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## ORIGINAL ARTICLE

# Expression of basement membrane components in odontogenic cysts

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**OBJECTIVE: To compare the expression of basement membrane components (BMCs), including laminins 1 and 5, collagen type IV, and fibronectin in odontogenic keratocysts (OKCs) with dentigerous cysts (DCs) and radicular cysts (RCs).**

**MATERIALS AND METHODS: Basement membrane components were analysed in 20 OKCs, 20 DCs and 20 RCs using an immunohistochemical technique.**

**RESULTS: Odontogenic keratocysts, DCs and RCs showed positive reaction to all BMCs studied, with different distributions and intensity. OKCs showed continuous linear deposits for laminins 1 and 5 but two staining patterns (continuous and discontinuous) for collagen type IV and fibronectin. DCs exhibited continuous linear deposits for laminins 1 and 5 and collagen type IV but a discontinuous linear deposit for fibronectin. RCs displayed similar results to DCs for laminin 1, collagen type IV and fibronectin. Laminin 5 in RCs had two staining patterns. Constant results in all cysts were strong intensity for laminin 1 and moderate intensity for laminin 5.**

**CONCLUSIONS: Substantial differences in the expression of BMCs among studied cysts were not observed, suggesting that the separation of the epithelial lining in OKCs is not associated with the existence of these proteins.**

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**Keywords:** collagen type IV; fibronectin; laminin 1; laminin 5; odontogenic cysts

## Introduction

Basement membrane (BM) is the organized extracellular matrix (ECM) that separates the epithelium from the fibrous connective tissue in various tissues, e.g. skin, mucosa and blood vessels. Apart from its role in mechanical function, BM also modulates the behaviour of the cells present on it. Several components including collagens (type I, III, IV, V, VII and XVII), laminins 1, 5 and 6, fibronectin, nidogen and heparan sulphate proteoglycan are present in the BM zone (for review, see Timpl, 1996; Burgeson and Christiano, 1997).

The essential function of BM has been demonstrated in some autoimmune diseases such as mucous membrane pemphigoid, epidermolysis bullosa and linear IgA disease. These diseases are characterized by the separation of the whole thickness of the epithelium from the underlying connective tissue, resulting in vesicles visible in the oral mucosa. BM is the target area as demonstrated by deposition of immunoglobulin (Ig)G and complement components in this region. For example, there is an accumulation of IgG and C3 at laminin 5 and bullous pemphigoid antigen 180 (BP 180 or type XVII collagen) in mucous membrane pemphigoid. Collections of IgG at collagen type VII are detected in the epidermolysis bullosa acquisita (Regezi and Sciubba, 1999).

Separation of the epithelial lining from the underlying connective tissue wall is frequently observed in odontogenic keratocysts (OKCs) (Browne, 1971; Danoff *et al*, 1972), but not in other odontogenic cysts (OCs). Besides the distinct histopathological features, OKCs also have a relatively higher recurrent rate compared with most OCs (Brannon, 1976; Vedtofte and Praetorius, 1979; Kondell and Wilberg, 1988; Crowley *et al*, 1992). Furthermore, the idea that OKC may be a benign tumour rather than a simple cyst has been suggested because some OKCs penetrate cortical bone (Jackson *et al*, 1993) and involve surrounding soft tissue (Emerson *et al*, 1972; Partridge and Towers, 1987). Ultrastructural study has demonstrated that deep to the lamina densa, the collagen shows signs of dissolution and often completely disappears (Philipsen *et al*, 1976). This result suggests that the defect in collagens may be responsible for the separation of the epithelial lining in OKCs. Nevertheless, it is not known whether this phenomenon contributed to the recurrence of these cysts.

