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# Runx2 expression is associated with pathologic new bone formation around radicular cysts: an immunohistochemical demonstration

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**BACKGROUND: Radicular cysts are the most common cysts in human jaw bones. These lesions induce bone remodeling of the surrounding alveolar bones, which was termed 'condensing osteitis', and was suggested to be related to cells of the osteoblastic lineage. The Runx2 (core-binding protein [cbfa]1/polyoma enhancer-binding protein [pebp]2αA) was shown to be a DNA-binding transcriptional molecule expressed in osteoprogenitor cells.**

**METHODS: We confirmed the specificity of anti-Runx2 antiserum, using Western blotting analysis. We investigated the expression and localization of Runx2 in 32 radicular cyst cases with bone tissue fragments, immunohistochemically.**

**RESULTS: Signals for Runx2 were seen in 18 cases (56.3%) of radicular cysts with bone formation. These signals were immunolocalized in the nuclei of the spindle-shaped osteoprogenitor cells in the cyst walls, whereas only a few signals were seen in the cuboidal osteoblastic cells near the fibrous bones. Signals for type I collagen were immunolocalized in the dense collagen fibers in the cyst walls and in the matrix of the fibrous bone around the radicular cysts, whereas no signals were seen on the inner portions with inflammatory cell infiltration of the cyst walls. Very weak signals for transforming growth factor (TGF)-β1 were infrequently seen in the osteoblasts of the fibrous bone, whereas signals for TGF-β2 were observed in young osteocytes in the fibrous bones, in B-cell lymphocytes infiltrating into the inner portions, and on the cellular membranes of the lining epithelium.**

**CONCLUSIONS: The nuclear expression of Runx2 in spindle-shaped cells in the outer portions may play an essential role in the induction of fibrous bone tissue around radicular cysts. TGF-β2 may play a role in the production of type I collagen, which acts as a template for pathologic new bone formation, in radicular cysts.**
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**Keywords:** immunohistochemistry; new bone formation; radicular cyst; Runx2(cbfa1/pebp2αA[AML3]); transforming growth factor-β

## Introduction

Radicular cysts (apical periodontal cysts) are the most common form of inflammatory cysts in human jaw bone. These lesions result from the necrotizing pulp complex of the tooth. Histologically, radicular cysts show chronic inflammatory cell infiltration, including macrophage-derived foamy cells, in the cyst walls, and lining by non-keratinizing squamous epithelium (1). Frequently, the alveolar bones around the radicular cysts reveal osteosclerosis, which has also been termed condensing osteitis. In the alveolar bone, osteoprogenitor cells around the lesions may be related to pathologic new bone formation. Type I collagen is a major component of extracellular matrices in the connective tissue and bone, and may become the template for bone formation (2). Also, the lamellar bone matrix includes large amounts of transforming growth factor (TGF)-β proteins. TGF-β plays an important role in bone remodeling. In animal fracture models, TGF-βs are secreted from platelets in the hematoma, after which they induce the differentiation of mesenchymal stem cells into osteoprogenitor cells and chondroblasts (3). TGF-β superfamily includes bone morphogenetic proteins (BMPs), which are also well known to be associated with differentiation from the mesenchymal stem/progenitor cells to osteoblasts.

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The Runx2 (core-binding factor [cbfa]1/polyoma enhancer-binding protein [pebp]2αA: AML3) gene, which encodes a DNA-binding transcriptional molecule, was cloned as a member of the *runt* domain family (4). This molecule binds to the core site of DNA through co-binding with cbfa/pebp2β protein (5). Mice homozygous for the Runx2 gene showed complete inhibition of skeletal bone formation, and histologic examination revealed no ossification of the cartilaginous anlagen (6). Therefore, Runx2 has been suggested to be the most important differentiation transcription factor of bone formation. *In situ* hybridization analysis showed strong expression in the osteoblasts, and in the lower hypertrophic chondrocytes in the growth plates of long bones (6). Runx2 is a deciding factor determining the differentiation from mesenchymal stem cells to an osteoblastic lineage.

These findings suggest that the pathologic new bone formation around the radicular cysts may be induced via the binding of Runx2 to the DNA core site in osteoprogenitor cells. To investigate this hypothesis, we examined the expression and localization of Runx2 in pathologic new bone formation in cases of radicular cysts with bone fragments.

