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RESEARCH ARTICLE

Prognostic value of transforming growth factor beta 1 [TGF-β1] and matrix metalloproteinase 9 [MMP-9] in oral squamous cell carcinoma

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Abstract

Objectives: The prognostic value of MMP-9 and TGF-β1 in oral squamous cell carcinoma (OSCC) is not clear. This study aimed to assess this subject.

Methods: After immunohistochemistry staining of 48 OSCC biopsies with MMP-9 and TGF-β1 markers, marker expression in the stroma was estimated, and the correlations with OSCC prognosis determinants were analyzed (α = 0.05).

Results: Mode of invasion was associated with metastasis (rho = 0.449, $p = 0.008$). MMP-9 was positively associated with metastasis and mode of invasion. TGF-β1 was negatively correlated to tumor histologic grade but was positively associated with metastasis.

Conclusions: Unlike TGF-β1, MMP-9 might be useful for prognosis determination.

Keywords: Matrix metalloproteinase 9 (MMP-9), transforming growth factor beta 1 (TGF-β1), oral squamous cell carcinoma (OSCC), prognosis

Introduction

Squamous cell carcinoma (SCC) is considered a major problem worldwide due to its extremely high prevalence (90%) among oral cancers (O-Charoenrat et al., 2001; van der Waal et al., 2011). The high rate of relapse in this tumor indicates the inadequacy of current prognostic predictors, that is, histological and clinical assessments, in predicting metastatic potential (Cortesina & Martone, 2006), as well as the need to investigate additional determinants among which are matrix metalloproteinase 9 (MMP-9) and transforming growth factor beta 1 (TGF-β1).

TGF-β1 is a pleiotropic polypeptide with various and paradoxical effects (Bierie & Moses, 2006; Chen et al., 2008; Tian & Schiemann, 2009; Yang, 2010). It might be a tumor suppressor due to its antiproliferative and fibrogenic effects which might reduce metastasis potential (Takiuchi et al., 1992; Wakefield & Roberts, 2002). On the contrary, it can be a proto-oncogene with aggravating effects on malignancy, tissue invasion, and metastasis (Wakefield & Roberts, 2002) via several mechanisms such as the expression of MMPs (Chen et al., 2008; Overall et al., 1991), contributing to tumor angiogenesis and neoplastic transformation of epithelial cells, immune suppression, and epithelial hyperproliferation (Lewis et al., 2004; Sinpitaksakul et al., 2008; Tian & Schiemann, 2009; Wakefield & Roberts, 2002; Yang, 2010; Yang & Moses, 2008). Whereas some investigators suggest that disruption of TGF-β1 signaling pathways might be approached to control the tumorigenesis (Wakefield & Roberts, 2002), according to some others it is useless for determining the prognosis (Logullo et al., 2003).

MMPs are a family of homologous extracellular calcium- and zinc-dependent endopeptidases with enzymatic activity against almost all protein components of the extracellular matrix (ECM) and capable of degrading the basement membrane (Chen et al., 2008; Cortesina

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& Martone, 2006; Dai et al., 2007; Kurahara et al., 1999; O-Charoenrat et al., 2001; Patel et al., 2005, 2007). By removing physical barriers in the ECM, these can be involved in both physiological events such as cell proliferation and angiogenesis as well as pathological conditions including tissue invasion and metastasis (Chen et al., 2008; Cortesina & Martone, 2006; Dai et al., 2007; Görögh et al., 2006; Katayama et al., 2004; Kurahara et al., 1999; O-Charoenrat et al., 2001; Patel et al., 2005, 2007). There is strong evidence that overexpression of multiple MMPs and tissue inhibitors of MMPs (TIMPs) are characteristic of head and neck SCC (HNSCC). Nevertheless, the prognostic value of these regulators remains unclear, and analysis of specific MMPs, *MMP-9 in particular*, might be useful for evaluating the malignant potential of HNSCC (Cortesina & Martone, 2006; Dai et al., 2007; Verstappen & Von den Hoff, 2006).

