

Original Article

Mel-CAM Expression in Common Oral Carcinomas

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KEY WORDS

Mel-CAM;
Mucoepidermoid
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Oral squamous cell
carcinoma;
Salivary glands;

ABSTRACT

Statement of the Problem: Mel-CAM (CD₁₄₆, MUC₁₈) is a 113-kD heterophilic cell-cell adhesion glycoprotein found in normal and tumoral tissues. The biologic functions and role of the Mel-CAM can be employed as a diagnostic marker in pathology.

Purpose: The aim of this study was assessing the expression of Mel-CAM in common oral carcinomas like salivary gland mucoepidermoid carcinoma (MEC) and oral squamous cell carcinoma (OSCC) to differentiate the OSCC from high-grade MEC.

Materials and Method: This study was performed on 19 specimens of MEC and 17 specimens of OSCC, which were retrieved from the archive of Department of Pathology of Taleghani Hospital, Tehran, Iran. Immunohistochemical staining was performed by using antibody against CD₁₄₆. The data were analyzed using SPSS software through Mann-Whitney, Spearman's correlation coefficient, and Kruskal-Wallis tests.

Results: Mel-CAM was expressed in all MEC samples and 10 OSCC cases. The two groups were significantly different regarding the CD₁₄₆ expression ($p= 0.035$). Furthermore, the CD₁₄₆ expression was found to be significantly correlated with the invasion mode ($p= 0.002$), tumor size ($p= 0.012$), and histological grade ($p= 0.024$) in OSCC group. No significant correlation existed between the expression, intensity, and location with the histological grade of MEC ($p> 0.05$) nor was any significant correlation detected between the CD₁₄₆ expression and lymph node metastasis in neither group.

Conclusion: Regarding the significant correlation between the CD₁₄₆ expression and the prognostic factors in OSCC, this marker may predict the prognosis in OSCC patients, but not the MEC lesions. It cannot be used for differentiating high-grade MEC and OSCC.

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Introduction

Mucoepidermoid carcinoma (MEC) is one of the most common salivary gland malignancies that mainly affect the parotid. The tumor occurs within the second to the seventh decades of life, and is the most common malignant salivary gland tumor in children [1]. The biologic behaviors of MEC range from slow-growing mass to destructive rapidly growing mass [2]. Prognosis of the MEC is usually related to the clinical stage and histological grade [3]. Despite the advancements in diagnosis

and treatment of high-grade MEC over the last two decades, the 5-year survival rate is still less than 50% [4-5]. High-grade MEC can be misdiagnosed for oral squamous cell carcinoma (OSCC), which is the most common malignancy of the oral cavity and accounts for almost 2% of the cancer burden worldwide. The overall 5-year survival rate has not significantly increased in the last few years despite the advanced treatment modality [6]. Mel-CAM (CD₁₄₆, MUC₁₈) is a 113-kD heterophilic cell-cell adhesion glycoprotein, which belongs to the

immunoglobulin supergene family [7]. It was initially identified as a marker of melanoma progression and metastasis [8]. This marker is primarily expressed by vascular endothelium and smooth muscle; but has also been detected in subpopulation of activated T lymphocytes, bone marrow, Schwann cells, ductal, and myoepithelial cells of the salivary glands [9].

Expression of Mel-CAM in tumor tissues is related to the tumor size, progression, metastatic potential, and aggressiveness [9]. Indeed, the biologic functions and role of the Mel-CAM as a diagnostic marker in pathology are now being recognized. The present study aims to assess the expression of Mel-CAM in salivary gland MEC and OSCC to find its possible correlation with the histological grade, tumor size, lymph node, and metastasis, besides its utility to differentiate the OSCC from high-grade MEC.

Materials and Method

Sample selection

The samples of this cross-sectional study were 36 formalin-fixed, paraffin-embedded tissue blocks including 17 cases of OSCC and 19 cases of MEC, which were obtained from the archive of the Department of Pathology of Taleghani Hospital, affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran. Anonymity of the patients' records was strictly respected.

Hematoxylin and eosin (H&E) stained sections were used to confirm the diagnosis. Clinicopathologic information of each case including age, sex, tumor location, and histological grade were collected from the patients' records and reviewing slides. For OSCC samples, mode of invasion was also identified. Cases with incomplete data, insufficient paraffin-embedded tumor material, inappropriate fixation, and incisional biopsy were excluded.

