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ORIGINAL ARTICLE

Prognostic Value of Elevated SHIP2 Expression in Laryngeal Squamous Cell Carcinoma

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Background and Aims. SHIP2, an important negative regulator of insulin signaling, has recently been found to be involved in cancer development and progression.

Methods. In this study, immunohistochemistry was performed to investigate SHIP2 expression in laryngeal squamous cell carcinoma (LSCC) clinical samples. Additionally, the relationship of SHIP2 expression to clinicopathological parameters and prognosis was investigated.

Results. SHIP2 expression was detected in 42 (42/54, 77.8%) primary tumor samples but only in three (3/16, 18.75%) adjacent normal samples ($p < 0.001$). Moreover, SHIP2 expression was closely associated with T classification ($p = 0.006$), clinical stage (I + II/III + IV) ($p = 0.001$), metastasis ($p = 0.002$) and recurrence ($p = 0.004$). Survival analysis revealed that high SHIP2 expression was significantly associated with shorter disease-free and overall survival (both $p < 0.001$). When lymph node status and SHIP2 expression were combined, lymph node-positive patients with SHIP2 overexpression had both poorer disease-free and overall survival than the others (both $p < 0.001$). Multivariate analysis further demonstrated that SHIP2 was an independent prognostic factor for patients with LSCC.

Conclusions. Collectively, these results support the hypothesis that SHIP2 may play a critical role in the initiation and progression of LSCC and may serve as both a prognostic marker and a potential therapeutic target in patients with LSCC.

Key Words: Laryngeal squamous cell carcinoma, SHIP2, Metastasis, Recurrence, Prognosis.

Introduction

Laryngeal squamous cell carcinoma (LSCC), the most frequent malignant neoplasm of the upper airway in adults, accounts for ~1.5% of all cancers (1,2). Although patients benefit from multimodality treatment including surgical management, chemotherapy, radiotherapy and biological therapy, limited improvement in 5-year survival has been achieved during the last few decades. Cancer metastasis and recurrence account for the low survival rates after surgical resection of LSCC (3,4). Therefore, discovery of LSCC-related metastasis genes and their mechanisms is of great importance for the development of novel strategies in the prevention and treatment of patients with LSCC.

Phosphatidylinositol lipids are membrane-bound second messengers that transmit intracellular signals triggered by a variety of external stimuli such as growth factors [e.g., epidermal growth factor (EGF)], hormones (e.g., insulin) and extracellular matrix (ECM) proteins, and therefore play significant roles in both diabetes and cancer (5). Phosphatidylinositol-3,4,5-trisphosphate (PIP3), produced by phosphoinositide 3-kinases (PI3Ks), controls a variety of functions including proliferation, survival, migration and differentiation of many cell types (6,7). A 3′-phosphoinositol

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Table 1. Clinicopathological features of LSCC patients

Variables	No. of patients	Percentage (%)
Age (years)		
<58	32	59.3
≥58	22	40.7
Gender		
Female	3	5.6
Male	51	94.4
Alcohol intake		
Yes	31	57.4
No	23	42.6
Tobacco use		
Yes	30	55.6
No	24	44.4
Tumor site		
Supraglottic	19	35.1
Glottic	33	61.1
Subglottic	2	3.8
Grade		
G1	29	53.7
G2	17	31.5
G3	8	14.8
T classification		
T1	9	16.7
T2	19	35.2
T3	22	40.7
T4	4	7.4
Clinical stage		
I	8	14.8
II	13	24.1
III	23	42.6
IV	10	18.5
Lymph node metastasis		
Yes	20	37.0
No	34	63.0
Recurrence		
Yes	31	57.4
No	23	42.6

LSCC, laryngeal squamous cell carcinoma.

phosphatase, phosphatase homologous to tensin (PTEN) is both a tumor suppressor and a negative regulator of insulin action that dephosphorylates PIP3 and suppresses Akt signaling (8). However, SH2-containing 5′-inositol phosphatase-2 (SHIP2) regulates multiple oncogenic signaling pathways by dephosphorylating PIP3 to produce phosphatidylinositol-3, 4-bisphosphate (PI-3,4-P2). These pathways include the phosphatidylinositol 3-kinase (PI3K) and Akt cascade (9) as well as the Ras and mitogen-activated protein (MAP) kinase cascade (10) which, in turn, regulate cell proliferation, differentiation, and apoptosis.

Given the oncogenic effect of the PI3K-Akt pathway and the tumor suppressor function of PTEN, a role for SHIP2 in cancer development and progression seems highly plausible. Recent publications show that SHIP2 silencing in MDA-231 breast cancer cells reduces cell migration and proliferation *in vitro* and tumor growth and spontaneous lung metastases *in vivo* (11,12). Furthermore, high expression of SHIP2 strongly correlates with poor prognosis of breast cancer (13). These studies indicate that SHIP2 may not only be a potential tumor marker for clinical diagnosis and prognosis in breast cancer but also a candidate therapeutic target that may be used in developing a novel treatment strategy. However, little is known about the role of SHIP2 protein expression in LSCC, particularly with respect to clinical outcome.

To gain better insight into the clinical relevance of SHIP2 in LSCC, the present study aimed to investigate the expression pattern of SHIP2 in LSCC tissue samples and to assess whether SHIP2 expression was correlated with clinicopathological parameters and prognosis in LSCC patients.

Materials and Methods

Patients and Tissue Preparation

A total of 54 patients with LSCC who underwent partial or total laryngectomy at the Department of Otolaryngology Head and Neck Surgery at the Third Xiangya Hospital of Central South University from January 2000–September 2004 were enrolled in this retrospective study. None of the patients had a history of previous malignancies, radiotherapy or chemotherapy. Informed consent was obtained from all patients prior to surgery, and the Research Ethics Committee of Central South University, Changsha, China approved this study.

The main clinical and pathological variables of the patients are described in Table 1. There were 51 males and three females with a mean age of 56.48 years (range: 27–76 years, standard deviation/SD = 10.507). According to the 2002 TNM classification of malignant tumors by the International Union Against Cancer, 19 cases were supraglottic, 33 were glottic and two were subglottic. There were eight stage I cases (T1N0M0), 13 stage II cases (T2N0M0), 23 stage III cases (T3N0M0: 12 cases; T1N1M0: one case; T2N1M0: five cases; T3N1M0: five cases) and 10 stage IV cases (T2N2M0: one case; T3N2M0: five cases; T4N0M0: one case; T4N1M0: two cases; T4N2M0: one case). Using pathological grading criteria, 29 were staged as well differentiated (G1), 17 as moderately differentiated (G2) and eight as poorly differentiated (G3). Twenty patients with lymph node metastasis were validated by conventional postoperative pathological examinations. Thirty one patients experienced tumor recurrence after surgery.

Immunohistochemistry

Immunohistochemical staining was performed using the UltraSensitive S-P IHC Detection Reagent following the manufacturer's recommended protocol (ZhongShan Goldenbridge Bio, Beijing, China). Briefly, antigen retrieval was performed in 10 mmol/L citrate buffer (pH 6.0) for 15 min at 100°C in a microwave oven. Endogenous