Clinicopathological Significance of CD105 Expression in Squamous Cell Carcinoma of the Oral Cavity

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Abstract

Background: Angiogenesis is essential for the growth, invasion and metastasis of solid tumors. Research on related factors such as microvessel density can be helpful in predicting a tumor's behavior. CD105 has been introduced as a marker of angiogenesis which stains vessels that are in the proliferating stage. There are some controversies about the relation of microvessel density and clinicopathological features of oral squamous cell carcinoma. The aim of this study is to determine the CD105 expression in oral squamous cell carcinoma and its relation to the clinicopathological features of this disease.

Methods: We studied a total of 42 patients who had oral squamous cell carcinoma. The control group consisted of 15 cases with normal oral epithelium. CD105 immunostaining was performed on 4 μm thick tissue sections. Intratumoral and peritumoral microvessel density in ten areas of the sections were recorded by two pathologists.

Results: There was a significantly higher CD105 microvessel density value in the tumoral tissues compared with normal tissues. In addition, there was more expression of this marker in the invasive front area. The CD105 microvessel density value had a positive relation with lymph node metastasis. There was an association between tumor size and CD105 microvessel density in the invasive front region. A negative association between tumor grade and CD105 microvessel density value in the intratumoral region was observed. In both areas, CD105 expression was higher in cases with advanced clinical stage. There was no association between this marker and patients' ages or gender.

Conclusion: CD105 microvessel density can be a useful factor for predicting the course of oral squamous cell carcinoma.

Keywords: Angiogenesis, Microvessel density, CD105, Oral squamous cell carcinoma

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Introduction

Angiogenesis is defined as a new blood formation[1,2] that is essential for growth, invasion and metastasis of solid tumors.[3,4] Numerous experiments have concluded that solid tumors cannot grow more than 2-3mm without induction of neovasculature,[5] thus the rate of endothelial cell proliferation is 20 to 2000 times faster than normal tissue.[6] Microvessel density (MVD) is a good predictor of tumor growth, invasion and metastasis.[7] Since 1991 numerous markers have been introduced to stain the vessels. These markers, however, cannot distinguish between neovasculature and preexisting vessels, with the exception of CD105.[6]

CD105, also known as endoglin,[8] is a good marker for the measurement of MVD.[2] It is a 180 kDa homotypedipolymer[9] glycoprotein located in the endothelial cell membrane which modulates responses to TGFβ.[6] Its gene is located on chromosome 9q34.[9] CD105 is activated under hypoxic conditions[6,8] in tumoral cells but its expression in normal tissue is either low or absent.[10]

Some studies have used CD105 as a marker of angiogenesis in oral squamous cell carcinomas (OSCC) and its relation to the tumor's clinico-pathological features, however the results are controversial.

The aim of this study is to determine CD105 expression in OSCC and its relation to clinico-pathological features of this disease.

Materials and Methods

Materials

This cross-sectional study analyzed the specimens from 42 patients with OSCC (28 males and 14 females). The mean age of patients was 54.47 (range: 35-81) years. Specimens were obtained from the archives of Oral Pathology Department of School of Dentistry between 2008 to 2012. The control group consisted of 15 cases of normal oral epithelium.

Immunohistochemical (IHC) staining and analysis

Initially we reviewed hematoxillin and eosin slides of the available blocks. Cases with a definite diagnosis and adequate cellular tissue depth were selected for immunohistochemical staining (IHC). Immunohistochemical staining was performed by using the Envsion Labled Peroxidase System (DAKO, Carpentaria, CA, USA). All samples were fixed in 10% buffered formalin and embedded in paraffin. We prepared 4 μm-thick sections which were deparaffinized in xylene, rehydrated in graded alcohol and washed with distilled water. Antigen retrieval was performed by using a DAKO cytomation target retrieval solution(pH=9) for 20 minutes. Internal peroxidase activity was inhibited by 3% H_2O_2.

