IL-1α regulates osteogenic differentiation and osteoclastic activity of dental follicle cells via JNK and p38 MAPK pathways

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Research

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Abstract

Background

The expression of interleukin-1α (IL-1α) is increased in tooth with periapical lesion and the eruption of the permanent successor is accelerated in the presence of an infected deciduous tooth with abscess formation. Dental follicle (DF) plays a central role in tooth eruption through regulating alveolar bone resorption and formation. However, whether the increased IL-1α can disturb functions of DF and influence tooth eruption remains unknown. Herein, we studied the effect and possible mechanism of IL-1α on osteogenic differentiation, osteoclastic activity and matrix remodeling of the DF cells (DFCs).

Methods

Rat DFCs were treated with IL-1α. Quantitative real-time PCR and western blot were performed to analyze the expressions of osteogenesis, osteoclast formation and matrix remodeling related genes and proteins. DFC–bone marrow cell co-cultures, osteogenic differentiation and transwell matrigel invasion assay were used to determine the effect of IL-1α on DFCs further. Phosphorylation of JNK, ERK, p65 and p38 were evaluated in the IL-1α-treated group. Specific inhibitors of signal pathways were added to confirm their roles in this process.

Results

IL-1α decreased the expression of osteogenesis-related genes and proteins, and increased the expression of MMP9 and ratios of RANKL/OPG. DFCs cultured with IL-1α showed weaker osteogenic capacity, higher osteoclastogenic and invasive ability. Moreover, phosphorylation of JNK and p38 were up-regulated and pretreatment with specific inhibitors reversed the effect of IL-1α on DFCs.

Conclusions

IL-1α suppresses osteogenic differentiation, promotes osteoclastogenic ability and matrix remodeling of DFCs by activating JNK and p38 MAPK signaling pathways.

Background

Tooth eruption means the movement of the tooth from its site of development within alveolar bone to its position of function in the oral cavity. It is a complicated physiological process involving bone resorption in the coronal portion of the alveolar bony crypt and osteogenesis at the base of the socket. Dental follicle (DF), a loose vascular connective tissue surrounding the tooth germ in early stages of tooth development, plays a central role in tooth eruption [1–3]. Previous studies showed surgical removal of DF prevents the eruption of tooth and DF is essential for enlargement of the eruptive pathway and formation
of trabecular bone [4]. And, substituting the crowns with replacements and keeping the DF intact results in normal eruption of these replicas [2]. Cells isolated from DF expresses a heterogeneous assortment of makers associated with stemness and can differentiate toward osteoblastic phenotype and expresses osteoblastic markers [5]. Moreover, stem cells in DF are also capable of differentiating into adipocytes, and neurons [6]. As the tooth erupts into the oral cavity, DF formes the periodontal ligament, cementum and alveolar bone proper.

Multiple cytokines are involved in tooth eruption. Interleukin-1α (IL-1α) may be the initial promoter of tooth eruption. Studies show that IL-1α can enhance the gene expression of monocyte chemotactic protein-one (MCP-1) and colony-stimulating factor-one (CSF-1) [7–9]. These two cytokines are secreted by the DF cells (DFCs) and are expressed maximally at an early postnatal age, correlating with the monocyte influx into the follicle, mediating the recruitment of mononuclear cells into DF and stimulating osteoclast differentiation. IL-1α can also participate in the alveolar bone modeling process of tooth eruption through upregulation of receptor activator of nuclear factor –kappa B ligand (RANKL), tumor necrosis factor-alpha (TNF-α) [10, 11]. Furthermore, 31 proteins are upregulated and 7 proteins are downregulated in IL-1α-induced rat DFCs shown by proteomic analysis [12]. And null mice devoid of the IL-1-type 1 receptor (IL-1R I) gene exhibits delay of tooth eruption [13].

Eruption of successors and exfoliation of predecessors are complex process. The successors interact with and depend on their primary teeth, and vice versa. Inflammation of a primary tooth pulp may arise abnormal eruption the successor. The eruption of the permanent successor is accelerated in the presence of an infected deciduous tooth with abscess formation, sometimes with immature root development [14, 15]. And the expression of IL-1α is increased in tooth with periapical lesion [16, 17]. Whether the stimulated IL-1α in the periapical area of deciduous teeth will cause the premature eruption of permanent teeth through regulation of osteogenesis and osteoclastic activity of DFCs and its mechanism needs further study.

