Epithelial cell proliferation in the odontogenic keratocyst: a review

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Abstract

Odontogenic cysts have in common an origin from the tissues of tooth formation, with the majority of them occurring within the jaws. Despite considerable effects, little is known about the biological processes governing the initiation and growth of these lesions, particularly those of 'developmental' origin, i.e. odontogenic keratocysts, lesions are the most difficult to treat surgically and quite often recur. The proposed histogenic differences and the clinically more aggressive nature of odontogenic keratocyst (OKC) in comparison with that of other odontogenic cysts has prompted studies aimed at characterizing possible differences between their fluid aspirates, connective tissue walls, and epithelial linings. In the light of recent developments and current opinion, this paper intended to review a variety of research interests which have concentrated on, or related to, cell kinetics and differentiation of the OKC epithelium. Such studies have provided a great deal of useful information, and at the same time, raised new questions with respect to our present understanding of this clinically important entity.

Key words:

odontogenic keratocyst, Gorlin syndrome, cell kinetics, epithelial differentiation, pathogenesis, growth factors and receptors, p53 tumor suppressor gene.

I. Introduction

The proliferation of odontogenic epithelial remnants within the jaws to form cysts and tumors is a relatively common event. The term odontogenic keratocyst (OKC) was first introduced by Philipsen (1956) to describe a group of odontogenic cysts which shows a characteristic histological appearance[1]. Unlike other cyst types, OKC appears to express an intrinsic higher growth potential[2-4]. Its propensity to recur following surgical treatment, relationship to the Gorlin syndrome and putative increased risk of neoplastic change in comparison to other cyst types places the OKC in an unique position within the spectrum of odontogenic lesions. Indeed, it has been suggested that the odontogenic keratocyst should be regarded as a benign neoplasm[5,6] and active mural growth of the OKC lining epithelium could be one of the main factors contributing to the development and enlargement of this cyst type. The present paper is to put together an overview of the recent advances in respect of epithelial

cell proliferation and / or differentiation in OKC and their clinical / biological implications.

II. Clinicopathological and embryological background of OKC

The OKC is of particular interest because it is clinically more aggressive and tends to recur after surgery. Figures for the incidence of recurrence in various reported series have varied from 2.5 to 62% (Table 1)[5),7),8)].The reason for this great variation is partly dependent upon the varied nature of the cases published. For example, some series include cysts from patients with the Gorlin syndrome and others exclude them and other important variables include the duration of the follow-up periods and the methods of treatment employed. Although some studies have pointed to possible histological differences between recurrent and non-recurrent OKCs[9),10)], others have failed to identify them[11),12)]. As a consequence, increasing stress is being placed upon the method of clinical management as the main explanation of recurrence. The wall of OKC is very thin and fragile and the cyst is consequently often removed in fragments. Such fragmentation increases the risk of portions of the epithelial lining and / or satellite cysts being left behind, so increasing the risk of recurrence[13),14)]. Although OKC most commonly occurs as a single lesion in the jaw of an otherwise healthy person, about 4-5% of all OKC patients have multiple cysts with other features of the so called 'Gorlin syndrome'[3),6),15),16)]. The condition is an autosomal dominant disorder in which multiple basal cell carcinomas of skin, skeletal abnormalities and other neoplasms may develop[15),17),18)].

Table 1. Recurrence of the odontogenic keratocyst

Authors (Year)	No. of cases	Recurrent rate (%)
Pindborg & Hansen (1963)	16	62
Hansen (1967)	52	52
Toller (1967)	55	51
Busch (1969)	35	2.9
Cernea *et al* (1969)	28	18
Panders & Hadders (1969)	22	14
Rud & Pindborg (1969)	21	33
Browne (1970)	85	25
Ebling *et al* (1971)	24	38
Stoelinga (1971)	54	9.3
Rayne (1971)	42	23.8
Donoff *et al* (1972)	13	15.4
Klammt (1972)	32	21.9
Machtens *et al* (1972)	44	59.1
Payne (1972)	20	45
Rittersma (1972)	48	31.3
Toller (1972)	55	50.9
McIvor (1972)	43	5
Radden & Reade (1973)	25	48
Borg *et al* (1974)	25	24
Forssel *et al* (1974)	38	28.9
Butz (1975)	38	10.5
Eversole *et al* (1975)	70	18.6
Brannon (1976)	283	12
Donatsky *et al* (1976)	88	29.5
Shear (1976)	38	10.5
Hodgkinson *et al* (1978)	79	36.7
Vedtofte & Pratorius (1979)	75	50.7
Forssel (1980)	121	40.5
Anniko *et al* (1981)	14	50
Voorsmit *et al* (1981)	52(Gp 1)	14
Voorsmit *et al* (1981)	40(Gp 2)	2.5
Choung *et al* (1982)	23	17.4
Farmand & Makek (1983)	21	38.1
Reff & Donath (1983)	158	12
Ahlfors *et al* (1984)	116	25.8
Reff-Eberwein et al (1985)	82	56
Niemeyer *et al* (1985)	64	36
Zachariades *et al* (1985)	16	25
Partridge & Towers (1987)	45	27
Forssel *et al* (1988)	75	43
Kondell & Wiberg (1988)	29	24
Stoelinga & Bronkhorst (1988)	27	10

Data adapted from references[5),7),8)]

The three kinds of odontogenic epithelial residues, thought to give rise to OKC, dentigerous cyst and radicular cyst, are the remnants of dental lamina (glands of Serres), reduced enamel epithelium and the epithelial rests of Malassez respectively (Table 2). The potential for further differentiation and proliferation of these epithelial cells during formation of a cyst may differ and lead to differences in epithelial expression and biological behavior among different cyst types. There is a strong support for the concept that the epithelium of OKC arises from the dental lamina and its residues[5),7),17)]. The evidence in support of such a concept has arisen from several sources including the natural process of degeneration of the

Table 2. The embryological derivation of the epithelium in odontogenic cysts

Epithelial residue	Embryological origin	Odontogenic cyst
Remnants of dental lamina (glands of Serres)	Dental Lamina	Odontogenic keratocyst
Reduced enamel epithelium	Enamel organ	Dentigerous cyst
Epithelial rests of Malassez	Epithelial root sheath of Hertwig	Radicular cyst

remnants of the dental lamina[19),20)], changes in epithelial residues in the walls of odontogenic cysts[13),21),22)] and the relationship between the pattern of proliferation of the dental lamina and the incidence of OKC in different parts of the jaw[7)].

Ⅲ. Cell proliferation in the epithelial linings of OKC

Cell proliferation is one of the most fundamental biological processes. There can be little dispute over the importance of assessing cellular proliferation in the study of many biological and pathological conditions. A variety of identifiable cellular changes can be used to pinpoint cell proliferation. In simple terms, mitoses can be counted, the incorporation of nucleotides into newly synthesized DNA during the S phase can be identified, and the varying levels of structural or functional moieties associated with different aspects of the cell cycle can be assayed.

