PRMT5 inhibition ameliorates inflammation and promotes the osteogenic differentiation of LPS-induced periodontal stem cells via STAT3/NF-κB signaling

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Abstract. It has been reported that protein arginine methyltransferase 5 (PRMT5) serves a significant role in osteogenic differentiation and inflammatory response. Nevertheless, its role in periodontitis as well as its underlying mechanism remain to be elucidated. The aim of the present study was to explore the role of PRMT5 in periodontitis and whether PRMT5 could reduce liposaccharide (LPS)-induced inflammation of human periodontal ligament stem cells (hPDLSCs) and promote osteogenic differentiation through STAT3/NF-κB signaling. In the current study, the expression levels of PRMT5 were determined in LPS-induced hPDLSCs by reverse transcription-quantitative PCR and western blot analysis. ELISA and western blot analysis were employed to assess the secretion and expression levels of inflammatory factors, respectively. The osteogenic differentiation and mineralization potential of hPDLSCs were evaluated using alkaline phosphatase (ALP) activity assay, Alizarin red staining and western blot analysis. Additionally, western blot analysis was applied to determine the expression levels of the STAT3/NF-κB signaling pathway-related proteins. The results showed that the expression levels of PRMT5 were significantly enhanced in LPS-induced hPDLSCs. Additionally, PRMT5 knockdown reduced the contents of IL-1β, IL-6, TNF-α, inducible nitric oxide synthase and cyclooxygenase-2. PRMT5 depletion also enhanced ALP activity, improved the mineralization ability and upregulated bone morphogenetic protein 2, osteocalcin and runt-related transcription factor 2 in LPS-induced hPDLSCs. Furthermore, PRMT5 knockdown inhibited inflammation and promoted the osteogenic differentiation of hPDLSCs via blocking the activation of the STAT3/NF-κB signaling pathway. In conclusion, PRMT5 inhibition suppressed LPS-induced inflammation and accelerated osteogenic differentiation in hPDLSCs via regulating STAT3/NF-κB signaling, thus providing a potential targeted therapy for the improvement of periodontitis.

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Introduction

Periodontitis is a common clinical oral infectious disease and one of the main causes of adult tooth loss, severely affecting the health of patients (1). Periodontitis, which is characterized by the damage of periodontal supporting tissue, usually results from pathogenic microorganisms, thus contributing to the loss as well as the extraction of teeth (2). It has been previously reported that periodontitis is a chronic inflammation of the periodontal supporting tissue induced by multiple factors, manifesting with alveolar bone absorption, gingival inflammation and loss of periodontal attachment with pathological features (3,4). The association between microbiota and host can determine the occurrence and the advancement of periodontal diseases. In the aforementioned diseases, the induction of the immune response of the host against bacteria and their products is considered as the key factor resulting in the destruction of periodontal tissue. The periodontal tissue is commonly impaired by the excessive release of inflammatory factors (5-7). Additionally, periodontal ligament contains a group of pluripotent periodontal stem cells, periodontal ligament stem cells (hPDLSCs), which have significant involvement in the reconstruction, regeneration and fixation of periodontal tissue (8). Furthermore, a case from a previous study has shown that the periodontal inflammatory microenvironment can destroy periodontal tissue by suppressing the regeneration ability of hPDLSCs (9). Therefore, the suppression of inflammatory injury and improvement of the osteogenic differentiation capability of hPDLSCs could be a promising treatment approach for periodontitis.

Protein arginine methyltransferases (PRMTs) catalyze the methylation of arginine residues on several proteins, including histones and non-histone proteins (10). As a common post-translational modification, arginine methylation has been associated with several cellular processes, such as

DNA transcription, signal transduction and subcellular protein localization (11). PRMT5, a member of the PRMT family, serves a crucial role in several biological processes (12). A study showed that PRMT5 downregulation could suppress the differentiation capacity of osteoclasts and exerted a protective effect on bone in patients underwent ovariectomy via downregulating 10-kDa (CXCL10) and radical S-adenosyl methionine domain containing 2 (13). In addition, PRMT5 silencing promoted the osteogenic differentiation of mesenchymal stromal cells and repressed basal interferon stimulated gene expression (14). A previous study also found that PRMT5 was involved and promoted inflammatory responses of bronchial epithelial cells (15). However, the role of PRMT5 in inflammatory response and the osteogenic differentiation of hPDLSCs remains to be elucidated. Therefore, the present study aimed to investigate the biological role of PRMT5 and its potential underlying mechanism in liposaccharide (LPS)-induced hPDLSCs. It was hypothesized that PRMT5 inhibition can reduce inflammation and accelerate the osteogenic differentiation of LPS-induced hPDLSCs via regulating the STAT3/NF-κB signaling.

Materials and methods

Cell culture. hPDLSCs were obtained from ScienCell Research Laboratories, Inc. and cultured in DMEM (Hyclone; Cytiva) supplemented with 1% antibiotics and 10% FBS at 37˚C in the presence of 5% CO_2. hPDLSCs were treated with 0.1, 1 or 10 μM EPZ015666 (EPZ), a PRMT5 inhibitor, for three days. To establish a periodontitis model, hPDLSCs were induced with 10 μg/ml *P. gingivalis* LPS (MiliporeSigma) for 24 h. Osteoblast differentiation was induced using conditional medium containing 1% antibiotics, 10% FBS, α-MEM, 50 μg/ml l-ascorbic acid, 10 nM dexamethasone and 10 mM β-glycerophosphate (MiliporeSigma). Colivelin (1 μM), a STAT3 agonist, was used to activate the STAT3 pathway.

