Expression of transforming growth factor-beta 1 (TGF-beta 1) in odontogenic cysts

A. Piattelli[1], C. Rubini[2], M. Fioroni[2], L. Favero[3] & R. Strocchi[1]

[1]Dental School, University of Chieti, [2]Department of Pathologic Anatomy and Histopathology, University of Ancona, and [3]Dental School, University of Padua, Italy

Abstract

Piattelli A, Rubini C, Fioroni M, Favero L, Strocchi R. Expression of transforming growth factor-beta 1 (TGF-beta 1) in odontogenic cysts. *International Endodontic Journal*, **37**, 7–11, 2004.

Aim To evaluate the positivity to transforming growth factor-beta 1 (TGF-beta 1) in different types of odontogenic cysts.

Methodology A total of 30 radicular cysts (RCs), 27 follicular cysts (FCs) and 28 odontogenic keratocysts (OKCs) were evaluated for immunohistochemical analysis of TGF-beta 1. TGF-beta 1 was evaluated in blood vessels, stromal cells (fibroblasts) and pluristratified squamous epithelium. TGF-beta 1 expression was determined by evaluating the number of positive elements. TGF-beta 1 expression was determined by evaluating 1000 cells in the pluristratified squamous epithelium (500 in the basal and parabasal layers, and 500 in the superficial layer) and 500 cells (the fibroblasts in the stroma) for each specimen, and counting the number of positive cells. The number of positive vessels was evaluated in 10 high power fields (HPF). The Chi-square test was used to evaluate differences between the two groups (RC + FC and OKC). A P-value <0.05 was considered to indicate statistical significance.

Results A higher and statistically significant positivity was found in the basal–suprabasal epithelial layers ($P = 0.0011$), superficial epithelium ($P = 0.053$) and stromal cells ($P = 0.0002$) of orthokeratotic and parakeratotic OKC as compared with RC and FC.

Conclusions These differences suggest that control of the cell cycle may be abnormal in orthokeratotic OKCs. These OKCs may have an intrinsic growth potential not present in other cyst types.

Keywords: follicular cysts, odontogenic cysts, odontogenic keratocysts, radicular cysts, transforming growth factor-beta.

Received 4 April 2002; accepted 13 August 2003

Introduction

Odontogenic keratocysts (OKCs) are separated histologically from other cyst types by a characteristic microscopic appearance and by an increased epithelial mitotic activity in suprabasal layers (High *et al.* 1993). OKCs are clinically more aggressive and tend to recur with greater frequency than the other cyst types (Li *et al.* 1995). Orthokeratotic OKCs have a different clinical behaviour than parakeratinized OKCs and are not associated with Basal Cell Naevus syndrome; they are thus, probably, a distinct clinicopathological entity (High *et al.* 1993).

Correspondence: Prof. Adriano Piattelli, Via F. Sciucchi 63, 66100 Chieti, Italy (Fax: +39 0871 3554076; e-mail: apiattelli@unich.it).

OKC epithelium has been shown to contain the highest number of PCNA+ cells, most of which are located in suprabasal layers, with less than 5% in basal layers (Li *et al.* 1994). The total PCNA+ cell counts in OKC epithelia are significantly higher than those in follicular cysts (FCs) and radicular cysts (RCs; Li *et al.* 1994, 1995, Piattelli *et al.* 1998). The epithelial lining of OKCs may have some intrinsic growth potential not present in other odontogenic cyst linings (Li *et al.* 1994, 1995).

Transforming growth factor-beta 1 (TGF-beta 1) is a cytokine central to growth, repair and inflammation (Frenkel *et al.* 2000). TGF-alpha and TGF-beta 1 have been linked with the cellular processes for both soft- and hard-tissue wound healing (Tyler *et al.* 1999). The presence of wound repair cytokines such as TGF-alpha and TGF-beta 1 suggests a mechanism by which the host

inflammatory response may participate in the repair and remodelling of periapical tissues (Tyler *et al.* 1999). Moreover, the vast majority of eosinophils present in periapical granulomas and cysts demonstrated TGF-beta 1 mRNA and protein (Tyler *et al.* 1999).