Exploring the links between these molecules and determinants of OSCC cancerization, invasion, and metastasis seems to be of value for diagnosis/prognosis establishment as well as development of more efficient interceptive treatment modalities by targeting the involved factors. However, there are only few studies regarding relationships between some OSCC prognostic factors with TGF-β1 (Logullo et al., 2003) and MMP-9 (Chen et al., 2008; Dai et al., 2007; Kurahara et al., 1999), and the results are quite controversial, as the prognostic values of neither of these markers are well understood (Cortesina & Martone, 2006; Dai et al., 2007; Logullo et al., 2003; Verstappen & Von den Hoff, 2006; Wakefield & Roberts, 2002), which it might be in part because of the weaknesses in the statistical and sampling methods. Furthermore, the literature regarding OSCC lacks studies on potential associations between these markers and some key prognostic features such as histopathological grade (with MMP-9), lymph node metastasis, and mode of invasion (with TGF-β1). Thus we aimed to assess both controversial and overlooked associations between OSCC prognostic determinants and the two markers.

Materials and methods

This descriptive cross-sectional study was performed on 96 immunohistochemistry (IHC) slides prepared from 48 archival biopsies of initial oral SCC cases taken from the pathology laboratory of the cancer institute of the Imam hospital (Tehran). The specimens were included after investigating all available initial OSCC files archived during 1998 to 2008. Incomplete patient records and cases with SCC relapse or therapy histories were excluded. The protocol ethics were approved by the research committee of Shahid Beheshti University. The pathologic diagnoses of the included tumors were confirmed with histologic examination of the available hematoxylin-eosin (H&E) slides by two experienced pathologists.

The patients' age and gender, the presence of lymph node metastasis, and maximum lymph node diameters were read from the patient records. Mode of invasion (Stages I to IV of the Jacobson method; Bundgaard et al., 2002) and tumor histologic grades (Grades I to III of the IUCC system) were determined on H&E slides by the two pathologists.

IHC staining with MMP-9 and TGF-β1 markers

The staining procedures were performed according to the exact instructions of the material manufacturers. Sections (3-μm thin) of the paraffin-embedded formalin-fixed specimens were mounted on 3-amino-propyltriethoxysilane-coated slides (Silanized S3003, Dako, Poland). According to the standard procedures, they were dewaxed in 100% xylene, and dehydrated in graded ethanol (Chen et al., 2008; Nagel et al., 2004).

After performing antigen-retrieval procedures and then blocking the activity of endogenous peroxidase using Peroxidase Blocking Reagent, the slides were incubated in a metal box placed in humid air at 6°C for 1 h with TGF-β1 antibody (mouse monoclonal antibody, clone 17, Novocastra, England) diluted 1:20 in TBS at pH 7.2, and MMP-9 antibody (mouse monoclonal antibody, clone 15 W2, Novocastra) diluted 1:30 in TBS. Afterward, the slides were stored in TBS (pH = 7.6) for 5 min.

The secondary antibodies available in the IHC kit (Novocastra) were incubated for 30 min, and the slides were rinsed and stored in TBS at pH 7.6 for 5 min. The Novolink Polymer solution was dropped on the slides which were placed in a dark humid room at 37°C. After 30 min, the slides were rinsed and stored in TBS for 5 min.

The chromogen (available in the Novocastra kit) was diluted 1:20 and was incubated for 5 min; after rinsing and storing in TBS, the slides were counter stained with hematoxylin (30 s), and were stored in lithium carbonate for 5 min and then rinsed.

The external positive control consisted of sections of human liver (for MMP-9) and placenta (for TGF-β1) treated identically as described above. The external negative control sections of oral SCC were treated alike, apart from that, nonimmune serum in TBS was used instead of the primary antibodies (Chen et al., 2008). The stained cytoplasm of lymphocytes or myofibroblasts was also evaluated as the internal positive control. Poorly stained slides were excluded and replaced.

Scoring

After dehydration in graded ethanol and storing in xylene, the slides were mounted with Merck KGaA (64271 Darmstadt, Germany) and were evaluated using a light microscope at 200x magnification by two pathologists. The proportion and intensity scores of the stained cells in the carcinomatose stroma were determined on 10 randomly selected fields (by counting all cells in each field). The proportion scores (PS) of the MMP-9 marker comprised 0 (≤ 20%), 1 (21%–50%), 2 (51%–75%), and **3** (≥ 76%; Katayama et al., 2004). The TGF-β1 proportion scores were 0 (< 10%), 1 (11%–60%), and **2** (> 60%; Logullo et al., 2003). Based on the similarity with the stained

internal positive controls, the intensity scores (IS) of both markers were determined as 0, 1 (mild), 2 (moderate), and **3** (strong). Total scores (TS = PS + IS) were also calculated for the markers. Any inconsistencies were settled through discussion.

