Original Article

Clinicopathologic Significance of P63, Maspin and MMP-2 Expression in Mucoepidermoid Carcinoma

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Abstract

Background and Aim: Mucoepidermoid carcinoma (MEC) is the most common malignant neoplasm of the salivary glands with complex histopathologic features and clinical behavior. The aim of this study was to evaluate the clinicopathologic significance of p63, Maspin and matrix metalloproteinase (MMP)-2 expression in MEC.
Materials and Methods: In this retrospective study, immunohistochemical staining was performed to detect the expression of p63, Maspin and MMP-2 in 35 cases of MEC. In addition, to detect the presence of myoepithelial differentiation, smooth muscle actin (SMA) staining was performed. Data were analyzed using non-parametric tests.
Results: P63 expression, intensity of staining for p63 and MMP-2 total score showed significant correlations with histologic grade (P=0.008, P=0.035 and P=0.003, respectively). The association between p63 expression and MMP-2 total score was significant (P=0.033). P63 positive cases did not express SMA.
Conclusion: Findings suggest that p63 and MMP-2 may be useful prognostic indicators in MEC. MEC is devoid of myoepithelial cells.

Key Words: TP63 Protein, Human, SERPIN-B5, Matrix Metalloproteinase 2, Carcinoma, Mucoepidermoid

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Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor. The peak age of incidence is in the fourth and sixth decades of life [1]. Its clinical behavior varies from asymptomatic swelling to rapidly growing mass with invasion to the surrounding structures [2]. MEC is composed of epidermoid cells, mucus cells, intermediate cells and clear cells [2,3]. Participation of myoepithelial cells in tumorigenesis of MEC is still elusive. Controversial results have been reported by immunohistochemistry and ultrastructural studies [4].

P63 protein is expressed in the nuclei of different cells, such as normal stratified epithelial cells in the oral cavity, esophagus and cervix [1]. In malignant tissues, p63 is expressed by transitional and oral squamous cell carcinoma (OSCC) and several salivary gland tumors such as adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. To date, few studies have focused on the diagnostic role of p63 expression in malignant salivary gland tumors [1,5]. However,

the prognostic and clinicopathologic implication of p63 expression in malignant salivary gland tumors is not clear.

Maspin (mammary serine protease inhibitor) is a member of serine protease inhibitor (serpin) family. It is documented that Maspin is expressed in normal cells and down-regulated in neoplastic tissues. In vivo and in vitro studies demonstrate that Maspin suppresses tumor growth and metastasis by inhibiting tumor cell invasion and antiangiogenic effect through microvessel density reduction [6]. Loss of Maspin has been associated with unfavorable prognosis in OSCC, lung and prostate cancer [7-9].

Extracellular matrix (ECM) molecules are modified by matrix metalloproteinases (MMPs), a family of enzymes that can modulate cell density by creating space for migration, releasing ECM-bound growth factors and activating signaling molecules. MMP-2 and MMP-9 are type IV collagenases that degrade the major structural protein of extracellular matrix and basement membrane [10]. The prognostic significance of MMP-2, 9 expressions has been reported in prostate cancer and OSCC [11,12]. The aim of the present study was to determine the clinicopathologic significance of p63, Maspin and MMP-2 in MEC and their mutual correlation.

Materials and Methods

This retrospective descriptive study was conducted on 35 cases of MEC diagnosed in the Pathology Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Demographic and clinicopathologic information of each patient including age, sex, location of tumor, size of tumor, histologic grade and lymph node metastasis were obtained from the medical records of patients and slides. Brandwein system was used to determine the histological grade of MEC [3].

Immunohistochemistry:

Sections (3mm thickness) of formalin-fixed paraffin-embedded blocks were mounted on silane- coated slides (silanized S 3003; Dako, Copenhagen, Denmark), dewaxed in xylene, rehydrated in graded ethanol and treated with 3% hydrogen peroxide. Antigen retrieval was carried out in l0mM citrate solution (pH 6.0) in a microwave oven (750W) for l0 minutes.

The sections were incubated with mouse monoclonal antibody against p63 (ready-to-use; Dako, Glostrup, Denmark), Maspin (1:50; Novocastra, Newcastle, UK), MMP-2 (1:60; Novocastra, Newcastle, UK) and smooth muscle actin (SMA; 1:50; Novocastra, Newcastle, UK) and were then treated with Dako Envision TM. Finally, the sections were incubated with 3, 3 diaminobenzidine (K3468; Dako) for 2-5 minutes at room temperature and counterstained with Mayer's hematoxylin. Human breast tissue, ulcerative colitis, normal salivary gland and bowel wall tissues were used as positive controls for p63, MMP-2, Maspin and SMA, respectively. The primary antibody was omitted as negative control.

