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ORIGINAL ARTICLE

# MMP-13 expression in keratocyst odontogenic tumour associated with NBCCS and sporadic keratocysts

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**OBJECTIVE: To investigate the matrix metalloproteinase (MMP)-13 expression in associated and non-nevoid basal cell carcinoma syndrome (NBCCS) Odontogenic Keratocysts (OCKs) in order to contribute to a better understanding of the differences in the growth pattern between them.**

**MATERIALS AND METHODS: Thirty-nine paraffin-embedded blocks of OCKs, 26 sporadic OCKs and 11 NBCCS-associated KCOTs were studied by immunohistochemistry to evaluate MMP-13 expression both in epithelial and stromal layers. A semi-quantitative scale was used to evaluate immunostaining. Obtained data were compared between the two groups, using Fischer's exact test and the chi-square test.**

**RESULTS: Only 13 of 26 sporadic OCKs showed a positive immunostaining, whilst 11 KCOTs resulted in positive labelling for MMP-13 expression. Moreover, syndromic cysts displayed a more intense and diffuse MMP-13 labelling of the stromal tissue. Instead, in non-syndromic forms, the staining pattern of MMP-13 in stromal tissue was completely absent. Fisher's exact test showed a statistically significant greater prevalence of KCOTs-immunolabelled cysts with respect to sporadic OCKs.**

**CONCLUSIONS: Results from this study point out that the biological behaviour of these cysts could be related not only to the epithelial layer but also to stromal tissue in that... MMP-13 overexpression in stromal tissue of NBCCS-associated KCOTs could clarify the higher aggressiveness of these cysts.**

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**Keywords:** odontogenic keratocysts; MMP13; NBCCS; Gorlin-Gotz syndrome

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## Introduction

Odontogenic keratocysts (OCKs), currently designated as keratocyst odontogenic tumours (KCOTs) by the WHO (Madras and Lapointe, 2008), are aggressive, developmental cystic lesions with a high recurrence rate if not adequately treated (Shear, 2002b; Katase *et al*, 2007; Gonzalez Moles *et al*, 2008). OCKs can be classified into sporadic keratocysts (SKs), multiple OCKs and OCKs with satellite microcysts. Usually, there is just a single lesion arising in the tooth-bearing or retromolar regions of the jaw (Forssell *et al*, 1988). Multiplicity of KCOTs is well recognized in nevoid basal cell carcinoma syndrome (NBCCS) (also called Gorlin-Goltz syndrome) (Gorlin and Goltz, 1960; Gorlin *et al*, 1965) where the presence of OCKs is considered a major criterion for NBCCS diagnosis (Evans *et al*, 1993). Thus, OCKs can or cannot be associated with NBCCS syndrome. These two varieties of OCKs, i.e. associated with NBCCS (KCOTs) and not (SKs), do not show the same biological behaviour, growth speed and recurrence rate, as outlined in the previous studies. Indeed, the multiplicity of the cysts, the increased number of satellite cysts and epithelial islands in the (KCOTs) of NBCCS patients, suggested that the more aggressive behaviour of the syndromic forms was probably related to defective tumour suppression functions, which were reflected by the higher proliferative activity in their epithelial linings (Li *et al*, 1995; Lo Muzio *et al*, 1999). Thus differences in cellular proliferation rates and/or in the expression of oncoproteins and tumour suppressor genes such as PCNA (de Paula *et al*, 2000; Shear, 2002a), Ki-67 (Li *et al*, 1995; de Paula *et al*, 2000; Shear, 2002a) and p53 (Ogden *et al*, 1992; Lombardi *et al*, 1995; Shear, 2002a), which are more strongly expressed in NBCCs associated KCOTs, have been well described.

Recently, it has been claimed that matrix metalloproteinases (MMPs) have an important role in regulating the integrity and composition of the extracellular matrix (ECM) in odontogenic cysts (Kubota *et al*, 2002; Oka *et al*, 2005; Leonardi *et al*, 2005). MMPs are a family of

