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Increase of disintergin metalloprotease 10 (ADAM10) expression in oral squamous cell carcinoma☆

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Abstract

The A disintergin and metalloprotease (ADAM) superfamilies play important roles in angiogenesis, development, and tumorigenesis. Amyloid precursor protein (APP) is an important protein related to Alzheimer's disease. Recent research shows that *ADAM10* α-secretase activity can release the secreted form of APP. We have previously demonstrated an increase of APP expression in oral squamous cell carcinoma (OSCC) and related OSCC cell lines. The present study characterizes *ADAM10* expression in the neoplastic process of OSCC. RT-PCR analysis revealed a two-fold increase in *APP* mRNA expression in 50% of OSCC ($n=50$) relative to corresponding non-malignant matched tissues (NMMT). This increase in mRNA expression occurred at the preneoplastic stage. A significant correlation between mRNA expression of *ADAM10* and *APP* in OSCC was noted. A non-buccal subset of OSCC correlated with an increase of mRNA expression of both *ADAM10* and *APP*. The increase of ADAM10 protein expression in the majority of OSCC tissues and cell lines studies was confirmed by Western blot analysis. Additionally, an increase of ADAM10 immunoreactivity in OSCC relative to NMMT was noted. An antisense oligonucleotide against *ADAM10* reduced ADAM10 expression as well as growth in an OSCC cell line. However, this treatment did not reduce the secreted form APP. This study suggests that *ADAM10* expression plays a role in the carcinogenesis of OSCC and proliferation of OSCC cells, independent of APP processing.

Keywords: *ADAM10*; *APP*; Carcinogenesis; Mouth

☆ Oral squamous cell carcinoma (OSCC) is a common malignancy worldwide. In this study we specified the up-regulation of ADAM10 expression in OSCC. This up-regulation is correlated with that of APP; and confers growth advantage for OSCC cells.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is the third most common malignancy in developing countries [1,2]. The prognosis for OSCC remains dismal, as more than a half of victims succumb to the disease progression and its complications after treatment. Identification of pathogenetic mechanisms for OSCC should facilitate the monitoring or prevention of OSCC [3]. A disintergin and metalloproteases (ADAMs) play a role in cell–cell and cell–extracellular matrix adhesion in tumor cells, angiogenesis, fertilization, muscle fusion, and organ development [4–9]. *ADAM10* (Kuzbanian; KUZ) is an ADAMs family membrane located on chromosome 15 [10]. Recent reports indicate that ADAM10 is critical for embryonic morphogenesis of ectoderm [11] and exhibit collagenase activity for type IV collagen [12]. Additionally, ADAM10 mediates the transactivation of epidermal growth factor receptor (EGFR) through protein-coupled receptors [13] and plays a role in protein-ectodomain processing and cellular signaling [6].

Amyloid precursor protein (APP) is an important protein associated with Alzheimer's disease. APP can be digested by α-secretase and release non-amyloid ß peptide and the secreted form of APP (sAPPα) [14–16]. Recent researches have demonstrated that ADAM10 exhibits α-secretase activity and the ADAM10 overexpression elicits an increased level of sAPP [17–19]. sAPP is related to the growth of keratinocyte and thyroid cells [20–23]. Hoffmann et al [23] have demonstrated the growth-promoting effects of sAPP on skin keratinocytes. *APP* expression has also been linked to the malignant progression or growth of neuronal and colorectal [24–26] and oral carcinoma cells [27]. *ADAM10* is abundantly expressed in cells derived from haematological malignancies [28], and prostate cancers cell lines [29,30]. Elevated levels of *ADAM10* were present in gastric carcinoma [31,32]. ADAMs cleavage of EGFR ligands can be induced by inflammatory cytokines [33], and the mediated ectodomain shedding may have a role in gastric carcinogenesis [31,32] but the function of ADAM10 in cancer is still obscure.

Our previous study has demonstrated the increase of APP expression in OSCC and related OSCC cell lines [27]. ADAM10 has been reported to have α-secretase activity. Presently, we verified the increase in ADAM10 expression in OSCCs. Moreover, we identify that ADAM10 contributes to the growth of OSCC cells.

2. Materials and methods

2.1. Samples

Fifty pairs of OSCC and the corresponding non-malignant match tissue (NMMT) samples (age range 31–69 y) were obtained for mRNA expression analysis and protein as approved by an ethical review board. The most common primary site for OSCC was buccal mucosa (52%, 26 cases). A total of 20% (10 cases) of OSCC were well differentiated. Fifty-four percent (27 cases) and fifty-two percent (26 cases) of OSCC patients presented with lymph node metastasis and stage IV tumor, respectively. The mean follow-up period was 23.1 ± 2.5 months. The most cellular part of the OSCC, as confirmed by frozen section, was subjected to mRNA and protein analysis. NMMT was sampled around 1 cm away from the resection margin. No dysplasia or neoplasia was noted in NMMT by evaluation of frozen sections.

Tissue arrays containing 49 primary OSCC lesions, 19 of their corresponding NMMT and 12 metastatic lesions retrieved from patients' neck lymph nodes were used for immunohistochemistry. The arrays of tumor tissues had been generated from $0.3 \times 0.3\ cm^2$ rectangles of the most cellular tissue fraction of each case.

In addition, four pairs of oral preinvasive lesion (OPL) and corresponding NMMT as well as eight pairs of OSCC and corresponding NMMT were dissected near the tumor center using Pixecell II laser capture microdissector (Arcturus, Mountain View, CA) following protocols provided by manufacturers. Frozen section examination revealed that two OPLs were epithelial hyperplasia and hyperkeratosis. The other two OPLs exhibited mild epithelial dysplasia. No other oral lesion was found in OPL patients. Around 2000 and 3000 cells were dissected from frozen sections of OPL and OSCC, respectively, for quantitative (Q)-RT-PCR analysis.

2.2. RNA extraction, aRNA and cDNA synthesis

Total RNA was extracted using a Tri-reagent® RNA isolation kit (Molecular Research Center, Cincinnati, OH). Contaminated DNA was removed form the RNA with DNase I (Stratagene, La Jolla, CA). mRNA prepared from the microdissected cells was amplified by RiboAmp™ RNA Amplification Kit (Arcturus). Amplified antisense RNA (aRNA) was obtained and the concentration was determined by $OD_{260/280}$ reading. Two micrograms of aRNA from microdissected cells and 2 μg total RNA from non-microdissecetd tissue