Chapter 4
Signaling Pathways in Dental Stem Cells During Their Maintenance and Differentiation

Genxia Liu, Shu Ma, Yixiang Zhou, Yadie Lu, Lin Jin, Zilu Wang, and Jinhua Yu

4.1 Introduction

Dental stem cells (DSCs) residing in dental tissues possess the self-renewal and multipotential differentiation ability, and are essential in the process of tooth homeostasis, repair and regeneration. The maintenance, proliferation and differentiation of DSCs are directly or indirectly regulated by a variety of factors, such as microenvironment, growth factors and donor ages. The complex network of signaling pathways, including fibroblast growth factor (FGF), bone morphogenetic protein (BMP), Notch, nuclear transcription factor kappa-B (NF-κB), mitogen-activated protein kinases (MAPKs), transforming growth factor-β (TGF-β), mammalian target of rapamycin (mTOR), phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/AKT) and sonic hedgehog (SHH) signaling pathways, participate in regulating the formation, homeostasis, and differentiation of DSCs in the developing tooth and throughout the adulthood. Researches over the past years have given rise to the meaningful progress on the understanding of the signaling network.

The authors "Genxia Liu" and "Shu Ma" contributed equally to this manuscript.

G. Liu • S. Ma • Y. Zhou • Y. Lu • L. Jin • Z. Wang
Key Laboratory of Oral Diseases of Jiangsu Province and Stomatological Institute of Nanjing Medical University, 136 Hanzhong Road, Nanjing, Jiangsu 210029, China

J. Yu, PhD, DDS (✉)
Key Laboratory of Oral Diseases of Jiangsu Province and Stomatological Institute of Nanjing Medical University, 136 Hanzhong Road, Nanjing, Jiangsu 210029, China

Institute of Stomatology, Nanjing Medical University,
136 Hanzhong Road, Nanjing, Jiangsu 210029, China
e-mail: yujinhua@njmu.edu.cn

F. Şahin et al. (eds.), *Dental Stem Cells*, Stem Cell Biology and Regenerative Medicine, DOI 10.1007/978-3-319-28947-2_4

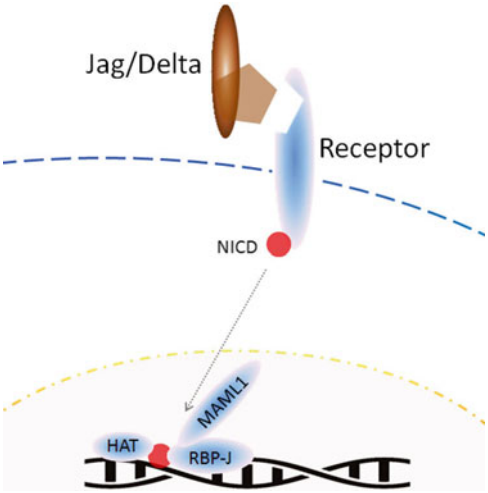

Fig. 4.1 Notch signaling pathway. Notch ligands (Jagged1, Jagged2, Delta1, Delta2 and Delta3) interact with Notch receptors and then initiate the Notch pathway. The activation of Notch subsequently gives rise to a release of Notch intracellular domain (NICD) into the cytoplasm where it translocates to the nucleus. In the nucleus, NICD binds to RBP-J and MAML1, recruits the transcriptional co-activators and leads to the transcription of target genes

4.2 Signaling Pathways in DSC Maintenance/Homeostasis

The signaling pathway is a series of cellular proteins that transfer a biological signal from a receptor on the cell membrane to the DNA in the cell nucleus. The pathway begins with a signaling molecule binding to the membrane receptor and ends when the nuclear DNA generates respective proteins and brings about some cellular changes (*e.g.,* cell differentiation).

During tooth development, DSCs can maintain the stable state, referred to as homeostasis, and many signaling pathways (*e.g.*, Notch, BMP-SHH, MAPK and Eph/Ephrin signaling pathways) control the maintenance of stem cells in tooth.

4.2.1 Notch Signaling Pathway

The Notch signaling pathway is a highly conserved signaling cascade and plays a key role in the stem cell maintenance and fate determination. There are usually four kinds of Notch receptors, i.e., Notch1, Notch2, Notch3 and Notch4. These receptors are single-pass transmembrane receptors. Notch ligands (Jagged1, Jagged2, Delta1, Delta2 and Delta3) interact with these membrane-bound Notch receptors and directly initiate Notch signaling pathway and downstream molecules to mediate the expression level of target genes (Fig. 4.1).

Previous studies have shown that Notch receptors are absent in the adult rat pulp tissues but the expression level will be reactivated during the repair of tooth injury [1]. Notch signaling is also essential for the development of dental epithelium and enamel organ [2]. Notch receptors as well as Notch ligands are expressed in both dental epithelial and mesenchymal cells during the odontogenesis, and initiate the stage of epithelial-mesenchymal interactions for tooth morphogenesis [3, 4]. Notch and FGF signaling pathways are associated with dental epithelial stem cells in regulating their fate and FGF10 maintains the stem cell population during the development of mouse incisors [5, 6].

4.2.2 SHH Signaling Pathway

SHH signaling pathway is a chain of proteins that transfer the information to cells for proper embryonic development. In addition, it is highly active in cell proliferation and differentiation of both epithelial and mesenchymal stem cells (MSCs).

In mice, dental epithelial stem cells residing in the cervical loop at the proximal end of the labial side of incisors are maintained along with the MSCs, and they allow the incisors to grow continuously throughout life [7]. Researchers have focused on the molecular mechanisms of this phenomenon, and find that SHH signaling pathway is related to the stem cell homeostasis [8]. Moreover, BMP-Smad4-SHH signaling can regulate the epithelial stem cell maintenance in tooth development. $Sox2^{+}$ epithelial stem cells exist transiently during the molar development, and sonic hedgehog-glioma-associated oncogene 1 (Shh-Gli1) activity provides a niche for maintenance of these stem cells. However, loss of Smad4 results in ectopic SHH-Gli1 signaling and maintenance of $Sox2^{+}$ cells [9]. This study has proved the importance of crosstalk between BMP and SHH signaling pathways in the regulation of epithelial stem cell fate during odontogenesis. Moreover, SHH pathway can inhibit the osteo/dentinogenic differentiation of stem cells from apical papilla [10].

4.2.3 MAPK Signaling Pathway

MAPK signaling pathway (also known as the Ras-Raf-MEK-ERK pathway) contains several proteins, including MAPK (mitogen-activated protein kinases), that communicate by driving the phosphate groups into a neighboring protein (work as an "on" or "off" switch manner). This pathway is involved in cell apoptosis, survival, migration, proliferation, differentiation as well as other cellular processes. Three main MAPK family members (extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK) and p38) are distinctly referred to these processes [11].

Recent literatures have provided convincing evidences that MAPK signaling pathway plays a critical role in the maintenance, migration, proliferation and differentiation of DSCs. Two-hydroxyethyl methacrylate (HEMA), a kind of resin-based dental materials, can inhibit the cell migration of dental pulp stem cells (DPSCs) by phosphorylation of p38 but not ERK, or JNK MAPK pathways [12]. p38 MAPK and insulin-like growth factor 1 receptor (IGF-1R) are responsible for the mitotic quiescence of DPSCs. The inhibitors of IGF-1R can improve the sphere-forming capacity of DPSCs and decrease the colony-forming capacity without causing cell death, in contrast to the p38 inhibitors. IGF-1R and p38 MAPK signaling pathways are interrelated at the molecular levels in DPSCs. Signals from these pathways converge as signal transducers and activators of transcription 3 (STAT3), and oppositely modulate its activity to maintain the quiescence or enhance the self-renewal and differentiation of cells [13].

Previous studies have proposed that interleukin 8 (IL-8) might be involved in regulating the immune response of DPSCs and promoting the recruitment process of neighboring DPSCs to the site of injury [14]. Lipopolysaccharide (LPS), which mediates IL-8 expression in DPSCs, is associated with toll-like receptor 4 (TLR4), myeloid differentiation marker 88 (MyD88), MAPK and NF-κB signaling pathways. Overall results of the study indicate that NF-κB and MAPK signaling pathways are closely involved in dental pulp inflammation and maintaining of the homeostasis of DPSCs niche. Another study reveals that DPSCs may play important roles in the immune responses during the pulp infection via activating NF-κB signaling pathway [15].

4.2.4 Eph-Ephrin Signaling Pathway

Eph-Ephrin signaling pathway includes Ephs and their corresponding ephrin ligands (ephrins), which are both membrane-bound proteins. Thus, the activation of Eph-Ephrin intracellular pathways can only happen through the direct cell-cell interactions. Eph-Ephrin signaling regulates diverse biological processes during the embryonic development (*e.g.*, formation of tissue boundaries, cell migration, angiogenesis, and stem cell differentiation).

Tooth development occurs through interactions between cranial neural crest-derived mesenchymal and epithelial cells [16], while DPSCs reside mainly within the perivascular niche of dental pulp tissue. The Eph family of receptor tyrosine kinases and their ligands, ephrin molecules, are reported to play an imperative role in the migration of neural crest cells throughout the development and maintenance of stem cell niche (Fig. 4.2) [17].

