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Effects of sclerostin on lipopolysaccharide-induced inflammatory phenotype in human odontoblasts and dental pulp cells

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ABSTRACT

Previously we have demonstrated that sclerostin inhibits stress-induced odontogenic differentiation of odontoblasts and accelerates senescence of dental pulp cells (DPCs) Odontoblasts and DPCs are main functioning cells for inflammation resistance and tissue regeneration in dentine-pulp complex. Sclerostin is relevant for systemic inflammation and chronic periodontitis processes, but its effects on dental pulp inflammation remains unclear. In this study, we found that sclerostin expression of odontoblasts was elevated in lipopolysaccharide-induced inflammatory environment, and exogenous sclerostin increased the production of pro-inflammatory cytokines in inflamed odontoblasts. Furthermore, sclerostin activated the NF-κB signaling pathway in inflamed odontoblasts and the NF-κB inhibitor reversed the exaggerative effects of sclerostin on the pro-inflammatory cytokines production. Additionally, sclerostin promoted adhesion and migration of inflamed DPCs, while inhibiting odontoblastic differentiation of inflamed DPCs. Sclerostin also might enhance pulpal angiogenesis. Taken together, it can therefore be inferred that sclerostin is upregulated in inflamed odontoblasts under pulpal inflammatory condition to enhance inflammatory responses in dentine-pulp complex and impair reparative dentinogenesis. This indicates that sclerostin inhibition might be a therapeutic target for anti-inflammation and pro-regeneration during dental pulp inflammation.

1. Introduction

Dental pulp inflammation is often a sequel to dental caries or trauma which contributes to the penetration of pathogens and their released components through dentinal tubules towards the pulp (He et al., 2013). Odontoblastsare neural crest-derived mesenchymal cells that are responsible for synthetizing and secreting dentin matrix (Linde and Lundgren, 1995). Due to their specific location at the dentin-pulp interface and cytoplasmic processes into tubules, odontoblasts are the first cells exposed to bacterial products and are proposed as the initiation of pulpal inflammatory immune responses for host defenses (Durand et al., 2006; Farges et al., 2011; Veerayutthwilai et al., 2007). Inflammation and regeneration often interact in dentine–pulp complex. Odontoblasts can produce numerous cytokines and chemokines after mild inflammatory stimuli, followed by a series of inflammatory immune responses and reactionary dentin formation (Veerayutthwilai et al., 2007; Horst et al., 2011). When suffering from intense inflammation, odontoblasts become damaged or destroyed, which stimulates the migration of dental pulp cells (DPCs) to the pulp injury site (Tecles et al., 2005). The migrated DPCs would then differentiate into odontoblasts to generate a reparative dentin barrier (Sloan and Smith, 2007). This replacement is the goal of vital pulp therapy, which is the preferred treatment method for localized and reversible pulpitis now. However, the underlying mechanisms on the inflammatory responses of odontoblasts and the following replacement of DPCs upon dental pulp inflammation have not been well described.

Sclerostin, the protein product of SOST gene, is mainly secreted by osteocytes and acts as an osteogenesis inhibitor (Moester et al., 2010). Currently, it has been proved that pro-inflammatory factors could increase the expression of sclerostin in vitro through multiple intracellular signaling pathways (Baek et al., 2014; Vincent et al., 2009; Sakamoto et al., 2019). Researches on systemic inflammation showed that sclerostin inhibition could prevent or reverse bone loss in arthritis and colitis (Chen et al., 2013; Marenzana et al., 2013; Eddleston et al.,

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2009). In oral cavities, sclerostin expression was elevated in gingival samples from patients with chronic periodontitis (Balli et al., 2015; Sankardas et al., 2019), and sclerostin antibody treatment enhanced bone regeneration to repair alveolar bone loss in experimental periodontitis (Ren et al., 2015; Taut et al., 2013; Chen et al., 2015). These investigations revealed that sclerostin was quite relevant for inflammation processes and therapeutic management.

Our previous studies investigated that sclerostin could be expressed in dentine-pulp complex, serving as a mediator between odontoblasts and external environment to sense and transduce mechanical stimulation, and more importantly, accelerating senescence of DPCs as reflected by decreased proliferation and odontoblastic differentiation (Ou et al., 2018; Liao et al., 2019). Many investigations pointed out a close relationship between inflammation and senescence (Mei, 2009). Dental pulp inflammation could cause premature cellular senescence through oxidative stress and DNA damage responses (Feng et al., 2014; Dierick et al., 2002). On the other hand, the aging process often leads to chronic inflammation and senescent DPCs upregulated the expression of chronic inflammatory molecules (Mei, 2009; Cesari et al., 2004). In addition, a recent study showed that sclerostin deficiency could accelerate the formation of reparative dentin in a pulp injury model (Collignon et al., 2017). These accumulating evidences inspired us to hypothesize that sclerostin might play a critical role in dental pulp inflammation and regeneration.

In this study, lipopolysaccharide (LPS), a major gram-negative bacterial component that is strongly implicated in endodontic infections and pulpitis (Vianna et al., 2007), was applied to induce immunoresponses of odontoblast and DPCs in vitro. Firstly, we detected the alternation of sclerostin in LPS-treated odontoblasts and investigated the functions of sclerostin on inflammatory cytokines production in odontoblasts during LPS-induced inflammation. We then explored the effects of secretory sclerostin on adhesion, migration, differentiation and angiogenesis of LPS-treated DPCs. The results may help illustrate the underlying mechanisms of the interactions between inflammation and regeneration in dentine-pulp complex.

