



Expressions of Src homology 2 domain-containing phosphatase and its clinical significance in laryngeal carcinoma

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ABSTRACT. We investigated the expression of Src homology 2 domain-containing phosphatase (SHP-2) in laryngeal carcinoma and its clinical significance. Expression of SHP-2 was detected by immunohistochemical staining in normal mucosal tissues and various grades of laryngeal carcinoma. We looked for possible correlations between expression of SHP-2 in laryngeal carcinoma and clinical staging and lymph node metastasis. Immunohistochemical staining results revealed that the SHP-2 expression was significantly higher (88.24%) in laryngeal carcinoma than in normal mucosal tissue (25%). Additionally, the expression of SHP-2 was significantly correlated with lymph node metastasis, but not with clinical stage and gender of patients with laryngeal carcinoma. Therefore, SHP-2 may be useful as a prognostic marker for laryngeal carcinoma and as a therapeutic target in laryngeal carcinoma treatment.

Key words: Src homology 2 domain-containing phosphatase (SHP-2); Laryngeal carcinoma; Immunohistochemistry

INTRODUCTION

Src homology 2 domain-containing phosphatase (SHP-2), an important protein-tyrosine phosphatase (PTP), acts as a downstream intracellular signal molecule for a number of extracellular stimuli, including cytokines and growth factors. SHP-2 is widely expressed in tissues and plays an important role in the signal transduction associated with cell proliferation and survival (Qu, 2000).

The correlation between the expression of SHP-2 and non-small cell lung cancer, leukemia and prostatic cancer has been identified in a number of studies worldwide (Tartaglia et al., 2003, 2004; Bentires-Alj et al., 2004; Loh et al., 2004b). It has also been demonstrated that the expression of SHP-2 is increased in breast cancer, leukemia and solid skin tumors. Further molecular biology research has identified the conspicuous correlation between SHP-2 expression and tumor presentation, and it is now a hot spot for clinical diagnosis and tumor treatment.

Laryngeal carcinoma (LC) is a common malignant tumor of the head and neck, which has serious effects on human health. To the best of our knowledge, the function of SHP-2 in the progression and development of LC has not been studied. Our study begins to research this point with the aim of exploring the importance of variation in the expression of SHP-2 in the development and morbidity of LC.

MATERIAL AND METHODS

Source of specimens

LC tissue samples were collected from 17 patients seen by our hospital's Hematology Department and wards during January 2008 to May 2011. None of the patients had received chemotherapy and radiotherapy before operations. Thirteen cases were males and the other 4 were females, and the average age of patients was 57.8 years. LC was classified according to the Union Internationale Contre le Cancer (UICC) TNM classification of 2010. The clinical stage of LC were characterized as follows: I, 2 cases; II, 3 cases; III, 5 cases; and IV, 7 cases. Lymph node metastasis was pathologically confirmed in 7 cases and in the rest not. All isolated specimens were handled within 2 h; they were fixed in 10% neutral formalin for 24 h, paraffin-embedded and hematoxylin and eosin (HE)-stained, and the pathological properties and grade were ascertained. All patients gave informed consent before participating, and the study was approved by the local research ethics committee.

Reagents

SHP-2 rabbit antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The other reagents were conventional reagents from our office section.

Histological and immunohistochemical staining

The specimens were fixed and paraffin-embedded, and serial sections (5 μ m) were cut and submitted to HE staining and PTP antibody (anti-SHP-2) immunostaining according to the manufacturer instructions. The results were graded according to the scoring system used by

Zhang et al. (2009). Cells stained with brown granules in the cytoplasm were graded as SHP-2 positive. Thus, the degree of SHP-2 staining could be assessed using the intensity and the percentage of positively stained cells. The staining intensity was classified into four groups: 0, unstained; 1, weakly stained; 2, moderately stained; and 3, strongly stained. A total of 10 typical high-power fields were selected, and 100 cells were randomly counted to calculate the percentage of positively stained cells. The specimens were graded: 0, for less than 5%; 1, for 5 to 25%; 2, for 25 to 50%; and 3, for greater than 50%. Subsequently, we multiplied the staining intensity score by the grade for percentage of positive cells. A results of 0-1 was defined as negative (-), 2-3 as weak positive (+), 4-5 as positive (++), and 6 or greater as strong positive (+++).

