ACTA ODONTOLOGICA SCANDINAVICA
2019, VOL. 77, NO. 3, 205–212
https://doi.org/10.1080/00016357.2018.1536280

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ORIGINAL ARTICLE

Leptin induces IL-6 and IL-8 expression through leptin receptor Ob-Rb in human dental pulp fibroblasts

Lili Wei[a,b]*, Yangxi Chen[a,c]*, Chi Zhang[a,c], Mingwen Liu[a,c] and Haofei Xiong[a,c]

[a]The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, China; [b]Department of Oral Radiology, School and Hospital of Stomatology Wuhan University, Wuhan, China; [c]Department of Oral Emergency, School and Hospital of Stomatology Wuhan University, Wuhan, China

ABSTRACT

Objective: Leptin, through binding to its special receptor (Ob-Rb), has potent effects on immunity and inflammation. This study aimed to investigate the expression of leptin receptor Ob-Rb in human dental pulp fibroblasts (HDPFs) and the effects of leptin on the production of proinflammatory cytokines of IL-6 and IL-8 by HDPFs.

Methods: Ob-Rb expression was determined by quantitative real-time PCR (real-time PCR), Western blot and immunofluorescence analyses in cultured HDPFs. Small interfering RNA (siRNA) was transfected into HDPFs to down-regulate the expression of Ob-Rb. Real-time PCR and enzyme-linked immunosorbent assay (ELISA) were used to determine the proinflammatory cytokines of IL-6 and IL-8 levels in leptin-stimulated HDPFs. The involved signalling pathways that mediate the leptin-stimulated production of proinflammatory cytokines were investigated using Western blot and specific signalling inhibitor analyses.

Results: The expression levels of Ob-Rb mRNA and protein were detected in HDPFs. Leptin could stimulate mRNA and protein expression of IL-6 and IL-8 in HDPFs in a concentration-dependent and time-dependent manner. Transfection with siRNA targeting Ob-Rb resulted in remarkable reduction of IL-6 and IL-8 expressions by HDPFs. In accordance with the enhanced expression of proinflammatory cytokines, leptin stimulation resulted in rapid phosphorylation of STAT3, p38 MAPK, ERK and Akt in HDPFs. Inhibiting JAK2/STAT3, p38 MAPK or PI3K/Akt substantially decreased leptin-induced IL-6 production, whereas blocking ERK and p38 MAPK substantially suppressed IL-8 production from leptin-stimulated HDPFs.

Conclusions: Leptin may up-regulate IL-6 and IL-8 production through binding with Ob-Rb in HDPFs via the activation of different intracellular signalling pathways.

ARTICLE HISTORY
Received 20 August 2018
Revised 18 September 2018
Accepted 6 October 2018

KEYWORDS
Leptin; IL-6; IL-8; human dental pulp fibroblasts

Introduction

Leptin (Ob), encoded by obese (*ob*) gene, is a 146 amino acid non-glycosylated polypeptide hormone [1]. It is synthesized predominantly in white adipose tissue by mature adipocytes and minorly produced by brown adipose tissue, placenta, gastric fundic epithelium, skeletal muscle, joint and bone tissues [2]. Leptin has various physiological functions in the human body, primarily as a hypothalamic modulator for regulating appetite and energy expenditure [3]. In addition, it is critically involved in immune response, which attracted the interest of researchers. Because the structure of leptin is similar to the long-chain helical cytokines such as interleukin (IL)-6, IL-11 and leukaemia inhibitory factor, leptin has been increasingly demonstrated as a cytokine-like hormone with pleiotropic actions in modulating immune responses [4,5]. Furthermore, leptin levels were associated with the progression of several inflammatory and autoimmune disorders, such as rheumatoid arthritis (RA), osteoarthritis (OA), connective tissue diseases, vasculitis and periodontitis, thereby suggesting the pivotal role of leptin in immunomodulation and in the pathogenesis of inflammatory diseases [6,7].

Leptin's biological effect is mediated by its specific receptors (Ob-R), which belong to class I cytokine receptors and are located in various tissues throughout the body. At least six isoforms of Ob-R are generated by alternative messenger RNA (mRNA) splicing. However, in humans, only the long isoform Ob-Rb, with a full intracellular domain, can transduce its leptin-binding signal and is essential in exerting its most of the biological actions [8]. The ubiquitous distribution of OB-Rb in almost all tissues is in line with the pleiotrophic function of leptin [4]. The Ob-Rb-activated signalling pathways include the classic activating Janus kinases 2/signal transducers and activators of transcription 3 (JAK2/STAT3) pathway, as well as the extracellular signal-activated kinase

CONTACT Haofei Xiong wb000266@whu.edu.cn The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University. No.237, Luo Yu Road, Hongshan District, Wuhan City, Hubei Province, China
*These authors contributed equally to this article.

(ERK), p38 mitogen-activated protein kinase (p38 MAPK) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathways [9].