DPSCs exposed to EphB2-Fc and EphB1-Fc can exhibit a significantly rounder and smaller morphology than hDPSCs treated with human IgG-Fc controls. EphB2-Fc treated DPSCs present the same migration speed as human IgG-Fc treated DPSCs while the migration ability of EphB1-Fc treated DPSCs decreases significantly. The ERK inhibitor U0126 can partially reverse the reduction of migration speed of EphB1-Fc treated DPSCs [18]. These data suggest that EphB-EphrinB

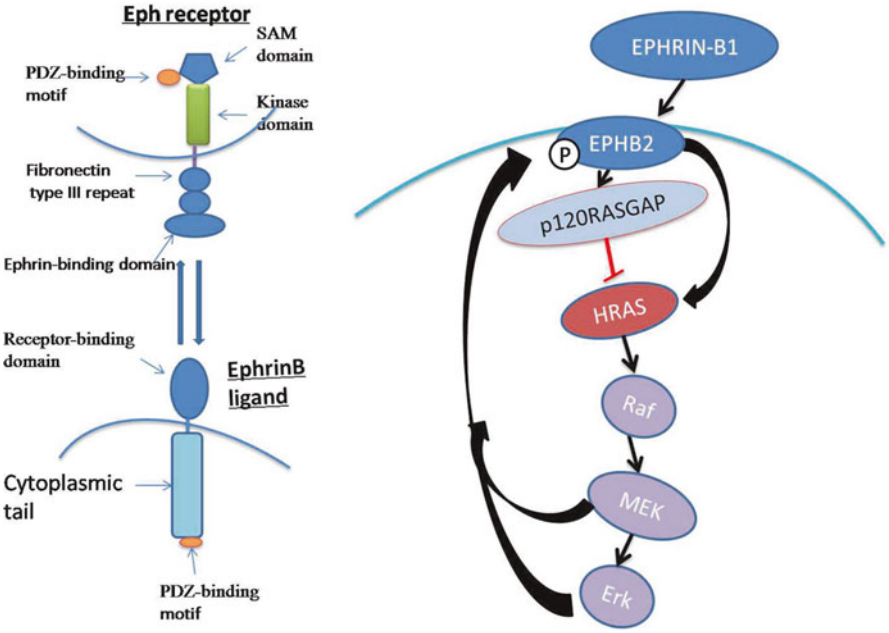

Fig. 4.2 Eph-Ephrin signaling pathway. Eph family is composed of receptor tyrosine kinases and their ligands. The activation of EphB2 bound by EphrinB1 stimulates the HRAS-Erk signaling pathway, and the increase in MEK and/or Erk activity, reversely enables the enhanced expression of EPHB2 under the stimulation of EphrinB1. The phosphorylation of EphB2 can also activate the expression of p120RASGAP, leading to the inhibition of HRAS

pathway also mediates human DPSCs attachment, spreading and migration in DPSC niche, in which ERK-MAPK signaling are involved in the regulation of these processes.

4.3 Signaling Pathways in DSC Migration

Stem cells can adhere, grow and migrate to the damaged areas during inflammatory response or wound healing. There are some critical signaling pathways that have great impacts on the migration of DSCs.

4.3.1 MAPK Signaling Pathway

ERK, JNK and p38 MAPKs can be activated by a variety of environmental factors. Activated ERK, JNK and p38 can translocate to the nucleus where they phosphorylate the transcription factors (c-Jun, c-Fos, Elk-1 and Sp1), and then regulate the downstream gene expression (Fig. 4.3).

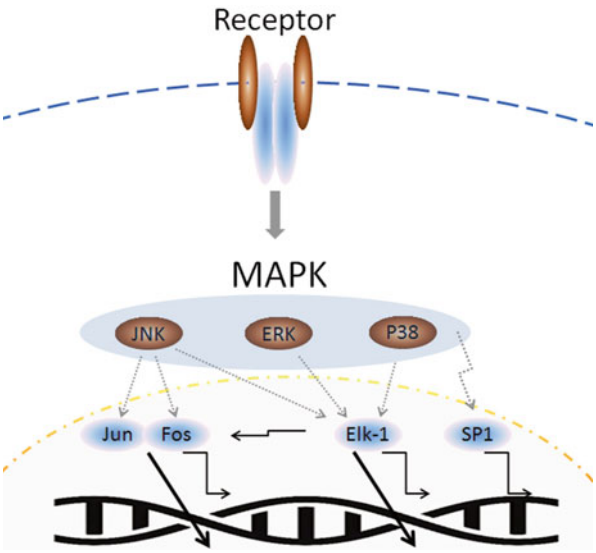

Fig. 4.3 MAPK signaling pathway. ERK, JNK and p38 MAPKs are members of MAPK family which can be activated by a variety of environmental factors. Activated ERK, JNK and p38 can translocate to the nucleus where they phosphorylate transcription factors (c-Jun, c-Fos, Elk-1 and Sp1) and then regulate the downstream gene expression

Previous studies have proved that MAPKs, including JNK, p38 and ERK are involved in the cell migration process [19]. In particular, JNK regulates cell migration by phosphorylating paxillin, doublecortin X-linked (DCX), Jun and microtubule-associated proteins. The antimicrobial peptide LL37 promotes the migration of DPSCs via activating the epidermal growth factor receptor (EGFR)-JNK signaling pathway, which may lead to the increased regeneration of pulp-dentin complexes [20]. MAPK regulates the directional migration of cells via the phosphorylation of MAPK-activated protein kinase 2/3 (MAPKAP 2/3). Some studies have demonstrated that HEMA inhibits the migration of DPSCs at non-toxic doses, and such inhibition is associated with the p38 signaling pathway [12]. Moreover, LPS can promote the adhesion and migration of DPSCs by upregulating the expression of adhesion molecules and chemotactic factors, while inhibition of MAPK and NF-κB significantly antagonizes LPS-induced adhesion and migration [21].

The inhibition of JNK or p38 pathways in DPSCs significantly decreases cell proliferation, alkaline phosphatase (ALP) activity, and mineralization ability stimulated by hepatocyte growth factor (HGF). JNK and p38 inhibitors can affect F-actin remodeling induced by HGF and thus, contribute to HGF-induced migration [22]. The activation of fibroblast growth factor receptor (FGFR), ERK, JNK, and AKT can modulate the upregulation of focal adhesion molecules, stress fiber assembly, and enhance cell migration induced by iRoot BP Plus [23]. ERK determines cell movement by the phosphorylation of myosin light chain kinase (MLCK), calpain or focal adhesion kinase (FAK). Overall, the different kinds of kinases in MAPK family all appear to be capable of regulating cell migration via particular mechanisms.

4.3.2 PI3K/AKT Signaling Pathway

The PI3K/AKT pathway mainly contains the phosphatidylinositol 3-kinase (PI3K) and AKT. The pathway begins with an activation of a membrane receptor and phosphorylation of PI3K. Then, PI3K phosphorylates the lipids and generates the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) which subsequently activates the AKT. Activated AKT mediates the downstream responses by phosphorylating a series of intracellular proteins.

PI3K/AKT signaling pathway is critical in cell growth and migration. Firstly identified in osteoblast-like cell line MC3T3-E1, periostin is a kind of matrix-cellular protein expressed in multiple tissues like bone, periodontal ligament, skin and various cancers [24, 25]. Periostin interacts with integrin molecule on the cell surface, mediating cell adhesion and migration of various kinds of cells. In periodontal tissues, periostin is localized between the cytoplasmic processes of cementoblasts/periodontal fibroblasts and the adjacent collagen fibers [26]. Periostin can induce cell proliferation and cell migration of periodontal ligament (PDL) cells by activating the PI3K/AKT signaling pathway (higher phosphorylation of AKT and the ribosomal protein S6) [27].

Cartilage oligomeric matrix protein (COMP) is another kind of matrix-cellular protein that is firstly detected in cartilage tissues [28]. Recent researches have revealed that COMP is essential in different diseases such as bone tissue disorders and atherosclerosis [29, 30]. Combination of recombinant angiopoietin 1 (Ang1), an important factor for endothelial survival and proliferation [31], COMP (COMP-Ang1) can promote the migration of periodontal ligament stem cells (PDLSCs) through the activation of PI3K/AKT signaling pathway [32]. Moreover, fibroblast growth factor-2 can stimulate the directed migration of PDLSCs via PI3K/AKT pathway [33].

4.3.3 Eph-Ephrin Signaling Pathway

The EphB-EphrinB family consists of contact-dependent molecules that mediate various inhibitory or repulsive cellular responses depending on the model of signaling. The EphB-EphrinB family has shown to be expressed in tooth development and plays critical roles in dental cell migration and tooth repair. EphrinB1 expression is downregulated in the dental pulp tissue of injured tooth, and it can inhibit the migration of DPSCs in vitro [18, 34]. EphB-EphrinB molecules are paramount for the perivascular DPSCs migration toward the dentin surfaces and differentiation into functional odontoblasts after the injury of dentin matrix [34]. The interaction between EphB and its corresponding ephrin ligand (EphrinB) is required for the attachment, spreading and migration of human DPSCs in its niche. However, the major role of EphB-EphrinB pathway in these processes is the induction of inhibitory responses [18].

Other signaling pathway may also interact with the Eph signaling pathway. EphrinB1-induced DPSCs migration inhibition can be partially reversed by the suppression of MAPK signaling pathway [18]. The actions of PI3K signaling pathway on endothelial cell migration and proliferation can be mediated by EphB receptors [35].

4.4 Signaling Pathways in DSC Proliferation

DSCs have a long-term proliferation capacity and generate many identical copies of themselves, which are regulated by several related signaling pathways.

