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Original Research

Evaluation and Comparison of Vascular Endothelial Growth Factor Expression between Ameloblastoma and Keratocystic Odontogenic Tumor

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Abstract:

Background: Odontogenic keratocyst (OKC) is a developmental odontogenic cyst with an aggressive clinical behavior suggesting a change in its terminology from a cyst to a tumor and has now been renamed as keratocystic odontogenic tumor (KCOT). The purpose of this study was to assess and compare angiogenesis in ameloblastoma and OKC.

Materials and Methods: Angiogenesis was assessed by studying the immunohistochemical expression of vascular endothelial growth factor (VEGF). The study samples included 15 ameloblastomas and 15 KCOTs. The immunoreactivity was statistically evaluated using Mann–Whitney *U*-test.

Results: VEGF expression was higher in ameloblastoma than KCOTs. However, a non-significant difference of VEGF expression was noted between ameloblastoma and KCOTs ($P = 0.345$).

Conclusion: The results suggest that tumor angiogenesis may play a significant role in aggressive biologic behavior of KCOT. Thus, angiogenesis could be a potent target for developing anatiangiogenic therapeutic strategies.

Key Words: Ameloblastoma, angiogenesis, keratocystic odontogenic tumor, vascular endothelial growth factor

Introduction

Angiogenesis or neovascularization is defined as the growth and development of new capillaries from pre-existing blood vessels, and is essential for tumors to grow beyond a minimal size.[1] This could occur either by branching and extension of adjacent blood vessels or by recruitment of endothelial progenitor cells from the bone marrow. Angiogenesis plays an essential part in a variety of physiological and pathological processes, including embryogenesis, wound healing, inflammation and tumor progression.[2] This process depends on tightly controlled endothelial cell proliferation, migration, and differentiation, and is controlled by a finely tuned balance of pro and anti-angiogenic factors and their receptors, most of which are polypeptide growth factors. Although the regulatory mechanisms in angiogenesis are not fully understood, evidence suggests that a cascade of events occur, and that intervention at one of the steps is sufficient to prevent or promote revascularization. Considerable evidence exists to indicate that solid tumor growth is dependent on angiogenesis, a hypothesis first proposed by Folkman in 1971.[3]

It is a well-accepted paradigm that tumors recruit new blood vessels from the existing circulation by secreting growth factors from the tumor cells. This has been shown in many human malignancies, especially lung, prostate and breast cancers. Tumor cells secrete angiogenesis-stimulating proteins, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), thymidine phosphorylase, basic FGF, hepatocyte binding growth factor, and others, whose specific receptors are found on the endothelial cells. In addition, tumor-associated macrophages, lymphocytes, and fibroblasts of the tumor stroma can also produce angiogenic factors, promoting tumor growth and spread. Though many growth factors exhibit angiogenic activity, most evidence points to a special role for VEGF. VEGF also known as vascular permeability factor, is a heparin-binding, dimeric glycoprotein with a selective mitogenic effect on vascular endothelial cells, and is the most potent and specific agent among the many molecules capable of initiating angiogenesis.[4] Actions of VEGF include enhancement of both angiogenesis and vascular permeability. Furthermore, expression levels of VEGF correlate with tumor status and also a useful biomarker for the prediction of response to therapy.[5]

A tumor may persist in a diffusion-limited state, usually not more than 2 mm in diameter, with cell proliferation balanced by cell death, for many months or years. It rarely causes significant damage in this dormant phase, and often goes undetected. A tumor may, however, emerge from dormancy by inducing the growth of new blood vessels (angiogenesis). This process

allows the tumor to progress from the avascular (lacking blood vessels) to the vascular (possessing a blood supply) state. There are a large number of pro-angiogenic and anti-angiogenic factors, some of which are produced by the tumor and some of which are produced by host cells in response to the tumor. It is a shifting of the balance from the anti- to the pro-angiogenic factors (the so-called "angiogenic switch") that causes the transition from the dormant to the angiogenic phase.