The expression of leptin has been suggested in both healthy and inflamed gingival and periodontal tissues [6,10]. In addition, both leptin and its receptors are expressed by healthy and inflamed human dental pulp cells, as well as periapical granulomas [11–15]. Human dental pulp fibroblasts (HDPFs), major dental pulp component, also have some features of immune cells. They are involved in local immune response by secreting great amounts of proinflammatory cytokines and chemokines, which provide signals that sustain local inflammatory response and tissue destruction [16]. Thus, leptin might participate in the pathogenesis of pulpitis partly through its effects on HDPFs.

Based on the abovementioned information, although the role of leptin has been demonstrated in pulp inflammation and it is well known that HDPFs are one of the important cells regulating cytokine cascades in pulpitis, the biological effects of leptin on HDPFs remains unclear. Therefore, the current study aimed to examine the Ob-Rb expression and explore the effect and signalling pathway involved in leptin-induced proinflammatory cytokine production in HDPFs.

Materials and methods

Cell culture and stimulation

Primary cultured HDPFs were isolated from healthy dental pulp tissue of the first premolar teeth of six individuals (14–20 years old) undergoing tooth extraction for orthodontic treatment. These HDPFs were cultured as described previously [17]. The study protocols were approved by the Ethics Committee of the School of Stomatology, Wuhan University in China. Cells between the third and eighth passages were used in the following experiments. The effects of leptin on IL-6 and IL-8 production were examined. After the serum was starved in Dulbecco's modified Eagle's medium (Gibco BRL, Gaithersburg, MD, USA) containing 1% foetal bovine serum (Gibco BRL) for 24 h, HDPFs were treated with recombinant human leptin (PeproTech Inc., USA) in different time and concentrations for mRNA examination and ELISA assay. For mRNA examination, HDPFs were seeded into 12-well cell culture plates at a density of 2×10^5/mL, whereas for ELISA assay, HDPFs were seeded into 24-well cell culture plates at a density of 1×10^5/mL.

Immunofluorescence analysis

HDPFs were cultured on slides at a low density. For immunofluorescence, the cells were rinsed with phosphate-buffered saline (PBS) and then incubated for 1 h at 37 °C with 10% non-immune goat serum. Cells were then incubated with Ob-Rb primary rabbit antibody (Boster Biological Technology, Wuhan, China) at a final dilution of 10 μg/mL at 4 °C overnight. Negative controls were obtained by the omission of primary antibodies, which were substituted with isotype IgG. For secondary staining, slides were incubated with a tetramethylrhodamine (TRITC)-conjugated goat antirabbit IgG (Boster Biological Technology) for 1 h at 37 °C. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Beyotime Biotechnology, Shanghai, China). Then, the cells were fixed with embedding medium on a microscope slide and photographed by fluorescent microscopy (Leica, Germany).

Western blot analysis

Cultured HDPFs were incubated with leptin (3,000 ng/mL) for 0–60 min. Primary antibodies to phosphorylated STAT3, STAT3, phosphorylated Akt, Akt, phosphorylated p38 MAPK, p38 MAPK, phosphorylated ERK and ERK were purchased from Cell Signaling Technology (Danvers, MA, USA). Cytoplasmic proteins were removed, as described in detail previously [17]. Cells were lysed, and the proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Moreover, the membranes were incubated with 5% non-fat milk, probed with primary antibodies and incubated at 4 °C overnight. Thereafter, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL, USA). Blots were examined with a Supersignal West Pico Chemiluminescent Substrate Kit (Pierce). To establish signalling pathway stimulation, phosphorylated STAT3, STAT3, phosphorylated p38 MARK, p38 MARK, phosphorylated ERK, ERK, phosphorylated Akt and Akt were determined.

Intracellular signalling inhibition of Ob-Rb

Intracellular signalling inhibitors (Sigma-Aldrich, St. Louis, MO, USA) included AG490, SB203580, PD98059 and LY294002, which antagonise the activation of JAK2/STAT3, p38 MAPK, ERK and PI3K/Akt, respectively. The inhibitors were used to determine the signalling pathway on leptin-induced IL-6 and IL-8 up-regulation in HDPFs. After pretreatment with the inhibitors for 1 h, HDPFs were treated with 3,000 ng/mL of leptin for 12 and 24 h for mRNA examination and ELISA assay, respectively.

RNA interference assay with Ob-Rb

An RNA interference assay was performed to assess the isoform Ob-Rb involved in the effects of leptin on IL-6 and IL-8 production. The special small interfering RNA (siRNA) targeting for Ob-Rb and negative-control siRNA were purchased from GenePharma (Shanghai, China). For gene knockdown, HDPFs were transfected with Ob-Rb siRNA (40 nM) or negative control siRNA (40 nM) by using Lipofectamine™ 2000 (Invitrogen Corp), following the manufacturer's instructions. After 48 h, cells were stimulated with 3,000 ng/mL of leptin for 12 and 24 h for mRNA examination and ELISA assay, respectively.