2. Materials and methods

2.1. Culture and treatment of cells

Healthy extracted third molars from 18 to 24-year-old patients were collected with informed consent according to the protocols approved by the Ethical Committee of the School and Hospital of Stomatology, Wuhan University, China (2015-C12). The pulp tissue was separated and the apical ends were removed to prevent contamination. The samples were then divided into two parts to generate human odontoblast-like cells (hOBs) and human dental pulp cells (hDPCs), respectively. Pulp explants were grown in α-MEM medium (HyClone, Laboratories, Inc., UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, NY, USA), 1% penicillin-streptomycin solution (HyClone), and odontoblastic inducing components including 10 mM β-glycerophosphate, 50 μg/mL ascorbic acid and 10 nM dexamethasone (Sigma-Aldrich Co., St. Louis, MO, USA). After 4 weeks, the resulting cells were used as hOBs in this study (Couble et al., 2000). Respectively, minced pulp tissue was digested in a solution containing 4 mg/ml dispase and 3 mg/ml collagenase type I (Roche, Mannheim, Germany) at 37 °C for 1 h. Cell suspensions were seeded in α-MEM medium supplemented with 10% FBS and 1% penicillin-streptomycin solution (Coppe et al., 2009). When reaching 90% confluence, hDPCs were harvested and passage 3–5 of hDPCs was used in this study. In the particular experiments, cells were treated with the following reagents: ultrapure *E. coli* LPS (Sigma), recombinant human sclerostin (rhSCL; Novoprotein, Shanghai, China), and the NF-κB inhibitor pyrollidine dithiocarbamate (PDTC; Selleck Chemicals, Shanghai, China).

2.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of cells was isolated using the TRIzol kit (Takara, Kusatsu, Japan). Then 1 μg of each RNA sample was transcribed into cDNA using PrimeScript TM RT reagent Kit with gDNA Eraser (Takara). qRT-PCR for SOST, interleukin (IL)-6, IL-8, IL-1β, intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, monocyte chemotactic protein (MCP)-1, chemokine receptor (CXCR)-4, dentin sialophosphoprotein (DSPP), bone sialoprotein (BSP), osteopontin (OPN), osteocalcin (OCN), vascular endothelial growth factor (VEGF)-A, VEGF receptor (VEGFR)-1 and placental growth factor (PlGF) was performed using an ABI 7900 thermocycle (Applied Biosystems, Foster City, CA, USA) with SYBR mix (Takara). GAPDH was used as the internal control and the ΔΔCT method was used to quantify the gene expression. The primer sequences are listed in Table. A.1 in Supplementary file.

2.3. Western blot

Cells were lysed with a RIPA buffer containing protease and phosphatase inhibitors (Roche). The extracted protein was heated at 95 °C for 10 min for denaturation separated by 10% SDS-PAGE electrophoresis and subsequently transferred onto a PVDF membrane (Roche). Primary antibodies against sclerostin (1:1,000, Abclonal, Boston, MA, USA), phosphorylated-P65^{S536} (1:1,000, Cell Signaling Technology, Danvers, MA, USA), P65 (1:1000, Cell Signaling Technology), DSPP (1:1,000, Abclonal), OPN (1:1,000, Abclonal), VEGFR-1 (1:1000, Santa Cruz Biotechnology, CA, USA) and β-actin (1:5,000, Santa Cruz Biotechnology) were used. The chemiluminescent signal was captured and analyzed using a chemiluminescent assay (LI−COR Biosciences, Lincoln, NE, USA USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

The supernatants of hOBs were collected and stored at −80 °C until use. Concentration of human IL-6, IL-8 and VEGF in the culture supernatants were quantitatively measured using a commercial ELISA kit according to the manufacturer's protocol (NeoBioscience, Shenzhen, China), respectively.

2.5. Adhesion assay

50 μl of type I collagen (Sigma) diluted in phosphate buffer saline (PBS) was added to each well of a 96-well plate and incubated overnight at 4 °C. After removal of unbound collagen, each well was rinsed with PBS and non-specific binding sites were blocked with 1% BSA at 37 °C for 1 h. hDPCs were starved in serum-free medium for 24 h, then dissociated and resuspended in serum-free α-MEM with or without LPS and rhSCL. Subsequently, 5×10^3 cells were placed in each well and allowed to adhere at 37 °C for 90 min. Non-adhered cells were rinsed off using PBS, and the remaining cells were fixed with 4% paraformaldehyde for 15 min. Then cells were stained with 0.5% toluidine blue for 10 min and finally rinsed in water. The number of adhered cells were counted from 5 randomly selected microscopic fields (100 × magnification).

2.6. Migration assay

Cells migration was evaluated using a two-chamber transwell system (8 mm pore size and 6.5 mm diameter). hDPCs were starved in serum-free medium for 24 h, then dissociated and resuspended in serum-free medium. Subsequently, 10^4 hDPCs were seeded onto the top chamber of transwell, and 600 μl of serum-free α-MEM with or without LPS and rhSCL was added to the lower chambers. After incubating for 24 h at 37 °C, the cells that migrated through the membrane were fixed in 4% paraformaldehyde for 15 min and stained with cresyl violet

acetate for 30 min. Photographs of the migrated cells in random fields were acquired using a microscope at 200 × magnification. Then 50 µl of 33% acetic acid was added to each top chamber and the absorbance of the colored solution was quantified at 570 nm.

2.7. Alkaline phosphatase (ALP) staining

For odontoblastic induction, hDPCs were cultured in α-MEM medium supplemented with 5% FBS and preceding odontoblastic inducing components. Then cells were rinsed with PBS and fixed in 4% paraformaldehyde for 15 min. ALP staining was performed with a BCIP/NBT kit (Beyotime, Shanghai, China) according to the manufacturer instructions.

2.8. Statistical analysis

All experiments were repeated at least three times, and the quantitative results are presented as the mean ± SEM from three independent experiments. Data were analyzed by *t*-test and Two-way analyses of variance using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered to be statistically significant.

3. Results

3.1. Sclerostin expression was elevated in inflamed hOBs

Alterations of sclerostin expression in inflamed hOBs were detected by qPCR and Western blot analyses. Firstly, hOBs were incubated at various LPS concentration (0, 0.1, 1, 5, and 10 µg/ml) for 3 h. The results showed that sclerostin expression was elevated for all concentration of LPS challenge, and was maximized under 1 µg/ml LPS treatment at both mRNA and protein levels (Fig. 1A, B). Based on these results and the related literatures (He et al., 2013; Soares et al., 2018; Park et al., 2011), 1 µg/ml was selected as the optimal concentration for subsequent experiments. hOBs were then incubated with LPS for various time (0, 3, 6, 12 and 24 h), and sclerostin expression was obviously increased for all time durations, despite not being in a time-dependent manner (Fig. 1C, D).

(A, B) qPCR and Western blot analyses of sclerostin expression in hOBs treated with LPS at different concentrations (0, 0.1, 1, 5 and 10 µg/ml) for 3 h. **(C, D)** qPCR and Western blot analyses of sclerostin expression in hOBs treated with 1 µg/ml LPS for different time (0, 3, 6, 12 and 24 h). Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05.