zinc-dependent, highly conserved, endopeptidases, basically capable of degrading all components of the Extra Cellular Matrix (ECM). At the moment, the MMPs family includes at least 24 well-characterized members, which can be classified into subgroups of collagenases, gelatinases, stromelysins, membrane-type MMPs and others, according to their structure and substrate specificity (Lynch and Matrisian, 2002; Nabeshima *et al*, 2002). The collagenases subfamily consists of collagenase-1 (MMP-1), collagenase-2 (MMP-8) and collagenase-3 (MMP-13), which are the only mammalian proteinases capable of degrading native collagen fibrils of types I, II and III. MMP-13 has been demonstrated in the basement membrane of OCKs (Wahlgren *et al*, 2003). In this investigation, it has been claimed that MMP-13 can induce epithelial migration and growth potential of OCKs compared with other odontogenic cyst, such as radicular and dentigerous cysts. However, no differences were reported between SK and KCOTs, contrary to a very recent study (Cavalcante *et al*, 2008) which described higher levels of some MMPs in NBCCS associated OCKs. Hence, keratocysts, and expecially NBCCS associated KCOTs, tend to activate and upregulate some metalloproteinases.

In light of the roles played by the MMP-13, and the distinct biological behaviour between sporadic OCKs and NBCCS-associated KCOTs, the present study aimed to analyse the immunohystochemical expression of MMP-13 in associated and non-NBCCS OCKs to contribute to a better understanding of the differences in the growth pattern between them.

## Materials and methods

After Institutional Ethics Committee approval, 39 paraffin embedded blocks of OCKs, from unrelated patients obtained from excisional biopsy, were randomly selected from the files of the School Of Dentistry laboratory, University of Birmingham, U.K. The lesions were classified as SKs ($n = 26$) and KCOTs ($n = 11$). None of the patients had been previously treated. They received curative surgical treatment. All diagnosis were made comparing the clinical, radiological and histological data and conformed to the parameters recommended by Kimonis (Kimonis *et al*, 1997) and Kramer as well as the World Health Organization classification of odontogenic cysts and tumours (Kramer, 1992; Barnes *et al*, 2005).

*Immunohistochemistry*

Four-micron serial sections, from formalin-fixed, paraffin-embedded blocks of cyst representative areas, were cut for each case. Only sections containing sufficient epithelium to assess the antibody reactivity with 200 cells were considered for the present study.

Immunohistochemistry was then performed on the remaining sections mounted on poly-L-lysine-coated glass slides. Deparaffined and rehydrated sections were incubated for 30 min in 3% $H_2O_2$/methanol to quench endogenous peroxidase activity, and then rinsed for 20 min with phosphate-buffered saline (PBS) (Bio-Optica M107, Milan, Italy). The sections were irradiated (5 min ×3) in capped polypropylene slide-holders with citrate buffer (pH 6), using a microwave oven (750 W) to unmask antigenic sites. Non-specific protein binding was attenuated by incubation for 30 min with 5% horse serum in PBS. Specimens were incubated overnight with monoclonal mouse anti-MMP-13 (NeoMarkers, Lab Vision, Fremont, CA, USA) (supplementary data about the antibody is available on http://www.labvision.com/ab.cfm?first = AntiBody&second = 825: MMP-13 (collagenase 3) Ab-1 clone VIIIA2 that recognizes pro and active forms of MMP-13 as assessed previously also in Cheng *et al* (2005) at 1:20 dilution. The antibody was applied directly to the section and the slides were incubated overnight (4°C) in a 'humidified chamber'. The sections were washed thrice with PBS at room temperature. Immune complexes were subsequently treated with the secondary byotinilated antibody and then detected using streptavidin peroxidase, both of them were incubated for 30 min at room temperature (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA). After rinsing with three changes of PBS the immunoreactivity was visualized by development for 2 min with 0.1% 3,30-diaminobenzidine and 0.02% hydrogen peroxide (DAB substrate kit, Vector Laboratories). Sections were counterstained with Mayer's haematoxylin, mounted with permanent mounting medium and examined with a light microscopy.

Positive controls consisted of tissue specimen sections of human skin basal cell carcinoma with known antigenic reactivity. A negative control was performed in all cases by substituting the primary antibody for normal mouse serum. Negative controls in all instances resulted in a negative immunoreactivity for MMP-13.

Both epithelial and stromal layers were evaluated. To evaluate the MMP-13 expression, a mean percentage of positive cells were determined from the analysis of 100 cells in 10 random areas at 40× magnification. The MMP-13 positivity was evaluated by an expert pathologist who had no knowledge of the clinical-pathological data and scored using a semi-quantitative scale, assigning cases to one of the three following categories: (a) score 0, when the stained cells were comprised from 0% to $< 5\%$ of the total; (b) score 1, when they were comprised from $> 5\%$ to $< 50\%$ of the total cell population; (c) score 2, when they amounted to $> 50\%$.

