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Association of nuclear localization of SHP2 and YAP1 with unfavorable prognosis in non-small cell lung cancer

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ABSTRACT

Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) is ubiquitously expressed in cytoplasmic localization, which in turn confers tumor malignancy and poor prognosis in various human cancers. YAP1 interacts with SHP2 to promote translocation of SHP2 to nucleus, which consequently promotes Wnt target activation. However, the oncogenic role of the nuclear localization of SHP2 in human cancers remains unclear. We hypothesized that nuclear SHP2 localization, in combination with nuclear YAP1 expression, could be associated with poor overall survival (OS) and relapse free survival (RFS) due to an increase in cyclin D1 and c-Myc mRNA expression following activation of Wnt/ß-catenin signaling. Immunohistochemical analysis of SHP2 and YAP1 protein expression in 102 tumors resected from patients with NSCLC revealed that nuclear SHP2 expression was well correlated with nuclear YAP1 expression ($P < 0.001$). Evaluation of cyclin D1 and c-Myc mRNA levels by the real-time reverse-phase polymerase chain reaction (RT-PCR) revealed that patients with high cyclin D1 and high c-Myc mRNA expressing tumors more commonly showed high nuclear YAP1 and high nuclear SHP2 (high/high) rather than the high/low, low/high, or low/low combinations ($P < 0.001$ for cyclin D1 and c-Myc). Kaplan-Meier and Cox-regression models showed OS and RFS to be poorer in patients in the high/high subgroup than in the low/low subgroup (OS: HR = 2.85, 95% CI, 1.52–5.35, $P = 0.001$; RFS: HR = 2.55, 95% CI, 1.37–4.72, $P = 0.003$). No prognostic significance was observed for the other two subgroups (low/high and high/low) when compared to the low/low subgroup in this study population. Therefore, we suggest that the prognostic value of SHP2 could reflect the nuclear localization of SHP2 and its interaction with nuclear YAP1, which led to subsequent upregulation of cyclin D1 and c-Myc mRNA expression via activation of the Wnt/ß-catenin signaling pathway.

1. Introduction

Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2), encoded by the *PTPN11* gene, is a non-receptor phosphotyrosine phosphatase [1]. SHP2 is localized in both the cytoplasm and nucleus at low cell density of AGS gastric epithelial cells but is excluded from the nucleus at high cell density of these cells [2]. SHP2 physically interacts with the transcriptional co-activators YAP and TAZ, which are targets of the cell-density-sensing Hippo signaling, which in turn stimulates gene regulation by TCF/LEF and TEAD via parafibromin dephosphorylation [2]. Non-phosphorylated YAP/TAZ promotes the nuclear translocation of SHP2, whereas YAP/TAZ phosphorylation by Hippo signaling sequesters SHP2 in the cytoplasm [2]. SHP2 is required for the full activation of the RAS/ERK signaling pathway in the cytoplasm, whereas, in the nucleus, it promotes Wnt target activation through dephosphorylation of parafibromin [2–4]. Therefore, the cytoplasmic and nuclear forms of SHP2 can act in oncogenic pathways through different mechanisms.

In studies of human tumor tissues, high SHP2 expression has been associated with unfavorable survival in patients with gastric, breast,

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oral, prostate cancers, melanoma, non-small cell lung cancer (NSCLC), and hepatocellular carcinoma (HCC) [5–11]. Surprisingly, low levels of SHP2 expression have also been associated with adverse outcomes in hepatocellular carcinoma and colorectal cancer [12,13]; however, the nuclear or cytoplasmic localization of the expressed SHP2 protein was not determined in these carcinomas. SHP2 showed a cytoplasmic localization in laryngeal, thyroid, and pancreatic duct adenocarcinoma, and cytoplasmic SHP2 overexpression was associated with poor prognosis in these carcinomas [14–16]. However, the prognostic value of nuclear expression of SHP2 expressed in tumor cells has not yet been reported.

A previous study indicated a possible association between nuclear YAP1 localization and unfavorable outcome in HCC [12]. Based on these previous studies, we speculated that nuclear YAP1 could promote the nuclear translocation of SHP2, and in turn, confer tumor malignancy and poor prognosis by activating the Wnt/ß-catenin signaling pathway [3,4]. We therefore hypothesized that the nuclear localization of both YAP1 and SHP2 proteins in lung tumors could be associated with poor prognosis in patients with NSCLC.