Quantitative real-time polymerase chain reaction (real-time PCR)

HDPFs were incubated with human recombinant leptin for the necessary concentrations and the amount of time. The total

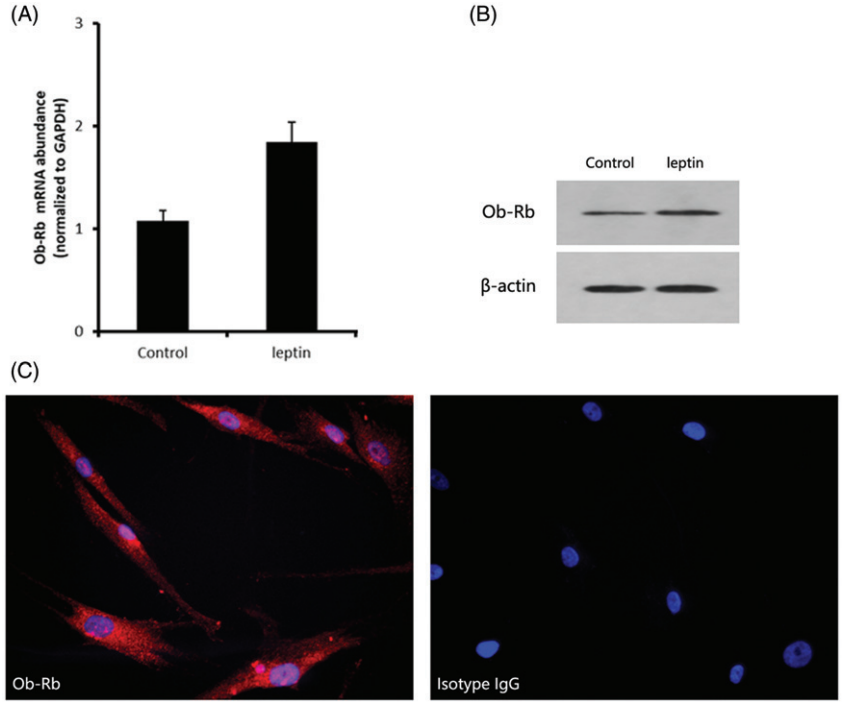

Figure 1. (A–B) HDPFs were incubated with or without 3000 ng/mL of leptin. The mRNA expression of Ob-Rb was determined through real-time PCR. Western blot was performed to establish Ob-Rb protein expression. (C) Fluorescent staining of Ob-Rb in HDPFs was conducted with rabbit Ob-Rb antibody or isotype IgG antibody, followed by TRITC-conjugated secondary antibody. Nuclear counterstaining was performed with DAPI (400 × objective). One of the three experiments with similar results is presented.

RNA was separated from HDPFs or leptin-stimulated cells by utilising an RNeasy mini kit (Qiagen, Valencia, CA), following the company's instructions. Then, cDNA was synthesised with a PrimeScript RT reagent kit (TAKARA, Otsu, Japan), according to the company's instructions. Quantitative real-time PCR was conducted on a real-time PCR system (Applied Biosystems) with SYBR Green Premix Ex Taq (TAKARA). The primers were planned as follows: Ob-Rb sense (5′- AAGCCAGTCTTTCCAGAGAATAACC-3′) and Ob-Rb antisense (5′- ATCCCAGTCCATCTAGCCTCTTACA-3′), IL-6 sense (5′- GGTCCAGTTGCCTTCTCCC-3′) and IL-6 antisense (5′- GTGCCTCTTTGCTGCTTTC-3′), IL-8 sense 5′-GACATACTCCAAACCTTTCCACCCC-3′) and IL-8 antisense 5′-CAAAAACTTCTCCACAACCCTCTGC-3′), and GAPDH sense (5′-TCAAGAAGGTGGTGAAGCAGG-3′) and GAPDH antisense (5′-TCAAAGGTGGAGGAGTGGGT-3′). The genes in this evaluation were investigated by establishing the genes' relative expression, based on their internal control GAPDH.

Enzyme-linked immunosorbent assay (ELISA)

HDPFs were activated at different leptin concentrations and time points. The levels of IL-6 and IL-8 in cell supernatants were quantified with commercially available ELISA kits (Elabscience Biotechnology, Wuhan, China), following the company's directions.

Statistical analysis

All of the measurements were listed as mean values ± SD. The values were determined with Kruskal–Wallis tests and then by the Mann–Whitney U-test. Statistical significance was established when the p value $< .05$.

Results

Expression of the Ob-Rb in HDPFs

Leptin functions by interacting with specific receptor Ob-Rb. The results of real-time PCR and Western blot showed that both Ob-Rb mRNA and protein were expressed in HDPFs. After treatment with leptin for 12 h, the mRNA and protein levels of Ob-Rb increased (Figure 1(A,B)). Immunofluorescence staining was performed to additionally examine the expression of Ob-Rb in HDPFs. A certain immunofluorescent signal was then noted in HDPFs stained with Ob-Rb antibody (Figure 1(C)).