*Statistical analysis*

Data were analysed using GraphPad Prism software version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). Significant differences ($P < 0.05$) between groups were determined using Fischer's exact test and the chi-square test.

## Results

A microscope examination of the H&E stained sections revealed that the epithelial lining consisted of stratified squamous epithelium, 8–10 layers thick. The basal layer was well-defined and consisted of palisaded cells with hyperchromatic nuclei that are polarized away from the

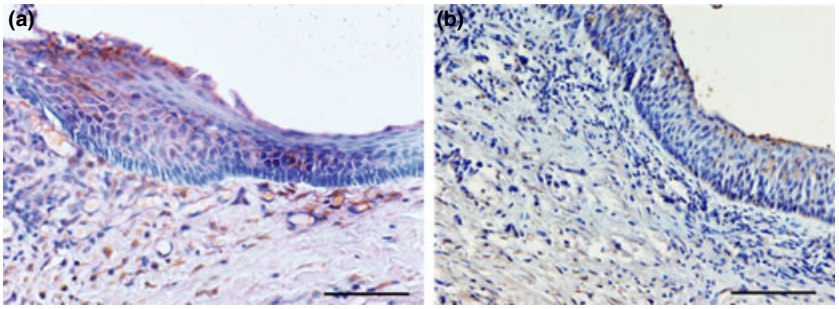


**Figure 1** Matrix metalloproteinase 13 expression in NBCCS-associated KCOTs **(a)** and sporadic OKCs **(b)**. MMP13 expression pattern in the epithelium of sporadic OKCs is weak and uneven, whereas in NBCCS-associated KCOTs is strong, original magnification ×100

basement membrane. The epithelial cells were para- or orthokeratinized with a different amount of keratin and produced a corrugate profile. The connective tissue component of the cysts wall was moderately thin. However, it is more prominent in syndromic cysts. Both SKs and KCOTs showed positive cytoplasmatic reaction to MMP-13 in the lining epithelium, but with some differences in the expression patterns between the two (Figure 1). Indeed, only 13 of 26 SKs showed a positive immunostaining, whereas 11 KCOTs resulted in positive labelling for MMP-13 expression (Table 1).

In SKs, MMP-13 expression, in the epithelial lining, was comparatively weak and uneven. Basal cells showed no to weak immunostaining. Suprabasal cells showed a moderate MMP-13 expression (Figure 1).

On the other hand, KCOTs showed a strong staining in suprabasal cell layers. Some basal cells were strongly immunolabelled with a spot-like pattern through the epithelium (Figure 2). However, the most striking difference between SKs and KCOTs was in the pattern of connective tissue immunostaining, which was always strongly immunolabelled in KCOTs and never in SKs (Figure 2). All in all, syndromic cysts displayed a more intense and diffuse MMP-13 labelling of the stromal tissue. Instead, in non-syndromic forms, the staining pattern of MMP-13 in stromal tissue was completely absent (Figure 3).

Regarding the semi-quantitative assessment of MMP-13 expression, in 46% of SKs the score was 0, 38% had score 1, and just 15% obtained score 2. In KCOTs, 30% of cases presented a semi-quantitative score of 1, whereas in 54% of cases the score was 2 (Table 2). This higher positivity of MMP-13 expression detected in KCOTs, when compared with SKs, was statistically significant according to Chi-square test ($P = 0.0306$).

Moreover, Fisher's exact test (Table 1 ) showed a statistically significant greater prevalence of KCOTs immunolabed cysts with respect to SKs ($P = 0.0449$).

**Table 1** Fisher's exact test

| *OKC* | *Positive* | *Negative* | *Total* |
|---|---|---|---|
| NBCCS | 11 | 2 | 13 |
| Non-NBCCS | 13 | 13 | 26 |
| Total | 24 | 15 | 39 |

OKC, odontogenic keratocysts; NBCCS, nevoid basal cell carcinoma syndrome; *P*-value = 0.0449.