Odontogenic tumors and cysts comprise a major portion of the pathology occurring in the orofacial region. The odontogenic epithelium, which is responsible for tooth development can also give rise to these tumors and cysts.[6] Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally invasive behavior with a high tendency to recur. On the other hand, odontogenic keratocyst (OKC) is a developmental epithelial odontogenic cyst most commonly occurring in the jaws, which could arise from the dental lamina or its remnants or extensions of basal cells from the overlying oral epithelium (basal cell off shoots). There has been a great deal of interest in the OKC since it became apparent that it may grow to a larger size before it manifests clinically (aggressive clinical behavior) and that unlike other jaw cysts, it has a particular tendency to recur following surgical treatment (high recurrence rate). There has been considerable evidence that has accumulated over the years that the OKC may be a benign cystic neoplasm, which has gone to the extent of a change in terminology from OKC to "keratinizing cystic odontogenic tumor" (KCOT).[7,8]

In the current study, we have compared the expression of VEGF between ameloblastoma and KCOT using immunohistochemistry in an attempt to analyze the property of angiogenesis in KCOT.

Materials and Methods

The study protocol was approved by SRM Institutional Ethical Committee. The samples diagnosed as multicystic ameloblastoma and solitary, sporadic KCOT was selected for the study. Clinical information regarding age, sex and location of the lesion was recorded. Formalin-fixed paraffin-embedded biopsy material of ameloblastoma and sporadic KCOT were retrospectively studied, the diagnosis in each case having been made on the basis of clinical, radiographic and histologic findings. A total number of 30 cases were selected, out of which 15 were cases of ameloblastoma (plexiform type) and 15 cases of KCOT. Hematoxylin and eosin sections of all cases were repeated to reconfirm the sign out diagnosis.

Immunohistochemistry

4 μm-thick sections were mounted on electrostatically charged glass slides. Sections were deparaffinized in xylene, hydrated through graded alcohol, and washed with tap water. Antigen retrieval was performed by immersing the slides in citrate buffer, pH 6.1 and run for two cycles in the biogenex antigen retrieval system at 97°C for 8 min each and then quenched in running tap water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 3 min. Sections were treated with power block for 15 min. Sections were incubated with rabbit antihuman VEGF polyclonal antibody for 1 h. Following this the sections were treated with super enhancer and poly horse radish peroxidase reagent for 30 min each. Each of these incubation procedures was preceded by 10-min rinse with phosphate buffer except before the addition of primary antibody. Reaction products were visualized using freshly prepared diaminobenzidine solution and counterstained with Harris hematoxylin. Sections of squamous cell carcinoma served as positive control. Negative controls were obtained by omitting the primary antibody and were confirmed to be unstained.

Evaluation of immunostaining

Immunoreactivity was evaluated by two independent observers. Immunohistochemical reactivity for VEGF was assessed according to the presence of positive staining and intensity of staining. Based on this, the immunoreactivity of VEGF was classified into four groups: (−) Negative (none of the epithelial or neoplastic cells staining), (±) weakly (<10% of epithelial or neoplastic cells) positive, (+) moderately (10-50% of epithelial or neoplastic cells) positive, and (+ +) strongly (more than 50% of epithelial or neoplastic cells) positive. Statistical evaluation was performed with Mann–Whitney *U*-test. Statistical analysis was performed using SPSS software and $P < 0.05$ was considered to indicate statistical significance.

Results

Demographic data have been summarized in Table 1. Immunoreactivity for VEGF was mainly seen in the cytoplasm of neoplastic epithelial cells in cases of ameloblastoma and in the epithelial lining of KCOT. Endothelial cells and inflammatory cells were also reactive for VEGF. Strong reactivity for VEGF was seen in the peripheral columnar cells in ameloblastoma and in cases of KCOT all the epithelial cell layers were positive

Table 1: Demographics and vascular endothelial growth factor expression data.

