Archives of Oral Biology 84 (2017) 37–44

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research Paper

Effects of connective tissue growth factor on human periodontal ligament fibroblasts

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ARTICLE INFO

Keywords:
Connective tissue growth factor
Human periodontal ligament fibroblasts
Periodontal regeneration
Concentrations

ABSTRACT

Objective: The aim of this study was to evaluate the effects of different concentrations of connective tissue growth factor (CTGF) on human periodontal ligament fibroblasts(HPLFs).
Design: HPLFs were cultured and identified. Then, different concentrations of CTGF (1, 5, 10, 50, 100 ng/ml) were added to the HPLF culture. Next, CCK-8 assays, alkaline phosphatase (ALP) assays, hydroxyproline determination, alizarin red staining methods, Transwell chambers and real-time PCR methods were applied to observe the effects of CTGF on the proliferation, ALP activity, synthesis of collagen, formation of mineralized nodules and migration. We also studied expression of ALP, fiber link protein (FN), integrin-binding sialoprotein (IBSP), osteocalcin (OC), and integrin beta 1 (ITGB1) mRNA by HPLFs. Statistical significance was assumed if $P < 0.05$ or $P < 0.01$.
Results: The addition of CTGF (1, 5, 10 ng/ml) remarkably promoted the proliferation and collagen synthesis of HPLFs compared with controls. CTGF (1, 5, 10, 50 ng/ml) improved ALP activity of HPLFs, and at all concentrations, CTGF (1, 5, 10, 50, 100 ng/ml) improved the expression of ALP, FN, IBSP and ITGB1 mRNA. In addition, CTGF (1, 5, 10, 50, 100 ng/ml) promoted the migration of HPLFs, which was dose-dependent, with maximal promotion in the 10 ng/ml group ($P < 0.05$ or $P < 0.01$).
Conclusions: Thus, in a certain range of concentrations, CTGF can promote the biological effects, including proliferation, migration and collagen synthesis of HPLFs, to promote the differentiation of HPLFs in the process of osteogenesis.

1. Introduction

As one of the most common infectious diseases, periodontal disease (PD) causes substantial destruction of alveolar bone, periodontal ligament (PDL), and gingiva, leaving the oral environment exposed and allowing initiation of root contamination and tooth loss. PDL, a type of tooth-supporting structure and non-mineralized connective tissue between alveolar bone and cementum, is composed of heterogeneous cell populations, including osteoclasts, cementoblasts, fibroblasts, osteoblasts, mast cells, mesenchymal cells, and phagocytes (Beertsen, McCulloch, & Sodek, 1997). PDL appears to be actively involved in remodeling of alveolar bone, has shock-absorbing properties against mechanical stress, and prevents the tooth and alveolar bone from being damaged during mastication. Thus, PDL has an important functional role in the maintenance and renewal of periodontal tissues (Wu, Zhang, Wang, Zhang, & Tan, 2015) and enables teeth to move via periodontal regeneration during orthodontic treatment (Yashiro et al., 2014). Among the cells present in the periodontium, periodontal ligament fibroblasts (PLFs) are the most abundant and play the most important role on the development, repair and regeneration of periodontal tissues (Choe et al., 2012). It has been proven that PLFs display biological characteristics similar to those of bone marrow-derived undifferentiated mesenchymal cells (Ren et al., 2015). In addition to constantly producing new dental cement and principal fibers and reconstructing alveolar bone, PLFs also synthesize the extracellular matrix, which holds the cells and plays special biological roles in cell conglutination, transportation and mineralization (Choe et al., 2012; Zhang et al., 2013). Being naturally osteogenic, PLFs are capable of differentiating into osteoblasts (Heo, Lee, & Lee, 2010). Furthermore, it has been found that PLFs are able to perceive the mechanical signals

