Stomatin-like protein 2 is overexpressed in cervical cancer and involved in tumor cell apoptosis

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Abstract. Stomatin-like protein 2 (SLP-2) is overexpressed in numerous types of human cancer and previous studies revealed that SLP-2 may function in mitochondria. The purpose of the present study was to evaluate the expression of SLP-2 in cervical cancer and the association between SLP-2 expression and clinical features, in addition to investigating the role of SLP-2 in the apoptosis of cervical cancer cells. The expression profile of SLP-2 was determined by quantitative polymerase chain reaction, western blotting and immunohistochemical staining. The effect of SLP-2 on cell apoptosis induced by chemotherapeutics in cervical cancer cells was evaluated using Annexin V staining and terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assays. The results indicated that SLP-2 expression in cervical cancer was significantly upregulated at the mRNA and protein levels, compared with that in normal cervical tissues. Immunohistochemical analysis revealed significant correlation between SLP-2 protein expression and clinical characteristics, including the squamous cell carcinoma antigen (P=0.003), deep stromal invasion (P=0.021), lymphovascular space involvement (P=0.044) and pelvic lymph node metastasis (P<0.001), which served as independent prognostic factors for predicting the shortening of overall survival time in patients with early-stage cervical cancer. In addition, TUNEL and Annexin V binding assays revealed that silencing SLP-2 expression significantly enhanced the sensitivity of cervical cancer cells to apoptosis induced by chemotherapeutics. Taken together, the results of the present study suggest that SLP-2 may be a progressive gene in the development of cervical cancer. Overexpression of SLP-2 serves an important role in the apoptosis of human cervical cancer cells.

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Introduction

Cervical cancer is the third most common type of cancer among females, worldwide (1). The annual global incidence of cervical cancer for 2008 was 529,800; the annual mortality rate was 275,100. Cervical cancer rates remain high within Hispanic/Latina, African descent and Asian female populations; additionally, cervical cancer is the second highest cause of cancer-associated mortality for females living in developing countries (2). Cervical cancer is characterized by local invasion, pelvic lymph nodes and distant organ metastasis (3). Following treatment, the five-year survival rate of early-stage cervical cancer may be as high as 90%. However, patients with local advanced or distant metastatic cervical cancer still have a poor prognosis; in particular, stage IV has a survival rate of ~20% (4). Therefore, understanding the molecular mechanisms underlying cervical cancer invasiveness would be of clinical value for the identification of effective therapeutic strategies and novel therapeutic targets.

Stomatin was first isolated from human erythrocytes and is an integral membrane protein, which is widely expressed in numerous types of cells (5). Stomatin is the founding member of a family of proteins that includes stomatin-like protein (SLP)-1, 2 and 3 in mammals (6,7). Unlike SLP-1 and SLP-3, SLP-2 does not share an N-terminal transmembrane domain with stomatin, which is a distinguishing feature (8). Therefore, SLP-2 may link stomatin or other integral membrane proteins to the peripheral cytoskeleton (8). The function of SLP-2

remains largely unknown. Previous studies have suggested that SLP-2 may serve a role in stabilizing the mitochondrial inner membrane, regulating ion channel conductance and the organization of sphingolipid and cholesterol-rich lipid rafts (9). Previous studies revealed that SLP-2 is overexpressed in numerous types of cancer tissues and is involved in the progression and development of cancer (10-12). Zhang *et al* (10) revealed that SLP-2 was upregulated ≥6 times in esophageal squamous cell carcinoma tissues, and that antisense transfection of the SLP-2 gene led to S-phase arrest and decreased expression of SLP-2 in the TE12 cell line (10). SLP-2 has been reported as overexpressed in laryngeal squamous cell carcinoma when compared with the adjacent normal laryngeal epithelium, and SLP-2 expression correlates with clinical stage (11). Zhang *et al* (12) demonstrated decreased cell growth, proliferation, tumorigenicity and cell adhesion in the antisense SLP-2 transfectants.

In the present study, the expression levels of SLP-2 in cervical cancer were evaluated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), western blotting and immunohistochemistry; to the best of our knowledge, this is the first study to do so. Thus, SLP-2 expression levels are correlated with pelvic lymph node metastasis, in addition to the prognosis of patients with early-stage cervical cancer. To investigate the possible biological function and underlying mechanisms of SLP-2, SLP-2 small interfering (si)RNA was transfected into HeLa-HCC94 cells, in the current study. Antisense transfection of SLP-2 in cervical cancer cells was identified to reduce the rate of apoptosis. Taken together, the results suggest that SLP-2 serves an important role in cervical cancer progression and pathogenesis.

Materials and methods

Cell lines. Cervical cancer cell lines, HeLa, CaSki, HCC94, SiHa and C33A, were obtained from the Cell Bank of Type Culture Collection, Chinese Academy of Sciences, Shanghai, China. HeLa, SiHa and C33A cells were maintained in Eagle's minimal essential medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), CaSki and Hcc94 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.), all supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA) in a humid incubator at 37˚C with 5% CO_2.

