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Immunohistochemical expression of epithelial cell adhesion molecule (EpCAM) in mucoepidermoid carcinoma compared to normal salivary gland tissues

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ABSTRACT

Objectives: Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor which displays biological, histological and clinical diversity thus representing a challenge for its diagnosis and management. Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein identified as a tumor specific antigen due to its frequent overexpression in the majority of epithelial carcinomas and its correlation with prognosis. It is considered to be a promising biomarker used as a therapeutic target already in ongoing clinical trials. The purpose of this study was to investigate the pattern, cellular characterization and level of EpCAM expression in MEC and demonstrate its correlation with histologic grading which may benefit future clinical trials using EpCAM targeted therapy.
Materials and methods: 48 specimens (12 normal salivary gland tissue and 36 MEC) were collected and EpCAM membranous expression was evaluated by immunohistochemistry. Total immunoscore (TIS) was evaluated, the term 'EpCAM overexpression' was given for tissues showing a total immunoscore >4.
Results: A highly significant difference was observed between TIS percent values in control and different grades of MEC ($p < 0.001$). High grade MEC (HG-MEC) was the highest EpCAM expressor. In addition, EpCAM expression pattern differed among the different grades.
Conclusion: EpCAM expression was detected in MEC, and its overexpression correlated with increasing the histological grade. The diffuse membranous expression in HG-MEC could be of diagnostic value in relation to the patchy expression observed in both low grade and intermediate grade MEC.

1. Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignancy of major and minor salivary glands of both children and adults (Janjua, Qureshi, Khan, & Alamgir, 2012). It represents 10–15% of all salivary gland tumors and 30% of salivary malignancies (Jayasooriya, Karunathilake, Siriwardena, Amaratunga, & Attygalla, 2013). In the major salivary glands, 65% to 80% of cases occur in the parotid (Arrangoiz, 2013). MEC shows a female predilection and has the highest incidence in adults during the fifth decade (Tekade, Chaudhary, Gawande, & Bagri, 2010; Janjua et al., 2012).

Surprisingly, MEC constituted over 85% of the salivary gland malignancies diagnosed in our department in the previous decade. Due to the highly variable biological behavior and grading systems of MEC, numerous difficulties are encountered with the proper grading and subsequently, the appropriate treatment.

Histologically, MEC is composed of 3 different cell types: mucous secreting cells, intermediate cells, and epidermoid cells. Patterns of growth vary from cystic, solid and infiltrative. MEC is graded and classified according to cellular, and cytologic features as well as to architectural pattern into 3 grades: low, intermediate, and high grade (Ellis & Auclair, 1996). Furthermore, several grading systems have been introduced for MEC and many studies proved the importance of the histological grading and how it aids in the prediction of the prognosis and determine the type of treatment (Khiavi, Vosoughhosseini, Saravani, & Halimi, 2012; Katabi et al., 2014). Although, variable prognostic factors correlate with the patient's clinical outcome and affect the mode of treatment, those associated with worse prognosis were large tumor size, high histological grade, perineural invasion, lymph node involvement, distant metastasis, and positive surgical margins (McHugh et al., 2012). LG-MEC shows a 92%–100% 5-year survival outcome while IMG-MEC shows 62%– 93% and the HG-MEC shows 0%–43% (Janjua et al., 2012; Katabi et al., 2014).

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EpCAM is a 40 kDa type I transmembrane glycoprotein expressed on most normal and cancerous epithelial tissues, as well as on cancer stem cells, embryonic stem cells and germ cells (Schönberger et al., 2013). In normal epithelium, EpCAM is expressed on the basolateral surface of simple, pseudostratified, and transitional epithelia with the exception of squamous epithelia, epidermal keratinocytes, myoepithelial cells, thymic cortical epithelium, gastric parietal cells and hepatocytes. It is also overexpressed in a majority of carcinomas, particularly adenocarcinomas.

Regarding diagnosis, EpCAM was used to differentiate tumors showing histopathologic resemblance such as malignant mesothelioma and pulmonary adenocarcinoma (Yaziji et al., 2006). Moreover, it was also used to distinguish hepatocellular carcinoma from metastatic adenocarcinoma or cholangiocarcinoma (de Boer, van Krieken, Janssen-van Rhijn, & Litvinov, 1999; Karabork, Kaygusuz, & Ekinci, 2010). Interestingly, some studies stated that skin basal cell carcinomas with squamous metaplasia demonstrated strong and diffuse EpCAM expression in contrast to the sporadic reactivity observed in basaloid squamous cell carcinoma (Linskey et al., 2013; Webb, Mentrikoski, Verduin, Brill, & Wick, 2015).

