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Clinicopathologic significance of EpCAM expression in squamous cell carcinoma of the tongue and its possibility as a potential target for tongue cancer gene therapy

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Summary Epithelial adhesion molecule (EpCAM) is a transmembrane glycoprotein involved in intercellular adhesion. In particular, EpCAM appears to be overexpressed by the majority of human epithelial carcinomas, including colorectal, breast, head and neck, and hepatic carcinomas. We therefore hypothesized that EpCAM would be a good molecular target for cancer gene therapy. EpCAM protein expression in 48 primary tongue cancers and 10 normal oral mucosa was evaluated using anti-EpCAM immunohistochemistry, and correlation was examined with the clinicopathologic factors. In four human tongue cancer cell lines (SAS, HSC-2, OSC19 and OSC20), we investigated EpCAM expression by reverse transcription-polymerase chain reaction (RT-PCR). The invasive potential of cancer cells was evaluated using Matrigel invasion assay. Moreover, the effect of EpCAM inhibition was analyzed using RNA interference (RNAi). EpCAM overexpression was detected in 30 of 48 tongue cancers (62.5%), and was significantly higher in primary squamous cell carcinoma (SCC) of the tongue than in normal oral mucosa. The expression of EpCAM was significantly associated with tumor size, regional lymph node metastasis, histological differentiation and invasion pattern. Cancer cell lines with higher EpCAM expression had more invasive potential. Moreover, RNAi-mediated EpCAM reduction decreased the invasion potential and proliferation activity. These results indicated that the overexpression

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of EpCAM was correlated with a more aggressive phenotype of tongue cancer. Moreover, we suggested that EpCAM could be a molecular target, and that RNAi targeting EpCAM could be useful for tongue cancer gene therapy.

Introduction

The primary modality for tongue cancer therapy is surgery. Although recent advancements in surgical techniques and anticancer agents have improved tumor regression and the survival rate, wide surgical resection of the tongue causes various oral dysfunctions; therefore, new treatment strategies are urgently needed.

The presence of neck regional lymph node metastasis is strongly related to a poor prognosis in squamous cell carcinoma (SCC) of the tongue.[1,2] Some biomarkers predicting the poor prognosis of tongue cancer patients have been reported[3] with several studies showing alterations of adhesion-related molecules in oral cancer.[4–6] The epithelial cell adhesion molecule (EpCAM) is a 39—42 kDa, transmembrane glycoprotein that consists of two epithelial growth factor-like extracellular domains, a cysteine-poor region, a transmembrane domain, and a short cytoplasmic tail.[7] EpCAM is detected at the basolateral membrane of the majority of epithelial tissues.[7,8] EpCAM overexpression has been reported in cancers including colorectal cancer and breast cancer.[7,9,10] EpCAM acts in Ca^{2+}-independent intercellular adhesion, and is not structurally related to the four major types of cell adhesion molecules, such as cadherins, integrins, selectins, and the immunoglobulin superfamily.[8,11] EpCAM overexpression in cancer cells induces the intracellular accumulation of cadherin—catenin complex, and cadherin-derived adhesion activity of cancer cells is consequently lost.[11] Since the intercellular adhesive activity of EpCAM is very weak, the intercellular adhesion of cancer cells decreases, which may result in the acquisition of invasiveness and metastatic ability.[8,11] In human breast cancer cells, a recent study suggested that the inhibition of EpCAM expression by short interfering RNA (siRNA) may decrease the availability of β-catenin for the Wnt pathway.[12] However, the correlation of EpCAM overexpression and invasiveness or metastatic ability in cancer cells and its underlying mechanisms still remain unclear.

From the clinical point of view, the EpCAM antigen has attracted major interest as a target for cancer immunotherapy. Practically, the use of monoclonal antibody against EpCAM has been successfully used in colorectal cancer[13] and breast cancer.[14]

In this study, we investigated the usefulness of EpCAM overexpression as a clinical molecular marker, and the possibility of EpCAM targeting therapy for SCC of the tongue.

Materials and methods

Patients

Paraffin-embedded sections were obtained from biopsy specimens of 48 patients with squamous cell carcinoma of the tongue who underwent radical surgery in our department. The tumor stage was classified according to the TNM classification of the International Union Against Cancer. Tumor histologic differentiation was defined according to the WHO classification. The pattern of invasion was classified according to Bryne's classification.[15] As controls, samples of normal oral epithelium were obtained after informed consent from ten patients undergoing routine surgical removal of their third molars.

Cell lines

The human tongue squamous cell carcinoma cell lines, SAS, HSC-2, OSC19 and OSC20 were obtained from the Human Science Research Resource Bank (Osaka, Japan). All of the cells were cultured under conditions recommended by their depositors.

Immunohistochemical staining and evaluation

Serial sections 3 μm thick were taken from the tissue blocks. Deparaffinized sections in xylene were soaked in target retrieval solution buffer (DAKO, Glostrup, Denmark) and placed in an autoclave at 121 °C for 5 min for antigen retrieval. Endogenous peroxidase was blocked by incubation with 0.3% H_2O_2 in methanol for 30 min. Immunohistochemical staining was performed using the Envision system (EnVision+, Dako, Carpinteria, CA). The primary antibody used was directed against EpCAM (HEA-125, GeneTex, San Antonio, TX; 1 μg/ml concentration). The sections were incubated with the monoclonal antibody overnight at 4 °C. Reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Meyer's hematoxylin and mounted. Negative controls were performed by replacing the primary antibody with phosphate-buffered saline. EpCAM expression was defined as the presence of specific staining on the surface membranes of tumor cells. EpCAM overexpression was evaluated by calculating the total immunostaining score as the product of the proportion score and the intensity score. The proportion score described the estimated fraction of positive-stained tumor cells (0, none; 1, <10%; 2, 10—50%; 3, 50—80%; 4, >80%). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). The total score ranged from 0 to 12. As described previously,[9] overexpression of EpCAM was defined as a total score >4.

RNA isolation and semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated with TRIzol Reagent (Invitrogen, Carlsbad, CA) and first-strand cDNA was synthesized from