ELISA. The secretion levels of IL-1β, IL-6 and TNF-α in cultured hPDLSCs were assessed using the IL-1β assay kit (cat. no. H002), IL-6 assay kit (cat. no. H007-1-1) and TNF-α assay kit (cat. no. H052-1) (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The optical density (OD) values at a wavelength of 450 nm were measured utilizing the xMark Microplate absorbance spectrophotometer (Bio-Rad Laboratories, Inc.). Finally, the concentration of the above inflammatory factors was calculated based on the corresponding standard curves.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from $1x10^4$ hPDLSCs using a TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, and its concentration was measured employing NanoDrop 2000 (Thermo Fisher Scientific, Inc.) at 260 and 280 nm. Subsequently, RNA was reverse transcribed into cDNA using the PrimeScript RT Master Mix (Takara Bio, Inc.) according to the manufacturer's instructions. qPCR was performed on the ABI PRISM 7900 Real-Time system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the SYBR Premix ExTaq kit (Takara Bio, Inc.) according to the manufacturer's instructions. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 3 min; followed by 40 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec. The primer sequences for PCR were: PRMT5: 5'-CTGACACACTAGGGGCTGTG-3' (forward) and 5'-ACTAGTCTGCCCTTCTCCGT-3' (reverse); GAPDH: 5'-GGGAAACTGTGGCGTGAT-3' (forward) and 5'-GAGTGGGTGTCGCTGTTGA-3' (reverse). The relative mRNA levels were calculated using the $2^{-\Delta\Delta Cq}$ method (16).

Alkaline phosphatase (ALP) activity assay. ALP activity was measured to assess the differentiation ability of hPDLSCs using an ALP Assay kit (Beyotime Institute of Biotechnology), according to the manufacturer's instructions. The OD values were measured at a wavelength of 405 nm using a microplate reader.

Alizarin red staining (ARS). The cells were cultured for two weeks and were then mineralized to form opaque calcified nodules. Subsequently, hPDLSCs were first treated with 95% ethanol for 10 min and were then stained with 0.1% ARS solution (MilliporeSigma) for 15 min at room temperature. To measure the degree of mineralization, ARS released from the cell matrix was visualized using an inverted light microscope.

Western blot analysis. Total proteins were isolated from hPDLSCs with RIPA buffer (Shanghai Yisheng Biotechnology Co., Ltd.) and the protein concentration was determined using a BCA Protein Assay Kit (Shanghai Fantai Biotechnology Co., Ltd.). Following separation by 10% SDS-PAGE (60 μg/lane), the proteins were transferred onto PVDF membranes. The overnight incubation of membranes, which were first blocked with 5% non-fat milk in 0.1% TBS-Tween-20 for 2 h at room temperature, was performed at 4˚C with primary antibodies against PRMT5 (cat. no. ab109451; 1:10,000; Abcam), inducible nitric oxide synthase (iNOS; cat. no. ab178945; 1:1,000; Abcam), cyclooxygenase-2 (COX-2; cat. no. ab179800; 1:1,000; Abcam), bone morphogenetic protein 2 (BMP2; cat. no. ab284387; 1:1,000; Abcam), osteocalcin (OCN; cat. no. ab133612; 1:1,000; Abcam), runt-related transcription factor 2 (Runx2; cat. no. ab92336; 1:5,000; Abcam), phosphorylated (p)-STAT3 (cat. no. ab267373; 1:1,000; Abcam), STAT3 (cat. no. ab68153; 1:1,000; Abcam), p-NF-κB (cat. no. ab239882; 1:1,000; Abcam), NF-κB (cat. no. ab220803; 1:1,000; Abcam) and GAPDH (cat. no. ab9485; 1:2,500; Abcam). Subsequently, the membranes were incubated with the corresponding HRP-labeled secondary antibody (cat. no. ab6759; 1:5,000; Abcam) for 1 h at room temperature. The protein blots were visualized using an ECL detection system (MilliporeSigma) and analyzed with ImageJ software (version 1.49; National Institutes of Health). The ratio of the target protein to GAPDH light density was considered as the relative protein expression.

Statistical analysis. All experiments were performed three times or more. All data were analyzed with SPSS 23.0 software (IBM Corporation) using one-way ANOVA followed by Bonferroni's multiple comparison test. Data are expressed as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

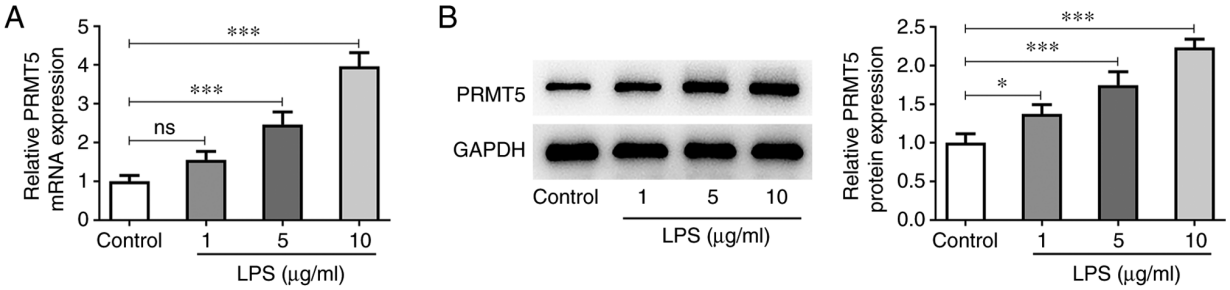

Figure 1. PRMT5 is upregulated in LPS-induced hPDLSCs. mRNA expression (A) and protein level (B) of PRMT5 in LPS-induced hPDLSCs were detected by reverse transcription-quantitative PCR and western blotting. Data are expressed as mean ± standard deviation. *P<0.05, ***P<0.001. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells; ns, not significant.

