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Original contribution

Prognostic significance of stomatin-like protein 2 overexpression in laryngeal squamous cell carcinoma: clinical, histologic, and immunohistochemistry analyses with tissue microarray☆

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Summary Stomatin-like protein 2 (*SLP*-2) is a novel cancer-related gene whose product promotes cell growth, tumorigenicity, and adhesion in human esophageal squamous cell carcinoma. The purpose of this study was to investigate whether *SLP*-2 is overexpressed in human laryngeal squamous cell carcinoma (LSCC) and, if so, the significance of its overexpression in relation to clinical parameters. By analyzing 124 cases of LSCC with a tissue microarray, we concluded that *SLP*-2 is overexpressed in LSCC as compared with the adjacent normal laryngeal epithelium ($P = .000$) and furthermore that *SLP*-2 expression correlates with clinical stage. Overexpression can be regarded as a significant prognostic factor, with higher expression being found in lymph node metastasis.

1. Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common malignant neoplasms of the head and neck. It has various rates of incidence and mortality around the world, with notably high rates in Southeast Asia and Eastern Europe; increasing trends have been observed in many previous studies [1]. Currently, prognostic evaluation is based mainly on tumor site, clinical stage, and histopathologic grade. Recent studies have suggested that other factors, such as the molecular and cellular characteristics of the primary tumor, may improve our ability to predict the clinical course of patients.

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The complete mapping of the human genome has revealed many sequences that were not previously recognized as being associated with cancer. The rapid development of gene array technology has also helped in the identification of genes that are upregulated or downregulated in different types of cancer [2]. These newly identified cancer-related genes may provide diagnostic or prognostic markers and even some new treatment methods for cancer.

In our previous study, complementary DNA (cDNA) microarray analysis was performed with 6 pairs of patient-matched esophageal squamous cell carcinoma (ESCC) tissues, and a cluster of differentially expressed genes was identified. One of them was human stomatin-like protein 2 (*SLP*-2), which was overexpressed 6-fold in cancer tissues as compared with their normal counterparts [3-5]. Human stomatin (band 7.2b) is a 31-kDa erythrocyte membrane protein of unknown function, although it is involved in the control of ion channel permeability, mechanoreception, and lipid domain organization. The *SLP*-2 gene is a novel and unusual member of the stomatin gene superfamily. Its product may link stomatin or other integral membrane proteins to the peripheral cytoskeleton and play a role in regulating ion channel conductance or the organization of sphingolipids and cholesterol-rich lipid rafts [6]. Our previous studies revealed that *SLP*-2 is overexpressed in many cancer tissues, including ESCC, lung cancer, endometrial adenocarcinoma, and LSCC. Antisense *SLP*-2 treatment has been found to decrease cell growth, tumorigenicity, and adhesion in human ESCC [7-9].

On the basis of our previous work, we investigated the expression of *SLP*-2 by reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot analysis in a larger number of samples (46 and 10 fresh paired LSCC tissues, respectively). Simultaneously, we performed immunohistochemistry (IHC) studies on a primary tumor tissue microarray (TMA) consisting of 104 laryngeal carcinoma and 20 adjacent normal laryngeal epithelial tissues to determine whether *SLP*-2 is upregulated in LSCC. We also investigated the relations between *SLP*-2 expression and the clinical characteristics of LSCC, including tumor site, clinical stage, lymph node metastasis, and outcome.

2. Materials and methods

2.1. Tissue specimens

Forty-six fresh paired specimens were taken from patients with LSCC as confirmed by pathologists. To remove differences in genetic background, we chose control tissues from 104 paraffin-embedded specimens of laryngeal carcinoma, from which we then chose 20 certain cases of adjacent normal epithelial tissue for making the TMA. One hundred twenty-four polyformaldehyde-fixed and paraffin-embedded tissue blocks were chosen from the files of the Cancer Hospital of the Chinese Academy of Medical Sciences and Tianjin Medical University Cancer Institute and Hospital covering patients treated from June 2003 through January 2005. None of the patients had received radiotherapy or chemotherapy before surgery. Fresh cancer tissues were dissected from the resected specimens, and the normal tissues were taken from the distal resection margin. All specimens were snap frozen in liquid nitrogen and stored at −80°C until used.

2.2. RNA isolation and semiquantitative RT-PCR

Paired tissues were minced, and total RNAs were extracted with the use of TRIZOL reagent (Invitrogen, Carlsbad, CA) and reverse transcribed to first-strand cDNA with the use of Transcriptase SuperScript II (Preamplification System for First-Strand cDNA kit, Invitrogen). Then, 0.5- to 1-μL aliquots of the cDNAs were used as the template to amplify the *SLP*-2 fragment with primers (forward 5′ -GTGACTCTCGACAATGTAAC-3′ and reverse 5′ -TGATCTCATAACGGAGGCAG-3′) under the following conditions: 95°C for 5 minutes; 28 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds; and, finally, 72°C for 5 minutes. The *GAPDH* gene was used as an internal control.

2.3. Antibody production, protein extraction, and Western blot analysis

Rabbit anti–*SLP*-2 antibody has been produced by our laboratory [8]. Paired tissues were ground and lysed with buffer consisting of 1% sodium dodecyl sulfate, 10-mmol/L Tris-Cl, pH 7.6, 150-mmol/L NaCl, 20-μg/μL aprotinin, 20-μg/μL leupeptin, and 1-mmol/L phenylmethanesulfonyl fluoride. The protein concentrations were determined with the use of a Bicinchoninic Acid Protein Assay kit (Pierce, Rockford, IL). Fifty micrograms of protein was separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. After blocking, the membrane was incubated at 4°C overnight with anti–*SLP*-2 antibody (1:1000 dilution) and anti-actin antibody (mouse 1:1000 dilution, Sigma, St Louis, MO) used as a loading control. After washing, the membrane was incubated with secondary antibody at a dilution of 1:3000 at room temperature for 1 hour. Proteins were detected with an enhanced chemiluminescence kit (Amersham Pharmacia Biotechnology, Inc, Piscataway, NJ).

2.4. Preparation of TMAs

The TMA block was constructed with a Beecher Instruments Tissue Array (Beecher Instruments, Silver Spring, MD) from archival paraffin blocks of radical laryngectomy cases. Recipient blocks were made with purified agar in frames. Holes of 0.6 mm were cut in the recipient blocks with a core needle, and the agar core was discarded. Donor blocks were prepared after thorough evaluation of hematoxylin-eosin–stained slides. After the identification of