

RESEARCH ARTICLE

Editorial Process: Submission:09/12/2017 Acceptance:06/04/2018

Immunohistochemically Detection of Angiogenesis in Oral Pre-Cancerous Lesions Compared with Oral Invasive Carcinomas

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Abstract

Background: Angiogenic activity is an important event in oral carcinogenesis. During transition of normal oral mucosa to different grades of dysplasia and to invasive carcinoma, significant increase of vascularity occurs. Angiogenesis can be determined by immunohistochemical assessment of several endothelial cell markers like Endogelin (CD 105), expressed in activated endothelial cells and associated with neovasculature, and the vascular endothelial growth factor (VEGF). This study was conducted to evaluate angiogenic activity in oral precancerous lesions compared with oral invasive carcinomas by immunohistochemical staining of VEGF and CD 105 proteins. **Methods:** In the present cross-sectional study, 20 normal, 20 pre-cancerous mucosa and 20 oral invasive carcinoma samples were immunohistochemically stained. Positive cells were counted in each section and micro vessel density (MVD) was determined. The data were statistically analyzed by Mann-Whitney and Kruskal-Wallis tests, with a P-value ≤ 0.05 considered significant. **Results:** The mean expression value for VEGF was 24.6 in oral invasive carcinoma, 16.4 in precancerous mucosa and 15.5 in normal mucosa, with no significant differences between the latter two. Endogelin was negative in all normal mucosa samples, but had scores of 7.58 for precancerous mucosa and 19.4 in oral invasive carcinoma specimens. MVD was significantly higher in SCC than in dysplastic mucosa. **Conclusion:** Oral invasive carcinoma has more angiogenic activity in comparison with pre-cancerous lesions and normal mucosa. Given the high expression of CD105 positive vessels in malignant lesions, we can argue that determination of mean vessel density (MVD) by application of the CD105 marker could be a useful parameter to differentiate cancerous from pre-cancerous lesions.

Keywords: Angiogenic activity- Immunohistochemistry- precancerous lesions- squamous cell carcinoma

Asian Pac J Cancer Prev, 19 (7), 1805-1808

Introduction

Oral carcinogenesis is a two-step process characterized by an initial precancerous lesion that will develop into cancer (Siar et al., 2009). According to World Health Organization (WHO), a precancerous lesion is defined as “altered epithelium with an increased potential for progression to squamous cell carcinoma (SCC)” (Neville, 2016, Ascani et al., 2005). The altered epithelium is characterized by morphologic and cytologic changes with termed dysplasia. Oral carcinogenesis is a two-step process characterized by an initial precancerous lesion that will develop into cancer (Neville, 2016).

Different researchers have shown that a significant vascularity increase occurs during transition from normal oral mucosa to different grades of dysplasia and to invasive carcinoma (Bouquot et al., 2006, Pazouki et al., 1997).

Angiogenesis is defined as the growth and

proliferation of blood vessels from the existing vasculature. This process is mediated by endothelial cells and a series of mediators (Siar et al., 2009, Hirsch, 2001, Hirsch et al., 2002). Studies have shown that tumoral endothelial cells have a higher proliferation rate than normal tissues (Marioni et al., 2005).

Angiogenesis is determined by immunohistochemical assessment of several endothelial cell markers in which Endogelin (CD 105) is an angiogenic factor expressed in activated endothelial cells and is associated with neovasculature. CD31, CD 34 and vascular endothelial growth factor (VEGF) are pan endothelial markers (Basnaker et al., 2014, Habson, 1984).

Neoangiogenesis is an important step in tumor progression. An important predictor for tumor behavior is intratumor microvessel density (MVD) (Basnaker et al., 2014, Taek, 2000).

Endogelin (CD105), a type 1 180KD homodynamic

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transmembrane glycoprotein, is a receptor for two types of transforming growth factor (TGE-1-TGF-3). It has been suggested that tumor-associated neovasculature endothelial cells proliferate more rapidly than endothelial cells of normal tissue (Basnaker et al., 2014).