A. Mitoses

The mitotic phase of the cell cycle is the only part that can be recognized by simple morphological examination. Early histological studies have shown that mitotic figures, usually of normal appearance, are a prominent feature of OKC epithelium[2),3),13)]. Main[2)] showed that the number of mitotic figures per centimeter length of basement membrane in OKC linings ranged from 0 to 19 with a mean of 8.0. This figure was similar to that in the ameloblastoma (7.0) and in dental lamina (8.4), but higher than that in non-odontogenic cysts (2.3) and radicular cysts (4.5). Browne[3)] also quantified the number of mitoses in OKC linings of a larger series. The number of mitotic figures per 1,500 cells varied from 0 to 5 with a mean of 0.74, which corresponded to a mean figure of 3.9 per centimeter length of basement membrane. The apparent discrepancy between the two studies may be partly due to the different size of the sample used. As mitoses only represents the shortest phase of the cell cycle[23)], its index may not be sensitive enough to reflect the cellular activity in the non-neoplastic lesion like OKC.

B. Autoradiography

Further evidence of a greater epithelial activity in OKC linings was produced by Toller[4)], who estimated tritiated thymidine uptake in explants of cyst wall by autoradiography. The method involved *in vitro* incubation of a fresh piece of cyst wall in tissue culture medium containing tritiated thymidine. The mean labelling indices, expressed as the mean percentage of labeled cells per 1,000 unlabeled basal cells, were 13.0% for a series of 6 OKC, which was approximately seven times greater than that for non-keratinizing jaws cysts (1.7%, n=5). Using a similar method, Scharfetter *et al* demonstrated that the mean labelling index (% of labeled cells in 770 cells counted per representative area) was higher in OKC (10.89%) than in radicular cyst (0.77%)[8)]. However, possibly due to the technical limitations, namely requirement of fresh tissue, *in vitro* incubation and autoradiography, both studies had only investigated a limited number of cases.

C. Flow cytometry

Flow cytometric analysis of DNA cellular content and cell cycle distribution has been shown to

be a good parameter reflecting cellular proliferation[24]. However, there has been only one study, to date, which relates to OKC. Using techniques that allow cytometry to be performed on paraffin tissue, High *et al* compared the DNA content of cells from ordinary OKC and one case which underwent epithelial dysplasia and malignant transformation[25]. The DNA distribution in control OKC had a major peak representing cells in the G0/G1 phase (i.e. diploid; 2N) and a small, less well defined peak representing cells that were tetraploid (4N), i.e. cells that had passed through S-phase and were in G2 or mitosis. By contrast, the OKC with epithelial dysplasia had a large additional peak representing an aneupoid G0/G1 cell component as did the subsequent carcinoma. It is tempting to speculate that the presence of aneuploidy in an OKC may predict its future biological behavior with respect to malignant transformation. However, flow cytometry technique requires to disrupt tissue specimens so that the spatial relation of cellular subpopulations is lost. In the present example, the cell suspension of OKC may contain a mixture of its lining epithelium as well as fibrous tissue components. Studies of larger series in conjunction with analysis of more specific cellular populations are required to ascertain the clinical implications of DNA cell cycle distribution of OKC epithelium.

D. Immunocytochemical labelling of PCNA and Ki67

Immunocytochemical methods of assessing cell proliferation have particular advantages over other techniques because of the maintenance of cellular and tissue architecture, the relative simplicity of the methodology and the rapidity of results. Over the years, many antibodies have been raised to the gene products or the relevant proteins associated with the cell cycle. Amongst those monoclonal antibodies to proliferating cell nuclear antigen (PCNA) and Ki67 are probably the most widely applied[24),26].

Using quantitative techniques based on a combination of TV image analysis for histomorphometric measurement of basement membrane and manual counting of PCNA and Ki67 immunostained cells, Li *et al* investigated the proliferative activity in the epithelial linings of various major- and / or sub-types of odontogenic jaw cysts[27)-29]. The results demonstrated that OKC linings exhibited a significantly higher level of labelling with a predominantly suprabasal location of positive cells in comparison to dentigerous and radicular cyst linings (Fig. 1, 2). Comparison of different subtypes of OKC linings indicated an increased number of cells expressing Ki67 in Gorlin syndrome related OKC than in those of solitary (non-recurrent) and recurrent lesions. Interestingly, no significant difference was found between recurrent and non-recurrent OKC (Fig. 3)[29].

The consistent higher level of PCNA and Ki67 labelling in the epithelial linings of OKC supports the hypothesis that active cell division of the lining epithelium or mural growth is more important in the pathogenesis of OKC than other types of odontogenic cyst. The characteristic predominant suprabasal location of the proliferating cells in OKC linings, in contrast to that in dentigerous and radicular cysts, suggests that a unique cellular proliferation and/or differentiation process occurs within this cyst type. It is proposed that the basal cells within OKC lining epithelium might have entered, at least to some extent, the pathway toward ameloblast differentiation possibly due to the inductive influences of underlying connective tissue wall[28),29]. This could explain the apparent low proliferating activity of the basal layer cells in OKC linings and may reflect its proposed embryological origin, e.g. remnants of dental lamina. These epithelial remnants are formed at an early stage of tooth development prior to histomorphogenesis of the enamel organ and may thus retain the potential for further differentiation/proliferation during the formation of OKCs.