In this study, the osteogenic differentiation, osteoclastic activity and matrix remodeling of the DFCs was evaluated under the treatment of IL-1α. And the molecular mechanisms of IL-1α on the function of DFCs were further investigated. The study may enhance our understanding the process of tooth eruption regulated by IL-1α via DFCs and facilitate the clinical treatment for guiding the normal eruption of permanent tooth.

Methods

Animals and tissue preparation

Sprague-Dawley (SD) rats used in this experiment were commercially purchased from the experimental Animal Laboratory of Sichuan University. All experimental procedures were approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University, Chengdu, China (WCHSIRB-D-2016-193). The first mandibular molars were removed from embryo rats at embryo (E) 16.5 and 18.5, and
newborn rats at the age of postnatal days (PN) 1, 10 and 20. The tissues were then fixed in freshly prepared 4% paraformaldehyde for 24 h at 4 °C and decalcified by soaking in 10% ethylene diamine tetraacetic acid in phosphate buffered saline (PBS) for 30 days. Afterwards, fully decalcified samples were dehydrated in a graded ethanol series and embedded in paraffin. Paraffin sections (5 mm thick) were mounted on poly-L-Lysine-coated glass slides (Sigma–Aldrich).

**Cells isolation and culture**

DFCs were isolated and cultured according to a method described in previous study [18]. Primary DFCs were cultured in 25-cm² plastic flasks with the standard medium consisting of minimum essential medium alpha basic (1×, a-MEM, GIBCO), 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C maintained in 5% CO₂. Medium was refreshed every 3 d. Cells were digested with 0.25% trypsin (GIBCO) and routinely passaged. Cells of the 3rd passage were used. In some experiments, cell cultures were treated with IL-1α alone, or 1 uM SB203580 / 10uM SP600125 (Selleck) for 2 hours before IL-1α treatment.

**Identification of DFCs – immunofluorescence staining and transmission electron microscopy (TEM)**

To identify the DFCs, immunofluorescence staining was applied to characterize the expression of surface molecules. Antibodies included mouse anti- cytokeratin 14 (CK14) (1:100, Abcam) and mouse anti-vimentin (1:200, Millipore). TEM were also adopted to detect the homogeneous electron-dense granules. These procedures were performed according to the previous report [18].

**Cell proliferation assay (cell-counting kit-8 and colony-forming)**

To determine the most suitable concentration of IL-1α, DFCs were seeded into 96-well plates (Becton Dickinson) at a cell density of 1 × 10³ cells/well for 24 hours. Cells were treated with 1 ng/ml, 5 ng/ml, 10 ng/ml, 25 ng/ml, 50 ng/ml IL-1α (E. coli-derived, PeproTech, Rocky Hill, NJ, USA). After treatment, the cells were enumerated using a cell-counting kit-8 (CCK-8, Dojindo, Tokyo, Japan) according to the manufacturer's instructions every other day. Colony-forming was further applied to verify that the chosen concentration according to the result of CCK-8 had no effect on cells proliferation. It was performed as described previously [19]. Experiments were performed in triplicate.

**Osteogenic differentiation**

A total of 1 × 10⁵ DFCs were seeded into each well of a six-well plate. Osteogenic inducing medium containing 10% FBS, 5 mM L-glycerophosphate (Sigma), 100 nM dexamethasone (Sigma), and 50 mg/ml ascorbic acid was applied when the cells grew to 80% confluence. The experimental group contained IL-1α. The medium was changed every two days. After 7 days culture, total RNA and proteins were extracted and prepared for quantitative real-time polymerase chain reaction (qRT-PCR) analysis and western blot analysis. After 15 days culture, cells were washed twice in PBS after being fixed in 4% paraformaldehyde.
for 10 min and then incubated in 0.1% alizarin red solution (Sigma) in Tris–HCl (pH 8.3) for 10 min. The alkaline phosphatase (ALP) activity assay was also performed using BCIP/NBT ALP color development kit according to the manufacturer’s instructions (Beyotime Biotechnology, China). The numbers and areas of mineralization nodules were quantified. Experiments were performed in triplicate.

