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REVIEW ARTICLE

Wnt and BMP signaling crosstalk in regulating dental stem cells: Implications in dental tissue engineering

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Abstract Tooth is a complex hard tissue organ and consists of multiple cell types that are regulated by important signaling pathways such as Wnt and BMP signaling. Serious injuries and/or loss of tooth or periodontal tissues may significantly impact aesthetic appearance, essential oral functions and the quality of life. Regenerative dentistry holds great promise in treating oral/dental disorders. The past decade has witnessed a rapid expansion of our understanding of the biological features of dental stem cells, along with the signaling mechanisms

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governing stem cell self-renewal and differentiation. In this review, we first summarize the biological characteristics of seven types of dental stem cells, including dental pulp stem cells, stem cells from apical papilla, stem cells from human exfoliated deciduous teeth, dental follicle precursor cells, periodontal ligament stem cells, alveolar bone-derived mesenchymal stem cells (MSCs), and MSCs from gingiva. We then focus on how these stem cells are regulated by bone morphogenetic protein (BMP) and/or Wnt signaling by examining the interplays between these pathways. Lastly, we analyze the current status of dental tissue engineering strategies that utilize oral/dental stem cells by harnessing the interplays between BMP and Wnt pathways. We also highlight the challenges that must be addressed before the dental stem cells may reach any clinical applications. Thus, we can expect to witness significant progresses to be made in regenerative dentistry in the coming decade.

Introduction

Tooth decay and tooth loss are common dental disorders. There is an increasing clinical need to develop predictable and effective therapeutic strategies to regenerate dental tissues and restore their morphology and functions.[1,2] Thus, dental tissue engineering is an attractive therapeutic strategy that complements traditional restorative techniques and benefits from recent advances in stem cell biology, molecular biology, genomics, and proteomics.[3] One of the most important components of successful regenerative dentistry involves in the use of adequate stem cells that are stimulated with appropriate biological factors for tissue/lineage-specific differentiation. Adult mesenchymal stem cells (MSCs) have been isolated from tooth or tooth-related tissues. Such repertoires of stem cells provide gold opportunity for effective regeneration of oral tissues.[4] In this review, we aim to provide an overview of the current knowledge of utilizing dental stem cells and harnessing the crosstalk between bone morphogenetic protein (BMP) and Wnt signaling pathways as novel and efficacious therapeutic strategies in dental tissue regeneration.

Tooth development and dental stem cells

Tooth development

Teeth arise from sequential and reciprocal interactions between oral ectoderm-derived oral epithelium and underlying cranial neural crest (CNC)-derived mesenchyme.[5] Tooth-specific hard tissues include enamel, dentin and cementum, which are formed by differentiated ameloblasts, odontoblasts and cementoblasts at the junction between the epithelium and mesenchyme (Fig. 1). The earliest morphological sign is the primary dental lamina giving rise to a thickening oral epithelium at the site of the future tooth row, which is followed by formation of dental placodes along the dental lamina.

During early tooth development, interactions between oral epithelium and underlying mesenchyme govern dental morphogenesis through successive bud, cap and bell stages.[6] Then, the tooth formation shifts from oral epithelium to underlying mesenchyme prior to the bud stage.[7] The inner dental epithelial cells differentiate into enamel-producing ameloblasts. The dental papilla mesenchymal cells differentiate into dentin-secreting odontoblasts, while the remaining dental papilla cells (DPCs) form dental pulp.[7] Mechanistically, the differentiation of ameloblasts and odontoblasts are a well-coordinated process (Fig. 1).

Once the crown is formed, root formation begins in most teeth with the formation of cementum by dental follicle mesenchyme-derived cementoblasts. Both root elongation and tooth eruption require resorption of surrounding alveolar bone.[6] Eventually, majority of epithelial tissue is lost when teeth erupt into oral cavity and reach final length. Interestingly, the rodent incisor grows continuously throughout the life, although the regenerative capacity of mammalian teeth is generally limited. Continuous growth of the incisor throughout life requires adult epithelial stem cells that give rise to enamel-forming ameloblasts,[8] and MSCs that lead to the dentin and cementum regeneration.[9–11]

The soft-hard tissue connection of periodontal ligament–cementum complex (PLCC) is originated from interactions between epithelial cells of Hertwig's epithelial root sheath (HERS) and mesenchymal cells of dental follicle (DFCs).[12] DFCs surrounding enamel organ differentiate into cementoblasts that line the root, as well as fibroblasts and osteoblasts that generate the periodontal ligament and alveolar bone supporting the tooth.[13] Thus, reciprocal interactions between epithelial and mesenchymal compartments are critical for tooth morphogenesis and maintenance of dental stem cell niches.

Dental stem cells

Epithelial and mesenchymal cells are necessary to produce a new functional tooth (Fig. 1).[14] Cells forming the tooth are derived from ectodermal epithelium and neural crest ectomesenchyme.[15] During development and regeneration, the stem cells derived from ectodermal epithelium give rise to ameloblasts and produce enamel after the first round of dentin formation by odontoblasts (Fig. 1). Neural crest ectomesenchyme stem cells consist of dental-derived MSCs