Statistical analysis

A forward-selection multiple regression analysis and a Spearman's correlation coefficient of the SPSS statistical package (version 17, SPSS, Chicago, US) were used to analyze the data. The level of significance was set at 0.01 for the correlations between the two markers, and at 0.05 for the remainder of the analyses.

Results

All of the included slides were successfully stained (Figures 1 and 2). The patients' mean age was 55.9 ± 14.5 years (range: 24–83). Of them (n = 48) 29.2%, 56.3%, and 14.6% were less than 50, between 50 and 70, and greater than 70 years old, respectively; and 70.8% of them were males (male:female ratio = 2.4). The prevalence rates of well-, moderately-, and poorly-differentiated tumors were 25.0%, 58.3%, and 16.7%, respectively. The distributions of modes of invasion I to IV were 22.9%, 31.3%, 20.8%, and 20.8%, respectively. Of the tumors, 50.0% and 2.1% showed unilateral and bilateral nodal metastases, respectively. The most frequent tumor site was tongue (22.9%), followed by maxillary alveolar mucosa, mouth floor, lower lip mucosa (16.7% each), mandibular alveolar mucosa (14.6%), palate (12.5%), upper lip mucosa, buccal mucosa (4.2% each), and retromolar (2.1%). The average tumor size was 22.45 ± 29.61 cm^3 (min = 0.38, Q1 = 3.75, median = 9.0, Q3 = 28.0, max = 126.0 cm^3). The average maximum node diameter was 1.924 ± 0.616 cm (min = 1, median = 2, max = 3 cm).

The score frequency distributions are demonstrated in Figure 3. There were no significant correlations between the IS and the PS of the different markers. The correlations between the proportion scores and between the intensity scores of the two markers were statistically significant. However the Spearman's rho values (< 0.4) indicated weak associations (Table 1). Only the associations between the IS and PS of each marker were moderate to strong (> 0.6, Table 1). Mode of invasion was significantly correlated with both metastasis (rho = 0.449, p = 0.008) and maximum node diameter (rho = 0.349, p = 0.023). Tumor size was not associated with any of the two markers' PS, IS, and TS (all rho values < 0.155, all p values > 0.06).

In the multiple regression analysis performed (Table 2), the F statistics and p values for the models were computed using an analysis of variance (ANOVA), by comparing the beta values in each model. Betas are correlation coefficients indicating the magnitude and direction of associations between the covariables and the dependent variables (the scores). During the forward-selection procedure, a covariable entered

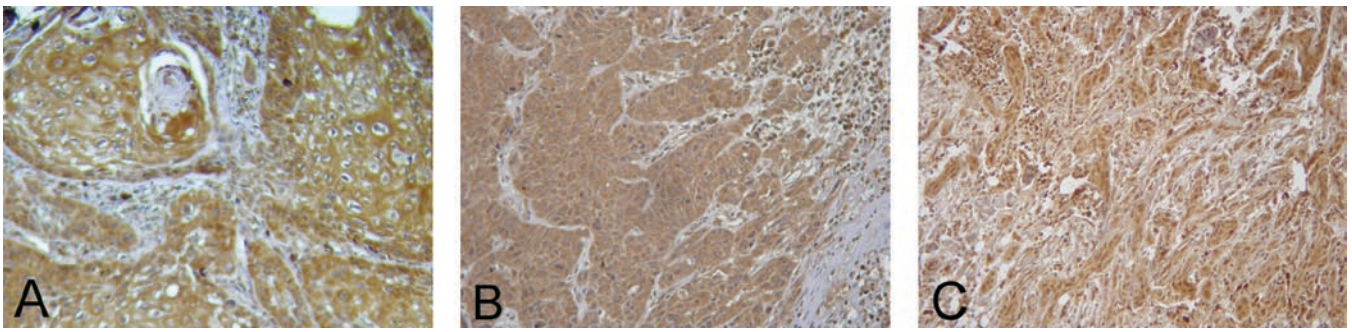

Figure 1. (**A**) IHC staining of TGF-β1 in a well-differentiated oral SCC at 200× magnification (PS = 2, IS = 3); **B** and **C** respectively represent 200× micrographs of IHC of TGF-β1 in moderately- and poorly differentiated oral SCC biopsies (**B**: [PS = 2, IS = 3], **C**: [PS = 2, IS = 3]).

