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ORIGINAL ARTICLE

Comparison of angiogenesis in keratocystic odontogenic tumours, dentigerous cysts and ameloblastomas

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OBJECTIVE: The aim of the present study was to evaluate and compare angiogenesis in keratocystic odontogenic tumours, dentigerous cysts (DCs) and ameloblasomas using monoclonal antibody against CD34.

MATERIALS AND METHODS: Microvessel density was assessed in a total of 53 cases including 20 keratocystic odontogenic tumours, 13 DCs and 20 ameloblastomas (14 solid and six unicystic variants). Microvessel density was expressed as the mean number of microvessels per high-power-field.

RESULTS: Statistically significant differences in mean microvessel density were observed between keratocystic odontogenic tumours, DCs and solid ameloblastomas ($P < 0.001$). Mean microvessel density was significantly higher in solid ameloblastomas compared with both keratocystic odontogenic tumours and DCs; and was also significantly higher in keratocystic odontogenic tumours than in DCs.

CONCLUSION: Within the limitations of the present study, it can be suggested that angiogenesis may be one of the mechanisms possibly contributing to the different biological behaviours of keratocystic odontogenic tumours, DCs and solid ameloblastomas.

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Keywords: keratocystic odontogenic tumour; dentigerous cyst; ameloblastoma; angiogenesis; immunohistochemistry

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Introduction

The odontogenic keratocyst, recently known as keratocystic odontogenic tumour (KOT), 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 (Shear, 2002a,b; Kolár *et al*, 2006; Neville *et al*, 2008). In addition to a distinctive biological behaviour, the expression of various proliferation markers in the epithelial lining and mutations in p53 and PTCH genes (tumour suppressor genes) have led several investigators to consider KOT as a benign cystic neoplasm (Shear, 2002a,b; Thosaporn *et al*, 2004; Tsuneki *et al*, 2008). Ameloblastoma is a benign locally aggressive epithelial odontogenic tumour exhibiting a marked infiltrative potential leading to multiple recurrences following enucleation or curettage (Neville *et al*, 2008). The dentigerous cyst (DC) is the most common type of developmental odontogenic cyst, which demonstrates an indolent behaviour and seldom recurs following removal (Neville *et al*, 2008).

The KOTs, similar to DCs, appear as cystic lesions, but their invasiveness and destructive growth are comparable to ameloblastoma (Kichi *et al*, 2005; da Silva *et al*, 2008). It has been suggested that unknown factors integrated in the epithelium or fibrous capsule of KOTs may be responsible for their specific biological behaviour (Shear, 2002a,b; Neville *et al*, 2008). Microscopic epithelial and stromal features have been the centre of attention in several investigations comparing KOTs, DCs and ameloblastomas (Piattelli *et al*, 1998; Thosaporn *et al*, 2004; Vered *et al*, 2005; da Silva *et al*, 2008).

Odontogenesis is controlled by interactions between the epithelial and mesenchymal components of developing dental tissues (Thesleff *et al*, 1990). Regarding the fact that odontogenic cysts and tumours arise from tissue remains of odontogenesis, these interactions have been considered to play an important role in the

tumourigenesis of odontogenic lesions (de Oliveira *et al*, 2004). 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 (De Wever and Mareel, 2003).

Neoplastic tissues require oxygen and nutrients for survival and growth; therefore, they induce the formation of new blood vessels through angiogenesis or neovascularization (Carmeliet and Jain, 2000; Kumar *et al*, 2005). Angiogenesis has been evaluated in various human lesions including breast cancer, melanoma, lichen planus and ameloblastoma (Weidner *et al*, 1991; Kumamoto *et al*, 2002; Mahabeleshwar and Byzova, 2007; Tao *et al*, 2007). Microvessel density (MVD) represents neovascularization in tumoral tissues and can be observed by immunohistochemical staining of endothelial cells with markers such as CD34 (Gong *et al*, 2005). The aim of the present study was to evaluate and compare MVD in KOTs, DCs and ameloblastomas using monoclonal antibody against CD34. The number of immunohistochemical studies focusing on angiogenesis in KOT is limited, but we were not able to find a comparison of angiogenesis among these three lesions in the literature in English language.