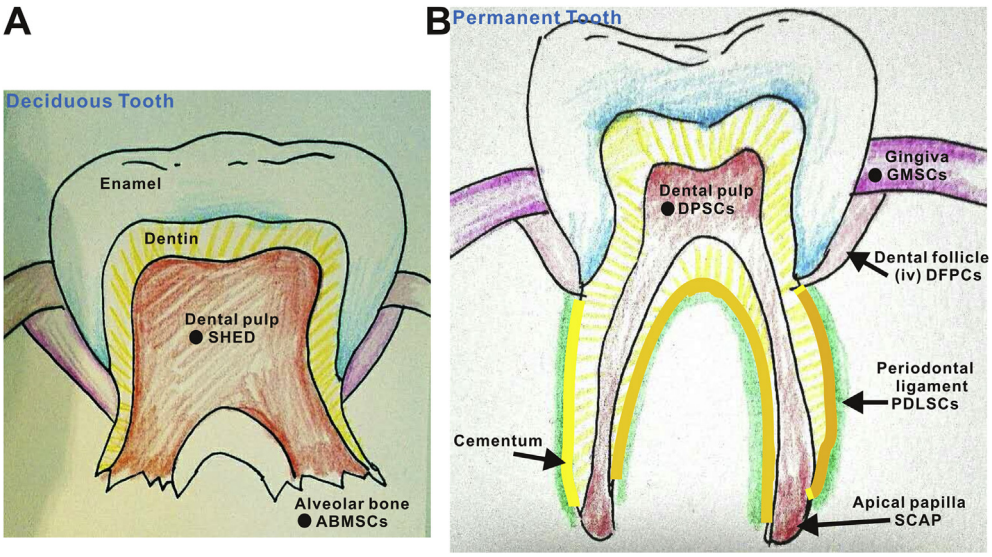

Figure 1 Anatomical locations of different types of dental stem cells in deciduous tooth (**A**) and permanent tooth (**B**). DPSCs, dental pulp stem cells; SCAP, stem cells from apical papilla; SHED, stem cells from human exfoliated deciduous teeth; DFPCs, dental follicle precursor cells; PDLSCs, periodontal ligament stem cells; ABMSCs, alveolar bone-derived mesenchymal stem cells; and GMSCs, MSCs from gingiva.

(DMSCs) and non-dental-derived MSCs (NDMSCs), both of which possess the capacity for dental and periodontal regeneration under certain conditions.[16] Subsets of neural crest-derived cells (NCDCs) also remain as stem cells into adulthood.[17] NCDCs in adults may be used as a stem cell source for tissue regeneration.

Dental-derived mesenchymal stem cells (DMSCs)

DMSCs are isolated from various dental tissues (Fig. 1). These cells can undergo multi-lineage differentiation including osteogenic and odontogenic differentiation, providing an alternative source of MSCs for tissue engineering.[18] Although DMSCs present some common markers, such as CD105, CD146 and STRO-1, stem cells derived from various tissues exhibit heterogeneous capabilities of proliferation, clonogenicity, and differentiation potential *in vitro* and *in vivo*.[19] Currently, DMSCs populations are composed of at least seven types of stem cells: (a) dental pulp stem cells (DPSCs), (b) stem cells from apical papilla (SCAP), (c) stem cells from human exfoliated deciduous teeth (SHEDs), (d) dental follicle precursor cells (DFPCs), (e) periodontal ligament stem cells (PDLSCs),[19–24] (f) alveolar bone-derived MSCs (ABMSCs), and (g) MSCs from gingiva (GMSCs) (Fig. 1).[25]

(1) DPSCs are multipotent stem cells that reside in the cell-rich zone of both adult pulp tissue and deciduous tooth pulp and apical papilla.[26,27] DPSCs can be readily isolated from discarded or removed teeth, offering a promising and attractive source of autologous stem cells.[28] They can differentiate along multiple cell lineages and promote the regeneration of dental pulp, dentin, and cementum,[29] such as the generation of complete or partial tooth structures as biological implants.[28] DPSCs can also actively proliferate, repair bone and give rise to other tissues.[30,31] Interestingly, human DPSCs also exhibit major neuroregenerative activities, demonstrating that tooth-derived stem cells may provide therapeutic benefits for treating spinal cord injury.[32] Nonetheless, it was postulated that dental pulp may be the only source of progenitor cells with dentinogenic potential for regenerating dentin–pulp complex.[33]

(2) SCAPs are isolated from soft tissue at the apices of developing permanent teeth and share significant similarities with DPSCs. Clinical attempts to preserve the remaining DPSCs and SCAPs lead to canal revascularization and completion of root maturation in young permanent teeth.[31]

(3) SHEDs are derived from the pulp tissue of an exfoliating deciduous tooth. They represent a postnatal stem cell population with high proliferative capacity, easy accessibility, high viability and multi-lineage differentiation potential (e.g., osteoblasts, neural cells, and odontoblasts).[34,35] Therefore, SHEDs have been widely used for oromaxillofacial bone regeneration.[36]

(4) DFPCs are derived from ectomesenchymal tissue surrounding enamel organ and dental papilla of developing tooth prior to eruption.[37] DFPCs are potential stem cells for cementoblasts, osteoblasts and periodontal ligament cells. They interact with HERS cells during tooth root formation.[38] When co-cultured with HERS cells, DFPCs exhibited a greater tendency to form mineralized nodules and higher levels of cementoblast/osteoblast differentiation.[12]

(5) PDLSCs are derived from DFPCs and are isolated from the mixed cell populations in the periodontal ligament space. Human PDLSCs possess high osteogenic and cementogenic differential ability.[37]

(6) ABMSCs also originate from DFPCs and are dental progenitor cells of alveolar osteoblasts.[37]
(7) GMSCs are ideal stem cells for repairing damaged periodontal tissues, muscle, and tendon,[25] although it remains unclear if PDLSCs and GMSCs could form a dentin—pulp-like structure.