P63 immunostaining was graded semi-quantitatively, with negative indicating less than 10% of cells staining. Positive nuclear staining was graded as follows: 10-25% of tumor cells stained weakly positive; 26-75% of tumor cells stained moderately positive, and 76-100% of tumor cells stained highly positive. The staining intensity was classified as: 0, no staining; 1, weak staining; 2, moderate staining and 3, strong staining [13].

Maspin immunoreaction was scored in three groups according to the percentage of positive tumor cells: high (≥50% of cells stained); intermediate (20-49% of cells stained), or low (up to 20% of cells stained) [14]. Staining intensity was evaluated similar to that for p63.

MMP-2 expression was determined using a semi-quantitative scoring system from 0 to 6 based on the percentage of positive tumor cells (0-3) and staining intensity (0-3). Finally, the two scores were multiplied, providing a final score: 0-1, negative (-); 2-3 (+); and ≥4 (++) [15]. Identification of p63 positive cells as myoepithelial cells was confirmed by SMA immunoexpression.

Statistical analysis:

The data were analyzed using SPSS version 18 Software (SPSS Inc., Chicago, IL, USA). The Kruskal Wallis test was used to assess the correlation of p63, Maspin and MMP-2 expression with histologic grade. In order to evaluate the correlation of p63, Maspin and MMP-2 expression with lymph node metastasis and tumor size, the Mann Whitney test was applied. In addition, the Kruskal Wallis and Mann Whitney tests were used

to assess the correlation of MMP-2 total score with histologic grade and metastasis, respectively. The Spearman's correlation coefficient was used to assess the correlation of expression of markers. P<0.05 was considered statistically significant.

Results

Thirty-five patients with MEC (22 females and 13 males) were evaluated. The mean age of patients was 42.14±17.13 years (range 12-74 years). Tumor size ranged from 0.9 to 7cm (mean of 3.07±1.49cm). The distribution of histologic grade 1 to 3 was 14 (40%), 6 (17.1%) and 15 (49.9%), respectively. Perineural invasion was seen in 3 (8.6%) cases and lymph node metastasis was seen in 1 (2.9%) case (Table 1).

P63 immunoexpression:

P63 was expressed in all cases as nuclear staining mostly in epidermoid cells and sparsely in intermediate cells (Figure 1). Nineteen cases (54.3%) showed high expression, 10 (28.6%) cases showed moderate staining and the remaining six (17.1%) cases showed weak p63 expression. Evaluation of staining intensity showed strong intensity in 20 (57.1%) cases. P63 positive cell percentage and staining intensity showed significant correlations with histologic grade (P=0.008 and P=0.03, respectively; Table 2). The correlation of p63 expression and intensity of staining and lymph node metastasis was not significant (P=0.629 and P=0.571, respectively). P63 positive cells did not express SMA.

Maspin immunoexpression:

Maspin was expressed in 88.6% (31/35) of the cases by epidermoid and intermediate cells (Figure 2). Most of the cases (45.2%, 14/35) showed high expression of Maspin. Regarding staining intensity, most of the cases (67.7%, 21/35) showed moderate intensity; 26 (83.9%) cases expressed Maspin in both nucleus and cytoplasm, 4(12.9%) cases in cytoplasm and 1 (3.2%) case in nucleus. No significant correlation between Maspin expression and intensity of staining with histologic grade (P=0.133 and P=0.447, respectively) or lymph node metastasis (P=0.914 and P=0.800, respectively) was found (Table 3).

MMP-2 immunoexpression:

MMP-2 was expressed in 74.3% (26/35) of cases (40% of grade 1, 17% of grade 2, 43% of grade 3) as cytoplasmic staining (Figure 3). Data analysis showed a significant correlation between MMP-2 total score and histologic grade (P=0.003). No association between lymph node metastasis and Maspin expression was seen (Table 4).

Relationship between p63, Maspin, and MMP-2 expression:

Our results showed significant correlations between p63 and MMP-2 expression (r=0.36, P=0.033). No association between p63 and Maspin expression (r=173, P=0.321), or Maspin and MMP-2 expression (r=0.425, P=0.134) was noted.

Discussion

MEC comprises 12-15% of malignant salivary gland tumors, with peculiar histopathologic features [2,3]. In the present study, we investigated p63, Maspin and MMP-2 expression in MEC.

The role of p63 in tumorigenesis is debatable. Overexpression of p63 has been reported in many solid tumors in particular, head and neck squamous cell carcinoma, urothelial carcinoma and adenoid cystic carcinoma [16-18]. P63 expression has been recommended as a reliable indicator of histologic grade and prognosis in breast cancer, meningioma and OSCC. It may be due to oncogenic role of one p63 isotype, ΔNP63, in proliferation and differentiation [19-21]. Additionally, p63 has been demonstrated as a helpful marker in distinguishing low-grade MEC from papillary cystadenoma of salivary glands [1].