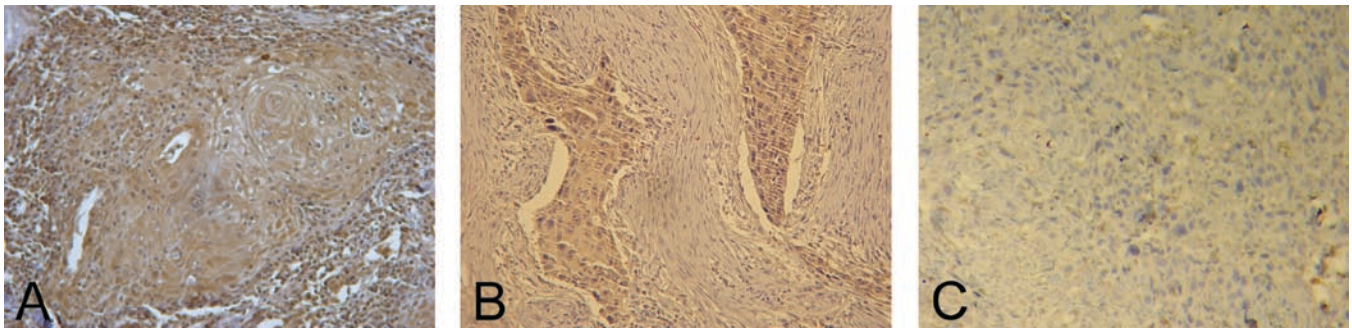

Figure 2. (**A**) a 200× IHC micrograph demonstrating MMP-9 expression in a well-differentiated specimen (PS = 3, IS = 2); (**B**) a moderately differentiated OSCC specimen stained with MMP-9 marker at 200× (PS = 1, IS = 2); (**C**) a poorly differentiated OSCC at 200× (MMP-9 PS = 0, IS = 0).

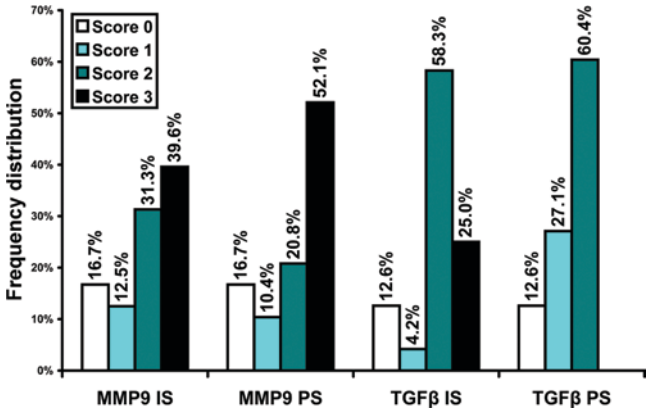

Figure 3. Frequency distributions (%) of MMP-9 and TGF-β1 proportion and intensity scores.

Table 1. The Spearman's correlation coefficients for the associations between the two markers.

Variables		rho	*p*
MMP-9 PS	MMP-9 IS	0.713	0.000
	TGF-β1 PS	−0.296	0.009
	TGF-β1 IS	−0.218	0.052
MMP-9 IS	TGF-β1 PS	−0.071	0.535
	TGF-β1 IS	−0.330	0.005
TGF-β1 PS	TGF-β1 IS	0.647	0.000

Note: $\alpha \leq 0.01$.

into each model and its role in predicting the score was estimated. The covariable incorporated the subsequent model only if its predicting role was statistically significant. The adjusted *r*-square value denotes the whole correlation between each model and the score. The change in the *r*-square of each model compared to the preceding one corresponds to the total effect of entered and eliminated covariables. The highest *r*-square for a score indicates the highest total correlation with that score, and thus identifies the best model for prediction.

Predictors of MMP-9 expression

PS

No significant regression models emerged to predict the MMP-9 PS (all ANOVA's *p* values > 0.09). The small sizes of the highest adjusted *r*-square and *F* statistics (= 0.081 and 2.921, respectively) revealed an absence of any correlations between the MMP-9 PS and the covariables.