Abbreviations: CTGF, connective tissue growth factor; HPLFs, human periodontal ligament fibroblasts; ALP, alkaline phosphatase; FN, fiber link protein; IBSP, integrin binding sialoprotein; OC, osteocalcin; ITGB1, integrin beta 1; PDL, periodontal ligament; PLFs, periodontal ligament fibroblasts; ECM, extracellular matrix; TGF-β, transforming growth factor-β

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http://dx.doi.org/10.1016/j.archoralbio.2017.09.010
Received 9 January 2017; Received in revised form 28 August 2017; Accepted 16 September 2017

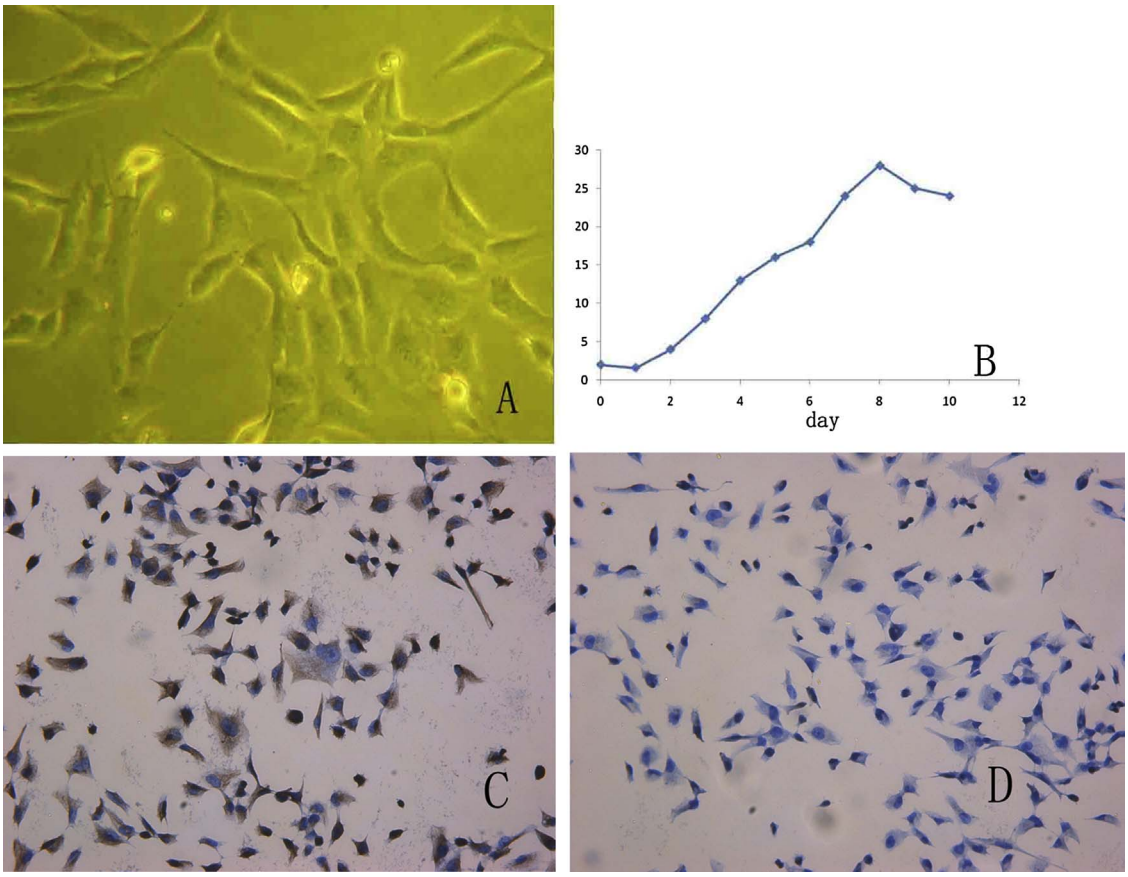

Fig. 1. The morphology, immunohistochemical staining and proliferation of HPLFs. A: HPLFs were either fusiform or dendroid in shape, with full cell bodies, clear nuclei, and two or three fine cytoplasmic processes. (40×). B: The proliferation curve of HPLFs. It was similar to an upside down 'S' with the arrest, lgarithmic growth, and plateau phases. C: Vimentin was positive in the cytoplasm of HPLFs (20×). D: Keratin was not expressed in HPLFs (20×).