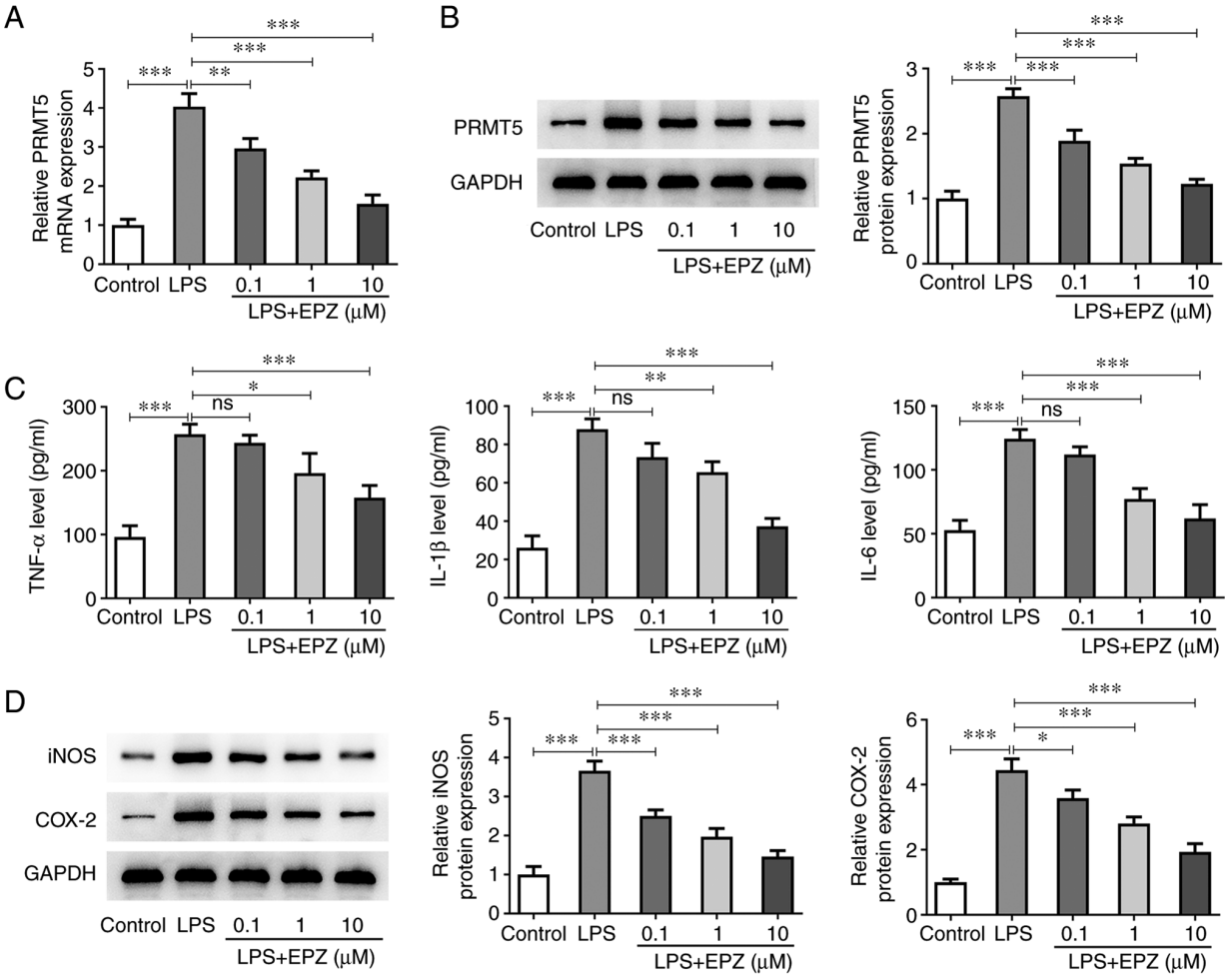

Figure 2. Downregulation of PRMT5 reduces LPS-induced hPDLSCs inflammation. (A) mRNA expression and (B) protein level of PRMT5 in LPS-induced hPDLSCs with 0.1-10 μM EPZ were detected by reverse transcription-quantitative PCR and western blotting. (C) The levels of TNF-α, IL-1β and IL-6 were evaluated by ELISA. (D) Western blot assay was used to assess protein levels of iNOS and COX-2. Data are expressed as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001. ns, not significant. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells; EPZ, EPZ015666; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.

Results

PRMT5 is upregulated in LPS-induced hPDLSCs. To explore the biological role of PRMT5 in LPS-induced hPDLSCs, the expression levels of PRMT5 in treated hPDLSCs were initially assessed. As shown in Fig. 1A and B, the mRNA and protein expressions levels of PRMT5 in LPS-induced hPDLSCs (5-10 μg/ml) were both markedly elevated compared with the control cells. Therefore, LPS concentration of 10 μg/ml was selected for the following assays.

