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Upregulation of Src homology phosphotyrosyl phosphatase 2 (Shp2) expression in oral cancer and knockdown of Shp2 expression inhibit tumor cell viability and invasion in vitro

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Objective. This study investigated the clinical significance of Shp2 protein expression in oral squamous cell carcinoma (OSCC) and elucidated its biologic significance in OSCC cells.
Study Design. A total of 88 OSCC cases were used to assess Shp2 expression, out of which 70 were for immunohistochemistry and 18 paired tumors vs normal tissues were for Western blot of Shp2 expression. OSCC cells were used to assess the effects of Shp2 knockdown for cell viability, apoptosis, invasion, and protein expressions.
Results. Expression of Shp2 protein was significantly upregulated in OSCC tissues compared with the normal tissues, and Shp2 overexpression was associated with advanced tumor clinical stages and lymph node metastasis ex vivo. Knockdown of Shp2 expression in vitro inhibited OSCC cell viability and invasion but induced apoptosis by regulating expression of the apoptosis-related proteins.
Conclusions. The data indicated that Shp2 may play an important role in OSCC progression. Further studies will investigate whether a target of Shp2 expression could be a novel therapeutic strategy for clinical control of OSCC. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:234-242)

Head and neck squamous cell carcinoma (HNSCC) is a devastating disease, although it accounts for only 3% of all malignancies.[1] HNSCC primarily affects the oral cavity, hypopharynx, oropharynx, larynx, and salivary glands, but more than 90% of HNSCCs are oral squamous cell carcinoma (OSCC), which remains a lethal disease in over 50% of the cases diagnosed annually[2] and affects the quality of life. In the clinic, because most OSCCs are diagnosed at the advanced stage, treatment fails, or patients develop a second primary cancer, local recurrence, and distant metastasis.[3] To date, treatment of OSCC involves radical surgical resection with adjuvant chemotherapy, radiotherapy, or both. However, in the past 30 years, the long-term survival rate for OSCC has not improved, and only 60% of OSCC patients survive for 5 years.[4,5] Thus, we should pay more attention to the discovery of novel and effective treatment options to improve the survival and quality of life of patients, in addition to identifying novel biomarkers for early detection and prediction of tumor progression.[6-10]

Cell activities, such as survival, proliferation, and differentiation, are tightly controlled by intracellular signaling initiated by extracellular factors, in which protein phosphorylation (conducted by protein kinases) and dephosphorylation (conducted by protein phosphatases) play an important role.[11] Src homology phosphotyrosyl phosphatase 2 (Shp2), encoded by the *PTPN11* gene in humans, is a ubiquitously expressed protein tyrosine phosphatase (PTP) with 2 N-terminal Src homology 2 (SH2) domains (N-SH2 and C-SH2, respectively) and a catalytic (PTP) domain that acts as an important transducer of the Ras-Erk pathway to promote cell growth and production of cytokines and hormones.[12] Depending on the specific cell type or receptor, Shp2 could also increase or decrease Rho activity,[13] enhance or antagonize PI3K-Akt activation,[14] and possibly affect the NFAT (nuclear factor of activated T cells) or NF-κB (nuclear factor kappa B) pathways.[15] Autosomal-dominant mutations in human gene *PTPN11* have been detected in nearly 50% of patients with Noonan syndrome who have higher risk of suffering juvenile myelomonocytic leukemia.[16] Somatic mutations constitutively activating Shp2 have also been detected in several types of leukemia,[17] and increased Shp2 expression has been reported in different human cancers, including breast cancer,[18] gastric cancer,[19] cervical cancer,[20] prostate cancer,[21] melanoma,[22] and thyroid cancer.[23] These data suggest that *PTPN11* functions as a proto-oncogene.[24]

Statement of Clinical Relevance

The study suggested that Shp2 should be further evaluated as a novel biomarker for prediction of OSCC progression and be further developed as a therapeutic target for OSCC.

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Discordant findings have been reported; for example, other studies found that Shp2 may be a tumor suppressor in hepatocellular carcinoma.[25,26] These contradictory results suggested that Shp2 may have dual roles in tumorigenesis, and elucidating the functions of Shp2 could lead to better understanding of tyrosine phosphorylation in different cell contexts.