We explored the possibility that the localization of SHP2 and YAP1 in the nucleus versus the cytoplasm of tumor cells could have different prognostic values in patients with NSCLC by subjecting 102 surgically resected tumors to immunohistochemistry. Kaplan-Meier analysis showed that NSCLC patients with tumors displaying a combination of nuclear and/or cytoplasmic SHP2 and nuclear YAP1 exhibited poorer overall survival (OS) and relapse free survival (RFS) when compared with patients with SHP2- or YAP1-negative tumors. Cox-regression analysis further confirmed the prognostic significance of nuclear and/or cytoplasmic SHP2 or nuclear YAP1 expression in this study population. More interestingly, the combination of nuclear SHP2 and nuclear YAP1 expression in tumors appeared to be an independent predictor of poor OS and RFS in patients with NSCLC.

2. Material and methods

2.1. Human study subjects

This study enrolled of 102 patients with NSCLC. The inclusion criteria for patients were a primary diagnosis with lung carcinoma; no metastatic disease at diagnosis; no previous diagnosis of carcinoma; no neoadjuvant treatment before primary surgery; and no evidence of disease within one month of primary surgery. Tumor specimens were collected from patients who underwent resection at the Department of Thoracic Surgery, Taichung Veterans General Hospital (Taichung, Taiwan), between 1998 and 2004. The resected tissues were stored at -80 ℃ until analysis. The study was approved by the Institutional Review Board of Chung Shan Medical University Hospital (CSMUH No: CS11177).

The tumor stage of each specimen was histologically determined according to the World Health Organization (WHO) classification system (4th edition of 2015). Cancer relapse data were obtained from chart review and confirmed by the surgeons. The clinical parameters of the patients and their overall survival (OS) data were collected from chart review and the Taiwan Cancer Registry, Department of Health, Executive Yuan, Taiwan, ROC. The survival time of each patient was taken as the period from the date of primary surgery to the date of death. The median follow-up time was 26.3 months (range from 1 to 165.3 months) and the end of the follow-up period was Dec. 2007. The relapse results were available for 91 patients. Forty patients had tumor relapse and 51 patients had no tumor relapse.

2.2. Immunohistochemical analysis

The immunohistochemical procedures and quantification methods were described previously [17]. The primary SHP2 (GTX29214) and YAP1 (GTX129151) antibodies were purchased from GeneTex (Irvine CA, USA). Specimens were formalin fixed, paraffin embedded, and cut into 3 μm sections, which were mounted on glass and dried overnight at 37 ℃. All sections were then deparaffinized in xylene, rehydrated through a graded alcohol series, and washed in phosphate-buffered saline. This buffer used for all subsequent washes. The sections were heated in a microwave oven twice for 5 min in citrate buffer (pH 6.0) and then incubated with the primary antibody for 60 min at room temperature. Nuclear and cytoplasmic SHP2 antibodies were blocked by blocking peptides of SHP2 (GTX29214-PEP) in serial sections under the same condition (Supplementary Fig. 1A). Negative controls were obtained by leaving out the primary antibody (Supplementary Fig. 1B). The sections were then subjected to a conventional streptavidin peroxidase treatment (LSAB Kit K675, DAKO, Carpinteria, CA, USA). The signals were developed with 3, 3′-diaminobenzidine for 5 min and the sections were counter-stained with hematoxylin. A total of 102 cases were analyzed by three observers. There were 88–90% cases with complete agreement of three observers for SHP2 and YAP1 expressions (Supplementary Table 1). The degree of agreement between two pathologists was calculated using kappa coefficient. The very good agreement (concordance) was seen in SHP2 and YAP1 expression. The internal consistency between three observers was calculated with Cronbach's alpha value. The excellent overall consistency was seen in SHP2 and YAP1 expression. The intensities of the signals were evaluated independently by three observers. Immunostaining scores were defined as the cell staining intensity (0 = nil; 1 = weak; 2 = moderate; and 3 = strong) multiplied by the percentage of labeled cells (0–100%), leading to scores from 0 to 300. A score over 150 was rated as "high" immunostaining, while a score of less than 150 was rated as "low" immunostaining.