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Recently, an expression of tenascin and fibronectin at the epithelial mesenchymal junction in OCs including, 10 cases of OKCs, 10 cases of dentigerous cysts (DCs) and 10 cases of radicular cysts (RCs), has been studied by the immunohistochemical procedure (de Oliveira *et al*, 2004). It was found that the expression of these two proteins, particularly in the cystic wall, was more intense in OKCs than the rest. They suggest that these differences may contribute to the aggressive behaviour of OKCs. Although the pivotal role of BM has been demonstated in some diseases showing separation of the epithelium, the expression of BM components in OKCs has received little attention. This study investigated the expression of major BM components, namely laminins 1 and 5, collagen type IV and fibronectin in OCs using immunohistochemistry.

## Materials and methods

The sample in this study consisted of 20 OKCs, 20 DCs and 20 RCs from the files of the Department of Oral Pathology, Faculty of Dentistry, Mahidol University. Haematoxylin and eosin-stained sections of these cysts were reviewed by two oral pathologists to confirm the diagnoses based on the criteria in the World Health Organization (WHO) *Histological Typing of Odontogenic Tumors* (Kramer *et al*, 1992). New sections of 5 $\mu$m thickness were cut from the formalin-fixed paraffin-embedded blocks and mounted on glass slides coated by aminopropyltriethoxysilane (APES; Sigma Chemical Co., St Louis, MO, USA).

Immunohistochemical analyses were performed using the standard streptavidin–biotin peroxidase technique. Sections were deparaffinized and dehydrated. Endogenous peroxidase activity was blocked with 10-min incubation in 3% $H_2O_2$. Antigen retrieval was done by immersing the sections in 0.4% pepsin (Sigma Chemical Co) in 0.01 M HCl at 37°C, 1 h for antibodies against laminin 1, collagen type IV and fibronectin and by covering the sections with proteinase K (Dako Corporation, Carpinteria, CA, USA) at room temperature, 15 min for antibody against laminin 5. After washing with 0.1% Tween 20 (MERCK-Schuchardt, Hohanbrunn, Germany) in phosphate-buffered saline (PBS), the sections were treated with 2% bovine serum albumin (BSA; Sigma Chemical Co) in PBS for 30 min and then treated with primary antibodies for 2 h at room temperature. The primary antibodies used in this study were against laminin 1 (Sigma Chemical Co) diluted at 1:60, laminin 5 (Chemicon International, Tamecula, CA, USA) diluted at 1:300, collagen type IV (Dako S/A, Glostrup, Denmark) diluted at 1:25 and fibronectin (Dako S/A) diluted at 1:400. The primary antibodies were diluted in PBS. After thorough washing in 0.1% Tween 20 in PBS, biotinylated IgG and streptavidin–biotin peroxidase complex (strepABComplex/HRP Duet kit; Dako S/A) were applied to the sections for 30 min each and followed by three washes of 0.1% Tween 20 in PBS. Colour was developed in freshly prepared diaminobenzidine (DAB Chromogen tablets; Dako Corporation). Sections were washed briefly in running tap water and lightly stained with Mayer's haematoxylin. Negative controls were done by omission of the relevant primary antibody. Sections of normal oral mucosa were stained at the same run as positive controls for comparison. Additionally, blood vessels present within each section served as an internal control for staining with antibodies against laminin 1, collagen type IV and fibronectin.

The immunoreactivity was evaluated in term of location, pattern, and intensity. The degree of intensity was categorized into three grades: weak, moderate and strong.

## Results

Odontogenic keratocysts, DCs and RCs expressed all BM components, including laminins 1 and 5, collagen type IV and fibronectin. The immunoreactivity of these proteins was observed as a linear deposit at the epithelial–connective tissue junction. Nevertheless, continuity, intensity and staining patterns varied, depending on the type of cysts and BM components (Tables 1 and 2).

Sections of normal oral mucosa that were stained for comparison expressed all BM components. A distinct linear deposit of strong intensity at the BM junction was seen in normal oral mucosa stained with laminins 1 and 5. Collagen type IV was localized at the junction with moderate intensity compared with its expression at the periphery of blood vessels and those of laminins 1 and 5. Fibronectin was present as a continuous linear deposit at the basal aspect of basal cells as well as at the periphery of blood vessels.

