ORIGINAL ARTICLE

Immunohistochemical analysis of CD34 expression in salivary gland tumors

Saede Atarbashi Moghadam, Ayda Mohammad Abadi, Sepideh Mokhtari
Department of Oral and Maxillofacial Pathology, Dental School of Shahid Beheshti University of Medical Sciences, Tehran, Iran

Address for correspondence:
Dr. Sepideh Mokhtari,
Department of Oral and Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Velenjak Street, Tehran, Iran.
E-mail: sepidemokhtary@yahoo.com

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ABSTRACT

Background: Tumor growth depends on angiogenesis which is assessed by measuring the tumor microvessel density (MVD) through CD34 immunostaining. The present study was performed to evaluate the situation of angiogenic activity in salivary gland neoplasms. The possible role of CD34 in progression and invasion of salivary gland tumors is also investigated. **Materials and Methods:** Tissue specimens of 15 pleomorphic adenoma (PA) and 15 malignant salivary gland tumors including mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (AdCC) and salivary duct carcinoma (SDC) were immunostained for CD34 protein. The most vascularized areas at low power magnification (hotspots) were selected for vessel counting at ×400 magnification. Then, the mean number of microvessels in three fields within the tumor mass was calculated. **Results:** MVD in PA and malignant salivary gland tumors were 10.93 ± 5.95 and 26.46 ± 7.32, respectively. Tumor angiogenesis in PA was much lower than other lesions ($P < 0.05$). No significant difference was observed between malignant tumors ($P > 0.05$). **Conclusion:** Salivary gland carcinomas demonstrated higher vascular density than benign PA despite of cell types and architecture. The reason for this higher angiogenic activity could be related to metabolic characteristics of malignant cells.

Key words: Angiogenesis, CD34, salivary gland tumors

INTRODUCTION

Salivary gland neoplasms make up 1–4% of all human tumors. The majority of these lesions are pleomorphic adenoma (PA). PA although classified as benign, can cause problems in clinical management since it has tendency for recurrence and malignant transformation.[1] Mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (AdCC) are the most common salivary gland malignancies. Salivary duct carcinoma (SDC) is a high-grade tumor that should be reserved only for tumors that histologically resemble ductal carcinoma of breast.[2]

Tumor growth and metastasis depends on its angiogenic activity. New blood vessels are essential for clonal expansion and formation of macroscopic tumors.[3] The properties of tumor cells to release angiogenic and antiangiogenic factors are essential for tumor-induced neovascularization.[4] Counting tumor blood vessels by immunohistochemistry (IHC) is a common method for evaluating the angiogenic activity.[5] CD34 is a pan-endothelial marker that stains both "newly formed" blood vessels and normal ones trapped within tumor tissues.[6] Tumors with high vascular density are associated with an increased metastatic potential and decreased survival.[3] Thus, antiangiogenic drugs are considered as a potential target for cancer therapy.[4] In the present study, we investigated CD34 expression and its possible role in progression and invasion of salivary gland neoplasms.

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MATERIALS AND METHODS

Thirty paraffin-embedded blocks of salivary gland tumors from archives of our hospital, including 15 benign PA and 15 malignant tumors (MEC: Six, AdCC: Five and SDC: Four) were selected. All benign and malignant tumors were primary. Clinical information was also recorded. EnVsion labeled peroxide systems (Dako, Carpentaria, CA, USA) was used for IHC staining and antigen retrieval was performed by Dako Cytomation target retrieval solution with pH = 9, for 20 min. Tissue sections were incubated for 30 min with anti-CD34 monoclonal antibody (mouse, Dako Corporation, Denmark) at

a 1/10 dilution. Pyogenic granuloma and omission of primary antibody were employed as positive and negative controls, respectively. Brown cytoplasmic staining for CD34 was considered positive.

Immunohistochemical results were interpreted by two observers. Three fields of the most vascularized areas at ×40 magnification were selected as hotspots and tumor vessels in each field were counted. Microvessel density (MVD) for each sample was considered as the mean number of vessels in these areas. Single endothelial cells or clusters of these cells, with or without lumen, were considered individual vessels. Vessels with muscular walls were excluded. Intensity of staining was not considered for evaluation.

