RESEARCH ARTICLE

Protein Expression of Stromelysin-2 in Head and Neck Squamous Cell Carcinomas

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Abstract

Background: Some matrix metalloproteinases (MMPs) are involved in invasion and metastasis of head and neck squamous cell carcinoma (HNSCC). However, there are few studies on association between stromelysin-2 (ST-2) and invasive behavior of HNSCC. The purpose of this study was to investigate Stromelysin-2 expression by immunohistochemistry. Materials and Methods: This study was conducted on 81 specimens, including 61 HNSCC and 20 non neoplastic epithelium. Sections with 5 micron thickness were prepared and stained with immunohistochemistry technique. Then expression of ST-2 was evaluated according to percentage of stained cells and intensity of staining. Data were analyzed by SPSS (V.21) using Kruskal-Wallis and Tukey tests (P<0.05). Results: The 61 HNSCC specimens were grades I 36.1%, II 34.4% and III 29.5%. The level of ST-2 expressions were moderate (++) and intensive (+++) in 21.3% and 78.7% of tumors, respectively. The ST-2 expression level was only significant between the tumors with grade I and grade III (P=0.016). Tumors presented ST-2 expression with staining intensity of mild 6.6%, moderate 26.2% and strong 67.2%. Staining intensity of ST-2 in grade I tumors was significantly lower than grade II and grade III (P<0.05), and there was no significant difference between grades II and III (P=0.99). Conclusions: According to this study, the expression of ST-2 is associated with histopathological grade and tumor differentiation in HNSCCs.

Keywords: Head and neck squamous cell carcinoma - immunohistochemistry - stromelysin-2

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Introduction

Cancer is one of the major cause of death in the world (Iizuka et al., 2014) and head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent cancers in the body with an annual incidence of 500,000 new cases in the world (Mao et al., 2004; Deraz et al., 2011; Barakat, 2015). HNSCCs clinically trend to develop local recurrence or metastatic spread to regional lymph nodes. In fact, the curing failure of local and regional lesions is the most important cause of morbidity and mortality of the tumors. Metastasis usually occurs in the late stages of the disease, particularly in cases with locally or regionally invasion. Metastatic lesions are indicative of the rapid clinical progress resulting in incurable fatal disease (Ruzevick et al., 2013).

Despite advances in treatment strategies, high recurrence rate, inadequate response to treatment, and a high mortality associated to invasion and metastasis are properties of these tumors (Stokes et al., 2010). Therefore, the identification of new molecules involved in invasion and metastasis, and a better understanding of the mechanisms involved in invasion of tumoral cells can play critical roles to innovate therapeutic strategies (Deraz et al., 2011).

MMPs are a family of zinc-dependent proteolytic enzymes with at least 26 members (Specenier and Brouwer, 2015). They are able to destroy the basement membrane around of transformed keratinocytes and epithelial blood vessels and lymphatic ducts (Lee et al., 2011). MMPs are known to degrade extracellular matrix in physiological and pathological processes. A variety of MMPs have an important role in invasion and metastasis of tumors.(Kerkelä et al., 2001; Iizuka et al., 2014). As one of the mechanisms of tumor progression, tumoral cells by producing MMP or inducuction of stromal cells to secrete these enzymes, help tumor invade regional tissues or perform metastatic foci (Boyd et al., 2009).

A member of MMPs, MMP-10 or stromelysin-2 (ST-2) has been shown that degrade gelatin, collagen type 3 and 4, elastin, fibronectin and laminin, in laboratory conditions (Boyd et al., 2009). However, because of high level detection of ST-2 in a variety of tumors, keratinocytes, human alveolar macrophages, fibroblasts, and carcinomas of the head and neck, lung and liver, it seems to participate in tumor invasion through degradation of extracellular matrix (Mathew et al., 2002; Liu et al., 2012). But precise role of ST-2 (MMP-10) in the invasion and metastasis of

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human cancers is conflicting (Liu et al., 2012).

