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Original Research

Expression of CD105 in tumor angiogenesis a comparative study (ameloblastoma, keratocystic odontogenic tumor and dentigerous cyst)

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Abstract:
Background: Demonstrate the expression of CD105 (angiogenetic marker) in ameloblastoma (AM), keratocystic odontogenic tumor (KCOT) and dentigerous cyst (DC).
Material and Methods: Assessment of microvessel density (MVD) in 70 cases, including 20 KCOT, 20 DC and 20 solid AMs. Assessment of MVD should be done as the mean number of microvessels per high-power-field.
Results: AM and KCOT demonstrated a higher mean value of 7.98 (±2.70) and 6.25 (±2.88) respectively while DC demonstrated a lower mean of 3.75 (±1.42). There was no statistically significant difference between AM and KCOT ($P > 0.05$). The difference between AM and DC; and between KCOT and DC were statistically significant ($P < 0.05$).
Conclusion: The present study suggested that angiogenesis may be one of the mechanisms possibly contributing to the different biological behaviors of KCOT, DCs and solid AMs.

Key Words: Ameloblastoma, angiogenesis, dentigerous cyst, keratocystic odontogenic tumor

Introduction

Keratocystic odontogenic tumor (KCOT), is regarded as a developmental abnormality and is generally known for its aggressive nature and high recurrence rate, especially in comparison with other developmental odontogenic cysts. In addition to a distinctive biological behavior, the expression of various proliferation markers in the epithelial lining and mutations in p53 and patched genes (tumor suppressor genes) have led several investigators to consider KCOT as a benign cystic neoplasm.[1]

Ameloblastoma (AM) is a locally invasive benign epithelial odontogenic tumor that may arise from rests of dental lamina, enamel organ rests, cell rests, the epithelial lining of an odontogenic cyst or from the basal cell layer of oral mucosa.[1] It is considered as the most common benign epithelial odontogenic tumors, which are locally invasive tumor that has a tendency to grow continually and invade the surrounding tissues.[2]

The dentigerous cyst (DCs) is the common odontogenic cysts, which is of developmental in origin and is suggested to be caused by accumulation of exudate between the tooth crown and reduced enamel epithelium, which demonstrates an indolent behavior and seldom recurs following removal.[1,2]

The KCOTs, similar to DCs, appear as cystic lesions, but their invasiveness and destructive growth are comparable to AM. It has been suggested that unknown factors integrated in the epithelium or fibrous capsule of KCOTs may be responsible for their specific biological behavior .[1]

The connective tissue stroma has an essential role in preservation of epithelial tissues and minor alterations in the epithelium, which are followed by corresponding changes in the stroma, such as angiogenesis.[1] Blood supply is an essential factor for the growth of odontogenic epithelium. Because there is no vascular system in the epithelium, apoptosis will happen if the connective tissue does not provide the necessary blood supply. Myofibroblasts (MF), blood vessels, and inflammatory cells are present in the tumoral stroma. The invasion and metastasis of tumors requires the presence of MF and blood vessels, which increases during tumorigenesis.[3] Neoplastic tissues require oxygen and nutrients for survival and growth; therefore, they induce the formation of new blood vessels through angiogenesis or neovascularization.[1,4]

Angiogenesis is the growth of new blood vessels from pre-existing vessels, which is a physiological process, like cancer it is a complex multi-stage process, including degradation of extracellular matrix, proliferation and migration of endothelial

cells, capillary differentiation and anastomosis .[3] There is a large spectrum of physiological and pathological processes in which angiogenesis occurs, ranging from tissue hypertrophy, wound healing, and inflammation to tumours.[2]

Folkman showed preliminary evidence that tumors could not enlarge beyond 1-2 mm diameter without recruiting new capillary blood vessels (microvessels).[5] An ideal marker for angiogenesis should detect the newborn vessel for its quality as well as its quantity. Tumor angiogenesis is regulated by various molecules such as vascular endothelial growth factor (VEGF), CD31, CD34, Von Willebrand factor, and CD105, also called as endoglin. Legan stated that pan-endothelial markers (CD31, CD34, Factor VIII) and CD105 are differentially expressed in angiogenic and normal vessel endothelial cells.[2,6]

CD105 is a component of the receptor complex of transforming growth factor (TGF-b), which is disulfide glycoprotein that modulates angiogenesis by the regulation of different cellular functions. The expression of CD105 predominantly restricted to vascular endothelial cells on which majority levels of proteins are detectable, whereas it is scarcely expressed on lymphatic endothelial cells. CD105 expression is a prominent feature of newly formed blood vessels, but minimally expressed in pre-existing tumor vessels. CD105 have shown greater specificity for the tumor vasculature than pan endothelial markers.[2,7,8]

In odontogenic cysts and tumors, the connective tissue stroma has an essential role in the preservation of epithelial tissues and minor alterations in the epithelium are followed by corresponding changes in the stroma, such as angiogenesis.[3,9]

In the present study, we intend to evaluate and compare microvessel density (MVD) in KCOTs, DCs and AM using monoclonal antibody against CD105.

