

RESEARCH ARTICLE

Expression of Matrix Metalloproteinase-10 at Invasive Front of Squamous Cell Carcinoma and Verrucous Carcinoma in the Oral Cavity

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Abstract

Background: Matrix metalloproteinases (MMPs) are a family of zinc metalloproteinases capable of degrading components of connective tissues. MMP-10 is frequently expressed in human cancers. The aim of this study was to immunohistochemically evaluate its expression in oral squamous cell carcinoma (OSCC) and verrucous carcinoma (OVC). **Materials and Methods:** A retrospective analysis of 73 samples (31 OSCC, 22 OVC and 20 non-neoplastic epithelium) was performed. All samples were immunohistochemically stained with monoclonal MMP-10 antibody and expression levels and staining intensity were evaluated with respect to microscopic features. Data were analyzed by SPSS (V.21), Mann-Whitney and Kruskal Wallis tests. **Results:** MMP-10 was detected in all OSCC and OVC cases. The expression of MMP-10 in OSCC was intensive (score 3) and in OVC was low and moderate (score 1 and score 2) more frequently. Non-neoplastic epithelium did not show MMP-10 expression. Differences between groups was statistically significant ($p < 0.05$). However, the expression of MMP-10 was not obviously different between various grades of OSCC. **Conclusions:** According to our study, MMP-10 protein can be important possible factor in the transformation of normal oral epithelium to OVC and OSCC, also the level of MMP-10 expression at invasion front of the lesions can be helpful in the differentiation of OVC and OSCC.

Keywords: MMP-10 - oral squamous cell carcinoma - oral verrucous carcinoma - immunohistochemistry

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Introduction

Oral Squamous Cell Carcinoma (OSCC) is the most common oral cavity malignancy, which forms 94% of all oral malignancies and about 38% of all head and neck malignant tumors (Henriques et al., 2012; Mohtasham et al., 2013). OSCC is a highly invasive tumor whose prognosis is associated with the activity of proliferative tumor, degree of differentiation, invasion and its metastatic potential. The last two processes include several stages such as destruction of basement membrane and extracellular matrix, change in cell adhesion, and tumoral cells motility. Studying the factors effective in these processes is important for understanding tumor behavior and anticancer treatments development (Impola et al., 2004; Henriques et al., 2012).

Oral Verrucous Carcinoma (OVC) is a low-grade and well-differentiated variant of OSCC that forms 1-10% of OSCC. This lesion has a benign microscopic feature, which is characterized by long rete ridges with abundant

production of keratin. OVC has a strong tendency to local invasion whereas metastasis is rarely seen (Klieb and Raphael, 2007; Zargaran et al., 2012; Mohtasham et al., 2013). Due to some pathological similarities with OSCC, it is sometimes difficult to distinguish between them while they have different treatments (Mohtasham et al., 2013).

Degradation of extracellular matrix and basement membrane is a major mechanism in local invasion and tumors metastasis (Mashhadiabbas et al., 2012). Matrix metalloproteinases (MMPs) include a group of zinc-dependent proteases capable of degradation extracellular matrix components such as collagen and proteoglycans (Tongtawee et al., 2015). Therefore, they play a major role in normal development and tissue injury in various pathological conditions such as arthritis, wound healing, angiogenesis, and cancer (Deraz et al., 2011; Liu et al., 2012; Hadler-Olsen et al., 2013). MMPs are classified into four big groups including gelatinases, collagenases, stromelysins, and membrane-bound type (Deraz et al., 2011; Mashhadiabbas et al., 2012; Specenier and Brouwer,

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MMP-10 or Stromelysin-2 is one of the members of this family, which breaks down different extracellular compounds such as proteoglycans, laminin, fibronectin and collagen types III and IV (Miyata et al., 2007; Liu et al., 2012). The increase of MMP-10 expression has been found in several tumors including head and neck SCC (Deraz et al., 2011; Iizuka et al., 2014), Hepatocellular carcinoma (García-Irigoyen et al., 2015) lung cancer (Gill et al., 2004), cutaneous SCC (Kerkelä et al., 2001), bladder transitional cell carcinoma (Seargent et al., 2005; Zhang et al., 2014) and renal cell carcinoma (Miyata et al., 2007). It may be involved in degradation of extracellular matrix during tumor progression and inflammation (Mathew et al., 2002) but exact role of MMP-10 in tumor invasion and metastasis in human cancers is contradictory (Liu et al., 2012). Previous studies have revealed effects of some members of MMPs including MMP2 and MMP9 in invasion and metastatic behavior of OSCC (Lotfi et al., 2014; Jafarian et al., 2015).

Tumor Invasion Front (TIF) is a major dynamic area for differentiation of malignancies. In fact, this area can be considered as an invasion front of epithelium in the connective tissue in epithelial-mesenchymal transition (EMT). The molecular biomarkers, which are found in TIF, have been introduced as the stronger predictors for tumor prognosis as compared with other tumoral areas (Mohtasham et al., 2013).

