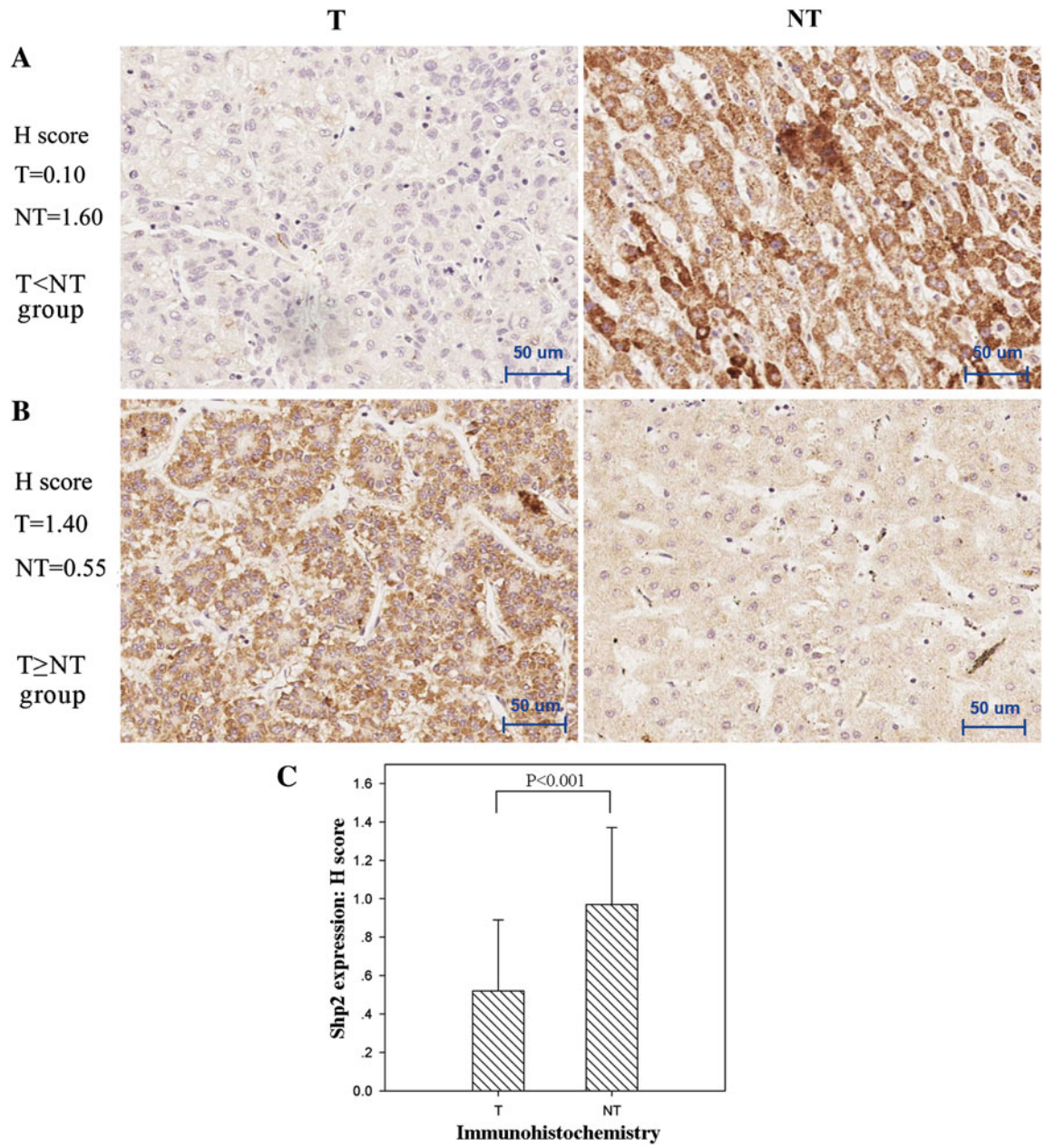

Fig. 1 Decreased Shp2 expression in hepatocellular carcinoma (HCC) was detected by immunohistochemistry staining using a tissue microarray of 333 pairs of HCC tumor tissues (T) and self-paired adjacent non-tumor tissues (NT). The immunostaining was located in cytoplasm and was graded by the *H*-score system. **a** Representative immunohistochemical photomicrographs of the T < NT group (decreased Shp2 expression in T compared with self-paired NT): total 235 cases. **b** Representative immunohistochemical photomicrographs of the T ≥ NT group (without decreased Shp2 expression in T compared with self-paired NT): total 98 cases. **c** The lower Shp2 expression in T compared with NT was detected by immunohistochemistry of the 333 HCC cases. Means, standard deviation (SD), and *P* value were given (*T* test)

metastasis ($P < 0.001$), cellular differentiation ($P < 0.001$), AJCC tumor stage ($P < 0.001$), peplos infiltration ($P = 0.031$), peripheral fatty degeneration ($P = 0.024$), and P53 expression ($P = 0.022$). Furthermore, although with less clinical significance, decreased Shp2 expression in NT (data not shown) was associated with female gender ($P = 0.020$), high serum AFP level ($P < 0.001$), and high P16 expression ($P < 0.001$).

Low and the decrease Shp2 expression was associated with short overall survival

To evaluate the prognostic significance of Shp2 expression, overall survival analysis of the 333 HCC cases was conducted using the Kaplan–Meier method. Presented in Fig. 3a, low Shp2 expression in T was significantly associated with short overall survival time (log rank = 17.7,