4.4.1 MAPK Signaling Pathway

MAPK pathway consists of many signaling molecules that can be activated by diverse extracellular stimuli. Activation of MAPK pathway can give rise to a variety of physiological effects, including cell apoptosis and proliferation. Many studies have revealed that chemical and mechanical stress can affect the proliferation of DSCs via activation of MAPK signaling pathway. For instance, cisplatin, a commonly used chemotherapeutic agent, can induce a greater genotoxic stress response in DPSCs in comparison to human dermal fibroblasts (HDFs). Cisplatin in higher concentrations can initiate the activation of all three main MAPK families (*e.g.*, ERK, JNK and p38) and cell apoptosis in DPSCs [36]. Dental tissues are subjected to various kinds of mechanical stress such as compression fluid-sheer stress and uniaxial vertical and horizontal stretch during jaw movement and occlusal forces. Mechanical stress can activate several intracellular signals such as MAPK through mechanoreceptors [37, 38]. Mechanical stretch can enhance the proliferation while suppressing the osteogenic differentiation of DPSCs. The stretch significantly enhances the phosphorylation of AKT, ERK1/2, and p38 MAPK as well as upregulating the proliferation of DPSCs [39].

Epiregulin (EREG), a member of epidermal growth factor family, can enhance the proliferation ability of stem cells from apical papilla (SCAPs) by activating JNK MAPK pathway [40]. In addition, mechanical stress stimuli can augment the proliferation of SCAPs by activating ERK 1/2 and JNK pathway [41]. Some researchers have established PDL tissue model under compression, and found that the prolonged compression can inhibit the cell proliferation by the activation of MAPK pathway [42].

4.4.2 PI3K/AKT Signaling Pathway

PI3K pathway is one of the key pathways in the regulation of crucial cellular processes such as cell survival, growth, migration, apoptosis, transcription and translation. Stem cell factor (SCF), one of the prominent homing factors, can bind to c-Kit receptor (CD117) and recruit stem cells toward homing sites [43]. Both SCF and c-Kit are highly expressed in differentiation of DPSCs. SCF treatment in dental pulp progenitors may enhance the phosphorylation of ERK and/or AKT, and stimulate the cyclin D3 and CDK4 (cell cycle proteins) expression in DPSCs [44]. In addition, the increasing fluid shear stress (FSS) and periostin may regulate the proliferation of human PDLSCs via the PI3K/AKT/mTOR signaling axis [27, 45].

4.4.3 NF-κB Signaling Pathway

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that modifies the transcription of DNA in almost all animal cell types. Canonical NF-κB pathway is regulated by the inhibition of IκB kinase complex (IKK-a, IKK-b and IKK-c). The IKK complex phosphorylates/degrades the IκB, and releases NF-κB subunits, mainly p65 and p50. These phosphorylated subunits enter the cell nucleus and bind to DNA, which subsequently bring about a variety of biological processes including cell proliferation, cell apoptosis, and cell differentiation. Moreover, NF-κB signaling pathway is greatly involved in the process of DSC proliferation. DPSCs derived from injured pulps present a lower proliferative capacity than normal DPSCs, and this process is proposed to be related with NF-κB signaling pathway [46]. Moreover, donor sodium nitroprusside (SNP) can induce nitric oxide (NO) production, and downregulate the proliferation of hPDLSCs. Blockade of NF-κB signaling suppresses the SNP-induced growth inhibition, showing that the influence of NO on the proliferation of hPDLSCs is conducted by NF-κB signaling pathway [47].

4.4.4 Notch Signaling Pathway

Notch signaling governs the cell fate determination of adult and embryonic tissues. The Notch ligand, Delta1, is known to affect the proliferation and differentiation of various tissue specific stem cells. Studies have revealed that Notch receptors and Delta1 ligand are identified and expressed in DPSCs. The proliferation index (PI) and colonies of dental pulp cells are significantly upregulated in Delta1 transduced DPSCs than the control groups (wt- and vector transduced DPSCs). Therefore, it can be proposed that Notch-Delta1 signaling is essentially associated with the proliferation of DSCs [48].

4.4.5 Wnt/β-Catenin Signaling Pathway

The canonical Wnt pathway is a key component in the induction of epithelial-mesenchymal interactions, and actively participates in tooth morphogenesis and development. WNT10A, a member of Wnt family, can promote the proliferation ability and negatively regulate the odontoblastic differentiation of DPSCs [49]. Moreover, the canonical Wnt/β-catenin pathway can facilitate the proliferation of SCAPs [50]. In addition, bioactive scaffolds containing lithium ions can enhance the proliferation of PDLSCs via the activation of Wnt/β-catenin pathway [51]. Recent studies have revealed that stress-associated periodontal disturbance may be due to GC-induced changes in PDLSCs. Dexamethasone treatment can induce the expression of several genes including dickkopf-1 (DKK-1) in PDLSCs, and then inhibit Wnt-mediated activation of β-catenin signaling as well as their growth rate [52].

4.4.6 Other Signaling Pathways

TGF-β2 may influence the growth and differentiation of DPSCs through an autocrine way via the activation of ALK/Smad2/3-signal transduction pathways [53]. Small molecules (Pluripotin (SC1), 6-bromoindirubin-3-oxime and rapamycin) can decrease the DPSC proliferation, which may be mediated by mTOR signaling pathway [54]. ITGA5 down-regulation inhibits the proliferative capacity of hDPSCs, and promotes their odontogenic differentiation, suggesting that ITGA5 signaling pathway can negatively affect the odontogenic differentiation of hDPSCs and may help hDPSCs to remain in a proliferative and undifferentiated state [55].

4.5 Signaling Pathways in DSC Differentiation

DSCs are undifferentiated cells that have a special capacity to differentiate into specialized cell types. More and more studies have found that many kinds of signaling pathways are involved in the multiple differentiation abilities of DSCs.

4.5.1 TGF-β Signaling Pathway

TGF-β1 is a multifunctional cytokine and intimately involved in the metabolism of several tissues, including dental pulps. TGF-β signaling pathway is crucial for epithelial-mesenchymal interactions, especially in those vital interactions during tooth morphogenesis. Interaction of TGF-β with the membrane TGF-β receptor I

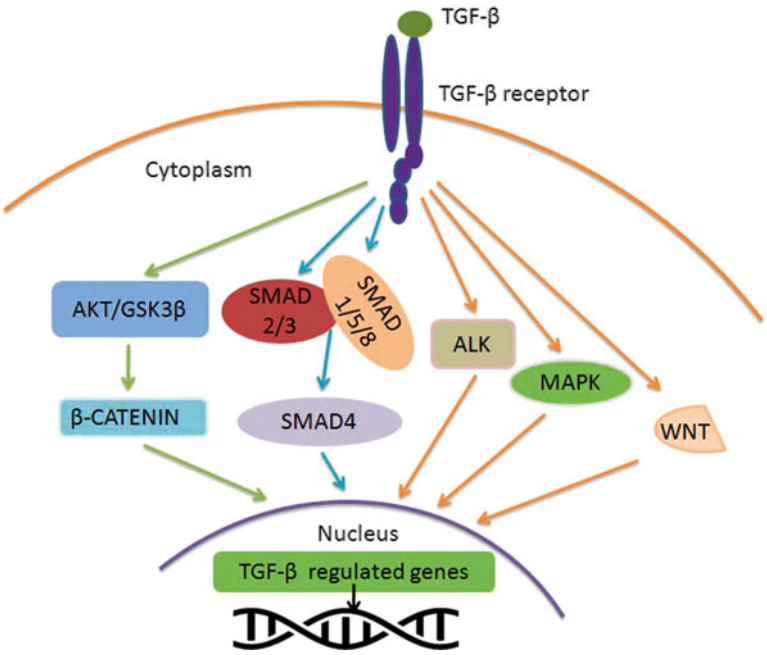

Fig. 4.4 TGF-β signaling pathway. TGF-β signaling pathway is crucial for the tooth morphogenesis and repair. Interaction of TGF-β with the membrane TGF-β receptor I and II mediates the activities of multiple kinds of signaling pathways (*e.g.*, MAPK, Wnt, Smad and PI3K/AKT pathways), and then regulates the expression levels of TGF-β related genes via the cascade interactions among these pathways

and II mediates the activities of multiple kinds of signaling pathways, and then regulates the expression levels of TGF-β related genes via the cascade interactions among these pathways (Fig. 4.4). TGF-β1, TGF-β2, and a small quantity of TGF-β3 mRNAs are expressed in DPSCs [53]. TGF-β receptors I/II are both expressed in odontoblasts and pulp cells, and they response to subtle variations in expression levels and participate in the tissues' response to injury [56].

Exogenous TGF-β2 can upregulate the expression levels of nestin and dentin sialophosphoprotein (DSPP) in DPSCs, indicating that TGF-β signaling controls the odontoblast differentiation and dentin formation ability during tooth morphogenesis [57]. TGF-β2 possibly mediates the differentiation of DPSCs at specific stages, which cooperates with other factors through multiple signaling pathways, especially with the ALK/Smad2/3-signal transduction pathways [53].

TGF-β signaling also participates in nerve growth factor (NGF) regulation during pulp tissue repair. TGF-β can up-regulate NGF in hDPSCs via p38 and JNK MAPK pathways [58]. Some studies suggest that TGF-β1 can inhibit the proliferation of SCAPs and their mineralization by decreasing the osteogenic/dentinogenic gene expressions [59]. In detail, TGF-β1 promotes the cell growth, collagen content

and ALP activity at lower concentrations (0.1–1 ng/mL) but down-regulates the activity at higher concentrations (>5 ng/mL) by regulating ERK1/2 and Smad2 signaling pathways [60].