PRMT5 inhibition attenuates inflammation in LPS-induced hPDLSCs. To investigate the function of PRMT5 in LPS-induced hPDLSCs, cells were treated with 0.1-10 μM EPZ. The inhibitory effect of EPZ on LPS-induced hPDLSCs is shown in Fig. 2A and B. LPS treatment of hPDLSCs significantly

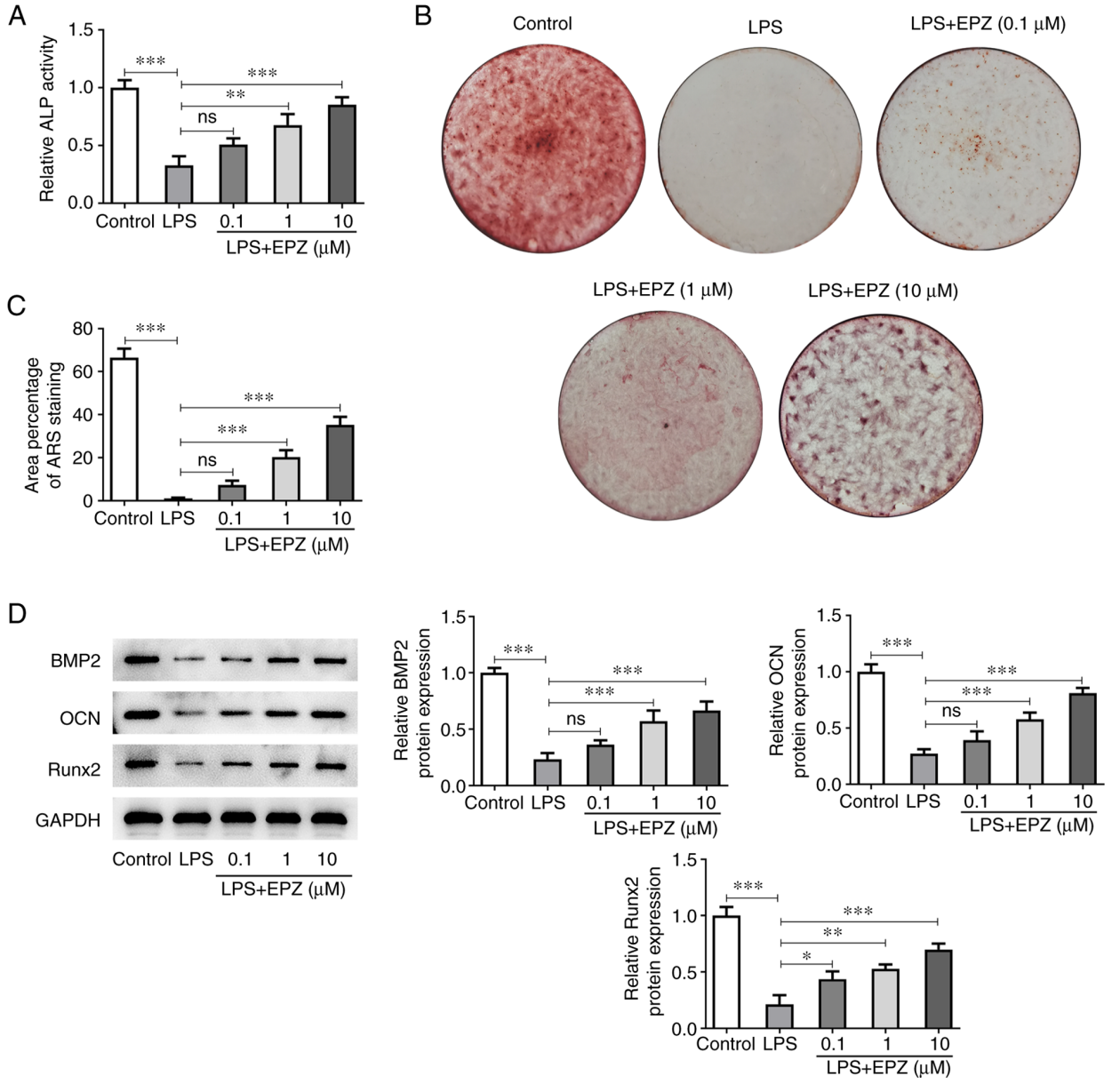

Figure 3. Inhibition of PRMT5 promotes LPS-induced osteogenic differentiation of hPDLSCs. (A) ALP activity in LPS-induced hPDLSCs with 0.1-10 μM EPZ. (B and C) The area of Alizarin red staining in LPS-induced hPDLSCs with 0.1-10 μM EPZ. (D) Western blot assay was used to assess protein levels of BMP2, OCN and Runx2. Data are expressed as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ns, not significant. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells; ALP, alkaline phosphatase; ARS, Alizarin red staining; EPZ, EPZ015666; BMP2, bone morphogenetic protein 2; OCN, osteocalcin; Runx2, runt-related transcription factor 2.

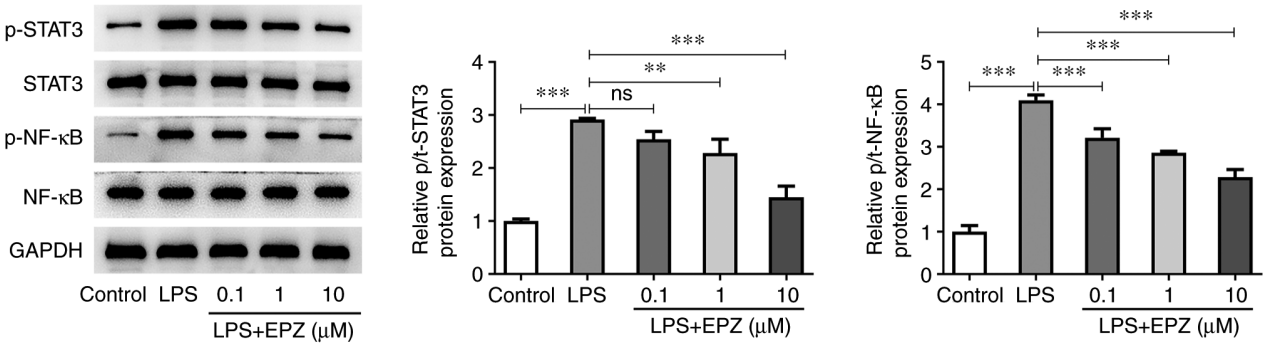

Figure 4. Inhibition of PRMT5 suppresses the activation of STAT3/NF-κB pathway in LPS-induced hPDLSCs. Western blot assay was used to measure the protein levels of STAT3, NF-κB, p-STAT3 and p-NF-κB in LPS-induced hPDLSCs with 0.1-10 μM EPZ. Data are expressed as mean ± standard deviation. **P<0.01, ***P<0.001, ns, not significant. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells; p-, phosphorylated; EPZ, EPZ015666.

