Archives of Oral Biology 73 (2017) 129–135

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Cyclic-stretch induces apoptosis in human periodontal ligament cells by activation of caspase-5

Dan Zhao[a,b,1], Yaqin Wu[a,b,1], Chun Xu[a,b,*], Fuqiang Zhang[a,b,*]

[a] Department of Prosthodontics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Shanghai Key Laboratory of Stomatology & Shanghai Research Institute of Stomatology, Shanghai, China

ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 24 August 2016
Accepted 11 October 2016

Keywords:
Apoptosis
Caspase-5
Cyclic stretch
Periodontal ligament cell

ABSTRACT

Background and objective: As caspase-5 plays a role in apoptosis, the present study aimed to identify the expression and activation of caspase-5 in human periodontal ligament cells in response to cyclic stretch and the role of caspase-5 in stretch-induced apoptosis.
Methods: Human PDL cells were exposed to 10% or 20% stretch strain for 6 or 24 h, and the mRNA and protein expressions of caspase-5 were analyzed with real-time PCR and Western blot, respectively. The caspase-5 activation was detected by colorimetric assay. Then the influence of the inhibition of caspase-5 on the stretch-induced apoptosis and caspase-3 activation were analyzed with flow cytometry and colorimetric assay, respectively.
Results: Both 6 and 24 h stretches increased mRNA, protein expression and activation of caspase-5 in human PDL cells. Inhibition of caspase-5 inhibited the stretch-induced apoptosis and caspase-3 activation in human PDL cells.
Conclusion: This study for the first time identified the expression and activation of caspase-5 in cyclic stretched human PDL cells and found that the stretch-induced apoptosis and caspase-3 activation were caspase-5 dependent.

1. Introduction

Mechanical stresses caused by occlusal load and orthodontic tooth movement act on the periodontal ligament (PDL), which is a connection between cementum and alveolar bone (Kaku & Yamauchi, 2014). Physiological mechanical stimulations generated during mastication play an important role in periodontal homeostatic process, while abnormal mechanical stimulations aroused from occlusal overloading or improper orthodontic treatment induce PDL cell death, which has a crucial role in physiological and pathological process (Kaneko, Ohashi, Soma, & Yanagishita, 2001; Kaku, Uoshima, Yamashita, & Miura, 2005; Krishnan & Davidovitch, 2006).

It is considered that cell death has two major mechanisms: necrosis or programmed cell death (PCD). Being a type of PCD, apoptosis plays an important role in protecting organisms from damage (Kerr, Wyllie, & Currie, 1972). Caspases are a family of proteins, which regulate cell death and inflammation to maintain tissue homeostasis. Accordingly, caspases have been broadly sorted by their roles in the cell death including caspase-3, −6, −7, −8, and −9, and in inflammation including caspase-1, −11, and −12 in mice and caspase-1, −4, −5, −12 in human (McIlwain, Berger, & Mak, 2013). Caspase-3 is one of the most important members of the family due to its core position in the apoptotic process (Mukai et al., 2005).

In the past few years, in vivo studies suggested that apoptosis was induced in PDL cells by mechanical stress during orthodontic tooth movement (Hatai et al., 2001). Apoptosis of MG-63 osteoblast-like cells was also reported to be induced by in vitro application of compressive force via the caspase-8 signaling cascade (Goga, Chiba, Shimizu, & Mitani, 2006). Recently, the link between different types of PCD and periodontal diseases has been pointed out (Tsuda, Ning, Yamaguchi, & Suzuki, 2012). Our recent studies also revealed that apoptosis was induced in cultured human PDL cells by cyclic stretch, probably via the caspase-9 signaling cascade (Zhong et al., 2008; Hao, Xu, Sun, & Zhang, 2009).

Abbreviations: PDL, periodontal ligament; PCD, programmed cell death; CSU, cell strain unit; DMEM, Dulbecco's modification of Eagle's medium; FBS, fetal bovine serum; PC, personal computer; PBS, phosphate buffered saline; PCR, polymerase chain reaction; ANOVA, analysis of variance; LSD, least-significant difference.

* Corresponding authors at: Department of Prosthodontics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China.
E-mail addresses: imxuchun@163.com (C. Xu), fqzhang@vip.163.com (F. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.archoralbio.2016.10.009
0003-9969/

Several force-sensing genes implicated in apoptosis pathway, including those encoding the caspase family members (CASP5 and CASP7), were also identified (Xu et al., 2011).

Caspase-5 has been known to be a member of the caspase-1 subfamily and its function has not yet been fully characterized (Nicholson, 1999). The expression of caspase-5 is very low in many normal tissues of human body, while its expression has been reported to be regulated by bacterial lipopolysaccharide (LPS) stimulation (Lin, Choi, & Porter, 2000). It is suggested that caspase-5 is involved in apoptosis, supported by the report that over-expression of caspase-5 induced apoptosis in Rat-1 cell (Kamada, Funahashi, & Tsujimoto, 1997). In another study, hypoxia-induced apoptosis was reduced by the inhibition of caspase-5 through specific siRNA, suggesting a potential role of caspase-5 in apoptosis (Zhu et al., 2012).

As noted above, apoptosis in cultured human PDL cells has been reported to be induced by cyclic stretch, and caspase-5 has been suggested to be involved in apoptosis. Whether caspase-5 plays a role in stretch-induced PDL cell apoptosis remains an interesting while unsolved question. To probe into this problem, the present study examined the mRNA and protein expressions of caspase-5 as well as the influence of the inhibition of caspase-5 on the stretch-induced apoptosis and caspase-3 activation, in cultured human PDL cells in response to in vitro cyclic stretch. To our best knowledge, this is the first report probing the role of caspase-5 in stretch-induced PDL cell apoptosis. Although it's just a beginning, the results in the present study should facilitate further studies toward the mechanisms underlying the force-driven PCD and help us understand the force-related periodontal homeostasis and remodeling better.

