Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2023 24(5):373-386
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2200576

Overview of the main biological mechanisms linked to changes in periodontal ligament stem cells and the inflammatory microenvironment

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Abstract: Periodontitis is a complex chronic inflammatory disease. The invasion of pathogens induces the inflammatory microenvironment in periodontitis. Cell behavior changes in response to changes in the microenvironment, which in turn alters the local inflammatory microenvironment of the periodontium through factors secreted by cells. It has been confirmed that periodontal ligament stem cells (PDLSCs) are vital in the development of periodontal disease. Moreover, PDLSCs are the most effective cell type to be used for periodontium regeneration. This review focuses on changes in PDLSCs, their basic biological behavior, osteogenic differentiation, and drug effects caused by the inflammatory microenvironment, to provide a better understanding of the influence of these factors on periodontal tissue homeostasis. In addition, we discuss the underlying mechanism in detail behind the reciprocal responses of PDLSCs that affect the microenvironment.

Key words: Inflammatory microenvironment; Inflammatory regulation; Osteogenic differentiation; Periodontal ligament stem cells; Periodontitis

1 Introduction

Periodontitis, as a chronic inflammatory disease characterized by periodontal tissue inflammation and alveolar bone resorption, is the main cause of tooth loss in adults (Tonetti et al., 2018). From 1990 to 2019, the number of severe periodontitis cases accounted for 67.9% of the global population growth, which amounted to 1.1 billion such cases in the world in 2019 (Chen et al., 2021). As a common chronic infectious disease in human beings, the inflammatory state of periodontitis not only destroys the microenvironmental balance around periodontal ligament stem cells (PDLSCs), but also affects the regulation of their endogenous signals, leading to abnormality in the restoration of periodontal tissue, such as alveolar bone absorption and tooth loss (Ezhilarasan and Varghese, 2022).

Stem cells proliferate and differentiate to compensate for the destruction of injured tissue when stimulated by exogenous cytokines or damaged cells. The functions of stem cells are usually static, and they retain their progenitor properties by self-renewal to maintain homeostasis in vivo (Naji et al., 2019). Resident stem cells can also actively communicate with the tissue microenvironment, especially by regulating inflammatory components (Shi et al., 2018). PDLSCs are natural mesenchymal stem cells (MSCs) within periodontal tissue that possesses various functions, including the formation of alveolar bone, periodontal ligament, and cementum. Also, PDLSCs play an important role in maintaining the dynamic balance of normal periodontal tissue and reconstructing damaged periodontal tissue.

The microenvironment is critical to maintain cell survival, and is composed of tissue cells, intercellular

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Received Nov. 14, 2022; Revision accepted Jan. 19, 2023;
Crosschecked Mar. 14, 2023; Published online Apr. 15, 2023

substances, and body fluid components, such as nearby blood vessels, immune cells, inflammatory factors, exosomes, chemokines, complement components and metabolites, signal molecules, and the extracellular matrix (Shi et al., 2018; Yu et al., 2020). The normal functional activity of tissue cells is dependent on the relative stability of the microenvironment that inhibits the differentiation of stem cells. However, substances released by necrotic cells may induce the differentiation of stem cells to repair tissue damage (Shi et al., 2018). Immune cells and inflammatory factors in the microenvironment may also have an impact on the proliferation and differentiation of stem cells (Huang et al., 2022; Wang and Yang, 2022). As an important stem cell type in periodontal tissue, PDLSCs are closely related to and constitutively interact with the microenvironment.

Conventional periodontal therapy in clinical practice involves removing inflammation irritants, while the achievable effect is limited by surgical technique and compliance. In addition, it is difficult for traditional periodontal therapy to regenerate destroyed periodontal tissue under inflammation. With the development of research on periodontal-derived MSCs, the regeneration effects of PDLSCs on periodontal tissue under inflammatory conditions have been demonstrated (Chen et al., 2016; Guo et al., 2017; Tang et al., 2017; Chew et al., 2019; Liu JY et al., 2019). Therefore, exploring the roles of PDLSCs in reducing inflammatory responses and initiating osteogenic differentiation in periodontal inflammatory microenvironments may help to reveal new best practice guidelines for periodontal treatment. This review aims to provide a framework to improve the understanding of the vast emerging literature in recent years on the interaction between PDLSCs and the inflammatory microenvironment and its possible mechanisms; we summarize the key strategies and emerging therapeutic applications of PDLSCs in periodontal regeneration treatment.

2 Immunomodulatory effect of PDLSCs on the inflammatory microenvironment

The inflammatory microenvironment includes various immune cells, such as macrophages, neutrophils, dendritic cells (DCs), and lymphocytes, which all contribute to the inflammation response. Apart from their remarkable regenerative potential, PDLSCs possess the capacity to regulate the inflammatory microenvironment by modulating immune cells (Zhou et al., 2020).