2.3. Real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) analysis

Total RNA was extracted by homogenization in 1 ml TRIzol reagent, followed by chloroform extraction and isopropanol precipitation. A 3 μg sample of total RNA from colorectal tumor tissues was reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen Life Technologies) and oligo(dT) 15 primer. The primer sequences for detecting cyclin D1 expression were the forward primers, 5′- CTGGAGG TCTGCGAGGAACA-3′, and the reverse primer, 5′- TGCAGGCGGCTCT TTTTC-3′. The primer sequences for detecting c-Myc expression were the forward primers, 5′- GGCCGACCAGCTGGAGAT-3′, and the reverse primer, 5′-TCCTGGATGATGATGTTTTTGATG-3′. The gene expression were calculated with $2^{-\Delta Ct}$, where ΔCt = Ct of target gene –Ct of GAPDH. The mRNA levels in lung tumors that were higher than the median value were defined as "high", while levels lower than the median value were defined as "low".

2.4. Statistical analysis

Statistical analysis was performed using the SPSS statistical software (Version 18.0; Chicago, IL.). The associations between clinical parameters and the nuclear and cytoplasmic expression of SHP2 and the nuclear expression of YAP1 were analyzed with the chi-square test. The degree of agreement between two pathologists was calculated using kappa coefficient. The internal consistency between three observers was calculated with Cronbach's alpha value. Survival plots were generated using the Kaplan-Meier method, and differences between patient groups were determined by the log-rank test. Cox-regression analysis was performed to determine OS and RFS. The analysis was stratified for all known variables (age, gender, smoking status, and tumor stage) and nuclear and cytoplasmic expression of SHP2 and nuclear expression of YAP1.