Previous studies have revealed effects of some family members of MMPs including MMP2 and MMP9 on invasion and metastatic behavior of HNSCCs (Deraz et al., 2011; Mohtasham et al., 2013; Lotfi et al., 2014). The aim of this study was to evaluate ST-2 expression in head and neck SCC by immunohistochemistry.

Materials and Methods

Patient selection

In this retrospective, Formalin-fixed, paraffin-embedded specimens of HNSCC were obtained from the departments of Pathology in Khatam-Alanbia Hospital and Dental School of Zahedan. The diagnoses were confirmed by experienced pathologist. Eighty one specimens including 61 SCC and 20 non neoplastic epithelium were collected. Clinicopathological data including age, gender, and location of samples were obtained from patient records.

Immunohistochemical staining

For immunohistochemical staining, the paraffin blocks were sectioned into 5-micron thickness, which were then mounted on silicon-coated glass slides and air dried at room temperature. Then the sections were deparaffinized with xylene and rehydrated using graded ethanol. For the block endogenous peroxidase activity, the slides were immersed in 3% hydrogen peroxidase /methanol for 30 minutes and were washed with phosphate buffered saline (PBS). For antigen retrieval specimens were immersed in citrate buffer (pH= 6) and maintained in microwave for 30 minutes. Sections were incubated for 1 hour at room temperature with the primary antibody. The sections were washed with PBS three times, and then secondary antibody was used. The immune complexes were incubated with streptavidin peroxidase (Novo Link Polymer Detection system). For the immune reactivity the slides were submerged in 0.02% diaminobenzidine solution for 10 min. Then sections were rinsed, counterstained with Mayer hematoxylin, finally the section were dehydrated with graded ethanol and cleared in xylene, and slides were mounted (Mohtasham et al., 2013).

Mouse monoclonal anti-human antibody MMP-10 (ST-2) [Code NCL-MMP-10-6016706, Novocastra, United Kingdom Dilute 1:50] were used according to the manufacture instruction (Novocastra), Sections of ulcerative colitis were used as positive control and as a negative control, primary antibody was omitted.

Evaluation of immunohistochemically stained sections

For assessment of ST-2 positivity, the number of positive-stained cells was counted in 1000 cells of each sample at 400 magnifications using light microscope (Nikon, Type2, Tokyo, Japan). Cell staining was scored according to other studies (Freitas et al., 2011; Mashhadiabbas et al., 2012): including Negative: no stain, low expression (+): less than 10%, moderate expression (++): more than 10% and less than 50%, intensive expression (+++): more than 50% positive staining. Also positive specimens for the staining intensity 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 were analyzed using SPSS 21(SPSS Inc, Chicago, IL), Kruskal-Wallis and Tukey tests. P-value less than 0.05 were considered statistically significant.

Results

Sixty-one HNSCC specimens taken from 61 patients (males 50.8%, women 45.9% and unknown gender 3.3%) with age range of 33 to 83 years (mean age: 59.2±11.2) were evaluated. Of the 61 cases, 22 (36.1%) cases had grade I, 21 (34.4%) cases grade II and 18 (29.5%) cases had grade III.

ST-2 expression was positive in all HNSCC samples and negative in non-neoplastic epithelium (Figure 1). The immune expression of this protein was confirmed by the presence of brown stained cytoplasm in tumor cells.

The maximum number of HNSCC samples 48(78.7%) showed intensive (+++) expression of ST-2, 13(21.3%) cases showed moderate (++) expression, while none of

Table 1. Expression Levels of ST-2 in Different Histological Grade of HNSCC

Study group	Number	Levels of ST-2 expression N (%)				
		-	+	++	+++	P- Value*
Low grade HNSCC	22	0	0	9(69.2)	13(27.1)	0.017
Moderate grade HNSCC	21	0	0	3(23.1)	18(37.5)	
High grade HNSCC	18	0	0	1(7.7)	17(35.4)	