2.1 Innate immune cells

Recent studies have established that PDLSCs regulate macrophages and neutrophils, and that their effects are essentially anti-inflammatory. Macrophages are a major component of periodontal disease development, and they consist of two subgroups: the proinflammatory phenotype (M1) and the anti-inflammatory phenotype (M2), which are closely related to periodontal status. PDLSCs promote the polarization of macrophages from M1 to M2 by enhancing the release of anti-inflammatory factors interleukin-10 (IL-10), transforming growth factor-β (TGF-β), chemokine (C-C motif) ligand 18 (CCL18), arginase-1 (Arg-1), and cluster of differentiation 163 (CD163), thus inhibiting the release of inflammatory factors IL-6 and tumor necrosis factor-α (TNF-α) (Liu JY et al., 2019; Liu JN et al., 2022). Moreover, conditioned medium derived from PDLSC (PDLSC-CM) was found to inhibit *Tnf-α* gene expression in macrophages pretreated with interferon-γ (IFN-γ) (Fairley et al., 2013). However, PDLSCs may also be pro-inflammatory under certain conditions. In force-induced inflammatory bone remodeling, the intracellular autophagy protein microtubule-associated protein light chain 3 (LC3) was upgraded and the protein kinase B (AKT) signaling pathway was inhibited in PDLSCs, improving M1 polarization (Jiang et al., 2021). Likewise, exosomes derived from PDLSCs pretreated with lipopolysaccharide (LPS) also induced M1 polarization (Kang et al., 2018; Wang YZ et al., 2022).

PDLSCs have a remarkable capacity to recognize microbial antigens, improve neutrophil viability, and enhance neutrophil chemotaxis, but not to induce the proinflammatory cytokine responses of increased neutrophils. PDLSC-CM can inhibit the production of reactive oxygen species (ROS) generated by neutrophil precursor cells and thus protect the surrounding tissues. Furthermore, PDLSCs modulate the recruitment of immune surveillance cells to the site of infection. In fact, PDLSC-CM treated with the total protein extract of *Porphyromonas gingivalis* may increase the neutrophil chemotaxic capacity. On the contrary, untreated PDLSCs have little effect on the chemotaxis

of neutrophils. PDLSCs also reduce neutrophil apoptosis through direct contact and the secretion of soluble factor IL-6 (Wang et al., 2017; Misawa et al., 2019).

2.2 Adaptive immune cells

PDLSCs have the potential to regulate T cells and B cells directly or indirectly. The development of periodontitis and the aging of $CD4^+CD28^-$ T cells could break the disruption of T helper cell 17 (Th17)/regulatory T cell (Treg), leading to the destruction of periodontal tissue and the absorption of alveolar bone (Deng et al., 2022; González-Osuna et al., 2022). Exosomes derived from PDLSCs can influence the balance of Th17/Treg through miR-155-5p targeting sirtuin-1 downstream of $CD4^+$ T (Zheng et al., 2019). Furthermore, the supernatant of human PDLSCs under heavy mechanical force can upregulate the number of Th17 cells (Lin et al., 2022). The results above demonstrate that PDLSCs can regulate the number and function of T cells through paracrine secretion.

PDLSCs could also regulate T cells indirectly through DCs. Mature DCs are the most effective antigen presenting cells to stimulate T cell activation. PDLSCs indirectly suppress T cell activation by restraining DC maturation (Ashour et al., 2020). $STRO\text{-}1^+CD146^+$ PDLSCs reduce the proliferation of T cells by decreasing the expression of non-classical major histocompatibility complex (MHC)-like glycoprotein CD1b on myeloid DCs (mDCs) (Shin et al., 2017). Moreover, osteogenic PDLSCs inhibit the proliferation of T cells by secreting prostaglandin E2 (PGE2) in vitro (Tang et al., 2014). It also has been reported that curcumin can improve PDLSCs-mediated T cell immunosuppression by activating the PGE2-indoleamine 2,3-dioxygenase (IDO) cascade (Arora et al., 2022).

PDLSCs also regulate B cells; they inhibit the proliferation of B cells through the interaction of programmed cell death protein-1 (PD-1) and programmed cell death ligand-1 (PD-L1) via direct contact between cells. In addition, they suppress the chemotaxis ability of B cells through the C-X-C motif chemokine receptor 4 (CXCR4), CXCR5, and C-C chemokine receptor 7 (CCR7), and decrease their apoptosis by secreting IL-6 (Liu OS et al., 2013). Furthermore, PDLSCs could inhibit the production of immunoglobulin M (IgM), IgG, and IgA by B cells (Liu OS et al., 2013).

3 Effects of inflammatory microenvironment on the biological characteristics and osteogenic differentiation of PDLSCs

Although inflammation is considered the main reason for tissue damage, it is still an essential factor in the early healing process. During periodontal tissue regeneration/repair activities, PDLSCs interact with the surrounding inflammatory microenvironment, and this interaction is bidirectional. On the one hand, PDLSCs have immune regulation ability regarding their local inflammatory surroundings. On the other hand, the stemness, proliferation, migration/homing, differentiation, and immunomodulatory properties of PDLSCs are subject to the regulation of the nature, intensity, and duration of the inflammatory challenge (El-Sayed et al., 2019). In the above process, the roles of Toll-like receptor (TLR) expression profiles on PDLSCs (Li and Wu, 2021), cytokines (El-Sayed et al., 2021), and even the signaling pathways (Wang DX et al., 2022) are significant.