Category	Ameloblastoma	KCOT
Subjects (*n*)	15	15
Age range (years)	21-65	25-53
Sex		
Male	11	13
Female	4	2
VEGF expression (%)		
Negative	0 (0)	1 (6.67)
Weak	1 (6.67)	3 (20)
Moderate	5 (33.3)	4 (26.67)
Strong	9 (60)	7 (46.67)

The intensity of VEGF expression in ameloblastoma and KCOT is represented.
Mann–Whitney test was used to compare the significance between the groups.
A $P<0.05$ is considered statistically significant, VEGF: Vascular endothelial growth factor, KCOT: Keratocystic odontogenic tumor

with the exception of the parakeratin layer. VEGF expression in ameloblastoma and KCOT is summarized in Table 1. Strong expression was seen in 60% of ameloblastoma (Figures 1 and 2) and in 46.67% of KCOT (Figure 3), while weak expression was seen in 6.67% of ameloblastoma and 20% of KCOT. VEGF expression was stronger in ameloblastoma when compared to KCOT, but there was no statistically significant difference between ameloblastoma and KCOT (P = 0.345, Mann–Whitney).

Discussion

The term "OKC" was first introduced by Philipsen over 50 years ago to describe a group of odontogenic cysts which showed a characteristic histological appearance. As compared with other types of odontogenic cysts, KCOTs appear to have an intrinsically higher growth potential.[9-11] A propensity to recur following surgical treatment and the potential risk of neoplastic change place KCOTs in a unique position within the spectrum of odontogenic lesions and has been suggested that KCOTs should be regarded as benign neoplasms.

Numerous investigations have pointed this distinctive behavior of KCOT toward the nature of its epithelium. The epithelium of the KCOT has been reported to show a higher rate of proliferation than other cyst types indicated by strong expression of p53, proliferating cell nuclear antigen and Ki-67 in KCOT.[12-14]

However, very little attention has been paid to the connective tissue of odontogenic cysts and stroma of odontogenic tumors. Browne (1975) was one of the first to suggest that the connective tissue wall may have a significant role in the pathogenesis of KCOT. Interaction between the fibrous capsule and epithelium of this cyst has also been confirmed in other studies. A number of connective tissue elements such as tenascin and fibronectin; RANK, RANKL and osteoprotegerin; laminins and collagen 4 have also been studied in this lesion.[15-18]

Li *et al*. studied the expression of epidermal growth factor receptor (EGFR) in epithelial cells of odontogenic cysts, including KCOT and determined that high levels of EGFR expression in KCOT supported the view that they have an intrinsic growth potential. It was reasoned that KCOT epithelium is derived from dental lamina remnants and this may reflect the potential for epithelial-mesenchymal interactions and growth factor/receptor modulations. Also transforming growth factor alpha, EGF, EGFR expression suggested involvement of growth factors in the pathogenesis of KCOT, via autocrine and paracrine mechanism.[19]

Angiogenesis is one of the best known stromal factors participating in tumor progression and has been extensively investigated in various lesions.[20-22] Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels and, like cancer, is a complex multi-stage process including degradation of extracellular

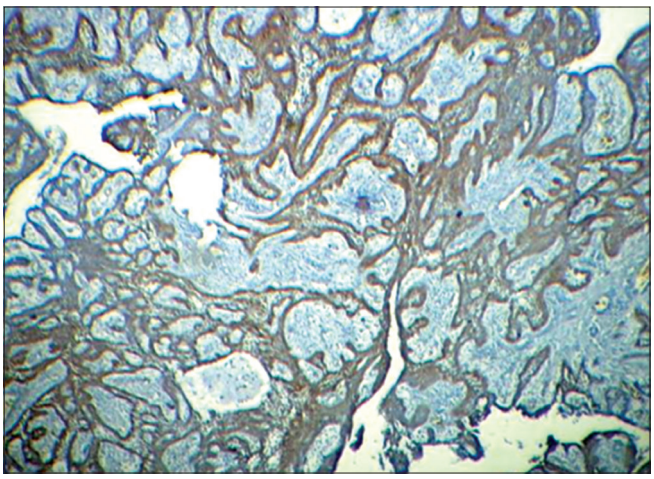

Figure 1: Immunohistochemical staining with vascular endothelial growth factor in ameloblastoma (×10). Staining of tumor cells and also blood vessels adjacent to the odontogenic epithelium.