Although recurrence could be due to incomplete

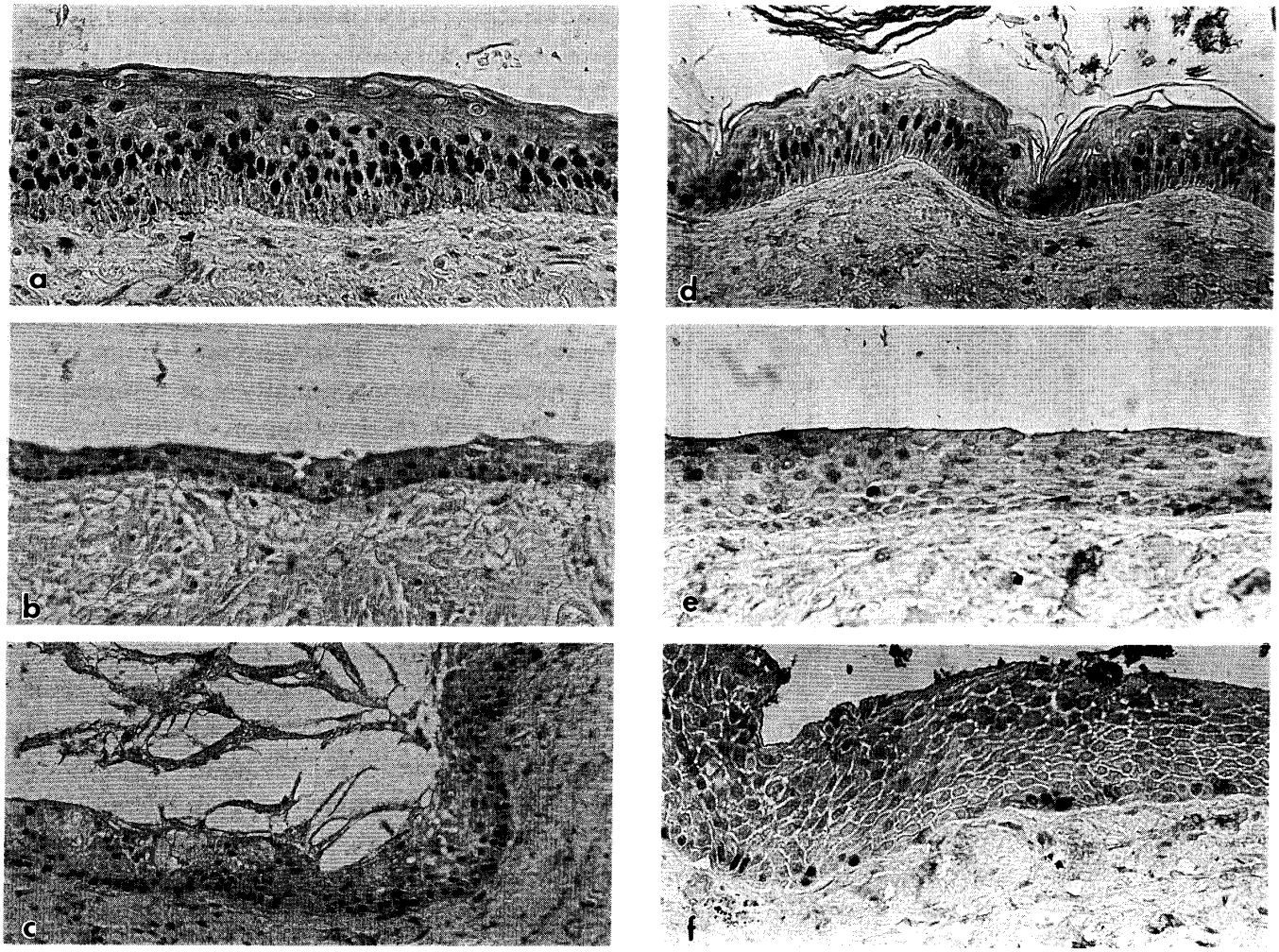

Fig. 1 PCNA (a, b, c) and Ki67 (d, e, f) expression by odontogenic keratocysts (a, d, x400), dentigerous (b, x300; e, x600) and radicular cysts (c, x300; f, x600). OKC linings show a predominantly suprabasal cell labelling pattern in contrast to the linings of dentigerous and radicular cysts.

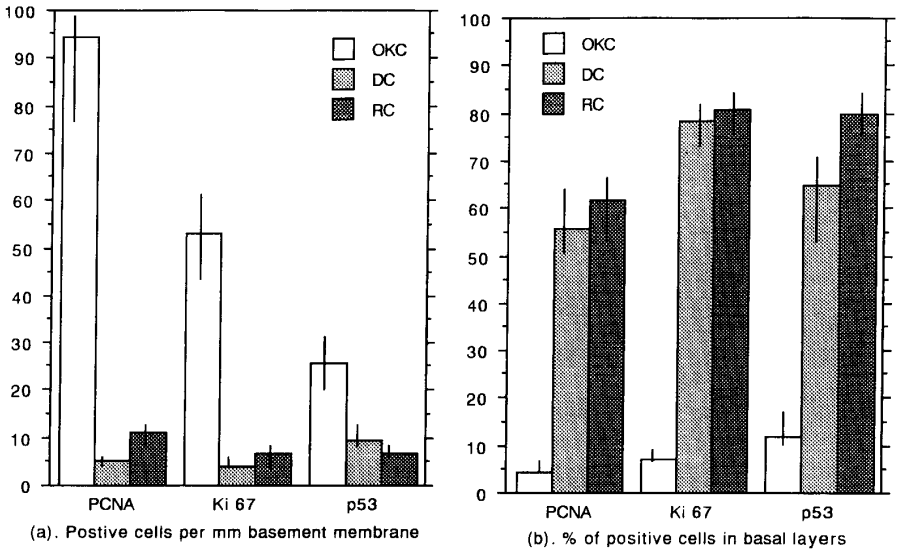

Fig. 2 Histograms showing PCNA, Ki67 and p53 positive cell counts (a) and the percentage of their positive cells in basal cell layers (b) within the epithelial linings of OKC, dentigerous cyst (DC) and radicular cyst (RC). Note the consistently higher labelling (a) and the predominant suprabasal location of the three markers in OKCs.

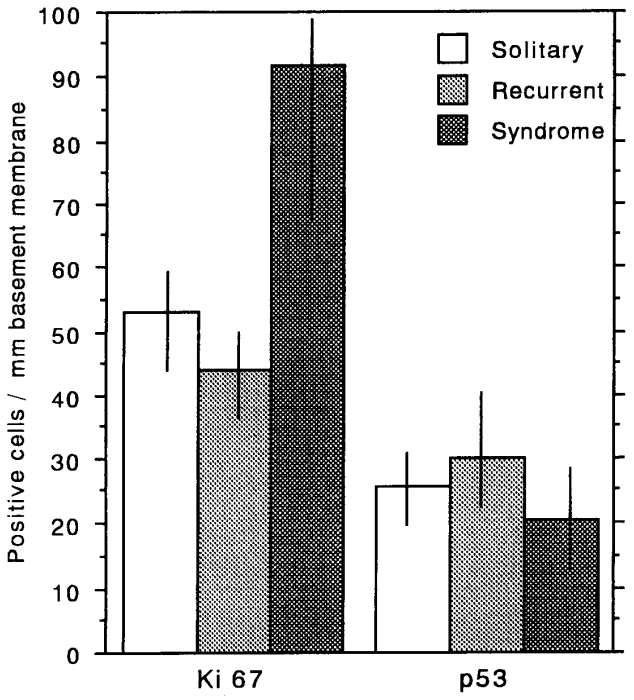

Fig. 3 Histogram showing differences of Ki67 and p53 positive cell counts between solitary, recurrent and Gorlin syndrome associated OKC epithelium.

removal it is also possible that there are intrinsic differences between recurrent and non-recurrent lesions[9),10)]. The demonstration of a similar Ki67 labelling index in the linings of recurrent and non-recurrent OKC (Fig. 3) indicates that recurrence is not associated with a subgroup of lesions showing increased proliferation, supporting the concept that inappropriate surgery of the original cyst is the most plausible reason for recurrence[29)]. The heightened proliferative activity of Gorlin syndrome related OKC could reflect the underlying genetic abnormalities in this group of individuals, as it has been recently demonstrated that this syndrome is caused by mutations in a tumor suppressor gene on chromosome 9, which plays an important role in normal development and in the growth control of precursor cells for basal cell carcinomas and other tumors[30)].

Ⅳ. Expression of the p53 tumor suppressor gene in OKC epithelium

The preceding section have stated that the level of epithelial cell proliferation in odontogenic cysts is highest in OKCs. The distribution of proliferating cells within OKC epithelium is predominantly suprabasal in sharp contrast to that in other cyst types. These qualitative and quantitative differences in proliferative activity suggest that control of the cell cycle may be abnormal in OKC. The p53 gene product is thought to control cell growth, with its wild-type form arresting the cell cycle at the G1 phase and its mutant forms promoting cell proliferation and/or malignant transformation[31),32)]. It is therefore interesting to determine whether abnormalities of p53 gene are associated with the development of OKCs.