## Materials and methods

*Antibodies*

An anti-Runx2 mouse monoclonal antibody was generated by standard procedures against recombinant mouse Runx2 (cbfa1/pebp2αA) protein, and then selected by a supershift assay with a nuclear fraction of mouse MC3T3E1 cells (7). This monoclonal antibody was confirmed to cross-react with human Runx2 protein on Western blotting analysis (K. Sasaguri, unpublished data). Antihuman type I collagen monoclonal antibody (clone I-8H5) was purchased from Fuji Yakuhin Kogyo (Toyama, Japan). Anti-TGF-β1 (cat# sc-146) and anti-TGF-β2 (cat# sc-90) polyclonal antisera were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The antigens of the anti-TGF-β antisera were each C-terminal polypeptides. The specificity of these antisera was confirmed in our previous study (8), and the lack of cross-reactivity between the anti-TGF-β1 antiserum and the anti-TGF-β2 antiserum was confirmed by Western blotting and immunohistochemical analyses of human vertebral bone. An anti-CD20 (clone L-26) mouse monoclonal antibody as a B-cell marker, and an anti-CD45RO (clone UCHL-1) mouse monoclonal antibody as a T-cell marker, were purchased from Dako Japan Co. (Kyoto, Japan). An anti-CD31 (clone JC70A) mouse monoclonal antibody as a endothelial marker was purchased from Dako Japan Co. (Table 1).

**Table 1** Antibodies used in the present study

| *Antigen* | *Clone (animal)* | *Dilution* | *Source* |
|---|---|---|---|
| Runx2 | Mono (m) | 1:50 | Broncker *et al.* |
| Type I collagen | Mono[I-8H5] (m) | 1:100 | Fuji Yakuhin Kogyo |
| TGF-β1 | Poly (r) | 1:100 | Santa Cruz Biotechnology |
| TGF-β2 | Poly (r) | 1:100 | Santa Cruz Biotechnology |
| CD20 | Mono[L-26] (m) | 1:50 | Dako Japan Co. |
| CD45RO | Mono[UCHL-1] (m) | 1:100 | Dako Japan Co. |
| CD31 | Mono[JC70A] (m) | 1:100 | Dako Japan Co. |

TGF-β1, transforming growth factor-β1; TGF-β2, transforming growth factor-β2; Mono, monoclonal antibody; Poly, polycolonal antibody; m, mouse; r, rabbit.

*Western blot analysis*

The protein samples used in the Western blot included recombinant mouse Runx2 protein, nuclear extracts from MC3T3E1 (mouse calvarial cells) and C3H10T1/2 (embryonic fibroblasts) cells, nuclear extracts from C3H10T1/2 cells transfected with mouse Runx 2/pebp2αA, Runx 1/pebp2αB (from Dr Y. Ito, Department of Viral Oncology, Institute for Virus Research, Kyoto University, Kyoto, Japan), and cbfa1 vector (from Dr Kersenty, Baylor College of Medicine, Houston, TX, USA), respectively. These samples were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, and electrotransferred onto a Hybond-P membrane (Amersham Biosciences, Piscataway, NJ, USA). The membrane was pre-incubated with 5% skim milk in Tween-phosphate-buffered saline (T-PBS; 0.1% Tween 20, 50 mM Tris, pH 7.6, 150 mM NaCl) for 1 h at room temperature (RT) and then incubated with anti-Runx2 monoclonal antibody in T-PBS at dilution of 1:100 000 for 1 h. After washing with T-PBS three times, 10 min each, the membrane was incubated in T-PBS with antimouse immunoglobulin (Ig)G conjugated with horseradish peroxidase (1:3000; Bio-Rad, Hercules, CA, USA). It was then washed again with T-PBS for 3 min and signals were detected on Hyperfilm$^{TM}$MP, Amersham Life Science, Piscataway, NJ, USA) using an ECL system (Amersham, Piscataway, NJ, USA).

*Tissues*

Thirty-two cases of radicular cysts with alveolar bone fragments were chosen from the pathologic files of the Dental Hospital, Tokyo Medical and Dental University, Tokyo, Japan, from 1997 to 1998. Of this population, 45% were females Japanese patients, the mean age being 54 years (±23). Neonatal vertebral tissue was used as a positive control. All human specimens were obtained after approval by the Local Ethical Committee of our institution. The radicular cyst specimens were fixed in 10% buffered formalin, routinely processed, decalcified with Planck-Rychlo decalcification solution (formic acid, hydrochloric acid, and aluminum chloride hexahydrate) at RT for 1–2 days, and embedded in paraffin wax. Serial sections of 3-μm-thick were prepared. One section from each specimen was stained with hematoxylin and eosin (H & E).