IS

Regarding MMP-9 IS, the small *r*-square values indicated that the associations between MMP-9 IS and the covariables were generally poor (Table 2). Only two models were useful to predict the MMP-9 IS (Table 2).

TS

The covariables gender (beta = –0.381, $p = 0.030$), maximum lymph node diameter (beta = 0.430, $p = 0.022$), and lymph node metastasis (beta = 0.314, $p = 0.050$) predicted MMP-9 TS in a regression model with the greatest adjusted *r*-square value.

Predictors of TGF-β1 expression

PS

Among the predictors, gender's effect was extremely poor as it appeared with a low beta value only in one model with a reduced *r*-square (Table 2).

IS

Five usable regression models (with small *r*-square values though) emerged to predict the TGF-β1 IS (Table 2).

TS

Tumor grade (beta = –0.514, $p = 0.001$), maximum lymph node diameter (beta = 0.357, $p = 0.030$), and age (beta = –0.296, $p = 0.05$) predicted the TGF-β1 TS in a model with the highest *r*-square.

Discussion

Nodal metastasis is considered the most principal prognostic predictor in HNSCC (Cortesina & Martone, 2006). The overexpression of MMP-9 can facilitate the infiltration of tumor cells into the stroma and then into the lymphatic or blood systems by degrading the basement membrane components (Dai et al., 2007; O-Charoenrat et al., 2001; Patel et al., 2005). Several studies have reported a significant positive correlation between MMP-9 and lymph node metastasis (Görögh et al., 2006; Katayama et al., 2004; Kurahara et al., 1999; O-Charoenrat et al., 2001). Such a significant correlation was not however detected in some other researches (Ruokolainen et al., 2004; Sinpitaksakul et al., 2008). In the present study, a marginal relationship was seen. Some reasons such as the influence of the staining techniques on the measurements (Logullo et al., 2003), the comparative inefficacy of categorical statistical tests in detecting moderate continuous correlations, as well as probable variations of the marker behaviors in different cancers studied might account for the dispute. Furthermore, it is the balance between MMPs and TIMPs which is relevant to the digestion of basement membranes (Cortesina & Martone, 2006; Dai et al., 2007; Kurahara et al., 1999; O-Charoenrat et al., 2001; Patel et al., 2005; Verstappen & Von den Hoff, 2006), not only the MMP expression. Therefore, the associations might be confounded by inhibitory (and sometimes boosting) influence of TIMPs which are also elevated in HNSCC, coexpressed with MMPs or as a reciprocal regulation by endogenously expressed cytokines and growth factors including TGF-β1 (O-Charoenrat et al., 2001; Verstappen & Von den Hoff, 2006).

The extremely complex role of TGF-β1 in metastasis is not clearly understood. It might play direct or indirect roles in either suppressing or inducing metastasis. It can reduce metastasis potential by inhibiting cell proliferation, maintenance of tissue architecture, and

Table 2. The results for the stepwise multiple regression analysis, assessing the associations between the independent covariables and the scores of the markers.

Markers	Model No.	*F*	*p*	Adjusted r^2	Predictor covariables	Beta	*p*
MMP-9 IS	5	2.654	0.047	0.112	Lymph node diameter	0.604	0.026
	6	3.260	0.031	0.133[a]	Gender[b]	−0.319	0.038
					Mode of invasion	0.351	0.035
					Lymph node metastasis	0.555	0.031
					Lymph node diameter	0.658	0.013
TGF-β1 PS	1	5.683	0.000	0.471	Age	−0.323	0.013
					Lymph node metastasis	0.455	0.044
					Tumor grade	−0.428	0.010
	2	6.539	0.000	0.480	Age	−0.316	0.014
					Lymph node metastasis	0.456	0.042
					Tumor grade	−0.388	0.010
	3	7.766	0.000	0.492[a]	Age	−0.316	0.013
					Lymph node metastasis	0.481	0.025
					Tumor grade	−0.359	0.007
	4	8.624	0.000	0.476	Age	−0.362	0.004
					Tumor grade	−0.328	0.014
					Gender[b]	−0.255	0.037
TGF-β1 IS	1	3.452	0.004	0.313	Lymph node metastasis	0.532	0.038
	2	4.000	0.004	0.328	Lymph node metastasis	0.532	0.036
					Tumor grade	−0.285	0.022
	3	4.623	0.002	0.336[a]	Lymph node diameter	−0.467	0.048
					Lymph node metastasis	0.573	0.021
					Tumor grade	−0.407	0.014
	4	5.348	0.001	0.336[a]	Lymph node diameter	−0.520	0.025
					Lymph node metastasis	0.563	0.023
					Tumor grade	−0.389	0.017
	5	6.190	0.000	0.326	Lymph node diameter	−0.497	0.032
					Lymph node metastasis	0.495	0.040
					Tumor grade	−0.348	0.009