TGF-beta 1 is a modulator of cellular phenotype, and its increased expression may result in increased production of collagen and organization into an ordered matrix (Bhatnagar *et al.* 1999, Adamo *et al.* 2001). Collagen and other components of the extracellular matrix are known to act synergistically with growth factors in promoting differentiation (Bhatnagar *et al.* 1999). TGF-beta has been also implicated in tooth development and tumorigenesis of odontogenic neoplasms, possibly as a signalling regulator of epithelial differentiation, and its distribution in different cyst types may be important (Heikenheimo *et al.* 1993, Li *et al.* 1997).

The aim of this study was to evaluate the presence and concentration of TGF-beta 1 in different types of odontogenic cysts.

Materials and methods

A total of 30 RCs, 27 FCs and 28 (18 orthokeratotic and 10 parakeratotic) OKCs were evaluated. All the RCs were asymptomatic and presented as a round or ovoid radiolucenct lesion connected with a root. FCs were discovered during routine radiographic examinations or in cases where a tooth had failed to erupt, and were always associated with the crown of an unerupted tooth. Radiographically, all FCs presented as a well-defined multilocular radiolucency. All the RCs and FCs were single lesions. The OKCs presented as well-circumscribed unilocular or multilocular radiolucencies with distinct margins. Most of the patients were asymptomatic, and only in a few cases were pain and swelling present. All OKCs were primary nonsyndromic (not part of the nevoid basal cell carcinoma syndrome), occurring as a single solitary lesion in otherwise healthy patients. The diagnosis for all cysts was made comparing the clinical, radiological and histological data.

Under local anaesthesia, a mucoperiosteal flap was elevated. Bone was removed with a bur, and after having opened a bony window, the cysts were removed completely.

All the biopsies were fixed in formalin (10% neutral buffered formalin) and embedded in paraffin. Sections of 4 μm were cut and mounted on poly L-lysine-coated slides and then heated in a microwave oven (700 W) for 10 min. Endogenous peroxidase was blocked by the incubation of samples in 3% hydrogen peroxide in methanol. After being washed with phosphate-buffered saline (PBS), the samples were incubated overnight with anti-TGF-beta 1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were then incubated with Envision-labelled polymer reagent (Dako, Copenhagen, Denmark) for 60 min at room temperature. Envision-labelled polymer reagent is a peroxidase-labelled polymer conjugated to goat antirabbit and goat antimouse immunoglobulins in Tris–HCl buffer. The reaction products were visualized with diaminobenzidine as the chromogen, and the sections were counterstained with haematoxylin. Normal rabbit immunoglobulin G was used instead of the primary antibodies for negative controls. Human breast carcinoma was used as a positive control tissue. TGF-beta 1 was evaluated in blood vessels, stromal cells (fibroblasts) and pluristratified squamous epithelium. TGF-beta 1 expression was determined by evaluating the number of positive elements. TGF-beta 1 expression was determined by evaluating 1000 cells in the pluristratified squamous epithelium (500 in the basal and parabasal layers and 500 in the superficial layer) and 500 cells (the fibroblasts in the stroma) for each specimen, and counting the number of positive cells. The number of the positive vessels was evaluated in 10 high power fields (HPF). In almost all cases, the cellular positivity was cytoplasmatic. All measurements were made by a blinded examiner, with no prior knowledge of the experimental design, and without knowledge of control and test specimens.

The correlation between the cyst histopathological features (RC + FC vs. OKC), and stromal-epithelial TGF-beta 1 expression was analysed by the Chi square test. A P-value <0.05 was considered to indicate statistical significance.

Results

Radicular cysts

These cysts were lined by nonkeratinized stratified squamous epithelium with different thickness. Frequently, an inflammatory cell infiltrate, mainly composed by polymorphonuclear leucocytes and lymphocytes, was found. Russell bodies, foci of dystrophic calcification and cholesterol clefts were found in some of the cyst walls. Almost all the RCs presented an epithelium that was negative to TGF-beta 1 (Fig. 1). A slight positivity was present in the stromal cells (11 of 30 cases; Fig. 1). Vessels were positive in all cases.