3.2. Sclerostin increases pro-inflammatory cytokines production in inflamed hOBs

Subsequently, rhSCL was added to LPS-treated hOBs, and the expression level of various pro-inflammatory cytokines were measured by qPCR and ELISA analyses. After exposing hOBs to LPS for 3 h, sclerostin was observed to enhance LPS-induced the production of IL-6, IL-8 and IL-1β mRNA expressions (Fig. 2A–C). After exposure for 24 h, IL-6 and IL-8 proteins in the culture supernatants was also increased in the presence of sclerostin (Fig. 2D, E). This functional impact of sclerostin was confirmed by lentivirus plasmid transduction, which showed that sclerostin overexpression causes a robust increase of IL-6, IL-8 and IL-1β mRNA expressions in inflamed hOBs whereas sclerostin knockdown attenuated LPS-induced upregulation of pro-inflammatory cytokines production (Fig. A.1 in Supplementary file).

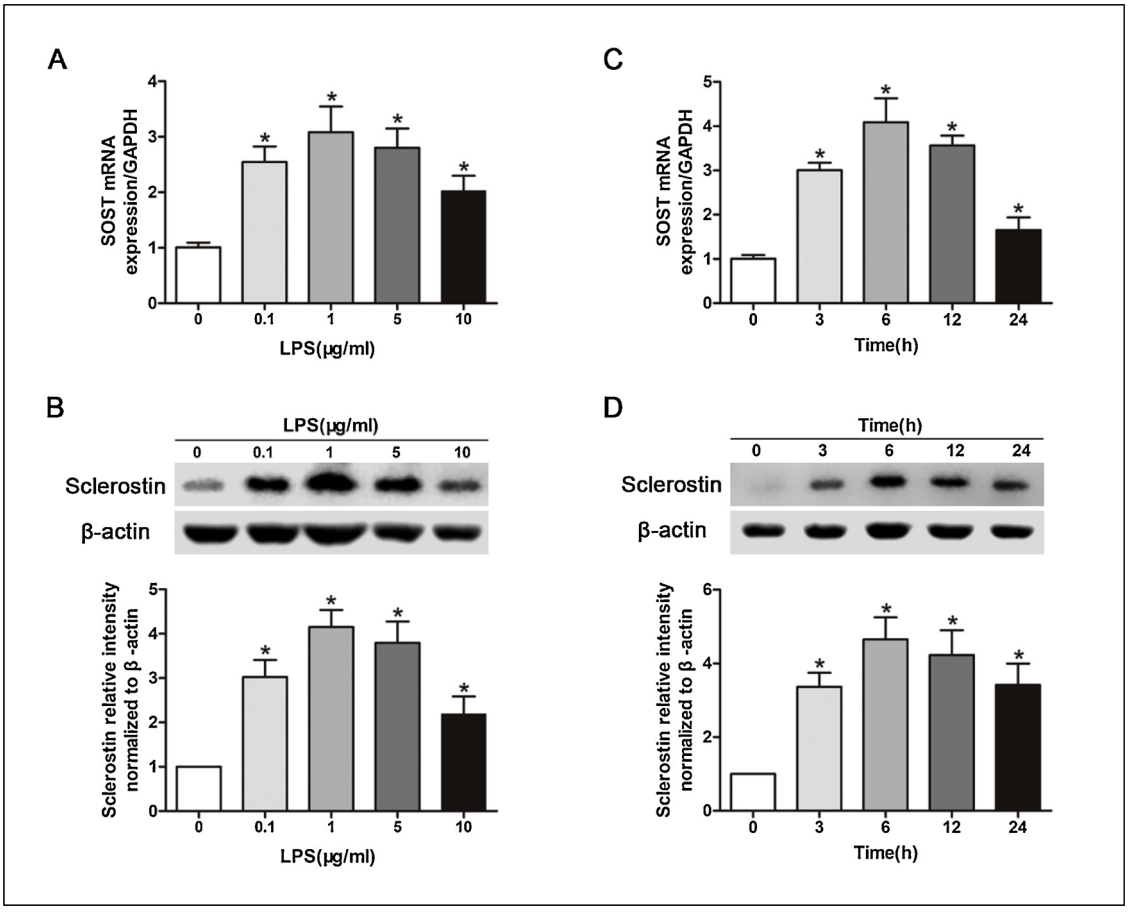

Fig. 1. The expression of sclerostin in LPS-treated hOBs.

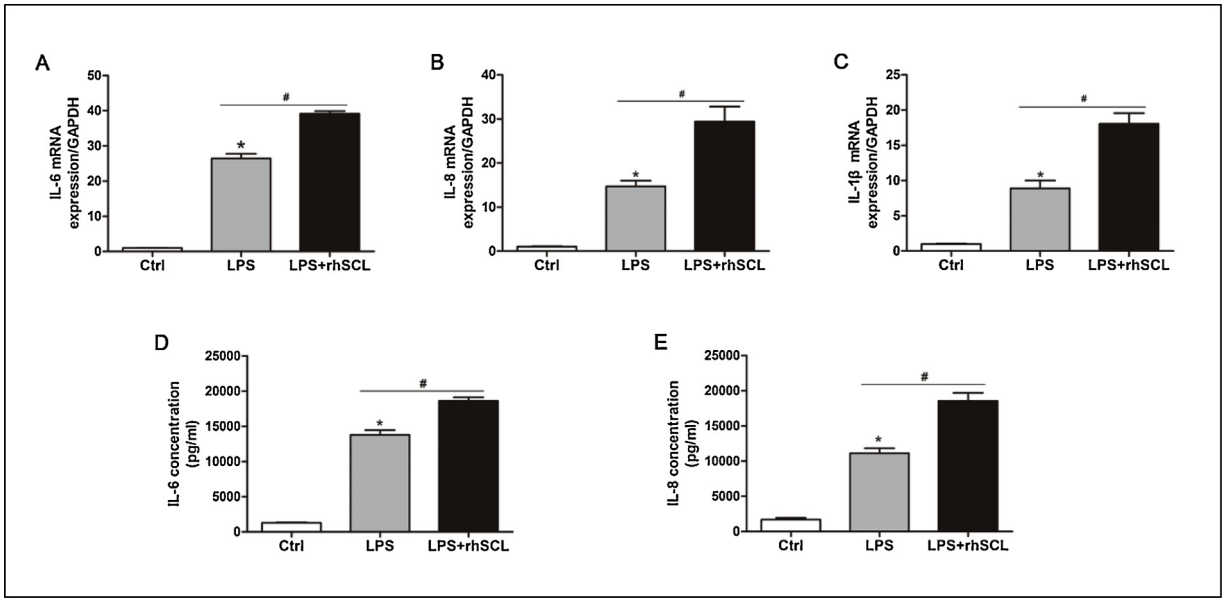

Fig. 2. Effects of sclerostin in LPS-induced inflammatory cytokines production in hOBs.