Non-dental-derived mesenchymal stem cells (NDMSCs)

The main stem cell populations of NDMSCs include BMSCs,[20] MSCs from peripheral nerve-associated glia, adipose tissue-derived MSCs (ADSCs), and induced pluripotent stem cells (iPSCs).[39—42] BMSCs exhibit capacity for adipogenic and osteogenic differentiation.[43] Additionally, they have demonstrated the ability to undergo odontogenic differentiation in the context of a pulp extracellular matrix (ECM) scaffold.[44] Peripheral glial cells can produce pulp cells and odontoblasts.[45] ADSCs are particularly attractive and have been shown to differentiate into teeth, bone, or cartilage.[46]

Oral stem and mucosal cells may also serve as an ideal source for reprogrammed cells including iPSCs.[34,47,48] iPSCs have characteristics similar to embryonic stem cells, and the use of patient-derived iPSCs may avoid host immunological rejection and ethical controversy.[49] Thus, successful generation of iPSCs would provide great promise in the development of regenerative medicine, including tooth regeneration.[50]

Differentiation and immunomodulatory properties of MSCs

As dental stem cells share many characteristics with those of MSCs, there has been considerable interest in their wider applications to treat disorders using mesenchymal cell derivatives.[28] Dental stem cells express various markers previously thought to be specific for MSCs, embryonic stem cells and neural cells.[23] In addition, other embryonic stem cell features have been reported in both DPSCs and SHEDs although specific conditions to maintain the ability of DMSCs to initiate whole tooth formation may be required.[51] These cells have a vast repertoire of differentiation (e.g., osteogenic, odontogenic, adipogenic, and neurogenic), and are even capable of generating corneal cells and pancreatic islet cells,[52] endothelial cells,[53] and dentin—pulp complex.[25] The tooth must be vascularized, innervated and appropriately anchored in the bone,[14] while pulp revascularization is dependent on the differentiation capability of residual pulp and apical and periodontal stem cells.[54] When stimulated with Wnt1, the DPSCs were prone to neural differentiation and expressed higher levels of neurogenic markers.[55]

These MSC-like cells exhibit some unique characteristics, including immunomodulatory properties[56] and paracrine processes.[57] MSCs, including those of dental origin, can host immune response[58—60] by inhibiting T cells, inducing regulatory T cells and converting dendritic cells (DCs) and macrophages into regulatory phenotype. MSCs were shown to inhibit cell proliferation of T cells, B cells, natural killer (NK) cells and DCs, leading to division arrest anergy.[58—60] Moreover, MSCs can inhibit a variety of other immune cell functions.[61]

BMP signaling in dental stem cells

BMPs and BMP receptors (BMPRs) regulate the development of calcified tissues by directing mesenchymal stem cell differentiation.[62—68] A complex network of BMP signaling pathways and transcription factors regulates the differentiation of MSCs during development and throughout adulthood. These signaling pathways include BMP, Wnt, sonic hedgehog (Shh), Notch, fibroblast growth factor (FGF), and retinoic acid pathways, and the homeobox gene superfamily, which are key players in epithelial-mesenchymal signaling loops driving tooth development.[69,70]

BMPs signaling mechanism in dental stem cells

BMPs belong to the TGF-β superfamily of proteins,[68,71] and play a critical role in skeletal development and stem cell differentiation. More than 20 BMP-like molecules have been identified in vertebrates and invertebrates,[5,72] several of which are of great importance to dental engineering. TGF-β/BMP plays an essential role in bone development by activating BMP receptor (BMPR) serine/threonine kinases.[68] Mutations of TGF-β/BMP activity are linked to many clinical disorders, such as skeletal, extra skeletal anomalies, autoimmune, cancer, and cardiovascular diseases. Tooth development requires synchronous and spatially different BMPs expression and interaction.[73]

BMPs exert their biological functions through both canonical and non-canonical pathways. In the canonical signaling pathway, BMPs initiate the signal transduction cascade by binding to BMPRs and forming a heterotetrameric complex comprised of two dimers of type I and type II serine/threonine kinase receptors.[71] There are seven type I receptors (ALK1-7) for the TGF-β family of ligands, three of which bind BMPs: type 1A BMPR (BMPR-1A or ALK3), type 1B BMPR (BMPR-1B or ALK6), and type 1A activin receptor (ActR-1A or ALK2).[74] The heterotetrameric signaling complex formation can vary and is less well defined. For example, BMP6 and BMP7 interact with BMPR-2 and recruit BMPR-1, whereas BMP2 and BMP4 preferentially bind BMPR-1 and recruit BMPR-2.[75] Phosphorylation of TGF-β (I/II) or BMPRs activates Smads. The signaling network in skeletal development and bone formation is complex and tempo-spatial specific.[76]

BMP signaling plays an essential role in early tooth development and disruptions of BMP signaling cause early arrested tooth development.[77] The essential role of BMPR-1A and BMPR-1B in tooth development is demonstrated in a tissue-specific manner.[78] CNC-specific inactivation of BMPR-1A arrests tooth development at the bud/early cap stages.[78] Substitution of BMPR-1A by constitutively active form of BMPR-1B in neural crest cells rescues molar and maxillary incisor development although the rescued teeth exhibit delayed odontoblast and ameloblast differentiation.[78] BMPR-1B, -2, and the ActR-1 are detected in dental follicular and HERS cells at day 6 of periodontal development and later more diffusely in the periodontium,[79] while