3.1 Effects of inflammatory microenvironment on the proliferation and apoptosis of PDLSCs

Besides the influence of PDLSCs on immune cells, the components within the inflammatory microenvironment interact with PDLSCs during the onset and development of periodontitis (El-Sayed et al., 2019). As most of these healing stages occur under inflammatory periodontal micro-environmental conditions, studying the impact of inflammatory cytokines on periodontal disease and the biological characteristics of PDLSCs is crucial to develop clinical regenerative approaches. Amongst the most potent pro-inflammatory cytokines that appear during periodontal inflammation are IL-1β, IL-6, TNF-α, and IFN-γ (Stadler et al., 2016; Pan et al., 2019).

The proliferative ability of PDLSCs depends on the degree of inflammation (Huang et al., 2017; Tomasello et al., 2017; Zhang et al., 2017; Zhou et al., 2017; Kong et al., 2018; Li et al., 2018; Dong and Shu, 2022). On the one hand, when exposed to inflammatory factors, such as IL-1 (1 μg/L), TNF-α (5–10 μg/L), and IFN-γ (100 μg/L), or low concentration of LPS derived from *P. gingivalis*, the proliferative ability of PDLSCs is significantly enhanced (Zhu et al., 2013; Tomasello et al., 2017; Zhang et al., 2017; Zhou et al., 2017; Meng CL et al., 2018; Dong and

Shu, 2022). The activation of classical Wnt signaling pathway leads to the formation and regulation of dozens of lymphoid enhancer-binding factor 1 (LEF1)-β-catenin complexes, as well as the target gene *cyclin D1*, altering the cell growth cycle and fostering the proliferation of PDLSCs (Liu et al., 2021). Likewise, increased histone deacetylase (HDAC) in PDLSCs breaks the original balance of acetylation, further affecting the transcription of inflammatory factors (Li et al., 2018), and the upregulation of C-X-C motif chemokine ligand 8 (CXCL8) and CCL5 is also an important reason for the rapid proliferation of PDLSCs (Yang et al., 2013). On the other hand, PDLSCs have a lower proliferation capacity under 10 μg/mL LPS derived from *P. gingivalis* or high concentration of TNF-α (20 μg/L) (Zhu et al., 2013; Huang et al., 2017; Wang et al., 2019; Su et al., 2020; Dong and Shu, 2022). High glucose aggravates the TNF-α-induced inhibition of PDLSC proliferation (Zhu et al., 2020). In summary, the proliferation of PDLSCs in an inflammatory microenvironment is related to the degree of periodontal inflammation. In mild inflammatory microenvironments, PDLSCs proliferate prominently; however, this phenomenon is inhibited by high levels of inflammatory factors.

The apoptosis of PDLSCs is affected by the inflammatory microenvironment primarily through two signaling pathways. First, the activated non-apoptotic signal of TNF receptor superfamily member 6 (Fas) can trigger the caspase-3 and caspase-8 pro-apoptosis-related pathways, stimulated by high levels of TNF-α and IFN-γ, reducing the nuclear factor-κB (NF-κB) in PDLSCs, activating apoptosis signals, and leading to the apoptosis of PDLSCs (Liu et al., 2011). Previous studies have demonstrated that necroptosis leads to the apoptosis of PDLSCs in the inflammatory microenvironment, and the receptor-interacting protein kinase-3 (RIP3)/caspase-8 signaling pathway is involved in necroptosis and regulates the immune response of PDLSCs (Yan et al., 2018a, 2018b). In fact, the inhibition of RIP3/caspase-8 promotes the regeneration of inflammatory periodontal tissue. Second, the level of autophagy is significant in the periodontal ligament tissue of periodontitis patients and is another way to avoid apoptosis. The inflammatory microenvironment can stimulate numerous autophagy-related markers in PDLSCs, such as LC3, Beclin-1, autophagy-related protein 7 (Atg7), and Atg12. In TNF-α-treated PDLSCs, LC3, Beclin-1, and B-cell lymphoma-2 (Bcl-2) are increased, whereas P62 and caspase-8 are reduced, meaning that autophagy has been activated. On the one hand, TNF-α enhances autophagy and suppresses apoptosis rapidly (An et al., 2016; Wang P et al., 2020). Meanwhile, the long-term effect of inflammation reduces the LC3 and Bcl-2 proteins, thus increasing the level of ubiquitinated protein P62. These data indicate that the inflammatory environment that causes autophagy gradually fades away and then stimulates apoptosis (Wang P et al., 2020). As mentioned above, necroptosis and autophagy are two ways that can affect PDLSC apoptosis by the inflammatory microenvironment, as well as different inflammatory factors.

3.2 Effects of inflammatory microenvironment on the osteogenic differentiation of PDLSCs through various signaling pathways

Many signaling pathways play important roles in controlling the osteogenic differentiation of PDLSCs within the inflammatory microenvironment. Reports have revealed the function of different signaling pathways in the process of osteogenic differentiation of PDLSCs (Fig. 1).