Tissue sections were then incubated for 30 minutes with the anti-CD105 monoclonal antibody (mouse, Dako Corporation, Denmark) at a 1:10 dilution. Brown cytoplasmic staining for CD105 was considered positive. Omission of primary antibody was employed as the negative control and liver tissue was the positive control.

Immunohistochemical results were interpreted by two pathologists.

Intratumoral and peritumoral MVD was quantified according to a recent consensus statement.[11]

Briefly, with the use of an optical microscope,we initially identified hot-spot areas for CD105 expression in discrete blood vessels by scanning the entire tumor at low power (40✕). The number of CD105 highlighted vessels in ten of these areas was then counted at high power magnification (400✕).

Statistical analysis

The t-test, independent samples test, paired t-test, Pearson, Spearman and receiver operating characteristic (ROC) curve were used to compare the results between the two groups and the relation with clinicopathological features. We used SPSS15 software for data analyses. $P \leq 0.05$ was considered significant for all statistical analyses.

Results

Table 1 shows the clinicopathological features

Table 1: Clinicopathological features and microvessel density (MVD) of study patients.

		MVD		P-value	
	Frequency (%)	Intra tumoral	Invasive front	Intratumoral	Invasive front
Sex				0.756	0.516
Male	28(66.7)	15.11±5.5	24.21±7.97		
Female	14(33.3)	15.64±4.61	25.86±6.98		
Tumor size				0.058	0.001
T1	13(31)	13.62±3.57	21.00±5.95		
T2	19(45.2)	15.63±5.62	24.16±7.04		
T3	8(19)	17.13±6.64	29.75±7.63		
T4	2(4.8)	15.50±0.70	35.00±7.07		
Lymph node involvement				0.007	0.000
N0	21 (50)	13.19±2.96	19.38±2.76		
N1	15(35.7)	17.27±6.45	30.53±7.54		
N2	5(11.9)	18.40±5.85	30.00±6.70		
N3	1(2.4)	14±4.65	25±5.87		
Grade				0.034	0.938
G1	27(64.3)	16.67±5.89	24.85±8.02		
G2	12(28.6)	12.75±2.05	25.33±7.69		
G3	3(7.1)	13.00±1.00	21.67±3.05		
Stage				0.000	0.000
I	9(21.4)	12.22±1.48	18.00±1.58		
III	10(23.8)	13.60±4.00	20.00±3.33		
III	14(33.3)	16.21±5.13	28.07±7.09		
IV	9(21.4)	18.78±6.68	31.67±6.89		

of the study patients. The mean age of the investigated patients was 54.4±12.44 (range: 35-81) years. There were 28 (66.7%) males and 14 (33.3%) females with OSCC.

The mean CD105MVD value was significantly higher in tumoral tissue (20.02±8.03) compared to normal tissues (8.67±1.75). CD105 expression was significantly higher in the invasive front (24.76±7.61) region compared to the intratumoral region (15.29±5.17; Figure 1). Microvessel density in the intratumoral ($P=0.008$) and invasive front ($P=0.00$) regions were associated with lymph node metastasis. There was no relation between the CD105MVD value and tumor size in the intratumoral region ($P=0.058$) though there was a positive association in the invasive front region ($P=0.001$). Statistical analysis showed a negative association between tumor grade in the intratumoral region ($P=0.034$) which was not observed in the invasive front region ($P=0.938$). In both regions CD105 expression was significantly higher in patients with an advanced clinical stage ($P=0.00$). We did not observe any correlation with age in the intratumoral ($P=0.40$)

and invasive front ($P=0.72$) regions. Similarly, there was no relation with gender for the intratumoral region ($P=0.75$) and invasive front ($P=0.51$) region. Correlation of the CD105MVD value with clinicopathological data is shown in Table1.

