Porphyromonas gingivalis-derived lipopolysaccharide inhibits brown adipocyte differentiation via IncRNA-BATE10

FAXIANG ZHENG1,2*, LINGKAI SU3*, NING ZHANG4, LONGJIE LIU3, JINGYI GU3 and WENHUA DU1

1Department of Stomatology, Affiliated Xiaoshan Hospital, Hangzhou Normal University, Hangzhou, Zhejiang 311202; 2Cosmos Wisdom Mass Spectrometry Center, Zhejiang University Medical School, Hangzhou, Zhejiang 310058; 3Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Cancer Center of Zhejiang University, Hangzhou, Zhejiang 310000; 4Department of Stomatology, Emergency General Hospital, Beijing 100028, P.R. China

Received July 8, 2022; Accepted September 29, 2022

DOI: 10.3892/etm.2022.11654

Abstract. Epidemiological studies have suggested an association between obesity and periodontal disease. Brown adipose tissue (BAT) has an anti-obesity effect. However, the effects of periodontitis on obesity and BAT remain unclear. Therefore, the present study aimed to determine the effects of lipopolysaccharide derived from Porphyromonas gingivalis (P. gingivalis LPS) on brown adipocytes. For this purpose, the present study examined the effects of the intravenous administration of P. gingivalis in mice, the treatment of brown adipocytes with P. gingivalis LPS during differentiation, and the administration of small interfering RNA targeting interferon on brown preadipocytes by assessing the expression of genes involved in differentiation, using a long non-coding (lnc)RNA, and pro-inflammatory factors using reverse transcription-quantitative PCR. In addition, the accumulation of lipid droplets was examined using Oil Red O staining. P. gingivalis LPS reduced the expression of uncoupling protein 1 (UCP1) and IncRNA-BATE10 in brown adipocytes during differentiation. Consistent with this finding, P. gingivalis reduced UCP1 and IncRNA-BATE10 expression in the BAT of mice. IncRNA-BATE10 may thus be involved in the regulation of UCP1 expression that occurs during the differentiation of brown adipocytes treated with P. gingivalis LPS. Thus, P. gingivalis LPS may inhibit BAT differentiation by reducing IncRNA-BATE10 expression.

Introduction

Periodontal disease has become a global public health concern, with a high prevalence (1). Periodontal disease is defined as the chronic inflammation of the periodontal supporting tissue, caused by chronic infection with bacteria, including Porphyromonas gingivalis (P. gingivalis) (2). Lipopolysaccharide (LPS) derived from Porphyromonas gingivalis (P. gingivalis LPS) is responsible for a substantial proportion of its systemic effects. When a host is invaded by a periodontal pathogen, the LPS released is recognized by the immune system, leading to a robust inflammatory response, and this can cause alveolar bone resorption (3). In addition, the inflammation may extend from the gingiva into the periodontal membrane, alveolar bone and cementum, leading to periodontitis. Chronic periodontal inflammation is also associated with the entry of host and bacterially-derived factors into the circulation (4). In addition, periodontal bacteria may colonize the gut via the oral route (5,6). Thus, periodontal bacteria can cause or affect systemic disease.

Epidemiological research has demonstrated an association between obesity and periodontal disease (7). In addition, a number of previous studies have demonstrated a link between periodontal inflammation and obesity (4,8-10). Obesity is associated with a higher incidence of tooth loss over a period of 5 years, and the periodontal conditions of individuals with obesity are significantly worse following periodontal treatment than those of individuals without obesity (11). Furthermore, the periodontal inflamed surface area index is positively associated with body mass index (BMI) (4).
Periodontal disease may affect glucose metabolism via low-grade inflammation (12). Accordingly, diabetes mellitus (DM) has been identified as a risk factor for the progression of periodontal disease (13,14). Furthermore, obesity predisposes towards type 2 DM (4). Host pro-inflammatory factors released by immune cells activated by bacterial products may reach the adipose tissue via the circulation in patients with periodontal inflammation. Therefore, local inflammation may have widespread effects on the body through effects on adipose tissue (4,15). However, the effects of periodontitis on obesity remain unclear.

Brown adipocytes are thermogenic, helping to maintain body temperature by increasing basal metabolism in cold environments. Thermogenesis in brown adipocytes is induced by the uncoupling of mitochondrial oxidative phosphorylation by uncoupling protein 1 (UCP1) (4), and this has been shown to protect against obesity and obesity-related disease (16). Periodontopathic bacteria affect the development of obesity, glucose intolerance and hepatic steatosis, and also alter lipid metabolism and the thermogenesis of brown adipose tissue (BAT) (17,18). In addition, P. gingivalis administration has been shown to modify gene expression in the BAT of pregnant mice (17).