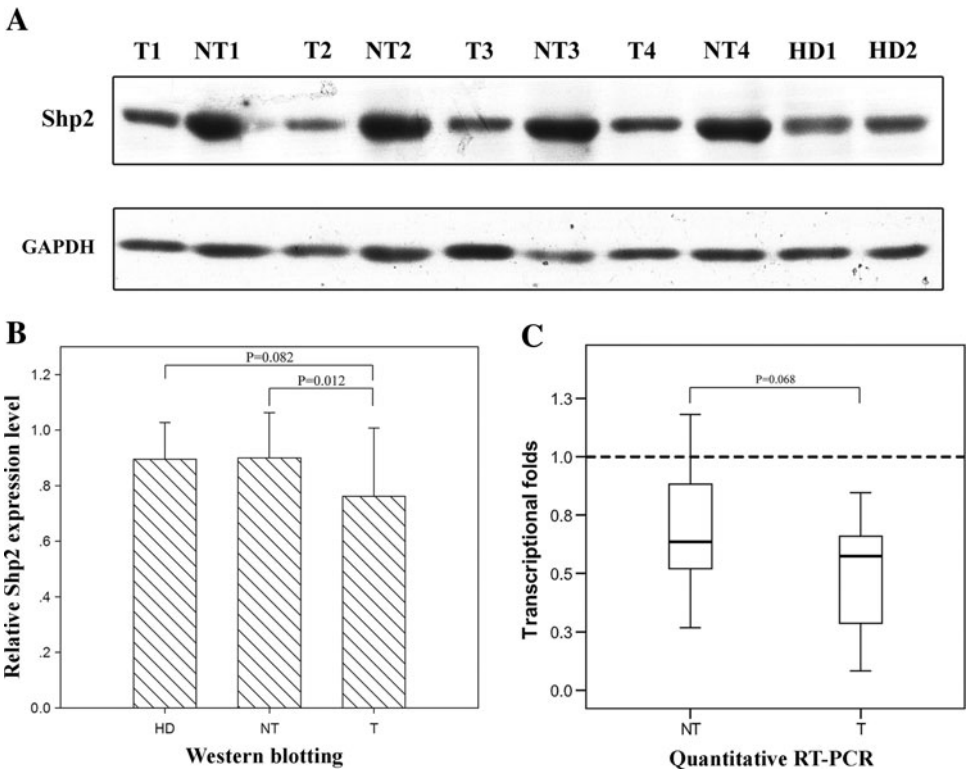

Fig. 2 Decreased Shp2 protein and mRNA levels in fresh hepatocellular carcinoma (HCC) specimens. **a** Representative Western blotting of Shp2 protein level in HCC tissues (T), self-paired adjacent non-tumor tissue (NT), and healthy donors (HD). **b** Semiquantitative Western blotting of 31 self-paired HCC samples showed significantly decreased Shp2 protein level in T compared with NT. GAPDH was used as internal control. Means, standard deviation (SD), and *P* values were given (*T* test). **c** Adjacent significantly decreased Shp2 mRNA level ($P = 0.068$, Mann–Whitney test) in T compared with NT was detected by real-time PCR of 19 self-paired HCC samples. GAPDH was used as internal control. The transcriptional fold change was calculated using liver samples from 5 healthy donors as a reference (*horizontal lines* represent the median; the *bottom* and *top* of the *boxes* represent the 25th and 75th percentiles, respectively; the *vertical bars* represent the range of data)

$P < 0.001$). The 5-year survival rate for low and high Shp2 cases was 42.6 and 70.1%, respectively (mean survival time was presented in Table 1). No prognostic significance of Shp2 expression in NT could be detected (Fig. 3b). The role of the low Shp2 expression in T and NT was further evaluated, and cases were divided into 4 subgroups (Fig. 3c). For the 2 subgroups of $T < 0.8$ ($NT < 0.8$ or not), surprisingly adjacent significance could be detected favoring the $NT < 0.8$ subgroup (log rank = 2.914; $P = 0.088$; figure not shown). By the calculated variable of ΔShp2, the role of Shp2 as a tumor suppressor could be detected (log rank = 15.7; $P = 0.001$; Fig. 3d). The 5-year survival rate for $T < N$ and $T \geq NT$ cases was 39.7 and 67.3%, respectively (mean survival time was presented in Table 1).

Multivariate analysis and the decrease in Shp2 expression was an independent prognostic marker in HCC

To determine the independent prognostic marker of HCC, all variables of $P < 0.10$ in univariate survival analysis were included in the Cox regression model. Presented in Table 2, of the 13 variables included, 6 were identified as independent prognostic markers, including ΔShp2 expression ($P = 0.033$; HR: 0.527; 95% CI: 0.293–0.950), tumor size, tumor nodules, preoperative local infiltration and metastasis, peripheral inflammation, and Ki67 index. Moreover, even if ΔShp2 was excluded from the Cox model, the T Shp2 alone still could not be an independent prognostic marker. The independent prognostic role of ΔShp2 could also be identified by the subgroup analysis using Kaplan–Meier method that the tumor suppressor role of ΔShp2 was independent of the variables ΔShp2 or Shp2 expression related to (data not shown). On account of the above data, we concluded that the decrease in Shp2 expression (ΔShp2) could be a better prognostic marker than Shp2 expression in T alone.

Discussion

Reversible tyrosine phosphorylation, which is governed by the balance of PTKs and PTPs, regulates important

