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ORIGINAL ARTICLE

Mast cell density and angiogenesis in oral dysplastic epithelium and low- and high-grade oral squamous cell carcinoma

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Abstract

Objective. Oral squamous cell carcinoma (OSCC) is one of the 10 most common malignant tumors and SCC accounts for ≈94% of all oral malignancies. The risk of malignant transformation in dysplastic lesions is greater than that of normal oral mucosa. The definite roles of mast cells and angiogenesis in OSCC have been under debate. The aim of this study was to compare mast cell count (MCC) and microvessel density (MVD) among normal oral mucosa, oral dysplastic epithelium and low- and high- grade OSCC. ***Material and methods.*** A total of 42 specimens of OSCC (21 high- and 21 low-grade) were collected, along with six normal and 22 dysplastic oral mucosa. The mean MCC and MVD, as well as the correlation between them, were evaluated by immunohistochemical staining. ***Results.*** Statistically significant increases in mean MCC and MVD were observed between normal oral mucosa and epithelial dysplasia, normal oral mucosa and OSCC and epithelial dysplasia and OSCC ($P < 0.05$), but there were no statistically significant differences in MCC and MVD between low- and high-grade OSCC. Also, the Spearman's correlation coefficient showed a significant correlation between MCC and MVD ($r = 0.727$, $P < 0.001$). ***Conclusions.*** The significant correlation found between MCC and MVD is in agreement with the idea that mast cells promote tumor progression via upregulation of angiogenesis. MCC and the degree of angiogenesis can potentially be used as indicators of the evolution of SCC from epithelial dysplasia.

Key Words: *Angiogenesis, mast cell, oral cavity, squamous cell carcinoma*

Introduction

Oral squamous cell carcinoma (OSCC) is one of the 10 most common malignant tumors. It shows variable biological behavior, leading to different degrees of aggressiveness that are not predictable according to histological grade or T or N stage [1].

The definite role of mast cells and angiogenesis in OSCC is an issue of debate [2]. For tumor growth, the required blood supply is provided via angiogenesis as a result of tumor-mediated induction or overexpression of angiogenic factors. Neovascularization, which promotes the progression and metastasis of malignant tumors [3], is mediated by several agents, including fibroblast growth factor (FGF), transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF) and inhibitors such as platelet factor V. By producing these factors, tumors stimulate angiogenesis, as manifested by accumulation of endothelial cell precursors or new vessel budding from the existing vessels, which is similar to physiologic angiogenesis. Mast cells have been proposed as angiogenesis promoters and the mast cell count appears to be a reliable prognostic marker in some tumors [4,5]. Mast cells cause neovascularization by producing angiogenic factors, such

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as VEGF, or substances with angiogenic properties, such as tryptase, FGF, TNF, interleukin (IL)-8, histamine and heparin. The accumulation of mast cells is a harbinger of the growth and invasion of several kinds of malignancy [2].

It is also instructive to take a look at the other side of the coin. The presence of mast cells around the tumoral cells may hamper tumor growth by producing IL-1, −4 and −6 and TNF [6]. Fibrosis induced by tryptase secreted by mast cells may have a dual role, i.e. forming a stroma for metastasis and also restricting tumor growth [2]. CD34 is a marker for the detection of endothelial cells and, consequently, angiogenesis [4]. The aim of this study was to compare the mast cell count (MCC) and microvessel density (MVD) among normal oral mucosa, oral epithelial dysplasia and low- and high-grade OSCCs.

Material and methods

Seventy paraffin-embedded specimens were obtained from the archives of the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Mashhad University of Medical Sciences and Dental Research Center, Mashhad, Iran. They included normal oral mucosa (n = 6), dysplastic oral mucosa (n = 22; 14 males, eight females), low-grade OSCC (n = 21; eight males, 13 females) and high-grade OSCC (n = 21; 12 males, nine females). Specimens containing necrotic areas were excluded. Normal oral mucosa was obtained from areas in the vicinity of oral lesions which had not shown any inflammation or hyperplasia.

For immunohistochemistry, two slices with a thickness of 4 μm were prepared from each paraffin-embedded section. Each section was deparaffinized, rehydrated in xylene and graded ethanol and washed in distilled water. Incubation of the slides in 1% H_2O_2 with methanol for 30 min blocked endogenous peroxidase activity. Antigen retrieval was performed by microwaving the sections in citrate solution (0.01 M, pH 6.0) for 35 min. After turning off the microwave, the sections were retained in the same solution for 15 min and finally the slides were washed in distilled water for 5 min. Sections were assessed for MCC using mouse anti-mast cell tryptase primary monoclonal antibody (IgG1, Kappa; code: NCL-MCTRYP-428; Novocastra, Newcastle upon Tyne, UK) at a dilution of 1:100 (Diluent Solution Code: RE10729; Novocastra) for 60 min at room temperature. For the evaluation of MVD, anti-CD34 monoclonal antibody (IgG1, Kappa; code: M7165; Dako, Glostrup, Denmark) was applied for 60 min at room temperature. Sections were washed with Tris-buffered saline–tween. For immunohistochemical staining of tryptase, the Novo Link Polymer Detection System (code: RE7140-K; Leica Microsystems Inc., Bannockburn, IL) was used and for CD34 staining, the Dako LSAB (code: K0679) was applied.