Materials and methods

The medical charts of approximately 7600 patients referred to the oral and maxillofacial pathology departments of our institutions were reviewed from 1998 to 2008 and all KOTs, DCs and ameloblastomas were retrieved. The protocol for this project was approved by the Ethics Committee of our university. The haematoxylin and eosin stained archival slides were re-examined and diagnoses were confirmed based on the World Health Organization (WHO) classification (Barnes *et al*, 2005). An average of three slides (range, 2–4) were available for the cystic lesions and nine (range, 5–14) for the solid ameloblastomas. All lesions with inflammation, incisional biopsies and recurrences were excluded from the study sample. The two most common variants of ameloblastoma, follicular and plexiform, were considered for evaluation. Only KOTs with parakeratinized surfaces and cases that were not associated with basal cell carcinoma syndrome were included in the present investigation. Formalin-fixed paraffin-embedded blocks of all selected cases were obtained and immunohistochemical staining was performed. In brief, 5 μm sections were dewaxed in xylene, rehydrated in graded alcohol and treated with 3% hydrogen peroxide in phosphate-buffered saline (PBS) for blocking endogenous peroxidase activity. Antigen retrieval was achieved by treating the sections with 10 mmol l^{-1} Tris buffer, 1 mmol l^{-1} EDTA (pH 9.0) in a microwave oven for 10 min at 120°C. The sections were then incubated with anti-CD34 monoclonal antibody (QBEnd 10; Dako, Glostrup, Denmark) for 30 min at room temperature with a working dilution of 1:50. This was followed by incubation with secondary biotinylated antibody and streptavidin for 15 min each. Diaminobenzidine was used for colour development followed by counterstaining with Mayer's haematoxylin. Positive and negative controls were run simultaneously for each section. Human tonsillar tissue served as positive control and large thick-walled vessels with erythrocytes, if present, were considered as internal positive control. Non-immune serum was used instead of primary antibody to obtain negative control. MVD quantification was carried out using an Olympus BH2 microscope (Olympus, Tokyo, Japan) according to the method suggested by Weidner *et al* (1991). The definition of a microvessel used in the present study was according to that described previously (Sharma *et al*, 2005). Three areas with the highest amount of vascularization (hotspots) were selected under a magnification of ×100. Microvessels were counted in each of the three fields at ×400 magnification and the mean density was reported. MVD was expressed as the mean number of microvessels per high power field (HPF). All slides were simultaneously evaluated by two observers using a double-headed microscope and both had to agree on each of the individual microvessels before being included in the count. The field size for ×400 magnification (×40 objectives and ×10 ocular) was approximately 0.18 mm^2. 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 significant.

Results

A total of 133 keratocystic odontogenic tumours, DCs and ameloblastomas were obtained from the aforementioned archives, but 80 cases were excluded mostly due to various degrees of inflammation or lack of an adequate amount of specimen in the paraffin block. Ultimately, the study sample consisted of 53 cases including 20 keratocystic odontogenic tumours, 13 DCs and 20 ameloblastomas (14 solid and six unicystic forms). Maximum and minimum MVDs were 20.66 and 7.33 per HPF in KOTs (Figure 1); 10.33 and 1.66 per HPF in DCs (Figure 2); 33 and 7.33 per HPF in solid ameloblastomas (Figure 3) and 14.66 and 5.33 per HPF in unicystic ameloblastomas (UAs), respectively. The mean (s.d.) microvessel density of the studied lesions is shown in Table 1.