**Table 2** Semi-quantitative evaluation of MMP-13 in odontogenic keratocysts

| *OKC* | *Score 0* | *Score 1* | *Score 2* | *Chi-square P* |
|---|---|---|---|---|
| NBCCS | 2 | 4 | 7 | 0.0306 |
| Non-NBCCS | 12 | 10 | 4 | |

OKC, odontogenic keratocysts; NBCCS, nevoid basal cell carcinoma syndrome.
Score 0: < 5%; Score 1: > 5% < 50%; Score 2: > 50%.

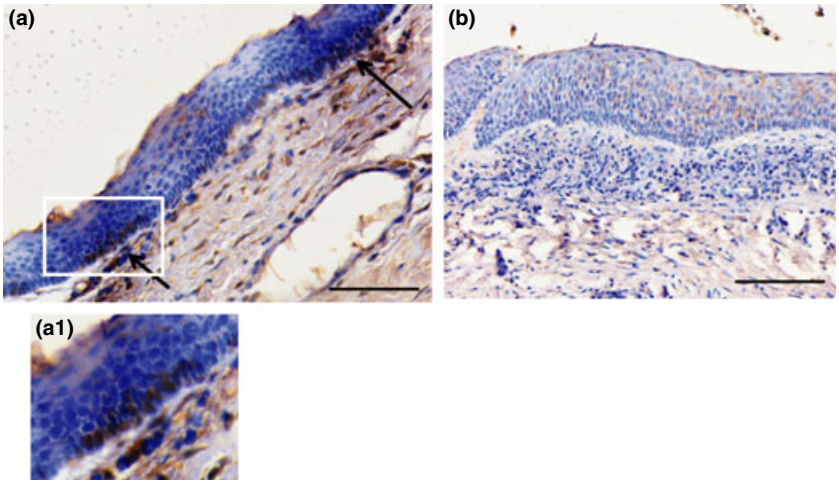


**Figure 2** Matrix metalloproteinase 13 expression in NBCCS-associated KCOTs **(a)** and sporadic OKCs **(b)**. Spot-like areas of positivity for MMP13 are observed in the basal layer of epithelium of NBCCS-associated KCOTs **(a)**, in the square at higher magnification **(a1)**, on the other hand any immunolabelling can be appreciated in sporadic OKCs **(b)**, original magnification ×100

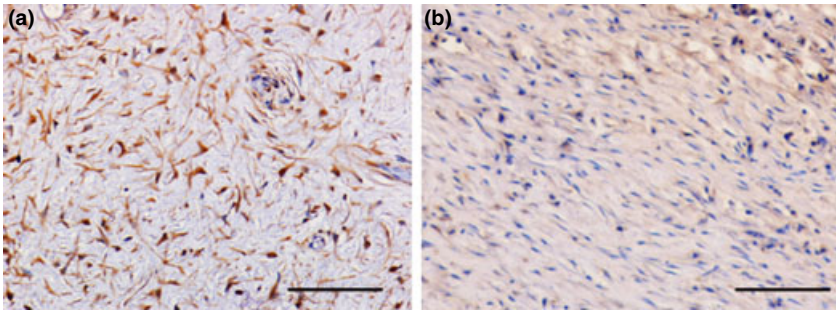


**Figure 3** Mesenchymal cells of NBCCS-associated KCOTs **(a)** reveal a cytoplasmatic positivity, contrary to sporadic OKCs **(b)**, original magnification ×100

## Discussion

Odontogenic keratocysts display a more aggressive biological behaviour, with a marked tendency to recurrence, if compared with other types of cysts, such as follicular or radicular cysts. These clinical differences could be explained by an increased epithelial mitotic activity in suprabasal layers. To date, several studies appear to confirm this hypothesis. Indeed, in the epithelial layer of OCKs could be detected enzyme immunoreactivity that had a central role in their development (Kimi *et al*, 2001; Wahlgren *et al*, 2003).

However, studies on OKCs focused on the epithelial layer without considering stromal tissue. Different studies have reported the bimolecular activity of stromal tissue in many neoplasms such as human breast carcinoma (Nielsen *et al*, 2001). The importance of epithelial-stromal interaction influence on the carcinogenetic potential of breast and epithelial skin cancers has been emphasized by many reports dealing with alterations in the regulation of matrix-degrading proteases, which are considered essential players in the process of cancer invasion and metastasis (Mueller and Fusenig, 2002). Stromal cells are the main source of MMPs and extracellular enzymes. There is strong evidence that the MMPs and the respective tissue inhibitors (TIMPs) play an important role in that process (Krecicki *et al*, 2003). They also participate in tumour vascularization as well as in foetal development, tissue repair and other physiological remodelling processes (Llano *et al*, 1997).