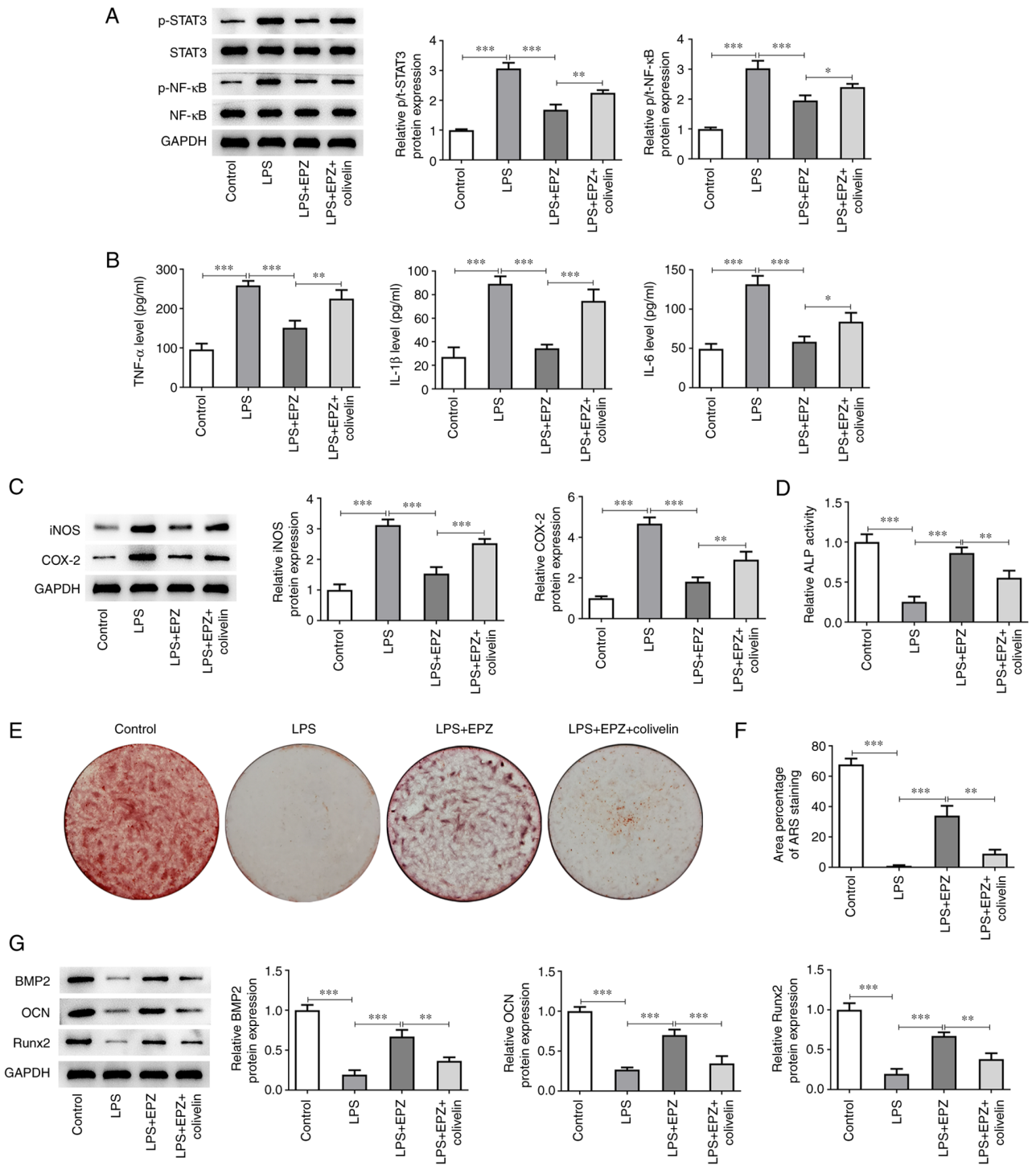

Figure 5. Downregulation of PRMT5 restrains LPS-induced hPDLSCs inflammation and promotes osteogenic differentiation by blocking the activation of STAT3/NF-κB pathway. (A) Western blot assay was used to measure the protein levels of STAT3, NF-κB, p-STAT3 and p-NF-κB in LPS-induced hPDLSCs with EPZ or colivelin. (B) The levels of IL-1β, IL-6, TNF-α were evaluated by ELISA. (C) Western blot assay was used to assess protein levels of iNOS and COX-2. (D) ALP activity in LPS-induced hPDLSCs with EPZ or colivelin. (E and F) The area of Alizarin red staining in LPS-induced hPDLSCs with EPZ or colivelin. (G) Western blot assay was used to assess protein levels of BMP2, OCN and Runx2. Data are expressed as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells; p-, phosphorylated; EPZ, EPZ015666; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2 ALP, alkaline phosphatase; ARS, Alizarin red staining.

increased the secretion levels of IL-1β, IL-6 and TNF-α, which were reduced following co-treatment with 1-10 μM EPZ (Fig. 2C). In addition, western blot analysis revealed that the protein expression levels of iNOS and COX-2 were increased by LPS, which were also restored following co-treatment of LPS-induced hPDLSCs with 0.1-10 μM EPZ (Fig. 2D).