Long non-coding RNAs (lncRNAs) are RNA transcripts of >200 nucleotides in length that do not encode proteins and exhibit poor sequence conservation (19,20). lncRNAs play roles in a number of physiological and pathological processes, including development and differentiation. They regulate gene expression by functioning as microRNA sponges and by affecting transcription, splicing, and translation (20). Recent research has also demonstrated that lncRNAs are involved in brown adipogenesis, the browning of white adipose tissue, and brown adipose thermogenesis (21). These lncRNAs include lncRNA-BATE1, lncRNA-BATE10, 5′-GAG UAC UGA UCA UCA UUA AdT 3′ (antisense); and NC, 5′-UUC UCC GAA CGU GUC ACG UdT 3′ (antisense). To date, research into the effects of periodontitis on obesity has mainly focused on P. gingivalis-induced endotoxemia; however, it remains unclear whether there are direct effects of P. gingivalis LPS on brown adipocytes, and whether these are mediated by lncRNAs. Therefore, the present study aimed to determine the effects of P. gingivalis LPS on BAT.

Materials and methods

*Mice.* C57BL/6J mice (n=10, male, 6–8 weeks old, weighing 20–22 g) were purchased from Shanghai Laboratory Animal Center, housed under standard environmental conditions at a temperature of 22±2°C and 55–60% humidity, with free access to food and water and a 12-h light/dark cycle and were allocated into two groups as follows: The first was administered a sonicated *P. gingivalis* suspension in PBS buffer (*P. gingivalis* group, n=5) via the tail vein, and the second was administered PBS alone (control group, n=5). According to a previous study (18), after 18 h, the mice were euthanized, and samples of BAT were collected for use in reverse transcription-quantitative PCR (RT-qPCR). The mice were monitored after 18 h after the *P. gingivalis* infection. All mice were euthanized using 30% vol/min CO2 inhalation. Death was verified by confirming the following: The cessation of respiratory and cardiovascular movements by observation at room air for at least 10 min. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Zhejiang University (Approval no. ZJU20170237,2017-02-24).

Culture of *P. gingivalis.* *Porphyromonas gingivalis* [donated by Dr Peihui Ding (22)] was cultured on trypticase soy agar (Qingdao Hope Bio-Technology Co., Ltd.), containing 10% defibrinated horse blood, hemin and menadione (Qingdao Hope Bio-Technology Co., Ltd.), under anaerobic conditions at 37°C. The bacteria were collected in PBS buffer (pH 7.4) (Shandong Victoryx Biotechnology Co., Ltd.; http://www.vxbiotech.com/en/) and 10⁶ CFU/ml of the bacterial suspension was sonicated at 20 kHz for 5 min on ice using a Vibra cell sonicator (Sonic & Materials, Inc.).

**Brown adipocyte culture in vitro.** Preadipocytes obtained from the BAT of mice according to a previously described method (23) [donated by Professor Zhuoxian Meng (24)] were cultured in high-glucose Dulbecco's modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Inc.). To induce the adipogenic differentiation of the preadipocytes, they were cultured in induction medium containing 20 nM insulin (cat. no. 15500, MilliporeSigma), 1 μM dexamethasone (cat. no. D1756, MilliporeSigma), 0.5 mM 3-isobutyl-1-methylxanthine (cat. no. I-5879, MilliporeSigma), 1 nM triiodothyronine (T3) (cat. no. T2877, MilliporeSigma), 125 μM indomethacin (cat. no. I-7378, MilliporeSigma) and 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Inc.) for 2 days and then in differentiation medium containing 20 nM insulin, T3, and 10% FBS for an additional 2 days. Subsequently, the differentiation medium was replaced every 2 days until day 7. *P. gingivalis* LPS (cat. no. lrl-pgmps, InvivoGen) or LPS from *Escherichia coli* (*E. coli* LPS) (cat. no. L4391, MilliporeSigma) was added to the induction and differentiation media.