Among the six cases of UA, two were mural- and four were luminal- and intraluminal- subtypes. Due to the limited number of cases and the fact that mural and luminal/intraluminal types may differ in their biological behaviours, UAs were not included in the statistical analysis and it seems that stating our descriptive findings would suffice. All UA subtypes contained ameloblastomatous lining in at least one area of the cystic epithelium, which was in accordance with the definition suggested by Reichart and Philipsen (2004). Mean vessel count was higher in the stromal tissue surrounding the intramural ameloblastic islands in comparison with that found in the connective tissue adjacent to the cystic epithelium. MVD did not differ between the ameloblastic and non-ameloblastic cystic lining.

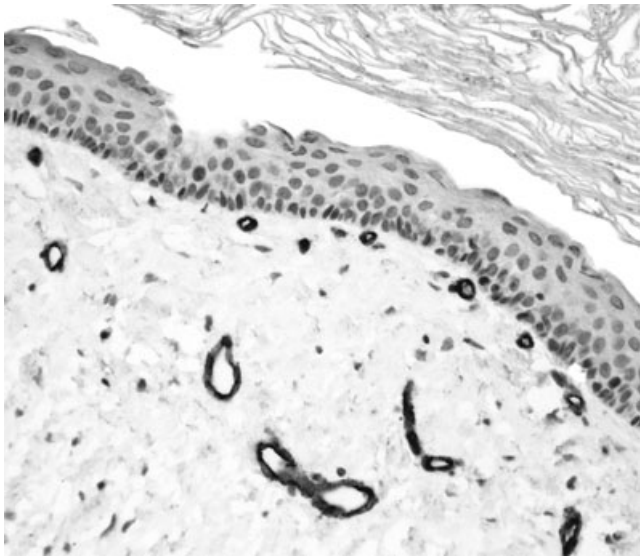

Figure 1 Microvessel density in keratocystic odontogenic tumour detected by immunohistochemistry using monoclonal antibody against CD34 (original magnification ×400)

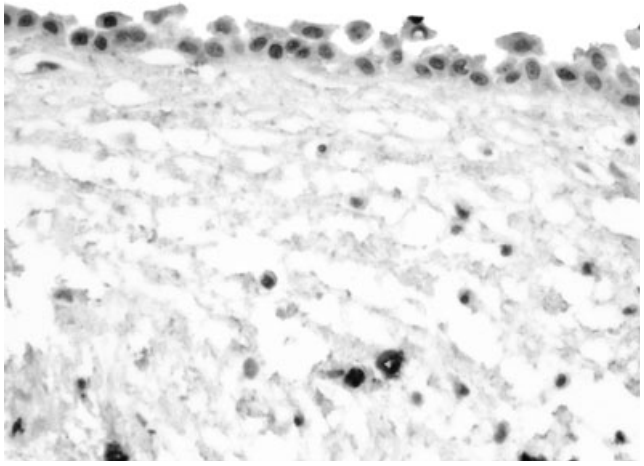

Figure 2 Microvessel density in dentigerous cyst detected by immunohistochemistry using monoclonal antibody against CD34 (original magnification ×400)

The Kruskal–Wallis test showed a significant difference in mean MVD between the KOTs, DCs and solid ameloblastomas ($P < 0.001$). Using the Mann–Whitney test, the mean MVD was significantly higher in solid ameloblastoma compared with both KOT (adjusted $P = 0.008$) and DC (adjusted $P < 0.001$). In addition, a significantly higher mean MVD was observed in KOT in comparison with DC (adjusted $P < 0.001$).