2. Material and methods

2.1. Preparation of human PDL cells

Human PDL cells were obtained from healthy premolars extracted from children aged 11–13 year for orthodontic reasons, after informed consents were obtained from their parents. The protocol for harvesting human periodontal tissue from extracted teeth was approved by the Ethics Committee of Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Reference: [2008]17). Human PDL cells were cultured as previously described (Xu et al., 2012). PDL on the middle of the root was acquired with a sterile scalpel. Then pieces of PDL were attached to a cell culture flask and cultured in Dulbecco's modification of Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 20% (v/v) fetal bovine serum (FBS, Hyclone, Logan, UT, USA) and five-fold reinforced antibiotics (500 U/mL penicillin and 500 μg/mL streptomycin, Sigma, St. Louis, MO, USA) at 37 °C in a humidified atmosphere of air containing 5% CO2. Cells that grew out from the extracts were passaged in DMEM supplemented with 10% (v/v) FBS and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Cells at passage 4–6 were used in the present study.

2.2. Stretch loading

Human PDL cells were stretched by using a cell strain unit (CSU) which has been described previously (Hao et al., 2009; Xu et al., 2011; Xu et al., 2012; Zhong et al., 2008). The CSU includes a strainer, a controller and a personal computer (PC). Cells were seeded in a flexible-bottomed culture dish (diameter 60 mm) whose bottom is made of elastic silicon rubber (Q7-4750, Dow Corning Co., Midland, MI, USA). A spherical cap moves up and down repeatedly and stretches cells attached on the bottom of culture dish by deforming the elastic silicon bottom. All changes in stretch strain and movement of the spherical cap are controlled by the controller and PC. Cells were seeded in the flexible-bottomed culture dishes at a concentration of 1.5×10^6 cells per dish and reached confluence following 3 d of culture, and were then exposed to 10% or 20% stretch strain for 6 or 24 h at a frequency of 6 cycles/min, each cycle consisting of a 5 s stretch period followed by a 5 s relaxation period. The treatments were repeated three times for every combination of stretch strain (10% or 20% stretch strain) and loading duration (6 or 24 h). Cells cultured in similar condition but without stretch served as non-stretching controls. It is believed that a stretch strain no higher than 24% is reasonable for cultured PDL cells to mimic the strain which may be confronted by in vivo PDL cells (Yamaguchi et al., 1994). Our recent studies discovered that 6 and 24 h cyclic stretches with 10% and 20% strains induced apoptosis in cultured human PDL cells (Xu et al., 2012; Zhong et al., 2008). Therefore, 10% and 20% stretch strains were chosen to load cells for 6 and 24 h in the present study. The loading frequency of 6 cycles/min (5s stretch and 5s relaxation) was the same to that in our previous studies (Xu et al., 2012).

2.3. RNA isolation and cDNA synthesis

Total RNA from cells in each group was isolated by using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After stretch loading, the medium was removed. Cells were washed with phosphate buffered saline (PBS) once and an appropriate amount of Trizol reagent (1 mL/10 cm^2 area of culture dish) was added. The cell lysate was incubated at room temperature for 5 min and then transferred to a 1.5 mL Eppendorf tube. Then 0.2 mL chloroform per 1 mL Trizol reagent was added in, and the tube was shaken vigorously by hand for 15 s. A further incubation of 2–3 min at room temperature was conducted for the lysate prior to a centrifugation at 12,000 *g* for 15 min at 4 °C. Following centrifugation, the clear aqueous phase was transferred to a fresh tube and mixed with an equal volume of isopropyl alcohol and incubated at room temperature for 10 min to precipitate the RNA. After centrifugation at 12,000 *g* for 10 min at 4 °C, the RNA pellet was washed once with 75% ethanol and centrifuged at 7500 g for 5 min at 4 °C. And then before dissolved in Rnase-free water, the RNA pellet was air-dried for 5–10 min. Contaminating genomic DNA was removed from total RNA samples by Dnase I digestion. The RNA samples were then purified by means of the RNeasy® MinElute™ Cleanup Kit (Qiagen, Valencia, CA, USA) in accordance with the instructions. The RNA concentration was determined by using a ND-1000 Spectrophotometer (Nanodrop, Rockland, DE, USA), and the RNA purity was confirmed by 260/280 optical density value of 1.8–2.0. The RNA samples were assessed for degradation status by denaturing agarose gel electrophoresis. First-strand cDNA synthesis was performed with the RT^2 First Strand kit (SABiosciences, Frederick, MD, USA) in accordance with the manufacturer's instructions.

2.4. Real-time PCR analysis

The mRNA level of caspase-5 was quantitated by Real-time PCR. The mRNA Level of β-actin served as internal control. RT^2 qPCR Primer Assay for Human CASP5 (PPH00108E) and RT^2 qPCR Primer Assay for Human β-actin (PPH00073E) (SABiosciences) were used as the primers for CASP5 and β-actin respectively. The Real-time PCR was performed in an ABI 7900 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA) with cDNA samples, primers and RT^2 Real-Time™ SYBR Green PCR Master Mix (SABiosciences), according to manufacturer's protocols. Amplification was performed as follows: 95 °C, 10 min followed by 35 cycles of denaturation at 95 °C for 15s, annealing at 60 °C for 60s. The