3.2.1 Wnt/β-catenin signaling pathway

The inflammatory microenvironment inhibits both the Wnt/β-catenin signaling pathway and the osteogenic differentiation of PDLSCs. Interestingly, the effect of Wnt/β-catenin signaling pathway is not only favorable for the osteogenic differentiation of PDLSCs, but also negative from another aspect. In the classical β-catenin-activated Wnt pathway, glycogen synthase kinase-3β (GSK-3β) is inactivated by phosphorylation to prevent the degradation of β-catenin, which subsequently builds up in the cytoplasm, enters the nucleus, binds with T cell factor/lymph enhancer-binding factor (TCF/LEF), and stimulates downstream target genes (Ling et al., 2009). Wnt3a, a Wnt pathway agonist, can activate the classical Wnt/β-catenin pathway and promote the osteogenic differentiation of PDLSCs (Wu et al., 2019). Furthermore, under the stimulation of *P. gingivalis*, the application of Wnt3a could reverse the inhibition of osteogenic differentiation of PDLSCs (Shen et al., 2021; Zhang XS et al., 2021). LPS from *Escherichia coli* promotes the osteogenic differentiation of PDLSCs through Wnt/β-catenin-induced transcriptional co-activator with PDZ-binding motif (TAZ)

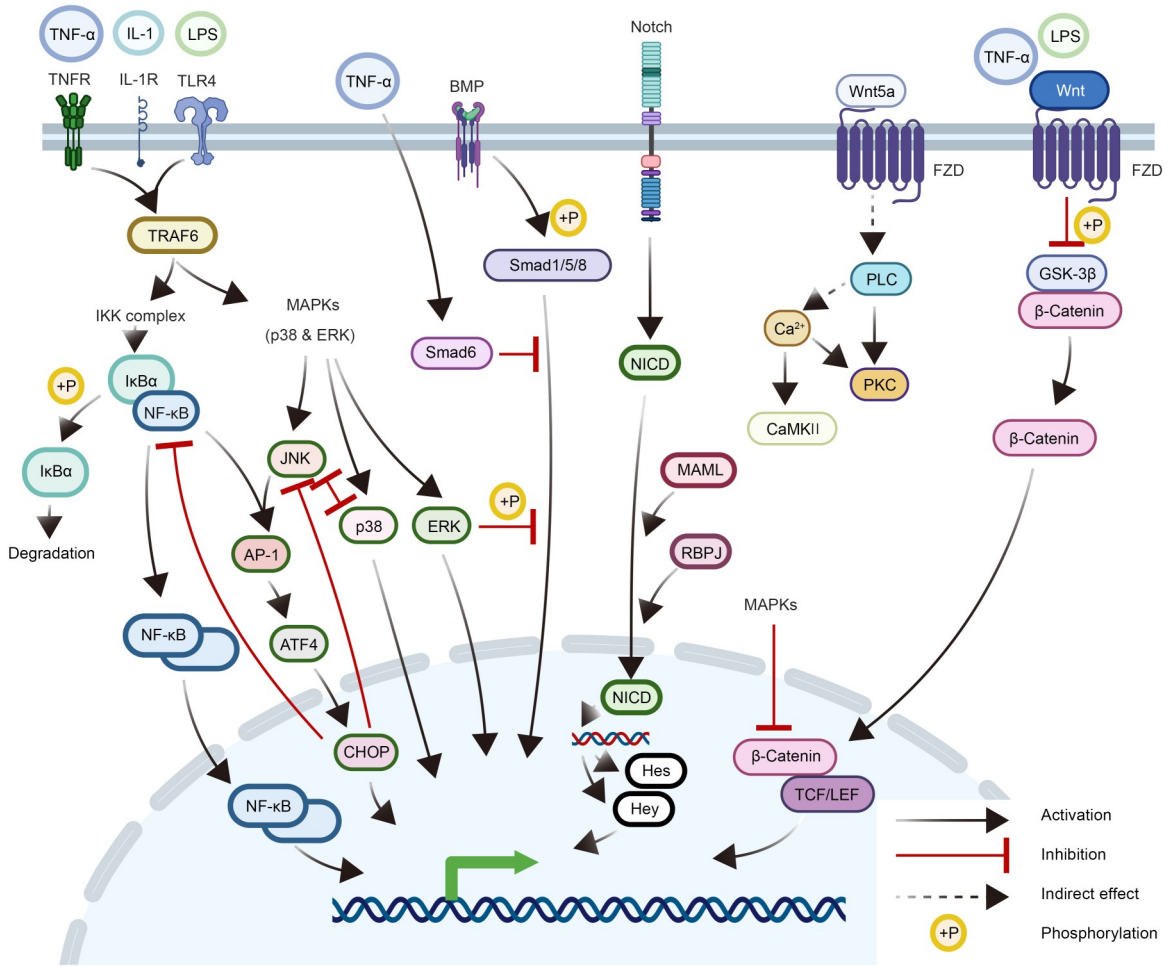

Fig. 1 Osteogenic signaling pathway of PDLSCs within the inflammatory microenvironment. PDLSCs: periodontal ligament stem cells; TNF-α: tumor necrosis factor-α; TNFR: tumor necrosis factor receptor; IL-1: interleukin-1; IL-1R: IL-1 receptor; LPS: lipopolysaccharide; TLR4: Toll-like receptor 4; TRAF6: TNFR-associated factor 6; NF-κB: nuclear factor-κB; IKK: inhibitor of NF-κB kinase; IκBα: inhibitor of NF-κB α; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; AP-1: activator protein-1; ATF4: activating transcription factor 4; CHOP: CCAAT/enhancer-binding protein homologous protein; BMP: bone morphogenetic protein; Smad: mothers against decapentaplegic homolog; NICD: Notch intracellular domain; MAML: mastermind-like protein; RBPJ: recombination signal sequence-binding protein J; FZD: frizzled; PLC: phospholipase C; PKC: protein kinase C; CaMKII: calcium/calmodulin-dependent protein kinase II; GSK-3β: glycogen synthase kinase-3β; TCF/LEF: T cell factor/lymph enhancer-binding factor; Hes: hairy and enhancer of split; Hey: Hes-related with YRPW motif.

elevation (Xing et al., 2019). On the contrary, it was shown that the classical Wnt signaling pathway also exerts a negative regulatory effect on the osteogenic differentiation of PDLSCs in the inflammatory microenvironment (Kong et al., 2015). The Wnt/β-catenin pathway commonly improves the osteogenic differentiation of PDLSCs; however, it has the opposite effect during the osteogenic induction of PDLSCs (Liu W et al., 2013). TNF-α, LiCl, and Wnt3a inhibit the activity of GSK-3β, thereby activating the Wnt/β-catenin signaling pathway and blocking the osteogenic differentiation of PDLSCs, which is manifested by the reduction in osteogenic factors (Kong et al., 2015). Some studies have revealed that GSK-3β and β-catenin of PDLSCs isolated from inflamed periodontal tissues are higher than those from healthy periodontal tissues. Either knockdown of β-catenin or overexpression of GSK-3β can restore the osteogenic differentiation ability of PDLSCs (Liu et al., 2014, 2016). Moreover, mitofusin (Mfn) takes part in the Wnt/β-catenin pathway. Provided that the Wnt/β-catenin pathway is triggered, *Mfn1* and *Mfn2*, endoplasmic reticulum (ER)-mitochondrial coupling, and mitochondrial fusion are increased in PDLSCs. Downregulation of *Mfn1* and