**Transfection with small interfering RNA (siRNA).** 50 nM LncRNA-BATE10-siRNA or scramble siRNA [negative control (NC)] were provided by Biomics Biotechnologies Co., Ltd. and mixed with transfection reagent (INVI DNA RNA, 20 μg/μl; Invigentech) and added to the preadipocytes; the mix of siRNA and the transfection reagent were kept at room temperature for 15 min before transfection (50 nM siRNA) into the cells, and then after 48 h, the cells were induced to differentiate. The siRNA duplex sequences were as follows: LncRNA-BATE10, 5′-GAG UAC UGA UCA UCA UUA AdT 3′ (sense) and 5′-UUA AUG AUA UCA UCA UUA UdT 3′ (antisense); and NC, 5′-UUC UCC GCA ACC UGC UAD TdT 3′ (sense) and 5′-AGG ACA CCG UCG GAG AAdTdT 3′ (antisense).

**RT-qPCR.** RNA was extracted using TRIzol reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.) from BAT following the manufacturer's instructions. cDNA was synthesized using the WCGENE mRNA cDNA kit (cat. no. WC-SJH0001; WCGENE Biotech), at 37°C for 15 min and 85°C for 5 sec. qPCR (Wgene mRNA qPCR mix; cat. no. WC-SJH0002; WCGENE Biotech) was performed using the Bio-Rad CFX96 Touch Real-Time PCR Detection System
present study examined the effects of an intravenous injection of 10^8 CFU E. coli suspension in 100 µl saline and 100 µl PBS on the BAT UCP1 expression of mice, and it was found that the bacterial administration reduced UCP1 expression (Fig. 1C).

P. gingivalis LPS reduces IncRNA-BATE10 expression in differentiating brown adipocytes and differentiated BAT in mice. In a previous study, it was shown that IncRNA-BATE10 may be involved in brown adipocyte thermogenesis (26). Therefore, the present study measured the expression of IncRNA-BATE10 in differentiating brown adipocytes treated with P. gingivalis LPS and BAT from mice administered P. gingivalis. As the concentration of P. gingivalis LPS increased, IncRNA-BATE10 expression decreased during brown adipocyte differentiation (Fig. 2A). E. coli LPS exerted a similar effect on IncRNA-BATE10 expression during brown preadipocyte differentiation (Fig. 2B). Consistent with P. gingivalis LPS, IncRNA-BATE10 expression was lower in the BAT of mice administered P. gingivalis (Fig. 2C).

IncRNA-BATE10 is involved in the differentiation of brown adipocytes. To better understand the role of IncRNA-BATE10 in brown adipocyte differentiation, the effects of siRNA targeting this IncRNA on UCP1 expression were assessed. IncRNA-BATE10 siRNA (Fig. 3A) was added to brown preadipocytes, differentiation was induced and UCP1 expression was then measured. It was found that UCP1 expression was decreased following the knockdown of IncRNA-BATE10 expression (Fig. 3B). Thus, IncRNA-BATE10 may be involved in brown adipocyte differentiation. In addition, after IncRNA-BATE10 was knocked down using siRNA, the effects of P. gingivalis LPS on UCP1 expression during the differentiation of brown adipocytes were less pronounced (Fig. 3C). A comparison of the ratios of UCP1 expression in differentiating brown adipocytes transfected with negative control siRNA ± P. gingivalis LPS also revealed that the inhibition of brown adipocyte differentiation by P. gingivalis LPS was suppressed by IncRNA-BATE10 knockdown (Fig. 3D). Thus, IncRNA-BATE10 may be involved in the effects of P. gingivalis LPS on brown adipocyte differentiation.

Discussion

The present study examined the effects of P. gingivalis and P. gingivalis LPS on brown adipocytes and mouse BAT. It was found that P. gingivalis decreased UCP1 expression and IncRNA-BATE10 expression in BAT, and that P. gingivalis LPS decreased the expression of UCP1 and IncRNA-BATE10 in differentiating brown adipocytes. In addition, the present study provided evidence that IncRNA-BATE10 may be involved in the effects of P. gingivalis LPS on brown adipocyte differentiation.

Periodontitis is a local form of inflammation that may have a systemic effect on obesity. Immune cells are activated in the adipose tissue of individuals with obesity. In addition, certain bacterial products, such as LPS, danger associated molecular patterns, bacterial flagellar protein, etc., activate immune cells (4,27,28), which may be transported to the adipose tissue via the circulation. Thus, local inflammation may have whole-body effects through effects on obese adipose tissue. Thus, obesity may be associated with periodontal disease and the presence of periodontal disease may also exacerbate the inflammation that characterizes obesity (4,15).