This procedure was followed by counterstaining using Meyer's hematoxylin, followed by dehydration by soaking the sections in 95% ethanol twice, each for 10 s, then 100% ethanol twice for 10 s each and then three times in xylene, each for 10 s. Human tonsil and hemangioma were used as positive controls for tryptase and anti-CD34 staining, respectively. MCC and MVD around the tumors were assessed by counting cells positive for tryptase and CD34 under a light microscope at a magnification of ×400 in five hot-spot fields (the areas most populated by mast cells and vessels) equivalent to an area of 0.2 mm^2 by two pathologists [3].

Data analysis was performed using SPSS software (SPSS Inc, Chicago, IL). The Kruskal–Wallis test was used for comparing MCC (tryptase-positive) and MVD in samples with different histopathologic grades and the Mann–Whitney U-test was used for intergroup comparisons. The correlation between MCC and MVD was assessed by means of Spearman's rank correlation coefficient. $P < 0.05$ was considered statistically significant.

Results

The mean MCC and MVD in normal and dysplastic oral mucosa as well as low- and high-grade OSCCs are illustrated in Figures 1 and 2 and Table I.

The mean MCC and MVD were significantly higher in OSCCs than dysplastic and normal oral mucosa and corresponding P-values are shown in Table I. However, there was no statistically significant difference between low- and high-grade OSCCs (P = 0.649). Also, the Spearman's correlation

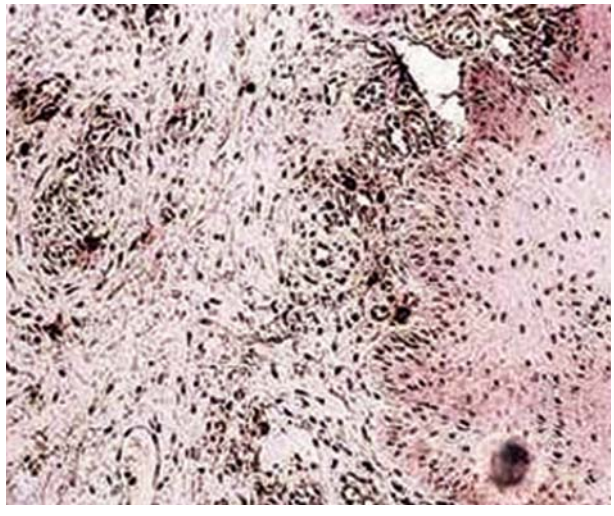

Figure 1. CD34-positive blood vessel in OSCC connective tissue. Original magnification ×400.

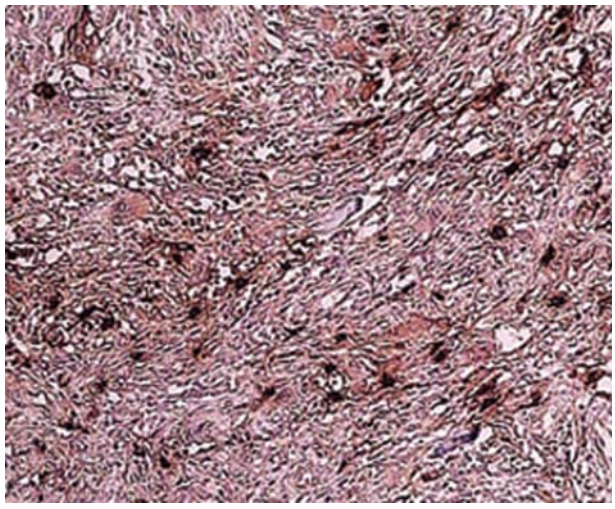

Figure 2. Tryptase-positive mast cells in oral squamous cell carcinoma connective tissue. Original magnification ×400.

coefficient showed a significant correlation between MCC and MVD ($r = 0.727$, $P < 0.001$) (Figure 3).

Discussion

To date, tumor thickness and metastasis to the lymph node are considered well-known predictors of the survival of patients with OSCC. Also, significant associations have been found between MVD and the recurrence and growth of cervical metastases [7]. In the current study, MCC and MVD showed a significant linear increment from normal oral mucosa to epithelial dysplasia and OSCC.