BMPR-1A expression is restricted to alveolar bone, consistent with a report indicating that STRO-1 positive DFCs may be targets of BMPs secreted by HERS.[79]

While BMP signaling plays a pivotal role in craniofacial organ and tooth development, canonical BMP signaling may not operate in early developing tooth. Although pSmad1 is highly expressed in the dental follicle, HERS, and the periodontium,[79] the absence of pSmad1/5/8-Smad4 complex may be caused by saturation of Smad4 by pSmad2/3 in the dental mesenchyme.[77] Silencing Smad2/3 or overexpression of Smad4 leads to the formation of pSmad1/5/8-Smad4 complexes, and subsequently activates canonical BMP signaling in dental mesenchymal cells.[77]

BMPs are potent regulators of not only bone, but also cartilage formation and repair, cell proliferation during development and adult bone homeostasis.[80] In tooth development, tight regulation of BMPR-1A signaling is essential.[81] Juglone-mediated inhibition of PIN1 augments the osteogenic medium (OM)-induced activation of BMPs, Wnt/β-catenin, ERK, JNK, and nuclear factor-kappa B (NF-κB) pathway, suggesting that PIN1 may function as an important modulator of odontogenic and adipogenic differentiation of DPSCs.[82] However, these odontogenic and osteogenic effects by BMPs can be reversed by deletion of key regulators in BMPs signaling pathways. First, deletion of Smad4 leads to defective odontoblast differentiation and dentin formation.[83] Melatonin-induced BMP2 expression and Smad1/5/8 phosphorylation can be blocked by noggin. Furthermore, melatonin activates p38MAPK, ERK, and NF-κB in hDPSCs, and these actions can be attenuated by inhibitors of BMP.[84]

BMP signaling is modulated by various factors and pathways.[76] ADSCs from caALK2$^{+/-}$ mice had increased BMP signaling and activated pSmad 1/5.[85] Mothers against Smad and MAPK pathways are also involved in BMP signaling, and their actions are regulated by intracellular and extracellular proteins and small molecules. Extracellular phosphate (Pi) regulates BMP2 expression via cAMP/PKA and extracellular signal-regulated kinase (ERK)1/2 pathways in human DPSCs.[86] Negative regulators of BMP signaling can block the signal transduction at multiple levels, including decoy receptors, inhibitory intracellular binding proteins, and inducers of BMP ubiquitination.[87] Furthermore, several non-canonical, Smad-independent signaling pathways for BMPs have been identified. For example, BMP4 was found to activate TAK-1 signaling.[88] It has also been demonstrated that BMPR-1 and BMP2 may principally regulate Fshb expression in LβT2 cells via noncanonical activation of Smad2/3 signaling.[89]

Diverse roles of BMPs in regulating osteogenic/odontogenic differentiation

BMP2 can give rise to osteogenic and odontogenic differentiation in autologous or allogeneic DMSCs/NDMSCs-based engineering dentistry. BMP signaling was shown to significantly accelerate in SHEDs.[90] BMP2 has been used as a surface coating on scaffolds, decreasing pore size and causing better adhesion and reduced proliferation of BMP-MSCs.[91] Recombinant human (rh)BMP2 is effective in establishing complete regeneration of a boney defect by 4–6 months, as assessed by intraoperative observations and histologic studies.[92] Histomorphometric analysis indicates that the use of rhBMP2 in bone repair without the use of bone grafting materials should offer new strategies for osseous reconstruction of facial bone defects.[92] Osteogenic differentiation in 3D micro-tissues is enhanced by strong integrin-ECM interactions and by stronger autocrine BMP2 signaling.[93] BMP2 has been detected in HERS cells, dental follicular cells, and in differentiated periodontal cells.[79] Local application of BMP4 in epithelium of molar territories either stimulates Islet1 expression, while inhibition of BMP signaling results in a loss of Islet1 expression.[94] BMP7 has been detected in HERS cells, dental follicular cells, and in differentiated periodontal cells.[79]

Through a comprehensive analysis of the 14 types of human BMPs, we demonstrated that BMP9 (aka, GDF2) is one of the most potent BMPs in promoting osteoblastic differentiation of MSCs both *in vitro* and *in vivo*.[62,63,66,68,95,96] Nonetheless BMP9 is one of the least studied BMPs and we have demonstrated that BMP9 interacts with ALK1 and ALK2 type I receptors[97] and upregulates a panel of critical downstream mediators that are involved in promoting the early stage of progenitor expansion and then late stage of terminal osteogenic differentiation of MSCs.[98–103] For example, we demonstrated that growth hormone (GH) is a direct early target of and upregulated by BMP9 signaling.[102] Furthermore, exogenous GH synergizes with BMP9 on inducing osteogenic differentiation through insulin-like growth factor 1 (IGF1) signaling, which can be significantly blunted by JAK/STAT inhibitors.[102] One potential mechanistic explanation of BMP9's potent osteogenic activity is that BMP9 can outcompete BMP antagonist noggin much more effectively than other osteogenic BMPs such as BMP2, BMP4, BMP6 and BMP7.[104] Furthermore, we demonstrated that BMP9 synergizes with several important signaling pathways, including Wnts, IGFs, EGF, Notch, and retinoic acid signaling pathways, in promoting osteogenic differentiation of MSCs.[39,66,105–109] More recently, we demonstrated that BMP9 effectively induces osteo/odontoblastic differentiation of the stem cells of dental apical papilla (SCAPs).[110]