Mfn2 conversely changes the decline in osteogenic differentiation caused by the activation of Wnt pathway (Zhai et al., 2018). Some scholars believe that these two perspectives are not contradictory. In other words, the effect of activated Wnt/β-catenin signaling pathway on the osteogenic differentiation of PDLSCs during inflammatory conditions is determined by the differentiation status of PDLSCs. At the beginning of the differentiation of PDLSCs into osteoblasts, the Wnt/β-catenin signaling pathway shows a promotive effect. However, towards the end of differentiation, the Wnt/β-catenin signaling pathway inhibits the differentiation and maturation of osteoblasts (Eijken et al., 2008).

3.2.2 Wnt/Ca^{2+} signaling pathway

The non-canonical Wnt/Ca^{2+} and the classical Wnt signaling pathways not only co-regulate but also compete with each other during the osteogenic differentiation of PDLSCs. However, in the inflammatory microenvironment, the classical Wnt signaling pathway is more active. The non-canonical Wnt/Ca^{2+} pathway is activated by Wnt5a and Wnt11. Instead of binding to β-catenin, the ligand binds to frizzled (FZD), thus activating phospholipase C (PLC) and protein kinase C (PKC) through G protein activation. Next, it increases intracellular Ca^{2+} concentration and activates Ca^{2+} sensitivity signal components (Shi et al., 2014). Moreover, the non-canonical Wnt5a/Ca^{2+} pathway antagonizes the classical Wnt/β-catenin signal by initiating the TGF-β-activated kinase 1-neuroleukin (TAK1-NLK) mitogen-activated protein kinase (MAPK) cascade (Ishitani et al., 2003). This mechanism might be related to the Wnt/Ca^{2+} signaling pathway, involving the activation of calcium/calmodulin-dependent protein kinase II (CaMKII) and NLK to phosphorylate TCF/LEF, blocking β-catenin, and triggering the transcription of Wnt target genes (Baksh et al., 2007). On the one hand, certain studies demonstrated that Wnt5a increases in the inflammatory microenvironment; specifically, the growth of Wnt5a continues until the end of osteogenic induction (Nanbara et al., 2012; Zhang et al., 2019). On the other hand, the inflammatory microenvironment controls the non-canonical pathway and triggers the Wnt classic pathway, inhibiting the late stage of PDLSC osteogenic differentiation (Liu et al., 2015). Additionally, the Wnt/Ca^{2+} non-canonical signaling pathway also plays an essential role in the osteogenic differentiation of PDLSCs. Knockdown of β-catenin via small interfering RNA (siRNA) can enhance the non-canonical pathway. The activity of Wnt/Ca non-canonical signaling pathway promotes the osteogenic differentiation of PDLSCs (Liu et al., 2016). Therefore, we infer that the Wnt/Ca^{2+} signaling pathway is advantageous to the osteogenic differentiation of PDLSCs under inflammatory conditions.