Patients and tissue specimens. Between January 1999 and December 2005, 300 cancerous and corresponding adjacent normal tissues from patients (20-71 years old) with early-stage (FIGO stage Ib-IIa) cervical cancer as well as 10 normal cervical tissues from patients with hysteromyoma were collected by the Department of Gynecologic Oncology (Cancer Center, Sun Yat-sen University, Guangzhou, China). For RT-qPCR, western blotting and immunohistochemistry, 19 surgically resected cervical cancer and paired adjacent tissues were obtained by the Department of Gynecologic Oncology (Cancer Center, Sun Yat-sen University, Guangzhou, China) and stored in -80˚C for further use. The present study was approved by the Medical Ethics Committee of the Cancer Center (Sun Yat-Sen University). Written informed consent was obtained from all patients prior to enrollment in the present study. Clinicopathological information for the tissue samples is presented in Table I.

RNA extraction, reverse transcription and RT-qPCR. Total RNA was isolated using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA was generated from 2 µg pretreated RNA with an iScript cDNA Synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RT-qPCR was used to determine SLP-2 mRNA expression levels in tissues and cell lines, using a Bio-Rad CFX96 sequence detection system with SsoFast® EvaGreen™ Supermix (Bio-Rad Laboratories, Inc.). The transcript amount for SLP-2 was normalized to the housekeeping gene GAPDH to control the variability in expression levels and analyzed using the $2^{-\Delta\Delta Cq}$ method described by the previous study (13). Sequences of RT-qPCR primers were designed using the Primer Express Software version 2.0 (Applera Corporation, Norwalk, CT, USA). Primers were as follows: SLP-2 forward, 5'-GTGACTCTCGACAATGTAAC-3' and reverse, 5'-TGA TCTCATAACGGAGGCAG-3', with annealing conditions of 57˚C for 30 sec; GAPDH forward, 5'-AATCCCATCACCATC TTCCA-3' and reverse, 5'-CCTGCTTCACCACCTTCTTG-3', with annealing conditions of 55˚C for 30 sec.

Western blotting. Cells and ground frozen tissues were harvested and lysed in sampling buffer [62.5 mmol/l Tris-HCl (pH 6.8), 2% SDS, 10% glycerol and 5% 2-h-mercaptoethanol]. Protein concentration was determined using a Bradford assay (Bio-Rad Laboratories, Inc.). A total of 20 µg of proteins were separated by 10% SDS-PAGE, prior to being transferred onto a polyvinylidene fluoride membrane (GE Healthcare Life Sciences, Chalfont, UK). Subsequent to being blocked in 5% non-fat dry milk, the membrane was incubated for 12 h at 4˚C with an anti-SLP-2 rabbit polyclonal antibody (dilution, 1:3,000; cat. no. AP20280c; Abgent, Inc., San Diego, CA, USA), then the membranes were washed with PBST and exposed to a horseradish peroxidase-conjugated anti-rabbit secondary antibody (dilution, 1:2,000; cat. no. NA934; GE Healthcare Life Sciences) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence kit (GE Healthcare Life Sciences). An anti-a-tubulin polyclonal antibody (dilution, 1:1,000; cat. no. SAB4500087; Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) was used as the loading control.

Immunohistochemistry. Immunohistochemical staining was performed using Histostain-Plus kits (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Briefly, 4 µm paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in ethanol and rinsed in distilled water. Endogenous peroxidase activity was blocked with 3% H_2O_2 and antigen retrieval was performed with 1 mM EDTA buffer (cat. no.7011 V; pH 8.0; Cell Signaling Technology, Inc., Danvers, MA, USA) for 10 min at 100˚C. Following incubation with an anti-SLP-2 rabbit polyclonal primary antibody (dilution 1:100; cat. no. 10348-1-AP; ProteinTech Group, Inc., Chicago, IL, USA) at 4˚C overnight, and a biotinylated anti-rabbit secondary antibody (dilution, 1:200; cat. no. TA130017; OriGene Technologies, Inc., Rockville, MD, USA) at 37˚C for 15 min, the tissue sections

Table I. Clinical/pathological characteristics of the study cohort (n=300).

Clinical/pathological characteristics	No. patients (%)
Age (years)	
<40	142 (47)
≥40	158 (53)
SCCA, ng/ml	
≤1.5	189 (67)
>1.5	92 (33)
FIGO stage	
Ib1	170 (56)
Ib2	71 (24)
IIa1	27 (9)
IIa2	32 (11)
Histological type	
Squamous cell carcinoma	264 (88)
Adenocarcinoma	20 (7)
Adenosquamous carcinoma	16 (5)
Differentiation grade	
G1	22 (8)
G2	117 (39)
G3	159 (53)
Tumor size (cm)	
<4	192 (66)
≥4	99 (34)
Deep stromal invasion	
Negative	134 (47)
Positive	152 (53)
LVSI	
Negative	286 (95)
Positive	14 (5)
Positive parametrium	
Negative	293 (98)
Positive	7 (2)
Pelvic lymph node metastasis (+)	
Negative	253 (84)
Positive	47 (16)
Recurrence	
Negative	259 (86)
Positive	41 (14)
Vital status at follow-up	
Alive	267 (89)
Cervical cancer associated mortality	32 (11)