*Immunohistochemistry*

Paraffin-embedded sections were deparaffinized in xylene and rehydrated. For Runx2 staining, deparaffinized sections were heated in citrate buffer, pH 6.0, for 15 min in a microwave oven (500 W) for antigen retrieval. For

TGF-β1 and -β2 staining, deparaffinized sections were pre-treated in 0.1% hyaluronidase (H6254; Sigma Chemical Co., St Louis, MO, USA) for 30 min at 37°C, and then, after two washes with PBS, the sections were heated in citrate buffer, pH 6.0, for 15 min in a microwave oven for antigen retrieval. For type I collagen staining, deparaffinized sections were pre-treated with 0.1% trypsin (0152-13-1; Difco Laboratories, Detroit, MI, USA) in PBS for 30 min at 37°C. For CD31 staining, deparaffiinized sections were heated in citrate buffer, pH 6.0, for 15 min in a microwave oven for antigen retrieval. After two washes in PBS, all sections were incubated for 30 min in 0.3% $H_2O_2$ in methanol to inactivate endogenous peroxidases. The sections were then washed twice more in PBS, and pre-incubation was carried out with normal rabbit serum or goat serum (Dako Japan Co.), followed by incubation with a primary antibody. After incubation for 18 h at 4°C, sections were washed twice in PBS and incubated with a biotinylated antimouse or rabbit immunoglobulin antibody (Dako Japan Co.). After two additional washes, they were incubated in peroxidase-conjugated streptavidin (P397; Dako Japan Co.), followed again by two washes in PBS. Bound peroxidase was developed with 0.02% 3,3′-diaminobenzidine in 0.1 M Tris buffer, pH 7.6, in 0.005% $H_2O_2$ for 10 min, and counterstained with 5% methyl green. As negative controls, we omitted the first antibody or replaced the antibody with normal rabbit or mouse serum.

## Results

*Western blot analysis*

The immunoreactivity of the Runx2 monoclonal antibody was determined by Western blot analysis (Fig. 1). The antibody reacted with the immunogen, the recombinant murine Runx2 (pebp2αA) protein, showing a band at 60 kDa, which served as a positive control (lane 1). The antibody specifically detected Runx2 and cbfa1 proteins in the nuclear extracts of the MC3T3E1 cells and the transfected C3H10T1/2 cells, with the Runx2 and cbfa1 expression vectors, showing the co-migration of three bands at approximately 63 kDa (lanes 2, 4, and 6). However, no Runx2 or cbfa1 protein was detected in the nuclear extracts from the C3H10T1/2 cells transfected with the empty vector or the Runx1 (pebp2αB) expression vectors (lanes 3 and 5).

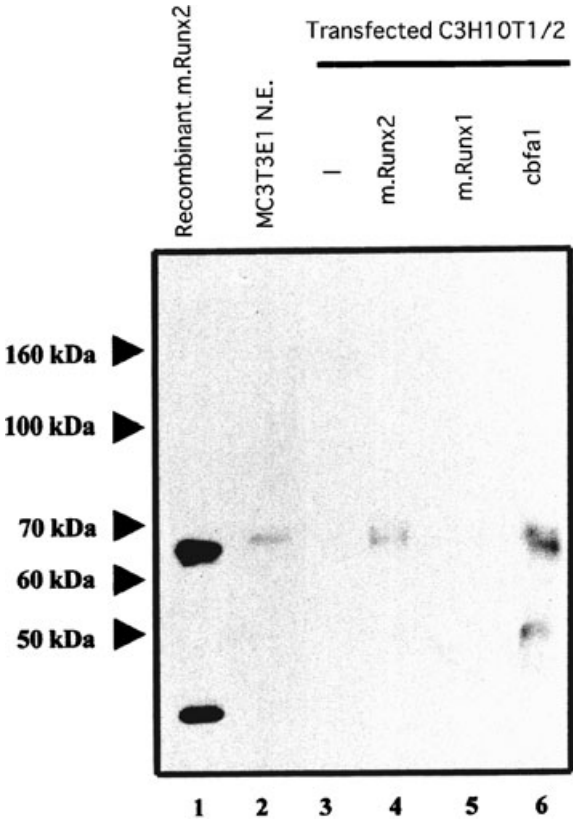


**Figure 1** Western blot analysis of the immunospecificity of the cbfa1/pebp2αA monoclonal antibody. The presence of the protein immunoreactivity, shown by Western blot analysis performed with recombinant mouse pebp2αA protein (lane 1), served as a positive control. Also applied were nuclear extracts from MC3T3E1 (lane 2), C3H10T1/2 (lane 3), and C3H10T1/2, transfected with mouse Runx2[pebp2αA], mouse Runx1[pebp2αB], and cbfa1, respectively (lanes 4–6). Immunoreactivity was seen in lanes 1, 2, 4 and 6. No bands were detected in the samples from the C3H10T1/2 cells (lane 3), or the C3H10T1/2 cell transfected with Runx1[pebp2αB] (lane 5), indicating the immunospecificity of the newly produced monoclonal antibody. m, mouse; NE, nuclear extract.

