MTA1 Expression in Salivary Mucoepidermoid Carcinoma: with Special Emphasis on Grading Systems

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Abstract

Metastasis-associated protein-1 (MTA1) has been recently identified as a unique gene which plays a key role in tumorigenesis and progression of cancer cells. The object behind this study is to evaluate MTA1 immunoexpression and its relationship with predictive value of point- based grading systems in salivary mucoepidermoid carcinoma. A total of 22 formalin-fixed, paraffin-embedded specimens of mucoepidermoid carcinoma were prepared for immunohistochemical staining with MTA1 antibodies. Assessment of MTA1 immunostaining was achieved by counting the proportion of positively-stained tumor cells in 5 high power fields; and staining was analyzed in relation to tumor grading systems and other clinicopathologic features. MTA1 showed nuclear and cytoplasmic expression in varying intensity in 95% of cases. Non- significant correlation was found between MTA1expression and age, gender, site of the tumor ($p > 0.05$). However, statistically significant correlation was observed between MTA1expression and clinical stage, as well, to nodal involvement ($p = 0.009$ and 0.007; respectively). Regarding histologic grade, high MTA1 level was significantly associated with grade of tumors categorized by Auclair and Brandwein systems ($p = 0.001$ and 0.009; respectively). MTA1 expression significantly correlates with tumor grade and progression, and it has a potential role to predict the prognosis of salivary MEC.

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Introduction

Mucoepidermoid carcinoma is the most common salivary gland malignancy; approximately accounting for 30% of these tumors.[1] MEC exhibits a complex histopathologic features; and it is generally categorized into low, intermediate, or high grade.[2,3]

Metastasis-associated protein-1 (MTA1), a unique member of the MTA proteins, which is specifically involved with more aggressive cell phenotype and tumor metastastasis.[4] MTA1-protein is transcriptional co-repressors involved with complex-containing deacetylation molecules that may inactivate the p53 protein.[4,5] Moreover, MTA1 may induct aberrant tumor neovascularization via stabilizing hypoxia-inducible factor-1α.[6] It has been suggested that the high MTA1 expression is significantly correlated with tumor invasion and progression.[7,8] However, high immunoexpression has been ascertained for MTA1protein in different tumor types, and it has statistically significant correlation with tumor grade and angiogenesis.[9,10] The purpose of this study is to evaluate the correlation between MTA1 immunoexpression and grade of salivary MEC categorized by Auclair and Brandwein systems.

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Materials and Methods

This study included 22 formalin-fixed, paraffin-embedded specimens of salivary MEC that retrieved from the archives of the Department of Oral Diagnosis/College of Dentistry/ Baghdad University. Clinical data concerning the age, gender, site of tumor and lymph node metastasis was obtained from patients' medical records for the period extending from 2009 to 2017. For all specimens, 4μm thick sections were prepared and stained with hematoxylin and eosin (H&E) stain to confirm the diagnosis. Clinicopathologic characteristics of the

overall series are summarized in (Table 1). The histological grade of tumors was evaluated according to criteria of Auclair et al. (1992), and that proposed by Brandwein et al, (2001), which are approved by The WHO tumor classification.[11,12] Moreover, all cases were staged according to the 7th edition of the American Joint Committee on Cancer (AJCC).[13]

Variables	Total	
	No.	(%)
Age group (years)		
<40	5	22.7
>40	17	77.3
Gender		
Female	12	54.5
Male	10	45.4
Site		
Major	11	50
Minor	11	50
Clinical stage		
I	9	40.9
II	7	31.8
III	3	13.6
IV	3	13.6
Lymph node status		
-ve	16	72.7
+ve	6	27.3
Auclair grading system		
Low grade	15	68.1
High grade	7	31.9
Brandwein grading ststem		
I	6	27.3
II	6	27.3
III	10	45.4

Table 1. Clinical-pathological Parameters of the Overall Series.