SCCA, squamous cell carcinoma antigen; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space involvement.

were immersed in streptavidin horseradish peroxidase (OriGene Technologies, Inc.) at 37˚C for 15 min and developed with diaminobenzidine (OriGene Technologies, Inc.). Slides were evaluated by two pathologists blind to the clinical characteristics. Immunoreactivity score was determined by adding the score of the percentage of positive cells (0, 0%; 1, 1-10%; 2, 11-50%; 3 51-70%; 4 71-100%) and the intensity of staining (0, no staining; 1, weak; 2, moderate; 3, strong). Tissue samples with an SLP-2 immunohistochemistry (IHC) final score >3 were defined as having high expression.

Transfection. Synthesis and purification of three siRNAs targeting SLP-2 was determined by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The siRNA sequences were as follows: siRNA#1, 5'-CCGTTATGAGATCAAGGATAT dTdT-3'; siRNA#2, 5'-GATGCAAGTCTTGATGAGGAA dTdT-3'; siRNA#3, 5'-GCAAATCGATGGAGTCCTTTA dTdT-3'. The negative control (NC)-siRNA sequence, 5'-UUC UCCGAACGUGUCACGUTT-3', was used as the control. Transfections in cells at ~70% confluency were performed with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol.

Cell survival. Cells 1x10^3 cells/well were seeded in 6-well plates and incubated in a humid incubator at 37˚C with 5% CO_2 for 24 h, followed by 25 μg/ml cisplatin (cat. no. 479306; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) treatment at 37˚C with 5% CO_2 for 48 h. Cell apoptosis was assessed by the TUNEL system (Promega Corporation, Madison, WI, USA) and an Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection kit (EMD Millipore, Billerica, MA, USA), and then analyzed using flow cytometry (EPICS XL flow cytometer; Breckman Coulter, Inc., Brea, CA, USA) and a fluorescence microscope equipment with a digital camera. Three independent experiments were done, in triplicate.

Statistical analysis. Statistical analysis was performed using SPSS software standard version 18.0 (SPSS, Inc., Chicago, IL, USA). The association between the expression levels of SLP-2 and patient clinical features was analyzed by the χ^2 test. Factors predictive of pelvic lymph node metastasis were analyzed by Binary Logistic Regression. Survival analysis was carried out by the Kaplan-Meier method. The Cox proportional hazards model was used to explore possible prognostic factors. Cell apoptosis was analyzed by an independent-sample t-test (two-tailed). Data are presented as the mean ± standard error of the mean. P<0.05 was considered to indicate a statistically significant difference.

Results

Upregulation of SLP-2 in cervical cancer cell lines and cervical cancer tissues. To determine SLP-2 protein expression, western blotting, RT-qPCR and IHC assay were carried out in various cervical cancer cell lines (HeLa, SiHa, C33A, CaSki and Hcc94), normal cervical tissues and fresh cervical cancer tissues (T), with paired adjacent noncancerous tissues (ANT). As presented in Fig. 1A and B, SLP-2 proteins and mRNA expression was upregulated in the examined cervical cancer cell lines, by comparison with normal cervical tissues. Furthermore, comparative analysis revealed that SLP-2 was highly expressed in all cancer tissues from patients with cervical cancer, as compared with the paired adjacent noncancerous tissue