Statistical analysis

The statistical analysis was conducted using SPSS 10.0 (SPSS, Inc., Chicago, IL, USA) using one-way ANOVA. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Expression and distribution of SHP-2 in LC tissues

In normal mucosa tissues, a pale yellow staining was observed in transitional epithelial cells, representing a low expression of SHP-2. However, in LC tissues a simple light or dark brown colored staining was observed in all pathological stages, representing a high expression of SHP-2 (Figure 1).

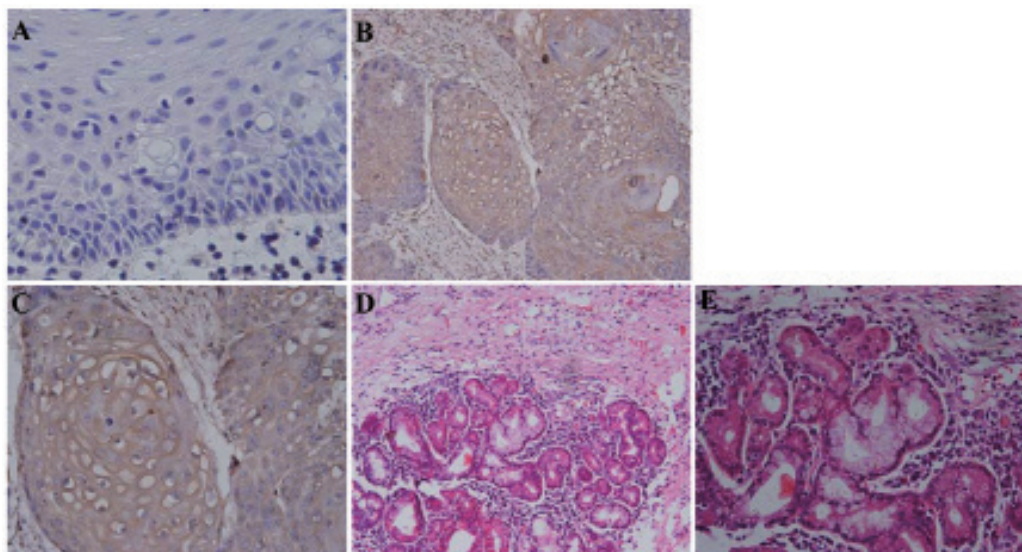


Figure 1. Histological and immunohistochemical staining detection of the expression and distribution of SHP-2 in laryngeal carcinoma (LC) tissues. **A.** Normal mucosa (400X magnification). **B.** and **C.** LC tissues (200X and 400X magnification, respectively). **D.** and **E.** LC tissues stained with hematoxylin and eosin (200X and 400X magnification, respectively).

Positive expression rate of SHP-2

Among the 17 LC tissue samples, a total of 15 cases were SHP-2 positive, yielding a positive expression rate of 88.24%. Among the 4 normal mucosa tissue samples, only 1 case displayed a positive expression of SHP-2, yielding a positive expression rate of 25%. The variant expression rate of SHP-2 between LC and normal mucosa tissues was analyzed using the Student *t*-test and the difference was considered to be statistically significant ($P < 0.05$; Table 1).

Table 1. Expression of SHP-2 in laryngeal carcinoma and normal mucosal tissues.

Tissue	Number of cases	Expression of SHP-2				Positive rate (%)	P value
		-	+	++	+++		
Laryngeal carcinoma	17	2	3	5	7	88.24	<0.05
Normal mucosal tissue	4	3	1	0	0	25	

Compared with patients without lymph node metastasis, the positive expression rate of SHP-2 was significantly higher in tissues of patients with lymph node metastasis ($P < 0.05$; Table 2). Additionally, the positive expression of SHP-2 was not significantly correlated with clinical stage or gender of patients with LC ($P > 0.05$; Table 2).

Table 2. Relationship between expression of SHP-2 and clinical stage, lymph node metastasis, and gender of laryngeal carcinoma.

	Number of cases	Expression of SHP-2			P value
		Negative	Positive	Positive rate (%)	
Clinical stage					
I-II	5	1	4	80	>0.05
III-IV	12	1	11	91.67	
Lymph node metastasis					
+	7	0	7	100	<0.05
-	10	2	8	80	
Gender					
Male	13	2	11	84.62	>0.05
Female	4	0	4	100	

Correlation between the expression of SHP-2 in LC tissues and the prognosis of patients

Among the 17 LC cases studied, only 7 mortalities occurred, all of which demonstrated a positive SHP-2 expression. Therefore, the survival rate for the SHP-2 positive tissues was 53.33% (7/15). A *t*-test demonstrated that there was a marked difference between the two groups ($P < 0.05$). The survival rate was significantly higher in patients with negative SHP-2 expression compared to patients with positive expression.