As an inhibitor of bone formation, BMP3 expression can be detected after day 13 of periodontal development. It is conceivable that BMP3 may arrest of this process by inhibiting cementogenic and osteogenic BMPs.[79] Nonetheless, epithelial stem cell proliferation in cervical loops is controlled by an integrated regulatory network consisting of activin, BMPs, FGFs, and follistatin within incisor stem cell niches.[10] Mesenchymal FGF3 stimulates epithelial stem cell proliferation, and BMP4 represses FGF3 expression.[10] Activin inhibits the repressive effect of BMP4 and restricts FGF3 expression to labial dental mesenchyme.[10] Follistatin limits the number of lingual stem cells and contributes to the asymmetry of mouse incisors.[10]

Wnt signaling in dental stem cells

Wnt family consists of at least 19 Wnt ligands encoded in both human and mouse genomes. Wnts are secreted proteins and are among the most potent factors regulating

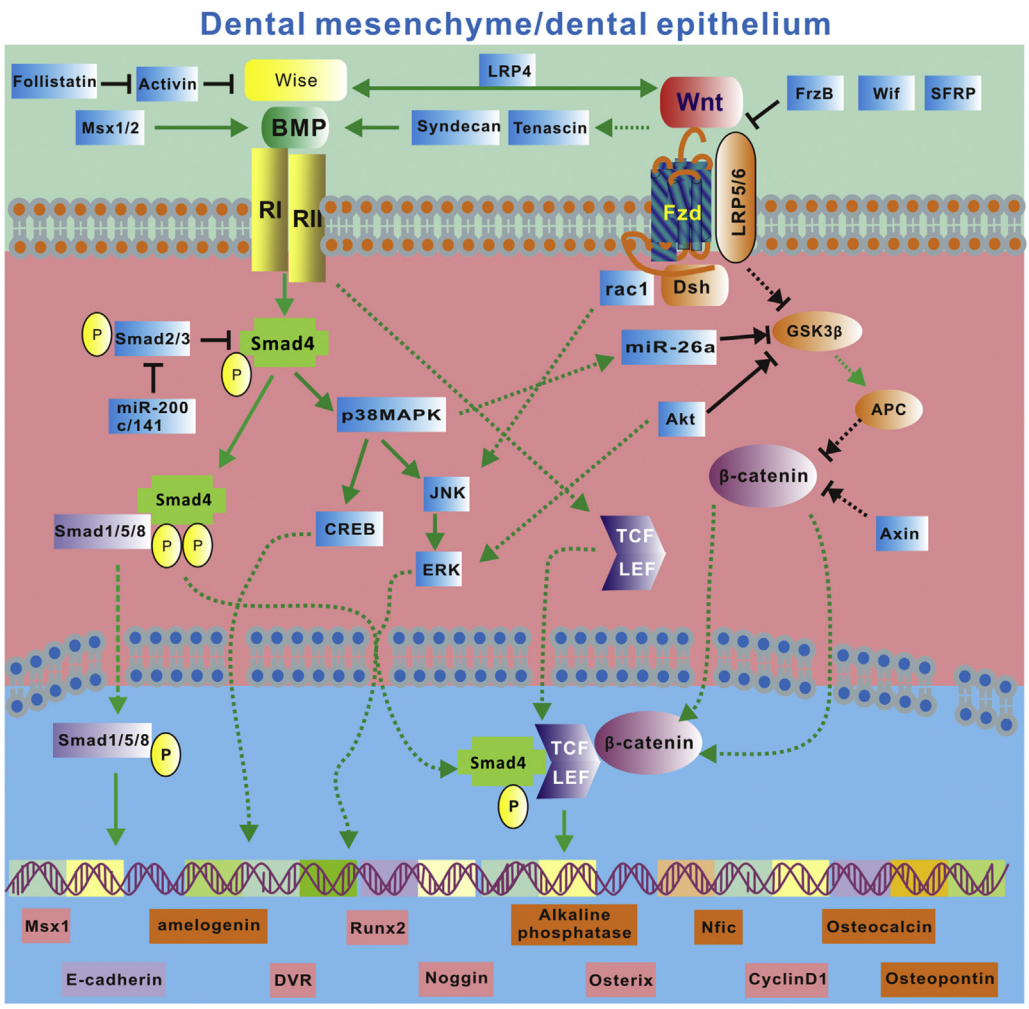

Figure 2 Wnt and BMP signaling crosstalk in regulating dental stem cell proliferation and differentiation. Green lines indicate stimulatory effects while black lines indicate inhibitory actions.

stem cell self-renewal and have tremendous potential for promoting human tissue regeneration[111–115] (Fig. 2). Wnt signaling regulates cell proliferation, migration, differentiation, apoptosis, and in epithelial-mesenchymal interactions involved in dental and periodontal tissue morphogenesis.[116] Wnt responsiveness in the craniomaxillofacial tissues was mapped and the patterns of Wnt signaling co-localize with stem cell populations in rodent incisor apex, dental pulp, alveolar bone, periodontal ligament, cementum, and oral mucosa.[117]