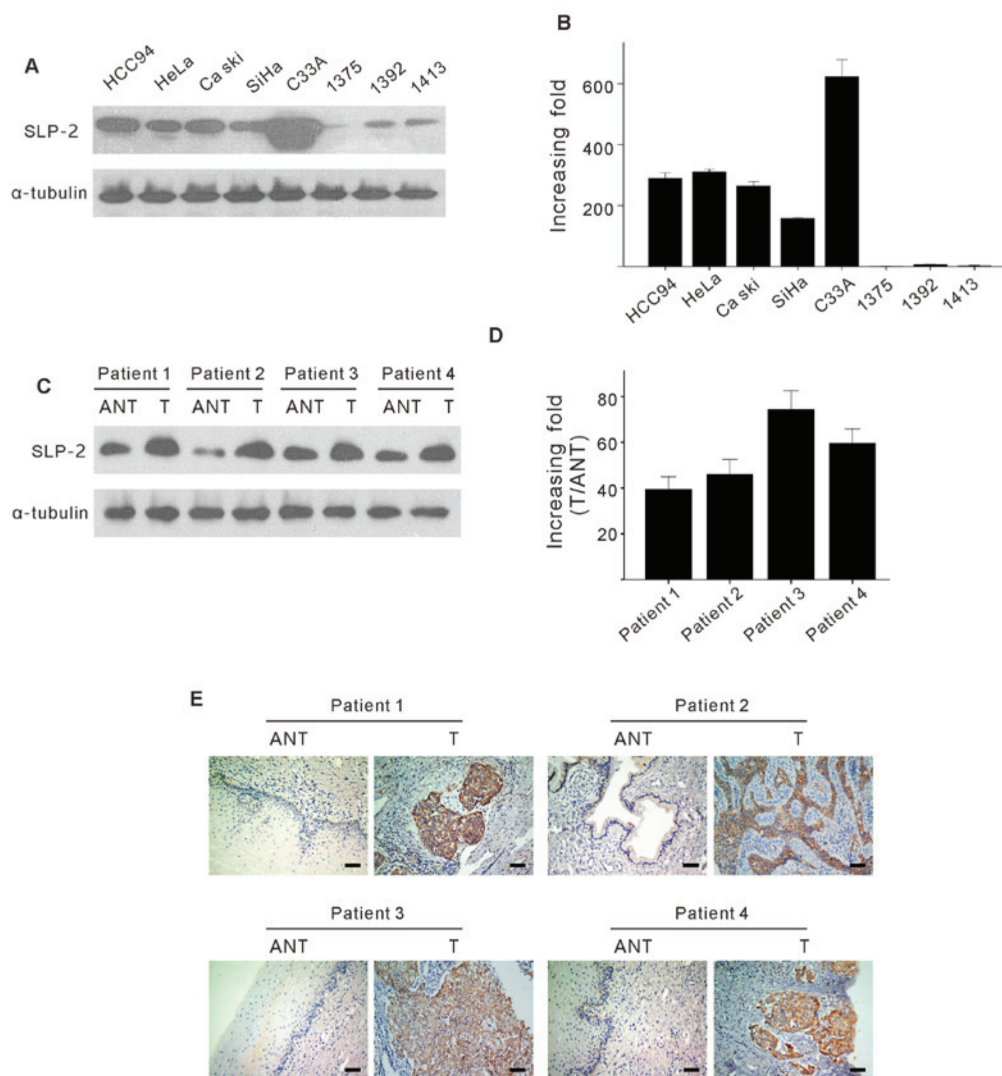

Figure 1. Expression level of SLP-2 was elevated in cervical cancer cell lines and normal cervical tissues. (A and B) Expression levels of SLP-2 in normal cervical tissues and cultured cell lines (HeLa, SiHa, C33A, CaSki and Hcc94) as determined by (A) western blotting and (B) quantitative polymerase chain reaction. (C-E) Comparative quantification of SLP-2 protein in T and ANT, with each pair obtained from the same patient, as examined by (C) western blotting, (D) quantitative polymerase chain reaction and (E) immunohistochemistry. Protein expression levels were normalized with α-tubulin. Expression levels of mRNA were normalized with β-actin. Scale bars represent 50 μm. SLP-2, stomatin-like protein 2; T, paired primary cervical cancer tissues; ANT, adjacent nontumor tissues.

expression levels (Fig. 1C and D). This was also confirmed by IHC in the four aforementioned paired tissues (Fig. 1E). Taken together, these results suggest that SLP-2 is upregulated at the protein and mRNA level in cervical cancer. Subsequently, IHC analysis was carried out to examine SLP-2 protein expression in 10 paraffin-embedded normal cervical tissue samples and in 300 cases of FIGO stage Ib-IIa sectioned cervical cancer tissues, including three histological types of cervical cancer (squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma). The detected expression levels of SLP-2 in paraffin-embedded cervical cancer tissues were as follows: SLP-2 strongly positive 24% (72/300), positive 48% (144/300), weakly positive 20.3% (61/300) and negative 7.7% (23/300). By contrast, SLP-2 was marginally or not detected in the normal cervical tissues or in areas surrounding the cancerous tissues in all tumor samples. As presented in Fig. 2, expression of SLP-2 was higher in patients with lymph node metastasis when compared with those with no lymph node metastasis. Immunostaining revealed that SLP-2 was localized to the cytoplasm.

SLP-2 is positively correlated with squamous cell carcinoma antigen (SCCA), deep stromal invasion, lymphovascular space involvement and, in particular, pelvic lymph node metastasis. As presented in Table II, statistical analysis of the IHC results demonstrated a significant correlation between SLP-2 protein expression and clinical characteristics, including the SCCA (χ^2=9.014; P=0.003), deep stromal invasion (χ^2=5.321; P=0.021), lymphovascular space involvement (χ^2=4.050; P=0.044) and pelvic lymph node metastasis (χ^2=38.668; P<0.001) of patients with cervical cancer, whereas it was not associated with age, gender, stage of cancer, differentiation grade, positive parametrium or histological type. Furthermore, in logistic regression analysis, including the variables of tumor size, deep stromal invasion, positive parametrium, lymph vascular space involvement, SCC and SLP-2 expression, revealed that SLP-2 protein expression (P<0.001; OR=6.810) and SCCA ≥1.5 ng/ml (P<0.001; OR=5.361) in cervical cancer was significantly associated with the lymph node metastasis (Table III).