This is the first study to examine the prognostic significance of p63 expression in MEC. P63 was expressed in all cases of MEC. Epidermoid and intermediate cells expressed p63. A significant correlation was found between p63 expression and intensity of staining with histologic grade as p63 expression was higher in less differentiated tumors compared to more differentiated ones. It was in agreement with previous studies conducted on breast cancer and prostate cancer (21) that suggested p63 expression as a prognostic marker.

Few studies on squamous cell carcinoma have addressed the correlation of p63 and MMPs expression. It seems that p63, through different pathways and mediators including snail-mediated epithelial-mesenchymal transition and repression of Wnt/β catenin response, regulates MMPs expression. These findings propose a peculiar role

Table 1. Clinicopathologic characteristics of patients with mucoepidermoid carcinoma

Characteristics	Total number of patients (%)
Patients	35
Age (years)	
Mean (SD)	42.14(17.13)
Range	12-74
Gender	
Female	22(62.9)
Male	13(37.1)
Location	
Major salivary gland	17 (48.6)
Minor salivary gland	18 (51.4)
Size (cm)	
<4	25(71)
≥4	10(29)
Grade	
1	14 (40)
2	6(17.1)
3	15(49.9)
Neural invasion	
No	32(91.4)
Yes	3(8.6)
Lymph node metastasis	
No	34(97.1)
Yes	1(2.9)

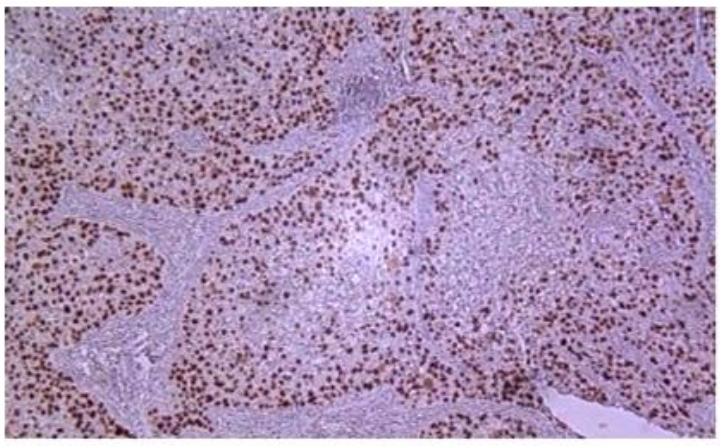

Figure 1. Nuclear p63 immunoexpression in mucoepidermoid carcinoma (x200)

Table 2. P63 expression profile: Association with grade and metastasis in mucoepidermoid carcinoma

	Percentage of positive cells				P-value					P-value
Parameter	0(n)	10-25% (n)	26-75% (n)	76-100% (n)		0 (n)	1(n)	2(n)	3(n)	
Grade										
1	0	4	5	5	0.008*	0	1	8	5	0.0358*
2	9	2	2	2		0	0	2	4	
3	0	0	3	12		0	0	4	11	
Metastasis										
No	0	6	9	19	0.629	0	1	14	19	0.571
Yes	0	0	1	0		0	0	0	1	

***: P<0.05**

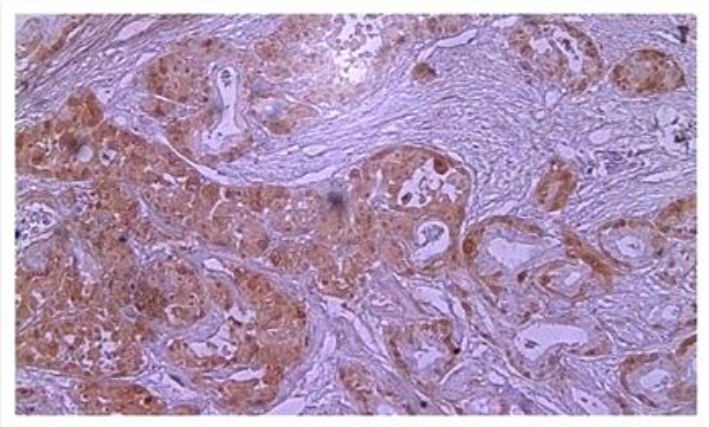

Figure 2. Nuclear-cytoplasmic Maspin immunoexpression in mucoepidermoid carcinoma (x100)

Table 3. Maspin expression profile: Association with grade and metastasis in mucoepidermoid carcinoma