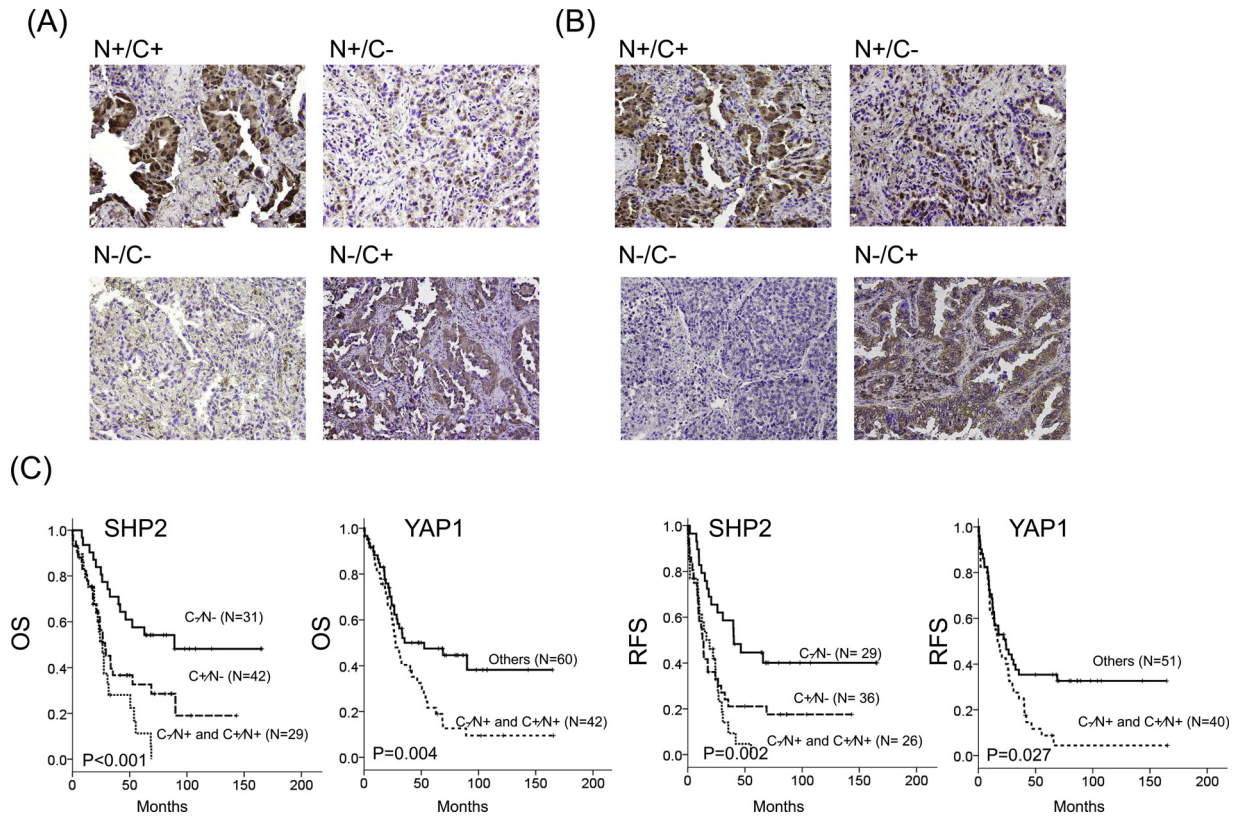

Fig. 1. High nuclear expression of SHP2 and YAP1 is associated with poor OS and RFS in patients with NSCLC. (A, B) A representative figure of nuclear and cytoplasmic SHP2 and YAP1 expression in lung cancer patients. (C) Lung cancer patients with tumors showing high nuclear SHP2 and YAP1 expression had poor outcomes.

3. Results

3.1. The relationships between the clinico-pathological parameters in NSCLC patients and nuclear and/or cytoplasmic SHP2 and YAP1 expression

In total, 102 tumors were surgically resected from NSCLC patients for immunohistochemical examination of SHP2 and YAP1 expression. SHP2 and YAP1 were expressed in the nucleus and/or the cytoplasm of the tumor cells (Fig. 1A and B upper panel). SHP2 and YAP1 were only expressed in the cytoplasm (Fig. 1A and 1B lower right panel). The negative immunostainings of SHP2 and YAP1 are shown in Fig. 1A and B (lower left panel). The relationships of SHP2 expression in the nucleus alone, in the cytoplasm alone, and in both the nucleus and cytoplasm with respect to the clinico-pathological parameters were analyzed using Pearson's Chi-square test for categorical variables. Tables 1 and 2 show that SHP2 and YAP1 expression in the nucleus, cytoplasm, or both the nucleus and cytoplasm of tumor tissues was not associated with age, genders, smoking status, tumor histologies, tumor sizes, nodal metastasis, or tumor stage (Tables 1 and 2). These results suggested that SHP2 and YAP1 expression in lung tumors was not associated with the clinico-pathological parameters in this study population.

Table 1
Relationships of SHP2 protein expression with clinico-pathological parameters in tumor tissues from patients with NSCLC.

		C-/N-	C+/N-	C-/N+ and C+/N+	P
Age					
≦65	43	14 (33)	14 (33)	15 (34)	0.280
> 65	59	17 (29)	28 (48)	14 (24)	
Genders					
Female	45	11 (24)	18 (40)	16 (36)	0.301
Male	57	20 (35)	24 (42)	13 (23)	
Smoking status					
Nonsmokers	64	16 (25)	29 (45)	19 (30)	0.293
Smokers	38	15 (40)	13 (34)	10 (26)	
Tumor type					
AD	56	14 (25)	23 (41)	19 (34)	0.285
SQ	46	17 (37)	19 (41)	10 (22)	
T					
1, 2	87	28 (32)	36 (41)	23 (27)	0.483
3, 4	15	3 (20)	6 (40)	6 (40)	
N					
0	48	17 (35)	20 (42)	11 (23)	0.421
1,2&3	54	14 (26)	22 (41)	18 (33)	
Stage					
I, II	61	19 (31)	26 (43)	16 (26)	0.833
III	41	12 (29)	16 (39)	13 (32)	

AD: adenocarcinoma; SQ: squamous cell carcinoma.
C-/N-: negative cytoplasmic SHP2/ negative nuclear SHP2; C+/N-: positive cytoplasmic SHP2/negative nuclear SHP2; C-/N+: negative cytoplasmic SHP2/ positive nuclear SHP2; and C+/N+: positive cytoplasmic SHP2/positive nuclear SHP2.