According to ROC curves in the intratumoral region, 11 was the acceptable cutoff point (sensitivity: 83.3%, specificity: 93.3%). In the invasive front region, 14 (sensitivity: 97.6%, specificity: 100%) seemed to be acceptable.

Discussion

Until now, numerous investigations have attempted to find an association between the prognostic factors and clinical manifestations of OSCC. Angiogenesis is an important parameter for tumor growth and metastasis, thus studying the related factors such as MVD can be helpful.[12] MVD is a good predictor of the tumor progression and survival rate;[1,7,13] however some investigations have failed to find such a result.[12,14,15] This discrepancy is a consequence of

differences in the research techniques used in different studies. Since 1991 many markers, such as CD31, CD34, VEGF and vWB, have been used to stain blood vessels. Although of benefit, their major problem is the inability to differentiate between new and preexisting blood vessels.[6] Other probable reasons are the lack of a direct method in assessing MVD[12,16] and the different MVD observed in different areas of the tumor.[5]

CD105 has been introduced as an angiogenesis marker that stains vessels which are in the proliferating stage.[2,9] This capability when compared with other markers can reduce false positive results.[17]

Intratumoral vascularization is necessary for growth as it provides nutrients for tumoral cells.[18] Peritumoral vasculature is essential for invasion and metastasis.[17] To reduce the posiibility of controversies we have used the CD105 marker to assess two regions within the tumor specimen, the intratumoral and invasive front. Two pathologists interpreted the specimens to decrease interobserver discrepancy. We inspected the relation of MVD to all of the prognostic factors of OSCC.

In the current study we analyzed specimens from 42 patients diagnosed with OSCC and 15 normal specimens as the control group. Our study proved that CD105MVD was significantly higher in tumoral tissue compared with normal tissue which was in line with previous studies.[5,16,19,20] These results have verified that CD105 is more expressed in tumor tissues and may have a major role in the tumor. The marker was also more expressed in the invasive front region when compared with the intratumoral region. Although this finding was consistent with studies performed by Mărgăritescu et al.[5,20] it differed from the results of Eshghyar et al.[19] who reported more expression of CD105 in the intratumoral region. We also observed a positive relation between CD105 expression and lymph node metastasis in both regions. This finding was compatible with previous investigations[7,10,21-23] and suggested that the marker could be helpful in predicting the possibility of metastasis. The other factor we studied, was the tumor size. Spearman's analysis showed that as the tumor size increased the level of CD105 expression in the invasive front area also increased. However this relation was not observed in the tumor bed. In 2008 Mărgăritescu et al. did not find any association between tumor size and CD105 expression however in a study in 2010 they found declining levels of CD105 in the T4 stage. Schimming and Marmé[16] observed an increasing level of CD105 only in the T1 stage. Miyahara et al.[7] reported higher levels of CD105 in the T3 and
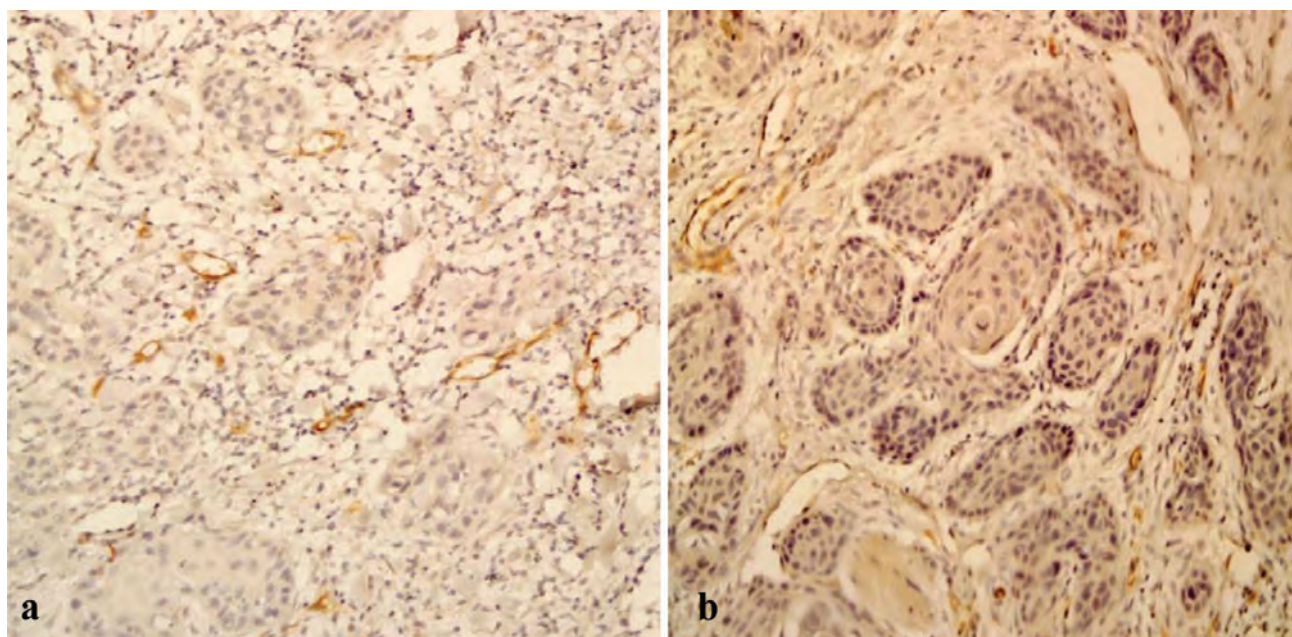