(A–C) qPCR analyses of IL-6, IL-8 and IL-1β expressions in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 3 h. **(D, E)** ELISA analyses of IL-6 and IL-8 expressions in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 24 h. Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05 compares to the control group. — demonstrates the comparison between groups, #P < 0.05.

3.3. NF-κB pathway mediates sclerostin-regulated inflammatory responses of hOBs

To explore the potential mechanisms on pro-inflammatory function of sclerostin, alterations of NF-κB signaling pathway were detected by Western blot analyses. Sclerostin enhanced LPS-induced phosphorylation of P65 in hOBs, revealing an activation of NF-κB pathway (Fig. 3A). Besides, sclerostin overexpression increased the phosphorylated-P65 and sclerostin knockdown decreased phosphorylated-P65 in inflamed hOBs, separately (Fig. A.2 in Supplementary file). Then hOBs were pretreated with the NF-κB inhibitor PDTC before other stimulation, and PDTC blocked sclerostin-induced P65 phosphorylation in inflamed hOBs (Fig. 3B). Besides, PDTC reversed the exaggerated effects of sclerostin on the production of IL-6, IL-8 and IL-1β in inflamed hOBs (Fig. 3C–G).

(A) Western blot analyses of phosphorylated-p65 and total P65 expression in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 40 min. hOBs were pretreated with 50 μM PDTC or DMSO

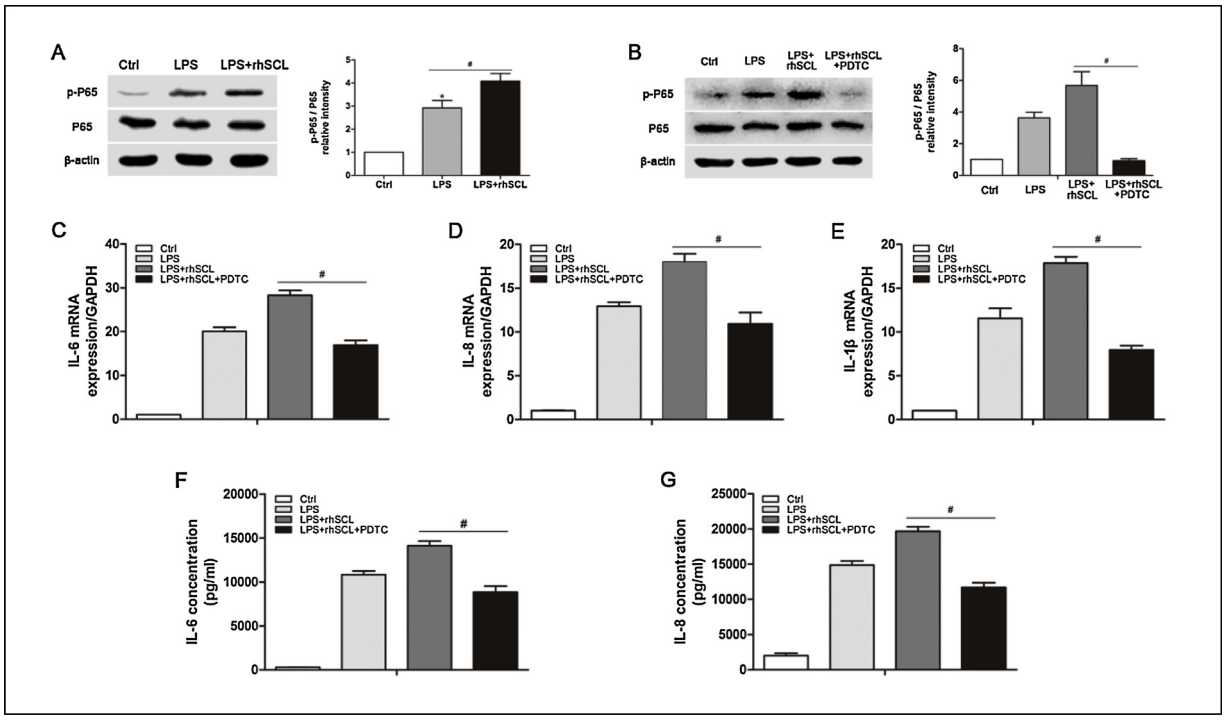

Fig. 3. Role of NF-κB pathway in sclerostin regulated inflammatory responses in LPS-treated hOBs.