4.5.2 BMPs Signaling Pathway

BMP2 and BMP4 genes are proved to be expressed and play essential roles during embryonic tooth development. The BMP2 gene is also expressed in post-natal odontoblasts and ameloblasts during tooth differentiation period from birth to approximately 3 weeks after birth. Dentin-derived BMP2 possesses the ability to drive the differentiation of DSCs from exfoliated deciduous teeth (SHEDs) into mature dentin-forming odontoblasts [61]. BMP2 transcripts are restricted in dental papillae, and remarkably upregulated during odontoblastic differentiation [62].

Both SHEDs and adult DPSCs express BMP receptors, including BMPR-IA, BMPR-IB and BMPR-II. The blockade of BMP2 signaling inhibits the expression of odontoblastic differentiation markers in SHEDs. Similarly, BMP2 drives the differentiation of SHEDs into odontoblasts [63]. Some studies suggest that lentiviral-mediated BMP2 gene transfection can accelerate the odontogenic differentiation capability of human SCAPs in vitro [64]. Meanwhile, hPDLSCs/rAd-BMP2 effectively promote the osteogenesis both in vitro and in vivo. Thus, hPDLSCs/rAd-BMP2 can be applied in a novel therapeutic approach for the regeneration of deteriorated bony defects [65].

BMP7 can induce the gene expression of several markers of cementoblasts and cementocytes, such as protein tyrosine phosphatase-like member/cementum attachment protein (PTPLA/CAP) and cementum protein 1 (CEMP1) [66]. BMP7 treatment upregulates the transcription of Sp7/Osterix and PTPLA/CAP by binding to specific short motifs termed as GC-rich Smad-binding elements (GC-SBEs) located in the human PTPLA/CAP and CEMP1 promoter. The gene expression levels of RUNX2 and ALP are increased afterward while the expression of odontogenic markers such as DSPP, bone sialoprotein (BSP) and dentin matrix acidic phosphoprotein 1 (DMP1) are not affected [67].

4.5.3 NF-κB Signaling Pathway

NF-κB signaling not only participates in regulating immune responses and inflammation, but also plays critical roles in differentiation of MSCs including DSCs (Fig. 4.5). NF-κB signaling pathway is activated in case of estrogen deficiency and subsequently decreases the osteo/odontogenic differentiation of DPSCs. Inhibitors of the NF-κB effectively rescues the down-regulated differentiation potential of DPSCs [68]. DPSCs derived from the injured pulps exhibit the robust osteogenic potential and weak odontogenic capacity as compared with healthy DPSCs. The

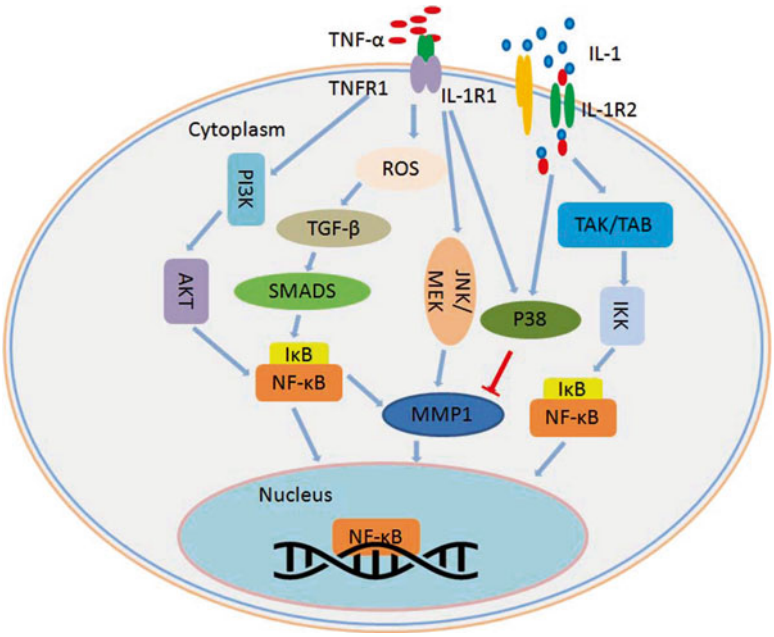

Fig. 4.5 NF-κB signaling pathway. Small molecules like TNF-α and IL-1 can induce PI3K/AKT, TAK/TAB/IKK and JNK/MEK/MMP1 signaling pathways in the cytoplasm. All these signalings will converge to the IκB/NF-κB at the cytoplasmic level and then translocate to the nucleus to regulate the expression of NF-κB target genes

inhibitors of NF-κB pathway can reverse the process that the osteogenic potential of DPSCs is significantly reduced while the odontogenic differentiation is enhanced. Therefore, the NF-κB signaling pathway can be proposed to be associated with the osteo/odentogenic differentiation of DPSCs [46].

LPS can activate TLR4, and regulate NF-κB pathway of human PDLSCs, leading to decrease in osteogenic potential. Thus, blockage of TLR4 or NF-κB pathway might provide a new approach for periodontitis treatment [69]. NF-κB pathway-activated SCAPs present higher proliferation/migration capacity and increased odonto/osteogenic ability than control cells. Likewise, NF-κB pathway-suppressed SCAPs inversely display lower proliferation/migration ability as well as decreased odonto/osteogenic ability than control group [70].

4.5.4 MAPK Signaling Pathway

p38-MAPK is involved in the inflammatory response of PDLSCs during the chronic periodontitis in which p38 is strongly induced in PDLSCs derived from the infected periodontal tissues. The p38 inhibition markedly suppresses the osteogenic differentiation of PDLSCs in a chronic inflammatory microenvironment [71].

Natural mineralized scaffolds (*e.g.*, demineralized dentin matrix-DDM, ceramic bovine bone-CBB) can induce DPSCs to exhibit higher levels of ALP activity and mRNA expression of osteo/odontogenetic markers than other scaffolds via the activation of MAPK signaling pathway. However, the inhibitors of ERK1/2 and p38 can down-regulate the odontogenic differentiation ability of DPSCs cultured on DDM and CBB [72]. BMP9 can promote the bone formation of PDLSCs. p38 and ERK1/2 MAPKs are involved in BMP9-induced osteogenic differentiation of PDLSCs. The inhibitors of ERK1/2 and p38 increase BMP9-induced osteogenic differentiation of PDLSCs [73]. Moreover, IGF-1can induce the phosphorylation of ERK and JNK in PDLSCs, and promote the osteogenic differentiation of PDLSCs, suggesting the involvement of MAPK signaling pathway in the IGF-1-based differentiation of PDLSCs [74]. Stretch can increase the proliferation rate of DPSCs via the activation of ERK pathway, and inhibit the osteogenic differentiation in which PI3K/AKT and ERK pathways are partly involved [39]. Mechanical stress can enhance the odonto/osteogenic differentiation of SCAPs via the activation of ERK 1/2 and JNK MAPK signaling pathways [75]. In addition, hypoxia can affect the osteogenic potential, mineralization and paracrine release of therapeutic factors from PDLSCs, and the process is closely related to ERK and p38 MAPK signaling pathways [76].

MAPK signaling pathway also plays an important role in the revascularization of dental-pulp complex. LPS stimulates the expression level of vascular endothelial growth factor (VEGF) in DPSCs and human dental pulp fibroblasts via ERK1/2 MAPK signaling pathway [77].

4.5.5 mTOR Signaling Pathway

mTOR kinase is the catalytic subunit of at least two distinct signaling complexes: target of rapamycin complex 1 and 2 (TORC 1 and 2) [78]. TORC 1 is a popular regulator of protein translation [79], and is essential for cell growth, cell proliferation, and cell cycle. On the other hand, TORC 2 is involved in the cytoskeleton reorganization and cell survival [78]. In the concept of DSC differentiation, the mTOR signaling pathway is activated in the process of osteogenic differentiation of hDPSCs [80].

Both TORC1 and TORC2 play critical roles in the modulation of DPSCs while TORC1 is essential in SHEDs differentiation. Inhibition of the TORC1 complex proteins (mTOR or raptor) can effectively decrease the mineralized matrix deposition of SHEDs. Conversely, when the TORC2 complex proteins are downregulated, both mineralization and differentiation markers are increased in SHEDs. Furthermore, the increased mineralization of SHEDs is dependent on functioning TORC1 complex [81].

Pluripotin can affect the maintenance of hDPSCs properties, decreasing cell proliferation, increasing the expression of STRO-1, NANOG, OCT4, and SOX2, and diminishing cell differentiation through various signaling pathways including mTOR-signaling pathway [54].