PRMT5 inhibition promotes the osteogenic differentiation of LPS-induced hPDLSCs. Subsequently, the current study aimed to evaluate the effect of PRMT5 on the differentiation ability of LPS-induced hPDLSCs. As shown in Fig. 3A, LPS stimulation dramatically repressed ALP activity in hPDLSCs, which was subsequently restored by PRMT5 inhibition

in a dose-dependent manner. Furthermore, cell treatment with LPS reduced the area of ARS in hPDLSCs. However, PRMT5 inhibition abrogated the effect of LPS on ARS area (Fig. 3B and C). Additionally, BMP2, OCN and Runx2 were downregulated after cell induction with LPS. However, the above effect was also reversed by PRMT5 inhibition (Fig. 3D).

Inhibition of PRMT5 suppresses the activation of the STAT3/NF-κB pathway in LPS-induced hPDLSCs. Subsequently, the present study aimed to uncover the potential mechanism underlying the regulatory effect of PRMT5 on LPS-induced hPDLSCs. As shown in Fig. 4, hPDLSCs treatment with LPS markedly enhanced the protein expression levels of p-STAT3 and p-NF-κB. However, PRMT5 inhibition abrogated the effect of LPS on the expression of p-STAT3 and p-NF-κB in LPS-induced hPDLSCs. The total expression levels of STAT3 and NF-κB remained unchanged.

PRMT5 inhibition restrains inflammation and promotes the osteogenic differentiation of LPS-induced hPDLSCs via blocking the activation of the STAT3/NF-κB pathway. To evaluate the role of the STAT3/NF-κB signaling pathway in PRMT5-mediated LPS injury, LPS-induced hPDLSCs were co-treated with PRMT5 inhibitor and the STAT3 agonist, colivelin. Western blot analysis showed that cell co-treatment with EPZ and colivelin upregulated p-STAT3 and p-NF-κB in LPS-induced hPDLSCs compared with EPZ-treated cells (Fig. 5A). Additionally, EPZ plus with colivelin elevated the contents of IL-1β, IL-6, TNF-α, iNOS and COX-2 in LPS-induced hPDLSCs compared with EPZ-treated cells (Fig. 5B and C). Furthermore, colivelin diminished the relative ALP activity and ARS area compared with EPZ-treated hPDLSCs (Fig. 5D-F). Finally, co-treatment with EPZ and colivelin markedly reduced the expression levels of BMP2, OCN and Runx2 in LPS-induced hPDLSCs compared with EPZ-treated hPDLSCs (Fig. 5G).

Discussion

Periodontitis may result in the damage of the alveolar bone. Therefore, bone regeneration is considered as one the most significant parts of the diagnosis and treatment of periodontal diseases (17). In addition, PDLSCs serve a significant role in bone regeneration due to their high proliferation, self-renewal and multi-directional differentiation abilities (18). hPDLSCs are derived from mesenchymal stem cells of the periodontal tissue and can differentiate into several tissues under *in vitro* culture conditions, which in turn can be used as the cell source of periodontal tissue regeneration (19). Nevertheless, changes in the microenvironment can affect the differentiation ability of hPDLSCs (20). A previous study demonstrated that the proliferation ability of hPDLSCs under an inflammatory environment was significantly increased, while their differentiation capability was greatly reduced (21). Therefore, it is of critical importance to attenuate the effects of inflammation on hPDLSCs in periodontitis. In the present study, PRMT5 inhibition decreased LPS-induced hPDLSCs inflammation and osteogenic differentiation via inactivating the STAT3/NF-κB signaling pathway, thus suggesting that PRMT5 could act as a potential target for attenuating LPS-induced periodontitis.

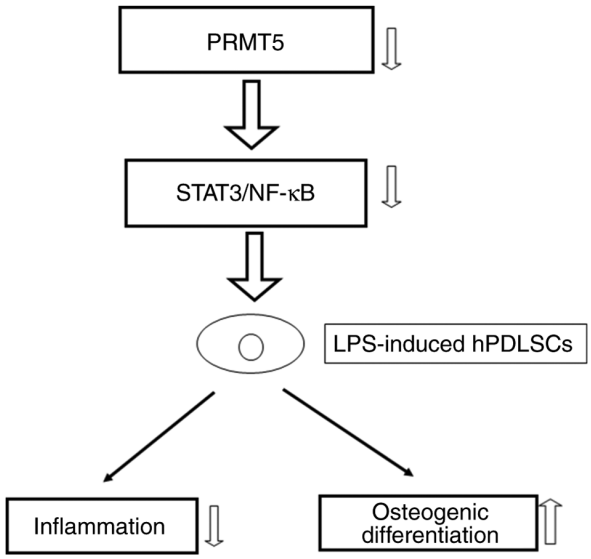

Figure 6. The graphical hypothesis of PRMT5 function mechanism in LPS-induced hPDLSCs. PRMT5, protein arginine methyltransferase 5; LPS, liposaccharide; hPDLSCs, human periodontal ligament stem cells.