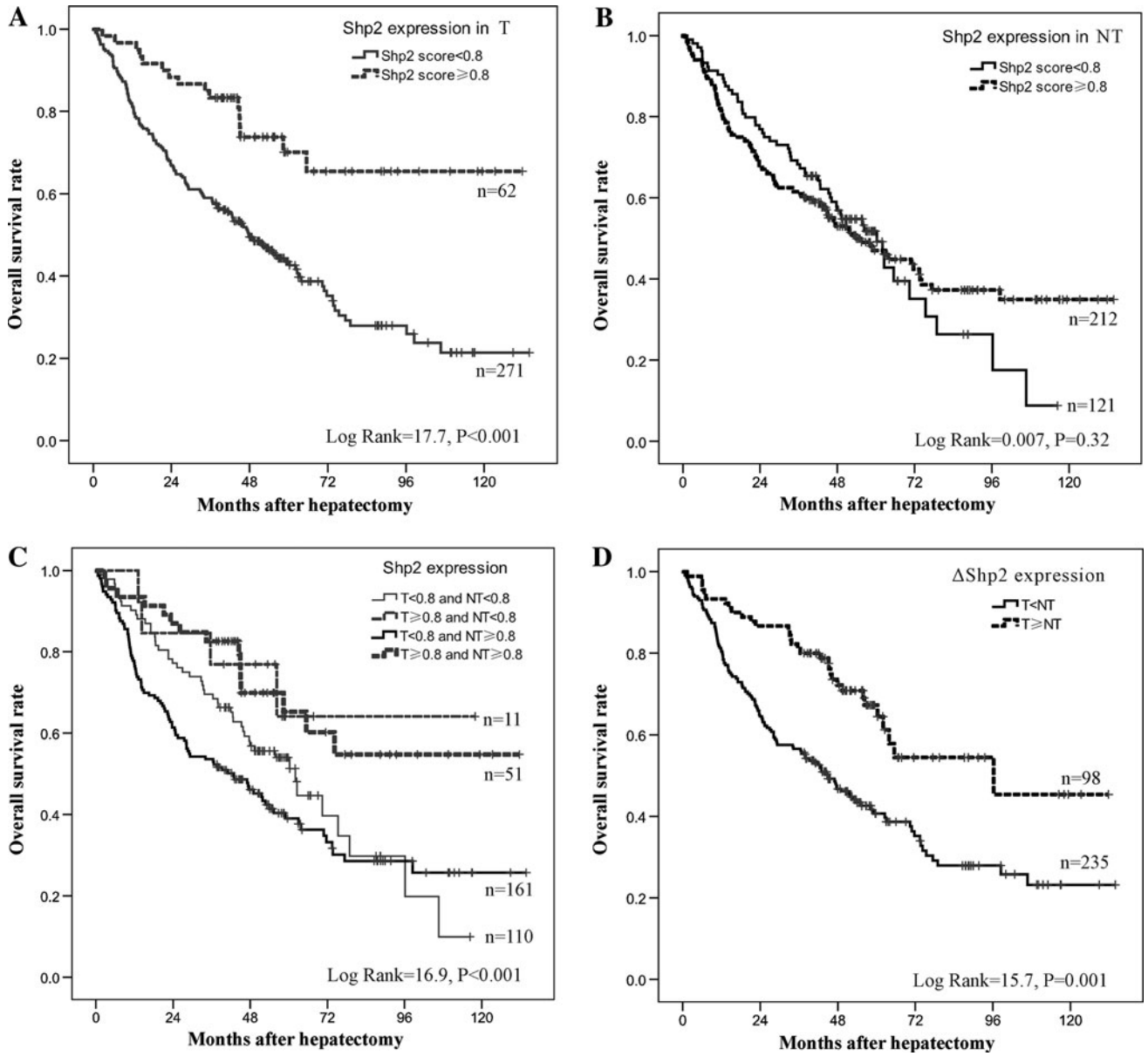

Fig. 3 Kaplan–Meier overall survival analysis of the 333 hepatocellular carcinomas underwent curative surgery (the *vertical bar* represented the cases censored). **a** Divided into 2 groups according to the Shp2 immunoreactivity in tumor tissues (T), low Shp2 expression was associated with poor overall survival. **b** Divided into 2 groups according to the Shp2 immunoreactivity in adjacent non-tumor tissues (NT), no prognostic difference could be detected. **c** Divided into 4 groups according to Shp2 immunoreactivity in both T and NT, decreased Shp2 expression in T was associated with poor prognosis in both the 2 NT subgroups. **d** Divided into 2 groups according to the decrease in Shp2 expression in T compared with self-paired NT (ΔShp2), ΔShp2 was significantly associated with poor overall survival