Immunohistochemistry (IHC) and analysis

The sections in these series were deparaffinized in xylene, rehydrated through graded alcohols, immersed in 0.3% H_2O_2 for 20 min to inhibit endogenous peroxidase activity, and antigen retrieval performed using citrate buffer with PH = 6. Nonspecific binding was blocked with 1% serum albumin at room temperature for 10 min, then the sections were incubated with anti-MTA1 rabbit-polyclonal antibody (1:500 dilution; Abcam, Cambridge, UK) overnight at 4 °C in a humidified chamber. Negative controls were achieved by omitting the primary antibody. After washing with phosphate buffer saline (PBS), the tissue sections were incubated with biotin-free, anti-rabbit secondary antibody conjugated with horseradish peroxidase (HRP) for 15 min, and then color was developed using 3,3'-diaminobenzidine as chromogen. then, it is counterstained with Mayer's hematoxylin, mounted and covered with cover slip for evaluation using microscope (OLYMPUS, Japan).

IHC scoring and Statistical analysis

The degree of IHC staining was separately evaluated by two pathologists who were blinded to the clinicopathologic information. MTA1 immunostaining was scored by calculating the proportion of positively stained tumor cells in 5 microscopic high power fields that reveal higher immunopositivity as follows: Score 0 (0-5% positive cells); Score I (6-25% positive cells); score II (26-50% positive cells); score III (51-75% positive cells) and Score IV (≥76 positive cells). A Mann-Whitney test, Kruskal Wallis test and Spearman´s correlation coefficient test were used to compare the result between groups and the relation with clinical-pathological parameters such as patient age, gender, tumor site, metastasis to lymph nodes, tumor grade and clinical stage. We used the SPSS version 24 software to statistically analyze the data. P-values <0.05 were considered statistically significant in all cases.

Results

Representative images of MTA1 immunohistochemical (IHC) expression in MEC are shown in (Figure 1A-F). Positive immunostaining of MTA1 was observed in both nuclear and cytoplasmic compartments in (95%) of the MEC tissues. While, negative or weak MTA1stanining was found in the adjacent non-cancerous ductal epithelial tissues in the same section (Figure 1 A).

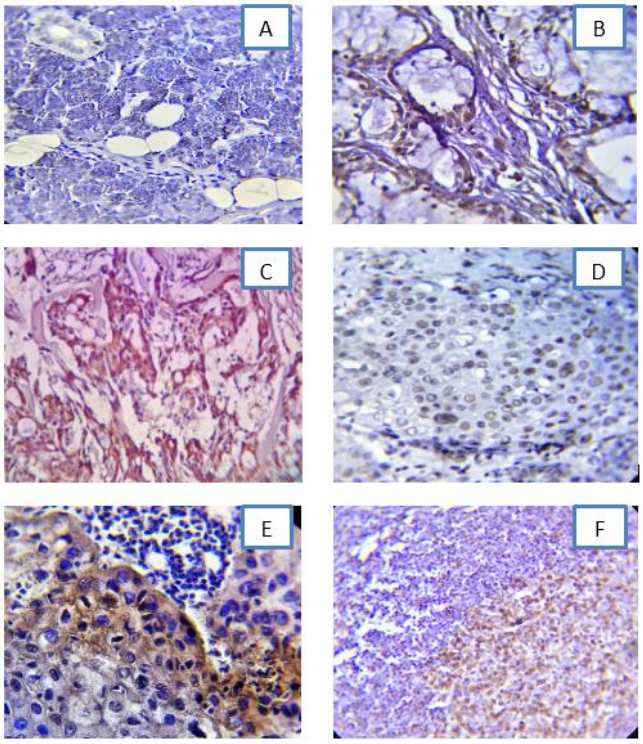

Figure 1. Immunohistochemical analysis of MTA1 protein in salivary MEC; (A) Negative staining in normal salivary ductal tissue (40x); (B) low grade tumor shows cystic components lined by mucous and intermediate cell: brown stain (40x); (C) MEC, with small islands of intermediate and epidermoid cell, mainly cytoplasmic expression (20x); (D) High grade MEC shows nuclear MTA1expression (40x); (E) Solid tumor with sheets of anaplastic epidermoid cells with cytoplasmic expression (40x); (F) lymph node infiltrated with tumor cells expressing nuclear MTA1 protein (20x).