Effect of leptin on IL-6 and IL-8 production in HDPFs

Next, we investigated how leptin affects the production of leptin-induced expression of IL-6 and IL-8 mRNAs in HDPFs using real-time PCR. The mRNA expression levels of IL-6 and IL-8 in HDPFs increased dose- and time-dependently by leptin. The HDPFs treated with low-leptin (10 ng/mL) concentrations were not substantially different with the untreated controls. IL-6 and IL-8 mRNA levels gradually increased and peaked when leptin was at 3,000 or 10,000 ng/mL concentration. Time course analysis revealed that the mRNA expression levels of IL-6 and IL-8 were both detected 3 h after leptin treatment, peaked at 12 h and then declined at 24 h (Figure 2(A–D)).

ELISA was conducted to additionally examine IL-6 and IL-8 protein expression in leptin-activated HDPFs. Consistent with the impact on mRNA production, leptin elevated the IL-6 and IL-8 protein expression of HDPFs dose- and time-

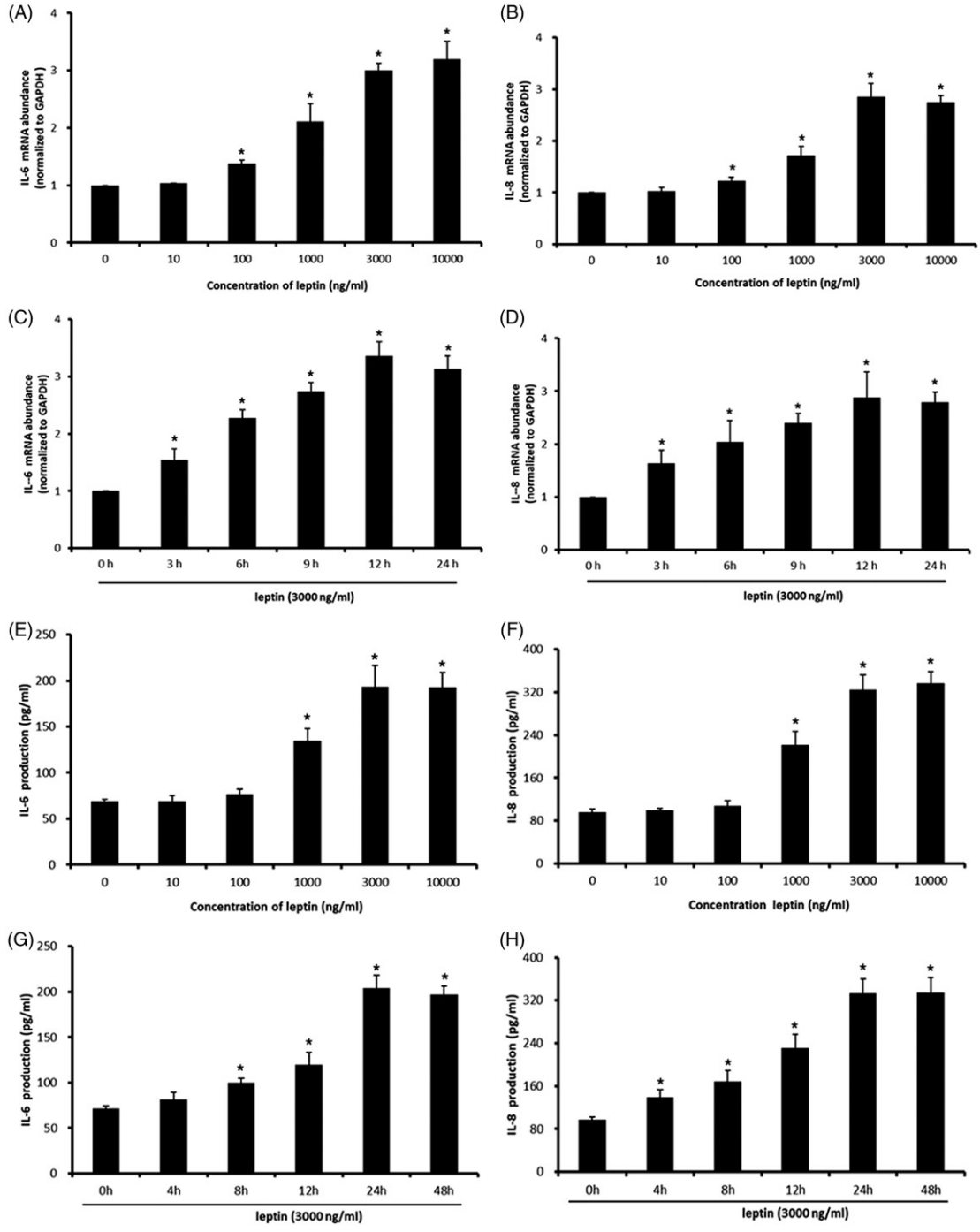

Figure 2. (A–D) Effect of leptin on IL-6 and IL-8 gene expressions in HDPFs. (A–B) Cultured HDPFs were treated with leptin for 12 h at the abovementioned concentrations. Gene expression levels of IL-6 and IL-8 were then assessed by real-time PCR. $^{*}p < .05$ vs. the untreated group. The data represent the mean ± SD of three separate experiments. (C–D) Time course of induction of IL-6 and IL-8 mRNA expression in HDPFs by leptin. Cultured