Table II. Association between SLP-2 expression and clinical/pathological characteristics (n=300).

Clinical/pathological characteristics	No. patients (%)		χ^2	P-value
	Low/no SLP-2 expression	High SLP-2expression		
Total no. of patients	228 (76)	72 (24)		
Age (years)				
<40	112 (49)	30 (42)	1.220	0.269
≥40	116 (51)	42 (58)		
SCCA (ng/ml)				
<1.5	154 (72)	35 (52)	9.014	0.003[a]
≥1.5	60 (28)	32 (48)		
FIGO stage				
Ib1	129 (57)	41 (57)	0.066	0.996
Ib2	54 (24)	17 (24)		
IIa1	21 (9)	6 (8)		
IIa2	24 (10)	8 (11)		
Differentiation grade				
G1	19 (8)	3 (4)	2.898	0.235
G2	92 (41)	25 (35)		
G3	115 (51)	44 (61)		
Tumor size (cm)				
<4	145 (66)	47 (66)	0.002	0.964
≥4	75 (34)	24 (34)		
Deep stromal invasion				
Negative	110 (51)	24 (35)	5.321	0.021[a]
Positive	107 (49)	45 (65)		
LVSI				
Negative	221 (97)	65 (90)	4.050	0.044[a]
Positive	7 (3)	7 (10)		
Positive parametrium				
Negative	225 (99)	68 (94)	2.656	0.103
Positive	3 (1)	4 (6)		
Positive surgical margin				
Negative	215 (94)	63 (87)	3.721	0.054
Positive	13 (6)	9 (13)		
Pelvic lymph node metastasis				
Negative	209 (92)	44 (61)	38.668	<0.001[a]
Positive	19 (8)	28 (39)		
Recurrence				
Negative	201 (88)	58 (81)	2.680	0.102
Positive	27 (12)	14 (19)		

[a]P<0.05. SLP-2, stomatin-like protein 2; SCCA, squamous cell carcinoma antigen; FIGO, International Federation of Gynecology and obstetrics; LVSI, lymphovascular space involvement.

SLP-2 expression is associated with the prognosis of patients with cervical cancer. Analysis of patient survival was conducted in order to determine whether SLP-2 expression was associated with the survival time. As presented in Fig. 3, the duration of survival was significantly different between the patients with low/none and high SLP-2 expression levels (log-rank test, χ^2=11.615; P=0.001), with the high SLP-2 expression group exhibiting a shorter overall survival time, indicating that the expression of SLP-2 was inversely correlated with survival time. The cumulative five year survival rate was 92% in the low/none SLP-2 expression group, whereas it was 78% in the high SLP-2 expression group. Furthermore, multivariate COX analysis was performed to determine whether the SLP-2 expression level is an independent prognostic factor of patient

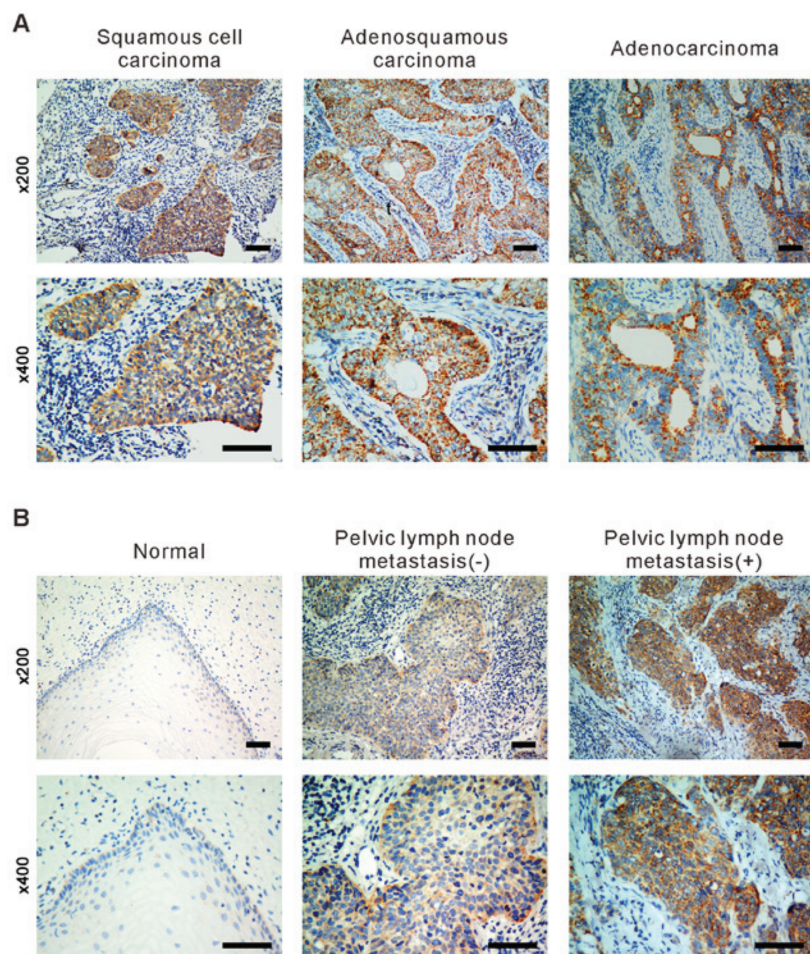

Figure 2. Overexpression of SLP-2 in cervical cancer specimens. (A) Representative immunohistochemistry images of SLP-2 expression in various subtypes of cervical cancer specimens (B) Representative immunohistochemistry images of SLP-2 expression levels in normal cervical tissues and in patients with lymph node metastasis, compared with those with no lymph node metastasis. Scale bars represent 50 μm. SLP-2, stomatin-like protein 2.