3.2.3 NF-κB signaling pathway

Under an inflammatory microenvironment, the NF-κB signaling pathway has a negative regulatory effect on the osteogenic differentiation of PDLSCs. NF-κB is a significant transcription factor of immune response. In the event of bacterial infection or inflammatory stimulation, inflammatory factors such as TNF-α, IL-1β, and IL-6 mediate the complex of the inhibitor of NF-κB kinase (IKK), resulting in the excessive phosphorylation and degradation of inhibitor of NF-κB α (IκBα). Then, the downstream p65:p50 freed from the NF-κB complex is translocated from the cytoplasm to the nucleus, upregulating inflammation-related genes and inhibiting osteogenic differentiation (Tak and Firestein, 2001). During the osteogenic differentiation of PDLSCs derived from inflammatory tissues, phosphorylated IκBα and p65 in the nucleus are largely upregulated (Chen et al., 2022). Furthermore, LPS stimulates TLR4 and the phosphorylation of NF-κB p65 on the membrane of PDLSCs, which weakens the osteogenic ability of PDLSCs (Guo et al., 2017; Duan et al., 2019; Yu et al., 2019; Wang W et al., 2020). Salvianolic acid C (SAC) effectively repairs the LPS-induced PDLSC osteogenic damage through the TLR4/NF-κB pathway (Duan et al., 2019). Furthermore, TNF-α stimulation significantly triggers the NF-κB signaling pathway of PDLSCs, and the osteogenesis-related genes and proteins are downregulated. As long as the NF-κB pathway is inhibited, the osteogenic differentiation is restored to some extent in PDLSCs (Chen et al., 2022). Knockdown of enhancer of zeste homolog 2 (*Ezh2*) or kruppel-like factor 5 (*Klf5*) considerably increases the expression of alkaline phosphatase (ALP), Runt-related transcription factor 2 (RUNX2), and osteocalcin (OCN) by inhibiting the NF-κB pathway (Wang et al., 2021; Li et al., 2022). Therefore, the NF-κB signaling pathway restricts the osteogenic differentiation of PDLSCs; however, this inhibitory effect is promoted during inflammatory conditions.

3.2.4 Notch signaling pathway

The inflammatory microenvironment restricts the Notch signaling pathway in PDLSCs. Previous research has reported that the low-level activation of Notch signaling may be beneficial for osteogenic differentiation in periodontal alveolar defects (Ma et al., 2018). In the nucleus, Notch intracellular domain (NICD) interacts with recombination signal sequence-binding protein J (RBPJ) and mastermind-like protein (MAML) to convert transcriptional repressors into activators, increasing downstream genes in the *Hes* and *Hey* families (Ongaro et al., 2016). Overexpression of NICD elevates the proliferation ability of PDLSC while suppressing its osteogenic differentiation ability (Qiu et al., 2019). Compared with PDLSCs derived from healthy tissue, the messenger RNA (mRNA) expression of *Notch1* and *Jagged1* in inflammatory PDLSCs is lower, indicating the inhibition of Notch signaling-related molecules (Ma et al., 2018). Therefore, during inflammatory conditions, the Notch signaling pathway in PDLSCs is inhibited. Vitamin C (Vc) blocks the aging of PDLSCs by reducing the expression of Notch3, suggesting an avenue to reverse the damage of Notch signaling under inflammatory conditions (Yang et al., 2021).

3.2.5 BMP-Smad signaling pathway

Under inflammatory microenvironments, the effects of the bone morphogenetic protein (BMP)-mothers against decapentaplegic homolog (Smad) signaling pathway in PDLSCs on osteogenesis depend on the severity of inflammation and the synergistic regulation of osteogenesis with other signaling pathways that regulate osteogenesis. BMPs belong to the TGF-β superfamily. BMP-2, -7, -6, and -9 promote bone formation (Peng et al., 2003), while BMP-3 has a negative effect on osteogenesis (Daluiski et al., 2001). The BMP signaling pathway regulates the transcriptional expression of target genes through the Smad. Low-dose IL-1β stimulates the BMP/Smad signaling pathway to enhance PDLSC osteogenesis; however, high-dose IL-1β decreases Smad1/5 phosphorylation by triggering NF-κB and MAPK signals, restricting the differentiation of PDLSCs into osteoblasts. The crosstalk among NF-κB, MAPK, and BMP/Smad signals mediates the osteogenesis of PDLSCs within the inflammatory microenvironment (Mao et al., 2016; Lin et al., 2023). Therefore, elevated inflammation significantly suppresses the BMP/Smad signaling pathway.