Most OKCs showed a strong continuous thick linear deposit of laminin 1 (Figure 1a). The staining pattern of laminin 5 in OKCs was very consistent, as only a continuous thin-sharp linear deposit of moderate intensity was found (Figure 2a). Unlike the pattern observed in those of laminins, two staining patterns were seen in OKCs stained with antibody to collagen type IV: a continuous linear deposit of strong intensity (10/18) (Figure 3a) and a discontinuous linear deposit of weak

**Table 1** Expression and continuity of basement membrane components in OCs

| *Type of basement membrane components* | *Type of odontogenic cysts* | *Expression (cases)* | | *Continuity (cases)* | |
|---|---|---|---|---|---|
| | | *Presence* | *Absence* | *Continuity* | *Discontinuity* |
| Laminin 1 | OKCs | 20 | 0 | 16 | 4 |
| | DCs | 20 | 0 | 16 | 4 |
| | RCs | 20 | 0 | 15 | 5 |
| Laminin 5 | OKCs | 20 | 0 | 20 | 0 |
| | DCs | 20 | 0 | 15 | 5 |
| | RCs | 20 | 0 | 10 | 10 |
| Collagen type IV | OKCs | 18 | 2 | 10 | 8 |
| | DCs | 20 | 0 | 15 | 5 |
| | RCs | 17 | 3 | 13 | 4 |
| Fibronectin | OKCs | 17 | 3 | 7 | 10 |
| | DCs | 18 | 2 | 0 | 18 |
| | RCs | 15 | 5 | 0 | 15 |

**Table 2** Staining pattern and intensity of basement membrane components in OCs

| *Type of basement membrane components* | *Type of odontogenic cysts* | | |
|---|---|---|---|
| | *OKCs* | *DCs* | *RCs* |
| Laminin 1 | Thick linear deposit/strong | Thick linear deposit/strong | Thick linear deposit to band/strong |
| Laminin 5 | Thin sharp linear deposit/moderate | Thin sharp linear deposit/moderate | Thin sharp linear deposit/moderate[a] or thin sharp linear deposit/weak[b] |
| Collagen type IV | Sharp linear deposit/strong[a] or thin irregular/weak[b] | Thin linear deposit/weak | Sharp linear deposit/strong |
| Fibronectin | Thin sharp linear deposit/moderate[c] | Thin sharp linear deposit/moderate | Thin sharp linear deposit/moderate to thick linear deposit/strong |

In case of two staining patterns: [a]continuous linear deposit, [b]discontinuous linear deposit, [c]continuous and discontinuous linear deposits.

intensity (8/18). The latter pattern could be seen as a very thin linear deposit and a ragged linear deposit. Fibronectin immunoreactivity was expressed either as a continuous (Figure 4a) or as a discontinuous thin linear deposit (Figure 5) of moderate intensity. Interestingly, cases that showed discontinuous linear deposits for collagen type IV also demonstrated discontinuous linear deposits for fibronectin.

Most DCs showed a continuous linear deposit at the BM junction for laminin 1 (Figure 1b), laminin 5 (Figure 2b) and collagen type IV (Figure 3b). However, the intensity was strong only for laminin 1 while it was moderate for laminin 5 and weak for collagen type IV. All DCs, that were positive for fibronectin, showed discontinuous linear deposits (Figure 4b).

The majority of RCs showed uninterrupted linear staining pattern for laminin 1 (Figure 1c) and collagen type IV (Figure 3c). The intensity of laminin 1 in these cysts was very strong. Additionally, in some cases, it had a band-like appearance (Figure 1c). For laminin 5 expression, half of the RCs showed a moderate intensity and continuous linear deposit (Figure 2c), while the rest had a weak irregular staining style. In RCs stained with fibronectin antibody, a discontinuous linear deposit of moderate intensity was often noticed; however, the thick linear deposit of strong intensity was also found particularly when the fibrous wall underneath was not heavily inflamed (Figure 4c).

