Maresin 1 regulates autophagy and inflammation in human periodontal ligament cells through glycogen synthase kinase–3β/β-catenin pathway under inflammatory conditions

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ABSTRACT

Objective: Accumulating lines of evidence suggest that maresin 1 (MaR-1) exerts anti-inflammatory effects in many cell types and plays beneficial roles in inflammatory disease, such as peritonitis and colitis. Moreover, it has been demonstrated that MaR-1 play protective roles against localized aggressive periodontitis. However, the function and mechanism of MaR-1 in human periodontal ligament cells (PDL) cells from periodontitis are poorly understood. The present study aimed to clarify the effects and molecular mechanism of MaR-1 in PDL cell survival and inflammation.
Methods: PDL cells were isolated from the middle third of the root surface of premolars from four healthy humans; MTT assay and cell death detection ELISA assay were used to detect cell survival and apoptosis; Inflammatory cytokines level was measured by ELISA assay; RT-PCR and western blot was used to measure the mRNA and protein expression in this study.
Results: Here we found that MaR-1 treatment markedly promotes survival and inhibits apoptosis in PDL cell treated by LPS. MaR-1 treatment strikingly suppressed the production of LPS-induced pro-inflammatory cytokines IL-6, IL-8, TNF-α and IL-1β. MaR-1 also promotes autophagy by increasing the ratio of LC3II/LC3I, the level of beclin-1 and reduced the expression of p62 in LPS treated PDL cells, which is beneficial to cell survival. Moreover, the results showed that MaR-1-mediated autophagy is dependent on the glycogen synthase kinase–3β(GSK-3β)/β-catenin signal pathway. The inhibitor of autophagy 3-MA and the inhibitor of the GSK-3β/β-catenin signal pathway LiCL both reverse the effects of MaR-1 on LPS-treated PDL cell survival and inflammation.
Conclusion: MaR-1 promotes cell survival and alleviates cell inflammation by activating GSK-3β/β-catenin-dependent autophagy. These results provide new insights into the mechanism of chronic periodontitis.

1. Introduction

Chronic periodontitis is a multifactorial inflammatory disease with an adverse impact on human health (Hajishengallis, 2015). Periodontitis, which affects the periodontium and gradually results in tooth loss, is initiated by colonization with microorganisms (Tonetti & Van Dyke, 2013). Virulent oral microbiota have been considered the main cause of periodontitis and accumulation of these bacteria affects the integrity of the supporting structures and causes bone loss (Bai, Wei, Wu, Wei, & Wang, 2016). Also, the bacteria could induce immune responses in periodontal tissues and mediate periodontal tissue destruction (Benakanakere & Kinane, 2012). The periodontium plays

supporting roles in the tooth and is composed of the mineralized bone-like cementum, the alveolar bone and the periodontal ligament (PDL), which is a connective tissue between the cementum and alveolar bone (El-Awady et al., 2010; Morsczeck et al., 2005). Evidence has shown that chronic inflammatory disease can lead to the progressive damage of cells in the periodontium, including PDL cells (Zhang et al., 2016). Hence, knowledge about the mechanisms related to PDL cell damage is needed to understand the pathogenic mechanism of, and find more effective therapeutic strategies for, human periodontitis.

Commonly, proper inflammation response is beneficial for the host defense; however, excessive inflammatory responses can result in serious damage, such as tissue destruction or organ failure. Periodontitis is

characterized by excessive inflammation and accumulating data has suggested that genetic variations that could affect immunological reactions or inflammation response may play an important role in periodontitis aggravation (Golz et al., 2015). Autophagy, a cellular degradation pathway that is dependent on lysosome, plays an essential role in the pathogenesis of multiple diseases, including inflammatory diseases such as periodontitis (Bullon et al., 2012). Autophagy has a large range of functions that have proved to have anti-inflammatory effects. It is now well established that the autophagy pathway and proteins play a crucial role in inflammation and immunity. Thus, we believe that a molecule or compound that affects autophagy may have a role in inflammation.

Emerging studies have revealed that maresin 1 (7R, 14S-dihydroxy-docosa-4Z, 8E, 10E, 12Z, 16Z, 19Z-hexaenoic Acid, MaR1) plays a role in cell inflammation and autophagy (Chatterjee et al., 2014; Laiglesia et al., 2016). Also, Wang et al. indicated that endogenous MaR1 is reduced in macrophages from localized aggressive periodontitis patients; moreover, the authors also suggested that administration of MaR1 was effective in localized aggressive periodontitis and other inflammatory oral diseases (Wang et al., 2015). MaR1, a potent proresolving lipid mediator, is the first identified member of the maresin family that is derived from docosahexaenoic acid (Serhan et al., 2009). The main function of MaR1 is anti-inflammatory. MaR1 has been shown to alleviate chemotherapy-induced neuropathic pain and formalin-induced inflammatory pain (Park, 2015), and it also promotes tissue regeneration. However, the function and mechanism of MaR1 in PDL cell inflammation and apoptosis are unknown. In the present study, we showed that MaR1 can activate the GSK-3β/β-catenin pathway and then mediate cell autophagy. MaR1 treatment increases LPS-induced PDL cell survival, inhibits its apoptosis and reduces the production of inflammatory cytokines through the GSK-3β/β-catenin pathway.