Note: Models and covariables with *p* values > 0.05 are not shown.
[a]The highest adjusted r^2 for each score.
[b]The negative beta values here point out higher levels of marker expression in females.

inducing TIMPs activity. Also being a major fibrogenic factor, it is capable of provoking fibroblast chemotaxis and synthesis of collagens, fibronectins, and proteoglicans (Takiuchi et al., 1992; Wakefield & Roberts, 2002). On the contrary, it might contribute to metastasis by promotion of cell proliferation and enhancing tumor cell invasion, immune suppression, angiogenesis, regulating the production of chemoattractant molecules (or being one of them), and triggering the detachment of cancer cells from the primary neoplasm (Cooper et al., 2003; Cortesina & Martone, 2006; Dranoff, 2004; Johansson et al., 1997; Lewis et al., 2004; Sinpitaksakul et al., 2008; Takiuchi et al., 1992; Tian & Schiemann, 2009; Verstappen & Von den Hoff, 2006; Yang, 2010; Yang & Moses, 2008). In addition, TGF-β1 might induce the expression of MMPs in human mucosal keratinocytes and OSCC (Chen et al., 2008; Salo et al., 1991; Overall et al., 1991). Among these oral SCC specimens, the TGF-β1 expression was positively correlated with the occurrence of metastasis while being negatively associated with the maximum lymph node diameter. This result was in contrast to the nonsignificant correlation observed in another study employing a comparable IHC method (Logullo et al., 2003). A high rate of nodal involvement existed in this sample which might justify the positive correlation seen between TGF-β1 and nodal metastasis; because, the tumor suppressor effects of TGF-β1 are likely to be absent in lymph node–positive OSCC cases, whereas they can exist in node-negative ones (Pasini et al., 2001). The negative association between node diameter and TGF-β1 in this sample might be explained by a possible increase in the number of nodes involved (which this was not assessed) or the existence of metastasis through other pathways of tumor progression. The complicated pathways of TGF-β1 effects might compromise its prognostic merit, supporting the findings of Logullo et al. (2003).

Tumor histopathologic grade

The effects of TGF-β1 might vary depending on numerous microenvironmental factors including its dosage, target cell types (normal or tumoral), TGF-β1 receptors (normal or diminished), and host immune response, in a way that its tumorigenesis effects such as MMP induction are present primarily in tumoral tissues whereas its

tumor-suppressor effects are often seen in normal cells (Chen et al., 2008; Tian & Schiemann, 2009; Wakefield & Roberts, 2002; Yang, 2010; Yang & Moses, 2008). Suppressor activities comprise apoptosis induction, maintenance of genomic stability and tissue architecture, induction of replicative senescence, and prevention of immortalization. However, its other effects like growth stimulation, enhancing epithelial to mesenchymal transition, and increasing motility and invasiveness might lead to cancerization (Lewis et al., 2004; Sinpitaksakul et al., 2008; Takiuchi et al., 1992; Tian & Schiemann, 2009; Wakefield & Roberts, 2002; Yang, 2010; Yang & Moses, 2008). In line with the results of Logullo et al. (2003), its scores were negatively correlated with tumor grade in this sample, implying a greater extent of its inhibitory activities in these OSCC cases. Nevertheless, no such relationships were distinguished between tumor grade and the ECM collagenase MMP-9, confirming the results of O-Charoenrat et al. (2001).