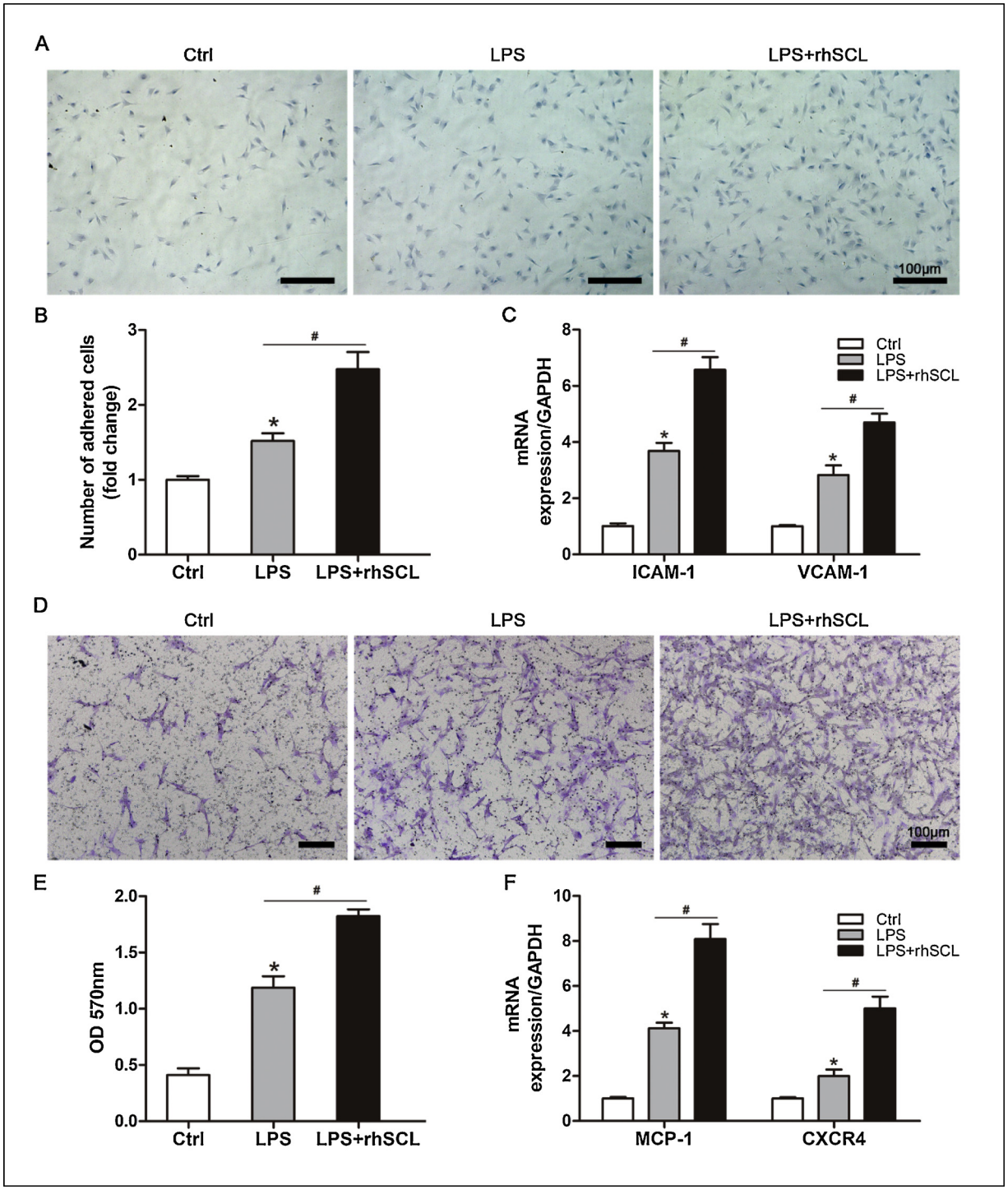

Fig. 4. Effects of sclerostin on adhesion and migration of LPS-treated hDPCs.

for 1 h, then stimulated with LPS or 200 ng/ml rhSCL. (B) Western blot analyses of phosphorylated-p65 and total P65 expression for 40 min. **(C–E)** qPCR analyses of IL-6, IL-8 and IL-1β expressions after 3 h stimulation. **(F, G)** ELISA analyses of IL-6 and IL-8 expressions after 24 h stimulation. Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05. *p < 0.05 compares to the control group. — demonstrates the comparison between groups, #P < 0.05.

3.4. Sclerostin promotes adhesion and migration of inflamed hDPCs

Effects of sclerostin on adhesion of inflamed hDPCs were examined using type I collagen-coated plates. The results suggested that LPS significantly increased the number of adhered cells, and sclerostin could further improve the adhesion ability of hDPCs (Fig. 4A, B). Separately, the key adhesion molecules ICAM-1 and VCAM-1 in hDPCs were increased at mRNA level in the presence of sclerostin under LPS challenge (Fig. 4C).

A two-chamber transwell system was used to investigate the effects of sclerostin on migratory motility of inflmamed hDPCs. As shown, LPS

stimulation upregulated the number of cells that migrated to the lower surface of the membrane, and sclerostin further enhanced the migratory ability of hDPCs (Fig. 4D, E). Detection for chemotactic factors also showed that sclerostin could increase the mRNA expression of MCP-1 and CXCR4 in LPS-treated hDPCs, respectively (Fig. 4F).

hDPCs were incubated with 1 μg/ml LPS or 200 ng/ml rhSCL and allowed to adhere for 90 min. **(A, B)** Representative photographs of the stained adhered cells and number of adhered cells number, scale bars:100 μm. LPS or rhSCL was added in the lower chambers of transwell system, then hDPCs were allowed to migrate for 24 h. **(D, E)** Representative photographs of the stained migrated cells and optical density values of the dissolved stain at 570 nm, scale bars: 100 μm. **(C, F)** qPCR analyses of ICAM-1, VCAM-1, MCP-1 and CXCR4 expressions in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 3 h from a separated experiment. Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05 compares to the control group — demonstrates the comparison between groups, #P < 0.05.

3.5. Sclerostin inhibits odontoblastic differentiation of inflamed hDPCs

To investigate the effects of sclerostin on odontoblastic differentiation under inflammatory condition, we cultured hDPCs in odontoblastic inducing medium with LPS and rhSCL treatments. The mRNA expression of the odontoblastic differentiation markers DSPP, BSP, OPN, and OCN was markedly upregulated in response to LPS, and sclerostin attenuated this upregulation on day 3 and 7 (Fig. 5A–D). The protein expression level of DSPP and OPN was also increased after LPS challenge, which was reversed in the presence of sclerostin on day 7 (Fig. 5E). Moreover, sclerostin reduced LPS-upregulated ALP activity on day 7 and day 10 (Fig. 5F).

Cells were cultured in odontogenic inducing medium with/without LPS and 200 ng/ml rhSCL. **(A–D)** qPCR analyses of DSPP, BSP, OPN and OCN expressions on day 3 and day 7. **(E)** Western blot analyses of DSPP and OPN expressions on day 7. **(F)** Photographs of ALP staining on day 7 and day 10. Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05 compares to the control group — demonstrates the comparison between groups, #P < 0.05.