The possible involvement of p53 gene in the growth and regulation of OKCs has been suggested by immunocytochemical demonstration of p53 protein overexpression by the OKC linings (Fig. 4)[33)-36)]. The only quantitative study of p53 immunoreactivity demonstrated a significantly higher level of p53 labelling and a predominantly suprabasal reactivity in OKC linings as compared to that in dentigerous and radicular cysts (Fig. 2)[36)], which significantly correlated with Ki67 labelling within the same series of cyst cases (Fig. 5). Interestingly, p53 expression was not further increased in the Gorlin syndrome associated OKC linings, which contrasts with the increased Ki67 labelling in this subgroup of lesions (Fig. 3). Unless molecular analysis of the p53 gene is performed, however, it is unknown whether the increased p53 labelling in OKC epithelium indicates

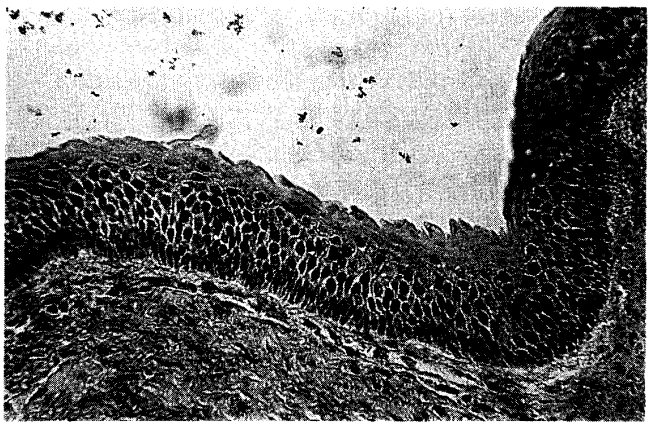

Fig. 4 p53 immunoreactivity (monoclonal antibody BP53-12) in OKC lining epithelium (x400).

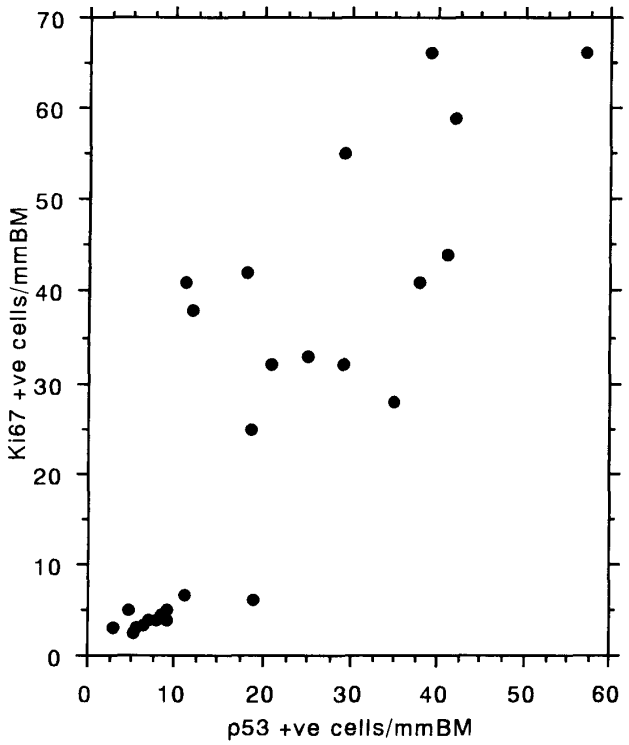

Fig. 5 Scatterplot showing the relationship between p53 and Ki67 positive cell counts in different types of odontogenic cysts (n=26), excluding Gorlin syndrome associated OKCs (r=0.55; P<0.01).

mutation of the p53 gene or overexpression of the wild-type product due to stabilization by other gene products, e.g. cdc2 protein kinase[37] or the mdm2 gene product[38]. Thus, Li *et al* further examined the status of the p53 gene in the immunopositive OKC cases using the technique of combined polymerase chain reaction and single-stranded conformation polymorphism (PCR-SSCP) and subsequent DNA direct sequencing[36]. The results indicated that DNA extracted from OKC, including Gorlin syndrome associated lesions, harbored no mutations within exons 5-9, which covers the reported 'hot spot' regions (codons 151-164, 171-181, 213-223, 234-258 and 270-286) of p53 mutations in various human tumors (Fig. 6).

Taken together, these evidences suggest that the epithelial linings of OKC express higher levels of p53 protein than other cyst types, which appears to correlate with cell proliferation. However, overexpression of p53 by OKC epithelium is not due to mutation of the p53 gene, but presumably

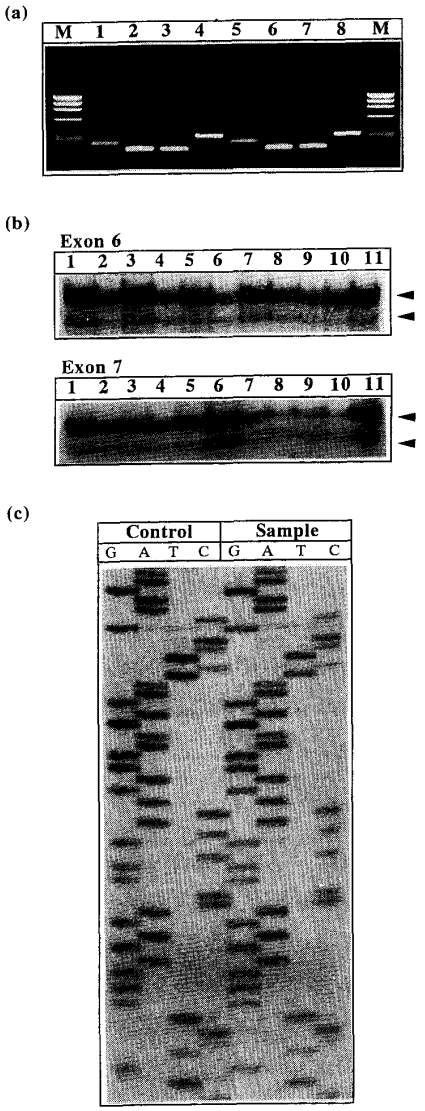

Fig. 6 Molecular analysis of p53 gene using PCR amplification of specific exons (a), single strand conformation polymorphism (SSCP) screening of the PCR products (b) and subsequent DNA sequence confirmation (c). Agarose gel electrophoresis (a) of PCR products obtained with DNA extracted from normal control cell line (lanes 1-4) and an OKC specimen (lanes 5-8). The amplified fragments of p53 gene include exons 5 (lanes 1, 5), 6 (lanes 2, 6), 7 (lanes 3, 7) and 8/9 (lanes 4, 8). The molecular size marker (lane M) is Hae III-digested ϕX174 replicative form DNA; SSCP gels (b) of PCR products (lanes 1-4, solitary OKC; lanes 5-6, recurrent OKC; lanes 7-10, Gorlin syndrome OKC) show similar banding patterns (arrow) with reference to normal control (lane 11) for exon 6 and exon 7 of p53 gene; Sequencing gel (c) shows that DNA sequences of part of exon 8 obtained with DNA extracts from a Gorlin syndrome associated OKC sample (Sample) are identical to the banding patterns of normal control (Control).