**Transwell migration assay**

Transwell migration assay was used to determine the invasion ability of DFCs under treatment of IL-1α. It was conducted as previously described [19]. Briefly, DFCs were seeded in upper inserts with serum-free medium, while a-MEM containing 10% FBS were placed in the lower chamber as a chemoattractant. The upper inserts in the experimental group contained 5 ng/ml IL-1α. After 24 h incubation, the invaded cells were fixed with 4% paraformaldehyde and stained with crystal violet, and then counted under a phase contrast microscope. Experiments were performed in triplicate.

**RNA extraction and qRT-PCR**

By using Trizol Reagent (Invitrogen, Carlsbad), RNA was split, collected, purified and extracted according to the manufacturer’s protocol. Synthesis of cDNA was performed with a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was carried out via ABI7300 real-time PCR System (Applied Biosystems, Inc, USA). Primer sequences for ALP, runt-related transcription factor 2 (RUNX2) and osteopontin (OPN), RANKL, osteoprotegerin (OPG), matrix metalloproteinase 9 (MMP9) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were synthesized by TsingKe Biotech (Beijing, China) listed in Table 1. Experiments were performed in triplicate.

| Target DNA | Forward primer | Reverse primer |
|------------|----------------|----------------|
| ALP        | CGTTGACTGTGGTTACTGCTGA | CTTCTTGTCGTGTCGCTCACA |
| RUNX2      | gag cac aaa cat ggc tga ga | tggagatggttgttgtgtcg |
| OPN        | AGCCAGGAGACACTACAAC | CTGAGTGTGGCTGTAAATGCG |
| RANKL      | TCGGGTTCCATAAGTCAG | CTGGGAATTTTGATGCTGGA |
| OPG        | CAGAGGACCACAATGGAACAGTG | TTTGTCACAAAGAGCTGACGTC |
| MMP9       | GACCTCAAGTGGCACCATCA | AGTCATCGATCGTCTCGC |
| GAPDH      | TATGACTCTACCCACGGCAAG | TACTCAGCACCAGCATCACC |

**Western blot analysis**

Total cellular protein was harvested with RIPA (Beyotime Biotechnology, China). Western blot was conducted as previously described [19]. The primary antibodies were rabbit monoclonal to ALP (1:500,
HuaAn Biotechnology), rabbit monoclonal to RUNX2 (1:1000, Cell Signaling Technology), rabbit polyclonal to OPN (1:1000, Abcam), rabbit monoclonal to MMP9 (1:500, HuaAn Biotechnology), rabbit polyclonal to heat shock protein 27 (HSP 27) (1:500, HuaAn Biotechnology), rabbit monoclonal to p38 mitogen-activated protein kinase phosphorylation (p-p38 MAPK), rabbit monoclonal to p38, rabbit monoclonal to JNK phosphorylation (p-JNK), rabbit monoclonal to JNK, rabbit monoclonal to ERK phosphorylation (p-ERK), rabbit monoclonal to ERK, rabbit monoclonal to p65 phosphorylation (p-p65), rabbit monoclonal to p65 (these eight, 1:1000, Cell Signaling Technology). Rabbit monoclonal to GAPDH (1:3000, Abcam) was used as the internal control. The intensity of each band was calculated after normalization to GAPDH. Experiments were performed in triplicate.

**In vitro osteoclasts formation**

This procedure was performed as previously described [19]. Bone marrow stromal cells got from 10-day-old SD rats were seeded into 12-well plates (10⁶ cells) with 20 ng/ml CSF-1. DFCs (20,000 cells) were seeded in 12-well hanging cell culture inserts (0.4um) to create DFCs-bone marrow stromal cells co-cultures. DFCs in the experimental group contained 5 ng/ml IL-1α. Six days later, the induced osteoclasts were detected by tartrate-resistant acid phosphatase (TRAP) staining. The TRAP staining of cells was done as previously described [19]. TRAP-kit (Beyotime Biotechnology, China) was applied according to the manufacturer’s instructions. TRAP-positive giant multinucleated (≥ 3 nuclei) cells were considered as osteoclasts. Osteo assay surface (Corning, NY, USA) was also conducted to directly assess the osteoclast activity in vitro under the instructions and the resorption area was obtained. Experiments were performed in triplicate.

