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Differentiated embryonic chondrocytes 1 expression of periodontal ligament tissue and gingival tissue in the patients with chronic periodontitis

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ABSTRACT

Objective: To evaluate the DEC1 expression of periodontal ligament tissue and gingival tissue in the patients with chronic periodontitis.

Methods: 20 non-smoking patients with chronic periodontitis and 20 healthy individuals were enrolled. Periodontal ligament tissue and gingival tissue samples from healthy subjects were collected during teeth extraction for orthodontic reason or the third molar extraction. The parallel samples from patients with chronic periodontitis were obtained during periodontal flap operations or teeth extraction as part of periodontal treatment. The DEC1 expression and the alkaline phosphatase (ALP) activity of both the periodontal ligament tissue and gingival tissue were determined by Western blot, Immunohistochemistry and ALP Detection Kit.

Results: The DEC1 expression of periodontal ligament tissue in the patients with chronic periodontitis decreased significantly along with the decreased ALP activity. On the contrary, the DEC1 expression of gingival tissue in the patients with chronic periodontitis increased significantly. Further study found that the DEC1 expression of gingival tissue increased mainly in the suprabasal layer of gingival epithelial cells but decreased in the gingival connective tissue of the patients with chronic periodontitis.

Conclusion: The DEC1 expression decreases in the periodontal ligament tissue which is related to the osteogenic capacity, whereas the DEC1 expression increases in the suprabasal layer of gingival epithelial cells which are involved in immune inflammatory response in the patients with chronic periodontitis. The findings provide a new target to explore the pathology and the therapy of periodontitis.

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Abbreviations: DEC1, differentiated embryo-chondrocyte 1; SHARP-2, split and hairyrelated protein-2; Stra13, stimulation of retinoic acid 13; ALP, alkaline phosphatase; OC, osteocalcin; PTH, parathyroid hormone; PTHrP, PTH-related peptide; TGF-β, transforming growth factor-β; BMP-2, bone morphogenetic protein-2; cAMP, cyclic adenosine monophosphate; IL-1, interleukin-1; TNF-α, tumour necrosis factor-α; RANKL, receptor activator of Nf Kb ligand; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate buffer saline; BSA, bovine serum albumin; MSC, mesenchymal stem cells.

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1. Introduction

Besides dental caries, periodontal disease is one of the most prevalent diseases in the world and around 5–15% of the population suffers from severe periodontitis.[1,2] Periodontitis is the result of complex interactions among periodontopathogenic bacteria, the host defence immune system, and environmental factors. A complex interaction between these bacteria and the host immune inflammatory response results in the loss of the collagenous structures which support the teeth.[3–5] Immune and inflammatory mediators, such as interleukin-1(IL-1), IL-6, IL-8, tumour necrosis factor-α (TNF-α), and receptor activator of NFκB ligand (RANKL) are involved in periodontal diseases.[6,7] These mediators may affect the activities of leukocytes, osteoblasts and osteoclasts and promote the tissue remodelling process systemically and locally,[8,9] ultimately leading to alveolar bone loss. However, the detail mechanisms of periodontitis progression are not well understood.

Human DEC1 (differentiated embryo-chondrocyte 1,DEC1), mouse Stra13 (stimulated with retinoic acid 13, Stra13), and rat SHARP2 (split and hairy related protein 2,SHARP2) constitute a new and structurally distinct class of basic helix-loop-helix (bHLH) proteins.[10–12] DEC1 is widely expressed in most normal tissues, including cartilage, lung, kidney, heart, liver, spleen, intestine.[13–15] DEC1 can be induced by extracellular stimuli, such as serum starvation, trans retinoic acid, growth factors, hormones and infection.[16–19] It is associated with chondrogenesis, neurogenesis, immune response, biological rhythm, lipogenesis, cell differentiation and carcinogenesis.[20–23] The transforming growth factor-β (TGF-β), bone morphogenetic protein-2 (BMP-2) and insulin which stimulated cartilage formation increased the DEC1 expression and cartilage formation.[24] On the contrary, PTH and the PTH-related peptide (PTHrP) suppressed the DEC1 expression and the differentiation of the ATDC5 cells.[24] DEC1 increased the chondrocyte differentiation via the cAMP (cyclic adenosine monophosphate) pathway.[25] The overexpression of DEC1 enhanced the osteocalcin (OC) expression and alkaline phosphatase (ALP) and induced matrix calcification. The knockdown of DEC1 with siRNA suppressed the expression of osteoblastic phenotype in the induced mesenchymal stem cells.[26] In addition, the DEC1 expression is highly elevated in response to environmental stimuli such as hypoxia (like periodontal pocket)[27] and cytokines such as IL-6,[28] and TNFα.[27,29] DEC1 can participate in the immune response by T cells.[30,31] These data imply that DEC1 is likely to be involved in bone metabolic diseases such as osteoporosis and periodontitis.

Our previous study shows that DEC1 increases the osteogenic capacity in the osteoblasts.[32] Based on the information mentioned above, we hypothesize that DEC1 is likely to play an important role in the development of chronic periodontitis. This study will help to further study the pathogenesis of the periodontitis.

2. Materials and methods

2.1. Chemicals and supplied

The antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Abcam (Cambridge, UK). The goat anti-rabbit IgG conjugated with horseradish peroxidase was from Pierce Chemical (Pierce, Rockford, IL, USA). Polyclone antibody (from rabbit) against DEC1 was donated by Dr. Yan Lab (University of Rhode Island). AKP/ALP Kit was from Nanjing Jiancheng Bioengineering institute (Jiancheng, Nanjing, China). Nitrocellulose membrane was from Bio-Rad Laboratories (Hercules, CA, USA). Bicinchoninic acid (BCA) protein assay reagent was from Pierce (Pierce, Rockford, IL, USA). All other reagents were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Study subjects

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by Ethics Committee of Jinling Hospital, Nanjing Medical University (Reg. no. 2013GJJ-027), Nanjing, China. All participants voluntarily provided written informed consent and recruited at the Department of Stomatology, Jinling Hospital, Nanjing Medical University.

Samples were collected from 20 patients with chronic periodontitis (9 males and 11 females from 30 to 59 years old) during routine periodontal flap operations which was done after the initial phase of periodontal therapy comprising conventional scaling and root planning or after teeth extraction as part of periodontal treatment. The diagnosis of chronic periodontitis was based on the pathological values for the gingival index (≥2), probing pocket depth ≥4 mm (measured at six sites per tooth) with bleeding upon probing, and clinical attachment level ≥3 mm combined with radiographic evidence of alveolar bone loss, but with no evidence of rapid progression of disease (Table 1, Fig. 1). Specimens from 20 healthy controls (10 males and 10 females from 19 to 59 years old) were obtained through teeth extraction as part of

Table 1 – The characteristics and clinical parameters in control subjects and periodontitis patients.

Clinic parameter	Gingival samples		Periodontal ligament samples	
	Control (n = 9)	Periodontitis (n = 9)	Control (n = 11)	Periodontitis (n = 11)
Gingival index(GI)	0.17 ± 0.22	2.36 ± 0.36***	0.14 ± 0.36	2.50 ± 0.38***
Probing pocket depth(PPD)	1.94 ± 0.46	7.19 ± 0.70***	1.97 ± 0.28	7.12 ± 0.80***
Clinical attachment level(CAL)	0.74 ± 0.51	7.21 ± 0.69***	0.64 ± 0.49	7.17 ± 0.95***

Data are expressed as mean ± SD.
*** $P < 0.001$, is considered as significant difference from control group.