reflects overproduction and/or stabilization of normal p53 protein. It has been hypothesized by some authors that a high proliferation rate may result in detectable concentrations of wild-type p53 protein in cells[39),40)]. This is supported by the experimental finding of detectable levels of wild-type p53 protein in phytohaemagglutinin stimulated, rapidly proliferating lymphocytes[39)] and by the occurrence of sporadic p53 positive cells in thymus and reactive lymphoid tissues[40)]. It is, therefore, reasonable to believe that the overexpression of p53 by OKC epithelium may represent a 'feedback' response to its high proliferative activity, rather than the cause of its intrinsic growth potential. Furthermore, the heightened proliferation in Gorlin syndrome associated OKC, which is not associated with alterations of p53 immunoreactivity and gene status, is probably related to abnormalities of the Gorlin syndrome gene locus mapped to chromosome 9q22.3-q31[30),41)].

V. Epithelial differentiation marker - cytokeratins in OKC linings

Cytokeratins are epithelial-specific intermediate filament proteins, made up of at least 20 different polypeptides in human epithelia[42)]. The functions of these proteins inside cells are not as yet completely understood. However, because of their differentiation-related expression patterns, they provide useful molecular markers for cell identification and differentiation[43)]. Unfortunately, the accumulated data so far do not appear to demonstrate consistent differences in keratin expression between types of odontogenic cysts[44)].

It appears that the major keratins consistently detected in odontogenic epithelium are keratins 13, 14, and 19[45)-48)]. Keratins 13 and 14 are known to be associated with noncornified and all stratified epithelia respectively, whereas keratin 19 is a major component in many simple epithelia as well as being present in basal cells of stratifying epithelia[46)]. This unusual combination of keratin expression may provide a potentially useful marker for the identification of odontogenic epithelium[45)].

Two research groups have consistently showed that OKC express keratins characteristic of cornified epithelium (keratins 1, 10)[45),46)]. By contrast two other groups have reported negative results when using the same monoclonal antibodies[48),49)]. Matthews *et al* ascribed the conflicting results to differences in the sensitivities of the immunocytochemical techniques used[45)]. In an attempt to examine possible changes in keratin expression that may accompany the development of OKC, one research group also indicated greater expression of keratins 1/10/11, 13, and 16 by OKC epithelium compared with the remnants of the dental lamina from which these lesions are believed to arise[44),45)]. Confirmation of these possible changes in keratin profiles associated with the transition of odontogenic epithelial remnants to their respective pathological lesions must await quantitative immunocytochemical studies and biochemical characterization of the keratins present.

The strong reaction of OKC lining for keratin 16[45),48)], which has been associated with high proliferative activity, is in accord with the known high proliferative potential of these lesions. The demonstration that keratin 13, a marker for differentiating prickle cells of stratified squamous epithelium, is expressed only by the superficial and basal layers of OKC epithelium but is absent in suprabasal cell layer[45)] provides further evidence to indicate an unique epithelial cell organization within OKC epithelium.

VI. Other studies related to the growth of OKC

A. Enzyme histochemistry

Enzyme histochemical studies on odontogenic cysts have provided somewhat varying results, probably due to the different techniques used. In general, both qualitative[50),51)] and quantitative[52)] studies have demonstrated that OKC epithelium exhibits high levels of oxidative enzymes whereas

linings of other cyst types show only weak activity. Thus high levels of oxidative enzymes were found throughout the whole length of OKC epithelium, with the basal cells[50] or the basal cells plus the granular layer[51] being particularly prominent. The non-keratinizing cyst epithelia (radicular and /or dentigerous cyst) showed uneven and consistently weaker oxidative activities. Furthermore, the *in situ* enzyme kinetic study of Mason & Matthews[52] demonstrated that OKC epithelial linings contained a significantly higher level of G6PDH and a lower level of LDH than radicular cyst linings. These findings suggest that the epithelial linings of OKC are metabolically more active, which is consistent with their higher level of mitotic and synthetic activity compared to other cyst types. The higher level of LDH in radicular cyst epithelium may reflect the anaerobic nature of these inflammatory lesions which may be associated with extensive tissue necrosis[52].

B. Growth factors and EGF/TGF-α receptor

Growth factors are small polypeptides that promote cell proliferation and metabolism through interaction with specific cell membrane-bound receptors and tend to act locally in a paracrine or autocrine fashion[53]. Members of the EGF family [e.g. epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α)] and their common receptor (EGF receptor) are thought to have a role in modulating cell growth and cell interaction during tooth development[54),55]. In view of the developmental origin of OKC and its possible pathogenic links to the cellular and molecular events occurred during normal odontogenesis, a series of studies has been undertaken to characterize the immunocytochemical location and differential expression of EGF, TGF-α and EGF receptor in different types of odontogenic cysts (Fig. 7)[56)-59]. Quantification of epithelial staining for EGF and TGF-α by measuring absorbance readings using TV image analysis showed that staining intensity of TGF-α was consistently higher than that of EGF (Fig. 7, 8). Similar weak or negative EGF reactivity in conjunction with consistent positive TGF-α staining has been reported in various human normal tissues[60] and tumors[61]. Thus, odontogenic cyst linings are similar to other tissues in that TGF-α appears to be the major player in the potential autocrine loop for stimulation of the EGF receptor. Furthermore, in comparison to other cyst types, a significantly higher level of coexpression of TGF-α and EGF receptor was detected in OKC epithelium (Fig. 7), suggesting that growth factor and receptor interaction (*via* autocrine and/or paracrine pathways) could contribute to the cell proliferation and/or differentiation in this cyst type. That such a relationship may exist was also suggested by the significantly higher levels of TGF-α detected in Gorlin syndrome-OKC compared