Wnts are secreted lipid-modified glycoproteins and short-range ligands to activate canonical and noncanonical signaling pathways.[115,118] The hallmark of canonical pathway is the activation of β-catenin-mediated transcriptional activity (Fig. 2). The canonical pathway is initiated by binding of Wnt ligand to receptor complex containing Frizzled (Frz) protein and co-receptor of low density lipoprotein receptor-related protein (LRP)5/6. Binding of Wnts to Frz and LRP-5/6 activates distinct signaling pathways.[105,115,118] Mutations in LRP-5 adversely affect skeletal development and bone mass.[119] Ligand–receptor interaction is transmitted through Dishevelled (Dsh) proteins, leading to the inhibition of a multiprotein complex containing proteins Axin, APC, PP2A, GSK3, and casein kinase 1α.[115,120] Without ligand binding, this complex facilitates phosphorylation of β-catenin, resulting in its degradation via ubiquitin–proteasome pathway. Thus, Wnt binding leads to an increase in cytoplasmic and nuclear β-catenin level, which complexes with T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors and other co-activators,[121,122] regulating downstream target genes.[115,118,123–125]

Many extracellular secreted inhibitors can modulate Wnt signaling by binding either to Wnt ligands, e.g., Wif and secreted frizzled-related protein (SFRP), or co-receptors LRP5/6 (e.g., Dkk, Wise/Sost).[121]

Canonical Wnt signaling regulates tooth number in mice and humans, while its role in tooth replacement remains to be elucidated.[118] Canonical Wnt target genes (e.g., Lef1 and Axin2) are continuously expressed in dental lamina tip and surrounding mesenchymal cells.[126] Canonical Wnt activity was shown to extend instead of thickening the python dental lamina.[126] We found that the canonical Wnt/β-catenin signaling pathway plays a critical role in BMP9-induced osteogenic differentiation of MSCs.[105] BMP9-induced ectopic bone formation and matrix mineralization are significantly inhibited by both FrzB overexpression or β-catenin knockdown,[105] suggesting that the canonical Wnt/

β-catenin pathway is a critical mediator of BMP9-mediated osteogenic signaling.

The non-canonical Wnt signaling of the planar cell polarity (PCP) pathway does not depend on β-catenin activity and is thought to transduce through NRH1, Ryk, PTK7, or ROR2 without interacting with LRPO5/6.[118] The PCP pathway is also activated via the binding of Wnt to Frz and its co-receptor. The receptor complex then recruits Dsh to interact with Dsh-associated activator of morphogenesis 1 (DAAM1). DAAM1 activates small G-protein Rho and then Rho-associated kinase (ROCK). Dsh also forms a complex with Rac1 and mediates profilin binding to actin. Rac1 activates JNK and leads to actin polymerization.[112,127] Nonetheless, it remains to be fully investigated whether non-canonical Wnt signaling plays any important roles in tooth development and in regulating the proliferation and differentiation of dental stem cells.

Crosstalk between Wnt and BMP signaling pathways in dental stem cells

Dedicatedly regulated interactions between epithelial and mesenchymal tissue components of developing teeth govern tooth morphogenesis and determine the key features of dentitions and individual teeth.[128] The development of individual tooth involves signaling networks, particularly in the BMP and Wnt signal pathways through positive and negative feedback loops (Fig. 2).[129]

At embryonic age (E) 9.5 days, BMP4 expression is detected in the epithelium of the dental lamina, follicle and papilla and decreases rapidly on E10.5 or E11.5. BMP2 expression is not prominent until E13.5, after which signal is widespread throughout the neural-crest mesenchyme.[130] BMP4 was shown to induce a translucent mesenchymal zone similar to that induced by dental epithelium, supporting a role of BMP4 in regulating epithelial-mesenchymal interactions during early tooth development.[131]

Msx, BMP, LEF1, and Activin βA were shown to co-express in coincidence of tooth phenotypes in knockout mice. Msx1 is required for BMP4 expression transition from dental epithelium to mesenchyme and for LEF1 expression.[132] Induced activity of canonical Wnt, FGF, and Shh signaling pathways rescues development of arrested mouse diastemal tooth germs as it was reported that BMP4 and Msx1 act in a positive feedback loop to drive sequential tooth formation.[133]

Physical interaction between Wise, an extracellular protein that binds BMP ligands, and Wnt-modulator LRP4 acts as a direct link the two pathways at extracellular level. Mutations in either Wise or LRP4 in mice produced similar abnormalities in tooth development that are associated with alterations in BMP and Wnt signaling.[134] During tooth development, LRP4 is expressed exclusively in epithelial cells while Wise mainly in mesenchymal cells. Wise and LRP4 act together to coordinate BMP and Wnt signaling activities in epithelial-mesenchymal cell communication during development.[134] Thus, LRP4 modulates and integrates BMP and canonical Wnt signaling during tooth development by binding Wise.[135]

During tooth and jawbone formation, TGFβ/BMP signaling regulates the fate of multipotent CNC cells and directs these cells differentiating into odontoblasts and osteoblasts. Deleting Smad4 led to defects in odontoblast differentiation and dentin formation and upregulation of canonical Wnt signaling,[83] indicating that Smad4 critically regulates crosstalk between TGFβ/BMP and Wnt signaling to ensure proper CNC cell fate.

Using iSCAP cells, we found that Wnt3A effectively induced early osteogenic markers, which was reduced by β-catenin knockdown.[136] While Wnt3A and BMP9 synergistically induced ALP activity in iSCAPs, knocking-down β-catenin diminished BMP9-induced osteo/odontogenic differentiation.[136] Furthermore, the iSCAPs stimulation with both BMP9 and Wnt3A exhibited more mature and highly mineralized trabecular bone, while knockdown of β-catenin in iSCAPs reduced BMP9 or BMP9/Wnt3A-induced ectopic bone formation *in vivo*,[136] suggesting that β-catenin may play an important role in BMP9-induced osteo/odontogenic signaling.