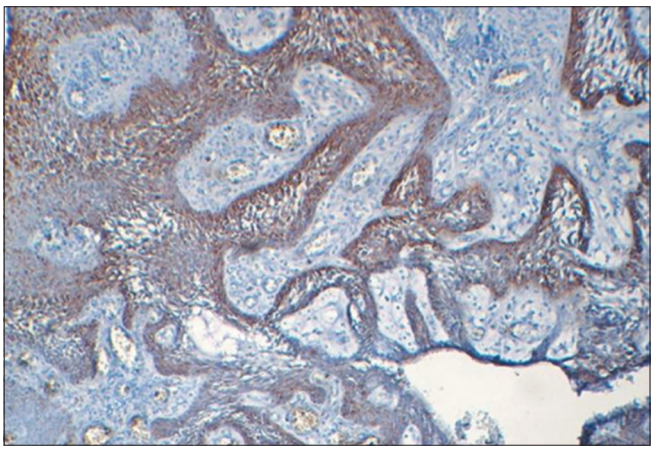

Figure 2: Immunohistochemical staining with vascular endothelial growth factor in ameloblastoma (×40). Staining of tumor cells.

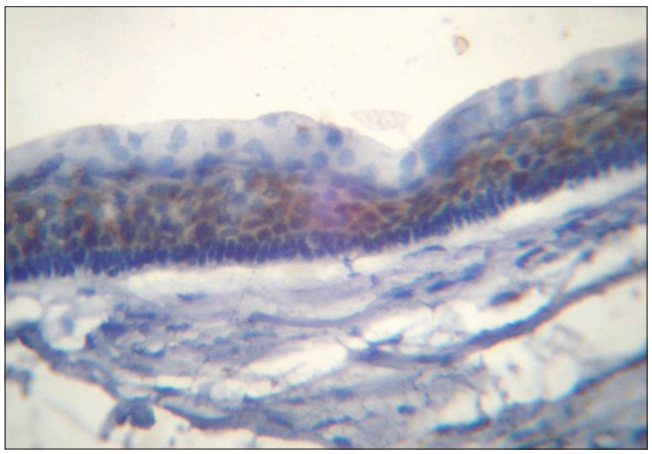

Figure 3: Immunohistochemical staining with vascular endothelial growth factor in keratocystic odontogenic tumor (×10). Staining of tumor cells except the parakeratin layer.

matrix, proliferation and migration of endothelial cells, capillary differentiation and anastomosis.[21] Among the very many angiogenic factors, VEGF has a selective mitogenic effect on the vascular endothelium and is the most potent agent among the many molecules capable of initiating the angiogenesis.[23,24] Also the expression levels of VEGF correlate with tumor status and prognosis.

All solid tumors such as ameloblastoma are composed of the neoplastic cell compartment and the stromal component. The stroma is composed of new blood vessels, inflammatory cells, connective tissues, and a fibrin-gel matrix. Tumor fibrin originates from the extravasation and extravascular clotting of plasma fibrinogen by tumor cell prothrombinase and is dependent on increased vascular permeability. Hyperpermeability of capillaries and the macromolecular transvascular transport are facilitated by VEGF produced by tumor cells.[25,26] However, the mechanism by which VEGF modulates the changes leading to an increase in capillary permeability is unknown.