3.2.6 MAPK signaling pathway

Under inflammatory microenvironments, the activated MAPK signaling pathway is more likely to inhibit the osteogenic differentiation of PDLSCs. Traditional MAPKs include the following subfamily members: extracellular signal-related kinase 1/2 (ERK1/2), ERK5, c-Jun N-terminal kinase 1/2/3 (JNK1/2/3), and p38. The MAPK signaling pathway is a vital transmitter delivering external stimuli to the nucleus (Arthur and Ley, 2013). First, activated ERK1/2 triggers the expression of RUNX2 protein, promoting osteoblast differentiation by regulating the expression of downstream target genes (Luo et al., 2017). However, in PDLSCs exposed to LPS, ERK1/2 may not directly participate in inhibiting osteogenesis but is more involved in stimulating cartilage production and adipogenesis. In addition, ERK1/2 activated by LPS can increase cyclooxygenase 2 (COX2) and IL-6 in PDLSCs (Kukolj et al., 2018). ERK5 is associated with adenovirus vector-mediated-BMP9 (Ad-BMP9), which is significantly expressed by PDLSCs transfected by adenovirus. Inhibition of ERK5 signaling causes the blockage of osteogenic differentiation of PDLSCs stimulated by Ad-BMP9 (Lei et al., 2021). In addition, p38 in PDLSCs within periodontal inflammatory tissues is higher than that in human PDLSCs extracted from healthy tissue (Nie et al., 2015). Nonetheless, the expression of phosphorylated p38 and phosphorylated JNK increases after osteoinduction, and the osteogenic differentiation ability is weakened. As long as the kinase activity of p38 is inhibited, the osteogenic differentiation ability of PDLSCs can be significantly restored (Mao et al., 2016). Moreover, IL-1β may play a bilateral regulatory role in the osteogenic differentiation of PDLSCs, acting as a p38 inhibitor and as a facilitator by activating ERK (Nie et al., 2015). However, both the p38 and ERK1/2 pathways are activated under high-glucose conditions. Metformin, a classical hypoglycemic drug, can inhibit the MAPK pathway and enhance the osteogenic differentiation of PDLSCs under high-glucose conditions (Zhang et al., 2022). In conclusion, the osteogenic differentiation of the MAPK signaling pathway in PDLSCs is significantly reduced in an inflammatory microenvironment.

3.2.7 PERK signaling pathway

The inflammatory microenvironment inhibits the osteogenic differentiation of PDLSCs by activating the protein kinase-like ER kinase (PERK) signaling pathway. PERK is a type of I transmembrane protein located on the ER membrane, which improves cellular apoptosis by inducing CCAAT/enhancer-binding protein homologous protein (CHOP). Furthermore, the PERK signaling pathway impairs the osteogenic differentiation ability of PDLSCs (Tan et al., 2016). In the gingival tissues of patients with periodontitis, recombinant activating transcription factor 4 (*Atf4*) and CHOP, as downstream factors of the PERK signaling pathway, both show high levels of expression (Zhang Y et al., 2021). The PERK signaling pathway-related factors PERK, CHOP, glucose-regulated protein 78 (GRP78), and *Atf4* in inflammatory tissues are remarkably elevated. In contrast, activated CHOP and PERK downstream factors can negatively regulate inflammation primarily through the activator protein-1 (AP-1) pathway, affecting JNK and NF-κB upstream (Guo et al., 2020). AP-1 transcribes a variety of inflammatory factors, such as IL-8, TNF, granulocyte-macrophage colony-stimulating factor, and inflammation-related cytokine receptors. Silenced PERK reverses the inhibitory effect on the osteogenic differentiation of PDLSCs caused by the inflammatory factors (Song et al., 2019). Consequently, the PERK signaling pathway is active in PDLSCs under inflammatory conditions.

4 Effects of drugs on the osteogenic differentiation of PDLSCs under inflammatory microenvironment

A wide range of drugs can modify the osteogenic differentiation of PDLSCs that have been damaged under an inflammatory microenvironment. This phenomenon is further confirmed by the fact that the inflammatory microenvironment is involved in the initiation and regulation of osteogenesis of PDLSCs. The effects of drugs on the osteogenic differentiation of PDLSCs in an inflammatory microenvironment are summarized in Table 1. Various drugs regulate osteogenic differentiation through different pathways. Tanshinone IIA (TSA) (Liu X et al., 2019), asaraldehyde (Hwang et al., 2021), and naringenin (NAR) (Wei et al., 2017) promote osteogenic differentiation through ERK-associated signaling pathways, while azithromycin (AZM) (Meng TT et al., 2018), asiaticoside (AC) (Fitri et al., 2018), exendin-4 (EX-4) (Liu HH et al., 2019; Liang et al., 2021), and quercetin (Zhang WJ et al., 2021) enhance osteogenic differentiation through Wnt- or NF-κB-associated signaling pathways. Moreover, in the field of osteogenic differentiation regulation, curcumin (acting through phosphoinositide 3-kinase (PI3K)/AKT/nuclear factor E2-related factor 2 (Nrf2) signaling pathway) (Xiong et al., 2020), rutin (acting through mammalian target of rapamycin (mTOR) signaling pathway) (Zhao et al., 2020a, 2020b), fraxinellone (acting through BMP2/Smad pathway) (Fu et al., 2021), and melatonin (acting through regulating the mitochondrial functions) (Zheng et al., 2020) also play important roles.

5 Summary

Researchers have been extremely interested in the biological characteristics and osteogenic differentiation potential of PDLSCs, which are ideal stem cells for periodontal tissue regeneration. The influence of the inflammatory microenvironment on PDLSCs formed by periodontitis is bidirectional, which is vital for periodontal tissue repair and regeneration. Mild inflammation can promote the proliferation and osteogenic differentiation of PDLSCs. With an increased level of inflammation, the osteogenic differentiation ability of PDLSCs is reduced. In fact, the influence between the inflammatory microenvironment and PDLSCs is also mutual. PDLSCs regulate the inflammatory microenvironment via various immune cells, and primarily show anti-inflammatory effects. In conclusion, the interaction between the inflammatory microenvironment and PDLSCs is complex and shows a delicate balance. Further elucidating the relevant mechanisms of action will help to improve the therapeutic effect of clinical drugs for periodontitis.

