Expression and Significance of SHP-2 in Human Papillomavirus Infected Cervical Cancer* 

Fei MENG (孟 斐), Xiaoyun ZHAO (赵晓云), Shulan ZHANG (张淑兰)

1Department of Obstetrics and Gynecology, Shengjing Hospital, China Medical University, Shenyang 110004, China
2Department of Microbiology and Cell Biology, Shenyang Pharmaceutical University School of Life Science and Biopharmaceutics, Shenyang 110016, China

Summary: This study investigated the expression and prognostic value of SHP-2 in cervical cancer caused by human papillomavirus (HPV) infection. Forty-five specimens from patients with cervical cancer (stage I-III), 32 specimens from patients with cervical intraepithelial neoplasia (CIN) (I, II) and 20 normal cervical samples from patients with hysteromyoma were collected in Department of Pathology for comparison. The expression levels of SHP-2 and IFN-β proteins were detected by using immunohistochemistry. The mRNA expression level of SHP-2 was detected by using quantitative real-time polymerase chain reaction (PCR). HPVs were detected by HPV GenoArray Test. The Spearman correlation was used to compare the expression level of SHP-2 in HPV infected cervical cancer vs non-HPV infected normal cervix. The level of SHP-2 protein expression in the cancer tissues (88.8%) was significantly higher than in CIN tissues (62.5%) and normal cervixes (45%) (P<0.05 and P<0.05, respectively). The SHP-2 mRNA levels in the cancer tissues were upregulated as compared with those in the normal cervixes (P<0.05). Twenty-one (46.7%) cervical cancers, 25 (78.1%) CINs and 17 (85%) normal cervixes showed IFN-β positive staining in cytoplasm. There was statistically significant difference in the expression rate of IFN-β between cervical cancer and normal cervix (χ²=8.378, P<0.05) as well as between cervical cancer and CIN (χ²=7.695, P<0.05). HPV16/18 infections could be found in normal cervixes (15%), CINs (68.7%) and cervical cancers (84.4%). There was a correlation between HPV infection and SHP-2 expression in cervical cancer (r=0.653, P<0.05). SHP-2 may be a useful prognostic and diagnostic indicator for HPV infected cervical cancer. In cervical cancers, SHP-2 mRNA and protein overexpression was associated with IFN-β lower-expression.

Key words: cervical cancer; cervical intraepithelial neoplasia; human papillomavirus; SH2-containing protein tyrosine phosphatase 2; type I interferon β

SHP-2 is an evolutionarily conserved protein tyrosine phosphatase, containing two SH2 domains at the N terminus, a central catalytic domain, and a C-terminal tail[1, 2]. SHP-2 positively regulates the signaling pathways of cytokines and growth factors, such as insulin, EGF, PDGF, FGF, IL-1, and IL-6[3-6]. Over 99% of cervical cancer cases are a result of human papillomavirus (HPV) infection[7], and of those approximately 70% are a result of infection with HPV16/18[8]. A vaccine targeting quadrivalent HPV types 6, 11, 16 and 18[8] (Human Papillomavirus L1 Viruslike Particle Vaccine from Merck & Co., Inc.) has been approved by the USA Food & Drug Administration to prevent HPV associated diseases. Despite the fact that a causal relationship exists between HPV infection and cervical cancer, the exact mechanism how HPV induces cervical cancer is still largely unknown. SHP-2 may also have an inhibitory role in activation of T and B lymphocytes[9].

It has been reported that infection by HPV may interfere with MAPK cellular signal transduction via Erk, JNK/SAPK, p38/RK and B MK1/E rk5 in keratinocytes[10]. Little is known about the role of protein phosphatases, i.e., SHP-2 in cervical cancer after HPV infection. SHP-2 (also known as PTPN11) is another member of the non-receptor protein tyrosine phosphatases[11], and is thought to participate in a variety of cytokine- and growth factor-initiated signal transduction processes[12-14]. SHP-2 acts as a downstream of receptor and cytoplasmic tyrosine kinases to propagate signal relay. SHP-2 plays a positive regulatory role in signal transduction, and has been reported to stimulate cell proliferation and differentiation[15-17]. In order to explore whether there are changes of SHP-2 in cervical cancer and examine their relationship to HPV infection, 45 cases of cervical cancer, 32 cases of cervical intraepithelial neoplasia (CIN), and 20 cases of normal cervix were enrolled in this study. HPV16/18 infections were identified by GenoArray Test. The expression of SHP-2 and IFN-B was examined by using immunohistochemistry. Results support a putative role of SHP-2 in cervical cancer pathogenesis after HPV infection.
1 MATERIALS AND METHODS

1.1 Tissue Specimens

Forty-five consecutive cases of cervical cancer (stage 1-Ⅲ) were entered in this study. There were 30 cases of squamous cell carcinoma and 15 cases of adenocarcinoma. Patient age ranged from 30 to 70 years, with an average age of 48 years. Cervical tissue was excised and the diagnosis was confirmed at Department of Pathology, Shengjing Hospital, Shenyang, China. Thirty-two cases of CIN (Ⅰ, Ⅱ) (age 30–60 years, mean 42 years), and 20 normal cervical samples from patients with myoma of uterus (age 30–62 years, mean 45 years) were collected in Department of Pathology for comparison. Before the selections, they have not accepted any treatment.