Furthermore, previous studies found that levels of *PTPN11* mRNA were significantly higher in hypopharyngeal SCC tissues compared with adjacent noncancerous hypopharyngeal tissues, implicating Shp2 involvement in OSCC.[27] Thus, we initiated this study by first investigating the expression of Shp2 protein in OSCC tissues ex vivo and then knocking down Shp2 expression in OSCC cells in vitro for assessment of changed tumor cell viability, apoptosis, invasion, and gene expression.

MATERIALS AND METHODS

Tissue specimens

A total of 88 tissue specimens were used in the study, of which 70 cases were retrospectively recruited for immunohistochemical analysis of Shp2 expression. For confirmation of immunohistochemical data, we prospectively recruited 18 paired tumors vs normal tissues for Western blot assessment of Shp2 expression. Specifically, 70 tissue specimens were obtained from patients who underwent surgical resection of primary OSCC in the Department of Oral and Maxillofacial Surgery, The Provincial Hospital Affiliated to Shandong University, between October 2008 and August 2011. These patients were not treated with any previous radiotherapy or chemotherapy. Clinicopathologic data, including gender, age, tumor (T) classification, node (N) classification, metastasis (M) classification, and overall TNM stage were collected from medical records and pathology reports. The TNM status was classified according to the 2002 American Joint Committee on Cancer Staging System.[28] Paraffin-embedded specimens of cancerous and normal tissues were retrieved from pathology archives and sectioned for reevaluation of pathologic diagnosis of OSCC and immunohistochemical analysis of Shp2 expression.

In addition, 18 cases of fresh cancerous and normal tissues were obtained from patients with OSCC at the Department of Oral and Maxillofacial Surgery, The Provincial Hospital Affiliated to Shandong University, between January and April 2012. These samples were histologically confirmed by frozen sections to have OSCC and were used for Western blot analysis of Shp2 expression. This study followed the Helsinki Declaration and Guidelines and was approved by the Medical Ethics and Human Clinical Trial Committee of The Provincial Hospital Affiliated to Shandong University. All patients provided a signed informed consent form to participate in this study.

Immunohistochemistry

Tissue sections were stained with hematoxylin-eosin and reevaluated by an independent pathologist to confirm the OSCC diagnosis. Paraffin sections (5 μm thick) were used for immunostaining of Shp2 protein. In brief, the sections were heated for 30 minutes at 68°C and then deparaffinized in xylene 3 times for 10 minutes each and rehydrated in a series of graded ethanol (100% to 50% ethanol and then in distilled water). Sections were incubated for 10 minutes in 3% H_2O_2 in phosphate buffered saline (PBS) and with normal serum for 30 minutes. A monoclonal anti-Shp2 antibody (Y478; Abcam, Cambridge, MA, USA) was applied to the sections at a dilution of 1:50 and incubated overnight at 4°C. On the next day, the sections were further incubated at room temperature for 1 hour and then incubated with a secondary antibody for 30 minutes. After the specimens were washed 3 times for 5 minutes each with PBS, the bound primary antibody was visualized by incubating the sections for 5 seconds in diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich, St Louis, MO, USA). The sections were rinsed in distilled water for 5 minutes, counterstained with Harris hematoxylin, dehydrated, cleared, and mounted. Finally, the sections were reviewed and scored by 2 pathologists who were blinded to patients' identification and clinical data. Normal and cancerous cells with an obvious membranous or cytoplasmic staining were defined as positive staining. The percentages of positive staining (P) were scored as 0 (<10%), 1 (10%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%), and the levels of intensity of staining (I) were determined as 0, negative; 1, light yellow (weak staining); 2, brown (moderate staining); and 3, dark brown (strong staining). The total scores (S) were designated as $P \times I$ for each section.

Cell line and culture

A specimen from a human tongue squamous cancer cell line, SCC-4, was obtained from the Department of Oral and Maxillofacial Surgery, The Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, having been originally purchased from ATCC, where it was characterized by mycoplasma detection, DNA fingerprinting, isozyme detection, and cell vitality detection. The cell line was refreshed every 3 to 4 months from a frozen vial of the same batch of cells for this study. Cells were cultured in high-glucose Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum with 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in 5% CO_2.