LPS is commonly used to induce periodontitis injury and it has been reported to induce cell inflammation, apoptosis, autophagy as well as endoplasmic reticulum stress in oral diseases (22). In the current study an *in vitro* periodontitis model was established following hPDLSCs stimulation with LPS. The results showed that cell treatment with LPS significantly aggravated inflammation and inhibited osteogenic differentiation in hPDLSCs. PRMT5 is an arginine methyltransferase that serves a significant role in osteogenic differentiation and inflammatory responses (23). A study showed that PRMT5 inhibition relieved cartilage degradation via inactivating MAPK and NF-κB signaling (24). Another study also revealed that PRMT5 inhibition prevented inflammation and migration of fibroblast-like synoviocytes in rheumatoid arthritis (RA). It was therefore considered as a promising treatment approach for RA (25). Qiao *et al* (26) suggested that RA was closely associated with periodontitis and that patients suffering from periodontitis could be more vulnerable to RA. Another study also showed that PRMT5 inhibition could alleviate the development of periodontitis via inhibiting the activation and metastasis of dendritic cells (27). Therefore, it was hypothesized that PRMT5 could be also involved in LPS-induced inflammatory response and osteogenic differentiation of hPDLSCs. The results of the present study demonstrated that PRMT5 was upregulated in LPS-induced hPDLSCs. Additionally, PRMT5 inhibition could attenuate LPS-induced cell inflammation and rehabilitate the osteogenic differentiation of hPDLSCs, thus supporting the protective effect of PRMT5 inhibition on LPS-induced hPDLSCs injury.

A previous study revealed that PRMT5 could act as a critical regulator of STAT3 activation. Therefore, PRMT5 depletion or inhibition could significantly inhibit the activation of STAT3 (28). Chen *et al* (29) also showed that suppression of deubiquitinase ubiquitin-specific peptidase 5 restrained the inflammatory response in chronic periodontitis via inhibiting STAT3 signaling. In addition, Jiang *et al* (30) revealed that

angiopoietin-like protein 2 (ANGPTL2) downregulation activated STAT3 and NF-κB signaling, and inhibited Akt signaling under inflammatory environment. However, treatment with a STAT3 inhibitor suppressed the inflammatory response of ANGPTL2-induced periodontal ligament cells. It was therefore hypothesized that STAT3/NF-κB signaling could be involved in PRMT5-mediated hPDLSCs. It is documented that LPS can activate the STAT pathways according to previous studies (31,32). In addition, some studies have reported LPS can specifically phosphorylate STAT3 (33-35). In the present study, LPS activated the phosphorylation of STAT3 and NF-κB in hPDLSCs. The activation of the STAT3/NF-κB pathway could reverse the effects of PRMT5 inhibition on LPS-induced hPDLSCs inflammation and osteogenic differentiation, which was consistent with previous studies. The present study found that PRMT5 inhibition can reduced the production of inflammatory cytokines, meanwhile the addition of STAT3 agonist colivelin enhanced the decreased inflammatory cytokine, which indicated that PRMT5 inhibition indirectly inhibited the activation of some inflammatory cytokine by inhibiting the pathway of STAT3/NF-κB. In addition, the present study did not explore the substrate PRMT5 enzyme catalyzed and the mechanism of PRMT5 and how to catalyze the substrate in LPS-induced hPDLSCs will continue to be studied.

In summary, the current study highlighted the essential protective effect of PRMT5 inhibition on LPS-induced cell inflammation and osteogenic differentiation. In addition, the results supported the important role of the PRMT5-regulated STAT3/NF-κB pathway in LPS-induced hPDLSCs (Fig. 6), thus suggesting that PRMT5 could be a promising therapy approach for improving periodontitis.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

KZ and XT designed the study, drafted and revised the manuscript. CL and JS analyzed the data and searched the literature. KZ and XT confirm the authenticity of all the raw data. KS, XT, CL and JS performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Slots J: Periodontitis: Facts, fallacies and the future. Periodontol 2000 75: 7-23, 2017.
2. Kwon T, Lamster IB and Levin L: Current concepts in the management of periodontitis. Int Dent J 71: 462-476, 2021.
3. Fischer RG, Lira Junior R, Retamal-Valdes B, Figueiredo LC, Malheiros Z, Stewart B and Feres M: Periodontal disease and its impact on general health in Latin America. Section V: Treatment of periodontitis. Braz Oral Res 34: e026, 2020.
4. Bartold PM: Lifestyle and periodontitis: The emergence of personalized periodontics. Periodontol 2000 78: 7-11, 2018.
5. Cecoro G, Annunziata M, Iuorio MT, Nastri L and Guida L: Periodontitis, low-grade inflammation and systemic health: A scoping review. Medicina (Kaunas) 56: 272, 2020.
6. Van Dyke TE and Sima C: Understanding resolution of inflammation in periodontal diseases: Is chronic inflammatory periodontitis a failure to resolve? Periodontol 2000 82: 205-213, 2020.
7. Verrusio C, Iorio-Siciliano V, Blasi A, Leuci S, Adamo D and Nicolò M: The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. Quintessence Int 49: 69-77, 2018.
8. Tran Hle B, Doan VN, Le HT and Ngo LT: Various methods for isolation of multipotent human periodontal ligament cells for regenerative medicine. In Vitro Cell Dev Biol Anim 50: 597-602, 2014.
9. Liu W, Konermann A, Guo T, Jäger A, Zhang L and Jin Y: Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 1840: 1125-1134, 2014.
10. Hwang JW, Cho Y, Bae GU, Kim SN and Kim YK: Protein arginine methyltransferases: Promising targets for cancer therapy. Exp Mol Med 53: 788-808, 2021.
11. Couto E Silva A, Wu CY, Citadin CT, Clemons GA, Possoit HE, Grames MS, Lien CF, Minagar A, Lee RH, Frankel A, *et al*: Protein arginine methyltransferases in cardiovascular and neuronal function. Mol Neurobiol 57: 1716-1732, 2020.
12. Stopa N, Krebs JE and Shechter D: The PRMT5 arginine methyltransferase: Many roles in development, cancer and beyond. Cell Mol Life Sci 72: 2041-2059, 2015.
13. Dong Y, Song C, Wang Y, Lei Z, Xu F, Guan H, Chen A and Li F: Inhibition of PRMT5 suppresses osteoclast differentiation and partially protects against ovariectomy-induced bone loss through downregulation of CXCL10 and RSAD2. Cell Signal 34: 55-65, 2017.
14. Kota SK, Roening C, Patel N, Kota SB and Baron R: PRMT5 inhibition promotes osteogenic differentiation of mesenchymal stromal cells and represses basal interferon stimulated gene expression. Bone 117: 37-46, 2018.
15. Kim JH, Yoo BC, Yang WS, Kim E, Hong S and Cho JY: The role of protein arginine methyltransferases in inflammatory responses. Mediators Inflamm 2016: 4028353, 2016.
16. 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.
17. Tomokiyo A, Wada N and Maeda H: Periodontal ligament stem cells: Regenerative potency in periodontium. Stem Cells Dev 28: 974-985, 2019.
18. Isola G, Polizzi A, Alibrandi A, Indelicato F and Ferlito S: Analysis of Endothelin-1 concentrations in individuals with periodontitis. Sci Rep 10: 1652, 2020.
19. Ren S, Yao Y, Zhang H, Fan R, Yu Y, Yang J, Zhang R, Liu C, Sun W and Miao L: Aligned fibers fabricated by near-field electrospinning influence the orientation and differentiation of hPDLSCs for periodontal regeneration. J Biomed Nanotechnol 13: 1725-1734, 2017.
20. Shang F, Liu S, Ming L, Tian R, Jin F, Ding Y, Zhang Y, Zhang H, Deng Z and Jin Y: Human umbilical cord MSCs as new cell sources for promoting periodontal regeneration in inflammatory periodontal defect. Theranostics 7: 4370-4382, 2017.
21. Yu B, Hu J, Li Q and Wang F: CircMAP3K11 contributes to proliferation, apoptosis and migration of human periodontal ligament stem cells in inflammatory microenvironment by regulating TLR4 via miR-511 sponging. Front Pharmacol 12: 633353, 2021.