1.2 Immunohistochemistry

Sections (4-6 μm in width) of the archival paraffin-embedded blocks were cut, deparaffinized, and stained using a streptavidin-biotin immunoperoxidase (SP) technique. Primary antibodies were applied: anti-IFN-β monoclonal antibody (BIOS Biotec, China) at a 1:200 dilution, anti-SHP-2 monoclonal antibody (Santa Cruz Biotec, USA) at a 1:500 dilution. 3, 3-Diaminobenzidine (DAB) was employed as a chromogen. Sections were counterstained with hematoxylin.

Staining evaluation was scored as negative, weak, or strong depending on the percentage and the intensity of the staining in the tumor cells (A: no staining, 0 score; light yellow staining, 1 score; moderate yellow staining, 2 score; strong yellow staining, 3 score. B: the positive percent in the respective lesions cells <5%, 0 score; between 5%-25%, 1 score; between 26%-50%, 2 score; between 51%-75%, 3 score; >75%, 4 score). A final score between 0 and 12 was achieved by multiplication of the extent of positivity and intensity. Scores of 9–12 were considered as “strong expression”, scores of 5–8 as “weak expression”, and scores of 0–4 as “no expression”, as mentioned by Hao[18]. SHP-2 expression was mainly detectable in the nucleus. Sections were counterstained with hematoxylin.

1.3 Quantitative Real-time PCR for SHP-2

The tissue samples were processed as the same with immunohistochemistry. They were all obtained from tissue specimens mentioned at the head of the article. Total RNAs were extracted by using Trizol method, and cDNA was synthesized.

The tissue samples were taken from surgical specimens after excision. Usually, it took about 10 min between the excision procedures and the tissue sample being kept in the liquid nitrogen. All samples were stored in the -80°C refrigerator within 6 months before QPCR manipulation. Each sample was kept independently in the aseptic vial, and there was little chance for contamination or degradation of those tissue samples.

RNA was reversely transcribed using a RNA PCR Kit (TaKaRa, Japan). Real-time quantitative PCR was performed using the Light Cycler PCR and detection system (Roche Diagnostics, Germany). In brief, the PCR amplification reaction mixtures (20 μL) contained cDNA, primer pairs, ddH2O, and Brilliant SYBR® Green QPCR Master mix (TaKaRa, Japan). The thermal cycle conditions were 45 cycles of 94°C for 20 s, 94°C for 20 s and 58°C for 20 s. PCRs were performed in triplicate. The relative fold changes were calculated with the following formula: \(2^{-\Delta \Delta Ct}\), \(\Delta Ct=Ct_{\text{target}}-Ct_{\text{β-actin}}\), which reflected the target gene expression normalized to β-actin levels. The mean expression level of SHP-2 in normal tissues was used as the control and considered as an value of 1.0, as mentioned by Jarboe[19]. The fold increase or decrease in SHP-2 expression was determined for each control and tumor sample and expressed as \(\Delta \Delta Ct\).

1.4 HPV GenoArray Test

HPV GenoArray Test is a routine inspection item for in-patients with cervical disease. The cytology samples were taken from the in-patients before surgery. The kit can detect 21 types of HPV, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 42, 43, 44, 53, 66, CP8304. The test uses a macroarray format with a nylon membrane onto which HPV genotype-specific oligonucleotides probes have been immobilized. The final results were detected by colorimetric change on the chip under direct visualization (table 3).

1.5 Statistical Analysis

SHP-2/IFN-β protein levels among cervical cancer, CIN and normal cervix were analyzed by using \(\chi^2\) test, with a value of \(P<0.05\) considered to be statistically significant. Spearman correlation was used to examine the correlation between HPV infection and positive expression of SHP-2. The SHP-2 mRNA levels among cervical cancer, CIN and normal cervix were analyzed by using analysis of variance, with a value of \(P<0.05\) considered to be statistically significant.

2 RESULTS

2.1 Expression of SHP-2 in Cervical Cancer, CIN and Normal Cervix

SHP-2 expression was mainly diffused in the cytoplasm, and weak expression was detectable in the nucleus. SHP-2 immunostaining was found in 40 (88.8%) cervical cancer samples. Only 20 (62.5%) CIN cases and 9 (45%) normal cervix cases showed SHP-2 positive staining in basal cells. There was statistically significant difference in the expression rate for SHP-2 between cervical cancer and normal cervix (\(\chi^2=12.105, P<0.05\)) as well as between cervical cancer and CIN (\(\chi^2=7.569, P<0.05\)). Though the positive SHP-2 expression rate in CIN was higher than that in normal cervix, the difference was not statistically significant (\(\chi^2=1.528, P>0.05\)) (table 1, fig. 1). There was no statistically significant difference in the SHP-2 expression in cervical cancer between squamous cell carcinoma and adenocarcinoma (\(P>0.05\)).

Table 1 The SHP-2 expression in normal cervix, CIN, and cervical cancer tissues

<table>
<thead>
<tr>
<th>Pathologic diagnosis</th>
<th>SHP-2 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Normal cervix</td>
<td>6</td>
</tr>
<tr>
<td>CIN</td>
<td>11</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>18</td>
</tr>
</tbody>
</table>

Data shown are the number of cases. “+”, “++” or “-” denotes “weak expression”, “strong expression” or “no expression” of SHP-2 in the indicated tissues, respectively.