3.6. Sclerostin might promote angiogenesis in inflamed hDPCs

We also evaluated the effects of sclerostin on angiogenesis in DPCs under LPS-induced inflammation. hDPCs were incubated with LPS and rhSCL for 24 h, and then a variety of pro-angiogenic cytokines were detected using qPCR, ELISA or Western blot analyses. As shown, LPS upregulated the expression of VEGF, VEGFR-1 and PlGF, and sclerostin exaggerated LPS-induced upregulation of these pro-angiogenic factors (Fig. 6A–E).

(A–C) qPCR analyses of VEGF-A, VEGFR-1 and PlGF expressions in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 24 h. **(D)** ELISA analyses of VEGF expression in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 24 h. (E) Western blot analyses of VEGFR-1 expression in control hOBs and LPS-treated hOBs with/without 200 ng/ml rhSCL for 24 h. Data are represented as the mean ± SEM and are based on three independent experiments. *p < 0.05 compares to the control group — demonstrates the comparison between groups, #P < 0.05.

4. Discussion

Dental caries or trauma, which allows pathogens and bacterial products to penetrate into dentine-pulp complex, often leads to dental pulp inflammation (He et al., 2013). Odontoblasts and DPCs are essential functioning cells for host defenses and regeneration. The present study demonstrates that under LPS-induced inflammatory environment, sclerostin enhances pro-inflammatory cytokines production in hOBs and inhibits odontoblastic differentiation of hDPCs, indicating that sclerostin inhibition might be propitious for pulpal inflammation resistance and vital pulp therapy.

Sclerostin, a glycoprotein negatively regulating osteogenesis, is proposed as being relevant for the processes and management of inflammation. Previous studies found that tumor necrosis factor (TNF)-α and LPS could elevate sclerostin expression in human osteoblasts and MLO-Y4 osteocytes (Baek et al., 2014; Vincent et al., 2009; Sakamoto et al., 2019). However, the role of sclerostin in inflammation is controversial. Several researches exhibited that sclerostin inhibition could reverse axial and appendicular bone loss in rheumatoid arthritis and colitis (Chen et al., 2013; Marenzana et al., 2013; Eddleston et al., 2009), and enhance alveolar bone regeneration in experimental periodontitis (Ren et al., 2015; Taut et al., 2013; Chen et al., 2015). But other researches showed that sclerostin deficiency promoted TNF-α-independent inflammatory joint destruction and pannus formation (Wehmeyer et al., 2016), and sclerostin protected against inflammation-induced cartilage damage in osteoarthritis (Warde, 2011; Chan et al., 2011). During dental pulp inflammation, odontoblasts are the first-line defensive cells against invading pathogens and their released components (Durand et al., 2006; Farges et al., 2011; Veerayutthwilai et al., 2007). In this study, we found that LPS increased sclerostin expression in hOBs, which suggested that sclerostin might affect the inflammatory immune responses of odontoblasts. Distinguished from previous investigations focusing on its function towards hard tissue damage, this study investigated the effects of sclerostin on cellular inflammatory cytokines production for the first time. Through exogenous stimulation with rhSCL (along with overexpression and knockdown of sclerostin exhibited in Fig. A.1 in Supplementary file), we elucidated that sclerostin could increase LPS-induced production of pro-inflammatory cytokines (IL-6, IL-8, and IL-1β) in hOBs, emphasizing the critical function of sclerostin on inflammatory immune responses of odontoblasts during dental pulp inflammation.

NF-κB is an important transcription factor involved in innate immune, oxidative stress and inflammation through inducing production of many cytokines, chemokines, and matrix metalloproteinases (Sen and Baltimore, 1986; Karin and Lin, 2002). Previous studies documented that LPS could promote phosphorylation of P65, a common activation form of the NF-κB signaling pathway, leading to production of pro-inflammatory cytokines, such as IL-6, IL-8 and IL-1β in odontoblasts-like cells and DPCs (He et al., 2013; Choi et al., 2009; Jung et al., 2017). Based on this background, we investigated the role of NF-κB pathway in sclerostin-mediated inflammatory responses of hOBs during LPS-induced inflammation. Our results showed that sclerostin promoted LPS-induced phosphorylation of P65, revealing the activation of NF-κB pathway in hOBs. Besides, sclerostin overexpression in hOBs increased phosphorylated-P65 and sclerostin knockdown decreased phosphorylated-P65 (exhibited in Fig. A.2 in Supplementary file). Furthermore, exaggerated effects of sclerostin on pro-inflammatory cytokines production in LPS-treated hOBs were blocked by NF-κB specific inhibitor PDTC. These results implied for the first time that sclerostin aggravated inflammatory responses of odontoblasts through the NF-κB signaling pathway under dental pulp inflammation.

When odontoblasts become damaged or destroyed, DPCs are capable of replacing denatured odontoblast for host defense and tissue regeneration. One work using tooth culture and BrdU labeling suggested that injured odontoblasts could stimulate dental pulp stem cells proliferating and migrating to pulp injury site (Tecles et al., 2005). LPS was reported to promote the adhesion and migration of DPCs and odontoblast-like cells (Park et al., 2011; Li et al., 2014; Liu et al., 2014; Wang et al., 2012). In this study, we found that sclerostin enhanced LPS-induced adhesion and migration of hDPCs, along with increased expression of adhesion molecules (ICAM-1 and VCAM-1) and chemokines (MCP-1 and CXCR4). These results revealed that sclerostin could improve the adherent and migratory abilities of hDPCs during dental pulp inflammation, and might function through upregulating these