Besides the staining at the epithelial–connective tissue junction, fibronectin was also present throughout the fibrous connective tissue wall of all studied cysts (Figure 4). Nevertheless, in some cysts (most were DCs), the staining at the juxtaepithelial area was lost; the absence was often found to be associated with hyalinization in this area (Figure 4b). The juxtaepithelial area demonstrated two staining patterns – including fibrillar and reticular patterns. The fibrillar style was the dominant pattern, while the reticular pattern was frequently detected in RCs. The staining pattern of the rest of the fibrous wall revealed either the fibrillar fashion or fibres that ran parallel to the epithelial lining. Most of studied cysts showed combined patterns.

## Discussion

Similar to the normal mucosa, OKCs, DCs and RCs expressed all BM proteins including laminins 1 and 5, collagen type IV and fibronectin. The presence of BM proteins studied in these OCs is consistent with the expression of BM in dental lamina of developing mouse and human teeth in previous studies (Thesleff *et al*, 1979, 1981; Sahlberg *et al*, 1992, 1998; Salo *et al*, 1999).

The expression of fibronectin at the junction in studied cysts is consistent with the study of de Oliveira *et al* (2004) in that, approximately half of the OKCs, and all the DCs and RCs, showed discontinuous linear deposits. Nevertheless, the present study found that it was in some RCs, and not OKCs as demonstrated by de Oliveira *et al* (2004), that staining intensity was stronger than in other cysts. de Oliveira *et al* (2004) proposed that the strong intensity of fibronectin expression found in the capsule of OKCs is responsible for the aggressive behaviour of OKCs. In addition to the difference in the fibronectin intensity, the staining pattern of this protein in the fibrous capsule was also inconsistently reported. da Silva *et al* (2002) demonstrated that fibronectin expression in the fibrous wall of OKCs was seen as a non-fibrillar substance. On the contrary, the present study and that of de Oliveira *et al* (2004) found that a fibrillar arrangement is the main feature of fibronectin in the fibrous wall of OKCs.

These conflicting findings in fibronectin expression are probably because of the difficulty of interpreting this protein in OCs. Fibronectin was seen both at the junction and throughout the cystic wall. In some cysts, staining was also present in the epithelial lining (data are not shown). Furthermore, the absence of fibronectin expression at the juxtaepithelial fibrous wall associated with hyalinization in many DCs makes interpretation and comparison of the staining intensity in the fibrous walls among these cysts questionable.

Because OKCs did not show marked differences in the expression of the investigated BM proteins compared with DCs and RCs, it is unlikely that the separation of the epithelial lining in OKCs is dependent on the presence of these proteins. Therefore, other BM components may be involved in this process. Another possibility is that the defect responsible for the separation is associated with the integrin receptors of the BM proteins examined, as demonstrated in junctional epidermolysis bullosa (JEB). The mutation of the $\beta 4$ integrin receptor for laminin 5 causes severe JEB (Niessen *et al*, 1996). $\beta 4$ knockout mice lacked hemidesmosome and showed widespread dermo–epidermal

