EXPRESSION OF CD147 (STEM CELL MARKER) IN ORAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA (IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

The majority of oral squamous cell carcinoma (OSCC) is preceded by oral premalignant lesions (OPL). The transition of premalignancy into invasive carcinoma necessitates the remodeling of the extracellular matrix (ECM) and basement membrane which may be the first step of local invasion. This remodeling is mediated by various soluble and cell surface molecules, including extracellular matrix metalloproteinase inducer (EMMPRIN).

Since EMMPRIN plays such an important role in epithelial-connective tissue interactions, the expression patterns of EMMPRIN were estimated in normal oral mucosa, epithelial dysplasia and in different grades of OSCC in order to evaluate its role in cancer progression.

In the current study, immunohistochemical staining using anti EMMPRIN antibody was conducted on 45 paraffin-embedded specimens of oral epithelial dysplasia (OED) and oral squamous carcinoma (OSCC) and compared with normal oral mucosa.

The positive immunoreaction of EMMPRIN was detected in both epithelium and underlying connective tissue. It was located at the cell membrane or appeared as a granular cytoplasmic reaction. Generally, the expression of EMMPRIN was significantly greater in OSCC followed premalignant lesions than in the normal oral mucosa.

As EMMPRIN is actively involved in tumor growth, invasion and metastasis, its measurement may be helpful in predicting patients’ prognosis.

INTRODUCTION

Squamous cell carcinoma which represents more than 90% of all head and neck cancers (Canto, 2004) arises as a result of multiple molecular events that develop from the combined influences of an individual’s genetic predisposition and exposure to environmental carcinogens (Califano et al, 1996).

The majority of oral cancers and a significant number of OSCC occur in a background of either a precancerous lesion or a precancerous condition (Tilakaratne, 2007). The transition from premalignancy to invasive cancer is preceded by the activation of local host stroma to be

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favorable for microinvasion of cancer cells. Therefore, the microenvironment of the host stroma is an active participant in this process (Vigneswaran et al., 2006). Therefore, the remodeling of the extracellular matrix (ECM) and basement membrane may be the first step necessary for local invasion (Yan et al., 2005).

Interactions between tumor cells and surrounding stromal cells are mediated by various soluble and cell surface molecules, including extracellular matrix metalloproteinase inducer (EMMPRIN) (Tang et al., 2005).

Extracellular matrix metalloproteinase inducer (EMMPRIN) is a $M_r \sim 58,000$ glycoprotein which is located on the outer surface of human tumor cells (Biswas et al., 1995). It contains a cytoplasmic domain and a transmembrane domain. The two domains are characteristic of the immunoglobulin superfamily (Sun, and Hemler, 2001).

The functional importance of EMMPRIN during tumor progression is mainly due to its stimulatory effects on host stromal fibroblasts to produce matrix metalloproteinases (MMPs) which in turn degrade the basement membrane and the ECM. It has also an additional role in tumor angiogenesis and growth as it stimulates the production of vascular endothelial growth factor (VEGF) by both tumor and stromal cells via MMP dependent and independent pathways (Tang et al., 2005). This increased VEGF results in increased proliferation, sprouting, migration, and tube formation of endothelial cells (Bougatet et al, 2009).

Therefore, EMMPRIN may be actively involved in cancer development and progression and its measurement may be helpful in predicting patients’ prognosis.

**MATERIAL AND METHODS**

**Tissue specimens**

A total of 45 OSCC, OPL and normal paraffin embedded tissue sections were studied. Normal oral mucosa was retrieved from cases diagnosed as a reactive or cystic lesion of salivary gland or connective tissue origin.

**Immunohistochemical staining procedure**

Immunohistochemistry was carried out using mouse monoclonal antihuman EMMPRIN antibody (2.5%, Santa Cruz, USA).

The four $\mu$m thick paraffin embedded tissue sections were microwaved in 1mM EDTA at 100$^\circ$C for 10 minutes. The slides were incubated with primary antibody overnight in the humidity chamber at room temperature. Then, the slides were incubated with biotinylated anti-mouse secondary antibody for 30 minutes followed by incubation with streptavidin-horseradish peroxidase conjugate for 30 minutes. The peroxidase activity was made visible with dianinobenzidine. Counterstaining was done with Mayer’s hematoxylin.