Design and construction of lentivirus carrying Shp2 shRNA and gene transfection

To knock down Shp2 expression in cell lines, we first designed and synthesized Shp2 shRNAs according to Shp2 cDNA sequences (Gen-Bank accession NM_002834) from Shanghai Genomeditech Co Ltd (Shanghai, China). Shp2 shRNA1 sequences were 5′-GTAACCCTGGAGACTTCAC-3′, shRNA 2, 5′-GATTCAGAACACTGGTGAT-3′; and shRNA3, 5′-CGCTAAGAGAACTTAAACTTT-3′. The sequence 5′-TTCTCCGAACGTGTCACGT-3′, which had no significant homology to any known human or mouse genes, was used as a negative control. These oligonucleotides were then subcloned into a lentiviral vector (pGMLV-SB1 RNAi; Shanghai Genomeditech Co Ltd, Shanghai, China), and the lentiviruses were produced in 293T cells. After estimating multiplicity of infection using a standard procedure, the authors used these viruses to infect SCC-4 cells using an Oligofectamine transfection reagent (Invitrogen, Carlsbad, CA, USA). Cells were divided into 3 groups, control (infection with PBS alone), mock (infection with negative control lentiviral vector), and Shp2-RNAi (Shp2-shRNA1, 2, or 3 lentiviral vector). They were subcultured at a density of 2×10^5 cells per well into 6-well cell culture plates. After 48 hours of culture, the cells were infected with Shp2 shRNA1, 2, and 3 or a negative control lentiviral vector at a multiplicity of infection of 1.5 according to the pre-experimental data. Thereafter, the cells were observed under a fluorescence microscope and harvested on the 4th, 7th, and 10th days after infection. Shp2 knockdown was confirmed by real-time polymerase chain reaction (PCR) and Western blot.

Real-time reverse transcription PCR

After infection with lentivirus for 4 days, real-time PCR was performed to detect expression of Shp2 mRNA in a human tongue squamous cancer cell line using the PrimeScript RT reagent kit (Takara, Japan) according to the manufacturer's instructions. Shp2 primers were 5′-GACTTTTGGCGGATGGTGTTCC-3′ and 5′-CGGCGCTTTCTTTGACGTTCCT-3′. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were 5′-GTCTCCTCTGACTTCAACAGCG-3′ and 5′-ACCACCCTGTTGCTGTAGCCAA-3′, which was used as an internal control for standardization. All PCR amplifications were performed in triplicate and repeated once. Data were analyzed using the comparative Ct method.

Protein extraction and Western blot

Total cellular protein from tissues and cells was extracted using an immunoprecipitation lysis buffer containing 50mM Tris-Cl (pH 7.5), 150mM NaCl, 10mM $MgCl_2$, 1mM ethylene diamine-tetracetate (pH 8.0), 1% Nonidet P-40, 100mM sodium fluoride, 1mM phenylmethylenesulfonylfluoride, and 2 μL/mL protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA). The protein concentration was determined by the BCA method (Pierce, Rockford, USA). Protein samples of 40 μg were separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis and electrophoretically transferred onto polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA). After blocking with 5% nonfat milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 1 hour at room temperature, the membranes were incubated with anti-Shp2 (Y478; Abcam), anti-p53, Bax, Bcl-2, or β-actin (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. The membranes were then washed with TBST thrice and further incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (Cell Signaling Technology, Beverly, MA, USA) secondary antibody for 1 hour at 37°C, and protein bands were visualized by using an enhanced chemiluminescence kit (Millipore, Bedford, MA, USA).

Cell viability MTT assay

SCC-4 cells were inoculated into a 96-well plate with 100 μL medium at a density of 5×10^3 cells per well. At 24 hours, 48 hours, 72 hours, and 96 hours after lentivirus infection, 20 μL MTT (5 μg/mL) solution was added to each well and incubated for 4 hours at 37°C. The medium was then discarded, and 150 μL dimethyl sulfoxide was added and oscillated. Optical density (OD) at a wavelength of 492 nm was read by enzyme-labeled analyzer (Labsystems Dragon Wellscan MK3, Finland). Cell viability was calculated by the following formula: % of control = (1 − OD value of the experimental group/OD value of the control group) × 100.