Correlation between MTA1 expression and the clinicopathologic features of salivary MEC

Table 2 showed the correlation between MTA1 expression and the clinicopathologic characteristics in all series. Accordingly, no statistically significant correlation between MTA1 protein expression and clinicopahtologic features, such as age, gender and tumor site ($P > 0.05$). However, the expression level of MTA1 protein was found to be significantly associated with clinical stage, showing a lower expression pattern in early-stage disease (I and II), and a stronger expression (median MTA1 score 4), in late stages (III and IV; $P = 0.009$). High MTA1 expression also was found to be significantly associated with positive nodal metastasis (p=0.007). Regarding both Auclair and Brandwein grading systems, the median MTA1 score was significantly higher among tumors with high grade (score-3) compared to those with low grade (median score = 2), thus a statistically significant correlation was observed between MTA1 expression and grade of tumors categorized by Auclair and Brandwein systems (p value < 0.001 and 0.009; respectively), as in (Figure 2).

Parameters	MTA1 scores*					Median MTA score	p-value†
	0	I	II	III	IV		
Age group (years)							
<40	0	1	3	1	0	2	0.15
>40	1	3	6	5	2	3	[NS]
Gender							
Male	0	1	4	3	2	3	0.10
Female	1	3	5	3	0	2	[NS]
Site							
Minor	0	1	6	3	1	2	0.45
Major	1	3	3	3	1	2	[NS]
Clinical Stage							
I-II	1	4	8	3	0	2	**0.009**
III-IV	0	0	1	3	2	4	
Lymph node metastasis							
N0	1	4	8	3	0	2	
N1	0	0	0	1	2	4	**0.007**
N2	0	0	1	2	0	3	
Auclair grading system							
Low	1	4	9	1	0	2	**0.001**
High	0	0	0	5	2	3	
Brandwein grading system							
Low	1	2	3	0	0	2	**0.009**
Intermediate	0	2	3	1	0	2	
High	0	0	3	5	2	3	

* Score 0 (0-5% positive cells); Score I (6 - 25% positive cells); score II (26 - 50% positive cells); score III (51 - 75% positive cells) and Score IV (≥ 76 positive cells). [NS] Non- significant p > 0.05; † A Mann-Whitney test, and Kruskal-Wallis test.

Table 2. Correlation Between MTA1 Expression and the Clinicopathologic Features.