signaling pathways that are involved in cell proliferation, adhesion, and migration (Ostman et al. 2006). To further characterize the clinical presentations and manifestations of Shp2 expression in HCC, we examined its expression in a HCC cohort of 333 cases in China, where hepatitis B is endemic and has a high prevalence of liver cancer (Xu et al. 2009). With the immunohistochemical approach using a TMA of 333 pairs of HCC tissues and self-matched adjacent NT tissues, our results showed significantly lower Shp2 expression in T compared with NT ($P < 0.001$), and the positive rate was 66.1 and 96.7%, respectively. The results were confirmed by Western blotting of 31 self-paired fresh HCC specimens ($P = 0.012$) and quantitative PCR of 19 self-paired specimens ($P = 0.068$). According to whether there were decreased Shp2 expression in T compared with self-paired NT or not (ΔShp2), we divided the 333 cases into 2 groups: T < NT and T ≥ NT. Survival analysis showed both the low Shp2 expression and T < NT

Table 2 Cox regression analysis of the overall survival in 333 HCC cases

Variables	Univariate analysis[a]	Multivariate analysis	
	P value	*P* value	Hazards ratio (95% CI)
ΔShp2 expression (T < NT or T ≥ NT)[b]	<0.001	0.033*	0.527 (0.293–0.950)
Tumor size (small, moderate or large)	<0.001	0.034*	1.419 (1.026–1.960)
Tumor nodules (single or multi)	<0.001	0.008*	2.104 (1.213–3.650)
Preoperative local infiltration and metastasis (absent or present)[c]	<0.001	0.001*	2.477 (1.475–4.159)
Peripheral inflammation (absent, mild, moderate or severe)	0.099	0.038*	1.382 (1.018–1.875)
Ki67 index (low or high)	0.016	0.015*	1.822 (1.123–2.958)
Shp2 expression in tumor (low or high)	<0.001	0.463	
Serum AFP level (<400 or ≥400 ng/mL)	0.035	0.899	
AJCC tumor stage (I, II, III, IV)	<0.001	0.944	
Cellular differentiation (well, moderate or poor)	0.001	0.666	
Venous infiltration (absent or present)	<0.001	0.688	
Peplos infiltration (absent, present or no peplos formation)	0.004	0.436	
HER1 expression (negative or positive)	0.068	0.259	

HCC hepatocellular carcinoma, *Shp2* Src homology phosphotyrosine phosphatase 2, *AJCC* American Joint Committee on Cancer, *AFP* α-fetoprotein, *CI* confidence interval, *HER1* human epidermal growth factor receptor 1

* Statistically significant

[a] Univariate Analysis was performed using Kaplan–Meier analysis

[b] ΔShp2: the decrease in Shp2 expression in T compared with self-paired NT. The two groups were divided according to whether they were decreased or not

[c] Preoperative local infiltration and metastasis indicate preoperative intra-hepatic metastasis, local lymphatic metastasis, and adjacent tissue infiltration

groups were significantly associated with short overall survival. Multivariate analysis by Cox regression model showed ΔShp2 was an independent prognostic marker in HCC ($P = 0.033$; HR: 0.527; 95% CI: 0.293–0.950). The decrease in Shp2 expression was significantly associated with hepatitis C states, tumor size, number of tumor nodules, venous infiltration, preoperative local infiltration and metastasis, cellular differentiation, AJCC tumor stage, peplos infiltration, peripheral fatty degeneration, and P53 expression. The 5-year survival rate for decreased Shp2 cases (T < N) was 39.7% and without decreased Shp2 cases (T ≥ NT) was 67.3%.