Material and Methods

A total of 70 archival specimens which were formalin fixed, processed and embedded tissues of prediagnosed 20 cases of SA (Group A), 20 cases of KCOT (Group B), 20 cases of DC (Group C) and 10 cases of pyogenic granuloma (control group) were retrieved from the Department of Oral Pathology and Microbiology, Government Dental College and Research Institute, Bangalore by random sampling. All the archival specimens that had been previously diagnosed as AM (only solid multicystic variant, no unicystic variant); KCOT and DC with no history of malignancy either orally and systemically were included in the study group. The study was approved by institutional review board of Government Dental College and Research Institute, Bangalore.

The study groups were segregated on the basis of histopathological classification. The cases were then stained with CD105 antibody using the methods of immunohistochemistry. Totally 10 cases of pyogenic granuloma were used as a positive control. The presence and frequency of microvessels were then assessed by observing the slides under the light microscope.

Histomorphometric evaluation of stained sections

Vascular endothelial cells which were stained by CD105, clearly identified by their brown cytoplasmic staining. Based on the criteria of Weidner *et al*. 1991, highlighted endothelial cell or a cell cluster clearly separate from adjacent microvessels and other connective tissue elements was regarded as a distinct countable microvessel. A lumen was not required, nor was the presence of red blood cells. Single cell sprouts were included in the counts. MVD quantification was carried out using an BH2 microscope Olympus according to the method suggested by Weidner *et al*. (1993).[10,11] 10 areas with the highest amount of vascularization (hotspots) were selected under a magnification of ×100 (×10 ocular and ×10 objective). Microvessels were counted in each of the 10 fields at ×400 magnifications (×10 ocular and ×40 objectives) and the mean density was reported. The blood vessels density was recorded as mean ± standard deviation.

MVD was assessed as the mean number of microvessels per high power field. All slides were simultaneously evaluated by two observers using a double-headed microscope, and both had to agree on each of the individual microvessel before being included in the count. The field size for 400 magnification (40 objectives and 10 ocular) was approximately 0.18 mm^2.

For cystic lesions, the fields were selected immediately beneath the cystic epithelium lining and for solid AM it was selected immediately adjacent to the tumoral islands/nests/cords.

Statistical analysis

Statistical analysis was performed using Kruskal–Wallis and Mann–Whitney tests. Bonferroni method was employed for the adjustment of *P* value in multiple comparisons and $P < 0.05$ was considered as significant. The statistical package for social sciences (SPSS 14) software was used for computations.

Results

In AM, islands of odontogenic epithelium were surrounded by layers of CD105 positive vessels (Figure 1). Endothelial cells showing fine CD105 positivity were located beneath and parallel to the basement membrane of the odontogenic epithelium of the cystic lesions (Figures 2 and 3). Whereas, the expression of CD105 is diffuse in pyogenic granuloma, which is taken as control (Figure 4).

AM and KCOT demonstrated a higher mean value of 7.98 (±2.70) and 6.25 (±2.88) respectively while DC demonstrated a lower mean of 3.75 (±1.42). There was no statistically significant difference between AM and KCOT ($P > 0.05$). The difference between AM and DC; and between KCOT and DC was

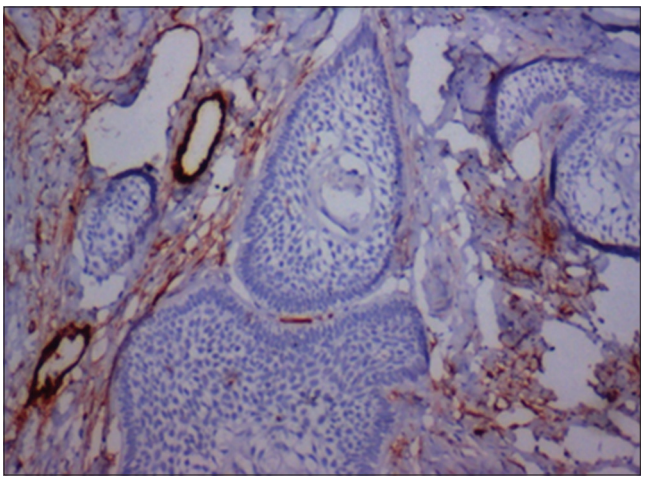

Figure 1: CD105 positive microvessels adjacent to the epithelial islands in ameloblastoma (original magnification ×200).