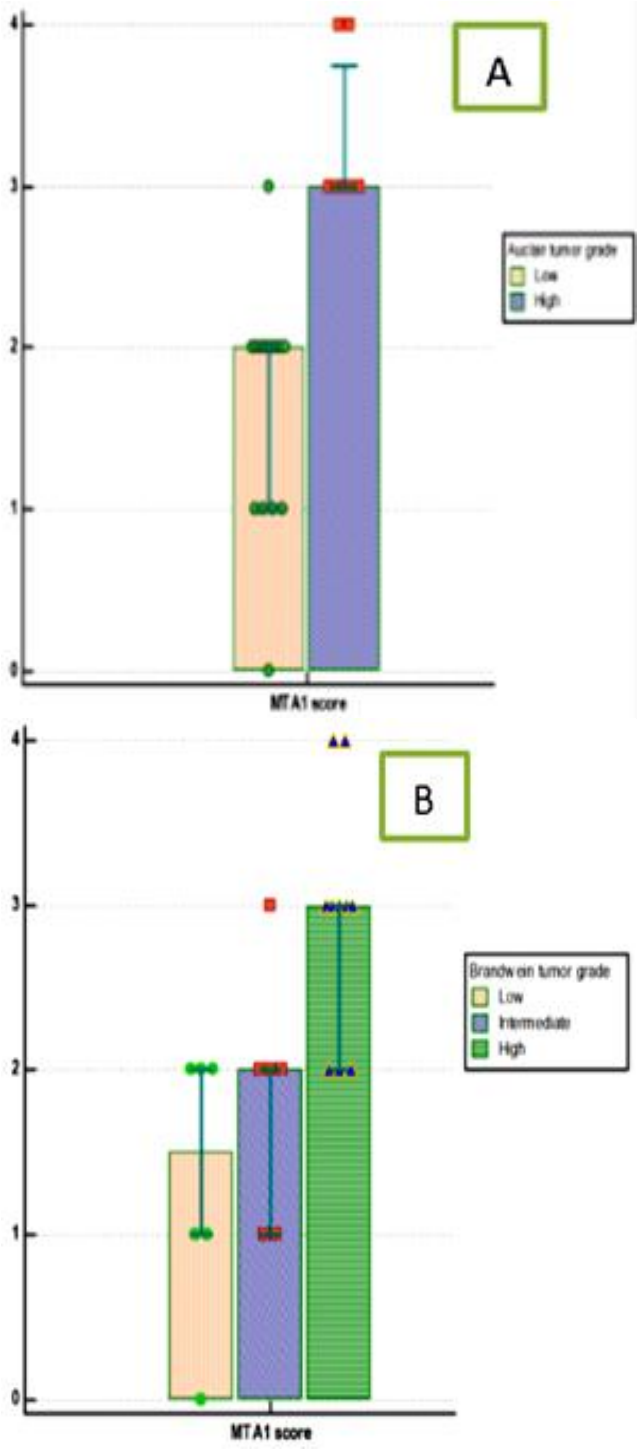

Figure 2. Diagram Shows MTA1 Expression (Median Scores) Regarding (A) Auclair; (B) Brandwein System.

Discussion

In the broad sense, all grading systems of MEC are prognostically effective and show a quite reproducibility among pathologists, although they are somewhat complex, time-consuming and each of systems vary regarding to the behavior of each particular grade. Although, a various methods were utilized as avenue in salivary cancer monitoring, it is necessary to find a new biomarkers that specifically predict tumor aggressiveness and behavior.[14,15,16] In our study, MTA1 exhibited different IHC and subcellular distribution which was in consistence with other researches.[17,18] In the present study, we evaluated MTA1 expression in salivary MEC, and there was no statistically significant correlation between MTA1 level and clinical findings, such as patient age, gender and tumor site. This finding was in agreement with previous studies.[19,20] In this study, also we observed that MTA1expression was significantly correlated with clinical stage and lymph node metastasis. In this context, our findings agree with other researches, in which IHC analysis indicated that MTA1 expression was significantly correlated with tumor aggressiveness, and positive nodal status in head and neck cancer.[21,22] However, our results were not in conformity with findings from Andishehtadbir et al. (2016) who found that MTA1 protein expression had no significant statistical correlation with clinical stage, lymph node status and metastasis of salivary MEC which may be attributable to an insufficient number of cases.[23] On the other hand, in this study, a significant cohort of cases showed a nuclear MTA1 expression compared to cytoplasmic one, and this suggest the invasive growth and metastatic potential of these tumors.[24] However, our explanation for cytoplasmic expression of MTA1 based on earlier investigations which have been stated that MTA1s, a short version of cytoplasmic MTA1 may bind to estrogen receptor-alpha (ER-α) and inhibit its nuclear function by non-genomic activity of (ER-α) that occurs in cytoplasm of cancer cells. Thus, this may rationalize the aggressive behavior of these tumors. [25,26] Regarding tumor grading, we found a significant up-regulation of MTA1 expression in higher grade tumors classified according to both Auclair and Brandwein system with strong positive linear correlation between MTA1 score (r = 0.7) and Brandwein tumor grade. This result indicated a strong association of MTA1 expression with the progression of MEC. In contrast to our findings, Andishehtadbir et al. (2016), found no correlation between MTA1 expression and histologic grade of salivary MEC, although high MTA1 expression

was seen in some cases with advanced tumor size and clinical stage.[23] The possible explanation behind this disagreement is that, in our analysis we have approved a standardized grading systems for tumor classification, with focus on more objective criteria, as compared to previous study which was built on a subjective morphologic and histologic features.[23] In conclusion, MTA1 expression significantly correlates with histologic grade and progression of salivary MEC, which means it may be prognostically effective and has a significant predictive value in salivary MEC.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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