Figure 1: a. CD105 positive vessels at the invasive front of oral squamous cell carcinoma (OSCC). Magnification: 200×. b) CD105 positive vessels in the intratumoral tissue of OSCC. Magnificantion:200×.

T4 stages. We studied the relation between the CD105MVD value and grade of differentiation. There was a decreasing level of CD105 at higher grades of differentiation. In contrast, Mărgăritescu et al.[5] did not report any correlation. In both the intratumoral and invasive front regions, as the clinical stage of the disease progressed, the levels of CD105 expression increased. Likewise, Chien et al.[10], Miyahara et al.[7] and Martone et al.[23] reported the same result. However Mărgăritescu et al.[5] did not observe any association.

According to ROC curves in the intratumoral area,11 (sensitivity: 83.3%, specificity: 93.3%), and in the invasive front,14 (sensitivity: 97.6%, specificity: 100%), seemed to be acceptable cut-off points. MVD values higher than the cut-off points could predict aggressive behavior and prognosis of the patients.

In summary, the results implicated that higher levels of MVD showed the aggressive course of the tumor and CD105 could be a good marker for determining the prognosis of the disease, particularly in the peritumoral region.

As mentioned above, conflicts exist among various studies which result from differences in sample size,[19] variable treatment protocols,[10] lack of direct methods in assessing MVD,[16] differences in selecting the hot spots to evaluate MVD among observers,[5,22] differences between immunohisto-chemical protocols, selection of the paraffin block and section within the block.[5] Unfortunately the small sample size and unspecified survival rate of the patients may be the reasons for differences between our results and previous studies.

By considering all the discrepancies among the results of various studies it is recommended to do more investigations with larger sample sizes, longer follow up and more advanced methods. These investigations would determine the relation between this marker and clinicopathological features of OSCC with the intent to achieve a reliable agreement.

Conclusion

CD105 is an angiogenic marker which specifically stains new blood vessels. It may have a role in tumor development. CD105 shows a relation to tumor size, lymph node metastasis and stage of OSCC therefore it can be a novel marker for predicting the prognosis of this tumor. However, whether prevention of angiogenesis can be an effective treatment for OSCC or not is still unclear and more studies on MVD are necessary.

Acknowledgment

Conflict of Interest

No conflict of interest is declared.

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