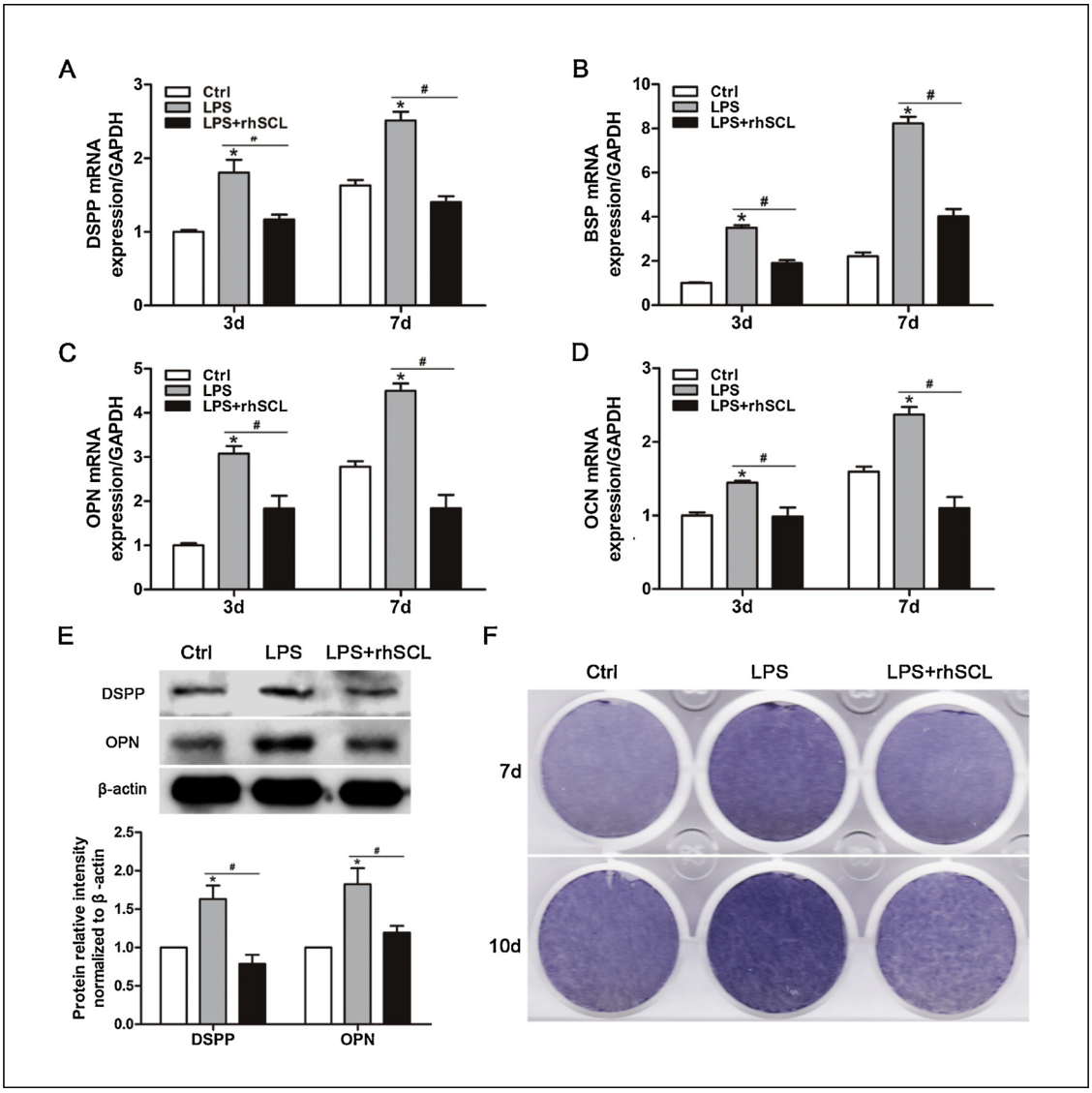

Fig. 5. Effects of sclerostin on odontoblastic differentiation of LPS-treated hDPCs.

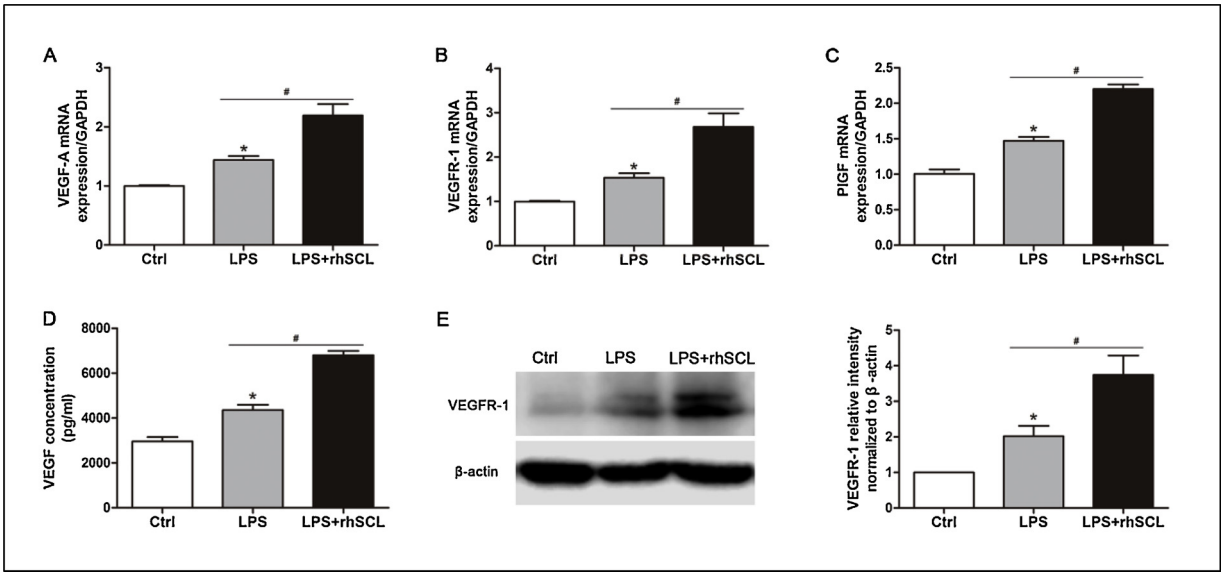

Fig. 6. Potential impacts of sclerostin on angiogenesis in LPS-induced hDPCs.