22. Pussinen PJ, Kopra E, Pietiäinen M, Lehto M, Zaric S, Paju S and Salminen A: Periodontitis and cardiometabolic disorders: The role of lipopolysaccharide and endotoxemia. Periodontol 2000 89: 19-40, 2022.
23. Motolani A, Martin M, Sun M and Lu T: The structure and functions of PRMT5 in human diseases. Life (Basel) 11: 1074, 2021.
24. Dong Y, Wang P, Yang Y, Huang J, Dai Z, Zheng W, Li Z, Yao Z, Zhang H and Zheng J: PRMT5 inhibition attenuates cartilage degradation by reducing MAPK and NF-κB signaling. Arthritis Res Ther 22: 201, 2020.
25. Chen D, Zeng S, Huang M, Xu H, Liang L and Yang X: Role of protein arginine methyltransferase 5 in inflammation and migration of fibroblast-like synoviocytes in rheumatoid arthritis. J Cell Mol Med 21: 781-790, 2017.
26. Qiao Y, Wang Z, Li Y, Han Y, Zhou Y and Cao X: Rheumatoid arthritis risk in periodontitis patients: A systematic review and meta-analysis. Joint Bone Spine 87: 556-564, 2020.
27. Mi W, Qiao S, Zhang X, Wu D, Zhou L and Lai H: PRMT5 inhibition modulates murine dendritic cells activation by inhibiting the metabolism switch: A new therapeutic target in periodontitis. Ann Transl Med 9: 755, 2021.
28. Cai C, Gu S, Yu Y, Zhu Y, Zhang H, Yuan B, Shen L, Yang B and Feng XH: PRMT5 enables robust STAT3 activation via arginine symmetric dimethylation of SMAD7. Adv Sci (Weinh) 8: 2003047, 2021.
29. Chen Q, Su J and Chen X: Role of ubiquitin-specific protease 5 in the inflammatory response of chronic periodontitis. Oral Dis 29: 1234-1241, 2021.
30. Jiang C, Yao S, Guo Y, Ma L, Wang X, Chen Y, Zhang H and Cao Z: Angiopoietin-like protein 2 deficiency promotes periodontal inflammation and alveolar bone loss. J Periodontol 93: 1525-1539, 2021.
31. Severgnini M, Takahashi S, Rozo LM, Homer RJ, Kuhn C, Jhung JW, Perides G, Steer M, Hassoun PM, Fanburg BL, *et al*: Activation of the STAT pathway in acute lung injury. Am J Physiol Lung Cell Mol Physiol 286: L1282-L1292. 2004.
32. Qin H, Wilson CA, Lee SJ, Zhao X and Benveniste EN: LPS induces CD40 gene expression through the activation of NF-kappaB and STAT-1alpha in macrophages and microglia. Blood 106: 3114-3122, 2005.
33. Lee SB, Lee WS, Shin JS, Jang DS and Lee KT: Xanthotoxin suppresses LPS-induced expression of iNOS, COX-2, TNF-α, and IL-6 via AP-1, NF-κB, and JAK-STAT inactivation in RAW 264.7 macrophages. Int Immunopharmacol 49: 21-29, 2017.
34. Kuriakose S, Muleme H, Onyilagha C, Okeke E and Uzonna JE: Diminazene aceturate (Berenil) modulates LPS induced pro-inflammatory cytokine production by inhibiting phosphorylation of MAPKs and STAT proteins. Innate Immun 20: 760-773, 2014.
35. Lee HH, Jang E, Kang SY, Shin JS, Han HS, Kim TW, Lee DH, Lee JH, Jang DS and Lee KT: Anti-inflammatory potential of Patrineolignan B isolated from Patrinia scabra in LPS-stimulated macrophages via inhibition of NF-κB, AP-1, and JAK/STAT pathways. Int Immunopharmacol 86: 106726, 2020.