3.2. Association of the nuclear localization of SHP2 with nuclear YAP1 expression and the correlation of nuclear expression of both proteins with mRNA expression of cyclin D1 and c-Myc

Table 3 shows that nuclear YAP1 expression was positively correlated with nuclear SHP2 expression ($P < 0.001$). Low or high nuclear YAP1 expression was commonly observed in tumors with low or high nuclear SHP2 expression, respectively (88% for low/low and 49% for high/high vs. 12% for low/high and 51% for high/low; Table 3). These results seemed to support a previous study indicating that nuclear YAP1 could promote nuclear translocation of SHP2 due to the physical interaction of YAP1 with SHP2 [2].

The interaction of SHP2 and YAP1 and nuclear localization of both proteins appeared to activate Wnt/ß-catenin signaling pathway to up-

Table 2
Relationships of YAP1 protein expression with clinico-pathological parameters in tumor tissues from patients with NSCLC.

		Nuclear YAP1		
		Low	High	P
Age				
≦65	43	20 (47)	23 (53)	0.104
> 65	59	37 (63)	22 (37)	
Genders				
Female	45	26 (58)	19 (42)	0.732
Male	57	31 (54)	26 (46)	
Smoking status				
Nonsmokers	64	36 (56)	28 (44)	0.923
Smokers	38	21 (55)	17 (45)	
Tumor type				
AD	56	31 (55)	25 (45)	0.906
SQ	46	26 (57)	20 (43)	
T				
1, 2	87	49 (56)	38 (44)	0.830
3, 4	15	8 (53)	7 (47)	
N				
0	48	28 (58)	20 (42)	0.638
1,2&3	54	29 (54)	25 (46)	
Stage				
I, II	61	34 (56)	27 (44)	0.971
III	41	23 (56)	18 (44)	

AD: adenocarcinoma; SQ: squamous cell carcinoma.
Immunostaining score of "low" or "high" was defined in the Materials and Methods section.

Table 3
Correlation of nuclear SHP2 with nuclear YAP1 protein expression in tumor tissues from patients with NSCLC.

		Nuclear SHP2		
		Low	High	P
Nuclear YAP1				
Low	57	50 (88)	7 (12)	< 0.001
High	45	23 (51)	22 (49)	

Immunostaining score of "low" or "high" was defined in the Materials and Methods section.

Table 4
Correlation of SHP2 protein expression with YAP1, cyclin D1 and c-Myc mRNA levels in tumor tissues from patients with NSCLC.

		Cyclin D1			C-MYC		
		Low	High	P	Low	High	P
SHP2							
C-/N-	31	26 (84)	5 (16)	< 0.001	16 (52)	15 (48)	< 0.001
C+/N-	42	23 (54)	19 (45)		29 (69)	13 (31)	
C+/N+ and C-/ N+	29	2 (7)	27 (93)		6 (21)	23 (79)	
Nuclear YAP1							
Low	57	34 (60)	23 (40)	0.028	45 (79)	12 (21)	< 0.001
High	45	17 (38)	28 (62)		6 (13)	39 (87)	
Nuclear SHP2/ YAP1							
Low/Low	50	32 (64)	18 (36)	< 0.001	40 (80)	10 (20)	< 0.001
Low/High	23	17 (74)	6 (26)		5 (22)	18 (78)	
High/Low	7	2 (29)	5 (71)		5 (71)	2 (29)	
High/High	22	0 (0)	22 (100)		1 (5)	21 (95)	

The mRNA levels in lung tumors that were higher than the median value were defined as "high", while levels lower than the median value were defined as "low". Immunostaining score of "low" or "high" was defined in the Materials and Methods section.

regulate mRNA expression of its downstream genes cyclin D1 and c-Myc [3,18,19]. As shown in Table 4, tumors that expressed nuclear and/or cytoplasmic SHP2 (C+/N + and C-/N+) more commonly showed high cyclin D1 and c-Myc mRNA expression when compared to C-/N- and C+/N- subgroups (cyclin D1: 93% vs. 16% for C-/N- and 45% for C+/N-, P < 0.001; c-Myc: 79% vs. 48% for C-/N- and 31% for C+/N-; P < 0.001). Higher expression of cyclin D1 and c-Myc was observed in tumors expressing high levels of nuclear YAP1 than low levels of nuclear YAP1 (P = 0.028 for cyclin D1; P < 0.001 for c-Myc). Tumors expressing high nuclear levels of both SHP2 and YAP1 (high/high) were also more likely to express high levels of cyclin D1 and c-Myc mRNA expression when compared with the high/low, low/high, or low/low expressing tumors (P < 0.01 for cyclin D1; P < 0.001 for c-Myc; Table 4). These results seemed to support previous studies indicating that nuclear YAP1 may promote SHP2 nuclear translocation, which in turn activates cyclin D1 and c-Myc mRNA expression through activation of the Wnt/ß-catenin signaling pathway [18,19].