In mice with diet-induced obesity, P. gingivalis has been shown to exacerbate weight gain and the expansion of adipose tissue (29). Endotoxemia associated with P. gingivalis also affects BAT function. The administration of P. gingivalis has been shown to increase the expression of inflammation-related genes and to reduce that of UCP1 and Cidea, as well as that of the genes related to lipolysis, Lipe, and Pparg. Adipoq has been found to be lower in BAT, but not in white adipose tissue from P. gingivalis-treated mice (18).
ZHENG et al.: Porphyromonas gingivalis-DERIVED LPS REGULATES BROWN ADIPOSE TISSUE FUNCTION

Figure 1. P. gingivalis LPS reduces UCP1 expression and lipid droplet formation in differentiating brown adipocytes. Preadipocytes were induced to differentiate into brown adipocytes, during which P. gingivalis LPS was added to the medium. (A) UCP1 mRNA expression in brown adipocytes (Diff) and preadipocytes. The brown preadipocyte group was used as the reference group and relative expression was calculated as the $2^{-\Delta\Delta Cq}$ value of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the brown preadipocyte group (as '1.0'). UCP1: P<0.0001 for ANOVA. Diff. vs. brown preadipocytes, P<0.0001; Diff vs. Diff + 10 ng/ml P. gingivalis LPS, P<0.0001; Diff vs. Diff + 100 ng/ml P. gingivalis LPS, P<0.0001; Diff vs. Diff + 1,000 ng/ml P. gingivalis LPS, P<0.0001. (B) Oil Red O-stained brown adipocytes (Diff) or preadipocytes. (C) Preadipocytes were induced to differentiate into brown adipocytes, during which E. coli LPS was present in the medium. UCP1 mRNA expression was measured in brown adipocytes (Diff) and preadipocytes. The brown preadipocyte group was used as the reference group and relative expression was calculated as the $2^{-\Delta\Delta Cq}$ values of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the brown preadipocyte group (as '1.0'). UCP1: P<0.0001 for ANOVA. Diff vs. brown preadipocytes, P<0.0001; Diff vs. Diff + 10 ng/ml E. coli LPS, P<0.0001; Diff vs. Diff + 100 ng/ml E. coli LPS, P<0.0001; Diff vs. Diff + 1,000 ng/ml E. coli LPS, P<0.0001. (D) UCP1 mRNA expression in the brown adipose tissue of mice injected with P. gingivalis 100 µl (10^8 CFU) or PBS 18 h previously. The PBS group was used as the reference group and relative expression was calculated as the $2^{-\Delta\Delta Cq}$ values of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the PBS group (as '1.0'). UCP1: PBS vs. P. gingivalis, P=0.0005; """P<0.001 and """"""""P<0.0001. P. gingivalis, Porphyromonas gingivalis; P. gingivalis LPS, lipopolysaccharide derived from Porphyromonas gingivalis; LPS, lipopolysaccharide; UCP1, uncoupling protein 1; E. coli LPS, LPS derived from Escherichia coli.
Figure 2. *P. gingivalis* LPS reduces lncRNA-BATE10 expression in differentiating brown adipocytes and *P. gingivalis* reduces lncRNA-BATE10 expression in the BAT of mice. (A) Preadipocytes were induced to differentiate into brown adipocytes, during which *P. gingivalis* LPS was added to the medium. lncRNA-BATE10 expression was examined in brown adipocytes (Diff) and preadipocytes. The brown preadipocyte group was used as the reference group and relative expressive was calculated as the $2^{-\Delta\Delta Cq}$ values of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the brown preadipocyte group (as ‘1.0’). P<0.0001 for ANOVA. Diff vs. preadipocytes, P<0.0001; Diff vs. Diff + 10 ng/ml *P. gingivalis* LPS, P<0.0001; Diff vs. Diff + 100 ng/ml *P. gingivalis* LPS, P<0.0001; Diff vs. Diff + 1,000 ng/ml *P. gingivalis* LPS, P<0.0001. (B) Preadipocytes were induced to differentiate into brown adipocytes, during which *E. coli* LPS was present in the medium, then lncRNA-BATE10 expression in brown adipocytes (Diff) and preadipocytes was measured. The brown preadipocyte group was used as the reference group and relative expressive was calculated as the $2^{-\Delta\Delta Cq}$ values of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the brown preadipocyte group (as ‘1.0’). P<0.0001 for ANOVA. Diff vs. preadipocytes, P<0.0001.; Diff vs. Diff + 10 ng/ml *E. coli* LPS, P<0.0001; Diff vs. Diff + 100 ng/ml *E. coli* LPS, P<0.0001; Diff vs. Diff + 1,000 ng/ml *E. coli* LPS, P<0.0001. (C, left panel) lncRNA-BATE10 expression in the BAT of mice intravenously administered *P. gingivalis* 100 µl (10^8 CFU) or PBS 18 h earlier. The PBS group was used as the reference group and relative expressive was calculated as the $2^{-\Delta\Delta Cq}$ values of the other groups/mean of $2^{-\Delta\Delta Cq}$ value of the brown preadipocyte group (as ‘1.0’). PBS vs. *P. gingivalis*, P=0.0001. (C, right panel) Estimation plot displaying the raw data and the confidence interval for the difference between the means. ***P<0.001 and ****P<0.0001. *P. gingivalis*, Porphyromonas gingivalis; *P. gingivalis* LPS, lipo-polysaccharide derived from *Porphyromonas gingivalis*; LPS, lipopolysaccharide; *E. coli* LPS, LPS derived from *Escherichia coli*. 
Periodontal bacteria have been identified in the gut of patients with inflammatory bowel disease. They may be transported to ectopically colonize the gut via the oral route (5,6). Thus, the systemic effects of periodontal inflammation may be mediated through \textit{P. gingivalis}.