Mode of invasion

Mode of invasion was associated with lymph node metastasis, indicating that it might be used for prognosis determination, as reported previously (Bundgaard et al., 2002), and that the markers associated with it might be used for the same purpose as well. MMP/TIMP imbalance in favor of MMPs plays a crucial role in tumor invasion and metastasis (Cortesina & Martone, 2006; Dai et al., 2007; O-Charoenrat et al., 2001; Patel et al., 2005; Verstappen & Von den Hoff, 2006). Some investigators have found significant correlations between OSCC mode of invasion and MMP-9 expression (Kurahara et al., 1999), and some others have failed to detect such a link (O-Charoenrat et al., 2001). Using a continuous statistical method, a significant but weak connection was observed in the current study.

Tumor cell invasion might be enhanced (Lewis et al., 2004; Wakefield & Roberts, 2002; Yang, 2010) or inhibited (Takiuchi et al., 1992; Wakefield and Roberts, 2002) by TGF-β1. No significant correlations were discerned in this study between mode of invasion and TGF-β1, although this is not necessarily against its potential enhancing effects on invasion. No similar studies were available to compare the results.

MMP-9 and TGF-β1

Chen et al. (2008) discovered a significant correlation between the two markers in OSCC. According to the findings of the present study, however, there were nonsignificant to weak negative associations between MMP-9 and TGF-β1. The contrast between the results of this study and those of Chen et al. (2008) might be caused by sampling and statistical method differences. Moreover, in their report, nodal involvement was absent, and out of their 15 cases, five were locally cancerated and 10 had developed OSCC mostly with shallow invasion, whereas this sample was composed only of OSCC cases, barely well-differentiated, with invasion into the stroma and a high prevalence of nodal involvement. It might justify the contrasting correlations appeared in terms of dissimilar roles of TGF-β1 at different levels of cancerization (Chen et al., 2008; Pasini et al., 2001; Tian & Schiemann, 2009; Wakefield & Roberts, 2002; Yang, 2010; Yang & Moses, 2008). Besides, TGF-β1 might directly down- and up-regulate the expression of MMPs while indirectly regulating them by upregulation of TIMPs (Chen et al., 2008; Overall et al., 1991). Therefore, much more than only a simple linear correlation between the two markers should be anticipated.

Age and gender

Consonant with the literature with regards to OSCC/HNSCC (Dai et al., 2007; Logullo et al., 2003; O-Charoenrat et al., 2001) as well as other SCC studies (Logullo et al., 2003; O-Charoenrat et al., 2001; Patel et al., 2005; Ruokolainen et al., 2004), in this study no connections were found between MMP-9 and age. The correlation between gender and MMP-9 was statistically significant but weak, with a higher MMP-9 expression in females which might be attributable to biologic differences. The associations between the two markers and SCC patients' gender have not been assessed before.

It has been suggested that TGF-β1 might not decline by aging in patients with HNSCC (Logullo et al., 2003). This study however showed that it might slightly decrease by aging. In addition, females had some higher levels of TGF-β1.

As limitations, the present cross-sectional study lacked a control group to explore the causations, and the measurements were semi-quantitative. Nonetheless, the authors tried to increase the reliability of the findings by selecting a rather large sample of initial tumors uniformly composed of OSCC specimens stained with two markers, using the regression analysis, adopting three methods of scoring, validating the staining procedures using both positive and negative controls as well as evaluating the slides by two examiners. The high positive correlations existed between IS and PS of each marker implied the consistency of the subjective intensity scores.

Conclusions

These markers showed changes in expression patterns proportional to the severity of OSCC, and thus they might be considered potential prognostic indicators. Nonetheless, most of the statistically significant associations emerged were not quite strong, implying a presence of several interrelated factors involved.

Although MMP-9 expression might not predict histopathological grade, it might be used as a prognostic indicator by being consistently correlated, although slightly, with nodal metastasis and mode of invasion. Nevertheless, taking into account its complex function, TGF-β1 might not be used as a prognostic marker at the present time; as it was negatively associated with tumor

histologic grade while being positively correlated with nodal metastasis. Moreover, it was not associated with mode of invasion.

There were weak negative associations between MMP-9 and TGF-β1 expressions. Mode of invasion was correlated with metastasis and hence it might be considered a prognostic determinant. TGF-β1 might be slightly higher in younger and female patients. MMP-9 was not associated with age; but it was higher in females.

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Declaration of interest

The authors report no declarations of interest.

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