3.3. Patients with tumors expressing SHP2 or YAP1 in the nucleus, the cytoplasm, or in both the nucleus and cytoplasm have poorer prognosis when compared to patients with SHP2- or YAP1-negative tumors

Kaplan-Meier analysis indicated that the OS and RFS periods were shorter for patients with nuclear and/or cytoplasmic SHP2-positive tumors than with SHP2-negative tumors (Fig. 1C left panel for OS, P < 0.001; Fig. 1B middle right panel for RFS, P = 0.002). Nuclear and/or cytoplasmic YAP1 expression also had a prognostic significance for OS and RFS when compared with cytoplasmic YAP1-positive and YAP1-negative tumors (OS: Fig. 1C middle left panel, P = 0.004; RFS: Fig. 1C left panel, P = 0.027). Cox-regression analysis indicated that patients with cytoplasmic SHP2-positive tumors exhibited higher hazard ratios (HR) of 2.38 and 2.56 for OS and RFS, respectively, when compared with patients with SHP2-negative tumors (95% CI 1.25–4.55, P = 0.009 for OS; 95% CI 1.36–4.80, P = 0.003 for RFS; Table 5). Patients with nuclear and/or cytoplasmic SHP2-positive tumors exhibited HRs of 3.27 and 2.78 for OS and RFS, respectively, when compared to patients with SHP2-negative tumors (95% CI 1.64–6.49, P = 0.001 for OS; 95% CI 1.44–5.38, P = 0.002 for RFS; Table 5). The expression of nuclear YAP1, cyclin D1, and c-Myc had a prognostic significance for OS and RFS in this study population (OS: HR, 1.89, 95% CI 1.14–3.13, P = 0.013; HR, 1.73, 95% CI 1.06–2.83, P = 0.028; Table 5). Patients with high cyclin D1 and high c-Myc expressing tumors exhibited poorer OS and RFS than their counterparts (Table 5). The expression of nuclear SHP2 plus nuclear YAP1 had a greater prognostic significance for tumors in the high/high subgroup than in the low/low subgroup (OS: HR, 2.85, 95% CI, 1.52–5.35, P = 0.001; RFS: HR, 2.55, 95% CI 1.37–4.72, P = 0.003; Table 5), but not in the low/high and high/low subgroups (Table 4). All HR values were adjusted by age, genders, smoking status, tumor types using SPSS software. These results suggest that nuclear SHP2 expression, in combination with nuclear YAP1, may act as an independent prognostic factor of OS and RFS in patients with NSCLC.

4. Discussion

The data provided here indicate that nuclear localization of SHP2 and YAP1 in tumor tissues may predict poorer OS and RFS in patients with NSCLC. The mechanism of action probably appears to involve activation of the Wnt/ß-catenin signaling pathway by nuclear SHP2 plus nuclear YAP1, and consequently to promote the expression of downstream c-Myc and cyclin D1 genes in these tumors. Therefore, we suggest that nuclear YAP1 may interact with SHP2 to promote the translocation of SHP2 into the nucleus for subsequent activation of Wnt/ß-catenin signaling. This suggestion is consistent with previous studies indicating that YAP/TAZ promoted nuclear translocation of SHP2, which in turn upregulated c-Myc and cyclin D1 mRNA expression via activation of the Wnt/ß-catenin signaling pathway [2–4,12].

Table 5
Cox regression analysis for the prognostic value of SHP2, YAP1, cyclin D1 and c-MYC expression on OS and RFS in lung cancer patients.