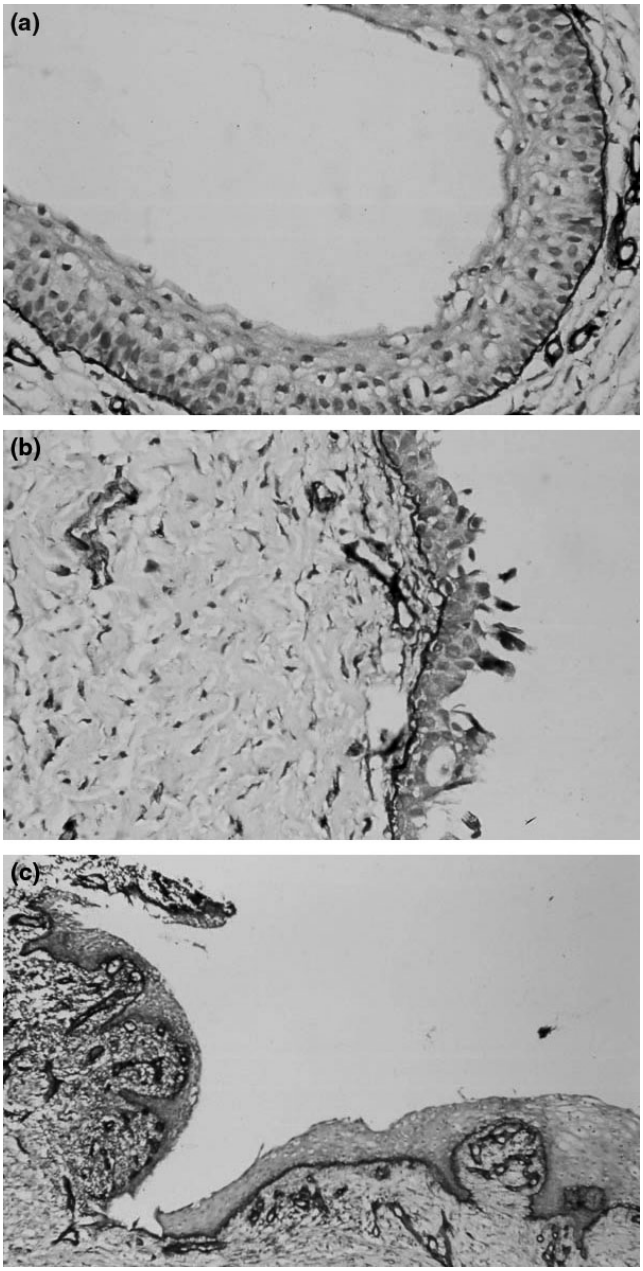


**Figure 1** Immunohistochemical staining of laminin 1 at the basement membrane zone in OKCs (**a**), DCs (**b**) and RCs (**c**). The strong and thick linear deposit was observed in all cysts. Note the band-like staining in RCs, allowing the visualization under the low power objective (streptavidin–biotin, original magnification: **a** and **b**: ×400; **c**: ×100)