and exhibit a number of the phenotypic characteristics of osteoblasts under stress stimulation, including osteocalcin (OCN), osteopontin (OPN), Osteriox (also known as SP7), sialoprotein (BSP), collagen type I (Col I), and activating transcription factor 4 (ATF4). The quality of PLFs is related to the differentiation of osteoblasts and remodeling of periodontal tissue (Ren et al., 2015).

Connective tissue growth factor (CTGF), also known as CCN2, is a member of the CCN family and is a secreted matricellular and multifunctional protein. The CCN family was named after the first three members, Cyr61 (CCN1), CTGF (CCN2) and Nov (CCN3), were identified. It also includes CCN4 (WISP-1), CCN5 (WISP-2) and CCN6 (WISP-3) (Klaassen, van Geest, Kuiper, van Noorden, & Schlingemann, 2015). It has been shown that CCN family members are involved in regulation of cell proliferation, differentiation, migration, extracellular matrix remodeling and apoptosis (Chen & Lau, 2009). CTGF was originally identified as a growth factor-inducible immediate early gene in mouse fibroblasts and in human vascular endothelial cells and is the most studied member of this family (Cheng, Chang, Fang, Sun, & Leung, 2015), which was found to promote the chemotaxis and mitosis of fibroblasts. While its expression level in various tissues under the normal physiological state is lower, CTGF expression can be up-regulated greatly in many diseases, especially in fibrotic and cancerous tissues or under high mechanical stress load stimulation (Chaqour & Goppelt-Struebe, 2006; Lomas et al., 2011; Reich, Maziel, Ashkenazi, & Ornan, 2010; Wang et al., 2011). Moreover, it was recently reported that CTGF/CCN2 gene expression in human PDL cells is up-regulated by stretch loading (Yuda et al., 2015). CTGF has been implicated in a number of more complex biological processes, including angiogenesis, osteogenesis, chondrogenesis, fibrosis, wound healing, and tumorigenesis (Arnott et al., 2011). It has been shown that CTGF is involved in regulation of various biological processes associated with fibrogenesis, including proliferation, differentiation, chemotaxis, cellular adhesion, migration, production of extracellular matrix (ECM) and angiogenesis, especially in promotion of deposition of several ECM proteins, such as fibronectin, collagen, and tenascin C (Yang et al., 2015; Zhang, Meng, Zhu, Liu, & Deng, 2014). Furthermore, CTGF also contributes to the proliferation and differentiation of mouse periodontal ligament-derived cells and a human periodontal ligament stem/progenitor cell line (Asano et al., 2005; Yuda et al., 2015).

However, to date, there has been no effort to determine the effects of different concentrations of CTGF, if any, on the biological activities of human PDL fibroblastic cells (HPLFs). This study was performed to investigate the biological effects of HPLFs, aiming to provide a new research direction for dental treatment of periodontal disease and to offer a new growth factor for periodontal disease clinical drug research and development.

2. Materials and methods

2.1. HPLFs culture and identification

Dental premolars that were free of dental caries or periodontal disease but extracted due to orthodontic treatment were collected. Immediately after extraction, the teeth were immersed in DMEM (Gibco, USA) containing double antibiotics (100 μg/ml penicillin G and 100 μg/ml streptomycin; Sijiqing Company, Hangzhou, China) and washed 10 times with PBS (pH 7.2) under sterile conditions. Then, the periodontal ligament tissues attached to the middle third of the roots were curetted gently by a surgical scalpel, minced and placed in 24-well plates. The plates were maintained at 37 °C in an atmosphere of 95% air