VEGF is one of the growth factors which directly affects vascular endothelial cells. It was first described by Folkman (1971) as the most important promoter of neovascularization by increasing vascular permeability and mitogen effects on endothelial cells (Stepan et al., 1971, 2012).

VEGF and CD105 are characterized for angiogenesis in oral carcinoma (Basnaker et al., 2014, Stepan et al., 2012). Given the fact that tumor angiogenesis is a critical step in tumor progression, this study was conducted to evaluate angiogenic activity in oral pre-cancerous lesions compared with oral invasive carcinoma by immunohistochemical staining of VEGF and CD 105 proteins.

Materials and Methods

This descriptive analytical cross-sectional study, is approved by Isfahan university ethics and research committee no:393123.393528 and389068. In a descriptive analytical cross-sectional study, to detect the angiogenic activity, the formalin fixed paraffin-embedded tissues of 20 normal, 20 pre-cancerous mucosa and 20 invasive carcinoma were immunohistochemically stained for specific antigen of CD105 and VEGF by envision method.

The normal mucosa were obtained from the patients who had undergone a dental implant with no mucosal pathology .precancerous mucosa and carcinomatous mucosa were obtained from the archives of department of oral pathology, dental school of Isfahan University of Medical Sciences. The study protocol was approved by research and ethical committee of Isfahan University of Medical Sciences.

The main procedure included serial sectioning (in 3-4 um sections), deparaffinization, rehydration and antigen retrieval. For VEGF staining, the tissues were incubated with primary antibody of VEGF clone VG1 (DAKO, Denmark). For CD105 staining, primary antibody CD105 clone SN6h was used (Dako, USA).

VEGF evaluation: five non-overlapping fields were

selected randomly and epithelial layers of each specimen was assigned a specific score based on endothelial stain intensity. Where the epithelial cytoplasm was stained stronger than endothelial cells, the field was given score 3, where it was in good harmony with endothelial cells, it was given score 2 and where it was weaker than endothelium, it was given a score of 1. The score 0 was recorded when it was not any staining (Stepan et al., 2012, Torabinia et al., 2014).

Finally, the mean intensity of five fields in one specimen was recorded. The data were statistically analyzed by Mann-Whitney and Kruskal-Wallis tests.

CD105 evaluation: in order to evaluate the CD105 protein expression, the sections were observed by two pathologists using light Olympus microscope (x400). The 10 fields with highest number of positively stained cells were selected and The stained cells were calculated and vessel density was determined in each section and the mean of calculated cells were reported as Mean Vessel Density (MVD)(Marioni et al., 2006). The data were statistically analyzed by Mann – Whitney and Kruskal-Wallis tests.

Results

VEGF protein had positive cytoplasmic expression in all cases of invasive carcinoma, pre-cancerous and normal mucosa. The mean expression of VEGF was 24.65 in oral invasive carcinoma, 16.35 in pre-cancerous mucosa and 15.5 in normal mucosa. VEGF expression was significantly higher in invasive carcinoma group than in pre-cancerous group ($p=0.002$) or normal group ($p=0.001$). On the other hand, there was no differences between pre-cancerous and normal mucosa in VEGF expression.

CD105 immunoreaction occurred in the cytoplasm of endothelial cells (Figure 1, 2). The immunostaining was present in the blood vessels with small diameter and irregular or collapsed lumen and was absent in large blood vessels.

Endoglin was reported to be negative in all normal mucosa samples, 7.58 in pre-cancerous mucosa and 19.35 in oral invasive carcinoma specimens. MVD was significantly higher in invasive carcinoma than in

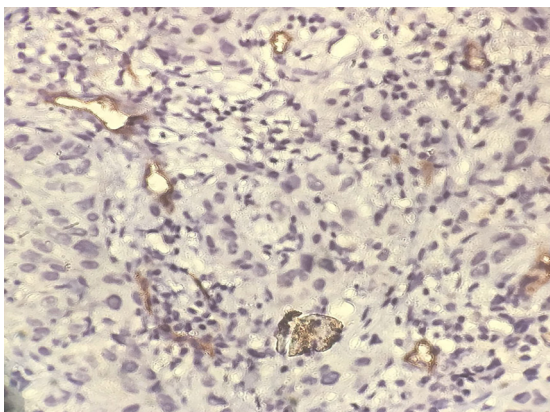


Figure 1. CD105 expression in precancerous mucosa (X400)