Flow cytometric apoptosis assay

Cells were collected after lentivirus infection for 72 hours, washed twice with PBS, and then resuspended with 100 μL prechilled 1× binding buffer from an Annexin V-APC/7-AAD kit (Becton Dickinson, San Jose, CA, USA). The cell density was adjusted to 1×10^6/mL, and then 5 μL PE Annexin V and 5 μL 7-ADD were added into cell solutions and incubated in the dark for 30 minutes. Afterward, 400 μL of 1× binding buffer was added and mixed thoroughly. The apoptotic rate was detected by flow cytometer (Becton Dickinson, San Jose, CA, USA).

Tumor cell Transwell invasion assay

Upper and lower Transwell chambers were separated by a polycarbonate membrane with 8 μm pores. The

membrane was coated with a diluted Matrigel solution (30 μL per chamber) and gelled at 37°C for 4 hours. The postinfected cells were added into the upper chamber at a density of 2×10^4 cells in serum-free medium, and the lower chamber was filled with 600 μL DMEM containing 10% fetal calf serum. The Transwell inserts were then incubated for 24 hours; at the end of experiments, cells on the upper side of the insert filter were removed with a cotton bud, and the cells invading the opposite side of the membrane were stained using 0.1% crystal violet for 30 minutes. Five visual fields were randomly selected and the number of cells counted under an inverted microscope. The experiment was repeated 3 times.

Statistical analysis

Data were expressed as mean ± standard deviation. All statistical analyses were carried out using SPSS software, Version 11.0 (SPSS Inc, Chicago, IL, USA). Comparisons between the relative expression value of Shp2 or the intensity of its expression in OSCC and adjacent epithelium were analyzed using a Wilcoxon nonparametric test. The Mann-Whitney *U* test was used for 2−independent sample analyses (such as comparison of Shp2 expression intensity in OSCC regarding gender, age, tobacco smoking, alcohol consumption, N stage, and clinical stage), whereas the Wilcoxon test was used to assess the paired samples (such as comparison between Shp2 expression intensity in OSCC and adjacent epithelium). Furthermore, the Kruskal-Wallis test was used to compare more than 2 samples that were independent or not related (such as comparison of Shp2 expression intensity in OSCC regarding T stage and pathologic differentiation grade). Other statistical comparisons were analyzed using *t* test and 1-way analysis of variance followed by the Tukey HSD (honestly significant difference) post hoc test. Differences were considered statistically significant at $P < .05$.

RESULTS

Upregulation of Shp2 protein in OSCC tissues and cell lines

In this study, we first assessed Shp2 expression in OSCC tissue samples. We found that expression of Shp2 protein was higher in OSCC tissue than in the adjacent nontumor tissue, except in 1 sample (Figure 1). Shp2 protein was mainly localized in the cytoplasm (Figure 2). Of these 70 cases, 46 cases had OSCC tissues and the corresponding adjacent normal epithelium in the same section. In the adjacent normal epithelium, Shp2 protein was either absent or weakly expressed, whereas the average total score of Shp2 protein levels in OSCC tissues was significantly higher than that in adjacent normal epithelium ($P = .031$; Table I).

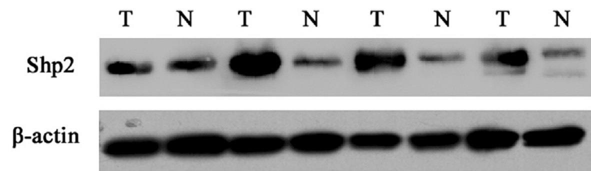

Fig. 1. Western blot analysis of Shp2 protein expression in OSCC tissues (T) and distant nonmalignant tissues (N). (*OSCC*, oral squamous cell carcinoma.)

Association of Shp2 expression with clinicopathologic data from patients

Table II summarized the parameters of 70 patients with OSCC. Specifically, there was no significant association between Shp2 expression and patient age ($P = .738$), sex ($P = .773$), tobacco smoking ($P = .773$), alcohol drinking ($P = .211$), tumor size ($P = .189$), anatomical location ($P = .811$), tumor stage ($P = .109$), or histologic grade ($P = .094$; Figure 2, *B, C, D*). In contrast, there was significant association of Shp2 expression with tumor clinical stage ($P = .003$; Figure 2, *G, H*) and lymph node metastasis ($P = .010$; Figure 2, *E, F*).