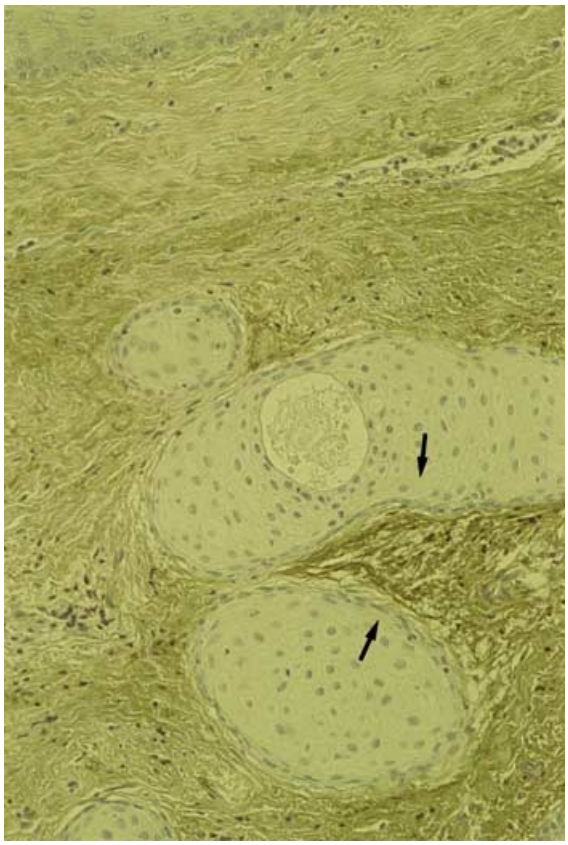

Figure 1 RC: the epithelium is negative to TGF-beta 1. A slight positivity to TGF-beta 1 of the stromal cells was present (arrows) ×200.

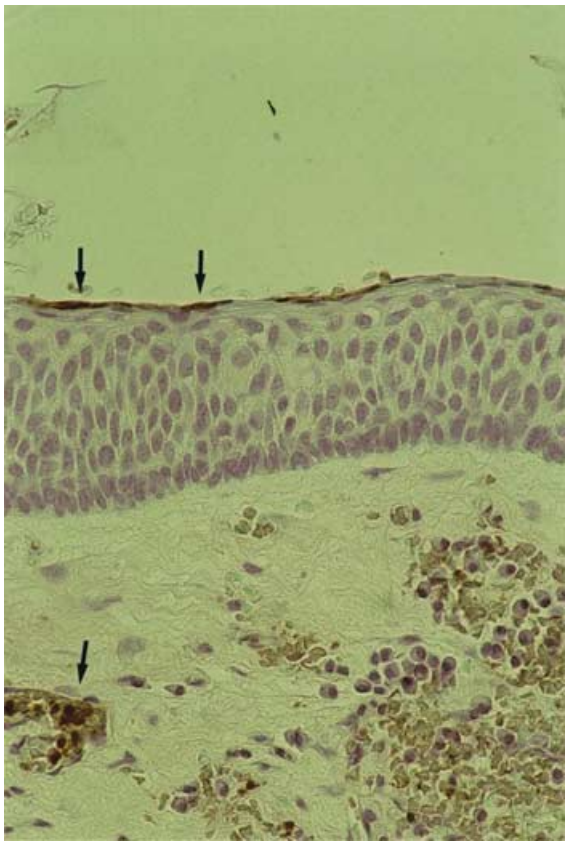

Figure 2 Parakeratotic OKC: strong positivity to TGF-beta 1 of the superficial layers of the epithelium (arrows) ×200.

Follicular cysts

The tissue wall of these cysts was lined by stratified squamous epithelium. The epithelial lining was nonkeratinized and was about four to six cell layers thick. Only in a few cases, ciliated and sebaceous cells were found in the lining epithelium. In FC, in 13 out of 27 specimens, there was a cytoplasmatic positivity of the superficial epithelial layer, whilst the basal–suprabasal layers were consistently negative. In 7 of the 27 cases, the stromal cells were positive. Vessels were positive in 24 out of 27 cases.