* Kruskal Wallis Test

Table 2. Staining Intensity of ST-2 in Different Histological Grade of HNSCC

Study group	Number	Levels of ST-2 expression N (%)			
		Mild	Moderate	strong	P- Value*
Low grade HNSCC	22	3(75%)	9(56.3)	10(24.4)	0.02
Moderate grade HNSCC	21	0	5(31.3)	16(39)	
High grade HNSCC	18	1(25)	2(12.5)	15(36.6)	

* Kruskal Wallis Test

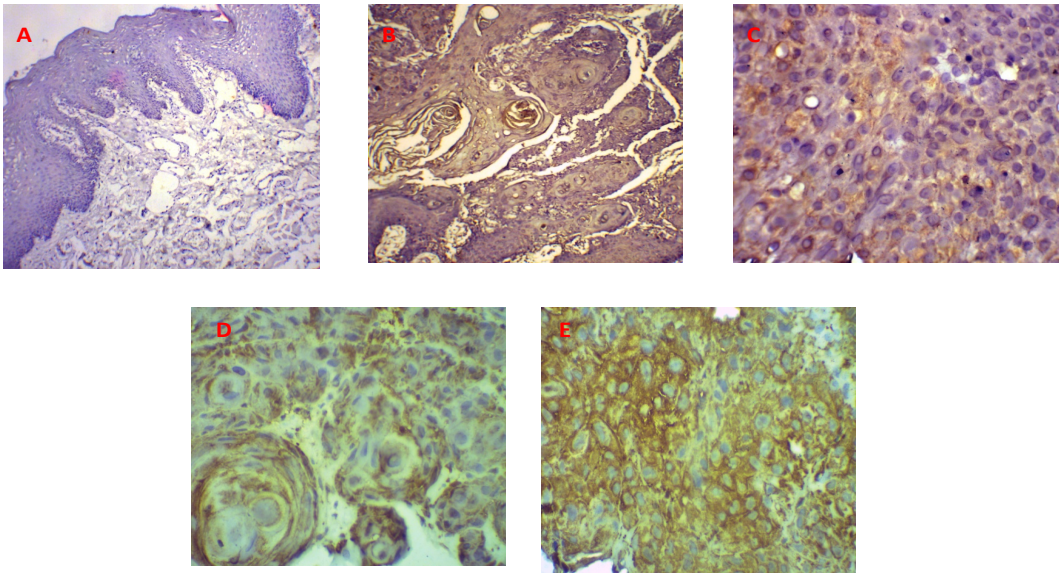

Figure 1. Immunohistochemical Expression of MMP-10 in Normal Epithelium and HNSCC. A: normal epithelium is negative for MMP-10 compared to HNSCC (×100). B: mild expression Of MMP-10 in HNSCC (×100). C: moderate expression of MMP-10 in HNSCC (×400). D and E: Strong expression of MMP-10 in HNSCC (×400)

the samples did not show low expression (-).

According to Kruskal-Wallis test, there was a significant difference in level of ST-2 expression among tumors with grades I, II and III (P=0.017) (Table 1). Tukey test revealed that difference in level of ST-2 was only significant between the tumors with grade I and grade III (P=0.016).

About staining intensity of ST-2, in most of cases of HNSCC, was strong 41(67.2%), in 16(26.2%) of cases was moderate and in 4(6.6%) cases was mild intensity. Kruskal-Wallis test showed a significant difference in ST-2 staining intensity among tumors with I, II and III grades (P=0.020) (Table 2). Tukey test displayed that ST-2 staining intensity in grade I tumors was significantly lower than grade II (P=0.040) and grade III (P=0.042).

Discussion

HNSCCs have severe invasive behavior to regional or distant tissues (Stokes et al., 2010). Study of factors influence on the aggressive behavior of this malignancy can contribute to improvement the patients' survival. Among the various factors, the role of MMPs has been known in aggressive behavior of the tumors. Culhaci et al. Demonstrated that MMP-13 and TIMP-1 play important roles in the invasive ability of HNSCCs (Culhaci et al., 2004). Johansson et al. showed association of MMP-13 in HNSCCs to invasive behavior of tumoral cells (Johansson et al., 1997). Mohtasham et al. reported that expression pattern of the MMP-2 and MMP-9 is related to the infiltration pattern of OSCC at the invasive front (Mohtasham et al., 2013).