outcome. As presented in Table IV, SLP-2 expression levels were identified as independent prognostic factors for patients with cervical cancer (P=0.001; relative risk=3.881). Taken together, the data suggests that SLP-2 may be a novel and potentially useful independent biomarker for the prognosis of patients with cervical cancer.

Downregulation of SLP-2 enhanced cellular apoptosis. SLP-2 expression levels were revealed in the present study to correlate with SCCA, deep stromal invasion, LVSI and pelvic lymph node metastasis. The function of SLP-2 is closely associated with the mitochondrial membrane (9,14); therefore, it has been suggested by the present study that SLP-2 serves a role in the resistance of cervical cancer cells to apoptosis. Endogenous SLP-2 in HeLa and Hcc94 cells was first knocked down by using specific siRNAs and the sensitivity of the modified cells to apoptosis was evaluated, in the current study. As presented in Fig. 4A, three siRNAs knocked down endogenous SLP-2 protein in cervical cancer cell lines. For subsequent evaluation, siRNA#3 was selected due to its higher efficiency. The apoptotic nature of induced cell death was confirmed by TUNEL and Annexin V binding assays on SLP-2 knocked down cells and control cells treated with 25 μg/ml cisplatin, a widely used chemotherapeutic drug for cervical cancer treatment (Fig. 4B and C).

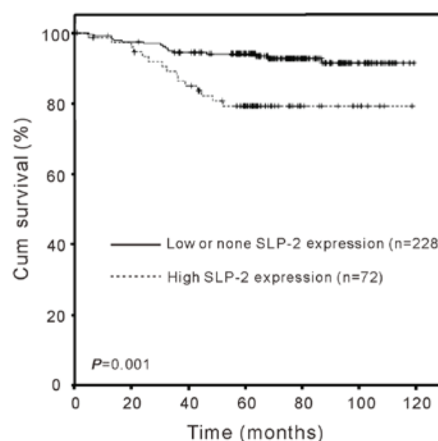

Figure 3. Overall survival rate of high expression level SLP-2 and low/no expression level of SLP-2 in 300 patients with cervical cancer. SLP-2, stomatin-like protein 2.

TUNEL and Annexin V binding assays revealed that the number of apoptotic cells, when treated with cisplatin in SLP-2 knocked-down cells was significantly higher than that in control cells. Taken together, the results indicated that cellular depletion of SLP-2 impaired the ability of cervical cancer cells to resist cisplatin-induced cell death.

Table III. Multivariate analysis of risk factors of lymph node metastasis.

Parameters	B	Wald	P-value	OR	95% confidence interval
SCCA	1.679	12.217	<0.001[a]	5.361	2.091-13.745
Tumor size	0.464	0.792	0.374	1.591	0.572-4.424
LVSI	1.084	2.004	0.157	2.956	0.659-13.253
Deep stromal invasion	0.372	0.665	0.415	1.451	0.593-3.553
Positive parametrium	2.788	3.252	0.071	16.255	0.785-336.589
SLP-2 expression level	1.918	19.797	<0.001[a]	6.810	2.925-15.855
Age	-0.147	0.115	0.734	0.863	0.369-2.017
FIGO stage	-0.196	0.548	0.459	0.822	0.489-1.382
Histological type	0.856	3.425	0.064	2.355	0.951-5.832
Differentiation grade	-0.064	0.033	0.856	0.938	0.469-1.877
Surgical margin	-1.110	0.925	0.336	0.329	0.034-3.166

[a]P<0.05. B, regression coefficient; OR, odds ratio; SCCA, squamous cell carcinoma antigen; LVSI, lymphovascular space involvement; SLP-2, stomatin-like protein 2; FIGO, International Federation of Gynecology and Obstetrics.

Table IV. Multivariate survival analysis of patients with cervical cancer.