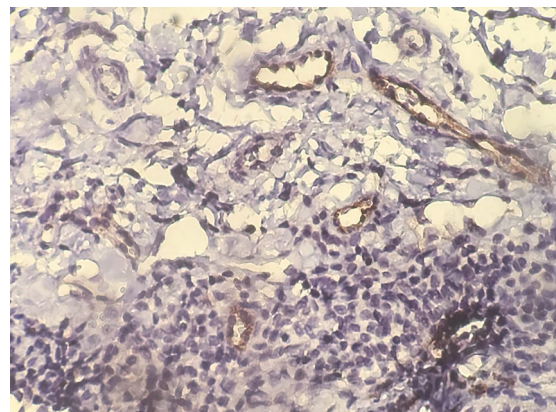


Figure 2. CD105 expression in oral invasive carcinoma (X400)

pre-cancerous mucosa.

Discussion

Angiogenesis has an important role in pre-cancerous progression to invasive carcinoma influencing local invasion as well as distant metastases (Thiem et al., 2017).

Angiogenesis is a process which new blood vessels develop from pre-existing blood vessels. This process is critical factor for cancer growth, progression and metastasis. It is generally known that VEGF is one of the most important proangiogenic factors (Subarnbhesaj et al., 2017).

Moreover, it has been suggested that VEGF overexpression plays a significant role in the primary transformation of tumors at earlier stages, but later other factors such as genetic factors have a greater influence on progression and metastasis (Wang, 2009).

In this study, there was a progressive increase of VEGF immunorexpression from normal mucosa to pre-cancerous mucosa and invasive carcinoma lesions. The highest value was reported in invasive carcinoma (24.65).

Concomitantly, the previous studies have shown the overexpression of VEGF in malignant lesions (Wang, 2009, Sun et al., 2005, Pazouki et al., 1997). Which is in line with the findings of the present study. Although the mean VEGF expression in pre-cancerous mucosa (16.35) was higher than that of normal mucosa (15.5), the difference was not statistically significant ($p=0.108$). However, the previous studies found a significantly higher VEGF expression in pre-cancerous mucosa than in normal mucosa. Thus, their findings were in line with our results (Subarnbhesaj et al., 2017).

In addition, Microvessel density (MVD) is a parameter for evaluation of new angiogenetic patterns, which has shown to be a prognostic factor for several malignant lesions including oral invasive carcinoma (Thiem et al., 2017). In the current study, we used anti-CD105 antibody because it reacts specifically with activated endothelial cells in angiogenic tissues with no reaction with the normal tissue (Khalili et al., 2015) in the present study we use MVD for quantitative angiogenesis assessment and evaluation of CD105 positive microvessels and it was significantly higher in oral invasion carcinoma than in pre-cancerous mucosa, and there was no expression in normal mucosa. There was a significant increase from pre-cancerous mucosa to malignant lesions. Therefore, evaluation of CD105 marker can be a helpful method to distinguish the premalignant lesions from malignant ones. The results of previous studies (Kyzas et al., 2006; Saad et al., 2003; Bellon et al., 2010; Randall et al., 2009) have shown that overexpression of CD105 in oral invasive carcinoma is associated with worse clinical features and lymph node metastasis.

In conclusion, oral invasive carcinoma has more angiogenic activity in comparison with pre-cancerous lesions and normal mucosa. Given the high expression of CD105 positive vessels in malignant lesions, we can argue that determination of mean vessel density (MVD) by application of CD105 marker can be a useful parameter to differentiate cancerous from pre-cancerous lesions

Acknowledgments

The authors would like to thank the Isfahan University of Medical Sciences for financial support and oral pathology department by giving the paraffin embedded tissues

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