This study aims to investigate MMP-10 expression in invasion front of OSCC and OVC by immunohistochemistry (IHC).

Materials and Methods

Patient selection

In this retrospective study, the specimens were obtained from the departments of Pathology in Khatam-Alanbia Hospital and Dental School of Zahedan. Seventy three specimens were collected, including 31 OSCC, 22 OVC and 20 non neoplastic oral epithelium. clinicopathological data including age, gender and location extracted from patients files. Specimens without clinicopathological data and sufficient paraffin- embedded tumor tissues were excluded. This study was approved by the ethics committee of Zahedan University of Medical Sciences (Project No. 5855).

Immunohistochemical staining

For IHC, the paraffin-embedded tissues were segmented into 4micron sections. Then the sections were deparaffinized and rehydrated in Xylene and graded ethanol respectively. To stop the endogenous peroxidase activity, the slides were incubated in 3% hydrogen peroxidase /methanol for 30 min and were then rinsed with phosphate-buffered saline (PBS).

For antigen retrieval, the sections were placed in citrate solution (PH=6) and were heated in a microwave oven for 30 min. Sections were incubated for 1 hour at room temperature with primary mouse monoclonal anti-human antibody MMP-10 [Code NCL-MMP-10-6016706, Novocastra, United Kingdom Dilute 1:50]according to the manufacture' instruction (Novocastra).

Then sections were rinsed with PBS at room temperature three times, the secondary antibody was applied. The immune complexes were incubated with streptavidin peroxidase (Novo Link Polymer Detection system). The immune reactivity was visualized with Diaminobenzidine and counterstained with Mayer hematoxylin, dehydrated and cleared in xylene, and slides were then mounted in Permount. Sections of ulcerative colitis were used as positive control and as a negative control, primary antibody was omitted.

Evaluation of immunohistochemically stained sections

All slides were observed by pathologist that who was blinded to the clinicopathological data for each specimen, using light microscope (Nikon, Type2, Tokyo, Japan). Expression of MMP-10 was considered positive if cytoplasmic staining was seen. Assessment of MMP-10 activity was performed in 5 microscopic fields in hot spot (the most populated areas by cells) with a magnification of 400, and cell staining was scored according to other studies (Freitas et al., 2011; Mashhadiabbas et al., 2012): including score 0: Negative, score 1 (low expression): less than 10%, score 2 (moderate expression): more than 10% and less than 50%, score 3 (intensive expression): more than 50% positive staining). Also for the staining intensity positive specimens were classified into 3 categories: strong (dark brown staining of the cells), mild (light or faint staining of the cells) and moderate (between strong and mild staining of the cells) (Mashhadiabbas et al., 2012).

Statistical analysis

Data analysis was performed using SPSS 21 (SPSS Inc, Chicago, IL) and relationship between groups was evaluated by Mann-whitney, Kruskal Wallis. P-value less than 0.05 were considered statistically significant.

Results

Clinical characteristics

OSCC patients ranged in age from 40 to 75 years (mean age: 56.92±9.55), Most patients were female (58%) and most of OSCC cases were located on the gingival and alveolar mucosa of mandible (55.17%) and others located on the buccal mucosa (17.24%), gingival and alveolar mucosa of maxilla (13.79%), Lip mucosa (6.89%), tongue (3.44%) and floor of mouth (3.44%) respectively, and two cases had unspecified location exactly. OVC patients ranged in age from 25 to 86 years (mean age: 55.8±17.67), most patients were female (59%) and most of cases were located on the gingival and alveolar mucosa of mandible (45%) lip mucosa (35%), buccal mucosa (10%) and tongue (10%), with two cases of unspecified location.

Immunohistochemical findings

MMP-10 was expressed in all cases of OSCC and OVC but non neoplastic epithelium did not show MMP-10 expression (Figure 1). The immune expression of this protein was confirmed by the presence of brown stained cytoplasm in tumor cells (Figure 2).

Most of cases of OSCC (87.1%) showed intensive expression (score 3) of MMP-10 whereas low expression (score 1) of this protein did not show. In OVC cases MMP-

10 staining was more low (40.9%) and moderate (40.9%) expression (score 1 and score 2). However, the number of MMP-10 positive epithelial cells increases continuously from the non neoplastic epithelium (control group) to VC and then toward SCC with significant differences between groups. ($p=0.000$). (Table 1)

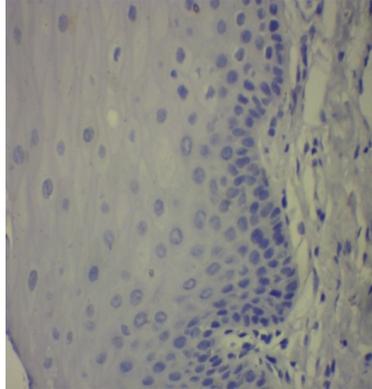


Figure 1. Normal Epithelium is Completely Negative for MMP-10 ($\times 100$)