Parameters	B	Wald	P-value	RR	95% confidence interval
Age	0.102	0.059	0.807	1.108	0.486-2.523
FIGO stage	-0.220	0.585	0.444	0.802	0.457-1.410
Tumor size	0.145	0.072	0.789	1.156	0.400-3.343
SCCA	0.206	0.188	0.665	1.229	0.483-3.127
SLP-2 level	1.356	10.276	0.001[a]	3.881	1.694-8.894
Histological type	0.526	1.874	0.171	1.693	0.797-3.597
Differentiation grade	-0.254	0.603	0.438	0.776	0.409-1.473
Deep stromal invasion	0.337	0.578	0.447	1.401	0.587-3.343
Positive parametrium	11.726	0.005	0.941	123,740.450	0.000-1.163E140
Surgical margin	-10.578	0.004	0.947	0.000	0.000-2.385E130
LVSI	0.457	0.392	0.532	1.579	0.378-6.603

[a]P<0.05. B, regression coefficient; Wald, wald odd-even space method; RR, relative risk; FIGO, International Federation of Gynecology and Obstetrics; SCCA, squamous cell carcinoma antigen; SLP-2, stomatin-like protein 2; LVSI, lymphovascular space involvement.

Discussion

In the present study, the expression of SLP-2 was revealed to be upregulated in cervical cancer at the mRNA and protein level, in comparison with normal cervical tissue expression levels. Meanwhile, western blotting and RT-qPCR analysis demonstrated the overexpression of SLP-2 in cervical cancer cell lines, when compared with their normal counterparts. A previous study revealed that SLP-2 is overexpressed in numerous types of human cancer tissues (10-12). In addition, the present study revealed that SLP-2 downregulation enhances the sensitivity to apoptosis inducers in cancer tissues. Therefore, it was concluded that SLP-2 may be fundamentally important in human tumorigenesis.

Based on the National Comprehensive Cancer Network (NCCN) guidelines, for patients with early stage disease who possess negative lymph nodes following surgery and pathologic risk factors including large primary tumor size, deep stromal invasion and LVSI, pelvic radiation is recommended (15-17). In the present study, it was demonstrated that the survival time was significantly different between patients with low/none and high SLP-2 expression. Additionally, multivariate COX analysis further confirmed that SLP-2 expression level is an independent prognostic factor of patient outcome. Furthermore, the present study revealed that SLP-2 protein expression correlated significantly with the previously mentioned pathologic risk factors including deep stromal invasion and LVSI. As a result, it was suggested that high SLP-2 expression is an important pathologic risk factor for determining the necessity of pelvic radiation following surgery.

Patients with stage IB or IIA tumors usually undergo surgery, however radiation therapy or concurrent chemoradiation may be the chosen method of treatment (18,19). A number of specialists suggest that if the lymph nodes are positive,