Since its discovery in the early 1990s, Shp2 has been established as a critical contributor to evolutionary conserved pathways, mostly are the downstream of PTKs and upstream of the Ras/Erk pathway (Chan et al. 2008). It appears to play an important role in regulating cell proliferation, differentiation, and migration (Miyamoto et al. 2008; Keilhack et al. 2005). Somatic missense PTPN11 gain-of-function mutations have been detected in 50% of Noonan syndromes and certain types of leukemias (Chan and Feng 2007; Kim et al. 2010; Keilhack et al. 2005). The frequency of PTPN11 mutations was much lower in human solid tumors (Chan et al. 2008; Ostman et al. 2006). Only one case of liver cancer was reported of PTPN11 mutation, and the T507 K Shp2 mutant showed altered substrate specificity and oncogenic Ras-like transforming activity (Miyamoto et al. 2008). However, the role of wild-type Shp2 in cancer progression was more tissue specific (Chan et al. 2008). The oncogenic role of Shp2 was reported in breast cancer due to the abundance of Gab-2 (Zhou and Agazie 2008), in gastric cancer associated with CagA-positive Helicobacter pylori (Matozaki et al. 2009; Kim et al. 2010) and in lung cancer might be associated with the pulmonary barrier function (Grinnell et al. 2010). The tumor suppressor role of Shp2 was also reported, such as in live cancer cell lines (Bard-Chapeau et al. 2011) and colorectal cancer (Yu et al. 2011). The results we presented here also suggested a tumor-inhibiting effect of Shp2 in HCC. Given that Shp2 functions as the downstream of many tyrosine kinases with transforming potential, biochemical, functional, and clinical studies of wild-type Shp2 could provide new insight into the pathogenesis of cancer as well as potential new targets for cancer treatment (Matozaki et al. 2009; Ostman et al. 2006). Further tissue- or event-specific signaling functions of Shp2 are sure to be uncovered and much still remains to be studied concerning the versatile protein.

In contrast to the leukemogenic effect of dominant-active mutants, the molecular mechanism of the tumor suppressor function of Shp2 in liver cancer was recently explored by Bard-Chapeau (Bard-Chapeau et al. 2011) and

found that hepatocyte-specific deletion of Shp2 would promote inflammatory signaling through the STAT3 pathway and hepatic inflammation/necrosis, resulting in regenerative hyperplasia and development of tumors in aged mice. Furthermore, Shp2 ablation could dramatically enhance the diethylnitrosamine-induced HCC development (Bard-Chapeau et al. 2011). However, in the present study, we identified no correlation of Shp2 expression with peripheral inflammation status and identified no Shp2-related survival changes due to the different peripheral inflammation status. In our work, all the Shp2 staining was cytoplasmic. Interestingly, the nuclear Shp2 was also reported in B cells although was much lower compared with the cytoplasm (Fridberg et al. 2008).

To the best of our knowledge, the present study is the first clinical evaluation of Shp2 expression in HCC tissues. The only information of Shp2 expression in HCC was reported by Bard-Chapeau (Bard-Chapeau et al. 2011) who had identified dramatically decreased Shp2 protein levels in 12/104 HCC specimens compared with their surrounding tissues; however, no further clinical evaluation had been provided. The primary limitation of the present work was the retrospective nature with all the problems inherent in the methodology. Furthermore, no disease-free survival data were available, and it will be interesting to determine whether there are deletions or point mutations at the PTPN11/Shp2 locus in HCC genomes that could result in functional inactivation. As the 333 cases of the present study were mostly associated with HBV infections (80.2%), the results reported here should be further confirmed in other populations.

In conclusion, the present study provides the first clinical evidence that Shp2 is a tumor suppressor and that the decrease in Shp2 expression was an independent prognostic marker in HCC patients. Although human gene PTPN11 is previously well interpreted as a proto-oncogene, for the oncogenic role of Shp2 was tissue specific, the therapeutic target of human gene PTPN11 should be reconsidered.

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Conflict of interest We declare that we have no conflict of interest.