Statistical analysis

To compare MVD between benign and malignant lesions, t-test was used [Table 1]. The results in three different groups of malignant tumors were analyzed by analysis of variance (ANOVA) [Table 2]. Data were presented as mean ± standard deviation (SD) and the results with $P < 0.05$ were considered significant. All the statistical procedures were performed using Statistical Package for Social Sciences (SPSS), WIN program package 13.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

CD34 was expressed in all studied cases ($n = 30$). The intensity of staining in all cases was strong. Figures illustrate CD34 expression in all groups [Figures 1-4]. In PAs, MVD was significantly ($P < 0.000$) lower than in malignant tumors [Figure 3]. CD34 positive vessels were more numerous in cell-rich areas than cell poor regions of PA. In addition, a lower number of vessels were detected in myxoid and chondroid areas.

MEC showed increased CD34 expression among malignant neoplasms, although the difference was not significant. The size of vessels in MEC was also smaller than other groups. In AdCC, MVD in tubular pattern was lower than cribriform areas and the newly formed vessels were frequently arranged as a rim of capillaries adjacent to the carcinomatous aggregates [Figure 1]. Tumor emboli within vessels were not found in specimens.

Table 1: Results of CD 34 immunohistochemical expression in benign and malignant salivary gland tumors

Salivary gland tumor	Number of specimens	Score	Min	Max
Benign	15	10.93±5.95	3.33	23.33
Malignant	15	26.46±7.32	17.67	40.67

Table 2: Results of CD34 immunohistochemical expression in individual malignant salivary gland tumors

Salivary gland tumor	Number of specimens	Score	Min	Max
MEC	6	29.94±9.18	18.00	40.67
AdCC	5	24.60±5.83	17.67	30.67
SDC	4	23.58±4.81	17.67	27.67

MEC=Mucoepidermoid carcinoma, AdCC=Adenoid cystic carcinoma, SDC=Salivary duct carcinoma

DISCUSSION

Recently, role of neoplastic microenvironment in proliferation, invasion and metastasis of tumor cells has been investigated. Vascularization is an important part of this process for cell nutrition in both malignant and benign tumors.[7] Increased MVD plays an important role in many physiologic and pathologic conditions. Angiogenesis is a multistep process, including basement membrane degradation, endothelial cell migration and sprouting into interstitial space, endothelial cell proliferation and lumen formation.[8] In macroscopic tumors, the major factor contributing to vessel density is metabolic demand, which frequently increases during tumor progression.[9,10]

In this study, malignant salivary gland tumors had higher levels of angiogenic activity than PA as a benign tumor, which is similar to the results of other investigations.[5,8,11-15] This indicates that development of blood vessels is associated with invasiveness and malignant behavior. It has also been stated that the stroma of PA always presents poor vascularization.[16] Soares *et al*.,[7] found that MVD is correlated with cellular density of PA as the cell-rich areas contain higher vascular density than cell-poor regions. This is probably because of higher metabolism and more oxygen demand. However, they mentioned that there is no correlation between vascularity and the risk of recurrence in PA. In our series, similar to other studies,[7,16] increased vessel density was observed in cell-rich areas.

Many salivary gland tumors have myoepithelial cells in their architecture. MEC and SDC rarely show myoepithelial differentiation, whereas AdCC and PA contain myoepithelial cells.[2] Several investigations have shown that tumors with myoepithelial phenotype are a distinct entity.[4,6] Costa *et al*.,[4] have stated that angiogenesisis is lower in carcinomas with myoepithelial differentiation. However, they could not find any correlation between MVD and number of myoepithelial cells. Myoepithelial microenvironment secretes high amounts of angiogenesis inhibitors and inhibits angiogenesis.[17] Moreover, salivary carcinomas with myoepithelial differentiation arising in PA have lower angiogenic activity than those without such differentiation.[6] It has been stated that these carcinomas meet their energy demands via an oxygen-independent process called glycolysis.[4] In addition, carcinoma cells in invasive tumors with myoepithelial differentiation frequently form large hypovascularized cellular aggregates that are surrounded by large vessels, whereas in those without such cellular differentiation, the carcinomatous aggregates are smaller and the vessels around them are thinner.[7] It is likely