	Percentage of positive cells				P-value					P-value
Parameter	0(n)	10-25% (n)	26-75% (n)	76-100% (n)		0 (n)	1(n)	2(n)	3(n)	
Grade										
1	1	2	3	8	0.133	1	3	7	3	0.447
2	0	2	2	2		0	3	3	0	
3	3	2	6	4		3	1	11	0	
Metastasis										
No	4	6	10	14	0.914	4	7	20	3	0.800
Yes	0	0	1	0		0	0	1	0	

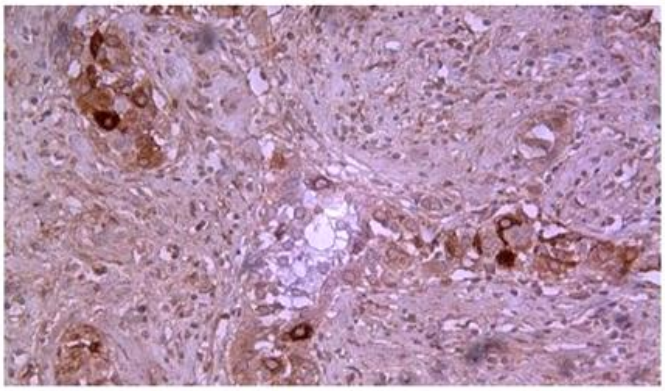

Figure 3. Cytoplasmic MMP-2 immunoexpression in mucoepidermoid carcinoma (x200)

Table 4. MMP-2 expression profile: Association with grade and metastasis in mucoepidermoid carcinoma

Percentage of positive cells					**P-value**	Intensity				**P-value**	**Total score**			**P-value**
Parameter	0(n)	1(n)	2(n)	3(n)		0(n)	1(n)	2(n)	3(n)		0(n)	+(n)	++(n)	
Grade														
1	7	2	2	3	0.001*	7	3	2	2	0.0358*	7	2	5	0.003*
2	0	0	1	5		0	1	4	1		0	0	6	
3	2	0	0	13		2	2	5	6		2	0	13	
Metastasis														
No	9	2	3	20	0.629	9	5	11	9	0.571	9	2	23	0.800
Yes	0	0	0	1		0	1	0	0		0	0	1	

***: P<0.05**

for ΔNP63α regulated by MMP-2 expression and invasiveness of squamous cell carcinoma cells through expression of inhibitor of differentiation-3 (id-3) protein [22,23]. It was of great interest to verify the association of p63 and MMP-2 expression in the present study. However, the underlying mechanism remains to be elucidated and needs further investigation.

Maspin is a 42KDa cytoplasmic protein, which functions as a tumor suppressor by inhibiting tumor cell invasion and cell motility. Of note, Maspin apoptotic effect is tumor-specific [24,25]. Regulation of Maspin expression may be due to transactivation of elements in the Maspin promoter and hormonal response [14]. Moreover, review of literature denotes that tumor suppressive effect of Maspin is related to Maspin cellular localization. Nuclear Maspin expression is indicative of favorable prognosis in non-small cell lung adenocarcinoma, pancreatic cancer and prostate adenocarcinoma [24,25], indicating the suppressive effect of Maspin. Most of the cases in our study expressed Maspin in both nucleus and cytoplasm. We did not observe a significant correlation between Maspin expression and histologic grade or lymph node metastasis confirming the previous studies on salivary gland tumors [26,27] and impact of Maspin cellular localization.

All ECM components can be degraded by MMPs and each element is cleaved by a specific member of the MMP family. The MMP family plays a critical role in the process of tumor cell invasion

into the tumor stroma and tumor-inducing vascularization [28-30]. The MMP activity is regulated at different levels, such as cellular receptors, presence of inhibitors, compartmentalization and gene expression. Among identified MMPs, MMP-2 has a significant role in tumor development and tumor invasion.

Up-regulation of MMP-2 in malignant salivary gland tumors compared to benign neoplasms and normal salivary gland tissue was reported by Zhou et al. [28]. However, no significant correlation was noted between MMP-2 expression and histologic grade in their study. Expression of MMP-2, MMP-9 and their tissue inhibitors in benign and malignant salivary gland tumors showed an imbalance between MMPs and their tissue inhibitors in malignant neoplasms and invasive properties related to MMP-2 [15]. Guan et al, [10] also evaluated MMP-2 expression in salivary gland adenoid cystic carcinoma. Their result emphasized on the role of high levels of MMP-2 expression in aggressive behavior of tumor. In accordance with previous studies, our findings showed the significant correlation of MMP-2 expression and histologic grade [15]. It disagrees with the findings of Taher et al [30]. The difference may be due to smaller sample size in the study by Taher et al, [30] which comprised of 15 cases of MEC.

In conclusion, the expression and intensity of staining of p63 and MMP-2 showed significant correlations with histologic grade and may be useful prognostic indicators for MEC. Moreover, all cases were devoid of myoepithelial cells.

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