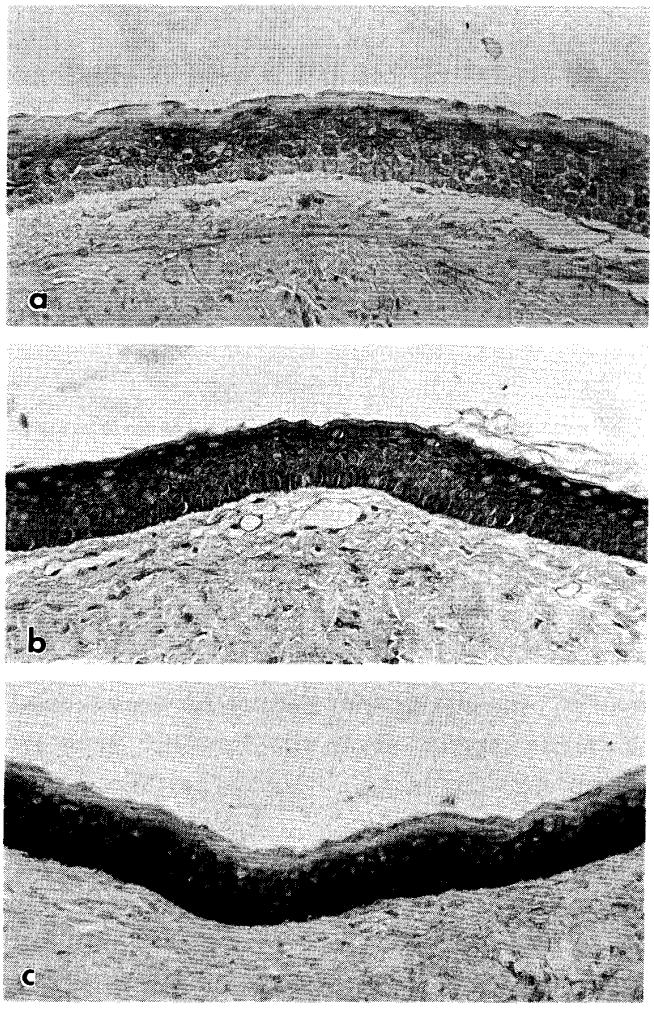

Fig. 7 Staining of OKC lining epithelium for EGF (a, x400), TGF-α (b, x300) and EGF receptor (c, x300).

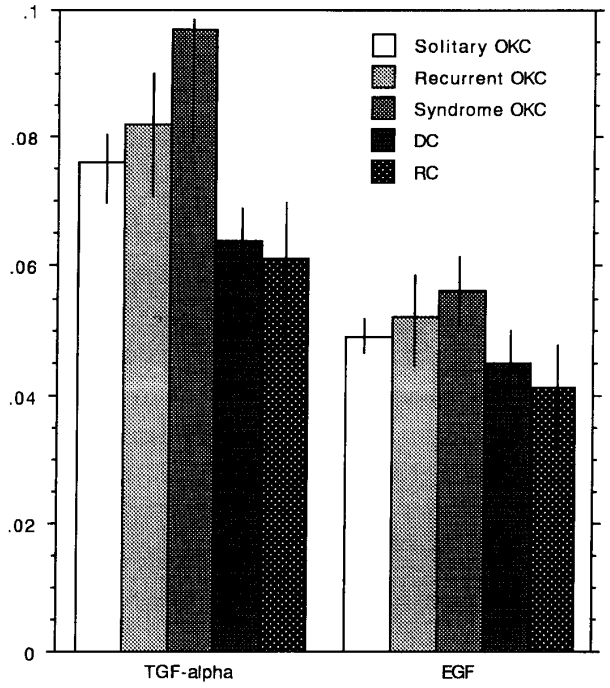

Fig. 8 Histogram showing TGF-α and EGF absorbance readings of the epithelial linings of odontogenic cysts measured by TV image analysis.

with solitary OKC (Fig. 8), as it is known that syndrome cysts exhibit higher mitotic[15] and Ki67[29] labelling indices.

C. Animal models and *in vitro* culture

The occurrence of odontogenic cysts in animals is rare and there is no convincing animal model that could simulate human situations. Whilst attempts to induce radicular cysts by exposing the pulp cavity to the oral environment and causing pulpal necrosis have provided some meaningful models similar to the periapical lesions in man[62),63)], the lesions that have so far been formed in animal experiments to simulate OKC have not shown any convincing similarity to those occurring naturally in man[64)-67)]. Transplantation of pieces of human OKC walls into nude mice resulted in epithelial proliferation, sometimes with new cyst formation[68)]. The typical histological features of an OKC were retained only when it was supported by its own fibrous tissue capsule, but an altered morphology was observed in epithelium overlying mouse connective tissue. These findings suggest that epithelial-mesenchymal interactions are essential for the growth and maintenance of the characteristic OKC epithelium. Stenman *et al* attempted to grow fresh tissue specimens from 3 OKCs, 3 dentigerous cysts and 3 ameloblastomas *in vitro*[69)]. Although none of the dentigerous cysts grew, all OKCs and ameloblastomas were cultured successfully, leading them to conclude that the behavior of the OKC is more like that of a neoplasm than that of a simple cyst. However, Hume *et al*'s recent study found little difference in the success rate of culturing epithelium from explants of dentigerous cysts or OKCs[70)]. The contradictory results between these two studies could be related to differences in culture conditions / techniques, but it does indicate that further *in vitro* experiments, i.e. analysis of growth with different calcium levels and ability to grow with anchorage independence, are required to establish any differences/similarities in the growth capacity between epithelial cells of OKCs and that of other cyst/tumor types.

Ⅶ. Concluding remarks

The classification of odontogenic cysts into two main groups, developmental and inflammatory, implies that lesions arise either from disturbances of normal tooth development or from acquired factors, such as the presence of inflammation. Proliferation of the epithelial rests of Malassez and formation of radicular cysts is known to be initiated by mediators (e.g. IL-1) released during local inflammatory and immune responses consequent upon pulpal necrosis of the associated tooth[71)]. By contrast, little is known about mechanisms underlying the initiation and growth of developmental cysts, in particular that of OKCs. Their epithelial origin and pathogenesis remains speculative. Whilst this review has mainly concentrated on various studies of the epithelial components of OKC, the possible role of mesenchymal influences requires further attention. Such reciprocal induction of dental mesenchyme is believed to be crucial for normal odontogenesis and may play an important role in the maintenance of

epithelial expression in odontogenic cysts.

Our knowledge of the molecular basis with respect to the development of OKCs is still rudimentary. It is, therefore, crucial to identify genes and /or their protein products that may have a role in the pathogenesis of this clinically important lesion. Data accumulated so far appear to indicate that the OKC is independent of mutations in the p53 gene, although the level of p53 protein expression is heightened in its epithelium[33)-36)]. The association of OKC with the inherited, autosomal dominant, Gorlin syndrome may imply pathogenic links or similarities in developmental pathways between isolated and syndrome-associated cases. Both the basal cell carcinoma and other tumors from patients with Gorlin syndrome showed loss of heterozygosity in the exact region to which the Gorlin syndrome gene maps[30)], suggesting that tumors are caused by loss of function of the putative suppressor gene on chromosome 9. However, whether the abnormality of this gene has a part to play in the development of OKC both in isolated cases and in syndrome patients remains to be clarified. The obvious questions are: 1). Does this genetic disorder affect OKC lesions from syndrome and non-syndrome patients? 2). If so, which tissue components (epithelial lining or fibrous capsule, or both) does it target?

Because of their origin within the jaws, majority of odontogenic keratocysts are well established at the time of their diagnosis. It is difficult, therefore, to obtain information concerning the early stages of their pathogenic processes using human surgical material alone. As the suitable animal model of OKC is currently lacking, further efforts in this area would provide opportunities to study in great detail the pathogenesis of early cyst formation.