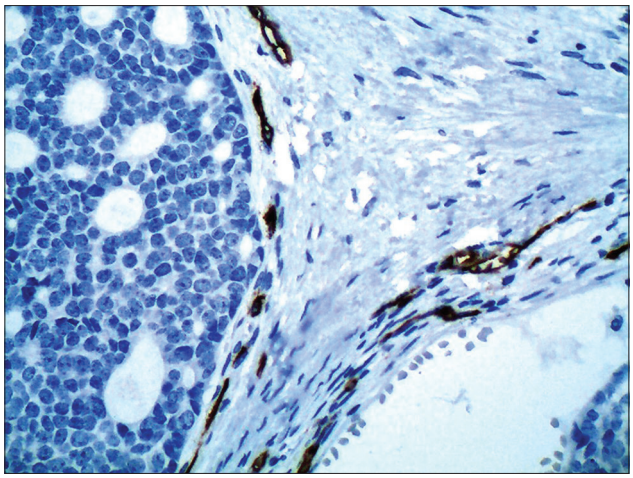

Figure 1: Immunohistochemical expression of CD34 in AdCC. The positive vessels frequently formed a rim of capillaries immediately adjacent to the carcinomatous aggregates (IHC stain, ×400). AdCC = Adenoid cystic carcinoma

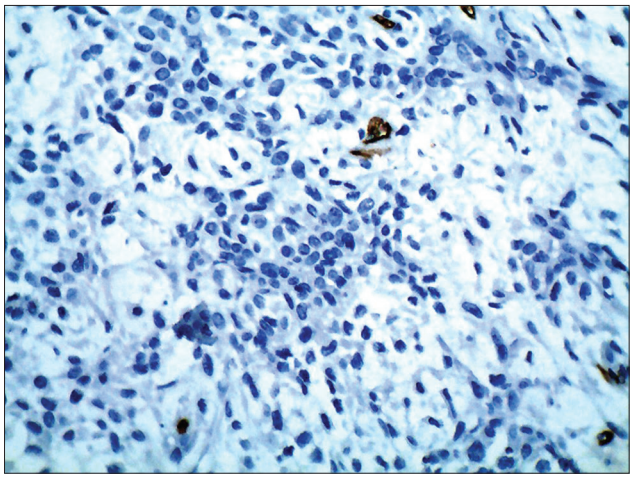

Figure 3: Immunohistochemical expression of CD34 in PA. Note the low MVD (IHC stain, ×400). MVD = Microvessel density, PA = pleomorphic adenoma

that large-sized blood vessels are needed for cellular nutrition in carcinomas with myoepithelial component to compensate for low angiogenesis inside the large cellular aggregates.[4,6]

In our study, MEC showed high MVD that is in agreement with other studies.[3,8,11] However, it is in contrast with Faur *et al*.,[18] investigation. They evaluated different types of salivary gland tumors and found low MVD in MEC and AdCC. They also found highest MVD in Warthin tumor, interestingly. Shi *et al*., mentioned that MVD in MEC was associated with clinical stage, histologic grade and tumor recurrence. They stated that high-grade MECs have higher MVD compared to low grade tumors.[8,15] They also found that MVD in MEC of minor salivary glands is significantly higher than in tumors of major salivary glands. In contrast, Gleber-Netto *et al*.,[11] stated that although angiogenesis is important in pathogenesis of MEC, it could not predict tumor behavior. In addition,