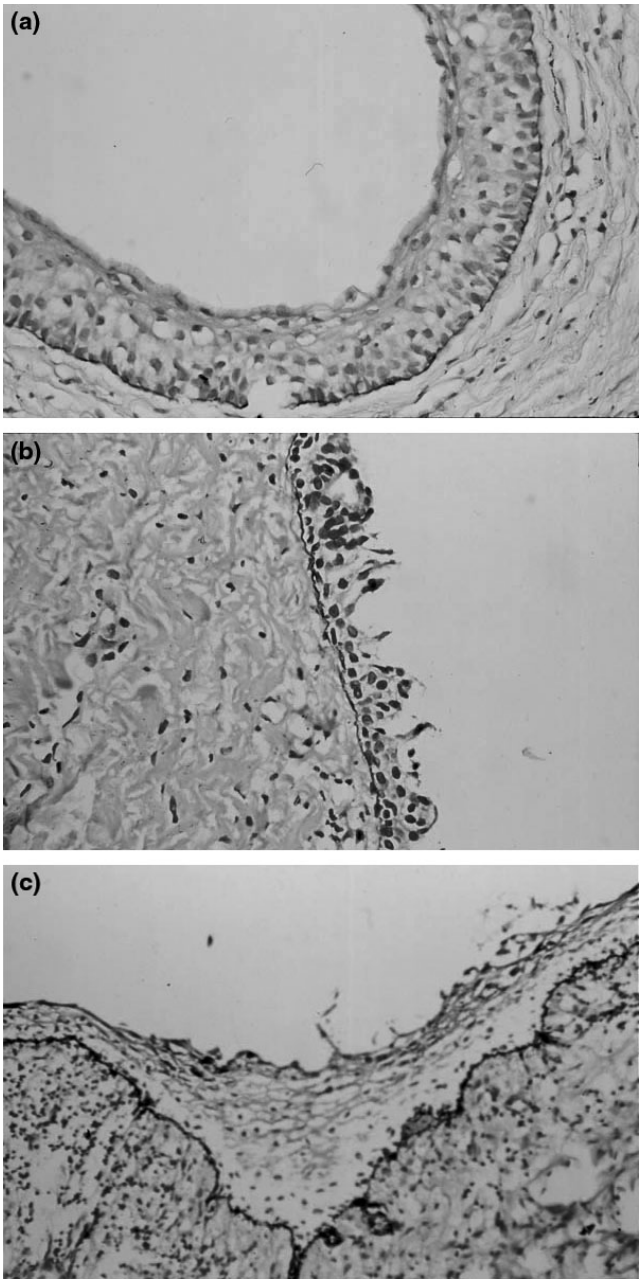


**Figure 2** A sharp linear deposit of laminin 5 at the basement membrane zone in OKCs (**a**), DCs (**b**) and RCs (**c**) (streptavidin–biotin, original magnification: **a** and **b**: ×400; **c**: ×200)

separation (Dowling *et al*, 1996; van der Nuet *et al*, 1996).

Compared with DCs and RCs, the discontinuity of collagen type IV staining and the continuity of fibronectin staining were more frequently observed in OKCs. As ECM can regulate cellular behaviour via integrins (Hynes, 1992), it is possible that the pattern of expression of collagen type IV and fibronectin at the BM in OKCs is involved in the aggressive behaviour of OKCs. This statement is consistent with some previous studies (da Silva *et al*, 2002; de Oliveira *et al*, 2004). de Oliveira *et al* (2004) suggested that the increased intensity of tenascin in the fibrous wall of OKCs than other OCs may cause instability in the cystic structure and may thus be responsible for the aggressive behaviour of OKCs. da Silva *et al* (2002) found that tenascin was expressed only in the subepithelial wall of OKCs, but not in the orthokeratinized odontogenic cysts that have a benign behaviour. Hirshberg *et al* (1999) reported that collagen fibres found in the fibrous wall of OKCs were structurally disorganized similar to those reported in odontogenic tumours, but were different from those seen in DCs and RCs.