Immunohistochemistry (IHC)

Sections of 4- μ m thickness were cut from all samples and mounted on silane-coated slides. The sections were deparaffinized with 100% xylene and rehydrated in graded ethanol series. Sections were immersed in Tris-buffered saline (TBS) with a pH of 6.0, and heated in a microwave oven at 750 watts for antigen retrieval. After cooling into room temperature, the sections were incubated with primary antibody (Anti-CD146, monoclonal mouse Anti-Human clone: AA1, Ready to use, Abcam,

USA) at 1:2000 for an hour. Having been washed in TBS, the sections were treated with Dako EnVision (Dako, Germany). The DAB chromogen was applied to visualize the antibody expression, and then, counterstained with Mayer's hematoxylin. Normal parotid salivary gland was used as positive control.

Evaluation of IHC

CD₁₄₆ immunoreaction in the tumoral cells was determined in 10 randomly-selected fields by counting all the positive cells in each field according to the median index of positive cells obtained from 10 high-power fields (HPF) and scored as negative (0-5%), weak (6-25%), moderate (26-50%) and strong (51-100%) [10]. The staining intensity was evaluated as 0=no positive cells, += mild, ++= moderate, +++= strong [11]. Mode of invasion in OSCC samples was assessed on the H&E slides according to Jacobson method (scored I to IV) [12].

According to WHO classification (2005), the histopathologic grade of OSCC samples was classified into well-, moderate- and poorly-differentiated. The histopathologic grade of the MEC was categorized as low, intermediate and high grade based on Auclair classification [10]. All slides were evaluated by two pathologists.

Statistical analysis

The statistical analysis was carried out on the tabulated data by using SPSS software, version 18.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney test was used to assess the correlation between CD₁₄₆ expression and the clinicopathologic variables including age, histological grade, nodal metastasis, and mode of invasion. Spearman's correlation coefficient and Kruskal-Wallis test were done to determine the correlation of CD₁₄₆ expression with the tumor size and expression location, respectively. The significance level of all tests was set at 0.05. All the procedures performed in the current study were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (#9204) in accordance with the Declaration of Helsinki (1964) and its later amendments. Formal written informed consent was not required with a waiver by the Ethics Committee of Shahid Beheshti University of Medical Sciences.

Results

The samples were 23 men and 13 women (Table 1). CD₁₄₆ was expressed in all MEC samples as cytoplasmic and membranous staining.

Table 1: Characteristics of MEC and OSCC patients

Variables	MEC	OSCC
Sex	Male	10
	Female	9
Age(Mean±SD)	45.58±14.31	64.76±9.28
Site of tumor	Alveolar mucosa	6
	Parotid	5
	Sublingual	1
	Tongue	7
	Hard palate	5
	Flour of the mouth	2
Histopathologic grade	High grade	7
	Moderate grade	10
	Low grade	2
Size(Cm) Range (mean)	2.68-3.56 (2.86)	3.5-6.12 (5.08)
Lymph node metastasis	Yes	2 (10.5%)
	No	17(89.5%)
Mode of invasion*	I	9
	II	0
	III	4
	IV	4

*Mode of invasion was classified only for OSCC

The CD₁₄₆ immunoeexpression was strong in 78.9% (n=15), moderate in 5.3% (n=1), and weak in 15.8% (n=3) of cases (Figure 1 and 2). In OSCC samples, 10 cases expressed CD₁₄₆, out of which 47.1% (n=8) was strong and 11.8% (n=2) were weak (Figure 3 and 4). The two groups were significantly different in terms of CD₁₄₆ expression ($p < 0.035$). No significant correlation was detected between the CD₁₄₆ expression, intensity, and location with the histological grade in MEC group ($p > 0.05$).