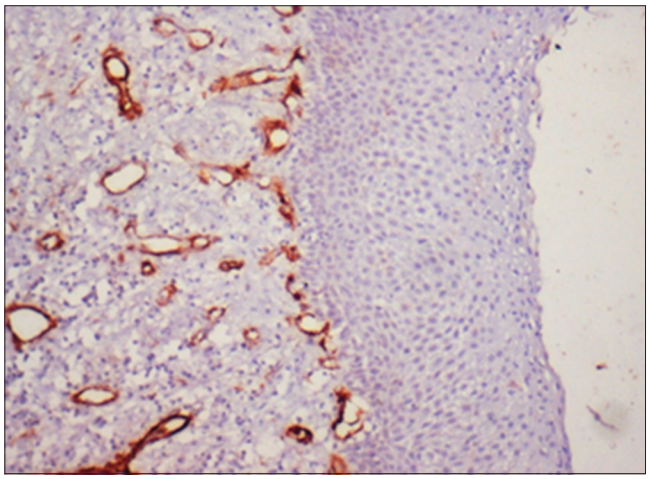

Figure 2: Microvessels beneath the epithelium in keratocystic odontogenic tumor expressed by CD105 antibody (×200).

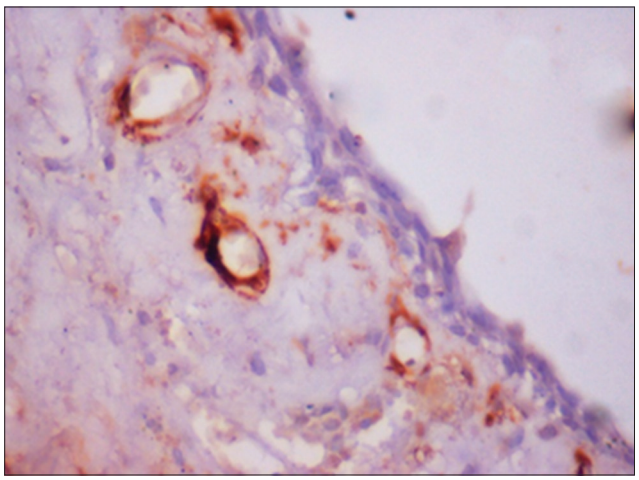

Figure 3: Microvessels in the connective tissue wall of dentigerous cyst (×400).

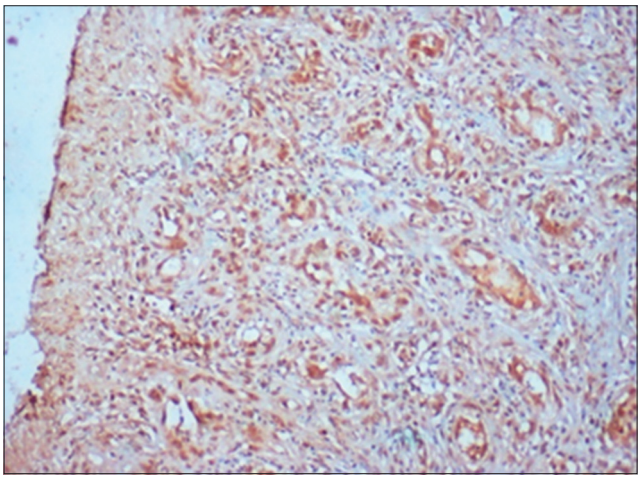

Figure 4: Microvessel density in the pyogenic granuloma expressed by CD105 antibody (×100).

statistically significant ($P < 0.05$). When AM, KCOT and DC were compared with pyogenic granuloma, the mean number of microvessels per field in PG was statistically significant from that of AM, KCOT and DC ($P < 0.05$) (Table 1).

Table 1: Mean number of CD105 positive microvessels in AM, KCOT, DC and PG.

