Stem Cell Marker Expression in Persistent Apical Periodontitis

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Abstract

Introduction: This study evaluated the expression of CD90 (mesenchymal stem cell) and Sox2 (progenitor stem cell) markers in persistent apical periodontitis (PAP) (n = 16) and primary periapical lesions (PPLs) (n = 10). **Methods:** All samples were classified histologically according to the intensity of inflammatory cell infiltrate in the periapical lesion. Immunohistochemistry was used to detect CD90 and Sox2 in PAP and PPLs. The Spearman correlation coefficient and the Mann-Whitney U test were used to analyze data at the 5% significance level. **Results:** CD90 expression was found in mesenchymal cells and vascular endothelial cells of 68.5% of all cases of PAP. There was no correlation between CD90 expression and histopathological diagnosis (P = .053) or inflammatory cell infiltrate intensity (P = .112). CD90 staining was predominantly found in the vascular endothelial cells of 30% (n = 3) of PPLs. CD90 expression was significantly higher in PAP than in PPLs (Mann-Whitney U test, P < .05). Sox2 expression was found in all cases of PAP. Eventually, all mesenchymal and chronic inflammatory cells exhibited Sox2 expression. There was no correlation between Sox2 expression and histopathological diagnoses (P = .749), inflammatory cell infiltrate intensity (P = .510), or acute or chronic inflammatory cell infiltrate (P = .256). Sox2 expression was found in 100% of PPLs. There was no difference in Sox2 expression between PAP and PPLs (P = .477). **Conclusions:** Mesenchymal stem cells may contribute to the immunosuppressive environment in PAP. Additionally, distinct stem cell sources may be associated with the chronic nature of PAP as well as with the development of PPLs. *(J Endod 2016;■:1–6)*

Key Words

Apical periodontitis, CD90, mesenchymal stem cells, persistent apical periodontitis, progenitor stem cells, Sox2

Significance

We evaluated the expression of CD90 (mesenchymal stem cell) and Sox2 (progenitor stem cell) markers in persistent apical periodontitis and primary periapical lesions. Mesenchymal stem cells may contribute to the immunosuppressive environment in PAP. Distinct stem cell sources may be associated with the chronic nature of PAP as well as with the development of PPLs.

The pathogenesis of apical periodontitis (AP) has been associated with inflammatory responses and the destruction of periapical tissues caused by bacterial agents from an infected root canal (1). Periapical radiolucencies that persist after root canal retreatment often suggest failure in eradicating intraradicular infection (2). Endogenous or exogenous nonbacterial causes, such as true cysts or foreign body reactions, may also favor the persistence of AP after retreatment (3). Primary periapical lesions (PPLs) are considered an initial response of the organism to the necrosis of dental pulp and root canal infection, whereas persistent apical periodontitis (PAP) lesions persist after the failure of root canal retreatment. The maintenance of intraradicular infection or the presence of extraradicular factors that may interfere with the healing process probably influence the persistence of AP (1). The study of the mechanism of AP associated with infected root canals may improve our understanding of periapical healing after root canal treatment (1, 4–7).

PAP is a biologically active lesion with high levels of receptor activator of nuclear factor kappa-B ligand and a potential for bone resorption. The inflammatory immune cells found in PAP may be indicative of a suppressive and regulatory periapical environment favorable to chronic clinical conditions (8).

Recently, new insights into the molecular mechanism of AP have been gained. Mesenchymal stem cells (MSCs) in the periapical region may contribute to the development of an immunosuppressive and prohealing environment associated with the stable nature of inactive apical lesions. In this case, the activation of the healing process may arrest the progression of chronic periapical lesions (9). Treg cells have been recently shown to have immunosuppressive and prohealing properties (8). In this scenario, the study of MSCs in PAP may improve our understanding of the molecular nature of PAP.

MSCs are multipotent progenitor cells capable of differentiating into mesenchymal and nonmesenchymal lineages (10), which are key determinants of healing (9). MSCs are found in several tissues and organs of the human body but not in high quantities in bone and other tissues (10). MSCs may be activated or emerge in the periapical region in the presence of inflammation (11, 12).

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CD90, also known as Thy-1, is 1 of the most recognized markers of various types of human MSCs, including bone marrow, periodontal ligament, adipose tissue, menstrual blood, saphenous vein blood, limbal stroma eye, lung, amniotic fluid, liver, placenta, spleen, umbilical cord vein (10), and periapical inflamed tissue (11). As critical regulators of cell-to-cell and cell-extracellular matrix interactions, they play an important role in tissue regeneration and fibrosis (13). CD90 expression has been recently found in inactive periapical granulomas (9) and inflammatory periapical tissues associated with osteogenesis (11), which suggests that the CD90 marker may be a good candidate for the study of the role of potential MSCs in the periapical environment associated with PAP.

Strong evidence indicates that the presence of viruses or microbial microorganisms in the epithelial cells of a periapical cyst may be associated with the persistence of an active stage of the inflammatory infiltrate (2, 14) and may contribute to the persistence of the biologically active status of periapical lesions and AP after root canal treatment. Therefore, molecular mechanisms may be involved in the activation of epithelial cells in periapical lesions, possibly associated with inflammatory stimuli.