It has been shown that over-activation of the Wnt/β-catenin pathway delays differentiation and growth of inner dental epithelium, resulting in adult teeth presenting with altered size, morphology and mineralization due to delayed differentiation and prolonged proliferation of the dental mesenchyme.[137] BMPR-1A depletion at differentiation stage switches differentiation of crown epithelia to root lineage, giving rise to ectopic cementum-like structures.[138] This phenotype is related to activated Wnt/β-catenin signaling and epithelial-mesenchymal transition (EMT). Epithelial β-catenin depletion during differentiation stage causes variable enamel defect and precocious/ectopic formation of fragmented root epithelia.[138] Concomitant epithelial β-catenin depletion was shown to rescue EMT and ectopic cementogenesis caused by BMPR-1A depletion, suggesting that BMP and Wnt/β-catenin pathways interact antagonistically in regulating root lineage differentiation and EMT.[138] Thus, proper crosstalk between BMP and Wnt signaling pathways is essential for tooth development.

Dental and periodontal tissue engineering

The current status

Tissue engineering in regenerative medicine is a therapeutic approach to restoring or repairing the functions of defective or damaged tissues through the use of scaffolds, signaling molecules, and progenitor cells.[19] The applications of effective regenerative approaches in dental clinics can potentially significantly improve patients' quality of life.[139–141]

Although current tissue engineering technology and the discovery of dental stem cells allow for regenerating pulp and dentin,[142] the development of reproducible animal models is essential to assess the efficacy and success of dental regeneration *in vivo*.[143] Nonetheless, several preclinical human models demonstrated the potential utility of tissue engineering-based strategies in regenerating pulp–dentin complex, particularly for necrotic or immature permanent teeth.[144] A recent study using pulpal MSCs showed promising results in pulp–dentin regeneration *in vivo* through autologous transplantation,[145] reinforcing

the notion that DPSCs may be used for successful dental tissue engineering.[146]

Regeneration of dental pulp de novo has proved difficult as the tissue is encased in dentin and is without collateral blood supply except from the root apical end. However, recent advances in this field may provide realistic means of replacing lost or damaged teeth.[147] Tooth enamel is incapable of self-repairing, but dentin and cementum can naturally regenerate with limited capacity. Regeneration of dentin depends on pulp tissue and the source of odontoblastic stem cells.[142] Recombinant human BMP7 and human TGF-β3 have been shown to induce cementogenesis and generate functionally oriented periodontal ligament fibers and interweaving Sharpey's fibres in non-human primate Class II and III furcation defects.[73]

Current preclinical studies have indicated that cell-based tissue engineered constructs induce more robust bone formation when compared with acellular constructs.[148] The advent of iPSC technology generates much of the excitement in dental/periodontal tissue engineering and regenerative medicine.[149] iPSCs show great promise in dental applications due to their proliferation and differentiation capacities, although it requires rigorous evaluation of their specificity and safety prior to any use in patients.[150] The potential of iPSCs to aid in the development of new treatments for various diseases is exciting, and researchers are only beginning to discover their potential benefits for humans. We recently demonstrated that conditionally immortalized SCAPs (iSCAPs) not only maintain long-term cell proliferation but also retain the ability to differentiate into multiple lineages, including osteoblasts and odontoblasts *in vivo*.[110] Thus, iSCAPs may serve as an important tool to study SCAP biology and SCAP translational use in tooth engineering.[110]

BMPs in dental tissue engineering

While the scaffolds and progenitor cells are critical components of tissue engineering, the addition of signal molecules, such as BMPs and Wnts, has proven more effective in promoting periosteal-mediated bone regeneration and dental/periodontal regeneration.[151,152] Successful extraction of growth factors and BMPs from mammalian teeth may offer an important alternative for effective tooth engineering.[64,153]

During crown formation, BMP2 is known as an inducer for tooth development. It was shown that BMP2 can accelerate amelogenesis.[154] Furthermore, there is a positive feedback mechanism linking some microRNAs, such as miR-200c and miR-203, and BMP signaling through regulating the expression of E-cadherin, amelogenin, and/or BMP antagonist BMPer.[8] BMP4 is secreted by MSCs and regulates cell differentiation in the dental epithelium during crown formation.[155] Mesenchymal BMP4 participates in signaling within the dental epithelium and maintains Shh and BMP2 expression in *Msx1* mutant dental epithelium.[156] During tooth development, HERS cells participate in root formation following crown development.[157] BMP4-positive cells are detected in dental papillae around HERS.[155] BMP4 is co-expressed with Sox2[158] and the mesenchymally expressed BMP4 promotes elongation and maintaining cell proliferation of HERS cells, suggesting BMP4 may be used as a root-formation agent in tissue engineering applications.[155] SHED cells transmit BMP signals through both the Smad and p38MAPK pathways.[159] Furthermore, BMP9 may be explored as a novel and efficacious osteogenic agent for odontogenic regeneration.[110]

BMPs within a collagenous matrix carrier are capable of inducing cementum and alveolar bone regeneration in the furcation defect model of non-human primates.[160] Morphological analysis of the resulting tissue reveals periodontal ligament formation and insertion of Sharpey's fibers into cementum.[160]

Low doses of BMP2 in combination with physiological doses of dexamethasone, ascorbic acid, beta-glycerophosphate, heparin, retinoic acid and vitamin D accelerate osteogenesis of mouse and human MSCs.[161,162] BMP7-transduced MSCs, AdBMP7 co-delivered with AdLMP3, or BMP7-transduced human oral keratinocyte cells are all also able to accelerate bone formation.[163–165] A comprehensive analysis of BMPs in bone formation identified BMP9 as the most osteogenic BMP and may present a more effective strategy for the augmentation of bone regeneration than the BMPs currently used in the clinical setting.[66]