	OS				RFS			
Variables	Patient No.	Adjusted HR*	95%CI	P	Patient No.	Adjusted HR*	95%CI	P
SHP2								
C-/N-	31	1			29	1		
C+/N-	42	2.38	1.25-4.55	0.009	36	2.56	1.36-4.80	0.003
C+/N+ and C-/ N+	29	3.27	1.64-6.49	0.001	26	2.78	1.44-5.38	0.002
Nucleus YAP1								
Low	57	1			50	1		
High	45	1.89	1.14-3.13	0.013	41	1.73	1.06-2.83	0.028
Nucleus SHP2/YAP1								
Low/Low	50	1			44	1		
Low/High	23	1.39	0.75-2.59	0.301	21	1.21	0.66-2.24	0.541
High/Low	7	0.96	0.27-3.42	0.956	6	0.71	0.23-2.15	0.538
High/High	22	2.85	1.52-5.35	0.001	20	2.55	1.37-4.72	0.003
Cyclin D1								
Low	51	1			45	1		
High	51	2.72	1.59-4.66	< 0.001	46	2.36	1.38-4.03	0.002
C-MYC								
Low	51	1			45	1		
High	51	1.92	1.15-3.21	0.013	46	1.72	1.05-2.83	0.033

OS: overall survival; HR: Hazard ratio; RFS: relapse free survival. *HR for all cases was adjusted by age, gender, smoking status and stage.

Moreover, activation of Wnt/ß-catenin signaling pathway by nuclear localization of SHP2 and YAP1 may promote tumor progression and, in turn, confer poor prognosis in patients with NSCLC [20–22].

SHP2 overexpression or loss plays oncogenic roles by different mechanisms. SHP2 loss suppressed liver tumorigenesis driven by the MET/ß-catenin or MET/PI3CA signaling pathways [3]. Interestingly, SHP2 deficiency triggered a tumor-promoting hepatic microenvironment [3]. SHP2 increased ß-catenin accumulation by inhibiting ß-catenin degradation mediated by glycogen synthase kinase 3ß in liver cancer stem cells [4], confirming that SHP2 could promote HCC cell dedifferentiation and liver cancer stem cell expansion by amplifying ß-catenin signaling [4]. Han et al. (2015) indicated that SHP2 promoted HCC growth and metastasis by coordinately activating the RAS/RAF/ERK signaling pathway and the PI3K/AKT/mTOR cascade [11]. Overexpression of SHP2 has also shown a good correlation with the malignant clinico-pathological characteristics of HCC and predicted the poor prognosis of patients with HCC; however, the localization of SHP2 was not determined in these patients [11]. Recently, an increase in YAP expression and a decrease in cytoplasmic SHP2 expression were reported in tumor tissues when compared to adjacent non-tumor tissues, and these expressions were significantly associated with the RFS and OS in patients with HCC [12]. The observation of an association between low SHP2 expression and poor prognosis in HCC was contrasted with previous studies indicating that SHP2 overexpression promoted tumor growth and metastasis and poor outcomes in HCC [3,4,11]. Therefore, we suspected that differences in the nuclear and cytoplasmic localization of SHP2 could lead to different responses in HCC tumorigenesis. As mentioned above, protein interactions between YAP1 and SHP2 may promote nuclear SHP2 translocation and subsequent tumor growth and metastasis by activation of the Wnt/ß-catenin signaling pathway.

In the present study, the association between nuclear localization of both YAP1 and SHP2 proteins with poor prognosis in NSCLC patients could be a result of increased c-Myc and cyclin D1 expression due to activation of the Wnt/ß-catenin signaling pathway. Consistent findings have been reported for an association of YAP1, Wnt/ß-catenin, c-Myc, and cyclin D1 expression and poor prognosis in patients with NSCLC [20–24]. Therefore, SHP2 expression in the nucleus, rather than its cytoplasmic localization, may activate Wnt/ß-catenin signaling via the interaction of nuclear YAP1 with SHP2 and, in turn, confer poor prognosis in patients with NSCLC.

In summary, the observations of tumors from patients with NSCLC support the findings in a previous cell model whereby YAP1 can promote SHP2 nuclear translocation to activate Wnt/ß-catenin signaling pathway and promote tumor growth, metastasis, and poor prognosis by up-regulation of cyclin D1 and c-Myc expression. Therefore, evaluation of the nuclear and/or cytoplasmic localization of SHP2 in tumor tissues could be valuable for assessing the prognostic value of SHP2 in patients with NSCLC.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prp.2019.01.027.

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