Effect of Shp2 silence on regulation of OSCC cell viability, apoptosis, and invasion capacity

After the steps outlined earlier, we produced lentiviruses carrying Shp2 shRNA to knock down Shp2 expression in OSCC cells. We first assessed the infection rate by determining the expression rate of lentiviruses carrying GFP (green fluorescent protein) under a fluorescent microscope 96 hours after infection and found that the infection efficiency of lentivirus vs the negative control (mock) in SCC-4 cells was more than 90% (data not shown). Real-time PCR data indicated that levels of Shp2 expression in Shp2 shRNA1, 2, and 3 subgroups were reduced by 39%, 47%, and 75%, respectively (Figure 3, *A*). Western blot analysis confirmed that the levels of Shp2 protein expression in these 3 subgroups were also significantly reduced (Figure 3, *B*).

The effect of Shp2 knockdown on regulation of SCC-4 cell viability was determined by using MTT assay. As shown in Figure 4, *A*, SCC-4/Shp2, Shp2 shRNA3 lentivirus-infected cells had a reduction in cell viability compared with control cells. Annexin V/7-ADD staining showed that the reduced cell viability was due to induction of apoptosis ($P < .05$; see Figure 4, *B, C*). Moreover, knockdown of Shp2 expression on SCC-4 cells significantly reduced tumor cell invasive capacity compared with control cells ($P < .05$; Figure 4, *D*).

Effect of Shp2 silence on regulation of apoptosis-related protein expression

We assessed expression of pro-apoptotic proteins after Shp2 knockdown and found that expression of p53

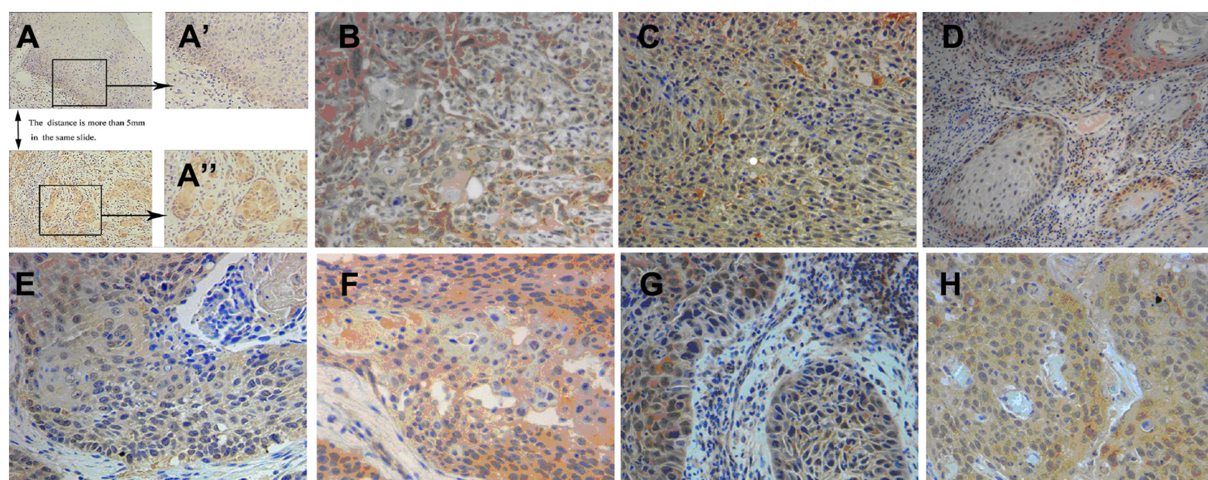

Fig. 2. Immunohistochemical detection of Shp2 expression in OSCC and normal tissues (diaminobenzidine tetrahydrochloride, brown color). A, Immunohistochemical staining of Shp2 in a section with both normal epithelium and OSCC tissue (original magnification ×100). A′, staining in the adjacent normal epithelium (magnification of the box). A″, staining in the OSCC (magnification of the box). B, Poorly differentiated OSCC tissue. C, Moderately differentiated OSCC tissue. D, Well-differentiated OSCC tissue. E, Primary tumor of OSCC without lymph node metastasis. F, Primary tumor of OSCC with lymph node metastasis. G, OSCC-early stage (T2N0M0). H, OSCC-advanced stage (T4N0M0) (original magnification ×200). (*OSCC*, oral squamous cell carcinoma.)