2. Methods

2.1. Cell isolation and culture

Human periodontal ligament cells (hPDLCs) were obtained according to the method previously described (Miron et al., 2013). PDL cells were isolated from the middle third of the root surface of premolars from four healthy humans who required orthodontic treatment. Approval was obtained from the Ethics Committee of Affiliated hospital of Yan'an University. All patients were approved and gave informed consent. The PDL cells used for the following studies were from passages 3–6. The cells were cultured at 37 °C with 5% CO2 in DMEM and supplemented with 10% FBS, and 1% penicillin/streptomycin.

2.2. LPS stimulation and maresin 1 treatment

Porphyromonas gingivalis (P. gingivalis) lipopolysaccharide (LPS) was obtained from Invivogen (Carlsbad, CA, USA). The PDL cells were seeded into 24-well plates at a density of 2×10^4 cells/well and stimulated with LPS (1 µg/mL) by cells grown to approximately 80% confluence (Bai et al., 2016). Inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8 in the PDL cell were determined to shown the inflammation conditions was established. After 24 h, the cells were treated with maresin 1 (concentration at 0.1, 1 and 10 nM, Cayman Chemical Company, Ann Arbor, MI, USA) for 24 h which dissolved in 0.1 ml normal saline, and then harvested for subsequent experiments.

2.3. Cell survival assay

Cell survival was measured using an MTT assay. PDL cells were seeded in 96-well plates at 5×10^4 cells/well and cells were stimulated by LPS for 24 h and then treated with MaR1 for 24 h. The surviving fractions were determined by using the MTT assay. The optical density (OD) of 570 nm was measured by using a microplate reader. The study

with five replicates was repeated at least three times.

2.4. Cell apoptosis

PDL cell apoptosis was determined by cell death detection ELISA assay according to the manufacturer's instruction. Induced cell death leads to mono- and oligo-nucleosomes produce in the

cytoplasmic fraction, and the cell death detection ELISA kit could determine the level of nucleosome by using antibodies against DNA and histones (Frankfurt & Krishan, 2001). Briefly, PDL cells were plated at in 96-well plates at 5×10^4 cells/well and stimulated by LPS for 24 h and then treated with MaR1 for 24 h. The nucleosome concentration was measured to indicate apoptosis according to the method described previously (Thacker, Robinson, Abel, & Tweardy, 2013). The study with five replicates was repeated at least three times.

2.5. Western blotting

Total protein from PDL cells was isolated by using RIPA. The same amount of total protein was separated by 10% SDS-PAGE. The following antibodies were used in this study: rabbit anti-GSK-3β monoclonal antibody (1: 5000), rabbit anti-β-catenin monoclonal antibody (1: 5000), mouse anti-β-actin antibody (1: 5000, Abcam, Cambridge, MA, USA). Horseradish peroxidase (HRP)-goat anti-rabbit IgG (1: 2000) and HRP-rabbit anti-mouse IgG antibody (1: 2000, Abcam) were used as secondary antibodies for 1 h at room temperature. β-actin was used as the internal control. The study with five replicates was repeated at least three times.

2.6. ELISA

PDL stimulated by LPS (1 µg/mL) for 24 h and then treated with MaR1 (0.1, 1 and 10 nM) for 24 h. The supernatants from the PDL cells were obtained, and the concentration of inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8 were measured. TNF-α, IL-1β, IL-6 and IL-8 in the PDL cell culture supernatants was measured by using an ELISA assay according to the manufacturer's directions. Inflammatory cytokine levels were quantified by using the standard curve. The study with five replicates was repeated at least three times.

2.7. Statistical analysis

Data in this study were expressed as means ± SD. Each study with five replicates was repeated at least three times. Statistical analysis was performed using one-way ANOVA followed by a Student's *t*-test. A P-value < 0.05 was considered significant.

3. Results

3.1. MaR1 treatment promotes survival and reduces apoptosis in human periodontal ligament (PDL) cells exposed to lipopolysaccharide (LPS)

In this study, PDL cells were stimulated with 1 µg/mL LPS to simulate the inflammatory microenvironment of the body. First, the effect of MaR1 on PDL cell survival and apoptosis was measured. As shown in Fig. 1, LPS stimulation remarkably inhibits PDL cell survival and induces its apoptosis compared with the control group. Also, compared with the LPS-treated group, the results indicated that administration with MaR1 promotes survival and reduces apoptosis in LPS-stimulated PDL cell at a dose-dependent manner. To verify the effects of MaR1 on LPS-induced PDL cell apoptosis, western blot assay was used. As shown in Fig. 1C, the results indicted that MaR1 treatment significantly curbed LPS-induced Bcl-2 decreased and Bax increased. Taken together, these results suggest that MaR1 treatment alleviates LPS-induced PDL cell damage.