**Assessment of immunostaining**

The ordinary light microscope was used to detect the positive and negative immunostaining and for localization of the positive reaction within the tissues.

The immunoreactivity was measured as area and area percent by an image analyzer computer system (Leica Quin 500, Germany). Data obtained from the computer image analysis were expressed as mean and standard deviation (SD) values and were used for statistical analysis using ANOVA test and for generation of a representative graph.

**RESULTS**

I- Assessment of immunostaining by ordinary light microscope:

The positive immunoreaction of EMMPRIN was detected as a brownish colour in the epithelium and in the underlying connective tissue (C.T). The immunoreactivity was membranous and/or cytoplasmic (Fig. 1-3).
Increased expression of EMMPRIN was also noted in all grades of OSCC specimens. EMMPRIN was mostly expressed in the invasive fronts of the tumor, while the more differentiated areas in the center of the tumor remained negative or only weakly positive (Fig. 3).

Submucosal and peritumoral fibroblasts, inflammatory and endothelial cells also expressed EMMPRIN (Fig. 2, 3).

II- Assessment of immunostaining by Computer Image Analysing:

Using the image analyzer computer system, the highest value was recorded in the poorly differentiated squamous cell carcinoma (27.823± 5.094), while the lowest value was recorded in normal oral mucosa (6.009±1.996), followed by the mild dysplasia (9.614± 3.269), (Table 1). Using ANOVA test, a highly statistically significant difference was detected (p= 0.000) between the studied groups.
TABLE (1) Area percentage of CD147 immunoexpression

<table>
<thead>
<tr>
<th></th>
<th>Normal mucosa</th>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia</th>
<th>Well differentiated SCC</th>
<th>Moderately differentiated SCC</th>
<th>Poorly differentiated SCC</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>6.009\textsuperscript{a}</td>
<td>9.614\textsuperscript{b}</td>
<td>11.584\textsuperscript{b}</td>
<td>20.581\textsuperscript{c}</td>
<td>16.752\textsuperscript{c}</td>
<td>23.634\textsuperscript{d}</td>
<td>27.823\textsuperscript{d}</td>
</tr>
<tr>
<td>SD</td>
<td>1.996</td>
<td>3.269</td>
<td>4.04</td>
<td>7.962</td>
<td>4.808</td>
<td>7.06</td>
<td>5.094</td>
</tr>
</tbody>
</table>

Means with different letters are statistically significantly different.

Using Tukey’s test for pairwise comparison, no significant difference could be detected between CD147 mean area % of immunoexpression in mild and moderate dysplasia. Similarly, severe dysplasia didn’t differ significantly from well differentiated squamous cell carcinoma. Moreover, the difference between the mean area % of CD147 immunoexpression between moderately and poorly differentiated squamous cell carcinoma was not statistically significant.

**DISCUSSION**

EMMPRIN has been found to stimulate fibroblasts to produce different types of MMPs which normally regulate a variety of physiological processes and signaling events including tissue remodeling and organ development (Page-McCaw et al., 2007), in the regulation of inflammatory processes (Parks et al., 2004) and represent key players in the molecular communication between tumor cells and stroma. (Braundmeier et al., 2006).

Several studies revealed the functional importance of EMMPRIN during tumor progression as well as an additional role in tumor angiogenesis and growth (Tang et al., 2005).

Since EMMPRIN plays such an important role in epithelial- connective tissue interactions, we studied the EMMPRIN expression patterns in normal oral mucosa, epithelial dysplasia and in different grades of OSCC to evaluate its role in cancer progression.

In the present study, immunohistochemical staining using anti EMMPRIN antibody revealed that EMMPRIN expression was generally low in normal oral mucosa and exclusively limited to the actively differentiating basal cells. This localized expression suggests that it may play a role in establishing basal cell polarity.