**Immunohistochemical (IHC) staining**

The paraffin-embedded sections were incubated with one of the following primary antibodies: rabbit polyclonal to IL-1α (1:200, Cloud Clone Corp), rabbit polyclonal to IL-1RI (1:800, Cloud Clone Corp). IHC was performed following the manual of the immunohistochemical assay kit (Zhongshan Jinqiao biological technology co., Beijing, China) (SP method). The sections were developed with diaminobenzidine tetrahydrochlorid (DAB). No primary antibody was applied in the negative controls.

**Statistical analysis**

All data are presented as means ± standard deviations. The statistical significance between two groups was assessed by t-test. P< 0.05 was considered statistically significant. And the statistical significance among multiple groups was assessed by ANOVA. The Bonferroni test was used to compare all pairs of means after the ANOVA. Values of P< 0.05/n were considered to have statistical significance (n means the number of groups brought into Bonferroni test). All statistical testing was performed with SPSS software (version 20.0; IBM, Armonk, NY).

**Results**
The expression of IL-1α and IL-1RI in rat mandibular first molar

Immunolocalization of IL-1α and IL-1RI in the first mandibular molars of SD rats from embryo and newborn rats showed that the protein was absent at bud (E16.5) and cap (E18.5) stage. At the age of PN 1, IL-1α and IL-1RI were markedly expressed in the ameloblast layer, dental follicle and bone around the tooth germ. There was some weak staining of IL-1α in the dental papilla, and the stellate reticulum was devoid of stain. No obvious expression of IL-1RI was detected in stellate reticulum and dental papilla. At the age of PN 10, the expression of IL-1α was still intense, especially in the ameloblast layer. And cells in dental follicle were also stained. But, the results of IL-1RI showed a faint staining in the ameloblast layer, while cells in the dental follicle showed a moderate staining. At the age of PN 20, prominent expression of IL-1RI was observed in the periodontal ligament (PDL). And relatively weaker staining of IL-1α was also detected in the PDL. There was also faint staining of IL-1α and IL-1RI in the odontoblasts and alveolar bone (Fig. 1).

Culture and identification of DFCs

After an attachment period of 24 hours, primary DFCs climbed out from the tissue blocks. The purified cells of the first passage displayed a fusiform shape mostly (Fig. 2a). Cell-surface antigen profiles obtained showed that DFCs were positive for vimentin, a marker of mesenchymal cells. Meanwhile, they were negative for CK14, a marker of epithelial cells (Fig. 2b). This result indicated that these cells were mesenchymal cells and were not contaminated by epithelial cells. TEM evaluations showed homogeneous electron-dense granules in DFCs, regarded as an identifying marker of DFCs (Fig. 2c).

Weakened osteogenic capacities, promoted osteoclast-inductive capacities and matrix remodeling of DFCs by IL-1α treatment

1 ng/ml IL-1α promoted the proliferation rate of DFCs, while 10 ng/ml, 25 ng/ml and 50 ng/ml IL-1α had an inhibition effect, shown by the CCK8 assay. The proliferation rate of 5 ng/ml IL-1α group was similar to that of DFCs without treatment, as evidenced by the optical density (OD) value in the CCK8 assay (Fig. 2d) and similar colony-forming efficiency (Figs. 2e and f). So we chose 5 ng/ml IL-1α in the following study to exert as little impact as possible on the proliferation rate. To evaluate the effect of IL-1α (5 ng/ml) on the osteogenic capacity of DFCs, alizarin red staining and ALP activity assay were applied. The results displayed that DFCs under the treatment of IL-1α exhibited lower osteogenic capacity (Figs. 3a, b, c and d). qRT-PCR and western blot also indicated prominently lower expression of osteogenic markers ALP, RUNX2 and OPN during DFCs culture no matter under the osteogenic induction (Figs. 3e to g) or not (Figs. 4a to c).

Data showed that the DFCs treated with IL-1α had higher gene expression levels of RANKL and OPG at 3, 6, 12 and 24 hr compared with the DFCs control group. And the ratios of RANKL/OPG were higher in DFCs
treated by IL-1α (5 ng/ml) (Fig. 4d). To further assess the effect of IL-1α on the osteoclast-inductive capacities of DFCs, DFCs-bone marrow stromal cells co-cultures were performed. The TRAP-positive multinucleated osteoclasts were nearly twice in the IL-1α treated DFCs than the group without IL-1α treatment (Fig. 4e). And more resorption area was detected on the osteo surface in the IL-1α treated DFCs (Fig. 4f).