*Histology*

In the present study, the radicular cysts consisted of cystic lesions lined with non-keratinizing squamous epithelium with elongated rete ridges, inflammatory granulation tissue, and fibrous tissue. The cyst walls showed various extents of fibrosis, plasma cell, and lymphocyte infiltration, aggregation of macrophage-derived foamy cells, cholesterin clefts, proliferation of capillaries, and hemorrhages. Pathologic new bone formation was seen around the cyst walls, where irregular trabeculae of the fibrous bone with plump osteocytes were frequently observed (Fig. 2). Fibrous bone was occasionally accompanied by a lining with cuboidal osteoblasts. Lamellar bone, however, was seen infrequently, which did not correspond with the plump osteoblasts. Dense fibrous tissue was seen on the outer portions of the cyst walls, whereas immature fibrous tissue, partly with myxoid stroma, was observed in the inner portions of the cyst walls. Occasionally, myxoid stroma was seen between bone trabeculae.

*Immunohistochemistry of type I collagen and Runx2*

Table 2 summarizes the results of the present immunohistochemical study. Signals for type I collagen were seen on the lamellar bones around the radicular cysts (100%), and also frequently on the dense fibrous tissues in the outer portions of the cyst walls (Fig. 3a). Signals for Runx2 were observed in the nuclei of the osteoprogenitor cells in the human neonatal vertebral tissue used as the positive control (Fig. 3b). Runx2 was immunolocalized in the nuclei of the spindle-shaped cells, which were considered to be osteoprogenitor cells, in the dense

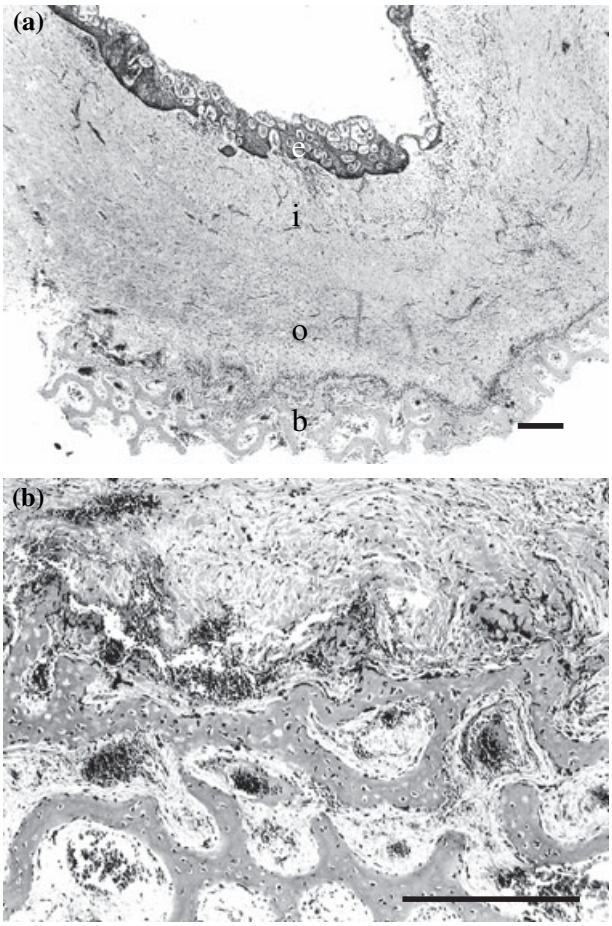


**Figure 2** (a) Typical histology of the radicular cysts with bone fragments. Non-keratinizing squamous epithelium (e) could be seen lining the cysts, mononuclear cell infiltration, and edematous granulation tissue were seen in the inner portion (i), and dense fibrous tissue with fibrous bone fragments (b) was observed in the outer portion (o). Scale bar = 25 μm (hematoxylin and eosin, H & E: ×25). (b) Bone trabeculae around the radicular cysts. Formation of irregular and immature fibrous bone trabeculae with young osteocytes and partly cuboidal osteoblasts were observed around the radicular cysts. Scale bar = 100 μm (H & E: ×100).

**Table 2** Immunohistochemistry of the radicular cysts with bone fragments

| *Bone tissue* | *Ep* | *IP* | *OP* | *OB* | *OCy* | *BM* |
|---|---|---|---|---|---|---|
| Runx2 | − | − | + | ± | − | − |
| Type I collagen | − | ± | + | − | − | + |
| TGF-β1 | − | − | − | ± | − | − |
| TGF-β2 | + | +[a] | − | − | + | − |
| CD20 | − | +[a] | − | − | − | − |
| CD45RO | − | ±* | − | − | − | − |
| CD31 | − | +[b] | +[b] | − | − | − |

Ep, lining epithelium; IP, inner portion of the radicular cyst; OP, outer portion of the radicular cyst; OB, osteoblasts lining the bone trabeculae; OCy, osteocytes in the bone trabeculae; BM, bone matrix; −, no expression; ±, focal or weak expression; +, diffuse expression; TGF, transforming growth factor.
[a]Lymphocytes were immunoreactive for each antibody.
[b]Endothelial cells were immunoreactive for anti-CD31 antibody.

fibrous tissues of the cyst walls (Fig. 3c). Although no signals for Runx2 were seen in the young osteocytes, signals for Runx2 were infrequently observed in the nuclei of the plump osteoblastic cells near the fibrous bones (Fig. 3d). Eighteen cases (56.3%) of radicular cysts with bone fragments showed positive signals for Runx2. No signals for Runx2 were observed in the squamous epithelium, foamy macrophages, plasma cells or lymphocytes. No signals were seen in the negative controls.