Odontogenic keratocysts

The epithelial lining was 8–10 cell layers thick. In the basal layer, a palisaded pattern with polarized nuclei was found. The luminal cells of the epithelium were para- or orthokeratinized, and produced a corrugated profile. The connective tissue component of the cyst wall was relatively thin. In 12 out of 18 orthokeratotic OKCs, there was a strong positivity of the cells of the basal layer of the epithelium, whilst the superficial layer was negative. On the contrary, in 9 out of 10 parakeratotic OKCs, there was a positivity of the superficial cells of the epithelium, whilst the basal–suprabasal layers were negative (Fig. 2). In 12 of 18 orthokeratotic cases, the stromal cells were positive (Fig. 3). The positivity of the vessels was different in orthokeratotic OKCs (13 out of 18) and in parakeratotic OKCs (9 out of 10; Table 1).

Statistical analysis

A higher and statistically significant positivity was found in the basal–suprabasal epithelial layers ($P = 0.0011$), superficial epithelium ($P = 0.053$) and stromal cells ($P = 0.0002$) of OKC as compared with RC and FC. No statistically significant differences were found in the positivity of the vascular component ($P = 0.6743$) of OKC as compared with RC and FC.

Figure 3 Orthokeratotic OKC: a focal positivity of the stromal cells is present (arrows) × 160.

Discussion

Transforming growth factor beta-1 is important for wound healing because it increases angiogenesis and fibroblast collagen formation (Wikesjo *et al.* 1998). It may play a pivotal role in many vital cellular activities, most significantly the regulation of cellular proliferation and differentiation, and synthesis of extracellular matrix components (Kim & Kim 1996, Pasini *et al.* 2001). Its ubiquitous presence in different tissues and strict conservation of nucleotide sequence down through the most primitive vertebrate organism underscores the essential nature of this family of molecules (Kim & Kim 1996). TGF-beta 1, moreover, increases production of basement membrane degrading enzymes (Saito *et al.* 1999) and it may play a role in epithelial cell survival (Shin *et al.* 2001).

Li *et al.* (1997) reported that PCNA+ cells had a predominant and consistent distribution in the suprabasal layers of OKC epithelium; this fact could mean a greater proliferative activity in OKC linings, and that is in accord with their more aggressive clinical behaviour. Moreover, OKC have been found to contain the highest number of p53+ cells, most of which were located in the suprabasal layers (Li *et al.* 1996). The distribution of proliferative cells within OKC epithelium is predominantly suprabasal in contrast to that of FC, RC and normal oral mucosa (Li *et al.* 1996). The fact that the orthokeratotic OKC in this study and also the TGF-beta 1 expression was mostly located in the basal–suprabasal layers, whilst in FC, DC and parakeratotic OKC, the expression was mainly found in the superficial epithelial layers is also striking. The higher positivity found in the vessels of the FC and RC, on the contrary, is explained by the presence of an inflammatory infiltrate in these two cysts. These quantitative and qualitative differences in proliferative activity in orthokeratotic OKC may suggest that control of the cell cycle may be abnormal (Li *et al.* 1996).

Li *et al.* (1997) found that the epithelial linings of all types of odontogenic cysts expressed TGF-alpha, with OKC showing the highest reactivity. This fact appears to be consistent with the higher levels of epidermal growth factor-receptor expression, PCNA and Ki-67 labelling in OKC, which could suggest that these cysts have an intrinsic growth potential not present in the other types. On the contrary, the most intense staining for TGF-beta was found by Li *et al.* (1997) to be in the fibrous tissue capsule, with the staining of the epithelial lining showing patchy weak reactivity.

Table 1 Comparison of TGF-beta 1 expression in odontogenic cysts

Clinical variants	No. of cases	Epithelial cells (basal–parabasal layer)	Epithelial cells (superficial layer)	Stromal cells	Vessels
Follicular cysts	27	–	13/27	7/27	24/27
Radicular cysts	30	4/30	3/30	11/30	30/30
Orthokeratotic keratocysts	18	12/18	–	12/18	13/18
Parakoratotic keratocysts	10	–	9/10	–	9/10

Acknowledgements

This work was partially supported by the National Research Council (C.N.R), Rome, Italy and by the Ministry of Education, University and Research (M.I.U.R), Rome, Italy.

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