p53 protein in odontogenic cysts: Increased expression in some odontogenic keratocysts

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Abstract

Aims: To assess p53 protein expression in a range of odontogenic cysts arising in the mouth, including those of developmental and inflammatory origin.

Methods: p53 protein was identified using the polyclonal antibody CM-1, together with a standard immunoperoxidase technique. A total of 36 cystic lesions were examined, all of which were histologically benign.

Results: Expression of p53 protein was identified within the lining of five of 12 odontogenic keratocysts but was not detected in the other cystic lesions in the series.

Conclusions: This is believed to be the first report that identifies increased expression of p53 protein in benign cystic epithelium. The increased expression of p53 protein in the nucleus is usually associated with malignant disease. These findings are relevant to the management of odontogenic keratocysts which have a tendency to recur, and also to Gorlin Goltz syndrome in which keratocysts and multiple basal cell carcinomas are features.

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genic cysts: odontogenic keratocysts, dentigerous cysts, and radicular cysts. Histologically, the odontogenic keratocyst is characterised by an epithelial lining five to eight cells thick, with a prominent basal cell layer of columnar or cuboidal cells (fig 1). The keratinisation of the epithelium is predominantly parakeratotic, although sometimes orthokeratosis is seen. The cyst wall is usually thin unless inflammation is present. The surrounding connective tissue may contain islands of epithelium or separate small cysts.¹⁴⁻¹⁶ This cyst is of particular interest because of the reported high rate of mitotic activity within its lining,¹⁷ its tendency to recurrence,^{14, 18} and the association with Gorlin Goltz syndrome,¹⁹ in which multiple naevoid basal cell carcinomas may develop. It is important to note also that some odontogenic keratocyst linings show epithelial dysplasia and it is generally accepted that keratinising cysts have a greater tendency to malignant transformation than others.¹⁵ Dentigerous cysts are found near the crown of an unerupted tooth. Histologically, the cyst wall is composed of a thin layer of connective tissue lined by stratified squamous epithelium or by epithelium that is only two to three cells thick. The epithelium may include mucus producing cells and sometimes ciliated cells. In some cases the cyst lining is keratinised.¹⁶ Odontogenic kera-

p53 protein was first reported by Lane and Crawford in 1979.¹ It is a product of the p53 gene which is now classified as a tumour suppressor gene.^{2, 3} The gene seems to be a frequent target for mutation,⁴ being seen as a common step in the pathogenesis of most human cancers.⁵ In normal cells p53 protein has a very short half life,⁶ such that it cannot be detected immunohistochemically.⁷ Mutation of the p53 gene often results in increased stability of the gene product (p53 protein).⁸ Thus the mutant form can be detected using immunohistochemical techniques; numerous studies report that it has been identified in malignant cells but not in normal cells.⁹⁻¹²

We examined a range of oral mucosal lesions and only identified p53 expression in patients with oral cancer.¹³ However, cystic lesions affecting the oral cavity have not been previously examined for the presence of p53. The epithelial cells which comprise the lining of odontogenic cysts are derived from the primitive oral epithelium of the oral mucosa and contribute to tooth formation. During and after this process such epithelia are a common source of cystic change within the jaw bones. This study examines three types of onto-

Figure 1 Classic histopathological features displayed by the odontogenic keratocyst obtained from case 4 (table 2) and illustrated in fig 2 and 3.

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Table 1 Expression of p53 in cysts of the oral cavity

Cyst type	Examined	Positive	Negative
Radicular cyst	12	0	12
Dentigerous cyst	12	0	12
Odontogenic keratocyst	12	5	7

tocyst and dentigerous cyst are both developmental cysts, but radicular cysts are inflammatory in origin. They arise as sequelae of dental caries from the effects of inflammation on epithelial residues situated in the apical portion of the periodontal ligament. They are the most common cysts found within the jaw, usually associated with a non-vital tooth. Histologically, most radicular cysts are lined by a non-keratinising stratified squamous epithelium. Other features include mucus metaplasia, hyaline bodies, cholesterol clefts with an associated foreign body giant cell reaction and an inflammatory cell infiltrate.¹⁶

Methods

This study was based on the examinations of 36 odontogenic cysts, all of which were routine surgical specimens fixed in 10% neutral buffered formalin. The cysts comprised 12 radicular cysts (three mandibular, nine maxillary), 12 dentigerous cysts (six each from the mandible and maxilla), and 12 odontogenic keratocysts (10 mandibular, two maxillary). For each specimen, sections were chosen randomly from paraffin wax embedded blocks which had been stained with haematoxylin and eosin p53 expression before. Four 5 µm sections were cut from each block and dried overnight at 37°C. The specimens were dewaxed and then washed in TRIS-buffered saline (TBS) and incubated overnight at 4°C, with the polyclonal antibody CM-1 (diluted 1 in 1000 of 10% fetal calf serum in phosphate buffered saline (PBS)). CM-1 is a rabbit polyclonal antibody raised against the whole p53 protein.²⁰ A biotinylated anti-rabbit immunoglobulin was applied for one hour before applying the avidin:biotin complex (Vectastain ABC Kit, Vector Labs, England) for one hour at room temperature. The specimens were then incubated with a solution of

diaminobenzidine and hydrogen peroxide in PBS for 10 minutes. A brown precipitate seen in the nucleus confirmed the presence of the p53 protein. The tissue specimens were not counterstained.