References

1) Philipsen, H. P.: Om Keratocyster (kolesteatom) i kaeberne. Tandlaegebladet. 60, 963-981, 1956.

2) Main, D. M. G.: The enlargement of epithelial jaw cysts. Odont. Revy., 21, 29-49, 1970.

3) Browne, R. M.: The odontogenic keratocyst. Histological features and their correlation with clinical behavior. Br. Dent. J., 131, 249-259, 1971.

4) Toller, P. A.: Autoradiography of explants from odontogenic cysts. Br. Dent. J., 131, 57-61, 1971.

5) Shear, M.: Cysts of the oral regions. 3rd ed., Wright P. S. G., Bristol, 1992.

6) Ahlfors, E., Larsson, A. & Sjogren, S.: The odontogenic keratocyst: a benign cystic tumor? J. Oral. Maxillofac. Surg., 42, 10-19, 1984.

7) Browne, R. M.: Investigative pathology of the odontogenic cysts. 1st ed., C.R.C. Press, Boston, 1991.

8) Scharffetter, K., Balz-Herrmenn, C., Lagrange, W., Koberg, W. & Mittermayer, C.: Proliferation kinetics-study of the growth of keratocysts. J. Cranio. Max. Fac. Surg., 17, 226-233, 1989.

9) Payne, T. F.: An analysis of the clinical and histological parameters of the odontogenic keratocyst. Oral.Surg., 33, 538-546, 1972.

10) Donatsky, O. & Hjorting-Hansen, E.: Recurrence of the odontogenic keratocyst in 13 patients with the naevoid basal cell carcinoma syndrome. Int. J. Oral. Surg., 9, 173-179, 1980.

11) Vedtofte, P. & Praetorius, F.: Recurrence of the odontogenic keratocyst in relation to clinical and histological features. A 20-year follow-up study of 72 patients. Int. J. Oral. Surg., 8, 412-420, 1979.

12) Woolgar, J. A., Rippin, J. W. & Browne, R. M.: A comparative study of the clinical and histological features of recurrent and non-recurrent odontogenic keratocysts. J. Oral. Pathol., 16, 124-128, 1987.

13) Brannon, R. B.: The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part II. Histological features. Oral.Surg., 43, 233-255, 1977.

14) Voorsmit, R.A.C.A., Stoelinga, P.J.W. &

van. Haelst, U.J.G.M.: The management of keratocysts. J. Maxillofac. Surg., 9, 228-236, 1981.
15) Woolgar, J. A., Rippin, J. W. & Browne, R. M.: A comparative histological study of odontogenic keratocysts in basal cell neavus syndrome and control patients. J. Oral. Pathol., 16, 75-80, 1987.
16) Brannon, R. B.: The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part I. Clinical features., Oral.Surg., 42, 54-72, 1976.
17) Browne, R. M.: The pathogenesis of odontogenic cysts. a review. J. Oral. Pathol., 4, 31-46, 1975.
18) Gorlin, R. J. & Goltz, R. W.: Multiple naevoid basal cell epithelioma, Jaw cysts and bifid rib: a syndrome. New. Eng. J. Med., 262, 908-912, 1960.
19) Browne, R. M.: The pathogenesis of the odontogenic keratocyst. Proc. 4th Int. Academy. Oral. Pathol., 28-38, 1969.
20) Saunders, I. D. F.: Bohn's nodules. A case report. Br. Dent. J., 132, 457-458, 1972.
21) Toller, P. A .: Origin and growth of cysts of the jaws. Ann. R. Coll. Surg. Engl., 40, 306-336, 1967.
22) Harris, M. & Toller, A. P.: The pathogenesis of dental cysts. Br. Med. Bull., 31. 159-163, 1975.
23) Quinn, C. M. & Wright, N. A.: The clinical assessment of proliferation and growth in human tumors: evaluation of methods and applications as prognostic variables. J. Pathol., 160, 93-102, 1990.
24) Hall, P. A. & Levison, D. A.: Review: Assessment of cell proliferation in histological material. J. Clin. Pathol., 43, 184-192, 1990.
25) High, A. S., Quirke, P. & Hume, W. J.: DNA-ploidy studies in a keratocyst undergoing subsequent malignant transformation. J. Oral. Pathol., 16, 135-138, 1987.
26) Li, T.-J., Browne, R. M. & Matthews, J. B.: Expression of proliferating cell nuclear antigen (PCNA) and Ki67 in unicystic ameloblastoma. Histopathology., 26, 219-228, 1995.
27) Li, T.-J., Browne, R. M. & Matthews, J. B.: Proliferating cells in odontogenic jaw cysts and unicystic ameloblastoma. J. Dent. Res., 72, 737A, 1993.
28) Li, T.-J., Browne, R. M. & Matthews, J. B.: Quantification of $PCNA^+$ cells within odontogenic jaw cyst epithelium. J. Oral. Pathol. Med., 23, 184-189, 1994.
29) Li, T.-J., Browne, R. M. & Matthews, J. B.: 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, 1995.
30) Gailani, M. R., Bale, S. J., Leffell, D. J., DiGiovanna,J. J., Peck, G. L., Poliak, S., Drum, M. A., Pastakia, B., McBride, O. W. & Kase, R.: Developmental defects in Gorlin syndrome related to a putative tumor suppressor gene on chromosome 9. Cell., 69, 111-117, 1992.
31) Levine, A. J., Momand, J. & Finlay, C. A.: The p53 tumor suppressor gene. Nature., 351, 453-456, 1991.
32) Lane, D. P. & Benchimol, S.: p53: oncogene or anti-oncogene? Genes. Dev., 4, 1-8, 1990.
33) Ogden, G. R., Chisholm, D. M., Kiddie, R. A. & Lane, D. P.: p53 protein in odontogenic cysts: Increased expression in some odontogenic keratocysts. J. Clin. Pathol., 45, 1007-1010, 1992.
34) Li, T.-J., Browne, R. M., Lawrence, G. M. & Matthews, J. B.: Epithelial cell proliferation in the odontogenic keratocyst. Histochemical. J., 26, 604A, 1994.
35) Slootweg, P. J.: p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J. Oral. Pathol. Med., 24, 393-397, 1995.
36) Li, T.-J., Browne, R. M., Prime, S. S., Paterson, I. C. & Matthews, J. B.: p53 expression in odontogenic keratocyst epithelium. J. Oral. Pathol. Med., 25, 249-255, 1996.