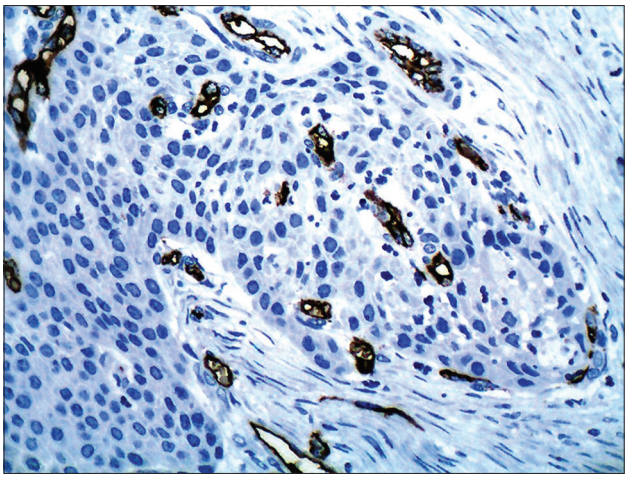

Figure 2: Immunohistochemical expression of CD34 in MEC (IHC stain, ×400). MEC = Mucoepidermoid carcinoma

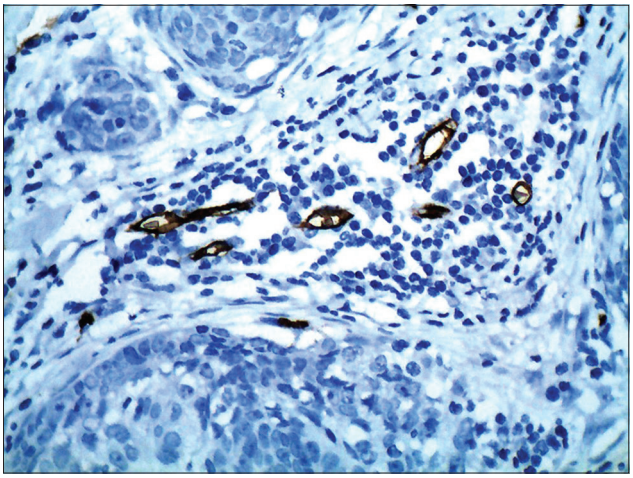

Figure 4: Immunohistochemical expression of CD34 in SDC (IHC stain, ×400). SDC = Salivary duct carcinoma

the size of vessels in MEC was smaller than other groups in our research. Andishe *et al*., mentioned that cell aggregates in MEC were smaller and had small blood vessels around them. They explained that the difference in shape, size and distribution of vessels in salivary carcinomas may be related to the characteristics of myoepithelial cells. These cells are able to produce large amounts of extracellular matrix that contains angiogenic inhibitors and is devoid of vessels.[3]

In the present study, vessels of AdCC frequently formed a rim of capillaries around tumor nests. This feature has also been found in MEC.[4] It seems that large blood vessels in AdCC are needed to compensate for decreased angiogenesis. Zhang and Peng stated that the microvessels around the solid tumor nests of AdCC were much denser than that around cribriform and tubular nests. MVD in AdCC had also significant correlation with tumor size, clinical stage, vascular invasion, recurrence, perineural invasion and distant metastasis.[12] However, a study by Costa *et al*.,[4] did not reveal any significant increase in MVD in metastatic group.

Angiogenesis has also been investigated in other salivary gland tumors.[18-20] Epithelial-myoepithelial carcinoma has lower MVD than MEC.[4] Luukkaa *et al*.,[19] stated that large vessel size, vessel irregularity and lower intensity of CD34 positivity may indicate unfavorable prognosis in acinic cell carcinoma (ACC). However, these results are in contrast to the study by Mărgăritescu *et al*.[20] They reported that there was an active angiogenesis in ACC and the highest MVD was in solid variant. Study on MVD in SDC has not been reported so far. Despite the fact that SDC has no myoepithelial cells, MVD was lower than other groups in our research. In addition, angiogenesis has not been investigated in relation to age and sex in previous studies. MVD in our series of cases was correlated with sex and was higher in female patients. Therefore, a hormonal influence seems a probable reason for such presentation. In addition, no association was found between age and MVD.

CONCLUSION

In conclusion, salivary gland carcinomas regardless of the pattern and cell type have significantly higher MVD than benign PA. This higher angiogenic activity may be associated with metabolic demand of malignant cells. In addition, MVD is correlated with cellular density of PA probably because cell-rich areas need more oxygen. Moreover, MVD is lower in carcinomas with myoepithelial differentiation. Therefore, antiangiogenic therapy should be preferentially considered in the treatment of highly vascular neoplasms without myoepithelial differentiation.

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