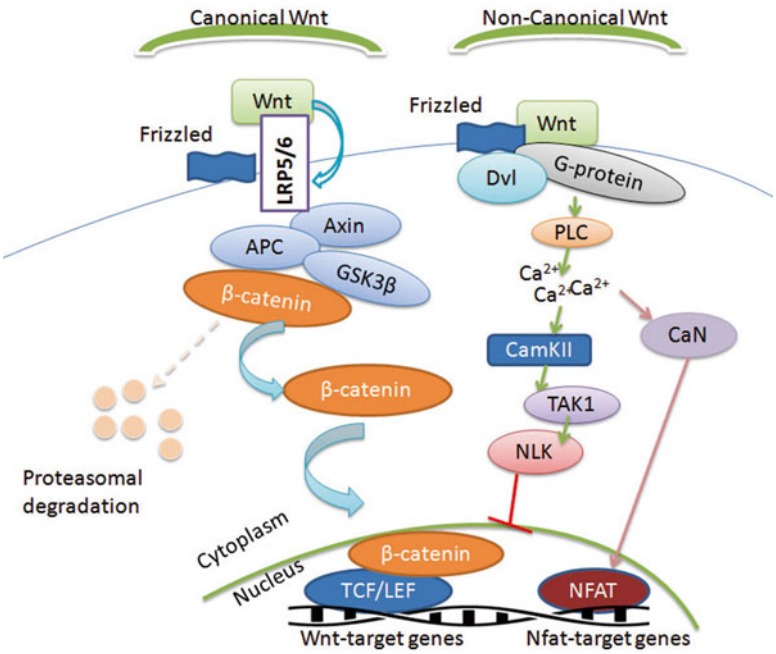

Fig. 4.6 Wnt/β-catenin signaling pathway. Wnt signaling pathway is divided into the canonical Wnt signaling and non-canonical Wnt signaling. The former plays a crucial role in tooth development. Wnt protein binds its receptor Frizzled and co-receptor LRP5/6, and stimulates the LRP5/6 phosphorylation. Phosphorylated LRP5/6 recruits Axin to the membrane and disrupts the Axin complex that containing APC and GSK3β. GSK3β phosphorylates β-catenin, subsequently, the phosphorylated β-catenin enters the nucleus, where it binds TCF/LEF and co-activators, and activates the downstream gene expression

4.5.6 Wnt/β-Catenin Signaling Pathway

Nineteen Wnt family proteins are divided into two main categories, canonical and non-canonical wnt signaling pathways, based on their role in cytosolic β-catenin stabilization [82]. Canonical Wnt signaling transduces their signals via regulation of β-catenin levels and is thought to be of much importance in the tooth development and self-renewal of stem cells (Fig. 4.6).

After transduction with canonical Wnt-1 by retrovirus-mediated infection, matrix-cellular protein osteopontin and type I collagen are upregulated while ALP activity and the mineralization of DPSCs are inhibited. Over-expression of β-catenin can effectively inhibit the differentiation and mineralization of DPSCs, indicating that DPSC differentiation is downregulated via the activation of Wnt/β-catenin signaling pathway [83]. Wnt3A effectively induces ALP activity in immortalized SCAPs (iSCAPs), and BMP9 also induces the expression of osteocalcin and osteopontin as well as matrix mineralization of iSCAPs. Moreover, BMP9 and Wnt3A

can act synergistically, and their ability to induce the osteo/odontogenic differentiation will be diminished by knockdown of β-catenin [84].

Zinc-bioglass (ZnBG) incorporated within calcium phosphate cements (CPC) can activate the odontogenic differentiation and promote the angiogenesis of DPSCs in vitro. ZnBG upregulates the integrins and their downstream signaling pathways including canonical and non-canonical Wnt signaling pathways [85].

After osteogenic genes in PDLSCs are increased by down-regulating anti-differentiation noncoding RNA (ANCR), the osteogenic differentiation of PDLSCs is improved. When the canonical WNT signaling pathway is suppressed, the osteogenic differentiation of PDLSC/ANCR-RNAi cells is inhibited too, indicating that Wnt/β-catenin signaling pathway may play a crucial role in the ANCR-mediated osteogenic differentiation of PDLSCs [86]. Nicotine and TNF-α can induce the osteogenic differentiation deficiency of PDLSCs by activating WNT signaling [87, 88], and down-regulation of β-catenin level can activate the non-canonical Wnt/Ca^{2+} pathway, leading to the promotion of osteogenic differentiation in PDLSCs [89]. The β-catenin also plays an important role in the osteo/odontogenic differentiation of SCAPs. Silencing of β-catenin in SCAPs can reduce BMP9/WNT3A-induced expression of osteocalcin/osteopontin and matrix mineralization in vitro and ectopic bone formation in vivo [90].

4.5.7 Other Signaling Pathways

Shh signaling pathway is related to cell differentiation and osteogenesis which is negatively modulated by BMP signaling. It can repress the osteo/dentinogenic differentiation of SCAPs [91]. Moreover, Notch signaling also participates in the odontoblastic differentiation of DSCs [2], which permits DPSCs differentiating into odontoblast-like cells in the appropriate inductive conditions. Notch signaling pathway is also important in maintaining the correct balance between proliferation and differentiation of DPSCs. Activation of Notch signaling by Delta1 ligand can enhance the proliferation and odontogenic ability of DPSCs due to the increasing of the proliferation index (PI), DSPP protein expression level and calcified nodules number in Delta1-DPSCs [48]. However, another study reports that the activation of Notch signaling by either Jagged1 or N1ICD can depress the differentiation of DPSCs into odontoblasts without interrupting cell proliferation [92]. In addition, Notch signaling pathway modulates the osteogenic differentiation of dental follicle stem cells (DFSCs) [93]. Therefore, we can conclude that distinct Notch ligand may induce different effects of Notch signaling on the differentiation of DSCs. The mechanism of these distinct effects remains puzzled and needs more explorations.

Trichostatin A (TSA) is an efficient histone deacetylase (HDAC) inhibitor with a wide spectrum of epigenetic activities known to mediate many kinds of cellular behaviors, including MSC differentiation. It can significantly upregulate the expression levels of phospho-Smad2/3, Smad4, and nuclear factor I-C, while specific

inhibitor of Smad3 suppresses TSA-based differentiation of hDPSCs, suggesting that Smad signaling pathway is also involved in the differentiation of DPSCs [94].

Basic FGF has been found to increase the neurosphere size and upregulate the expression of neurogenic markers of DPSCs. Inhibition of FGFR or Phospholipase Cγ (PLCγ) signaling can abolish the basic FGF-mediated neuronal differentiation of DPSCs [95].

4.6 Signaling Pathway Networks

Crosstalk between cellular processes and molecular signaling pathways is frequent in any biological system. Signaling pathways can affect each other synergistically in maintaining cell survival, apoptosis, proliferation, differentiation as well as other cellular processes of DSCs.

Some similar stem cell–related genes can be detected in DPSCs and PDLSCs during their odontogenic/osteogenic differentiation. The genes exhibit considerable overlap with minor difference between DPSCs and PDLSCs. Numerous regulatory genes in odonto/osteogenic differentiation interact or crosstalk through Notch, Wnt, TGF-β/BMP, and cadherin signaling pathways [96]. Extracellular phosphate (Pi) can regulate the BMP2 expression level by cAMP/protein kinase A and ERK1/2 MAPK signaling pathways in human DPSCs [97]. TGF-β1 can downregulate the differentiation ability of human DPSCs through ALK5/Smad2/3 signaling pathways [98]. Furthermore, p38 MAPK pathway is involved in regulating ALP activity of hDPSCs and may interact with Smad pathway [99]. As the main element of many pulp capping materials, calcium ions can upregulate the odontoblastic differentiation and mineralization of DPSCs. Calcium ions activate the BMP2-mediated Smad1/5/8 and ERK1/2 pathways to control the odontoblastic differentiation of DPSCs in which Smad1/5/8 and ERK1/2 signaling converge at Runx2 in DPSCs [100].

5' adenosine monophosphate-activated protein kinase (AMPK), AKT and mTOR signaling pathways act synergistically in the differentiation process of human DPSCs. AMPK, the upstream mechanism of AKT and mTOR signaling pathways, can regulate the osteogenic differentiation of human DPSCs via both early mTOR suppression-modulated autophagy and late activation of AKT/mTOR signaling axis. AKT inhibition restrains mTOR activation without influencing AMPK phosphorylation [101]. PIN1, a peptidyl-prolyl cis/trans isomerase, acts as an important modulator of odontogenic and adipogenic differentiation of hDPSCs. BMP, Wnt/β--catenin, MAPK and NF-κB pathway are involved in PIN1-mediated differentiation of hDPSCs [102]. Moreover, WNT5α mRNA and protein expressions rapidly increased in response to LPS treatment in a time- and dose-dependent manner. LPS-induced WNT5α expression is mediated through the TLR4/MyD88/PI3K/AKT signaling pathways, which subsequently activate NF-κB signaling pathway in hDPSCs [103].

4.7 Conclusions and Prospects

Overall data has shown that the maintenance, proliferation, migration, and differentiation of DSCs are regulated by a variety of signaling pathways. Although larger amount of recent studies have led to rapid expansion of knowledge of signaling molecular mechanisms in stem cell biology, this field is still full of confusions and challenges. The complex signaling networks participating in the homeostasis, migration, proliferation and differentiation of DSCs are still in its infancy. DSCs are thought to be an appropriate and sufficient candidate for tooth regeneration. However, their clinical applications remain much immature and difficult. Therefore, more laboratorial and clinical researches need to be conducted to explore the further pathway mechanisms, which are important to clarify the signaling-related behaviors of dental stem cells. Moreover, the upstream and downstream transcription factors as well as their detailed functions in these signaling pathways should be extensively investigated, so that we can easily and efficiently smooth the potential difficulties in stem cell-based tooth regeneration.

Acknowledgements This work was supported by National Natural Science Foundation of China (No. 81371144), Qing Lan Project (2014–23), Zhou Shan 5313 Project, Natural Science Foundation of Jiangsu Province (No. BK20131392), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, No. 2014–37).