HDPFs were treated with 3,000 ng/mL of leptin for 0, 2, 4, 8, 12 and 24 h. Real-time PCR was used to determine the effect of leptin on IL-6 and IL-8 gene expression in HDPFs. $^{*}p < .05$ vs. the untreated group. The data represent the mean ± SD of three separate experiments. (E–H) Effect of leptin on IL-6 and IL-8 protein expression in HDPFs. (E–F) Cultured HDPFs were treated with the abovementioned concentrations of leptin for 24 h. IL-6 and IL-8 protein expression levels were then assayed via ELISA. $^{*}p < .05$ vs. the untreated group. The data represent the mean ± SD of three separate experiments. (G–H) Time course of leptin-induced IL-6 and IL-8 protein generation by HDPF cells. Cultured HDPFs were treated with 3000 ng/mL of leptin for 0, 4, 8, 12, 24 and 48 h. IL-6 and IL-8 protein levels in the conditioned medium were then assayed by using ELISA. $^{*}p < .05$ vs. the untreated group. The data represent the mean ± SD of three separate experiments.

dependently. The HDPFs treated with low-leptin concentrations (10 and 100 ng/mL) were not substantially different with the untouched controls. The protein expression of IL-6 and IL-8 gradually increased and climaxed, when leptin was at 3,000 or 10,000 ng/mL. Time course evaluation showed that the protein expression levels of IL-6 and IL-8 initially happened 4 h after leptin treatment, climaxed at 24 h and then lowered afterwards at 48 h (Figure 2(E–H)).