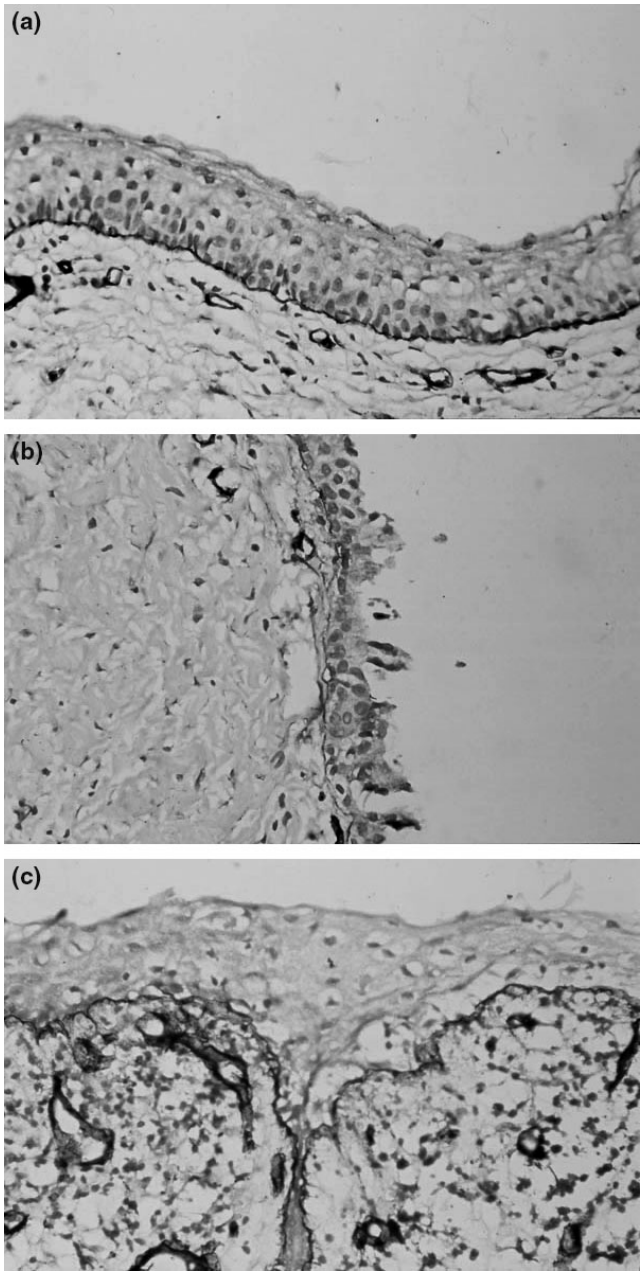


**Figure 3** A linear deposit of collagen type IV at the basement membrane zone in OKCs (**a**), DCs (**b**) and RCs (**c**). Note the weak and irregular pattern in DCs (streptavidin–biotin, original magnification: ×400)

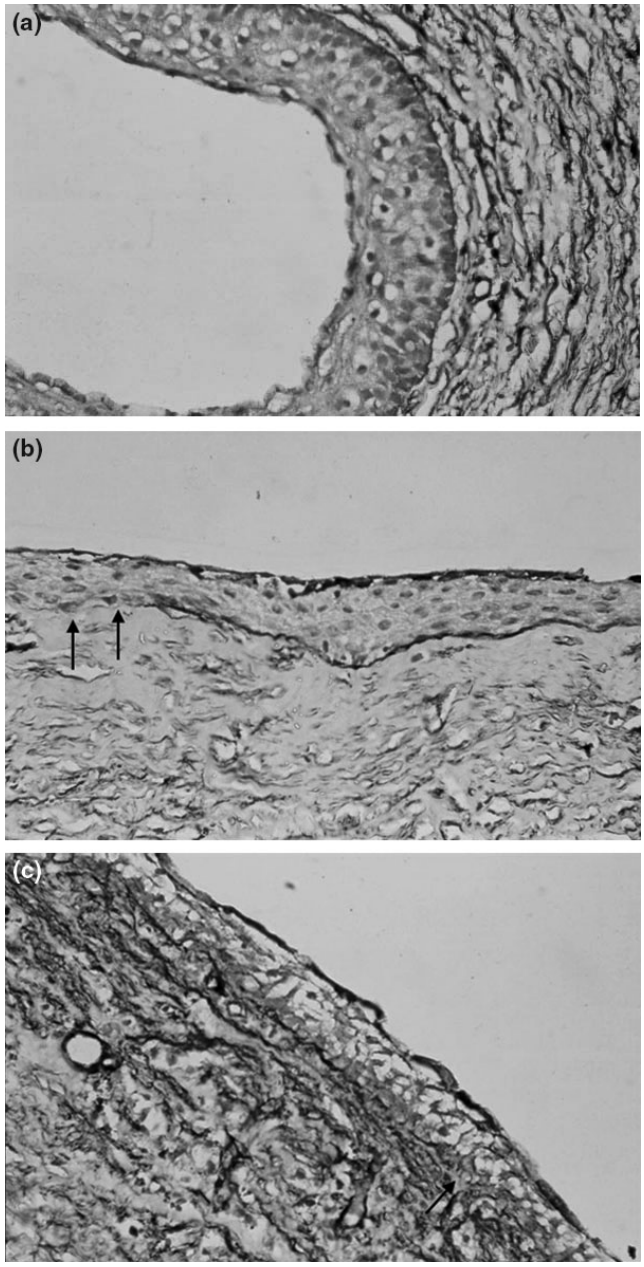


**Figure 4** Distributions of fibronectin in OKCs (**a**), DCs (**b**) and RCs (**c**). A linear deposit is seen at the basement membrane zone, while a fibrillar fashion is found in the fibrous wall. The discontinuous linear deposit is characteristic of DCs and RCs. Arrows indicate loss of expression. Note the staining in the fibrous wall is more intense in RCs (streptavidin–biotin, original magnification: ×400)

Besides the influence of ECM, several factors have been postulated to be involved in the aggressive behaviour of OKCs. Examples include an increase in the level of proliferative markers, e.g. proliferating cell nuclear antigen (Li *et al*, 1994; Piatteli *et al*, 1998), Ki-67 (Slootweg, 1995), argyrophilic nucleolar organizer regions (Hume *et al*, 1990; Allison and Spencer, 1993), alteration of p53 proteins (Matthews *et al*, 1995; Slootweg, 1995; Li *et al*, 1996), and the high recurrent rate partly because of the difficulty in the complete removal of the thin and friable wall of OKCs.