References

Bard-Chapeau EA, Li S, Ding J, Zhang SS, Zhu HH, Princen F, Fang DD, Han T, Bailly-Maitre B, Poli V, Varki NM, Wang H, Feng GS (2011) Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. Cancer Cell 19(5):629–639. doi:10.1016/j.ccr.2011.03.023

Chan RJ, Feng GS (2007) PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. Blood 109(3):862–867. doi:10.1182/blood-2006-07-028829

Chan G, Kalaitzidis D, Neel BG (2008) The tyrosine phosphatase Shp2 (PTPN11) in cancer. Cancer Metastasis Rev 27(2):179–192. doi:10.1007/s10555-008-9126-y

Fleming I, Cooper J, Henson D (1997) AJCC cancer staging manual, 5th edn. Lippincott-Raven, Philadelphia, pp 98–126

Fridberg M, Kjellstrom S, Anagnostaki L, Skogvall I, Mustelin T, Wiebe T, Persson JL, Dictor M, Wingren AG (2008) Immunohistochemical analyses of phosphatases in childhood B-cell lymphoma: lower expression of PTEN and HePTP and higher number of positive cells for nuclear SHP2 in B-cell lymphoma cases compared to controls. Pediatr Hematol Oncol 25(6):528–540. doi:10.1080/08880010802237054

Grinnell KL, Casserly B, Harrington EO (2010) Role of protein tyrosine phosphatase SHP2 in barrier function of pulmonary endothelium. Am J Physiol Lung Cell Mol Physiol 298(3):L361–L370. doi:10.1152/ajplung.00374.2009

Keilhack H, David FS, McGregor M, Cantley LC, Neel BG (2005) Diverse biochemical properties of Shp2 mutants. Implications for disease phenotypes. J Biol Chem 280(35):30984–30993. doi:10.1074/jbc.M504699200

Kim JS, Shin OR, Kim HK, Cho YS, An CH, Lim KW, Kim SS (2010) Overexpression of protein phosphatase non-receptor type 11 (PTPN11) in gastric carcinomas. Dig Dis Sci 55(6):1565–1569. doi:10.1007/s10620-009-0924-z

Li HY, Liu H, Wang CH, Zhang JY, Man JH, Gao YF, Zhang PJ, Li WH, Zhao J, Pan X, Zhou T, Gong WL, Li AL, Zhang XM (2008) Deactivation of the kinase IKK by CUEDC2 through recruitment of the phosphatase PP1. Nat Immunol 9(5):533–541. doi:10.1038/ni.1600

Matozaki T, Murata Y, Saito Y, Okazawa H, Ohnishi H (2009) Protein tyrosine phosphatase SHP-2: a proto-oncogene product that promotes Ras activation. Cancer Sci 100(10):1786–1793. doi:10.1111/j.1349-7006.2009.01257.x

McClelland RA, Finlay P, Walker KJ, Nicholson D, Robertson JF, Blamey RW, Nicholson RI (1990) Automated quantitation of immunocytochemically localized estrogen receptors in human breast cancer. Cancer Res 50(12):3545–3550

Miyamoto D, Miyamoto M, Takahashi A, Yomogita Y, Higashi H, Kondo S, Hatakeyama M (2008) Isolation of a distinct class of gain-of-function SHP-2 mutants with oncogenic RAS-like transforming activity from solid tumors. Oncogene 27(25):3508–3515. doi:10.1038/sj.onc.1211019

Ostman A, Hellberg C, Bohmer FD (2006) Protein-tyrosine phosphatases and cancer. Nat Rev Cancer 6(4):307–320. doi:10.1038/nrc1837

Xu MZ, Yao TJ, Lee NP, Ng IO, Chan YT, Zender L, Lowe SW, Poon RT, Luk JM (2009) Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. Cancer 115(19):4576–4585. doi:10.1002/cncr.24495

Yu SJ, Yu JK, Ge WT, Hu HG, Yuan Y, Zheng S (2011) SPARCL1, Shp2, MSH2, E-cadherin, p53, ADCY-2 and MAPK are prognosis-related in colorectal cancer. World J Gastroenterol 17(15):2028–2036. doi:10.3748/wjg.v17.i15.2028

Zhou XD, Agazie YM (2008) Inhibition of SHP2 leads to mesenchymal to epithelial transition in breast cancer cells. Cell Death Differ 15(6):988–996. doi:10.1038/cdd.2008.54