In particular, MMP-13 also plays a pivotal role in the MMP activation cascade, both activating and being activated by several MMPs (Leeman *et al*, 2002). Elevated MMP-13 expression has been found in a number of different malignancies, and expression has been related to tumour behaviour and patient prognosis (Freije *et al*, 1994; Johansson *et al*, 1997; Nielsen *et al*, 2001). In laryngeal cancer, MMP-13 overexpression was linked to an advanced tumour size but not with nodal status, which may confirm the participation of this metalloproteinase in ECM degradation and local invasion, but not in the formation of metastases (Cazorla *et al*, 1998). In the present study, both SKs and KCOTs showed MMP-13 expression in varying degrees.

Recent observations about MMP-13 activity in keratocysts (Wahlgren *et al*, 2003), raised the possible hypothesis that the biological behaviour of these cysts could be partially attributable to MMPs activation or overexpression. Furthermore, increased levels of MMP-1, -7 and -26 in KCOTs have recently been highlighted (Cavalcante *et al*, 2008). Besides, MMPs could exert a major role especially in syndromic cysts as suggested by the MMP-3 mRNA overexpression detected in basal cell carcinomas from NBCCS patients (Majmudar *et al*, 1994).

The findings in the present study seem to confirm these hypotheses. Indeed, KCOTs have been found to contain the highest number of MMP-13-positives cells, whereas sporadic forms occasionally show MMP-13 expression. Moreover, in KCOTs the protein expression was prominent also in the stromal tissue rather than in the epithelial layer; whereas in non-syndromic cysts the MMP-13 staining in stromal tissue was almost absent. This finding is consistent with MMP-3 mRNA overexpression in fibroblasts, isolated and cultured from uninvolved skin specimens of NBCCS patients, whereas lacking detection in fibroblast cultures isolated from normal skin in non-syndromic patients (Majmudar *et al*, 1994). Furthermore, in a recent study, a strong mesenchymal immunoreactivity to MMP-1 was observed in OCKs associated with Gorlin syndrome having a statistically significant difference with respect to that of non-syndromic OCKs (Cavalcante *et al*, 2008).

Matrix metalloproteinases-13 overexpression in stromal tissue of KCOTs could clarify the higher aggressiveness of these cysts. Stromal tissue represents a source of MMP-13. In this layer, the enzyme expression is up-regulated under conditions that could promote the in-growth of the cysts throughout the degradation of surrounding tissues. As matter of fact, the growth speed of KCOTs is higher than SKs, whereas there was no difference between primary and recurrent OCKs (Kimi *et al*, 2001) suggesting that KCOTs might be a distinguishable entity from solitary OCKs. In this respect, since the 90s, studies on NBCCS and sporadic KCOT have provided molecular evidence of a two-hit mechanism in the pathogenesis of these tumours, demonstrating allelic loss at two or more loci of 9q22 leading to the overexpression of bcl-1 and TP53 in the NBCCS. All these studies supported the concept that KCOT represents a neoplasm. On this basis WHO experts in 2005 suggested the use of KCOT (Li *et al*, 1995; Lombardi *et al*, 1995; Lo Muzio *et al*, 1999; Meara *et al*, 2000; Zedan *et al*, 2001; Shear, 2002a).

Recently, it has been suggested that a possible interaction between WNT/SHH intracellular signalling pathways can account for a higher MMPs expression in NBCCS-associated OCKs. Indeed, mutations in PTCH

gene were related to β-catenin overexpression (Dakubo *et al*, 2006). As β-catenin is able to bind to the LEF/TCF transcription factors activating target genes such as MMPs, β-catenin de-regulation could be involved in the local invasiveness and the aggressive biological behaviour of this kind of cyst. Furthermore, a study investigating Gli1, a transcription factors that mediates hedgehog signalling in mammalian cells, protein expression in BCC observed immunostaining of both mesenchymal and epithelial elements in BCC and in a subpopulation of mesenchymal cells in normal skin, corresponding to mesenchymal stem cells (Ghali *et al*, 1999). Thus, the possibility could be raised that MMPs overexpression in stromal tissue may happen in mesenchymal stem cells.