Acknowledgments

The work was supported by the Jilin Provincial Department of Finance (No. jcsz2020304-9) and the Guangzhou Medical University Student Innovation Ability Improvement Program (No. (2022)66-113), China. The authors thank AiMi Academic Services (https://www.aimieditor.com) for English language editing and review services.

Table 1 Summary of the effects of different drugs on the osteogenic differentiation of PDLSCs under inflammatory microenvironment

Drug	Abbreviation	Origin of drug	Pretreatment	Result	Target	Reference
Curcumin		Turmeric rhizome		Promotes the osteogenesis of hPDLSCs	PI3K/AKT/Nrf2 signaling pathway	Xiong et al., 2020
					EGR1	Shi et al., 2021
Tanshinone IIA	TSA	Roots of the herbal medicine *Salvia miltiorrhiza* Bunge		Induces the osteogenic differentiation of hPDLSCs	ERK1/2 signaling pathway	Liu X et al., 2019
Asaraldehyde		Plants such as *Acorus calamus* and *Fructus carotae*		Promotes the osteogenic differentiation of hPDLSCs	p38/ERK signaling pathway	Hwang et al., 2021
Naringenin	NAR	Orange, tangerine, grape fruit, raw lemon peels, and other citrus fruits		Promotes the osteogenesis of hPDLSCs	ERK1/2 signaling pathway	Wei et al., 2017
				Promotes the proliferation rate, osteogenic and endothelial differentiation of hPDLSCs	SDF-1/CXCR4 signaling pathway	Zhang L et al., 2021
Azithromycin	AZM	Clinically available macrolide antibiotic	TNF-α (100 ng/mL)	Promotes the osteogenic differentiation of hPDLSCs in an inflammatory microenvironment	Wnt and NF-κB signaling pathways	Meng TT et al., 2018
Asiaticoside	AC	Herb *Centella asiatica*		Induces the osteogenic differentiation of hPDLSCs	Wnt/β-catenin signaling pathway	Fitri et al., 2018
Exendin-4	EX-4	GLP-1 agonist that has similar properties to GLP-1	LPS (10 μg/mL)	Promotes the osteogenic differentiation of hPDLSCs in the inflammatory microenvironment	Wnt and NF-κB signaling pathways	Liu HH et al., 2019; Liang et al., 2021
Quercetin	Quer	Vegetables and fruits, such as apples, onions, and blueberries	TNF-α (20 ng/mL)	Reverses the inhibitory effects of TNF-α on the osteogenic differentiation of hPDLSCs	NF-κB/NLRP3 inflammasome pathway	Zhang WJ et al., 2021
Curcumin		Turmeric rhizome		Promotes the osteogenesis of hPDLSCs	PI3K/AKT/Nrf2 signaling pathway	Xiong et al., 2020
					EGR1	Shi et al., 2021
Rutin		Roots, stems, leaves, flowers, fruits, and seeds of plants	TNF-α (20 ng/mL)	Protects hPDLSCs from TNF-α-induced damage to osteogenic differentiation in an inflammatory environment	mTOR signaling pathway	Zhao et al., 2020a, 2020b
Fraxinellone		Root bark of *Dictamnus dasycarpus*	LPS (0.1, 1, 10 μg/mL)	Alleviates inflammation and promotes the osteogenic differentiation of hPDLSCs	BMP2/Smad pathway	Fu et al., 2021
Melatonin	MT	Pineal gland		Physiological concentrations inhibit osteogenic differentiation; lowest pharmacological concentrations promote the osteogenic differentiation of hPDLSCs	Associated mitochondrial functions	Zheng et al., 2020

PDLSCs: periodontal ligament stem cells; hPDLSCs: human PDLSCs; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; Nrf2: nuclear factor E2-related factor 2; EGR1: early growth response factor 1; ERK1/2: extracellular signal-related kinase 1/2; SDF-1: stromal cell-derived factor-1; CXCR4: C-X-C motif chemokine receptor 4; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor-κB; LPS: lipopolysaccharide; GLP-1: glucagon-like peptide-1; NLRP3: NOD-like receptor family, pyrin domain-containing protein 3; mTOR: mammalian target of rapamycin; BMP2: bone morphogenetic protein 2; Smad: mothers against decapentaplegic homolog.

Author contributions

Xuetao ZHAO: conceptualization, formal analysis, data curation, writing original draft, and visualization. Hongbing LIN: supervision. Tong DING: conceptualization. Yawei WANG: formal analysis. Na LIU: writing – review & editing. Yuqin SHEN: funding acquisition and writing – review & editing. All authors have read and approved the final version.

Compliance with ethics guidelines

Xuetao ZHAO, Hongbing LIN, Tong DING, Yawei WANG, Na LIU, and Yuqin SHEN declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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