In oral epithelial dysplasia, the expression of EMMPRIN rose, spreading to superficial epithelial layers, with increasing severity of OED seen in the premalignant lesions while remained restricted to the basal cells of reactive dysplasia associated with the inflammatory conditions.

These results suggest that immunohistochemical expression of EMMPRIN can be significant in differentiation between OED seen in the premalignant lesions and reactive inflammatory atypia.

Similarly, EMMPRIN expression was overexpressed in early or superficially invasive OSCC (micro-invasive SCC) and was noted in the most of tumor cells with strongest expression was detected in epithelial cells invading the basement membrane and the underlying connective tissue.

EMMPRIN remained overexpressed in primary and recurrent OSCC, but its overexpression was mostly localized to the invasive fronts of these tumors. Such expression was repressed and became negligible in most differentiated areas of these tumors. Such disassociated expression pattern suggests the role of EMMPRIN in invasion and metastasis.
The expression of EMMPRIN increased directly with increased grading of oral epithelial dysplasia as well as OSCC. The poorer histopathological differentiation, the more expression was detected.

Vigneswaran et al (2006) studied the correlation of EMMPRIN expression in breast and esophageal cancer with tumor size, stage and prognosis. Breast cancer cells, engineered to overexpress EMMPRIN, had demonstrated an accelerated growth rate and metastatic progression, confirming a causal role of EMMPRIN in tumor growth and metastasis.

In another study, Yang et al (2010) examined the expression of EMMPRIN in adenoid cystic carcinoma (ACC) of salivary glands and in normal salivary gland tissues. The positivity of EMMPRIN in ACCs was significantly higher than that in normal salivary gland tissues. It was positively correlated with tumor size, histopathological types, clinical stage, perineural invasion, vascular invasion and metastasis.

Huang et al (2010) also studied the expression of EMMPRIN in normal and neoplastic salivary gland tissues. The expression was significantly higher in mucoepidermoid carcinomas and adenoid cystic carcinomas than in normal salivary gland tissues and pleomorphic adenomas.

Within epithelial cells, the immunoreactivity of EMMPRIN was located at the cell membrane and within the cytoplasm.

Betsuyaku et al (2003) studied the expression of EMMPRIN in smokers’ lungs. It was found diffusely in the cytoplasm of bronchiolar epithelial cells, and bronchial glands.


Jung et al (2011) evaluated the expression EMMPRIN in normal colonic mucosa and in colorectal adenocarcinomas. It was expressed focally in normal colonic mucosa. Over expression of EMMPRIN was observed in colorectal carcinoma compared to normal mucosa. The staining patterns of EMMPRIN in tumor cells were seen in cell membrane and within the cytoplasm.

Peritumor stromal fibroblasts showed positive immunoreactivity to EMMPRIN which is consistent with the hypothesis that EMMPRIN can bind to stromal cells and induce their MMPs production which degrade the basement membrane and extracellular matrix facilitating epithelial cells invasion.


Moreover, some endothelial cells immunohistochemically reacted with EMMPRIN reflecting its stimulatory effects in the production of VEGF and new vessels formation (tumor angiogenesis).

Therefore, the expression of EMMPRIN in invading epithelial cells, fibroblasts, endothelial and inflammatory cells clarifies the integrated functions of CD147 in all stages of cancer as the distribution in peripheral (invading) malignant cells located near the connective tissue emphasizes its role in cell-to-ECM adhesion, degradation and remodeling of ECM and subsequently in cancer invasion and metastasis. This is further supported by its expression in stromal fibroblasts.

In addition, the positive immunoreactivity of endothelial cells to EMMPRIN highlights its role in angiogenesis, an additional crucial factor in cancer progression.

According to these findings, EMMPRIN may actively be involved in the growth, angiogenesis, invasion and metastasis. So, EMMPRIN can be used as a diagnostic and prognostic marker for different diseases including cancers and as a potential molecular target in cancer therapy.
CONCLUSION

EMMPRIN was found to play an important role in all stages of cancer development and progression. Consequently, EMMPRIN represents a diagnostic and prognostic marker as well as a potential molecular target for cancer therapy.

REFERENCES