Table 1. Expression Scores of MMP-10 in Invasive front Area of OSCC and OVC

Study groups	Score of MMP-10 expression N (%)				P- Value*
	Score 0	Score 1	Score 2	Score 3	
OSCC	0	0	4(12.9)	27(87.1)	0.000**
OVC	0	9(40.9)	9(40.9)	4(18.2)	

*Mann- whitney Test; ** $P<0.05$

There was no significant difference for MMP-10 expression in different histological grades of OSCC cases ($P=0.885$). (Table 2)

Also in most of cases of OSCC, staining intensity of MMP-10 was strong (70%) but in OVC was weak (68.2%) (Figure 3). There was a significant difference between

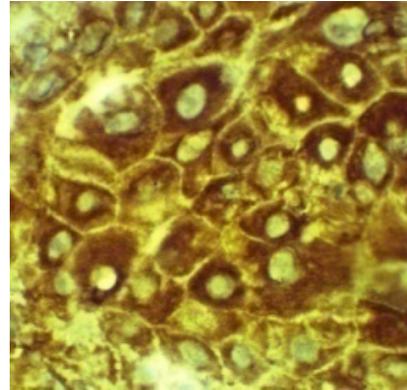


Figure 2. Positive Cytoplasmic Staining in Expression of MMP-10 ($\times 400$)

Table 3. Staining Intensity of MMP-10 in Invasive front Area of OSCC and OVC

Study group	Staining intensity N (%)			P- Value*
	Mild	Moderate	Strong	
OSCC	2(6.5)	7(22.5)	22(71)	0.000**
OVC	15(68.2)	5(22.7)	2(9.1)	

*Mann- whitney Test; ** $P<0.05$

Table 2. Expression Scores of MMP-10 in Invasive front Area in Different Histological Grade of OSCC

Study group	Score of MMP-10 expression N (%)					P- Value*
	Number	Score 0	Score 1	Score 2	Score 3	
Low grade OSCC	12	0	0	2(16.6)	10(83.4)	0.885
Moderate grade OSCC	10	0	0	1(10)	9(90)	
High grade OSCC	9	0	0	1(11.2)	8(88.8)	

* Kruskal Wallis Test

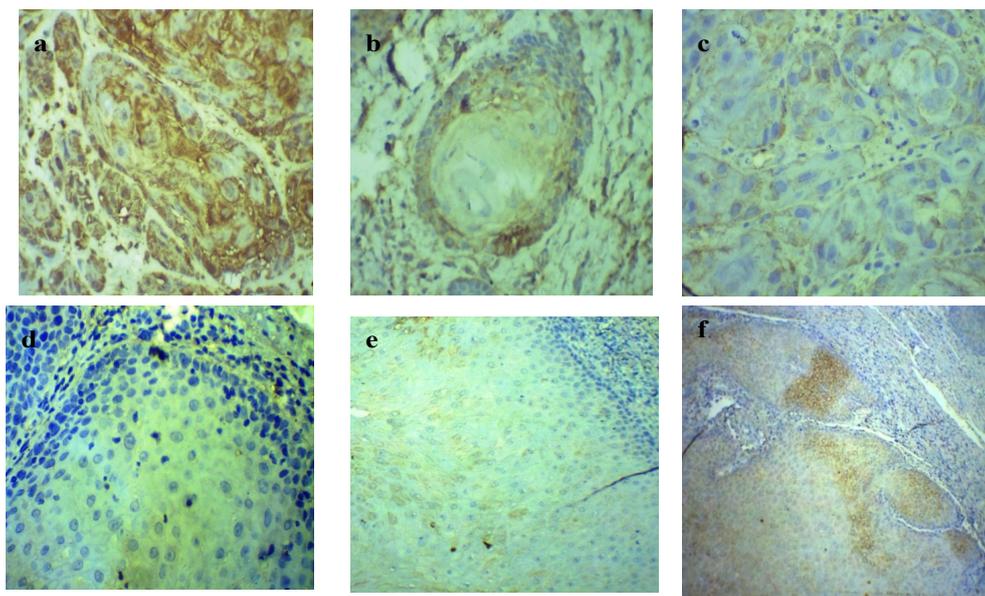


Figure 3. Immunohistochemical Staining of MMP-10 in OSCC and OVC. a, Strong staining intensity in OSCC ($\times 400$). b, Moderate staining intensity in OSCC ($\times 400$). c, Mild staining intensity in OSCC ($\times 400$). d, Mild staining intensity in OVC ($\times 400$). e, Moderate staining intensity in OVC ($\times 400$). f, Strong staining intensity in OVC ($\times 100$)

OSCC and VC. ($p=0.000$). (Table 3)

There was no significant difference for staining intensity of MMP-10 in different histological grades of OSCC cases. ($P=0.913$)

Discussion

A few studies have been done on biological behavior of OVC, and growth mechanism of this tumor in pushing borders is still questionable (Mohtasham et al., 2013). IF histological pattern can be used to determine the growth pattern, tumor invasion, and biological behavior of a tumor as shown for colorectal cancer (Spiro et al., 1999).