Table I. Differential expression of Shp2 protein in paired tumor vs normal tissues (n = 46)

	Cases	*Total scores (mean ± standard deviation)*	P
Adjacent non-malignant epithelium	46	2.43 ± 1.58	.031
Cancerous tissue	46	4.82 ± 2.52	

protein was increased after Shp2 knockdown in SCC-4 cells (Figure 5). Meanwhile, Shp2 knockdown was able to induce an increase in expression of Bax protein but decreased expression of bcl-2 protein (Figure 5).

DISCUSSION

In this study, we first detected Shp2 expression in OSCC tissue samples and found that Shp2 was upregulated in tumor tissue vs the adjacent normal or distant normal tissue. Upregulated Shp2 expression was associated with advanced tumor clinical stage and lymph node metastasis. Knockdown of Shp2 expression in vitro inhibited OSCC cell viability and invasion, but induced OSCC cells to undergo apoptosis. Knockdown of Shp2 expression also modulated expression of p53, Bax, and Blc-2 proteins in OSCC cells. These data indicated that Shp2 expression was associated with OSCC progression ex vivo. The in vitro study found that knockdown of Shp2 expression could inhibit tumor cell viability and invasion but induce tumor cell apoptosis. Overall, these data suggested that *PTPN11* may act as an oncogene in OSCC and should be further evaluated as a biomarker for prediction of tumor progression and as a novel therapeutic target for OSCC.

PTPN11 plays a regulatory role in various cells that is important for diverse cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration.[11-15] Regarding tumorigenesis, Shp2 was first identified as a proto-oncogene because activating mutations of the *PTPN11* gene are associated with leukemogenesis.[29] However, it has been found in recent studies that Shp2 acts as a tumor suppressor in hepatocarcinogenesis.[25,26] In this study, our data indicated that the expression of Shp2 protein was upregulated in OSCC and associated with advanced tumor clinical stage and lymph node metastasis, supporting the observation that Shp2 is a proto-oncogene in OSCC. In this study, our patients did not have any distant metastasis, even in patients with stage IV disease. Thus, we were not able to evaluate the association of Shp2 expression with tumor distant metastasis. This finding was consistent with those of previous studies examining other tumors.[18-23,30] For example, Zhou et al.[30] found that Shp2 protein was significantly elevated in breast cancer and associated with tumor lymph node metastasis, nuclear accumulation of hormone receptor, and higher tumor grade, suggesting that Shp2 may play a principal role in regulation of invasion and metastasis. Moreover, increase in tumor cell immunogenicity could inhibit tumor growth and metastasis.[31,32] Recently, Leibowitz et al.[33] found that Shp2 was overexpressed in head and

Table II. Association of Shp2 expression with clinicopathologic characteristics of OSCC (n = 70)

Classification	*Cases*	*Cancerous protein positive grade (mean ± standard deviation)*	*Nonparametric test*	P
Age (y)				
<60	27	4.66 ± 2.23	Z = −0.335	.738
≥60	43	4.60 ± 2.61		
Gender				
Female	29	4.65 ± 2.81	Z = −0.289	.773
Male	41	4.60 ± 2.21		
Smoking				
Yes	50	4.64 ± 2.52	Z = −0.341	.733
No	20	4.60 ± 2.37		
Drinking				
Yes	42	4.91 ± 2.56	Z = −1.250	.211
No	28	4.17 ± 2.27		
Tumor size				
<31 mm	32	4.48 ± 2.41	Z = −1.312	.189
≥31 mm	38	1.54 ± 0.50		
Anatomic locations				
Tongue	26	5.03 ± 2.04	χ^2 = 0.651	.986
Gingiva	17	4.76 ± 2.75	df = 5	
Mouth floor	8	5.00 ± 1.77		
Lip	7	4.71 ± 2.05		
Cheek	8	4.75 ± 1.83		
Soft palate	4	4.50 ± 2.51		
T stage				
T1	24	3.70 ± 1.73	χ^2 = 4.439	.109
T2	38	5.10 ± 2.67	df = 2	
T3	8	5.12 ± 2.74		
N stage				
N0	36	4.02 ± 2.47	Z = −2.572	.010*
N1-N2	34	5.26 ± 2.31		
Clinical stage				
Stage I/II	41	4.02 ± 2.39	Z = −2.940	.003*
Stage III/IV	29	5.48 ± 2.33		
Pathologic differentiation grade				
Well	30	4.60 ± 2.64	χ^2 = 4.719	.094
Moderately	36	4.86 ± 2.38	df = 2	
Poorly	4	2.75 ± 0.50		