A modified transwell matrigel invasion assays were used to evaluate invasive ability of the DFCs under the treatment of IL-1α (5 ng/ml). IL-1α promoted the invasive ability of DFCs significantly as shown in Fig. 5a and b. Consistently, the expression of MMP9 was also increased by IL-1α in DFCs, as evidenced by the qRT-PCR and western blot results (Fig. 5c to e).

**IL-1α participating in tooth eruption via JNK and p38 MAPK pathways**

Western blot analysis showed a significant increase in HSP 27, p-p38 and p-JNK in DFCs treated with 5 ng/ml IL-1α, while the expression of p-ERK and p-p65 did not change (Fig. 6a and b).

To further confirm the role of the p38 and JNK signaling pathways in the process of IL-1α participating in tooth eruption, the p38 MAPK inhibitor SB203580 and JNK inhibitor SP600125 were used. When pretreated with SB203580, the level of p-p38 was increased. But the expression of HSP27 was substantially decreased. SB203580 not only reversed the down-expression of ALP, RUNX2 and OPN, but also weakened the expression of MMP9 (Fig. 6c and d). The application of SP600125 before IL-1α treatment decreased the expression of p-JNK. Similarly, MMP9, ALP, OPN and RUNX2 also tended to come back to the level of control groups by the treatment of SP600125 (Fig. 6e and f). These results suggested that the effect of IL-1α was reversed by the treatment with the p38 MAPK inhibitor SB203580 and JNK inhibitor SP600125. This strongly implied that p38 MAPK and JNK signaling pathway were involved in this process.

**Discussion**

Tooth eruption is a fundamental developmental and physiologic process in the alveolar bone, involving a series of metabolic events. DF is essential for tooth eruption since it initiates and regulates osteoclastogenesis, osteogenesis and matrix remodeling. Stem cells isolated from DF are pluripotent and can differentiate into osteoblasts and cementoblasts, which may contribute to alveolar bone formation and tooth development. Cells from DF can also secrete a variety of cytokines, such as RANKL, CSF, and MCP-1, acting as molecular regulation of osteoclastogenesis during tooth eruption. In this study, we investigated the role of IL-1α in regulation of tooth eruption through in vitro culture of DFCs. Results showed that IL-1α could strengthen the matrix remodeling and osteoclast-inductive capacities, and weaken the osteogenic differentiation of DFCs. JNK and p38 MAPK signaling pathway were involved in regulation of osteogenesis and osteoclastic activity of DFCs by IL-1α.
IL-1α was immunolocalized in the epithelial stellate reticulum adjacent to the dental follicle in the first mandibular molars of rats from day 0–11 postnatally and dental follicle was devoid of stain [20]. Xu LX et al [21] illustrated that enamel-secreting ameloblasts, dentine-secreting odontoblasts and osteoclasts on surfaces of alveolar bone expressed IL-1RI and -II mRNA, and no transcripts were detected in stratum intermedium cells and other cells in dental follicle, stellate reticulum, dental papilla, or pulp. But results of immunostaining showed that IL-1RI was primarily localized in the dental follicle [22]. And qRT-PCR analysis revealed the expression of IL-1 receptor mRNA in the dental follicle [20]. Studies illustrated that IL-1α influenced synthesis of several genes and proteins of DFCs in vitro and in vivo [7, 8, 10, 11, 23], indicating the expression of IL-1RI in DFCs. Our research confirmed the expression of IL-1α and IL-1RI in the ameloblast layer, dental follicle and bone around the tooth germ at the age of PN 1 and 10. Staining of IL-1α and IL-1RI was also detected in the periodontal ligament at PN 20. Lossdörfer S [24] also found the positive staining of IL-1α and IL-1RI in the fibroblasts of PDL, suggesting both cytokines are of some importance in maintenance of tissue homeostasis and remodeling events involving in physiological tooth movement. After the application of mechanical forces, the expression of IL-1α in the bone and PDL along the roots of orthodontically moved molars was increased, implying its role in the alveolar bone-modeling process [25].