Discussion

Due to the unique clinicopathological features of odontogenic keratocyst, WHO has suggested the term keratocystic odontogenic tumour, as it better describes its neoplastic nature (Barnes *et al*, 2005). A considerable number of investigations attribute the distinctive behaviour of KOT to its epithelium (Piattelli *et al*, 1998; Thosaporn *et al*, 2004; Kolár *et al*, 2006; Tsuneki *et al*, 2008). Browne (1975) was one of the first to suggest that the connective tissue wall may have a significant role in the pathogenesis of KOT. Interaction between the fibrous capsule and epithelium of this cyst has also been confirmed in other studies (Vedtofte *et al*, 1982). A number of connective tissue elements such as tenascin and fibronectin (Amorim *et al*, 2004; de Oliveira *et al*, 2004); RANK, RANKL and OPG (da Silva *et al*, 2008); laminins and collagen 4 (Poomsawat *et al*, 2006) have also been studied in this lesion. Angiogenesis is one of the best known stromal factors participating in tumour progression and has been extensively investigated in various lesions (Weidner *et al*, 1991; Kumamoto *et al*, 2002; Mahabeleshwar and Byzova, 2007; Tao *et al*, 2007). It is quantified by the assessment of MVD in

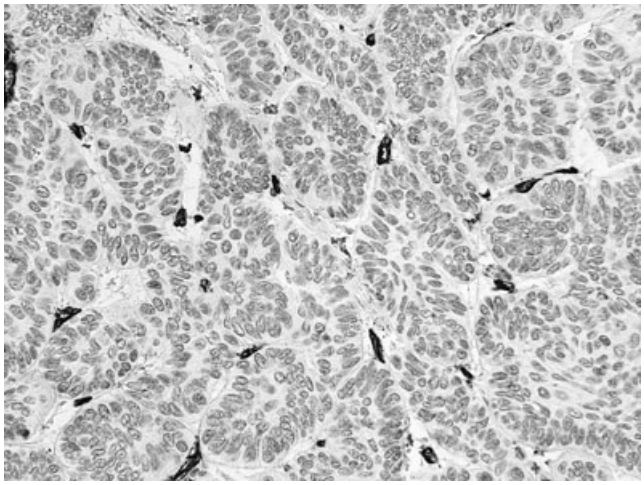

Figure 3 Microvessel density in ameloblastoma detected by immunohistochemistry using monoclonal antibody against CD34 (original magnification ×400)

Table 1 Comparison of microvessel density in keratocystic odontogenic tumour, dentigerous cyst and ameloblastoma

	Number of cases	*Microvessel density/HPF*[a] *(mean ± s.d.)*	P-*value*[b]	P-*value*[c]
Solid ameloblastoma	14	18.85 ± 6.71		0.008[d]
Keratocystic odontogenic tumour	20	12.57 ± 3.58	< 0.001	< 0.001[e]
Dentigerous cyst	13	5.07 ± 2.45		< 0.001[f]
Unicystic ameloblastoma[g]	6	9.99 ± 3.61		

[a]High power filed.
[b]Kruskal–Wallis.
[c]Mann–Whitney, adjusted *P*-value.
[d]Solid ameloblastoma *vs* keratocystic odontogenic tumour.
[e]Keratocystic odontogenic tumour *vs* dentigerous cyst.
[f]Dentigerous cyst *vs* ameloblastoma.
[g]Statistical analysis was not performed.

tumoral tissues using endothelial markers. Former investigations have shown that CD34, similar to CD31, is present on immature blood vessels, but demonstrates a stronger expression and a lower rate of staining failure (Uzzan *et al*, 2004). CD34 has also been previously employed for the assessment of MVD in different lesions (Kumamoto *et al*, 2002; Tao *et al*, 2007) and was therefore used for the evaluation of angiogenesis in the present investigation. According to our results, a significant difference was found in the expression of this protein between KOTs, DCs and follicular/plexiform ameloblastomas. It is noteworthy that previous studies (Reichart and Philipsen, 2004) have suggested the possibility of desmoplastic ameloblastoma presenting a separate clinicopathological entity and not merely a histological variant of solid ameloblastoma. Therefore, desmoplastic ameloblastoma were excluded from the present investigation and only the two most common subtypes, which are also similar in their biological behaviours, were studied. It seems that evaluation of angiogenesis in desmoplastic ameloblastoma and its comparison with other odontogenic lesions and ameloblastic variants could be helpful and contribute more data to future investigations.