References

1. Lovschall H, Mitsiadis TA, Poulsen K, Jensen KH, Kjeldsen AL (2007) Coexpression of Notch3 and Rgs5 in the pericyte-vascular smooth muscle cell axis in response to pulp injury. Int J Dev Biol 51(8):715–721. doi:10.1387/ijdb.072393hl
2. Cai X, Gong P, Huang Y, Lin Y (2011) Notch signalling pathway in tooth development and adult dental cells. Cell Prolif 44(6):495–507. doi:10.1111/j.1365-2184.2011.00780.x
3. Mitsiadis TA, Lardelli M, Lendahl U, Thesleff I (1995) Expression of Notch 1, 2 and 3 is regulated by epithelial-mesenchymal interactions and retinoic acid in the developing mouse tooth and associated with determination of ameloblast cell fate. J Cell Biol 130(2):407–418
4. Mitsiadis TA, Henrique D, Thesleff I, Lendahl U (1997) Mouse Serrate-1 (Jagged-1): expression in the developing tooth is regulated by epithelial-mesenchymal interactions and fibroblast growth factor-4. Development 124(8):1473–1483
5. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I (1999) Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 147(1):105–120
6. Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, Kato S, Sekine K, Ohuchi H (2002) FGF10 maintains stem cell compartment in developing mouse incisors. Development 129(6):1533–1541
7. Biehs B, Hu JK, Strauli NB, Sangiorgi E, Jung H, Heber RP, Ho S, Goodwin AF, Dasen JS, Capecchi MR, Klein OD (2013) BMI1 represses Ink4a/Arf and Hox genes to regulate stem cells in the rodent incisor. Nat Cell Biol 15(7):846–852. doi:10.1038/ncb2766
8. Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, Chai Y (2014) Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. Cell Stem Cell 14(2):160–173. doi:10.1016/j.stem.2013.12.013

9. Zhang J, Huang FF, Wu DS, Li WJ, Zhan HE, Peng MY, Fang P, Cao PF, Zhang MM, Zeng H, Chen FP (2015) SUMOylation of insulin-like growth factor 1 receptor, promotes proliferation in acute myeloid leukemia. Cancer Lett 357(1):297–306. doi:10.1016/j.canlet.2014.11.052
10. Jiang Q, Du J, Yin X, Shan Z, Ma Y, Ma P, Du J, Fan Z (2013) Shh signaling, negatively regulated by BMP signaling, inhibits the osteo/dentinogenic differentiation potentials of mesenchymal stem cells from apical papilla. Mol Cell Biochem 383(1–2):85–93
11. Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298(5600):1911–1912. doi:10.1126/science.1072682
12. Williams DW, Wu H, Oh JE, Fakhar C, Kang MK, Shin KH, Park NH, Kim RH (2013) 2-Hydroxyethyl methacrylate inhibits migration of dental pulp stem cells. J Endod 39(9):1156–1160. doi:10.1016/j.joen.2013.06.004
13. Vandomme J, Touil Y, Ostyn P, Olejnik C, Flamenco P, El Machhour R, Segard P, Masselot B, Bailliez Y, Formstecher P, Polakowska R (2014) Insulin-like growth factor 1 receptor and p38 mitogen-activated protein kinase signals inversely regulate signal transducer and activator of transcription 3 activity to control human dental pulp stem cell quiescence, propagation, and differentiation. Stem Cells Dev 23(8):839–851. doi:10.1089/scd.2013.0400
14. Kim SM, Kim DS, Jeong CH, Kim DH, Kim JH, Jeon HB, Kwon SJ, Jeun SS, Yang YS, Oh W, Chang JW (2011) CXC chemokine receptor 1 enhances the ability of human umbilical cord blood-derived mesenchymal stem cells to migrate toward gliomas. Biochem Biophys Res Commun 407(4):741–746. doi:10.1016/j.bbrc.2011.03.093
15. Chang J, Zhang C, Tani-Ishii N, Shi S, Wang CY (2005) NF-kappaB activation in human dental pulp stem cells by TNF and LPS. J Dent Res 84(11):994–998
16. Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127(8):1671–1679
17. Smith A, Robinson V, Patel K, Wilkinson DG (1997) The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. Curr Biol 7(8):561–570
18. Stokowski A, Shi S, Sun T, Bartold PM, Koblar SA, Gronthos S (2007) EphB/ephrin-B interaction mediates adult stem cell attachment, spreading, and migration: implications for dental tissue repair. Stem Cells 25(1):156–164. doi:10.1634/stemcells.2006-0373
19. Huang C, Jacobson K, Schaller MD (2004) MAP kinases and cell migration. J Cell Sci 117(Pt 20):4619–4628. doi:10.1242/jcs.01481
20. Kajiya M, Shiba H, Komatsuzawa H, Ouhara K, Fujita T, Takeda K, Uchida Y, Mizuno N, Kawaguchi H, Kurihara H (2010) The antimicrobial peptide LL37 induces the migration of human pulp cells: a possible adjunct for regenerative endodontics. J Endod 36(6):1009–1013. doi:10.1016/j.joen.2010.02.028
21. Li D, Fu L, Zhang Y, Yu Q, Ma F, Wang Z, Luo Z, Zhou Z, Cooper PR, He W (2014) The effects of LPS on adhesion and migration of human dental pulp stem cells in vitro. J Dent 42(10):1327–1334. doi:10.1016/j.jdent.2014.07.007
22. Su Y, Xie W, Wang C, Peng L, Zhou X, Ye L (2012) JNK/P38 mitogen-activated protein kinase used for hepatocyte growth factor-induced proliferation, differentiation, and migration in human dental papilla cells. J Endod 38(9):1207–1213. doi:10.1016/j.joen.2012.06.011
23. Zhang J, Zhu LX, Cheng X, Lin Y, Yan P, Peng B (2015) Promotion of dental pulp cell migration and pulp repair by a bioceramic putty involving FGFR-mediated signaling pathways. J Dent Res. doi:10.1177/0022034515572020
24. Takeshita S, Kikuno R, Tezuka K, Amann E (1993) Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J 294(Pt 1):271–278
25. Zhou HM, Wang J, Elliott C, Wen W, Hamilton DW, Conway SJ (2010) Spatiotemporal expression of periostin during skin development and incisional wound healing: lessons for

human fibrotic scar formation. J Cell Commun Signal 4(2):99–107. doi:10.1007/s12079-010-0090-2
26. Polizzotti BD, Arab S, Kuhn B (2012) Intrapericardial delivery of gelfoam enables the targeted delivery of Periostin peptide after myocardial infarction by inducing fibrin clot formation. PloS one 7(5), e36788. doi:10.1371/journal.pone.0036788
27. Padial-Molina M, Volk SL, Rios HF (2014) Periostin increases migration and proliferation of human periodontal ligament fibroblasts challenged by tumor necrosis factor -alpha and Porphyromonas gingivalis lipopolysaccharides. J Periodontal Res 49(3):405–414. doi:10.1111/jre.12120
28. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, Sommarin Y, Wendel M, Oldberg A, Heinegard D (1992) Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 267(9):6132–6136
29. Wang L, Zheng J, Du Y, Huang Y, Li J, Liu B, Liu CJ, Zhu Y, Gao Y, Xu Q, Kong W, Wang X (2010) Cartilage oligomeric matrix protein maintains the contractile phenotype of vascular smooth muscle cells by interacting with alpha(7)beta(1) integrin. Circ Res 106(3):514–525. doi:10.1161/CIRCRESAHA.109.202762
30. Das BR, Roy A, Khan FR (2015) Cartilage oligomeric matrix protein in monitoring and prognostication of osteoarthritis and its utility in drug development. Perspect Clin Res 6(1):4–9. doi:10.4103/2229-3485.148792
31. Abdel-Malak NA, Srikant CB, Kristof AS, Magder SA, Di Battista JA, Hussain SN (2008) Angiopoietin-1 promotes endothelial cell proliferation and migration through AP-1-dependent autocrine production of interleukin-8. Blood 111(8):4145–4154. doi:10.1182/blood-2007-08-110338
32. Kook SH, Lim SS, Cho ES, Lee YH, Han SK, Lee KY, Kwon J, Hwang JW, Bae CH, Seo YK, Lee JC (2014) COMP-angiopoietin 1 increases proliferation, differentiation, and migration of stem-like cells through Tie-2-mediated activation of p38 MAPK and PI3K/Akt signal transduction pathways. Biochem Biophys Res Commun 455(3–4):371–377. doi:10.1016/j.bbrc.2014.11.025; S0006-291X(14)02035-X [pii]
33. Shimabukuro Y, Terashima H, Takedachi M, Maeda K, Nakamura T, Sawada K, Kobashi M, Awata T, Oohara H, Kawahara T, Iwayama T, Hashikawa T, Yanagita M, Yamada S, Murakami S (2011) Fibroblast growth factor-2 stimulates directed migration of periodontal ligament cells via PI3K/AKT signaling and CD44/hyaluronan interaction. J Cell Physiol 226(3):809–821. doi:10.1002/jcp.22406
34. Arthur A, Koblar S, Shi S, Gronthos S (2009) Eph/ephrinB mediate dental pulp stem cell mobilization and function. J Dent Res 88(9):829–834. doi:10.1177/0022034509342363
35. Steinle JJ, Meininger CJ, Chowdhury U, Wu G, Granger HJ (2003) Role of ephrin B2 in human retinal endothelial cell proliferation and migration. Cell Signal 15(11):1011–1017
36. Seifrtova M, Havelek R, Cmielova J, Jiroutova A, Soukup T, Bruckova L, Mokry J, English D, Rezacova M (2012) The response of human ectomesenchymal dental pulp stem cells to cisplatin treatment. Int Endod J 45(5):401–412. doi:10.1111/j.1365-2591.2011.01990.x
37. Cook B, Hardy RW, McConnaughey WB, Zuker CS (2008) Preserving cell shape under environmental stress. Nature 452(7185):361–364. doi:10.1038/nature06603
38. Kanzaki M, Nagasawa M, Kojima I, Sato C, Naruse K, Sokabe M, Iida H (1999) Molecular identification of a eukaryotic, stretch-activated nonselective cation channel. Science 285(5429):882–886
39. Hata M, Naruse K, Ozawa S, Kobayashi Y, Nakamura N, Kojima N, Omi M, Katanosaka Y, Nishikawa T, Tanaka Y, Matsubara T (2013) Mechanical stretch increases the proliferation while inhibiting the osteogenic differentiation in dental pulp stem cells. Tissue Eng Part A 19(5–6):625–633. doi:10.1089/ten.tea.2012.0099
40. Cao Y, Xia DS, Qi SR, Du J, Ma P, Wang SL, Fan ZP (2013) Epiregulin can promote proliferation of stem cells from the dental apical papilla via MEK/Erk and JNK signalling pathways. Cell Prolif 46(4):447–456. doi:10.1111/cpr.12039