The eruption of first mandibular molar in rats undergoes a major burst of osteoclastogenesis on postnatal day 3 and a minor burst of osteoclastogenesis on postnatal day 10. RANKL is one essential molecule required for osteoclast development, activation, and survival [26]. The expression of RANKL in rat DFCs was significantly elevated on postnatal days 9–11, corresponding to the minor burst of osteoclastogenesis in the alveolar bone of the first molars [10, 27]. Failure of tooth eruption was observed in mice devoid of the RANKL gene [28]. OPG is a soluble decoy receptor, which can neutralize the effect of RANKL. The gene expression of OPG is reduced in the dental follicle of rats at day 3 post-natally, correlating with the days of maximal mononuclear cell influx and osteoclast numbers [29]. The ratios of RANKL/OPG are important signals regulating osteoclastogenesis and bone resorption. Researches showed that IL-1α could up-regulate RANKL, and down-regulate OPG in human dental pulp cells [30]. Meanwhile, IL-1α could also decrease the expression of osteoblast-related genes (ALP, RUNX2, et al) in the MC3T3-E1 cells [31]. In our study, IL-1α decreased the osteogenic differentiation of DFCs, upregulated molecular signals of osteoclast formation, suggesting the roles of IL-1α in tooth eruption through regulation of osteogenesis and osteoclastic activity of DFCs.

Inadequate expression of MMP9 in DFCs was presumably related to the delayed tooth eruption in patients with cleidocranial dysplasia (CCD), characterized by impaired tooth eruption in the permanent dentition stage [19]. Cerri PS et al [32] showed MMP9 was expressed in multiple cells in different phases of tooth eruption and it was involved in the complex process of degradation of the eruptive pathway extracellular components. It was also located in odontoclasts and played a role in deciduous tooth resorption [33]. Previous studies showed that IL-1α could increase expression of MMPs, such as MMP 1, 3, 7, 9, and 13 during infection or the formation of endodontic and periodontal osteolytic lesions [30, 34, 35]. In this study, MMP9 was upregulated in IL-1α-DFCs, which might be related to abnormal tooth eruption.
Myeloid differentiation factor 88 (MyD88) is an adaptor molecule involved in IL-1α signaling, linking the upstream pathways and triggering the subsequent activation of MAPK and nuclear factor-kappa B (NF-κB) [36–38]. MyD88 is expressed in DFCs and knockdown of MyD88 nullifies the up-regulatory effect of IL-1α on MCP-1 and RANKL gene expression [39]. In the present study, we explored the downstream of MyD88 and found that a pronounced level of p38 and JNK phosphorylation was elicited by IL-1α. The activation of p-JNK was impeded by SP600125. Using SB2035080 unexpectedly improved the activation of p-p38, but the expression of HSP 27, a specific physiological substrate of p38 MAPK was decreased. Kumar S et al [40] revealed that SB2035080 inhibited only the activity but not the activation of p38 MAPK. It can potently inhibit the activity of p38 MAPK as demonstrated by the inhibition of the activation of HSP27, but not its activation by upstream MAPK kinases regardless of stimuli or cell type. Further analysis showed that blocking the function of p38 using SB203580 and JNK by SP600125 actually prevented the upregulation of MMP9 and downregulation of ALP, OPN and RUNX2 by IL-1α alone. These results indicated that p38 MAPK and JNK signaling pathways are involved in the process of IL-1α influencing the tooth eruption.

**Conclusion**

Our findings demonstrated that IL-1α decreased the osteogenic differentiation of DFCs, upregulated molecular signals of osteoclast formation promoting and matrix remodeling. The p38 MAPK and JNK signaling pathways were involved in the process of IL-1α influencing the function of DFCs. Getting a better appreciation of the molecular and cellular events that regulate osteoclastogenesis and osteogenesis in eruption is not only central to our understanding of how these processes occur, but also is needed for ultimate development of the means to control them.

**Abbreviations**

IL-1α: interleukin-1α; DF: dental follicle; DFCs: DF cells; MCP-1: monocyte chemotactic protein-one; CSF-1: colony-stimulating factor-one; RANKL: receptor activator of nuclear factor –kappa B ligand; TNF-α: tumor necrosis factor-alpha; IL-1R I: IL-1-type 1 receptor; SD: Sprague-Dawley; E: embryo; PN: postnatal day; PBS: phosphate buffered saline; TEM: transmission electron microscopy; CK14: cytokeratin 14; CCK-8: cell-counting kit-8; qRT-PCR: quantitative real-time polymerase chain reaction; ALP: alkaline phosphatase; RUNX2: runt-related transcription factor 2; OPN: osteopontin; OPG: osteoprotegerin; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; MMP9: matrix metalloproteinase 9; HSP 27: heat shock protein 27; p-p38: p38 MAPK phosphorylation; p-JNK: JNK phosphorylation; p-ERK: ERK phosphorylation; p-p65: p65 phosphorylation; TRAP: tartrate-resistant acid phosphatase