*Immunohistochemical analysis of TGF-β, lymphocytic, and endothelial markers*

No signals for TGF-β1 were seen in the bone tissue around the radicular cysts, whereas very weak signals for TGF-β1 were seen in the lymphocytes infiltrating the inner portions of the cyst walls, on the extracellular matrix of the outer portions, which consisted of dense fibrous tissue, and in the osteoblasts lining the fibrous bone (data not shown). Signals for TGF-β2 were observed in the young osteocytes of the fibrous bones, in lymphocytes infiltrating into the inner portions of the cyst walls, and on the cellular membranes of the lining squamous epithelium: especially, strong signals for TGF-β2 were seen in the basal cells of the stratified squamous epithelium (Fig. 4a–c). Weak signals for TGF-β2 were infrequently seen in the cytoplasm of the spindle-shaped cells in the outer portions of the cyst walls. No signals for TGF-β2 were seen in the osteoblasts lining the trabeculae of the fibrous bones, or in the osteocytes of the lamellar bones around the radicular cysts. Almost all of the infiltrating lymphocytes were positive for CD20, and focally positive for CD45RO (Fig. 4d). The endothelial cells in the stroma around the radicular cysts were positive for CD31, but the spindle-shaped cells, which were immunolocalized for Runx2, did not indicate the positivity for CD31 (data not shown). No signals were seen in the negative controls.

## Discussion

Radicular cysts (apical periodontal cysts) are the most common inflammatory cysts of jaw bone cystic lesions. Histologically, various degrees of inflammatory granulation tissue formation are seen in such cysts. New immature fibrous bone formation is frequently observed around the cyst walls, which is involved by dense fibrous tissue. As radicular cysts become larger in cases where the infected tooth root is not treated, bone remodeling occurs around the cyst walls due to direct pressure from the cyst. We considered that this type of cyst was a good model of pathologic new bone formation.

Type I collagen is the major matrix protein of the soft connective tissue and bone (2). In the present study, type I collagen was deposited in the matrix of the fibrous bone and on the outer portions of the cyst walls, which consisted of dense fibrous tissue. As type I collagen was deposited on the outer portion of the cyst walls, this collagenous matrix molecule was the template for immature fibrous bone in pathologic new bone formation.