41. Mu C, Lv T, Wang Z, Ma S, Ma J, Liu J, Yu J, Mu J (2014) Mechanical stress stimulates the osteo/odontoblastic differentiation of human stem cells from apical papilla via erk 1/2 and JNK MAPK pathways. Biomed Res Int 2014:494378. doi:10.1155/2014/494378
42. Li Y, Li M, Tan L, Huang S, Zhao L, Tang T, Liu J, Zhao Z (2013) Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression. Arch Oral Biol 58(5):511–522. doi:10.1016/j.archoralbio.2012.10.006
43. Yu XY, Geng YJ, Li XH, Lin QX, Shan ZX, Lin SG, Song YH, Li Y (2009) The effects of mesenchymal stem cells on c-kit up-regulation and cell-cycle re-entry of neonatal cardiomyocytes are mediated by activation of insulin-like growth factor 1 receptor. Mol Cell Biochem 332(1–2):25–32. doi:10.1007/s11010-009-0170-x
44. Pan S, Dangaria S, Gopinathan G, Yan X, Lu X, Kolokythas A, Niu Y, Luan X (2013) SCF promotes dental pulp progenitor migration, neovascularization, and collagen remodeling - potential applications as a homing factor in dental pulp regeneration. Stem Cell Rev 9(5):655–667. doi:10.1007/s12015-013-9442-7
45. Qi L, Zhang Y (2014) The microRNA 132 regulates fluid shear stress-induced differentiation in periodontal ligament cells through mTOR signaling pathway. Cell Physiol Biochem 33(2):433–445. doi:10.1159/000358624
46. Wang Y, Yan M, Wang Z, Wu J, Zheng Y, Yu J (2013) Dental pulp stem cells from traumatically exposed pulps exhibited an enhanced osteogenic potential and weakened odontogenic capacity. Arch Oral Biol 58(11):1709–1717. doi:10.1016/j.archoralbio.2013.09.001
47. Lee SK, Choi HI, Yang YS, Jeong GS, Hwang JH, Lee SI, Kang KH, Cho JH, Chae JM, Kim YC, Kim EC (2009) Nitric oxide modulates osteoblastic differentiation with heme oxygenase-1 via the mitogen activated protein kinase and nuclear factor-kappaB pathways in human periodontal ligament cells. Biol Pharm Bull 32(8):1328–1334
48. He F, Yang Z, Tan Y, Yu N, Wang X, Yao N, Zhao J (2009) Effects of Notch ligand Delta1 on the proliferation and differentiation of human dental pulp stem cells in vitro. Arch Oral Biol 54(3):216–222. doi:10.1016/j.archoralbio.2008.10.003
49. Zhang Z, Guo Q, Tian H, Lv P, Zhou C, Gao X (2014) Effects of WNT10A on proliferation and differentiation of human dental pulp cells. J Endod 40(10):1593–1599. doi:10.1016/j.joen.2014.07.009
50. Wang J, Liu B, Gu S, Liang J (2012) Effects of Wnt/beta-catenin signalling on proliferation and differentiation of apical papilla stem cells. Cell Prolif 45(2):121–131. doi:10.1111/j.1365-2184.2012.00806.x
51. Han P, Wu C, Chang J, Xiao Y (2012) The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/beta-catenin signalling pathway by Li+ ions released from bioactive scaffolds. Biomaterials 33(27):6370–6379. doi:10.1016/j.biomaterials.2012.05.061
52. Choi SS, Park EK, Kwack MH, Sung YK (2015) Effects of dexamethasone, a synthetic glucocorticoid, on human periodontal ligament stem cells. Naunyn Schmiedebergs Arch Pharmacol. doi:10.1007/s00210-015-1151-0
53. Tai TF, Chan CP, Lin CC, Chen LI, Jeng JH, Chang MC (2008) Transforming growth factor beta2 regulates growth and differentiation of pulp cells via ALK5/Smad2/3. J Endod 34(4):427–432. doi:10.1016/j.joen.2008.02.007
54. Al-Habib M, Yu Z, Huang GT (2013) Small molecules affect human dental pulp stem cell properties via multiple signaling pathways. Stem Cells Dev 22(17):2402–2413. doi:10.1089/scd.2012.0426
55. Cui L, Xu S, Ma D, Gao J, Liu Y, Yue J, Wu B (2014) The role of integrin-alpha5 in the proliferation and odontogenic differentiation of human dental pulp stem cells. J Endod 40(2):235–240. doi:10.1016/j.joen.2013.08.011
56. Sloan AJ, Couble ML, Bleicher F, Magloire H, Smith AJ, Farges JC (2001) Expression of TGF-beta receptors I and II in the human dental pulp by in situ hybridization. Adv Dent Res 15:63–67

57. Oka S, Oka K, Xu X, Sasaki T, Bringas P Jr, Chai Y (2007) Cell autonomous requirement for TGF-beta signaling during odontoblast differentiation and dentin matrix formation. Mech Dev 124(6):409–415. doi:10.1016/j.mod.2007.02.003
58. Yongchaitrakul T, Pavasant P (2007) Transforming growth factor-beta1 up-regulates the expression of nerve growth factor through mitogen-activated protein kinase signaling pathways in dental pulp cells. Eur J Oral Sci 115(1):57–63. doi:10.1111/j.1600-0722.2007.00420.x
59. He W, Zhang J, Niu Z, Yu Q, Wang Z, Zhang R, Su L, Fu L, Smith AJ, Cooper PR (2014) Regulatory Interplay between NFIC and TGF-beta 1 in Apical Papilla-derived Stem Cells. J Dent Res 93(5):496–501. doi:10.1177/0022034514525200
60. Chang HH, Chang MC, Wu IH, Huang GF, Huang WL, Wang YL, Lee SY, Yeh CY, Guo MK, Chan CP, Hsien HC, Jeng JH (2015) Role of ALK5/Smad2/3 and MEK1/ERK signaling in transforming growth factor beta 1-modulated growth, collagen turnover, and differentiation of stem cells from apical papilla of human tooth. J Endod 41(8):1272–1280. doi:10.1016/j.joen.2015.03.022
61. Vainio S, Karavanova I, Jowett A, Thesleff I (1993) Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell 75(1):45–58
62. Lyons KM, Pelton RW, Hogan BL (1990) Organogenesis and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenetic protein-2A (BMP-2A). Development 109(4):833–844
63. Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nor JE (2010) Dentin-derived BMP-2 and odontoblast differentiation. J Dent Res 89(6):603–608. doi:10.1177/0022034510364487
64. Zhang W, Zhang X, Ling J, Liu W, Zhang X, Ma J, Zheng J (2014) Proliferation and odontogenic differentiation of BMP2 genetransfected stem cells from human tooth apical papilla: an in vitro study. Int J Mol Med 34(4):1004–1012. doi:10.3892/ijmm.2014.1862
65. Jung IH, Lee SH, Jun CM, Oh N, Yun JH (2014) Characterization of the enhanced bone regenerative capacity of human periodontal ligament stem cells engineered to express the gene encoding bone morphogenetic protein 2. Tissue Eng Part A 20(15–16):2189–2199. doi:10.1089/ten.TEA.2013.0648
66. Torii D, Konishi K, Watanabe N, Goto S, Tsutsui T (2015) Cementogenic potential of multipotential mesenchymal stem cells purified from the human periodontal ligament. Odontology 103(1):27–35. doi:10.1007/s10266-013-0145-y
67. Torii D, Tsutsui TW, Watanabe N, Konishi K (2014) Bone morphogenetic protein 7 induces cementogenic differentiation of human periodontal ligament-derived mesenchymal stem cells. Odontology. doi:10.1007/s10266-014-0182-1
68. Wang Y, Yan M, Yu Y, Wu J, Yu J, Fan Z (2013) Estrogen deficiency inhibits the odonto/osteogenic differentiation of dental pulp stem cells via activation of the NF-kappaB pathway. Cell Tissue Res 352(3):551–559. doi:10.1007/s00441-013-1604-z
69. Li CH, Li B, Dong ZW, Gao L, He XN, Liao L, Hu CH, Wang QT, Jin Y (2014) Lipopolysaccharide differentially affects the osteogenic differentiation of periodontal ligament stem cells and bone marrow mesenchymal stem cells through Toll-like receptor 4 mediated nuclear factor kappa B pathway. Stem Cell Res Ther 5. doi:Artn 67; Doi10.1186/Scrt456
70. Li J, Yan M, Wang Z, Jing S, Li Y, Liu G, Yu J, Fan Z (2014) Effects of canonical NF-kappaB signaling pathway on the proliferation and odonto/osteogenic differentiation of human stem cells from apical papilla. Biomed Res Int 2014:319651. doi:10.1155/2014/319651
71. Nie J, Zhang B, Gu B, Liu N (2015) Effects of p38 mitogen-activated protein kinase on osteogenic differentiation of human periodontal ligament stem cells in inflammatory microenvironment. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 37(1):1–7
72. Zhang H, Liu S, Zhou Y, Tan J, Che H, Ning F, Zhang X, Xun W, Huo N, Tang L, Deng Z, Jin Y (2012) Natural mineralized scaffolds promote the dentinogenic potential of dental pulp stem cells via the mitogen-activated protein kinase signaling pathway. Tissue Eng Part A 18(7–8):677–691. doi:10.1089/ten.TEA.2011.0269