37) Sturzbecher, H. W., Maimets, T., Chumakov, P., Brain, R., Addison, C., Simanis, V., Rudge, K., Philp, R., Grimaldi, M. & Court, W.: p53 interacts with p34 cdc2 in mammalian cells: implications for cell cycle control and oncogenesis. Oncogene., 5, 795-801, 1990.
38) Wu, X., Boyle, J. H., Olson, D. C. & Levine, A. J.: The p53-mdm2 autoregulatory feedback loop. Genes. Dev., 7, 1126-1132, 1993.
39) Mercer, W. E. & Baserga, R.: Expression of the p53 protein during the cell cycle of human peripheral blood lymphocytes. Exp. Cell. Res., 160, 31-46, 1985.
40) Villuendas, R., Piris, M. A., Orradre, J. L., Mollejo, M., Algara, P., Sanchez, L., Martinez, J. C. & Martinez, P.: p53 protein expression in lymphomas and reactive lymphoid tissue. J. Pathol., 166, 235-241, 1992.
41) Farndon, P. A., Del Mastro, R. D., Evans, D. G. R. & Kilpatrick, M. W.: Location of gene for Gorlin syndrome. Lancet., 339, 581-582, 1992.
42) Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. & Krepler, R.: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell., 31, 11-24, 1982.
43) Lane, E. B. & Alexander, C. M.: Use of keratin antibodies in tumor diagnosis. Semin. Cancer. Biol., 1, 165-179, 1990.
44) Smith, A. J. & Matthews, J. B.: Odontogenic epithelium and its residues., In: Investigative pathology of the odontogenic cysts, R. M. Browne, Ed, 54-85, Boston, C.R.C. Press, 1991.
45) Matthews, J. B., Mason, G. I. & Browne, R. M.: Epithelial cell markers and proliferating cells in odontogenic jaw cysts. J. Pathol., 156, 283-290, 1988.
46) Morgan, P. R., Shirlaw, P. J., Johnson, N. W., Leigh, I. M. & Lane, E. B.: Potential applications of antikeratin antibodies in oral diagnosis. J. Oral. Pathol., 16, 212-222, 1987.
47) Gao, Z., Mackenzie, I. C., Williams, D. M., Cruchley, A. T., Leigh, I. & Lane, E. B.: Patterns of keratin-expression in rests of Malassez and periapical lesions. J. Oral. Pathol., 17, 178-185, 1988.
48) Gao, Z., Mackenzie, I. C., Cruchley, A. T., Williams, D. M., Leigh, I. & Lane, E. B.: Cytokeratin expression of the odontogenic epithelia in dental follicles and developmental cysts. J. Oral. Pathol., 18, 63-67, 1989.
49) Hormia, M., Yipaavalniemi, P., Nagle, R. B. & Virtanen, I.: Expression of cytokeratins in odontogenic jaw cysts. J. Oral. Pathol., 16, 338-346, 1987.
50) Magnusson, B. C.: Odontogenic keratocysts: a clinical and histological study with special reference to enzyme histochemistry. J. Oral. Pathol., 7, 8-18, 1978.
51) Chomette, G., Mosadomi, A., Auriol, M. & Vaillant, J. M.: Histoenzymological features of epithelial cells in lesions of oral mucosa in cysts and ameloblastomas of jaws. Int. J. Oral. Surg., 14, 61-72, 1985.
52) Mason, G. I. & Matthews, J. B.: In situ determination of different dehydrogenase activity profiles in the linings of odontogenic keratocysts and radicular cysts. Histochemical. J., 28, 187-193, 1996.
53) Sporn, M. B. & Roberts, A. B.: Peptide growth factors and their receptors I. Berlin, Springer-Verlag, 1991.
54) Heikinheimo, K., Voutilainen, R., Happonen, R.-P. & Miettinen, P. J.: EGF receptor and its ligands, EGF and TGF-α, in developing and neoplastic human odontogenic tissues. Int. J. Dev. Biol., 37, 387-396, 1993.
55) Hu, C.-C., Sakakura, Y., Sasano, Y., Shum, L., Bringas, P., Werb, Z. & Slavkin, H. C.: Endogenous epidermal growth factor regulates the timing and pattern of embryonic mouse molar tooth morphogenesis. Int. J. Dev. Biol., 36, 505-516, 1992.
56) Li, T.-J., Wilson, C., Browne, R. M. & Matthews, J. B.: Expression of proliferating cell nuclear antigen and EGF receptors by odontogenic keratocyst epithelium. Proc. R. Microsc. Soc., 27, 32A, 1992.

57) Li, T.-J., Browne, R. M. & Matthews, J. B.: Expression of epidermal growth factor receptors by odontogenic jaw cysts. Virchows. Archiv. A. Pathol. Anat., 423, 137-144, 1993.
58) Li, T.-J., Browne, R. M. & Matthews, J. B.: TGFa and EGF in odontogenic jaw cysts. J. Dent. Res., 74, 891A, 1995.
59) Li, T.-J., Browne, R. M. & Matthews, J. B.: Immunocytochemical expression of growth factors by odontogenic jaw cysts. JCP., Clin. Mol. Pathol. 1996. (in press)
60) Terada, T., Ohta, T. & Nakanuma, Y.: Expression of transforming growth factor-alpha and its receptor during human liver development and maturation. Virchows. Archiv., 424, 669-675, 1994.
61) Barton, C. M., Hall, P. A., Hughes, C. M., Gullick, W.J. & Lemoine, N. R.: Transforming growth factor alpha and epidermal growth factor in human pancreatic cancer. J. Pathol., 163, 111-116, 1991.
62) Valderhaug. J.: A histologic study of experimentally induced radicular cysts. Int. J. Oral. Surg., 1, 137-147, 1972.
63) Torneck, C. D., Smith, J. S. & Grindall, P.: Biologic effects of endodontic procedures on developing incisor teeth. III. Effect of debridement and disinfection procedures in the treatment of experimentally induced pulp and periapical disease. Oral. Surg., 35, 532-540, 1973.
64) Soskolne, W. A., Bab, J. & Sochat, S.: Production of keratinizing cysts within mandibles of rats with autogenous gingival epithelial grafts. A histologic study. J. Oral. Pathol., 5, 122-128 1976.
65) Ramanathan, J. & Philipsen, H. P.: In vivo behavior of intraosseously implanted oral epithelium in rats. Int. J. Oral. Surg., 10, 180-188, 1981.
66) Atkinson, M. E.: A histological study of odontogenic cysts formed following mouse molar tooth transplantation. J. Oral. Pathol., 5, 347-357, 1976.
67) Al-Talabani, N. G. & Smith, C. J.: Experimental dentigerous cysts and enamel hypoplasia: their possible significance in explaining the pathogenesis of human dentigerous cysts. J. Oral. Pathol., 9, 82-91, 1980.
68) Vedtofte, P., Holmstrup, P. & Dabelsteen, E.: Human odontogenic keratocyst transplants in nude mice. Scand. J. Dent. Res., 90, 306-314, 1982.
69) Stenman, G., Magnusson, B., Lennartsson, B. & Juberg-Ode, M.: In vitro growth characteristics of human odontogenic keratocysts and dentigerous cysts. J. Oral. Pathol., 15, 13-145, 1986.
70) Hume, W. J., Moore, J. K. & Main, D. M. G.: Differences in in vitro growth of epithelium from inflammatory and developmental odontogenic cysts. Br. J. Oral. Maxillofac. Surg., 28, 85-88, 1990.
71) Bando, Y., Henderson, B., Meghji, S., Poole, S. & Harris, M.: Immunocytochemical localization of inflammatory cytokines and vascular adhesion receptors in radicular cysts. J. Oral. Pathol. Med., 22, 221-227, 1993.