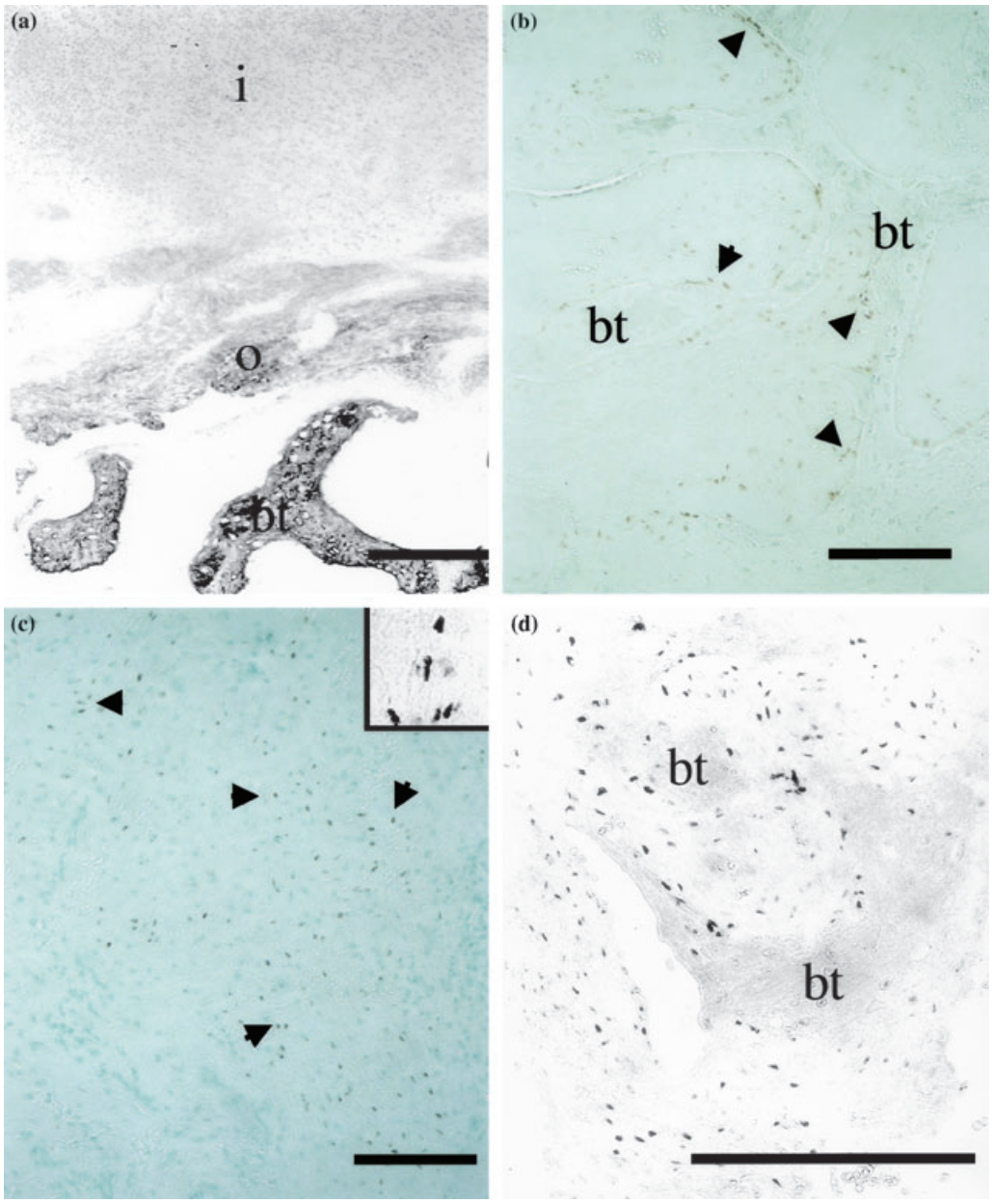


**Figure 3** (a) Immunohistochemistry for type I collagen in the radicular cysts. Strong signals for type I collagen were seen in the bone matrix, whereas moderate signals for type I collagen were observed in the connective tissue of the outer portion of the radicular cysts. i, inner portion of the cyst; o, outer portion of the cyst; bt, bone tissue. Scale bar = 100 μm (immunostaining: ×100). (b) Immunohistochemistry for Runx2 in human neonatal vertebral tissue as a positive control. Moderate signals for Runx2 were seen in the nuclei of the osteoprogenitor cells and osteoblasts (arrowheads) around the new bone trabeculae (bt, bone trabeculae). Scale bar = 100 μm (immunostaining: ×100). (c) Immunohistochemistry for Runx2 in the radicular cysts. Moderate to strong signals for Runx2 were observed on the nuclei of the spindle-shaped cells (arrowheads) in the outer portion of the radicular cysts, and these were considered to be osteoprogenitor cells that had differentiated from mesenchymal stem cells. Scale bar = 100 μm (immunostaining: ×100). Inset: note that the nuclear expression of Runx2 was observed in the spindle-shaped cells in the outer portion of the cyst walls (immunostaining: ×150). (d) Immunohistochemistry for Runx2 in the radicular cysts. Moderate signals for Runx2 were occasionally seen on the nuclei of the plump osteoblasts lining the fibrous bone trabeculae (bt). Scale bar = 200 μm (immunostaining: ×200).

Runx2-deficient mice were reported to reveal no ossification of the skeletal tissue, although cartilaginous anlagen were formed (6). Osteoblasts of the mutant mice expressed osteonectin, a low level of alkaline phosphatase, and barely detectable amounts of osteopontin and osteocalcin (6). These observations indicated that osteoblast maturation arrests at an early stage of differentiation in Runx2-deficient mouse embryos. In the case of Runx2-deficient mouse embryos, no subperiosteal bone was formed around the calcified cartilage of the tibia or radius. In the calvaria of these mouse embryos, a complete blockage of intramembranous ossification also accompanied the maturation arrest of the osteoblasts (6). This transcriptional molecule, therefore, is an essential factor in the differentiation from mesenchymal stem cells to osteoprogenitor cells. Runx2 is a member