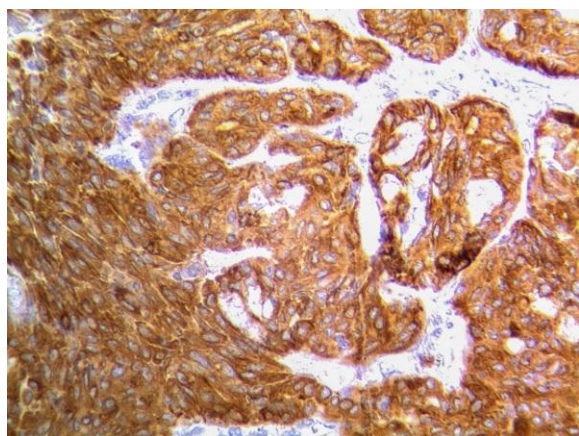


Figure 1: Cytoplasmic and membranous staining of epidermoid cells in mucoepidermoid carcinoma, moderate intensity (CD146 IHC stain, 400 X)

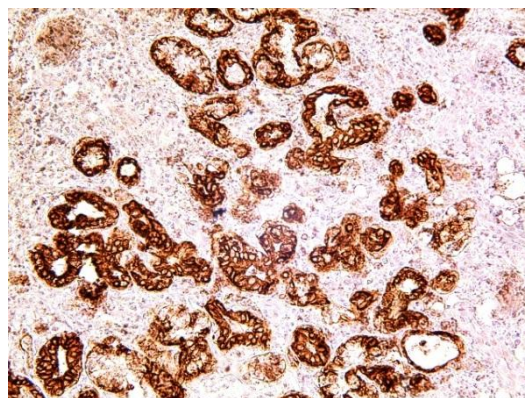


Figure 2: Cytoplasmic and membranous staining of mucous cells in mucoepidermoid carcinoma, strong intensity (CD146 IHC stain, 200 X)

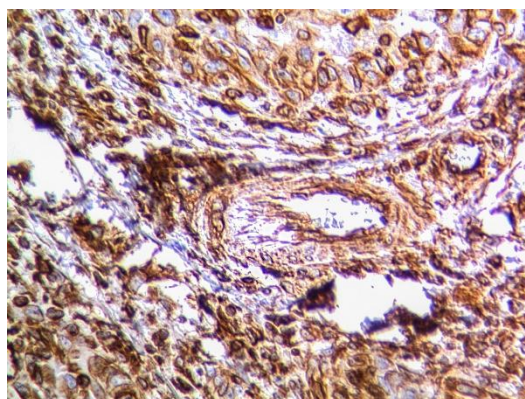


Figure 3: Cytoplasmic and membranous staining of squamous cells in squamous cell carcinoma, moderate intensity (CD146 IHC stain, 400 X)

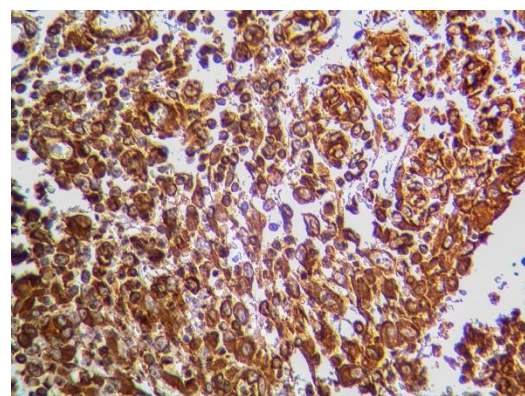


Figure 4: Cytoplasmic and membranous staining of squamous cells in squamous cell carcinoma, strong intensity (CD146 IHC stain, 400 X)

However, in the OSCC group, the intensity and expression of CD₁₄₆ was significantly correlated with the histopathological grade of OSCC. Meanwhile, the CD₁₄₆ expression was not significantly correlated with the lymph node metastasis in neither group. Moreover, a significant correlation existed between the CD₁₄₆ expression and the mode of invasion and tumor size in the OSCC cases ($p < 0.05$) (Table 2).

Table 2: Correlation of CD₁₄₆ expression in MEC and OSCC with clinicopathologic findings

Variables	CD ₁₄₆ expression in MEC	CD ₁₄₆ expression in OSCC
Metastasis	0.338	0.442
Size	0.649	0.012
Mode of invasion	-	0.002
Histologic grade	0.726	0.024

p < 0.05 is significant

Discussion

The correlation between the CD₁₄₆ expression and the clinical behavior and lymph node metastasis has been evaluated in some tumors such as melanoma, breast carcinoma, and prostate carcinoma. The present study tried to investigate if the expression of CD₁₄₆ in MEC and OSCC samples is associated with the histological grade, lymph node metastasis, tumor size, and mode of invasion in these tumors.