73. Ye G, Li C, Xiang X, Chen C, Zhang R, Yang X, Yu X, Wang J, Wang L, Shi Q, Weng Y (2014) Bone morphogenetic protein-9 induces PDLSCs osteogenic differentiation through the ERK and p38 signal pathways. Int J Med Sci 11(10):1065–1072
74. Yu Y, Mu J, Fan Z, Lei G, Yan M, Wang S, Tang C, Wang Z, Yu J, Zhang G (2012) Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways. Histochem Cell Biol 137(4):513–525. doi:10.1007/s00418-011-0908-x
75. Mu C, Lv T, Wang Z, Ma S, Ma J, Liu J, Yu J, Mu J (2014) Mechanical stress stimulates the osteo/odontoblastic differentiation of human stem cells from apical papilla via erk 1/2 and JNK MAPK pathways. Biomed Res Int 2014:494378
76. Wu Y, Yang Y, Yang P, Gu Y, Zhao Z, Tan L, Zhao L, Tang T, Li Y (2013) The osteogenic differentiation of PDLSCs is mediated through MEK/ERK and p38 MAPK signalling under hypoxia. Arch Oral Biol 58(10):1357–1368
77. Botero TM, Son JS, Vodopyanov D, Hasegawa M, Shelburne CE, Nor JE (2010) MAPK signaling is required for LPs-induced VEGF in pulp stem cells. J Dent Res 89(3):264–269. doi:10.1177/0022034509357556
78. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307(5712):1098–1101. doi:10.1126/science.1106148
79. Foster KG, Fingar DC (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. J Biol Chem 285(19):14071–14077. doi:10.1074/jbc.R109.094003
80. Feng X, Huang D, Lu X, Feng G, Xing J, Lu J, Xu K, Xia W, Meng Y, Tao T, Li L, Gu Z (2014) Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. Dev Growth Differ 56(9):615–624. doi:10.1111/dgd.12179
81. Kim JK, Baker J, Nor JE, Hill EE (2011) mTor plays an important role in odontoblast differentiation. J Endod 37(8):1081–1085. doi:10.1016/j.joen.2011.03.034
82. He B, Barg RN, You L, Xu Z, Reguart N, Mikami I, Batra S, Rosell R, Jablons DM (2005) Wnt signaling in stem cells and non-small-cell lung cancer. Clin Lung Cancer 7(1):54–60. doi:10.3816/CLC.2005.n.022
83. Scheller EL, Chang J, Wang CY (2008) Wnt/beta-catenin inhibits dental pulp stem cell differentiation. J Dent Res 87(2):126–130
84. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He TC, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154. doi:10.1016/j.biomaterials.2014.11.007
85. Zhang J, Park YD, Bae WJ, El-Fiqi A, Shin SH, Lee EJ, Kim HW, Kim EC (2014) Effects of bioactive cements incorporating zinc-bioglass nanoparticles on odontogenic and angiogenic potential of human dental pulp cells. J Biomater Appl. doi:10.1177/0885328214550896
86. Jia Q, Jiang W, Ni L (2015) Down-regulated non-coding RNA (lncRNA-ANCR) promotes osteogenic differentiation of periodontal ligament stem cells. Arch Oral Biol 60(2):234–241
87. Zhou Z, Li B, Dong Z, Liu F, Zhang Y, Yu Y, Shang F, Wu L, Wang X, Jin Y (2013) Nicotine deteriorates the osteogenic differentiation of periodontal ligament stem cells through alpha7 nicotinic acetylcholine receptor regulating Wnt pathway. PLoS One 8(12), e83102
88. Liu W, Konermann A, Guo T, Jager A, Zhang L, Jin Y (2014) 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(3):1125–1134
89. Liu N, Shi S, Deng M, Tang L, Zhang G, Liu N, Ding B, Liu W, Liu Y, Shi H, Liu L, Jin Y (2011) High levels of beta-catenin signaling reduce osteogenic differentiation of stem cells in inflammatory microenvironments through inhibition of the noncanonical Wnt pathway. J Bone Miner Res 26(9):2082–2095

90. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He T-C, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154
91. Jiang Q, Du J, Yin X, Shan Z, Ma Y, Ma P, Fan Z (2013) Shh signaling, negatively regulated by BMP signaling, inhibits the osteo/dentinogenic differentiation potentials of mesenchymal stem cells from apical papilla. Mol Cell Biochem 383(1–2):85–93. doi:10.1007/s11010-013-1757-9
92. Zhang C, Chang J, Sonoyama W, Shi S, Wang CY (2008) Inhibition of human dental pulp stem cell differentiation by Notch signaling. J Dent Res 87(3):250–255. doi:87/3/250 [pii]
93. Viale-Bouroncle S, Gosau M, Morsczeck C (2014) NOTCH1 signaling regulates the BMP2/DLX-3 directed osteogenic differentiation of dental follicle cells. Biochem Biophys Res Commun 443(2):500–504. doi:10.1016/j.bbrc.2013.11.120
94. Jin H, Park JY, Choi H, Choung PH (2013) HDAC inhibitor trichostatin A promotes proliferation and odontoblast differentiation of human dental pulp stem cells. Tissue Eng Part A 19(5–6):613–624. doi:10.1089/ten.TEA.2012.0163
95. Osathanon T, Nowwarote N, Pavasant P (2011) Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCgamma signaling pathway. J Cell Biochem 112(7):1807–1816. doi:10.1002/jcb.23097
96. Liu L, Ling J, Wei X, Wu L, Xiao Y (2009) Stem cell regulatory gene expression in human adult dental pulp and periodontal ligament cells undergoing odontogenic/osteogenic differentiation. J Endod 35(10):1368–1376. doi:10.1016/j.joen.2009.07.005
97. Tada H, Nemoto E, Foster BL, Somerman MJ, Shimauchi H (2011) Phosphate increases bone morphogenetic protein-2 expression through cAMP-dependent protein kinase and ERK1/2 pathways in human dental pulp cells. Bone 48(6):1409–1416. doi:10.1016/j.bone.2011.03.675
98. Lin PS, Chang MC, Chan CP, Lee SY, Lee JJ, Tsai YL, Tseng HC, Tai TF, Lin HJ, Jeng JH (2011) Transforming growth factor beta1 down-regulates Runx-2 and alkaline phosphatase activity of human dental pulp cells via ALK5/Smad2/3 signaling. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 111(3):394–400. doi:10.1016/j.tripleo.2010.09.079
99. Wang FM, Hu T, Zhou X (2006) p38 mitogen-activated protein kinase and alkaline phosphatase in human dental pulp cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102(1):114–118. doi:10.1016/j.tripleo.2005.08.007
100. Li S, Hu J, Zhang G, Qi W, Zhang P, Li P, Zeng Y, Zhao W, Tan Y (2015) Extracellular Ca2+ promotes odontoblastic differentiation of dental pulp stem cells via BMP2-mediated Smad1/5/8 and Erk1/2 pathways. J Cell Physiol 230(9):2164–2173. doi:10.1002/jcp.24945
101. Pantovic A, Krstic A, Janjetovic K, Kocic J, Harhaji-Trajkovic L, Bugarski D, Trajkovic V (2013) Coordinated time-dependent modulation of AMPK/Akt/mTOR signaling and autophagy controls osteogenic differentiation of human mesenchymal stem cells. Bone 52(1):524–531. doi:10.1016/j.bone.2012.10.024
102. Lee YM, Shin SY, Jue SS, Kwon IK, Cho EH, Cho ES, Park SH, Kim EC (2014) The role of PIN1 on odontogenic and adipogenic differentiation in human dental pulp stem cells. Stem Cells Dev 23(6):618–630. doi:10.1089/scd.2013.0339
103. He WX, Wang ZH, Zhou ZY, Zhang YQ, Zhu QL, Wei KW, Lin Y, Cooper PR, Smith AJ, Yu Q (2014) Lipopolysaccharide enhances Wnt5a expression through toll-like receptor 4, myeloid differentiating factor 88, phosphatidylinositol 3-OH kinase/AKT and nuclear factor kappa B pathways in human dental pulp stem cells. J Endod 40(1):69–75