In addition to diagnosis, EpCAM demonstrated a substantial prognostic significance. Some studies have reported that EpCAM overexpression correlated with a poorer prognosis in breast cancer (Spizzo et al., 2004), pancreatic (Fong et al., 2008), gall bladder cancers (Varga et al., 2004) and squamous cell head and neck cancer (Matsuda et al., 2014). Whilst other studies stated that EpCAM overexpression demonstrated a better prognosis in clear cell renal carcinoma (Went et al., 2005), moderately differentiated colon (Went et al., 2006), esophageal (Kimura et al., 2007) and gastric cancers (Songun et al., 2005). In ovarian cancer however, conflicting results were observed, where a poorer prognosis was demonstrated by Spizzo et al. (2006) and a more favorable prognosis was demonstrated by Woopen et al. (2014).

Furthermore, EpCAM was selected as target antigen for several immunotherapeutic approaches due to its frequent and high-level expression on carcinomas (Balzar, Winter, de Boer, & Litvinov, 1999). Hence, it is important to determine human cancers that are eligible for EpCAM-target therapy based on EpCAM expression according to intensity, frequency and disease stage.

Thus this study aimed to evaluate the pattern, cellular characterization and the level of expression of EpCAM in different grades of MEC, since limited research has been reported in this area. Furthermore, it aimed to understand the correlation between the histologic grade and EpCAM expression, which may benefit future clinical trials using EpCAM targeted therapy.

2. Materials and methods

2.1. Tissue specimens

Formalin fixed paraffin embedded specimens of 36 MEC (12 LG-MEC, 12 IMG-MEC, 12 HG-MEC) were retrieved from the archives of the Oral and Maxillofacial pathology department, Faculty of Oral and Dental medicine, Cairo University, Alexandria University as well as from the National Cancer Institute. Twelve specimens of normal salivary gland tissues were collected as archival blocks from donated autopsy tissues as control specimen. Data were collected from patients' files, for personal data (age and sex) and clinical data regarding the tumor site.

2.1.1. Positive control

Formalin fixed paraffin embedded colon adenocarcinoma was used as a positive control for EpCAM according to manufacturer's instructions and was prepared simultaneously with the other selected samples to ensure the validity of the technique.

2.1.2. Negative control

Negative control was prepared in the same method after omitting the step of the primary antibody application and using the isotype-matched mouse IgG1 to ensure the specifity of the technique.

2.2. Histopathological examination

The paraffin embedded specimens were cut into 5-micrometer-thick sections, mounted on slides, stained with hematoxylin and eosin (H&E), and examined by light microscopy. The histologic features observed in MEC were re-diagnosed to confirm the previously made diagnosis. The sections for grades of MEC were examined and scored by two independent pathologists according to the diagnostic criteria of Brandwein system (Brandwein et al., 2001).

The sections were cut at 4 μm thickness and placed on positively charged slides (Optiplus) ready for immunostaining procedures.

2.3. Immunohistochemistry

Immunostaining for EpCAM was done for all using Ventana Benchmark XT autostainer and the following steps occurred automatically: deparrafinization at 72 °C; antigen retrieval with Dako Cytomation Envision for 20 min at 95 °C; wash solution; peroxide blocking solution 3% H_2O_2/methanol for 5 min at room temperature; wash solution; treatment with the primary antibody for one hour (mouse monoclonal anti-human EpCAM, ((C-10): sc-25308), Santa-Cruz Biotechnology, USA, 50 μ/3 ml which recognizes an epitope between amino acids 24–93 in the extracellular domain of EpCAM, under incubation temperature at 37 °C for 44 min); wash solution; post-primary antibody treatment over 8 min at room temperature; wash solution; Leica BOND-MAX Polymer treatment over 8 min at room temperature; wash solution; application of DAB for 10 min at room temperature; wash solution; counter stain with Hematoxylin for 8 min at room temperature; washing, dehydration in alcohol and xylene and mounting on glass slides by DPX.

a) Transmitted light microscope

The method was used to assess the prevalence of immunopositivity of EpCAM in the studied cases.

b) Evaluation of immunoexpression

Immunohistochemistry results were independently evaluated by two pathologists with no knowledge of the patients' clinical or histopathological data. The immunohistochemical evaluation was performed by calculating the total immunostaining score (TIS) as the product of the proportion score (PS) and the intensity score (IS) according to Allred scoring system used in the evaluation of oestrogen receptor positivity.

2.4. PS described the estimated fraction of positively stained tumor cells

0 = none
1 = <10%
2 = 10–50%
3 = 51–80%
4 = > 80%

2.5. IS represented the estimated staining intensity

0 = no staining