Two negative controls were used in each case. For one section a solution of CM-1 previously blocked with p53 protein was applied, whilst normal goat serum was applied to the second section.

Further staining of the odontogenic keratocysts was carried out using the monoclonal antibody PC10, which recognises the proliferating cell nuclear antigen (PCNA). This was used at a dilution of 1 in 250. A biotinylated anti-mouse immunoglobulin was applied instead of the biotinylated anti-rabbit immunoglobulin used with CM-1.

To obtain an indication of the rate of cell division, the number of suprabasal mitoses was counted in a randomly selected area of 1500 epithelial cells in each specimen of odontogenic keratocyst, as described by Browne.⁴

Results

The results for p53 expression in a variety of oral cysts are given in tables 1 and 2. None of the inflammatory radicular cysts nor developmental dentigerous cysts expressed p53. In each case the histopathological features were typical and unremarkable. The only cyst type in which p53 was detectable was the odontogenic keratocyst (fig 2) and a positive result was obtained in five of 12. Staining with the PCNA antibody (PC10) indicated that the p53 positive cells were actively dividing, because similar regions were positive for both antibodies (fig 3). CM-1 positivity was identified in most of the basal cells; staining with PC10 was much more intense, being present in all basal cells and most parabasal cells. All odontogenic keratocysts were positive for PCNA. Two of the five cases positive for p53 were recurrent lesions compared with five of the seven p53 negative keratocysts. One female patient had features associated with the Gorlin Goltz syndrome (table 2), namely hypertelorism, spina bifida, and odontogenic keratocysts. However, to date, no basal cell neoplasms have

Table 2 Characteristics of the odontogenic keratocysts assessed for p53 expression

Cyst No	Patient			Multiple cysts	Recurrent cysts	Histopathological features	p53 expression
	Age	Sex	Site				
1	24	F	L maxilla (molar)	+	—	Moderate focal inflammation mild epithelial hyperplasia	+
2	14	F	L mandible (molar)	—	—	Mild focal inflammation	+
3	16	F	L maxilla (premolar)	—	—	Mild epithelial hyperplasia	+
4	75	M	L mandible (ramus)	+	+	Mild diffuse inflammation	+
5	48	F	L mandible (body)	—	—	Mild focal inflammation	+
6	21	F	L mandible (molar)	+	—	Moderate focal inflammation Mild epithelial hyperplasia Satellite cysts (Types 1 and 2)	—
7	45	F	L mandible (ramus)	+	—	Satellite cyst (Type 1)	—
8	59	F	R mandible (ramus)	—	—	Mild focal inflammation Cholesterol, hyaline	—
9	16	M	L mandible (ramus)	—	—	Mild focal inflammation Satellite cyst (Type 1), hyaline	—
10	35	M	R mandible (ramus)	+	—	Mild diffuse inflammation Cholesterol, hyaline	—
11	75	M	L mandible (angle)	+	+	Mild focal inflammation	—
12	63	F	L mandible (premolar)	+	+	Mild diffuse inflammation Satellite cysts (Types 1 and 3)	—

Figure 2 Identification of p53 positive cells using CM-1 in most basal cells in the lining of a recurrent odontogenic keratocyst (case 4).

presented in this case. Of the two cysts examined from this patient with Gorlin Goltz syndrome, the first, removed from the mandibular molar region, proved p53 positive. The other cyst, removed three years later from the molar region of the maxilla, was p53 negative.

Two cysts from the same elderly male patient, removed at one operation also behaved differently. The first, removed from the angle of the mandible, was p53 negative; the second, removed from the ramus of the mandible, on the same side as the first, was p53 positive. Three of the 12 cysts were recurrent, and of these one was p53 positive. A history of multiple cysts of the jaws was given by two of five patients with cysts showing p53 expression. This history was noted in five of seven patients with cysts that did not express p53.