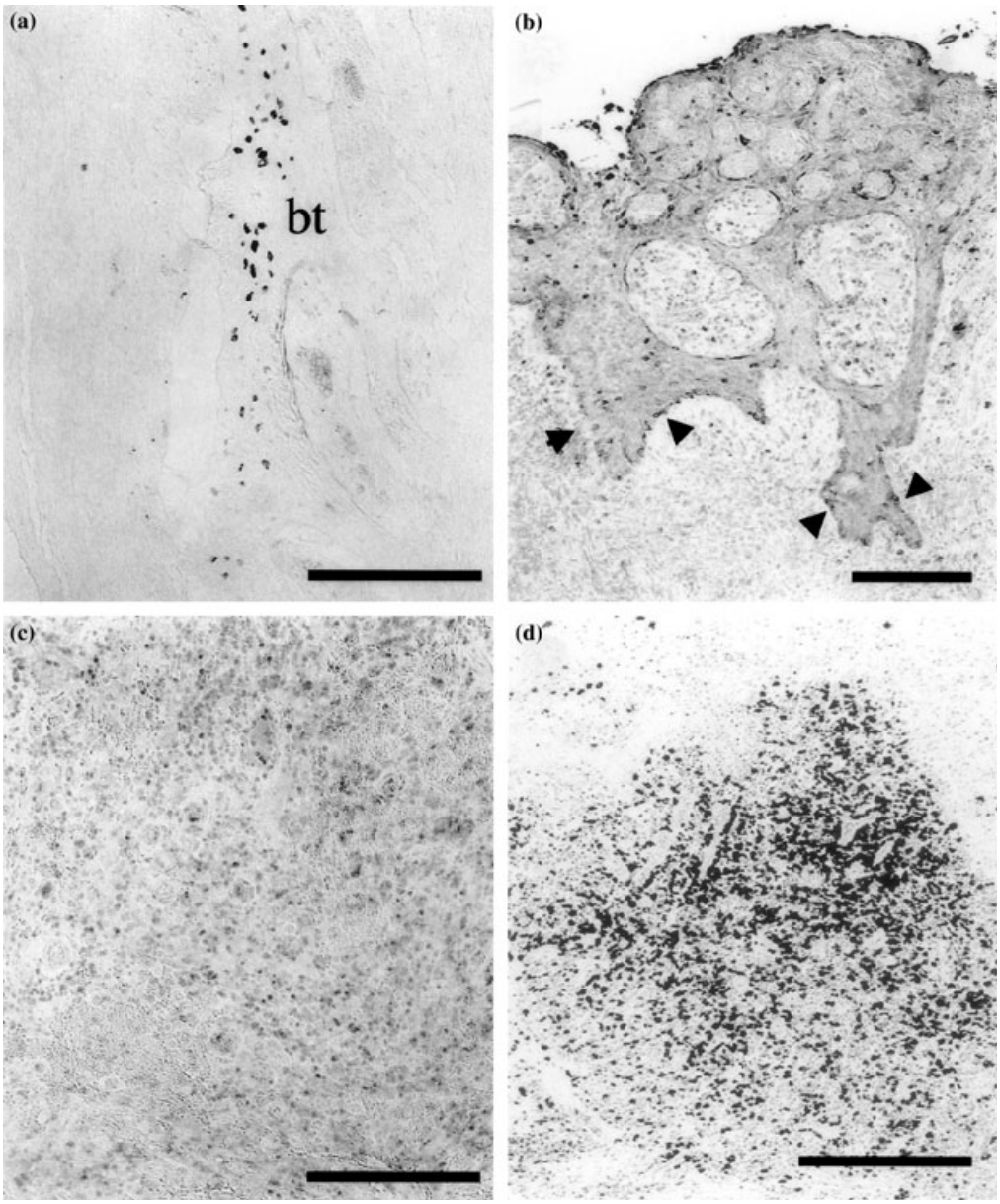


**Figure 4** (a) Immunohistochemistry for transforming growth factor (TGF)-β2 in the radicular cysts. Strong signals for TGF-β2 were observed in the cytoplasm of young osteocytes in the immature fibrous bone tissues around the radicular cysts. bt, bone trabeculae. Scale bar = 128 μm (immunostaining: ×128). (b) Immunohistochemistry for TGF-β2 in the radicular cysts. Moderate signals for TGF-β2 were seen in the cellular membrane of the squamous epithelium lining the radicular cysts. Note that strong signals for TGF-β2 were seen in the basal cells (arrowheads) of the squamous epithelium. Scale bar = 100 μm (immunostaining: ×100). (c) Immunohistochemistry for TGF-β2 in the radicular cysts. Moderate signals for TGF-β2 were observed in the cytoplasm of lymphocytes infiltrating into the inner portion of the radicular cysts. Scale bar = 128 μm (immunostaining: ×128). (d) Immunohistochemistry for CD20 in the radicular cysts. Strong signals for CD20, which is a marker of B-cell lineage, were seen in almost all lymphocytes infiltrating the inner portion of the radicular cysts. Scale bar = 128 μm (immunostaining: ×128).