However, present results pointed out that the biological behaviour of these cysts could be related not only to epithelial layer but also to stromal tissue. If not adequately removed, keratocysts, especially the syndromic forms, could reoccur because of the persistence of molecular factors that promote MMP-13 and other enzyme activity. Thus, it is possible to infer that MMP-13 should be considered as a main stromal mediator of tissue destruction in this variety of cysts.

In conclusion, syndromic keratocysts could show more aggressive behaviour than non-syndromic varieties because of its propensity for the activation and up-regulation of some metalloproteinases such as MMP-13.

## References

Barnes L, Eveson J, Reichart P, Sidransky D (2005). Pathology and genetics of head and neck tumours. In: *WHO classification of tumours series*. 2005 IARC Press, Lyon.

Cavalcante RB, Pereira KM, Nonaka CF, Nogueira RL, de Souza LB (2008). Immunohistochemical expression of MMPs 1, 7, and 26 in syndrome and nonsyndrome odontogenic keratocysts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **106:** 99–105.

Cazorla M, Hernandez L, Nadal A *et al* (1998). Collagenase-3 expression is associated with advanced local invasion in human squamous cell carcinomas of the larynx. *J Pathol* **186:** 144–150.

Cheng XW, Kuzuya M, Nakamura K *et al* (2005). Mechanisms of the inhibitory effect of epigallocatechin-3 gallate on cultured human vascular smooth muscle cell invasion. *Arterioscler Thromb Vasc Biol* **25:** 1864–1870.

Dakubo GD, Mazerolle CJ, Wallace VA (2006). Expression of Notch and Wnt pathway components and activation of Notch signaling in medulloblastomas from heterozygous patched mice. *J Neurooncol* **79:** 221–227.

Evans DG, Ladusans EJ, Rimmer S, Burnell LD, Thakker N, Farndon PA (1993). Complications of the naevoid basal cell carcinoma syndrome: results of a population based study. *J Med Genet* **30:** 460–464.

Forssell K, Forssell H, Kahnberg KE (1988). Recurrence of keratocysts. A long-term follow-up study. *Int J Oral Maxillofac Surg* **17:** 25–28.

Freije JM, Diez-Itza I, Balbin M *et al* (1994). Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J Biol Chem* **269:** 16766–16773.

Ghali L, Wong ST, Green J, Tidman N, Quinn AG (1999). 'Gli1 protein is expressed in basal cell carcinomas, outer root sheath keratinocytes and a subpopulation of mesenchymal cells in normal human skin'. *J Invest Dermatol* **113:** 595–599.

Gonzalez Moles MA, Mosqueda-Taylor A, Esteban F *et al* (2008). Cell proliferation associated with actions of the substance P/NK-1 receptor complex in keratocystic odontogenic tumours. *Oral Oncol* **44:** 1127–1133.

Gorlin RJ, Goltz RW (1960). Multiple nevoid basal-cell epithelioma, jaw cysts and bifid rib. A syndrome. *N Engl J Med* **262:** 908–912.

Gorlin RJ, Vickers RA, Kellen E, Williamson JJ (1965). Multiple basal-cell nevi syndrome. An analysis of a syndrome consisting of multiple nevoid basal-cell carcinoma, jaw cysts, skeletal anomalies, medulloblastoma, and hyporesponsiveness to parathormone. *Cancer* **18:** 89–104.

Johansson N, Saarialho-Kere U, Airola K *et al* (1997). Collagenase-3 (MMP-13) is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts during human fetal bone development. *Dev Dyn* **208:** 387–397.

Katase N, Nagatsuka H, Tsujigiwa H *et al* (2007). Analysis of the neoplastic nature and biological potential of sporadic and nevoid basal cell carcinoma syndrome-associated keratocystic odontogenic tumor. *J Oral Pathol Med* **36:** 550–554.

Kimi K, Kumamoto H, Ooya K, Motegi K (2001). Immunohistochemical analysis of cell–cycle- and apoptosis–related factors in lining epithelium of odontogenic keratocysts. *J Oral Pathol Med* **30:** 434–442.

Kimonis VE, Goldstein AM, Pastakia B *et al* (1997). Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. *Am J Med Genet* **69:** 299–308.

Kramer IR (1992). The World Health Organization: histological typing of odontogenic tumours: an introduction to the second edition. *J Dent Assoc S Afr* **47:** 208–210.