Angiogenesis has been previously investigated in keratocystic odontogenic tumours, but the number of studies in this field is limited. Previous investigators (Tete *et al*, 2005) found newly-formed vessels in maxillary KOTs and radicular cysts using CD31 immunostaining. They proposed a primary role for angiogenesis in the formation of gnathic cysts and suggested that CD31 could be employed as a diagnostic marker. Hayashi *et al* (2002) studied the efficiency of dynamic multislice helical CT in the differentiation of ameloblastoma and KOT. Their results showed significantly higher amounts of CD31-positive microvessels in ameloblastoma. Considering that CD34 and CD31 are similar in detecting neovascularization (Uzzan *et al*, 2004), our findings are in accordance with their results. el-Labban and Aghabeigi (1990) morphometrically and ultrastructurally evaluated blood vessels in KOTs and DCs. In contrast to the findings obtained in the present investigation, they failed to show a significant difference between the two cysts using histochemical techniques. This can be explained by the fact that immunohistochemical staining may be more accurate in the quantification of vasculature. On the other hand, their ultrastructural examination revealed the presence of thrombosis in KOTs, but rarely in DCs. The thrombosed vessels in KOT probably contain a large number of platelets, which can produce angiogenic and proangiogenic factors such as PDGF, VEGF, bFGF and TGF-β1 (Brill *et al*, 2004; Anitua *et al*, 2007). These proteins, especially VEGF, can induce endothelial cell proliferation, migration and vascular permeability at different levels (Kumar *et al*, 2005; Maharaj and D'Amore, 2007) leading to increased angiogenesis and microvessel formation. Therefore, their electron microscopic observations essentially confirm the elevated MVD of KOTs (compared to DCs) found in the current investigation.

Neovascularization is a complex process that involves the interaction and balance between pro- and anti-angiogenic factors. Several agents are known to influence this process indirectly by affecting different angiogenesis-related factors (Kumar *et al*, 2005), such as p53, TGF-α, TGF-β, V-CAM, matrix metalloproteinases and murine double minute-2 (MDM2); which have been investigated in keratocystic odontogenic tumours and either DCs or ameloblastomas (Li *et al*, 1997; Carvalhais *et al*, 1999; Shear, 2002a,b; Wang *et al*, 2003; Zhong *et al*, 2003; Kichi *et al*, 2005). One of the problems in the present study was that we were unable to evaluate these proteins and other angiogenic factors like VEGF in the selected cases. Simultaneous assessment of MVD and other factors in KOT, DC and ameloblastoma may provide additional information on the role of angiogenesis in odontogenic lesions. It should be noted that the epithelial lining of the studied cystic lesions demonstrated slight immunopositivity with CD34. However, we were not able to find a previous report of CD34 immunoreactivity in KOTs and DCs in the English literature. Therefore, it remains unclear as to whether the immunoreactivity was due to technical errors or other reasons.

In summary, our results suggest a significant difference in angiogenesis between KOTs, DCs and solid ameloblastomas. The highest mean MVD in the studied lesions was observed in ameloblastoma, followed by KOT, UA and DC, in that order. On the other hand, when treatment is based on enucleation, the recurrence rates of these lesions have been reported as 50–90% in solid ameloblastomas, 17–56% in KOTs, 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 behaviours of these lesions and at least to some degree can reflect their clinical features. However, we did not perform statistical analysis on the UA cases and the current results may differ if recurrent or nevoid basal cell carcinoma syndrome-associated KOTs or a larger number of UA cases are used for comparison. We cannot draw strong conclusions using the findings of this single study; therefore, further evaluation using more sophisticated methods and additional cases is recommended to help clarify the role of angiogenesis in odontogenic lesions.

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Author contributions

Drs. M Alaeddini and S Etemad-Moghadam contributed equally to all aspects of the research including study concept and design, literature research, data acquisition, experimental studies, statistical analysis and drafting, writing, editing and reviewing of the manuscript. Drs. S Salah and F Dehghan

contributed to literature research and data acquisition. Dr. N Eshghyar critically reviewed the manuscript.