Cancerous protein positive grade indicated the average score of immunohistochemical staining. The various nonparametric test values were calculated with SPSS software, version 11.0 (SPSS Inc).
OSCC, oral squamous cell carcinoma.
*$P < .05$.

neck cancer tissue and was responsible for basal suppression of pSTAT1 and consequent antigen processing machinery component-mediated immune escape in head and neck cancer cells from cytotoxic T lymphocytes recognition. Taken together, Shp2 expression should be further evaluated as a novel biomarker for early detection and as a predictor of OSCC progression.

You et al.[34] indicated that Shp2 was able to promote cell growth and survival. Activation of growth factor−initiated mitogenic pathways and suppression of cytotoxic effect induced by interferon were involved in mechanistic pathways. However, there are many unknowns about the Shp2 functions in OSCC. In the past few years, shRNA has been widely used to knock down expression of many genes because of its high specificity and apparent low toxicity.[35-37] In the current study, we produced lentivirus carrying Shp2 shRNA and knocked down Shp2 expression in OSCC cells. Our in vitro data further supported our ex vivo data, showing that knockdown of Shp2 expression reduced OSCC cell viability and invasion capacity but induced apoptosis. Apoptosis exerts a negative regulatory function in cancer development and progression by eliminating the rapid growth of tumor cells. However, a decrease in tumor cell apoptosis or arrest of the cell cycle accumulates a large number of abnormal cells and induces tumor progression and metastasis.[38] Tumor invasion and metastasis are characteristic features of OSCC, and those factors are important in the prognosis of OSCC patients.[39] The present study found that invasive capability of SCC-4

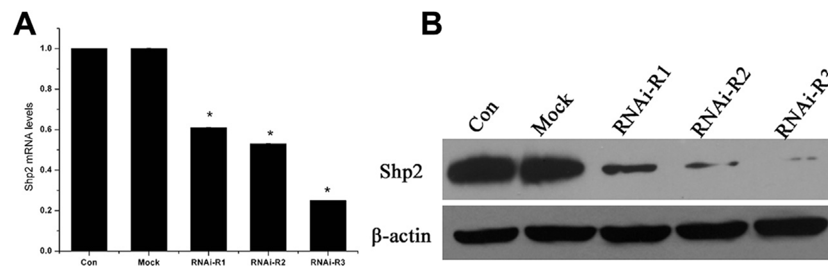

Fig. 3. Knockdown of Shp2 expression in SCC-4 cells after infection with lentivirus carrying Shp2 shRNAs. A, qRT-PCR. B, Western blot. SCC-4 cells were grown and infected with or without lentivirus carrying Shp2 shRNA or negative control shRNA for 4 days and then subjected to qRT-PCR and Western blot analyses. (*Con*, control; *Mock*, SCC-4 transfected with negative control lentivirus; *SCC-4*, a cell line; *qRT-PCR*, real-time reverse-transcription quantitative polymerase chain reaction.)