In this study, we examined the expression of MMP-10 protein in different histopathological grades of OSCC and OVC. The results showed that MMP-10 appeared in the epithelium of all the studied tumoral samples whereas its expression in normal epithelium was negative. Its expression in most OSCC cases was score 3 with a strong staining intensity, but its expression in OVC was score 1, 2 with a weak staining intensity. This shows that the lower growth nature of OVC invasion, as compared with that of OSCC, may be related to MMPs expression pattern. Moreover, MMP-10 protein may be one of the important possible factors in transforming a normal epithelium into verrucous carcinoma and then into OSCC.

Consistent with these results, the study of Tsang et al. showed that MMP-10 expression in tongue carcinoma tissues increased, compared with normal epithelium. Moreover, OSCC treatment (tongue area) using Curcumin, agent derived from the root of *Curcuma longa*, can reduce and inhibit cell migration and invasion in OSCC through reducing MMP-10 protein expression. (Tsang et al., 2012).

Mashhadiabbas et al. studied MMP-2, MMP-10, TIMP-1, and TIMP-2 markers in OSCC. Similar to the present study; MMP-10 was expressed in all OSCC samples and the protein's expression in most cases (97.8%) was score 3 with moderate staining intensity. In addition, a significant relationship was not observed between MMP-10 expression in OSCC and the histopathologic grade. Mashhadiabbas et al. also found a positive relationship between MMP-10 expression and LVD (lymphatic vessel density) in TIF; therefore, MMP-10 expression may be associated with the lymphatic metastasis in OSCC tumors (Mashhadiabbas et al., 2012).

In the study of Impola et al., MMP-10 protein expressed in the tumoral epithelium of more than 90% of OSCC samples and more than 50% of OVC cases. However, MMP-10 was not found in the IF of tumors, its expression was mostly superficial and MMP-10 expression was higher in the samples with higher sub epithelial inflammation, which is inconsistent with the present study. This is probably due to the types of the employed techniques; however, we used sensitive IHC technique but In situ hybridization technique was employed by Impola. It was finally proposed that the invasive behavior of oral cancer might be associated with the expression pattern of MMPs (Impola et al., 2004).

Yen et al. studied MMP-1-10-12 markers expression in OSCC and showed that MMP-10 is a potential oral cancer marker (Yen et al., 2009).

Also Deraz et al. examined MMP-10 expression in head and neck SCC. They observed the high expression of MMP-10 protein in 76.7% of SCC samples as compared with normal epithelium, which is similar to the present study. However, the relationship among MMP-10 expression, invasion pattern, disease stage, and metastasis to lymph nodes was significant. The lesions with poor differentiation had higher MMP-10 expression (Deraz et al., 2011), but this relationship was not found in our study. This may be due to the small sample size of the present study. In another study have been reported that MMP-10 plays an important role in head and neck cancer progression and that invasion created by MMP-10 is partially associated with p38 MAPK inhibition (Iizuka et al., 2014). Functionally, MMP-10 breaks down different extracellular matrix components, and consequently facilitates cells detachment and leads to migration of tumoral cells to surrounding tissues; however, its exact role in tumor invasion and metastasis in human cancers is contradictory (Liu et al., 2012). Some studies have shown that MMP-10 expression is not associated with the invasive behavior of tumors, such as lung cancer (Gill et al., 2004; Kren et al., 2006), epithelial skin cancers (Kerkelä et al., 2001), and bladder transitional cell carcinoma (Seargent et al., 2005). Others have discussed an anti apoptotic role for MMP-10, mentioning that it helps cells resist against apoptosis and consequently may cause further growth of tumoral cells of MMP-10 positive patients (Liu et al., 2012).

In conclusion, according to our study MMP-10 protein can be important possible factor in the transformation of non neoplastic oral epithelium to VC and SCC, also the level of MMP-10 expression at IF of the lesions can be helpful in the differentiation of VC and SCC. Based on this study MMP-10 was not a reliable marker for grading although this may due to the low number of samples. Since the studies on the role of MMP-10 on oral cancer, especially on OVC, are fewer than the other MMPs, it is proposed to conduct further studies with more sample size using other techniques to have a better understanding of the exact role of this protein. It is also proposed to discuss the relationship between the expression of this protein and the clinical behavior of tumors in future studies.

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