ST-2 (MMP-10), as one of MMPs, has been detected in a variety of SCC tumors in larynx, tongue and skin/mucosa (Culhaci et al., 2004). However, studies on ST-2 are fewer than the other MMPs and they provided contradictory results on the role of this factor in tumor invasion. Therefore, we investigated the expression of ST-2 in HNSCC samples.

Our study showed that the ST-2 expression level increased with increasing tumor grade. So that the ST-2 staining intensities in grades II and III were significantly higher than that in grade I. The rise was even higher in grade III to grade II, although there was no statistically significant difference between them. These findings suggest that ST-2 expression increases in tumors with higher histopathologic grades.

Consist with our study, in Deraz et al. study was observed high expression of ST-2 in 76.7% of the head and neck SCC samples whereas normal epithelium did not show expression of ST-2. Also the lesions with poor differentiation had higher expression of ST-2. In their study the expression of ST-2 was associated to the lymph node invasion and metastasis (Deraz et al.,2011). Fan et al examined expression of ST-2 in cutaneous SCC and similarly found that ST-2 expression was seen in all degree of differentiation in SCC and ST-2 expression was significantly higher in poorly differentiated SCC than well differentiated ones. But in their study there was no significant correlation between ST-2 and metastasis (FAN et al). Also T- Sang et al. showed that ST-2 over expressed in tongue carcinoma in comparison of normal epithelium that is similar to our study. Moreover they suggested that Curcumin treatment in tongue carcinoma reduce cell migration and tumor invasion through inhibition of ST-2 expression (Tsang et al., 2012).

In 2014 Iizuka et al. studied gene expression of some MMPs in head and neck cancer progression and they have been showed that ST-2 (MMP10) plays an important role in progression of HNSCC, also they reported that invasion which created by ST-2 is associated with p38 MAPK inhibition (Iizuka et al., 2014).

Mashhadiabbas et al. evaluated MMP-2, MMP-10, TIMP-1, and TIMP-2 proteins in OSCC, ST-2 (MMP-10) was expressed in all cases and this expression in most cases (97.8%) showed score 3 with moderate staining intensity. But in their study, was not observed a significant correlation between ST-2 expression in OSCC

and the histopathologic grade. That is inconsistent with the present study; this difference is probably due to the technical variation used in immunohistochemistry method and sample size differences. But Mashhadiabbas et al. found a positive relationship between ST-2 expression and LVD (lymphatic vessel density) in TIF; therefore, ST-2 expression may be associated with the lymphatic metastasis in OSCC tumors, which this has not been evaluated in our study. (Mashhadiabbas et al., 2012).

Additionally, Kerkelä et al evaluated differential pattern of ST-2 expression in epithelial skin cancers and reported no significant correlation between histological aggressive behavior of epithelial tumors of the skin and ST-2 expression (Kerkelä et al., 2001). In 2012 Liu et al. Examined expression of ST-2 in esophageal SCC (ESCC). They found that the increase in ST-2 expression was not significantly associated with disease-specific survival rate in patients, but correlated to inappropriate condition of patients during early stage (I-IIA) than that of advanced stage. They reported that expression of ST-2 in advanced stage of ESCC probably influenced by other factors for example tumor penetration depth and involvement of lymph node. Finally their study found that ST-2 plays an important role in the development of esophageal SCC during the initial stage of its development and its rising can be applied as a prognosis predictor in early clinical stage of ESCC (Liu et al., 2012).

In conclusion, the result of this study concluded that according to the intensive expression of ST-2 in HNSCC and negative expression in normal epithelium, ST-2 or MMP-10 can be possible factors in transformation of normal epithelium to carcinoma. Also it is associated with higher histopathological grade and to the tumor differentiation in HNSCC, especially significant in grades II and III.

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