of the *runt*-domain gene family (4). Three *runt*-domain genes (Runx1[cbfa2/pebp2αB], Runx2[cbfa1/pebp2αA], and Runx3[cbfa3/pebp2αC]) have been identified (5, 9). They have a DNA-binding domain, *runt*, which is homologous to the *Drosophila* pair-rule gene *runt*, and they form heterodimers with co-transcription factor cbfa/pebp2β and acquire enhanced DNA-binding capacity *in vitro* (5). In recent studies, cbf-related factors were shown to interact with the promoter region of the osteocalcin gene (10–12). Osf2/cbfa1 was reported to be one of the transcriptional activators of osteoblast differentiation (13). Forced expression of osf2/cbfa1 in non-osteoblastic cells induced the expression of the principal osteoblast-specific genes: α1(I) collagen, bone sialo-protein, osteopontin, and osteocalcin genes (13). A recent study indicated that cbfa1 has three isoforms: type I (Runx2 [cbfa1/pebp2αA]), type II (*til*-1), and type III (osf2/cbfa1); these isoforms have been shown to have different functions in a transient transfection assay. Although the transient transfection of types I and II

cbfa1 induced alkaline phosphatase activity in the mouse fibroblast cell line C3H10T1/2, transient transfection of type III cbfa1 cDNA induced no alkaline phosphatase activity in the same cell line (14). In the present study, the monoclonal antibody immunoreacted with the recombinant Runx2 [cbfa1/pebp2αA] at approximately 60 kDa in the Western blot analysis. The antibody also specifically detected the presence of Runx2 from the nuclear extracts of MC3T3E1 cells and transfected C3H10T1/2 cells with a Runx2 [pebp2αA] or cbfa1 expression vector at approximately 63 kDa. Using affinity-purified polyclonal antibodies, Banerjee et al. reported that AML3 was detected in mature differentiated osteoblast cells as two proteins of 60 kDa and 65 kDa, respectively, by Western blotting analysis (15). Our results, consistent with the previous findings, thus indicate the specificity of the monoclonal antibody. In future studies, we will examine the differential localization of these isoforms in pathologic new bone formation around radicular cysts. Recently, transient expression of Runx2 was reported in endothelial cells and vascular smooth muscle cells during vessel formation in skin, stroma of forming bones, developing periodontal ligament, developing skeletal muscle cell and differentiating adipocytes, and Runx2 has been recently considering to be not an exclusive marker for chondrogenic, osteogenic, and dentinogenic tissues (16). In the present study, although we found the expression of Runx2 in the spindle-shaped cells in the stroma of radicular cysts, we could not observe the Runx2 expression in the endothelial cells in the stroma. We believe that the spindle-shaped cell, which expressed Runx2, is an osteoprogenitor cells in the outer portion in the radicular cyst wall.

There are cbf sites in the regulatory regions of many T cell-specific genes, and cbfa1 binds to the T-cell receptor β-enhancer, and stimulates the enhancer activity *in vitro* (17). These observations led to the speculation that cbfa1 may be involved in T lymphocyte-specific transcriptional regulation (18). Although there were various degrees of infiltration of plasma cells, lymphocytes, and foamy macrophages, in the inner portion of the radicular cysts, in the present study no signals for Runx2 were seen in the infiltrating lymphocytes, such as the B-cell lineage, in the cyst wall.

TGF-β has been reported to have multiple functions, and this molecule has been shown to play an important role in new bone formation in animal fracture models *in vivo* (19–22). TGF-β is the most potent known inducer of collagen production in fibroblasts (23). In the present model of pathologic new bone formation, TGF-β1 was not expressed, but TGF-β2 was localized in young osteocytes of the fibrous bones, in lymphocytic cells of the inner portions of the cysts, and on the lining squamous epithelium. TGF-β2 produced by young osteocytes may play an important roles in the maturation of fibrous bone, and in the production of type I collagen as a bone template. TGF-β2 is also a glioblastoma-derived T-cell suppressor factor by virtue of its suppressive effects on interleukin-2-dependent T-cell growth (24). As the lymphocytes in the inner portions of the cyst walls were of the B-cell lineage, TGF-β2 produced by these cells may play a role in the suppression of T-cell infiltration into the cyst walls in a paracrine manner. In the present study, squamous epithelium, which is the lining epithelium of the radicular cysts, produced only TGF-β2 protein. TGF-β2 may be activated by plasminogen or extreme pH in the inflammatory cyst walls, and may play a role in stromal formation in the granulation tissue of the cyst walls in a paracrine manner.

In the present study, the nuclear expression of Runx2 protein was observed in spindle-shaped cells in the outer portions of the cyst walls. Although we could not confirm alkaline phosphatase activity, a marker of the osteoblastic lineage, in these spindle-shaped cells, we believe that these cells were osteoprogenitor cells, and that they may play an essential role in the induction of pathologic new bone formation around the cyst walls. TGF-β2 may also play an important role in the maturation of fibrous bone tissue and in the production of type I collagen as a template for bone tissue in radicular cysts.

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