DISCUSSION

LC, a common malignancy in the head and neck region, presents a serious health threat. It accounts for approximately 1.2~1.6% of the total malignant tumor cases. At the same time, it contributes to approximately 7.9~35% of ear, nose and throat (ENT) cancers. Statistics have shown that the incidence of LC is approximately 4/100,000 cases. The morbidity of LC

varies greatly in different regions of the world.

In certain countries, the morbidity level is up to 17.7/100,000 cases. Statistical reports have revealed that the incidence of LC is more common in urban areas compared to the countryside. In recent years, the morbidity of LC exhibited a rising trend as a result of the harmful effects caused by an increased exposure to various environmental carcinogenic factors. In reality, patients in stage III-IV, and those with recurrence or metastasis, do not respond well to existing treatments; thus, LC has become a malignant disease which is life-threatening for numerous individuals. With further research in molecular biology, the study of the correlation between tumors and SHP-2 is of particular interest and has become a significant factor in clinical diagnosis and oncotherapy (Qu et al., 1997; Guillemot et al., 2000; Su et al., 2001; Qu, 2002).

Complicated signal transduction pathways are involved in carcinogenesis and the growth and development of cells (Qu et al., 1998). A large number of signal molecules are implicated in these pathways, in which phosphorylation is a basic biochemical process. Studies have revealed that carcinogenesis increases in accordance with the rise in the levels of protein phosphorylation (Qu et al., 2001). Therefore, we conjectured that the protein phosphorylation level may be the key factor associated with the proliferation and differentiation mechanism of tumors. Additionally, the intracellular protein phosphorylation level is regulated by the mutual coordination of PTP and protein tyrosine kinase (PTK). Thus, it has been suggested that PTP is closely correlated with tumorigenesis. Studies have confirmed that SHP-1 and SHP-2 are first expressed in hematopoietic cells and lymphocytes. They play various roles in signal transduction as well as cell proliferation and differentiation; however, a disorder in cell proliferation and differentiation is a characterization of tumor cells.

The role of SHP-2 in signal transduction remains unclear; however, it may have certain upregulatory effects. It is possibility that the negative regulation of SHP-1 maintains a dynamic equilibrium with the positive regulation of SHP-2. Once the dynamic equilibrium is impaired, it would directly influence cell proliferation and signal transduction. The present study demonstrated that the expression of SHP-2 is significantly increased in LC tissues, with no marked signs of a shift in normal mucosa tissues. This suggests that when the normal transitional epithelial tissues near the LC undergo malignant transformation, the expression of SHP-2 significantly increases at the same time. Therefore, the expression of SHP-2 may be a clinical diagnostic index for the malignant transformation of transitional cells.

Our results revealed that the positive expression rate of SHP-2 is significantly higher in tissues of patients with lymph node metastasis compared with patients without lymph node metastasis, suggesting that the positive expression of SHP-2 is positively correlated with lymph node metastasis. However, further statistical analysis for the correlation between the expression of SHP-2 in LC and gender and clinical staging revealed that expression of SHP-2 was not significantly correlated with the clinical stage or gender of patients with LC. SHP-2, as an important PTP, is a vital molecule in cell proliferation and survival-related signal transduction (Henshall et al., 2001). Among those malignant tumors for example lung solid tumors, colon solid tumors, nervous system solid tumors and skin solid tumors, the activating mutations of SHP-2 is commonly found (Loh et al., 2004a).

Other studies have indicated that SHP-2 may be a useful target to regulate Bcr-Ab1-positive leukemia and chronic myeloid leukemia (Chen et al., 2007). Certain studies have revealed that SHP-2 may play a positive role in regulating the receptor-mediated signaling pathway of receptor tyrosine kinase. Additionally, SHP-2 may also partially block signal transduction via dephosphorylation of signal transduction molecules (Charest et al., 2006). Although the function of SHP-2 in the progres-

sion and development of laryngeal neoplasms has not been confirmed, there are a number of studies identifying its impact on other forms of tumorigenesis; for example, the association between changes in SHP-2 expression and non-small cell lung cancer, leukemia and prostate cancer (Lieskovska et al., 2006; Jamshidi et al., 2007). The present study tentatively explored the role of SHP-2 in LC, and it confirmed that the expression level of SHP-2 in LC tissues was significantly higher compared to that of normal tissues. In the future, the biological correlation between the increased expression of SHP-2 and cell proliferation and apoptosis in LC cells requires extensive studies.

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