By definition, all odontogenic keratocysts reported in this paper had typical histopathological features. A striking feature was the remarkable consistency of the histopathological appearance by the epithelium. All cyst capsules showed mild to moderate non-specific chronic inflammatory cell infiltration. Of the seven odontogenic keratocysts in which p53 expression was absent, six capsules or linings contained either cholesterol clefts, hyaline bodies, or satellite cysts. The latter feature

Figure 3 Identification of PCNA using PC10 in the basal and parabasal cells of the p53 positive recurrent odontogenic keratocyst illustrated in fig 2.

comprises three types¹⁴ and each, in turn, represents a proliferative, mature, or degenerative phase in the life cycle of the cyst. Interestingly, the five linings which were p53 positive lacked the presence of cholesterol, hyaline, and satellite cysts in the sections examined. However, a larger series will be necessary before a firm conclusion with regard to the clinical relevance of this finding can be drawn. The number of suprabasal mitotic figures per 1500 cells varied from 0 to 4 but no difference could be made between the groups p53 positive and p53 negative linings.

Thus the two groups of odontogenic keratocysts could not be distinguished in terms either of patient medical or dental history, including age and sex, nor recurrence or mitotic activity of the cyst lining.

Discussion

Many papers have recently reported the association of p53 protein with a variety of different malignant tumours.³⁻⁵ However, it is important to emphasise that the CM1 antibody recognises both wild and mutant forms of p53. Thus identification of p53 in the lining of some odontogenic keratocysts should not necessarily imply an association with malignant disease. It is known that the cellular environment can affect the stability of p53.⁶ For example, the enzymatic pathways for p53 degradation may be inactivated.²¹ Certain viruses, such as adenovirus, can also stabilise p53 in non-malignant tissue.²² Furthermore, because CM1 recognises the whole p53 protein it is more likely to detect its presence than monoclonal antibodies that are directed against specific epitopes of the p53 protein. CM1 seems to produce a much stronger "signal" than some of the earlier p53 monoclonal antibodies.²¹ Hence it seems to be more sensitive. Certainly the staining seen in odontogenic keratocysts is much weaker than that seen in oral cancers.¹³ Various factors peculiar to the keratocyst may help to explain the occurrence of p53 positivity. Firstly, the cyst has a tendency to recur, suggesting increased epithelial activity.¹⁴ Although the rate of recurrence may depend more on the method of treatment,²³ recurrence up to 60% has been reported.¹⁵ This may in part be due to its high mitotic rate. Main found an average of eight mitoses for every 1 cm of lining examined (range 0-22), which was much higher than the other cysts he examined¹⁷ (although Browne¹⁴ calculated a rate of 3.9 in his study of 130 primary and nine recurrent keratocysts). The position of these mitoses may also be influential. The number of mitoses situated in the basal region of oral epithelium from various sites has been estimated to be between 30-50%.²⁴⁻²⁶ However, only 10% were found in the basal region of odontogenic keratocysts as reported by Browne¹⁴ and the present study supports this view. If PCNA positivity can be related to cell proliferation,²⁷ then our results would support the finding of increased nuclear activity.

The other factor of possible importance is the association between odontogenic kerato-

cysts and Gorlin Goltz syndrome, an autosomal dominant genetic disorder with high penetrance.²⁸ The syndrome has variable expression, and includes odontogenic keratocysts, basal cell naevi/carcinomas, bifurcation of the ribs, rib or vertebral fusion, spina bifida, intracranial calcification, hypertelorism, mental retardation, cleft lip and palate and palmar and plantar pitting.^{18 19 29} The lack of consistency of these physical signs in the syndrome^{18 19 29} would tend to support the opinion of Browne¹⁴ that the occurrence of jaw cysts represents the minimal expression of the syndrome. However, this remark may have been based on his finding of no difference in the histology of single, multiple, or Gorlin Goltz syndrome associated keratocysts. Since then differences have been found in the histology of odontogenic keratocysts that presented as single cysts compared with those in the Gorlin Goltz syndrome,³⁰ principally an increase in mitotic activity in the lining of cysts from the Gorlin Goltz syndrome.

p53 expression may therefore be a marker for the syndrome particularly as p53 expression has previously been associated with malignancy, and the syndrome is associated with development of basal cell carcinomas. However, not all cysts removed from our one case of Gorlin Goltz syndrome were p53 positive. One possibility is that p53 positivity may be indicative of potential malignant change within the cyst lining. Malignant transformation in jaw cysts, although rare, is thought to occur more frequently in keratinising cysts^{16 30 31} and has been reported in odontogenic keratocysts.^{32 33} A more extensive study of keratocysts from Gorlin Goltz syndrome and, in particular, those cases associated with basal cell neoplasms is now required: particularly as other tumours may be associated with the Gorlin Goltz syndrome.³⁴ It has been suggested that there might be a hereditary embryological maldevelopment in these patients which predisposes them to further cysts and recurrences. It may be important, then, that of the odontogenic cysts examined in this study, only odontogenic keratocysts expressed p53 positive cells, a cyst whose characteristics have been compared with that of an aggressive tumour.³⁴

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