Krecicki T, Fraczek M, Jelen M, Podhorska M, Szkudlarek T, Zatonski T (2003). Expression of collagenase-1 (MMP-1), collagenase-3 (MMP-13) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in laryngeal squamous cell carcinomas. *Eur Arch Otorhinolaryngol* **260:** 494–497.

Kubota Y, Oka S, Nakagawa S, Shirasuna K (2002). Interleukin-1alpha enhances type I collagen-induced activation of matrix metalloproteinase-2 in odontogenic keratocyst fibroblasts. *J Dent Res* **81:** 23–27.

Leeman MF, McKay JA, Murray GI (2002). Matrix metalloproteinase 13 activity is associated with poor prognosis in colorectal cancer. *J Clin Pathol* **55:** 758–762.

Leonardi R, Caltabiano R, Loreto C (2005). Collagenase-3 (MMP-13) is expressed in periapical lesions: an immunohistochemical study. *Int Endod J* **38:** 297–301.

Li TJ, Browne RM, Matthews JB (1995). Epithelial cell proliferation in odontogenic keratocysts: a comparative immunocytochemical study of Ki67 in simple, recurrent and basal cell naevus syndrome (BCNS)-associated lesions. *J Oral Pathol Med* **24:** 221–226.

Llano E, Pendas AM, Knauper V *et al* (1997). Identification and structural and functional characterization of human enamelysin (MMP-20). *Biochemistry* **36:** 15101–15108.

Lo Muzio L, Staibano S, Pannone G *et al* (1999). Expression of cell cycle and apoptosis-related proteins in sporadic odontogenic keratocysts and odontogenic keratocysts associated with the nevoid basal cell carcinoma syndrome. *J Dent Res* **78:** 1345–1353.

Lombardi T, Odell EW, Morgan PR (1995). p53 immunohistochemistry of odontogenic keratocysts in relation to recurrence, basal-cell budding and basal-cell naevus syndrome. *Arch Oral Biol* **40:** 1081–1084.

Lynch CC, Matrisian LM (2002). Matrix metalloproteinases in tumor-host cell communication. *Differentiation* **70:** 561–573.

Madras J, Lapointe H (2008). Keratocystic odontogenic tumour: reclassification of the odontogenic keratocyst from cyst to tumour. *J Can Dent Assoc* **74:** 165–165h.

Majmudar G, Nelson BR, Jensen TC, Johnson TM (1994). Increased expression of matrix metalloproteinase-3 (stromelysin-1) in cultured fibroblasts and basal cell carcinomas of nevoid basal cell carcinoma syndrome. *Mol Carcinog* **11:** 29–33.

Meara JG, Pilch BZ, Shah SS, Cunningham MJ (2000). Cytokeratin expression in the odontogenic keratocyst. *J Oral Maxillofac Surg* **58:** 862–865.

Mueller MM, Fusenig NE (2002). Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. *Differentiation* **70:** 486–497.

Nabeshima K, Inoue T, Shimao Y, Sameshima T (2002). Matrix metalloproteinases in tumor invasion: role for cell migration. *Pathol Int* **52:** 255–264.

Nielsen BS, Rank F, Lopez JM *et al* (2001). Collagenase-3 expression in breast myofibroblasts as a molecular marker of transition of ductal carcinoma *in situ* lesions to invasive ductal carcinomas. *Cancer Res* **61:** 7091–7100.

Ogden GR, Chisholm DM, Kiddie RA, Lane DP (1992). p53 protein in odontogenic cysts: increased expression in some odontogenic keratocysts. *J Clin Pathol* **45:** 1007–1010.

Oka S, Kubota Y, Yamashiro T *et al* (2005). Effects of positive pressure in odontogenic keratocysts. *J Dent Res* **84:** 913–918.

de Paula AM, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA (2000). Cell proliferation markers in the odontogenic keratocyst: effect of inflammation. *J Oral Pathol Med* **29:** 477–482.

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.

Wahlgren J, Vaananen A, Teronen O *et al* (2003). Laminin-5 gamma 2 chain is colocalized with gelatinase-A (MMP-2) and collagenase-3 (MMP-13) in odontogenic keratocysts. *J Oral Pathol Med* **32:** 100–107.

Zedan W, Robinson PA, Markham AF, High AS (2001). Expression of the Sonic Hedgehog receptor 'PATCHED' in basal cell carcinomas and odontogenic keratocysts. *J Pathol* **194:** 473–477.