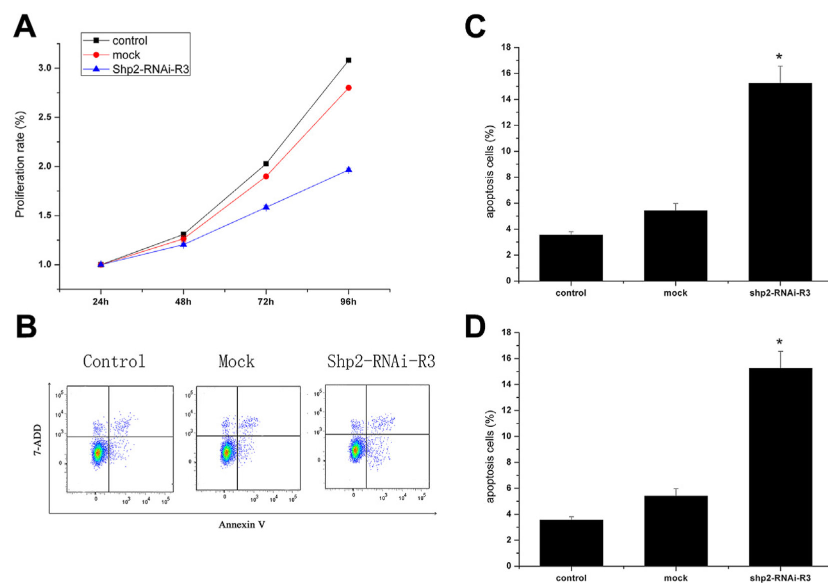

Fig. 4. Effects of Shp2 knockdown on regulation of OSCC cell viability, apoptosis, and invasion capacity in vitro. A, Cell viability MTT assay. B, Flow cytometric apoptosis assay. C, Summarized data for apoptosis assay (*$P < .05$). D, Tumor cell invasion assay (*$P < .05$). (*OSCC*, oral squamous cell carcinoma.)

cells was suppressed after Shp2 knockdown. Zhou and Agazie[18] also reported that inhibition of Shp2 could suppress epidermal growth factor−induced activation of the Ras-ERK and the PI3K-Akt signaling pathways, and induce reversion to a normal breast epithelial phenotype, which may be relevant to cell migration and malignant transformation. We speculate that the effect of Shp2 on OSCC cells could be attributed to 2 aspects, (1) upregulated expression of Shp2 protein could inhibit apoptosis that appeared to be responsible for reduction of cell death, and (2) Shp2 might downregulate expression of certain cell adhesion molecules to induce cell migration and invasion.[40,41] Future studies will investigate the possibility and feasibility of targeting Shp2 as a novel anticancer strategy in OSCC.

In addition, the present study also found that Shp2 knockdown regulated expression of apoptosis-related genes. Indeed, p53 can promote apoptosis through the mitochondrial pathway that shifts the balance of bcl-2 family proteins toward the pro-apoptotic members and induces caspase-mediated apoptosis. The altered bax/bcl-2 ratio could result in significant activation of caspases and apoptosis.[42] Recent studies found that Shp2 was a negative regulator of the Jak/STAT pathway that regulates cell proliferation, differentiation, and apoptosis.[34] Moreover, Leibowitz et al.[33] described the role of Shp2 in the immune escape of head and neck cancer cells from cytotoxic T lymphocyte recognition.

In summary, the results presented in this study indicate that Shp2 may be an oncogene and promote OSCC lymph node metastasis. These data also suggest that Shp2 might be further evaluated as a biomarker for prediction of OSCC progression and that targeting of Shp2 may provide a novel therapeutic strategy for clinical control of OSCC in the future.

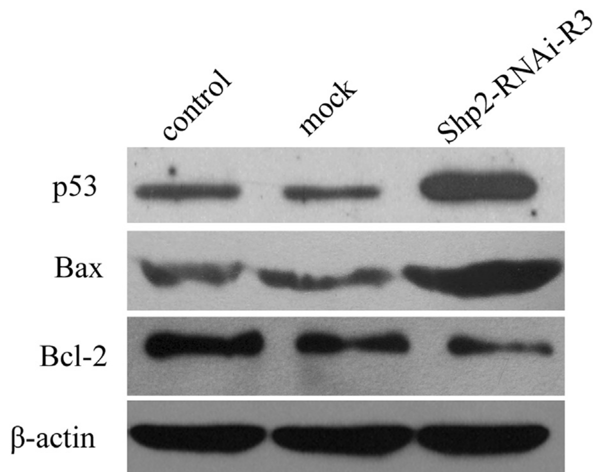

Fig. 5. Effect of Shp2 knockdown on regulation of apoptosis-related gene expressions. The cells were grown and infected with Shp2 shRNA3 for 72 hours and subjected to Western blot analysis of p53, Bax, and bcl-2 expression in SCC-4 cells. (*SCC-4*, a cell line.)

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