Ultrastructural findings on blood vessel architecture including volume and surface densities of odontogenic cysts (KCOT and dentigerous cyst) were compared based on a premise that angiogenesis is a feature of a benign neoplasm and evidence of this in KCOT may account for its characteristic behavior. Although there was no significant difference in the overall vascularity, there were differences in the ultrastructural findings between the two cysts. KCOT connective tissue wall showed fenestrated capillaries and degeneration of the endothelial lining with associated thrombosis. These features were not seen in a dentigerous cyst. It was also speculated that an increase in the number of platelets seen in the capillary thrombi of the KCOT may stimulate epithelial cell growth and viability through unspecified growth factors.[27]

The present study showed that VEGF is expressed strongly in ameloblastomas than in KCOT. Although ameloblastomas showed stronger expression of VEGF than KCOT, the results were statistically not significant, and there was a strong expression of VEGF in KCOT as well. This finding supports the fact that KCOT has an aggressive behavior like tumors though being a cystic lesion of odontogenic origin.

Seifi *et al*. studied the microvessel densities (MVD) in odontogenic lesions and suggested that intratumoral MVD has a prominent role in growth and clinical behavior of odontogenic lesions and hypothesized that both ameloblastoma and KCOT share similarities regarding angiogenesis accounting for its aggressive behavior.[28] Furthermore, KCOT exhibited increase in the total vascular area and mean vascular area in comparison with other development cysts and suggested that this angiogenesis with dilated and tortuous blood vessels in KCOT, which may be related to the increase in tissue metabolism, nutrition requirement of the proliferating epithelium and associated lymphatic drainage.[29]

Elevated expressions of VEGF was detected in benign and malignant ameloblastomas, suggesting that VEGF production by odontogenic epithelial cells was up regulated in association with neoplastic changes, malignant transformation or both. In addition, it was also noted that VEGF expression was markedly decreased in keratinizing, and granular cells and also VEGF may act differently according to the architecture of tumor tissues. This could partly account for the difference in the levels of expression of VEGF among ameloblastoma and KCOT in our present study.[21]

In summary, the results suggest that VEGF expression was strongly expressed in ameloblastoma than KCOT. However, the immunoreactivity between these two lesions does not show a significant difference, suggesting that angiogenesis in part could explain the aggressive behavior of these cystic neoplasms. These results suggest that VEGF is an important mediator of tumor angiogenesis and upregulation of VEGF might be associated with tumorigenesis. The results and hypothesis achieved from the study, proved to be consistent, and augments the already existing hypothesis regarding the concepts of its aggressive behavior.

Conclusion

Nevertheless, angiogenesis is a complex process that involves the interaction between pro and anti-angiogenic factors and several agents directly or indirectly influence this event. Hence, it is logical that other proteins associated with angiogenesis are concurrently evaluated in selected cases to arrive at a consensus. The role of VEGF in the pathogenesis of odontogenic cysts and tumors should be further evaluated using more sophisticated methods and additional cases, as angiogenesis could be a potential target for the therapeutic management of these lesions.

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References

1. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3(2):65-71.
2. Carmeliet P, Collen D. Molecular analysis of blood vessel formation and disease. Am J Physiol 1997;273(5 Pt 2):H2091-104.
3. Folkman J. Tumor angiogenesis: Therapeutic implications. N Engl J Med 1971;285(21):1182-6.
4. Bouck N, Stellmach V, Hsu SC. How tumors become angiogenic. Adv Cancer Res 1996;69:135-74.
5. Srivastava VK, Gara RK, Rastogi N, Mishra DP, Ahmed MK, Gupta S, *et al*. Serum vascular endothelial growth factor-A