In this study, the immunohistochemical expression of CD₁₄₆ was observed in all MEC samples; the staining was strong in 78.9% of samples, which was in accordance with Pires *et al.*'s findings [10]. The present study detected no significant correlation between the staining intensity and histological grade of the tumor. It was in agreement with Pires *et al.*'s study [10] and in contrast with Zhang's [11] and Li's [12] findings, who studied the CD₁₄₆ expression in breast carcinoma and malignant cervix tumor. The difference between the studies might be attributed to the type of investigated tumors.

In high-grade MEC, the staining intensity was moderate in 57.1% and strong in 42.9% of samples. Yet, the correlation between the CD₁₄₆ staining intensity and the histopathologic grade of MEC was not significant. The staining localization was mostly in the cytoplasm of mucous and epidermoid cells. No significant correlation was found between the staining localization and the histopathologic grade in MEC cases.

CD₁₄₆ was expressed in 58.9% of OSCC cases, which disagreed with what was found by Pires *et al.* [10], who reported the CD₁₄₆ expression in none of the OSCC samples in his study. This difference might be due to the different staining method and the smaller sample size in Fabio's study [10]. In OSCC samples, strong staining was seen in all tumors with histopathologic grade III, which was statistically significant and in line with Zhang *et al.*'s study [11].

No significant correlation was detected between the staining intensity and lymph node metastasis (*p* > 0.05).

Our findings were in contrast with Zhang *et al.* [11] and Wu *et al.* [13] studies on cervix, ovarian, and prostate tumors. The difference might be due to the different type of tumors and the smaller sample size in current study. Our findings revealed that the CD₁₄₆ expression cannot predict lymph node metastasis in MEC and OSCC but it did in prostate, ovarian tumors, and colorectal cancers [14]. In addition, Li *et al.* [15] noted that the CD₁₄₆ expression was an indicator of poor prognosis in esophageal SCC.

Some studies documented the role of α₇β₃ integrin as a ligand of Mel-CAM and accredited that α₇β₃ integrin in adults' normal tissues are limited to basolateral membrane cells in ductal epithelium of parotid glands [16]. Seemingly, the integrin is expressed in normal and benign tumors; but in malignant tumors, it either remains unexpressed or undergoes structural changes, which further lead to dysfunction and lack of connection between the neoplastic cells [16].

Mesenchymal and epithelial interactions are essential for glandular organ formation in salivary glands. Moreover, the role of CD₁₄₆ in epithelial-mesenchymal transition in breast cancer has been alluded [17]. Evidence shows that absence or decrease of CD₁₄₆ expression in breast cancer leads to repair connections between the normal cells and waste connections between the tumoral cells, and acts as a suppressor in breast tumor [18]. The low expression of CD₁₄₆ in normal tissue and benign tumor is used for differential diagnosis of some benign and malignant tumors with similar origin (malignant mesothelioma and reactive one) [19]. The present study detected a significant relation between the tumor size and CD₁₄₆ expression (*p* = 0.012). OSCC samples of larger than 6.1 cm showed higher expression, which was in accordance with Mills's *in vivo* study on melanoma cells [20]. Other *in vivo* studies have asserted the role of CD₁₄₆ in aggressive behavior of melanoma cells. Likewise, MMP-2 regulation and inhibition of CD₁₄₆ correlate with less aggressiveness and high apoptotic activity [18]. The current study detected a significant correlation between the histological grade of OSCC and CD₁₄₆ expression, staining intensity, and localization of staining. These variables had never been evaluated before. Additionally, the mode of invasion was significantly correlated with high expression and intensity of CD₁₄₆ in OSCC, indicating the imperative

role of CD₁₄₆ in tumor development and invasion. Regarding the correlation between CD₁₄₆ expression and mode of invasion, the present results suggest that CD₁₄₆ expression is a more determining indicator of mode of invasion in OSCC than MEC cases. However, further studies with larger sample sizes may introduce CD₁₄₆ as an advantageous prognostic marker in OSCC.

Conclusion

CD₁₄₆ expression is significantly correlated with the mode of invasion, tumor size, and histological grade in oral squamous cell carcinomas. It may help predict the prognosis in patients with OSCC but not MEC. CD₁₄₆ is not a useful marker for differentiating between the high-grade MEC and OSCC.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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