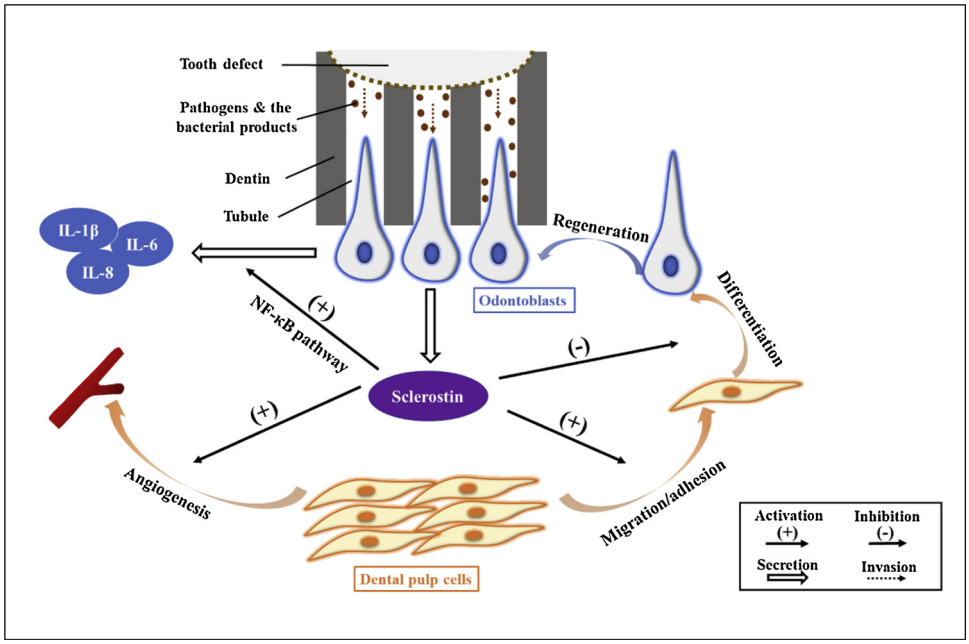

Fig. 7. Schematic diagram of the role of sclerostin in dentine-pulp complex under pupal inflammatory condition.

adhesion molecules and chemokines. Considering the elevated expression of sclerostin in inflamed hOBs, it could therefore be inferred that odontoblasts might promote the adhesion and migration of hDPCs for regeneration through sclerostin-independent manner, and sclerostin might serve as a mediator of the interactions between odontoblast and DPCs to orchestrate inflammatory processes in dentine-pulp complex.

Odontoblastic differentiation of the replacing DPCs is the most fundamental and vital process for dentin regeneration. In this study, we found that LPS improved odontoblastic differentiating capability of hDPCs, as reflected by increased ALP activity and upregulated expression of odontoblastic markers (DSPP, BSP, OPN and OCN), but sclerostin could attenuate LPS-induced odontoblastic differentiation of hDPCs. Differentiating potential of DPCs alters under inflammatory condition, manifested in that a lower concentration of TNF-α, IL-1β and LPS could stimulate DPCs toward an odontoblastic phenotype in vitro (Soares et al., 2018; Paula-Silva et al., 2009; Huang et al., 2015; Yang et al., 2011). Whilst in contrast, inflammation often causes bone loss and internal resorption of teeth, and an in vivo investigation showed that after surgical pulp injury, capping with calcium hydroxide for diminishing inflammation accelerated reparative dentin formation (Decup et al., 2000; Cochran, 2008). The discrepancy could be related to the degree of inflammation to some extent, but more importantly, monoculture of DPCs exposed to proinflammatory factors is a pure inflammatory environment, whereas dental pulp inflammation involves complex interactions between innate and adaptive immune responses of odontoblasts and DPCs. Therefore, our results that sclerostin secreted by inflamed odontoblasts could inhibit odontoblastic ability of inflamed DPCs may help explain the controversial evidences regarding reparative dentinogenesis under inflammatory condition in vivo and in vitro.

Dental pulp has a rich vasculature involved in nutrition/oxygen delivery and metabolic waste transport. But pulp is in low compliance under inflammatory condition, and vascular responses causes increased capillary permeability and intra-pulpal pressure, which may lead to pulp necrosis (Heyeraas et al., 2001). DPCs play an essential role in the process of angiogenesis, by directly differentiating themselves into endothelial cells or mediately expressing angiogenic factors to induce angiogenesis of endothelial cells (Sieveking and Ng, 2009; Bronckaers et al., 2013; Gandia et al., 2008; Marchionni et al., 2009). Previous investigation showed that sclerostin could serve as a regulator of angiogenesis-osteogenesis coupling because sclerostin stimulated angiogenesis to provide the route allowing monocyte, osteoclasts and its precursors to reach the bone resorbing sites (Oranger et al., 2017). In aforementioned results, we found that sclerostin promoted migration of inflamed hDPCs. Actually, cell migration interplays with revascularization, e.g. chemokines MCP-1 and CXCR4 can promote angiogenesis by producing chemotactic activity to endothelial progenitor cells (Bronckaers et al., 2013; Liekens et al., 2010). These inspired our explorations into the function of sclerostin on pulpal angiogenesis under inflammatory condition. VEGF is the master regulator of vascular permeability and angiogenesis (Ferrara and Davis-Smyth, 1997). Increased expression of VEGF is clearly evident in inflamed pulp tissue and LPS-treated odontoblast/DPCs (Botero et al., 2006; Güven et al., 2007; Botero et al., 2010). VEGFR-1 is a receptor of VEGF and is one of endothelial-specific proteins. Latest research showed that VEGFR-1 could be expressed in DPCs (Schertl et al., 2019). Our results showed that sclerostin enhanced the production of VEGF, its homologue PlGF, and VEGFR-1 in inflamed hDPCs, implying that sclerostin might promote pulpal angiogenesis during dental pulp inflammation by improving angiogenesis of endothelial cells and accelerating angiogenic differentiation of DPCs. Precise definition and the underlying mechanisms require further study to elucidate.

Upon dental caries or trauma causing tooth defect, odontoblasts are the first-line defensive cells against invading pathogens and their bacterial products. Inflammation results in increased secretion of sclerostin in odontoblasts, then sclerostin increases pro-inflammatory cytokines production in hOBs via NF-κB signaling pathway, and promotes adhesion, migration and angiogenesis whereas inhibits odontoblastic differentiation of hDPCs.

In conclusion, this study illustrates that inflammation results in upregulated expression of sclerostin in hOBs, then sclerostin increases pro-inflammatory cytokines production in hOBs via NF-κB pathway, and promotes adhesion, migration and angiogenesis, while inhibits odontoblastic differentiation of hDPCs (Fig. 7). The cumulative findings reveal that sclerostin may enhance the inflammatory responses of odontoblasts and impair dentin tissue regeneration, which provide novel inspirations towards therapeutic treatments for anti-inflammation and pro-regeneration during dental pulp inflammation.

Declarations of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2019.105628.

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