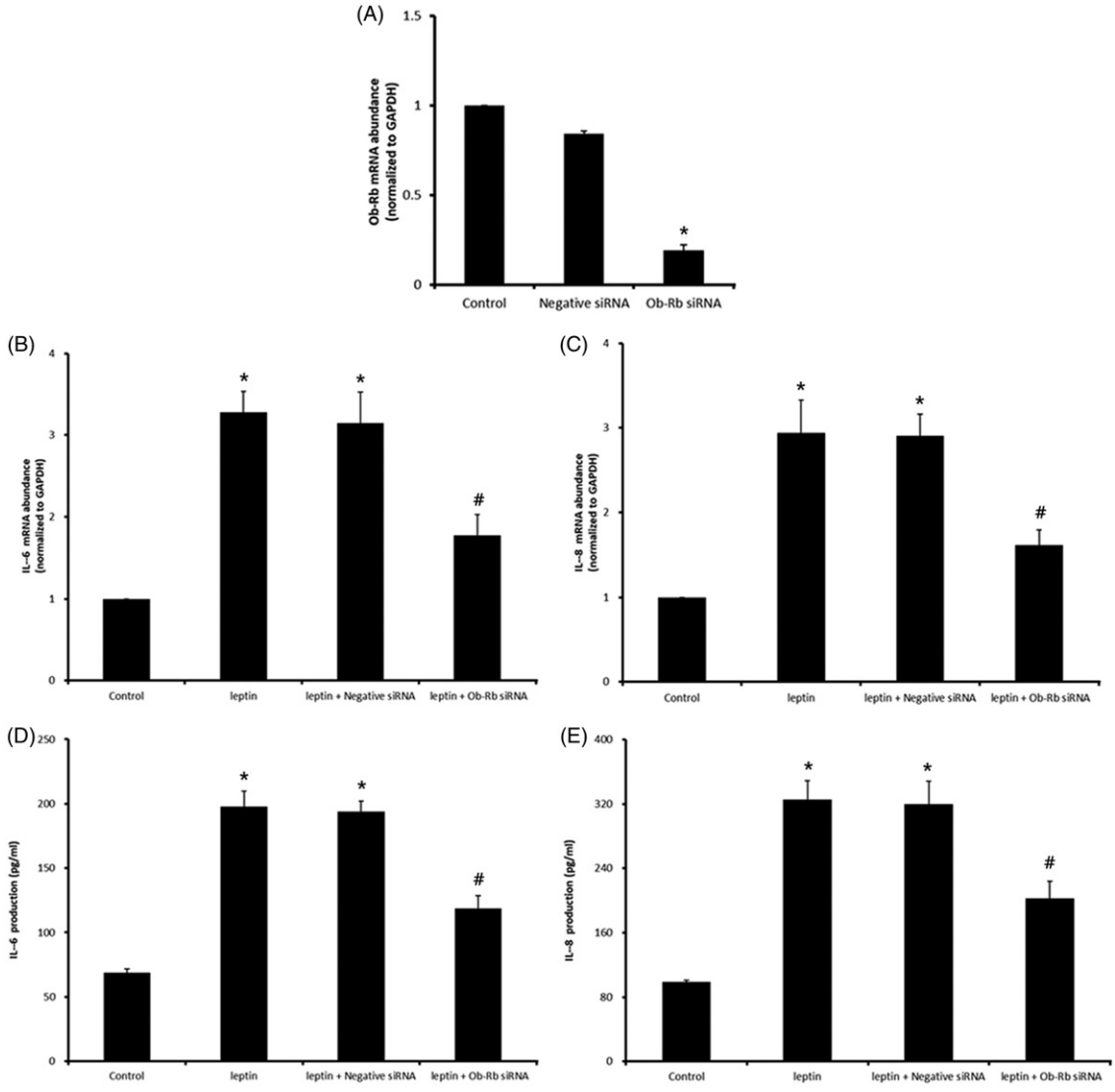

Figure 3. Effect of siRNA targeting the leptin receptor Ob-Rb during IL-6 and IL-8 production by HDPFs. (A) HDPFs were transfected with siRNA for Ob-Rb or negative siRNA. Ob-Rb mRNA levels were analysed by real-time PCR. $^{*}p < .05$ vs. the untreated group. The data represent the mean ± SD of three separate experiments. (B–C) After transfection with siRNA or negative siRNA, HDPFs were treated with 3000 ng/mL of leptin, and gene expression levels of IL-6 and IL-8 were assessed by real-time PCR. The data represent the mean ± SD of three separate experiments. $^{*}p < .05$ vs. the untouched group, $^{\#}p < .05$ vs. the negative siRNA group. (D–E) IL-6 and IL-8 protein secretion levels in the supernatant of HDPFs were assayed via ELISA. The data represent the mean ± SD of three separate experiments. $^{*}p < .05$ vs. the untouched group, $^{\#}p < .05$ vs. the negative siRNA group.

Effect of siRNA for Ob-Rb on IL-6 and IL-8 production by HDPFs

The Ob-Rb mRNA expression by HDPFs greatly decreased after exposure to the siRNA for Ob-Rb. The efficiency of Ob-Rb knockdown by RNA interference reached approximately 80%, compared with negative controls (Figure 3(A)). The production of IL-6 and IL-8 by HDPFs transfected with the siRNA targeting Ob-Rb was remarkably lower than that by HDPFs transfected with negative-control siRNA (Figure 3(B–E)).

The signalling pathways of JAK2/STAT3, p38 MARK, ERK and Akt are involved in the action of leptin stimulation by HDPFs

The effects of leptin were evaluated on STAT3, p38 MAPK, ERK and Akt phosphorylation in HDPFs by Western blot analysis. Leptin led to the rapid phosphorylation of STAT3, p38 MAPK, ERK and Akt in HDPFs within 10 min of induction (Figure 4(A)).

We examined the effects of signalling pathway inhibitors on leptin-induced IL-6 and IL-8 up-regulation in HDPFs. Compared with the control group, leptin-induced IL-6 production was substantially inhibited by AG490, SB203580 and LY294002 and not influenced by PD98059. Meanwhile, the mRNA and protein expression of IL-8 was remarkably inhibited by PD98059 and SB203580 and not influenced by AG490 and LY294002. Thus, leptin-induced IL-6 production in HDPFs was mainly through activating JAK2/STAT3, PI3K/Akt and p38 MAPK pathways, whereas leptin-induced IL-8 expression in HDPFs was mainly through the ERK and p38 MAPK pathways (Figure 4(B–E)).

Discussion

Since its discovery by Zhang et al. in 1994, leptin has become noteworthy and revealed a new correlation between nutrition absorption, energy metabolism and immunoregulation.

