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Short communication

Effects of substance P on osteoblastic differentiation and heme oxygenase-1 in human periodontal ligament cells

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Abstract

Although substance P (SP) is associated with osteoclast differentiation and bone resorption, little is known about the osteogenic differentiation-inducing effects of SP in periodontal ligament (PDL) cells. This study investigated whether PDL cells could differentiate into osteoblastic-like cells by SP. The expression of osteoblastic differentiation markers such as osteopontin (OPN), osteonectin (ON), osteocalcin (OCN) and bone sialoprotein (BSP) were evaulated by Western blotting. Additionally, SP-mediated heme oxygenase-1 (HO-1) pathways were further clarified.

SP increased HO-1 and osteogenic differentiation in concentration- and time-dependent manners, as determined by OPN, ON, OCN and BSP expression. Furthermore, treatment with inhibitors of p38, ERK MAPK, and NF-κB abolished SP-induced osteogenic differentiation and HO-1 expression. SP-induced translocation of Nrf-2 was also observed. The combined results suggest that SP activates the stress-response enzymes HO-1 and Nrf-2, subsequently leading to upregulation of osteogenic differentiation in human PDL cells.

Keywords: Substance P; HO-1; PDL cells; Nrf-2; Osteogenic differentiation

1. Introduction

The periodontal ligament (PDL) is a soft connective tissue that lies between the tooth cementum and the alveolar bone. PDL cells exhibit osteoblast-like features, such as high alkaline phosphatase (ALP) activity, osteonectin (ON), bone sialoprotein (BSP), osteocalcin (OCN), osteopontin (OPN), parathyroid hormone responsiveness, production of bone-like matrix proteins, and formation of mineralized nodules, and are capable of differentiating into osteoblasts or cementoblasts (Hayami et al., 2007). However, the molecular mechanisms that mediate osteoblastic or osteoclastic differentiation are not completely understood, especially in human PDL cells.

Substance P (SP), a neuropeptide present in primary sensory neurons, has important roles in many physiological, inflammatory and pathological processes including osteoblastic bone formation and osteoclastic bone resorption (Mori et al., 1999; Lerner, 2002). SP was previously reported to increase bone resorption in an experiment using mouse calvaria (Sherman and Chole, 1995). Mori et al. (Mori et al.,

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1999) demonstrated that the addition of SP to cultured rabbit osteoclasts induces an increase in intracellular calcium concentration, which could be abrogated by an SP receptor antagonist. Moreover, SP upregulates osteoclastogenesis by activating the receptor activator of nuclear factor-kappa B ligand (RANKL) in synovial fibroblastic cells (Shih and Bernard, 1997a,b), osteoclast precursors cells (Sohn, 2005), and human pulp cells (Kojima et al., 2006).

However, the effect of SP on bone formation remains unclear. Shih and Bernard (1997a,b) reported that treating bone marrow cells with SP increased the number and size of bone colonies. In contrast, SP inhibited osteoblastic cell differentiation in rat bone marrow-derived osteogenic cells (Adamus and Dabrowski, 2001) and rat calvarial cells (Azuma et al., 2004) by suppressing the expression of core-binding factor a-1 (Cbfa-1), bone matrix protein, SP receptors, and alkaline phosphatase. Recently, it was found that SP stimulates rat osteoblastic cell differentiation by inducing the expression of Cbfa-1, type I collagen, and osteocalcin (OCN) mRNA in the osteoblasts (Goto et al., 2007). Although the results were not uniform, it is certain that osteoblastic bone formation is affected by SP.

Furthermore, SP is present in the nerve fibers of dental pulp and periodontal tissues in rats, cats, monkeys, and humans (Lutherman et al., 1988). SP-immunopositive nerve fibers alter their patterns as a result of local pulp trauma, possibly indicating that SP-containing fibers are involved in the inflammatory process as well as in tissue injury and repair in human dental pulp (Casasco et al., 1990). Moreover, the expression of SP increased in dental pulp and PDL in response to orthodontic force in experimental studies (Norevall et al., 1998). Therefore, SP may be involved in the initial pulpal and periodontal inflammation that occurs in orthodontic tooth movement or periodontal remodeling.

Heme oxygenase (HO) is a rate-limiting enzyme involved in the catabolism of heme, yielding equimolar quantities of carbon monoxide (CO), free iron, and biliverdin. One of the 3 mammalian HO isoforms, HO-1 (also called heat shock protein 32), is a stress-response protein activated by various agents and involved in a variety of regulatory and protective mechanisms in cells (Otterbein and Choi, 2000). We previously reported that pro-inflammatory cytokines (Min et al., 2006a,b), nitric oxide (Min et al., 2006a,b), and H_2O_2 (Pi et al., 2007a,b) induced expression of HO-1, potentially playing a role in cytoprotection within human pulp and PDL cells. Regarding the mechanisms of HO-1 induction, several studies have suggested the involvement of mitogen-activated protein kinase (MAPK), nuclear factor-Kappa B (NF-κB), the phosphoinositide-3 kinase (PI3K)/Akt pathways and nuclear factor erythroid 2-related factor 2 (Nrf-2)(Alvarez-Maqueda et al., 2004). According to our previous study, HO-1 expression was induced by SP in human PDL cells (Lee et al., 2007). However, the countering of the SP-inductive effects of osteoblastic differentiation in PDL cells by HO-1 has not yet been reported.

This study investigates whether SP-treated human PDL cells could differentiate into osteoblastic cells. We further examined the HO-1 pathways involved in the expression of osteogenic differentiation markers such as OP, ON, OCN, and BSP induced by SP.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and other tissue culture reagents were obtained from Gibco BRL Co. (Grand Island, NY, USA). Zinc protoporphyrin IX (ZnPP IX) and hemin were purchased from Porphyrin Products (Logan, UT, USA). Anti-OPN, anti-OCN, anti-ON, anti-BSP and anti-HO-1 antibodies were purchased from Santa Cruz Biotechnology (Heidelberg, Germany). Antibodies against Nrf-2 were purchased from Cell Signaling Technology (Beverly, MA, USA). Pyrrolidine dithiocarbamate (PDTC) and the MAP kinase inhibitors SB203580, PD98059, and SP600125 were purchased from Calbiochem (La Jolla, CA, USA). SP and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

2.2. Cell culture

HPV16-immortalized human periodontal ligament cells (IPDL) were derived by transfecting normal human PDL cells with a PLXSN vector containing the E6/E7 open reading frames of HPV type 16, following previously described methods (Pi et al., 2007a,b). The IPDL cells were cultured in DMEM (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% FBS (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 in air at 37 °C. The study was approved by the institutional review board and ethical Committee at Wonkwang University.

2.3. Western blot analysis

Treated cells were subjected to lysis buffer (10 mM Na_2HPO_4 (pH 7.2), 0.9% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 0.2% sodium azide and 0.004% sodium fluoride). Protein concentrations were determined with the Bradford assay (Bio-Rad Laboratories, USA) using bovine serum albumin (BSA) as standards. Equivalent amounts of total protein from each cell extract sample were denatured and run on a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins in the gel were electrophoretically transferred to a PVDF membrane (Millipore Corp., Bedford, MA, USA), which was then blocked with blocking solution (Zymed, San Francisco, CA, USA), and then reacted with anti-ON (1:2500 dilution), anti-BSP (1:2500 dilution) and anti-HO-1 (1:2500 dilution) polyclonal antibodies for 1 h. After washing with PBS, membranes were incubated with each anti-rabbit IgG in blocking solution for 40 min. Reactive bands were visualized using an enhanced chemiluminescence (ECL) kit (Amersham Corp., Arlington