(VEGF-A) as a biomarker in squamous cell carcinoma of head and neck patients undergoing chemoradiotherapy. Asian Pac J Cancer Prev 2014;15(7):3261-5.
6. Shear M. Developmental odontogenic cysts. An update. J Oral Pathol Med 1994;23(1):1-11.
7. Agaram NP, Collins BM, Barnes L, Lomago D, Aldeeb D, Swalsky P, *et al*. Molecular analysis to demonstrate that odontogenic keratocysts are neoplastic. Arch Pathol Lab Med 2004;128(3):313-7.
8. Shear M. The aggressive nature of the odontogenic keratocyst: Is it a benign cystic neoplasm? Part 2. Proliferation and genetic studies. Oral Oncol 2002;38(4):323-31.
9. Li TJ, Browne RM, Matthews JB. Expression of epidermal growth factor receptors by odontogenic jaw cysts. Virchows Arch A Pathol Anat Histopathol 1993;423(2):137-44.
10. Li TJ, Browne RM, Lawrence GM, Matthews JB. Epithelial cell proliferation in the odontogenic keratocyst. Histochem J 1994a;26:604A.
11. Li TJ, Browne RM, Matthews JB. Quantification of PCNA+ cells within odontogenic jaw cyst epithelium. J Oral Pathol Med 1994;23(4):184-9.
12. Piattelli A, Fioroni M, Santinelli A, Rubini C. Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. Oral Oncol 1998;34(5):408-12.
13. Thosaporn W, Iamaroon A, Pongsiriwet S, Ng KH. A comparative study of epithelial cell proliferation between the odontogenic keratocyst, orthokeratinized odontogenic cyst, dentigerous cyst, and ameloblastoma. Oral Dis 2004;10(1):22-6.
14. Tsuneki M, Cheng J, Maruyama S, Ida-Yonemochi H, Nakajima M, Saku T. Perlecan-rich epithelial linings as a background of proliferative potentials of keratocystic odontogenic tumor. J Oral Pathol Med 2008;37(5):287-93.
15. Vedtofte P, Holmstrup P, Dabelsteen E. Human odontogenic keratocyst transplants in nude mice. Scand J Dent Res 1982;90(4):306-14.
16. Amorim RF, Godoy GP, Galvão HC, Souza LB, Freitas RA. Immunohistochemical assessment of extracellular matrix components in syndrome and non-syndrome odontogenic keratocysts. Oral Dis 2004;10(5):265-70.
17. da Silva TA, Batista AC, Mendonça EF, Leles CR, Fukada S, Cunha FQ. Comparative expression of RANK, RANKL, and OPG in keratocystic odontogenic tumors, ameloblastomas, and dentigerous cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105(3):333-41.
18. Poomsawat S, Punyasingh J, Weerapradist W. Expression of basement membrane components in odontogenic cysts. Oral Dis 2006;12(3):290-6.
19. Li TJ. The odontogenic keratocyst: A cyst, or a cystic neoplasm? J Dent Res 2011;90(2):133-42.
20. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 1995;147(1):9-19.
21. Kumamoto H, Ohki K, Ooya K. Association between vascular endothelial growth factor (VEGF) expression and tumor angiogenesis in ameloblastomas. J Oral Pathol Med 2002;31(1):28-34.
22. Mahabeleshwar GH, Byzova TV. Angiogenesis in melanoma. Semin Oncol 2007;34(6):555-65.
23. Toi M, Taniguchi T, Yamamoto Y, Kurisaki T, Suzuki H, Tominaga T. Clinical significance of the determination of angiogenic factors. Eur J Cancer 1996;32A(14):2513-9.
24. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18(1):4-25.
25. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, *et al*. Regulation of transport pathways in tumor vessels: Role of tumor type and microenvironment. Proc Natl Acad Sci U S A 1998;95(8):4607-12.
26. Monsky WL, Fukumura D, Gohongi T, Ancukiewcz M, Weich HA, Torchilin VP, *et al*. Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res 1999;59(16):4129-35.
27. Jordan RC. Histology and ultrastructural features of the odontogenic keratocyst. Oral Maxillofac Surg Clin North Am 2003;15(3):325-33.
28. Seifi S, Shafaie S, Ghadiri S. Microvessel density in follicular cysts, keratocystic odontogenic tumours and ameloblastomas. Asian Pac J Cancer Prev 2011;12(2):351-6.
29. Gadbail AR, Hande A, Chaudhary M, Nikam A, Gawande M, Patil S, *et al*. Tumor angiogenesis in keratocystic odontogenic tumor assessed by using CD-105 antigen. J Oral Pathol Med 2011;40(3):263-9.