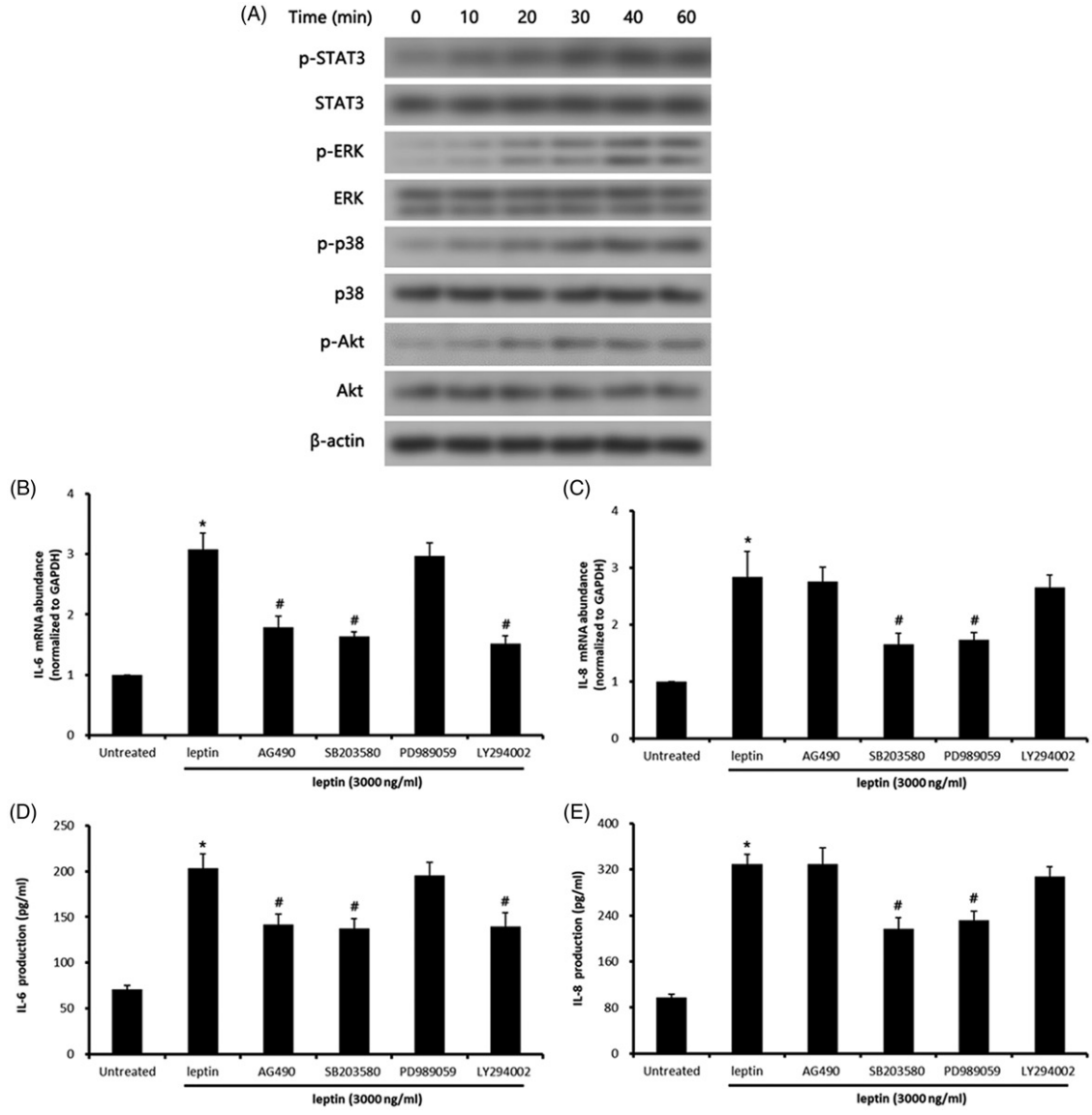

Figure 4. (A) Signalling pathways prompted by leptin activation in the HDPFs. Cultured HDPFs were incubated with leptin (3000 ng/mL) for 0–60 min. Cytoplasmic proteins were then removed. The extracts were subjected to SDS–PAGE, and then, Western blot was conducted with antibodies against phospho-STAT3 and STAT3, phospho-ERK and ERK, phospho-p38 MAPK and p38 MAPK, phospho-Akt and Akt; the expression level of β-actin was used as an internal control. One of the three experiments with comparable outcomes is shown. (B–C) Impact of different signalling inhibitors on leptin-induced IL-6 and IL-8 gene expression of HDPFs. HDPFs were pretreated for 1 h with 10 μM of AG490, 10 μM of PD98059, 10 μM of SB203580 or 10 μM of LY294002. Then, the cells were incubated with 3000 ng/mL of leptin for 12 h. Gene expression levels of IL-6 and IL-8 were then assessed by real-time PCR. The data represent the mean ± SD of three separate experiments. $^{*}p < .05$ vs. the untouched group, $^{\#}p < .05$ vs. the leptin group. (D–E) Impact of different signalling inhibitors on leptin-induced IL-6 and IL-8 protein expression of HDPFs. HDPFs were pretreated for 1 h with 10 μM of AG490, 10 μM of PD98059, 10 μM of SB203580 or 10 μM of LY294002, and then, the cells were incubated with 3,000 ng/mL of leptin for 24 h. IL-6 and IL-8 protein secretion levels in the supernatant of HDPFs were assayed via ELISA. The data represent the mean ± SD of three separate experiments. $^{*}p < .05$ vs. the untouched group, $^{\#}p < .05$ vs. the leptin group.

Currently, it is suggested as a pleiotropic hormone that may play an important role in regulating various physiological functions, such as immune homeostasis, angiogenesis, reproduction, inflammation and bone growth [1,2,4].

Dental pulp inflammation is mainly caused by microorganisms from caries, trauma or microabrasion around faulty restorations [18]. Such infection or inflammation nearly always leads to pain and suffering and likely causes serious fatal systemic infections if left untreated [19]. The pulp inflammatory process is mediated through a complex cytokine network [16]. The factors responsible for initiating and sustaining the inflammation and destruction of the pulp tissues are not yet completely elucidated. According to a recent study, the expression of leptin in the inflamed pulp group was significantly greater than in healthy teeth [12]. However, the exact role of leptin in the pathogenesis of pulp inflammation is unknown.

The present study showed that Ob-Rb expression in HDPFs, both mRNA and protein, increased with leptin stimulation. Moreover, immunofluorescent results also showed Ob-Rb expression in the HDPFs where the immunofluorescent signal was evident. Replacement of the HDPF antibody with an isotype IgG eliminated false-positive immunostained cells and showed the analytical specificity.