References

Amorim RF, Godoy GP, Galvão HC, Souza LB, Freitas RA (2004). Immunohistochemical assessment of extracellular matrix components in syndrome and non-syndrome odontogenic keratocysts. *Oral Dis* **10:** 265–270.

Anitua E, Sanchez M, Nurden AT *et al* (2007). Reciprocal actions of platelet-secreted TGF-beta1 on the production of VEGF and HGF by human tendon cells. *Plast Reconstr Surg* **119:** 950–959.

Barnes L, Eveson JW, Reichart P, Sidransky D (2005). *World Health Organization classification of tumours. Pathology and genetics of head and neck tumours*. IACR Press: Lyon, France.

Blanas N, Freund B, Schwartz M, Furst IM (2000). Systematic review of the treatment and prognosis of the odontogenic keratocyst. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **90:** 553–558.

Brill A, Elinav H, Varon D (2004). Differential role of platelet granular mediators in angiogenesis. *Cardiovasc Res* **63:** 226–235.

Browne RM (1975). The pathogenesis of odontogenic cysts: a review. *J Oral Pathol* **4:** 31–46.

Carmeliet P, Jain RK (2000). Angiogenesis in cancer and other diseases. *Nature* **407:** 249–257.

Carvalhais J, Aguiar M, Araújo V, Araújo N, Gomez R (1999). p53 and MDM2 expression in odontogenic cysts and tumours. *Oral Dis* **5:** 218–222.

De Wever O, Mareel M (2003). Role of tissue stroma in cancer cell invasion. *J Pathol* **200:** 429–447.

Gong Y, Sun X, Huo L, Wiley EL, Rao MS (2005). Expression of cell adhesion molecules, CD44s and E-cadherin, and microvessel density in invasive micropapillary carcinoma of the breast. *Histopathology* **46:** 24–30.

Hayashi K, Tozaki M, Sugisaki M, Yoshida N, Fukuda K, Tanabe H (2002). Dynamic multislice helical CT of ameloblastoma and odontogenic keratocyst: correlation between contrast enhancement and angiogenesis. *J Comput Assist Tomogr* **26:** 922–926.

Kichi E, Enokiya Y, Muramatsu T *et al* (2005). Cell proliferation, apoptosis and apoptosis related factors in odontogenic keratocyst and in dentigerous cysts. *J Oral Pathol Med* **34:** 280–286.

Kolár Z, Geierová M, Bouchal J, Pazdera J, Zboril V, Tvrdý P (2006). Immunohistochemical analysis of the biological potential of odontogenic keratocysts. *J Oral Pathol Med* **35:** 75–80.

Kumamoto H, Ohki K, Ooya K (2002). Association between vascular endothelial growth factor (VEGF) expression and tumour angiogenesis in ameloblastomas. *J Oral Pathol Med* **31:** 28–34.

Kumar V, Abbas AK, Fausto N (2005). *Robbins and Cotran pathologic basis of disease*. Elsevier Saunders: Philadelphia.

el-Labban NG, Aghabeigi B (1990). A comparative stereologic and ultrastructural study of blood vessels in odontogenic keratocysts and dentigerous cysts. *J Oral Pathol Med* **19:** 442–446.

Lau SL, Samman N (2006). Recurrence related to treatment modalities of unicystic ameloblastoma: a systematic review. *Int J Oral Maxillofac Surg* **35:** 681–690.

Li TJ, Browne RM, Mathews JB (1997). Immunocytochemical expression of growth factors by odontogenic jaw cysts. *Mol Pathol* **50:** 21–27.

Mahabeleshwar GH, Byzova TV (2007). Angiogenesis in melanoma. *Semin Oncol* **34:** 555–565.