The impact of the contribution of mast cells to tumor invasion has also been shown in several cancers, including carcinomas of the breast [8], stomach [9], esophagus [4], oral cavity [3], lung [10] and larynx [11] and melanomas [12]. Angiogenesis has been reported to be an unfavorable survival indicator in cancers of the breast [13,14], prostate [15], lung [16], skin [17], larynx [18], oral cavity [19] and bladder [20]. The significant correlation between MCC and MVD with progression of the disease from normal mucosa to dysplasia and SCC which was observed in the present study has also been reported in other malignant tumors, such as lung carcinoma [21,22] and SCC of the esophagus [4].

In previous studies, angiogenesis was the significant independent variable of recurrence in patients with OSCC [7,19]. However, a study by Ascani et al. [1] showed no statistically significant association between MVD and clinical variables such as age, sex, tumor size and site.

In another study of 100 paraffin-embedded oral mucosal tissues, vascularity was measured by two methods: first, highest MVD and second, microvascular volume. According to the first method, vascularity was significantly enhanced with disease growth from normal oral mucosa to mild, moderate and severe dysplasia and to early and late carcinoma. However, the second method did not distinguish between dysplastic oral mucosa and carcinoma [23].

It has been suggested that mast cells have a significant correlation with tumor angiogenesis in OSCC, and MVD has been considered to be a desirable prognostic factor in patients with OSCC [24,25]. This is in contrast to our results, where MVD increased with tumor progression, except for low- and high-grade OSCC.

It has been postulated that, in Hodgkin's lymphoma patients with a poor prognosis, tumoral tissue is infiltrated by many mast cells and shows a high rate of angiogenesis. Although mast cells have been suggested to be angiogenesis promoters in other types of lymphoma, no correlation has been found between MVD and MCC in Hodgkin's lymphoma [26]. The number of mast cells differs between a variety of primary malignant tumors, such as breast and colorectal cancers as well as liver metastases, and so the definite role of mast cells is still under debate. As previously reported [27], in liver metastasis a higher MCC is detected in tumors with a moderate or high grade of histological differentiation compared with those with a lower grade of differentiation, which differs from the results of our study, in which we did not detect such a significant difference between low- and high-grade lesions. This may be due to the different nature of the tumors or the low number of cases in the previous study. Contrary to other studies [26,27], which demonstrated better prognosis for a variety of tumors that contained fewer mast cells and less neovascularization, the 5-year survival rate for SCC of the tongue was significantly lower in patients with low MCC and MVD [28].

Table I. MCC and MVD in oral lesions.

			OSCC ($N = 42$)		P[a]		
	Normal ($N = 6$)	Dysplasia ($N = 22$)	Low grade	High grade	A	B	C
MCC	4.1 ± 1.7	9.3 ± 2.6	11.2 ± 2.3	12.2 ± 3.3	0.001	0.001	0.01
MVD	5.5 ± 2.5	8.3 ± 1.7	11.6 ± 3.1	11.5 ± 3	0.01	0.01	<0.001

[a]A = normal vs dysplasia; B = normal vs carcinoma (low and/or high grade); C = dysplasia vs carcinoma (low and/or high grade).

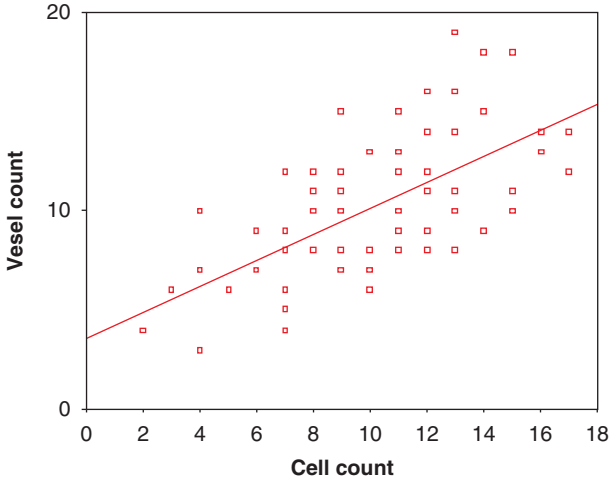

Figure 3. Relation between MCC and MVD.

Conclusions

The higher values for MCC and MVD found in OSCC and dysplastic oral mucosa in comparison with normal oral mucosa suggests these two factors as indicators for oral tumor progression, albeit this relationship was not found between low- and high-grade OSCC. Also, the significant correlation found between MCC and MVD is in agreement with the idea of considering mast cells for a role in tumor progression via the promotion of angiogenesis. Further studies are recommended to compare the number of mast cells and the severity of neovascularization between OSCC and OSCC-derived lymphatic metastases in the head and neck area. From the prognostic point of view, designing studies to determine the relationship between MCC, MVD and survival rate in oral cancers is to be encouraged. Therapeutically, given the prominent role of angiogenesis in the evolution of ominous oral cavity lesions towards those with a graver prognosis, it would be of great value to design studies to investigate the effect of inhibitors of angiogenic factors in stopping this process or for the treatment of malignant lesions.

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