Lesion	Mean MVD per field (±SD)
AM	7.98±2.70
KCOT	6.25±2.88
DC	3.75±1.42
PG (control group)	15.02±0.64

AM: Ameloblastoma, KCOT: Keratocystic odontogenic tumor, DC: Dentigerous cyst, PG: Pyogenic granuloma, MVD: Microvessel density

Discussion

Odontogenesis is controlled by interactions between the epithelial and mesenchymal components of developing dental tissues. Regarding the fact that odontogenic cysts and tumors arise from tissue remains of odontogenesis, these interactions have been considered to play an important role in the tumorigenesis of odontogenic lesions. The connective tissue stroma has an essential role in preservation of epithelial tissues and minor alterations in the epithelium are followed by corresponding changes in the stroma, such as angiogenesis.[2] In embryogenesis vasculogenesis is one of the earliest events. Mesodermal cells differentiate into hemangioblasts, progenitors of both endothelial cells and hematopoietic giving rise to blood vessels in the early part of embryonic development. After further differentiation, hemangioblasts produce angioblasts, aggregation of which results in the formation of blood islands. Blood islands, which are fused results in appearance of the primary blood vascular plexus consisting of fine capillaries formed by endothelial cells.[12]

An ideal marker for angiogenesis should detect the newborn vessel for its quality as well as its quantity. Tumor angiogenesis is regulated by various molecules such as VEGF, CD31, CD34, Von Willebrand factor, and CD105, also called as endoglin. Legan stated that pan-endothelial markers (CD31, CD34, Factor VIII) and CD105 are differentially expressed in

angiogenic and normal vessel endothelial cells. The expression of CD105 is predominantly featured on newly formed tumor vessels but minimally expressed in quiescent pre-existing tumor vessels. CD105 have shown greater specificity for the tumor vasculature than pan endothelial markers.[3,4,13]

The AM is characterized by local invasiveness and recurrence. Various stromal factors, like growth and angiogenic factors, extracellular matrix components and proteinases play an important role for the invasion, growth and progression of tumors. Several authors have shown that the elevated expression of matrix metalloproteinases 2 (MMP 2), MMP 9, TGF-b, fibronectin, tenascin, stromal MF is related to the invasive behavior of AM. This could indicate an increased metabolic activity in the connective tissue of AM. These findings are well supported by significantly increased MVD in AM compared with KCOT and DC in our study.[2,14,15]

The KCOTs, similar to DCs, appear as cystic lesions, but their invasiveness and destructive growth are comparable to AM. It has been suggested that unknown factors integrated in the epithelium or fibrous capsule of KCOTs may be responsible for their specific biological behavior. Microscopic epithelial and stromal features have been the center of attention in several investigations comparing KCOTs, DCs and AMs.

In our study, there is a significant difference in angiogenesis between KCOTs, DCs and solid AMs. The highest mean MVD in the studied lesions was observed in AM, followed by KCOT and DC. On the other hand, when treatment is based on enucleation, the recurrence rates of these lesions have been reported as 50-90% in solid AMs, 17-56% in KCOTs, 30.5% in UAs and usually none in DCs. (Blanas *et al*., 2000; Lau and Samman, 2006; Neville *et al*., 2008). Therefore, it seems that angiogenesis may be associated with the different biological behaviors of these lesions and at least to some degree can reflect their clinical features. This suggests that the angiogenesis have an important role in tumor progression and invasiveness of AM. Increased angiogenesis in KCOT may contribute to the locally aggressive biological behavior.[4]

Assessment of tumor angiogenesis may prove very valuable in predicting response to anti-angiogenic therapeutic strategies and also provide an objective assessment of post therapeutic response particularly in recurrent cases of AM and KCOT.

Conclusion

The findings of the present study indicated that the aggressive nature and locally invasive growth pattern of AM can be positively correlated to the CD105 expressed MVD. Though KCOTs appear as cystic lesions, but their invasiveness and destructive growth are comparable to AM. Thus our results do support the previous studies. Further investigation and studies in this field with a note on the treatment prospective will help in establishing the role of angiogenesis in the biological behavior of odontogenic lesions. Newer modalities of treatment like anti-angiogenic therapeutic agents can be used prior to surgical removal of these lesions in order to reduce the tumor size and postoperative morbidity because curative surgery of particularly aggressive odontogenic lesions could occasionally result in significant functional, esthetic and psychological damage.

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