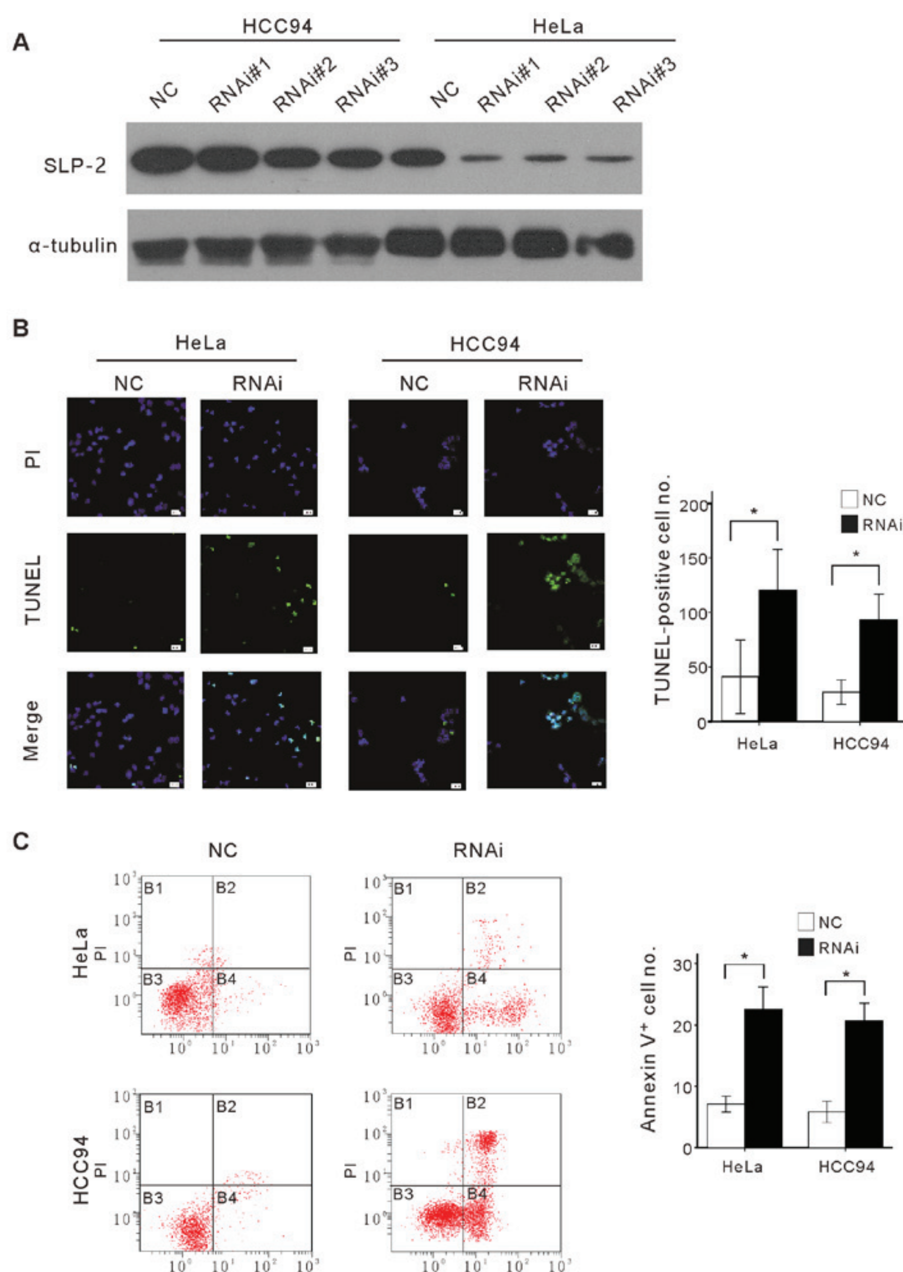

Figure 4. Downregulation of SLP-2 by siRNA enhances cisplatin-induced apoptosis of cervical cancer cells. (A) Expression of SLP-2 was examined in the indicated cells, α-tubulin was used as a loading control. (B) Representative immunofluorescent images (left) and quantification (right) of TUNEL stained cells, in the indicated cells. The number of TUNEL positive cells was counted from 10 random fields and presented as a percentage of the total cell numbers. *P<0.05. (C) Representative Annexin V^{+}/PI^{-} images (left) and quantification (right) of Annexin V^{+}/PI^{-} cells in the indicated cells. Data represents three independent experiments with similar results. *P<0.05. SLP-2, stomatin-like protein 2; PI, propidium iodide; NC, negative control; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling.

surgery should be abandoned and the patients should receive chemoradiation (20). Therefore, if pathological factors predict the pelvic lymph node metastasis, gynecologic oncologists may select the method of treatment to avoid unnecessary surgical intervention. Currently, there are no efficient techniques for diagnosing lymph node metastasis, particularly for those are smaller than 0.5 cm. (21-23) The present study demonstrated that high expression of SLP-2 was closely associated with lymph node metastasis. In addition, logistic regression analysis revealed that SLP-2 protein expression in cervical cancer was an independent risk factor for lymph node metastasis. Therefore, if SLP-2 expression levels are high, chemoradiation may be the better option for patients with suspected lymph node metastasis diagnosed by computed tomography or magnetic resonance imaging scans. Taken together, the results suggest that SLP-2 may be a novel and potential biomarker that may assist in guiding treatment.

SLP-2 is expressed in a number of normal types of cell in addition to cancer cells; it is associated with the inner mitochondrial membrane and faces the intermembrane space (9,24-27). The function of SLP-2 association with the mitochondrial membrane marks a suitable starting point for investigating the potential role of SLP-2 in tumorigenesis. It is well known that mitochondria serve an important role in the regulation of apoptosis, which indicates that mitochondria could determine cell outcomes (28,29). Four major

events are involved in this process, including the regulation of calcium concentration within the cytoplasm, modification of mitochondrial membrane permeability, dissipation of mitochondrial transmembrane potentials and the alteration of mitochondrial functions by Bcl-2 family members (29). Previous studies revealed that SLP-2 contributes to mitochondrial membrane stability and regulates the functions of its ion channels (24,25). SLP-2 interacts with the mitochondrial fusion mediators mitofusin 1, mitofusin 2 and optic atrophy 1, which may participate in mitochondrial fusion (9,26). Hajek *et al* (9) demonstrated that knockdown of SLP-2 by the siRNA approach reduced the mitochondrial membrane potential. Da Cruz *et al* (24) demonstrated that SLP-2 serves a role in the regulation and stability of mitochondrial proteins, including prohibitins and subunits of the respiratory chain complexes. Da Cruz *et al* (25) also revealed that SLP-2 negatively modulates the mitochondrial sodium-calcium exchange. As aforementioned, elevation of the calcium concentration participates directly in signal transduction and performance of early apoptosis via the Ras-mitogen-activated protein kinase (MAPK) signaling pathway (30,31). Mitochondria serve an important functional role at the intracellular calcium level but the underlying mechanisms require further investigation. SLP-2, located in the mitochondrial membrane, is able to stabilize the mitochondrial membrane and regulate mitochondrial ion channels (24-26). The present study revealed that silencing of SLP-2 induces apoptosis in cervical cancer cell lines, indicating that SLP-2 may inhibit apoptosis by affecting mitochondrial membrane permeability and regulating the internal flow of calcium ions. Further study into the regulation of calcium and the Ras-MAPK signaling pathway by SLP-2 is required.

In conclusion, SLP-2 serves an important role in apoptosis and is closely associated with the occurrence and development of cervical cancer; therefore, it may be a potent diagnostic marker and therapeutic target.

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