IncRNA-BATE10 is BAT-specific and is a member of the IncRNA-BATE family. IncRNA-BATE10 is transcribed from four exons in an intergenic region of mouse chromosome 18 and is \textasciitilde1.7 kb in length (21). IncRNA-BATE10 expression in white adipose tissue is increased by exposure to cold, \(\beta\)-adrenergic agonists and intense physical exercise (26). Accordingly, IncRNA-BATE10 expression is increased by exposure to cold in BAT and is lower at 30°C (26). During the differentiation of brown preadipocytes, the knockdown of IncRNA-BATE10 leads to a decrease in the expression levels of BAT-specific genes, including \textit{UCP1} and \textit{Pgc1a} (26,30). These findings demonstrate that IncRNA-BATE10 may play a role in BAT thermogenesis; therefore, it was hypothesized that \textit{P. gingivalis} LPS inhibits the expression of \textit{UCP1} during the differentiation of brown adipocytes by reducing IncRNA-BATE10 expression.

In conclusion, \textit{P. gingivalis} may have deleterious effects on BAT that are mediated by LPS. Specifically, \textit{P. gingivalis} reduces UCP1 expression, and IncRNA-BATE10 promotes a pro-inflammatory state. The results of the present study may enhance the current understanding of the association between periodontal disease and obesity.

Acknowledgements
The authors would like to thank Professor Zhuoxian Meng (Department of Pathology and Pathophysiology, Key Laboratory of Disease Proteomics of Zhejiang Province, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China) for providing the brown preadipocytes and Dr Peihui Ding (Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Clinical Research Center for Oral Diseases of Zhejiang Province, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Zhejiang, Hangzhou, China) for providing \textit{Porphyromonas gingivalis}.

Funding
The present study was supported by grants from the National Natural Science Foundation of China (grant no. 81700972), the Cao Guangbiao High Sci-Tech Development Fund of Zhejiang University (grant no. 2020QN026), and the Pre-Research Fund from School of Medicine, Zhejiang University (grant no. 519600-I52104/004).

Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions
WD conceived and designed the study. FZ, LS, NZ, LL and JG performed the experiments. FZ, LS, NZ, LL, JG and WD prepared a draft of the manuscript, and WD and FZ finalized the manuscript. FZ, LS and WD confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.
Ethics approval and consent to participate

The animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Zhejiang University (Approval no. ZJU20170237, 2017-02-24).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pihlstrom BL, Michalowicz BS and Johnson NW: Periodontal diseases. Lancet 366: 1809-1820, 2005.
2. Nassar H, Kantarci A and van Dyke TE: Diabetic Periodontitis: A model for activated innate immunity and impaired resolution of inflammation. Periodontol 2000 40: 233-244, 2007.
3. Zarric SS, Lappin MJ, Fulton CR, Landy FT, Coulter WA and Irwin CR: Sialylation of Porphyromonas gingivalis LPS and its effect on bacterial-host interactions. Innate Immun 23: 319-326, 2017.
4. Iwashita M, Hayashi M, Nishimura Y and Yamashita A: The link between periodontal inflammation and obesity. Curr Oral Health Rep 8: 76-83, 2021.
5. Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, Iwashita M, Hayashi M, Nishimura Y and Yamashita A: The link between periodontal inflammation and obesity. Cur 6. Qian F, Akkouch A, Su D, Amendt BA, Wang GX, Zhao XY, Meng ZX, Kern M, Dietrich A, Chen Z, Ohyama H, Hata M, Soga Y, Kushiyama A, Asano T, et al: Endotoxemia by Porphyromonas gingivalis administration induces gestational obesity, alters gene expression in the liver and brown adipose tissue in pregnant mice, and causes underweight in fetuses. Front Cell Infect Microbiol 11: 745117, 2022.
7. Yoshida S, Hatasa M, Ohsugi Y, Katagiri S, Yoshida S, Niimi H, Morita K, Shimohira T, Sasaki N, Maekawa S, et al: Porphyromonas gingivalis administration induces gestational obesity, alters gene expression in the liver and brown adipose tissue in pregnant mice, and causes underweight in fetuses. Front Cell Infect Microbiol 11: 745117, 2022.
8. Hatasa M, Ohsugi Y, Katagiri S, Yoshida S, Niimi H, Morita K, Tsuchiya Y, Shimohira T, Sasaki N, Maekawa S, et al: Endotoxemia by Porphyromonas gingivalis alters endocrine functions in brown adipose tissue. Front Cell Infect Microbiol 11: 745117, 2022.
9. Xie X, Huang CY and Oka SI: lLncRNA KCNQ1OT1 promotes Atg12-mediated autophagy via inhibiting miR-26a-5p in ischemia reperfusion. Int J Cardiol 339: 132-133, 2021.
10. Wang Z, Tang X, Xu W, Yang M, Wang W, Tang L, Tang D and Wang D: Cardamom exerts anti-gastric cancer activity via inhibiting lLncRNA-PVT1-STAT3 axis. Bioosci Rep 39: BSR20190357, 2019.
11. Lai S, Du K, Shi Y, Li C, Wang G, Hu S, Jia X, Wang J and Chen S: Long non-coding RNAs in brown adipose tissue. Diabetes Metab Syndr Obes 13: 3193-3200, 2020.
12. Kang S, Daji A, Wang H and Ding PH: Interaction between autophagy and Porphyromonas gingivalis-induced inflammation. Front Cell Infect Microbiol 12: 892610, 2022.
13. Klein J, Fasshauer M, Klein HH, Benito M and Kahn CR: Novel adipocyte lines from brown fat: A model system for the study of differentiation, energy metabolism, and insulin action. Bioessays 24: 382-388, 2002.
14. Wang GX, Zhao XY, Meng ZX, Kern M, Dietrich A, Chen Z, Cozaczek V, Zhou D, Okunade AL, Su X, et al: The brown fat-enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. Nat Med 20: 1436-1443, 2014.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Bai Z, Zhai XR, Yoon MJ, Kim HJ, Lo KA, Zhang ZC, Xu D, Siang DTC, Walet ACE, Xu SH, et al: Dynamic transcriptome changes during adipose tissue energy expenditure reveal critical roles for non-coding RNA regulators. PLoS Biol 15: e2002176, 2017.
17. Lucci C, Vieira E, Perchet T, Gual P and Golub R: Natural killer cells and type 1 innate lymphoid cells are new actors in non-alcoholic fatty liver disease. Front Immunol 10: 1192, 2019.
18. Cullender TC, Chassaing B, Janzon A, Kumar K, Muller CE, Werner JJ, Angenent LT, Bell ME, Hay AG, Peterson DA, et al: Innate and adaptive immunity interaction to quench microbiome flagellar motility in the gut. Cell Host Microbe 14: 571-581, 2013.
19. Rojas C, García MP, Polanco AF, González-Osuna L, Sierra-Cristancho A, Melgar-Rodríguez S, Cafferata EA and Vernal R: Humanized mouse models for the study of Periodontitis: An opportunity to elucidate unresolved aspects of its immunopathogenesis and analyze new immunotherapeutic strategies. Front Immunol 12: 663328, 2021.
20. Cui X, You L, Li Y, Zhu L, Zhang F, Xie K, Cao Y, Ji C and Guo X: A transcribed ultraconserved noncoding RNA, uc.417, serves as a negative regulator of brown adipose tissue thermogenesis. FASEB J 30: 4301-4312, 2016.