Maharaj AS, D'Amore PA (2007). Roles for VEGF in the adult. *Microvasc Res* **74:** 100–113.

Neville BW, Damm DD, Allen CM, Bouquot JE (2008). *Oral and maxillofacial pathology*. W.B. Saunders: Philadelphia.

de Oliveira MD, de Miranda JL, de Amorim RF, de Souza LB, de Almeida Freitas R (2004). Tenascin and fibronectin expression in odontogenic cysts. *J Oral Pathol Med* **33:** 354–359.

Piattelli A, Fioroni M, Santinelli A, Rubini C (1998). Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. *Oral Oncol* **34:** 408–412.

Poomsawat S, Punyasingh J, Weerapradist W (2006). Expression of basement membrane components in odontogenic cysts. *Oral Dis* **12:** 290–296.

Reichart PA, Philipsen HP (2004). *Odontogenic tumours and allied lesions*. Quintessence: London, UK.

Sharma S, Sharma MC, Sarkar C (2005). Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis. *Histopathology* **46:** 481–489.

Shear M (2002a). The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 1. Clinical and early experimental evidence of aggressive behaviour. *Oral Oncol* **38:** 219–226.

Shear M (2002b). The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 2. Proliferation and genetic studies. *Oral Oncol* **38:** 323–331.

da Silva TA, Batista AC, Mendonça EF, Leles CR, Fukada S, Cunha FQ (2008). Comparative expression of RANK, RANKL, and OPG in keratocystic odontogenic tumours, ameloblastomas, and dentigerous cysts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **105:** 333–341.

Tao X, Huang Y, Li R *et al* (2007). Assessment of local angiogenesis and vascular endothelial growth factor in the patients with atrophic-erosive and reticular oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103:** 661–669.

Tete S, Mastrangelo F, Grimaldi S *et al* (2005). Immunohistochemical evaluation of CD31 in human cystic radicular lesions and in keratocysts. *Int J Immunopathol Pharmacol* **18:** 39–45.

Thesleff I, Vaahtokari A, Vainio S (1990). Molecular changes during determination and differentiation of the dental mesenchymal cell lineage. *J Biol Buccale* **18:** 179–188.

Thosaporn W, Iamaroon A, Pongsiriwet S, Ng KH (2004). A comparative study of epithelial cell proliferation between the odontogenic keratocyst, orthokeratinized odontogenic cyst, dentigerous cyst, and ameloblastoma. *Oral Dis* **10:** 22–26.

Tsuneki M, Cheng J, Maruyama S, Ida-Yonemochi H, Nakajima M, Saku T (2008). Perlecan-rich epithelial linings as a background of proliferative potentials of keratocystic odontogenic tumour. *J Oral Pathol Med* **37:** 287–293.

Uzzan B, Nicolas P, Cucherat M, Perret GY (2004). Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. *Cancer Res* **64:** 2941–2955.

Vedtofte P, Holmstrup P, Dabelsteen E (1982). Human odontogenic keratocyst transplants in nude mice. *Scand J Dent Res* **90:** 306–314.

Vered M, Shohat I, Buchner A, Dayan D (2005). Myofibroblasts in stroma of odontogenic cysts and tumours can contribute to variations in the biological behavior of lesions. *Oral Oncol* **41:** 1028–1033.

Wang J, Zhong M, Zhang LZ, Wang Y, Wang ZY (2003). Expression of ICAM-1 and VCAM-1 in human ameloblastoma and odontogenic keratocyst. *Shanghai Kou Qiang Yi Xue* **12:** 273–276. (Abstract)

Weidner N, Semple JP, Welch WR, Folkman J (1991). Tumour angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* **324:** 1–8.

Zhong M, Han YP, Wang J, Li ZJ, Bao G, Yue YL (2003). Expression of matrix metalloproteinases and tissue inhibitor of metalloproteinase in ameloblastoma. *Shanghai Kou Qiang Yi Xue* **12:** 427–431. (Abstract)