The transcription factor sex-determining region Y-related high mobility group box 2 (Sox2) is specific to multiple tissue progenitor and adult stem cells. Sox2, which is associated with the persistence of pluripotent activity and reprogramming of pluripotent stem cells, also acts in the reprogramming of differentiated fibroblasts into pluripotent stem cells (15). Sox2 is expressed in dental epithelial stem and progenitor cells during tooth renewal, especially in epithelial stem cells in active growth (16). In addition, Sox2 seems to act together with inflammation in the initiation of some types of cancer (17, 18).

MSC and progenitor stem cell characteristics may be associated with AP activation or stabilization, which may contribute to AP persistence and outcome. This study evaluated the expression of the MSC CD90 and progenitor stem cell markers Sox2 in PAP and PPLs.

Material and Methods

Sample Selection

Periapical lesion samples were collected after periapical surgery (School of Dentistry, Federal University of Goiás, Goiânia, Brazil) and clinical or radiographic confirmation of lesion persistence after root canal retreatment. Only asymptomatic periapical lesions that persisted for more than 6 months after root canal retreatment without sinus tract involvement or radiographic signs of remission were included (PAP samples). PPLs associated with infected and untreated root canals were collected from teeth that were extracted after an indication of surgical removal. Symptomatic cases or cases associated with trauma or complications were not included. All patients in this study were clinically healthy and had no history of systemic diseases.

Periapical lesion samples that persisted more than 6 months after root canal retreatment without clinical symptoms and images of periapical radiolucency (PAP samples) and periapical lesions related with untreated teeth (as PPL) were used.

Study Design

Morphologic and immunohistochemical analysis of CD90 and Sox2 stem cell markers were conducted using 26 paraffin-embedded specimens of PAP (n = 16) and PPLs (n = 10).

Formalin-fixed paraffin-embedded specimens were used to obtain 5-μm histologic sections for routine hematoxylin-eosin staining and analysis under light microscopy to confirm the diagnosis of periapical lesions. After histopathological confirmation, the samples underwent morphologic analyses and immunohistochemical reactions. This study was approved by our institutional review board, and all participants signed an informed consent form.

Morphologic Analysis

All samples were histologically classified according to the intensity of the inflammatory cell infiltrate in the periapical lesions. The specimens were evaluated under light microscopy at 200× magnification according to the protocol described by Tsai et al (19). Each specimen was graded into 3 categories: grade 1, less than one third of inflammatory cells per high-power field; grade 2, between one third and two thirds of inflammatory cells per field; and grade 3, when the total of inflammatory cells exceeded two thirds per field. The degree of inflammation was analyzed on 3 histologic fields of each histologic specimen: the center, intermediate, and outer portions.

Immunohistochemistry

Immunohistochemistry reactions for anti-CD90 mouse monoclonal antibody (Abcam, Cambridge, MA, ref: ab181469) and anti-Sox2 mouse monoclonal (Santa Cruz Biotechnology, Santa Cruz, CA, ref: sc365823) were performed using 3-μm sections of paraffin-embedded tissues in a series on glass slides coated with 2% 3-aminopropyltriethoxysilane (Sigma-Aldrich, St Louis, MO). The sections were deparaffinized and rehydrated in decreasing concentrations of alcohol followed by antigen retrieval. In sections that received CD90 antibody, antigen was retrieved by means of immersion of the slides in a solution containing 1.21 g Tris, 0.3722 g EDTA, and 500 mL Tween (pH = 9.0) in a water bath at 95°C for 30 minutes. Sox2 sections were treated with a solution of 10 mmol/L monohydrated citrate buffer solution (pH = 6.0) in a water bath at 95°C for 30 minutes. Endogenous peroxidase activity was blocked with 6% hydrogen peroxide and a methanol solution (1:1) in 2 baths of 15 minutes each at room temperature followed by immersion in Tris buffer (pH = 7.6) 3 times.

Incubation of the primary antibodies, tertiary complex, revelation with diaminobenzidine (Liquid DAB + K3468; DAKO, Carpinteria, CA) and counterstaining with Mayer hematoxylin were performed automatically using an autostainer unit (DAKO). The slides were incubated with anti-CD90 (dilution 1:100) and anti-Sox2 (dilution 1:50) antibodies for 60 minutes at room temperature. Breast adenocarcinoma samples were used as the positive control for CD90, and adenoid cystic carcinoma samples for Sox2. The negative control was obtained by omitting the primary antibody during the reaction.

After that, the slides were exposed to an avidin-biotin complex (LSAB-Kit + HRP; Dakocytomation, Carpinteria, CA) and to 3,3′-diaminobenzidine chromogen (DAB+, Dakocytomation). The sections were counterstained using Meyer hematoxylin, dehydrated in ethanol, cleared in xylene, and mounted.

CD90 and Sox2 immunoexpression was analyzed according to the percentage of positive staining cells in the area under examination. Immunohistochemical scores were partially based on the method described by Pityński et al (20), which described 4 categories to estimate staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong) and 6 categories to estimate the proportion of positive cells (0, expression in <1%; 1, in 1%–5%; 2, in 6%–10%; 3, in 11%–25%; 4, in 26%–50%; and 5, in >50% of the cells). CD90 and Sox2 immunostaining was classified as positive in inflammatory cells and mesenchymal cells. Additionally, Sox2 staining was classified as positive in odontogenic epithelium cells of epithelialized periapical granulomas and periapical cysts.

CD90 and Sox2 final scores were calculated by multiplying the intensity values by the proportion values, which generated a score ranging from 0 to 12. Stem cell marker expression was evaluated in the "hot