**Additional File**

Figure S1. Model of the mechanisms by which IL-1α induced by apical periodontitis regulates osteogenic and osteoclastic activity of DFCs. (a) schematic diagram of apical periodontitis of deciduous tooth affecting DFCs. (b) IL-1α pathway.
Declarations

Ethics approval and consent to participate

All experimental procedures were approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University, Chengdu, China (WCHSIRB-D-2016-193). Consent to participate is not applicable.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

MMM, YDC, XLC, QZ, and XDZ contributed in the conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing. JZ and WHG contributed to the provision of study material, conception and design, and assembly of data. XDZ and JZ contributed to the conception and design, manuscript writing, financial support, and final approval of the manuscript. All authors reviewed the manuscript and approved it for publication.

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Figures
Figure 1

Immunolocalization of IL-1α (a~h) and IL-1RI (i~p) in rat first mandibular molars. (a, i): E16.5(X400); (b, j): E18.5(X100); (c, k): PN1(X100); (d, l): PN1(X400); (e, m): PN10(X100); (f, n): PN10(X400); (g, o): PN20(X100); (h, p): PN20(X400); E, embryo rats; PN, postnatal days; DP, dental papilla; AM, ameloblast; DF, dental follicle; AB, alveolar bone; PDL, periodontal ligament.
Figure 2

The culture, identification and proliferation of DFCs. (a) Culture of DFCs. (b) DFCs were positive for vimentin and negative for CK14. (c) TEM of DFCs, the lower picture was the enlargement of the boxed area in the upper one. The red arrows indicated homogeneous electron-dense granules in second-passage cells. (d) The proliferation rate of DFCs analyzed by CCK8 assay. (e and f) The proliferation rate of 5ng/ml IL-1α group was similar to that of DFCs without treatment, as shown by colony formation ability.
Figure 3

Osteogenic differentiation of DFCs under the treatment of IL-1α. (a to d) DFCs treated by 5ng/ml IL-1α exhibited weaker osteogenic capacity, as visualized by ALP activity (a, c) and alizarin red staining (b, d). (e to g) With osteogenic induction, IL-1α decreased the expression of ALP, RUNX2 and OPN shown by qRT-PCR results (e) and western blot analysis (f, g). *P<0.05
Figure 4

Weakened osteogenic capacities, promoted osteoclast-inductive capacities of DFCs treated by IL-1α. (a to c) The mRNA (a) and protein (b, c) of ALP, RUNX2 and OPN of DFCs were decreased by IL-1α at different time point. (d) The gene expression of RANKL and OPG, and the ratios of RANKL/OPG were increased in IL-1α treated group. (e) TRAP staining of DFC–bone marrow stromal cells co-cultures revealed that the osteoclasts were more in the IL-1α treated group than control group. (f) Resorption lacuna on osteo surface after co-cultures. More resorption lacuna (black arrows) was seen in group with 5 ng/ml IL-1α. *P<0.05.
Figure 5

Promoted matrix remodeling capacity of DFCs by IL-1α treatment. (a, b) DFCs treated by IL-1α exhibited significantly stronger invasive ability relative to DFCs without IL-1α at 24 h, as measured by invasion assays. (c to e) IL-1α increased MMP9 expression as detected by qRT-PCR (c) and western blot analysis (d and e). *P<0.05
IL-1α participating in tooth eruption via JNK and p38 MAPK pathways. (a, b) Western blot showed HSP27, p-p38 and p-JNK was increased in the IL-1α-treated DFCs, while p-ERK and p-p65 did not change. (c, d) SB203580 obviously inhibited the effect of IL-1α. SB203580 not only reversed the down-expression of ALP, RUNX2 and OPN, but also weakened the expression of MMP9. (e, f) SP600125 also impeded the effect of IL-1α. MMP9, ALP, OPN and RUNX2 trended to come back to the level of control groups.

**Supplementary Files**

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