Multiple OKCs are the most common findings in naevoid basal cell carcinoma syndrome (NBCCS), occurring in 65–100% of patients (Lovin *et al*, 1991; Reisner *et al*, 1994). A clinical significance is that OKCs in NBCCS have been reported to more likely recur than sporadic OKCs (Woolgar *et al*, 1987; Mustaciuolo *et al*, 1989; Gorlin, 1995; Stoelinga, 2001). However, the development of new cysts may be confused with recurrence (Stoelinga, 2001). With

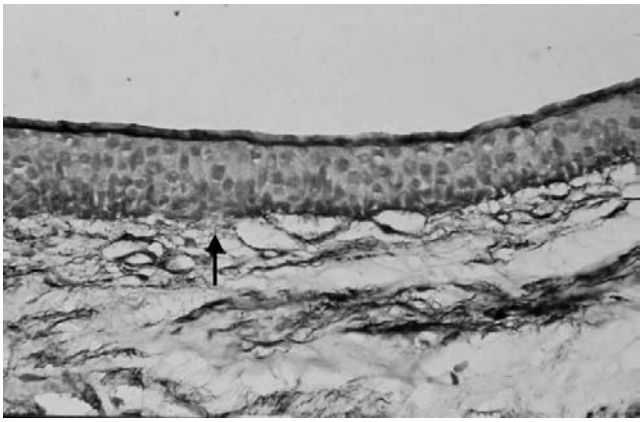

**Figure 5** The discontinuous staining pattern of fibronectin at the basement membrane zone in OKCs. Arrow indicates loss of expression. Note the staining in the fibrous wall is less intense than that of RCs (Figure 4c) (streptavidin–biotin, original magnification: ×400)

regard to histopathological features, budding of the basal cell layer of the cystic lining and the high number of daughter cysts in the connective tissue wall were more often observed in syndromic OKCs (Shear, 1992). Expression of cytokeratin 17 in syndromic OKCs was stronger and more uniform than sporadic OKCs (Meara *et al*, 2000). Thus, cytokeratin 17 may be a useful marker to separate syndromic OKCs from sporadic OKCs. As discussed previously, it appears that several aspects are different between sporadic OKCs and OKCs associated with NBSCC. We agree with de Oliveira *et al* (2004) and da Silva *et al* (2002) that ECM may contribute to the aggressive behaviour of OKCs. Therefore, it would be beneficial to compare the expression of ECM either at the BM zone or in the fibrous wall between sporadic OKCs and OKCs associated with NBCCS.

Interestingly, DCs demonstrated only one staining pattern of the investigated BM proteins, whereas OKCs and RCs exhibited variable expressions. It has been postulated that DC develops by accumulation of fluid between the reduced enamel epithelium and the crown of an unerupted tooth (Kramer *et al*, 1992). Therefore, the staining pattern seen in DCs, which indicates an origin from a one-cell source, supports this pathogenesis. Unlike DCs, OKCs showed two distinct patterns of expression of BM proteins, suggesting two different processes for their epithelial origin – different cell sources or the same cell type but with different stages of tooth development. A variable expression of BM proteins observed in RCs may be the result of degradation of these proteins by products of inflammation which are present to a high degree. However, the different cell sources of origin of RCs cannot be excluded.

In conclusion, OKCs, DCs and RCs did not demonstrate significant differences in the expression of laminins 1 and 5, collagen type IV and fibronectin. These suggest that separation of the epithelial lining from the fibrous wall in OKCs is not related to the presence of these BM components.

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