The immune response is the main cause of local inflammation and pulp tissue destruction in pulpitis. In RA and OA,

leptin has the potential role in inducing IL-6 and IL-8 production and promoting proteoglycan degradation in human synovial fibroblasts [20,21]. Immune-responsive HDPFs are the major cellular components of pulp tissue. They are pivotal targets for biological molecules to modulate tissue inflammation and regeneration. HDPFs play an important role in immune regulation as well, partially depending on secreting various soluble inflammatory mediators and cytokines. Among them, IL-6 is a multifunctional proinflammatory cytokine that participates in various physiologic and pathologic processes, including the response to infection and trauma stimuli, activation and amplification of the immune response and induction of angiogenesis, haematopoiesis and bone metabolism [22]. In addition, IL-8 is a well-known chemoattractant that exerts a pivotal effect in controlling neutrophil and monocyte chemotaxis towards infection and inflammation sites [23]. Both IL-6 and IL-8 are extremely important in the initiation and progression of pulp inflammation and are mostly up-regulated in inflammatory HDPFs [16]. Thus, the effects of leptin on IL-6 and IL-8 production in HDPFs were explored in this study. It was found that leptin promoted both mRNA and protein expression levels of IL-6 and IL-8 in HDPFs in concentration-dependent and time-dependent manner, which were in agreement with results of previous studies showing the potential role of leptin in inducing proinflammatory cytokines production [6,20,21]. The presence of leptin and its receptor in the dental pulp combined with our result indicate that leptin can significantly up-regulate proinflammatory cytokines in HDPFs. The low (10 ng/mL) and medium (100 ng/mL) concentrations of leptin used in this study cover serum leptin levels reported in clinical studies [24–27], whereas the high concentration (1,000–10,000 ng/mL) of leptin used in this study referred to several basic biology studies [6,28,29]. It turned out that only the high level of leptin could significantly up-regulate IL-6 and IL-8 expression in HDPFs. Furthermore, leptin can promote the expression of dentine sialophosphoprotein in human dental pulp and induce angiogenesis, odontogenic differentiation and mineralisation in HDPFs [29,30], thereby suggesting that leptin may have a dual effect in pulpitis. In inflammatory pulp tissue, up-regulated leptin could induce an adequate amount of proinflammatory cytokine production to participate in sustained inflammation, continuous migration and hyper-responsiveness of immunoinflammatory cells. Meanwhile, leptin has beneficial effects on angiogenesis, odontogenic differentiation and mineralisation in HDPFs that may provide the odontogenic conditions for pulp regeneration and healing [29]. Further studies will be needed to explore leptin for pulp inflammation, its dominant effect and the mechanism by which leptin influences.

Leptin receptors exist in at least six different isoforms with cytoplasmic domains of different length. The long-form Ob-Rb is the main functional isoform and has potential in activating intracellular signalling pathways [8]. In our evaluation, under physiological conditions or leptin stimulation, HDPFs expressed high levels of Ob-Rb mRNA and proteins. Moreover, after treatment with leptin for 12 h, the levels of Ob-Rb in HDPFs increased. Our results agree with other researchers with regard to Ob-Rb expression in numerous cells, including synovial fibroblasts and periodontal fibroblasts [6,21]. The roles of Ob-Rb and signalling pathway in leptin-induced IL-6 and IL-8 production were further investigated. With high-knockdown efficiency, transfection with siRNA targeting Ob-Rb resulted in remarkable reduction of IL-6 and IL-8 expressions by HDPFs. Thus, Ob-Rb is essential for leptin-mediated IL-6 and IL-8 production.

JAK2/STAT3 is a classic downstream effector of leptin in various kinds of human cells [9], such as colorectal adenoma cells [31], peripheral blood mononuclear cells and synovial fibroblasts [20,32]. However, alternative signalling pathways in immune cells have also been described, including ERK, p38 and PI3K/Akt [9,33]. We investigated whether these signalling pathways were affected by the leptin-mediated proinflammatory cytokine production in this study. Leptin elevated the phosphorylation levels of STAT3, ERK, p38 Mark and Akt within 10 min of induction. To verify the roles of these signalling pathways in the stimulation of IL-6 and IL-8 by leptin-mediated HDPFs, we used several signalling pharmacological inhibitors. The data suggested that leptin-stimulated IL-6 production by JAK2/STAT3, p38 Mark and PI3K/Akt. However, leptin-induced IL-8 production in HDPFs that was mediated by the ERK and p38 MAPK pathways rather than the JAK2/STAT3 pathway. Thus, leptin may selectively activate one of the signalling pathways above during the progression of pulp inflammation to preferentially exert corresponding effects.

In conclusion, this study demonstrated the expression of Ob-Rb in HDPFs and indicated that leptin, after binding to Ob-Rb, could stimulate IL-6 expression mainly through JAK2/STAT3, p38 Mark and PI3K/Akt pathways and stimulate IL-8 production mainly through p38 MAPK and ERK pathways in HDPFs. Therefore, leptin may play a role in regulating immune responses in pulp inflammation.

Disclosure statement

The authors deny any conflicts of interest related to this study.

Funding

This work was supported by grants from National Natural Science Foundation of China (no. 81100744 and 81500878).

ORCID

Haofei Xiong http://orcid.org/0000-0002-8896-9312

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