Wnt signaling molecules in dental tissue engineering

The canonical Wnt signaling pathway plays an important role in tooth development.[137,166] When tooth replacement is initiated, Wnt/β-catenin activity is presented in the budding successional lamina and adjacent mesenchyme but no active FGF signaling.[158] Interestingly a novel insertion/frameshift mutation in BMP target gene *Runx2* caused a typical cleidocranial dysplasia (CCD) phenotype, altered the biological function of Runx2$^{(+/m)}$ MSCs and the reduced ability of MSCs to differentiate into osteoblasts may explain defects of bone and teeth in CCD patients.[167]

During Wnt-induced enamel formation, Pitx2/β-catenin regulatory pathway is involved in epithelial cell differentiation and conversion of mesenchymal cells to amelogenin-expressing epithelial cells via miR-200a.[168] Pitx2 activates miR-200a-3p expression and miR-200a-3p reciprocally represses Pitx2 and β-catenin expression. Pitx2 and β-catenin interact to synergistically activate gene expression during odontogenesis, and miR-200a-3p attenuates their expression and directs differentiation.[168] Furthermore, Wnt activation is associated with superior pulp healing as pulp cells responded to Wnt stimulus by differentiating into secretory odontoblasts, improving pulp vitality and formation of more tertiary dentin.[169]

Hydroxyapatite bioceramics with micro-nano-hybrid surface (mnHA) was shown to stimulate gene expression of LRP5 and β-catenin in human PDLSCs.[170] Moreover, the stimulatory effect of mnHA bioceramics on ALP activity and cementogenic gene expression was repressed by Dkk1.[170] Conditional knockout of β-catenin in developing odontoblasts and cementoblasts resulted in rootless molars and incomplete incisors, indicating Wnt/β-catenin signaling is critical for root odontogenesis and cementogenesis.[171]

Wnt3a is expressed in HERS during mouse tooth root development but not in cultured dental mesenchymal cells.[12] Pretreatment of cells with Dkk-1 markedly attenuated Wnt3a-induced ALP expression.[12] Furthermore, Wnt3a induces Runx2 and osterix at gene and/or protein levels in HERS, suggesting that HERS may play an important role in stimulating cementoblast/osteoblast differentiation of DFCs via the Wnt/β-catenin signaling pathway.[38] Conversely, Dkk1 was shown to promote cementogenic differentiation of ADSCs.[172] Activation of endogenous canonical Wnt signaling with LiCl was shown to inhibit cementoblast differentiation and promote cell proliferation.[173]

Wnt/β-catenin upregulates the expression of dentine sialophosphoprotein, osteocalcin and ALP in SCAPs after incubation with mineralization induction medium, suggesting that canonical Wnt/β-catenin signaling may promote odonto/osteogenic differentiation of SCAPs.[136,174] Nonetheless, non-canonical Wnt5a was shown to stimulate hADSC osteogenic differentiation.[175] Interestingly, a blockade of canonical Wnt signaling by Dkk1 led to osteogenic differentiation of PDLSCs under inflammatory conditions, but activation by Wnt3a increased osteogenic differentiation of BMSCs.[43] Differentiation of dentin matrix-treated DFCs was enhanced by Wnt3a when in direct contact with HERSCs, indicating HERSCs induce osteogenic differentiation of DFCs involving Wnt signaling and dentin matrix during tooth root formation.[12]

Activation of Wnt signaling by acetylsalicylic acid, which also upregulates the telomerase reverse transcriptase (TERT), improved SHED-mediated bone regeneration.[36] Down-regulated anti-differentiation noncoding RNA (ANCR) promoted proliferation and osteogenic differentiation of PDLSCs while inhibition of canonical Wnt signaling also inhibited the osteogenic differentiation of PDLSCs/ANCR-RNAi cells, demonstrating that ANCR is a key regulator of PDLSC proliferation and osteogenic differentiation, and its function is regulated by canonical Wnt signaling.[176] Nevertheless, activation of ERK1/2 signaling by bFGF inhibits Wnt/β-catenin pathway and causes osteogenic deficiency of SHEDs, which was rescued by ERK1/2 inhibitors.[177]

It should be pointed out that a long-term aberrant activation of Wnt signaling, such as by expression of a stabilized form of β-catenin in Sox2-positive postnatal dental epithelial stem cells, was shown to induce odontoma, which contains multiple tooth-like structures and all dental tissue layers.[178] Activation of Wnt signaling in Sox2-positive embryonic progenitor cells promoted odontogenesis throughout the oral cavity.[178] Thus, certain precautions should be exercised when using canonical Wnt signaling molecules in tooth tissue engineering.

Concluding remarks and future directions

Our understanding of oral stem cells has expanded significantly for the past decade. Nonetheless, limited numbers of clinical trials are currently underway to evaluate the potential use of stem cells in the treatment of oral and dental diseases.[25] While there is a great need to establish cost-effective and safe protocols for exploiting DMSCs and NDMSCs in clinical use,[49] significant challenges must be addressed before the dental stem cells reach any clinical applications. The advantages for using dental tissue-derived stem cells for tooth regeneration include highly accessible attainment, reproducibility, capability of self-renewal and large-scale expansion, less immune rejection, avoidance of ethical controversy, and readiness for making iPCSs. However, the generation of fully functional teeth from the oral progenitor cells remains an elusive long-term goal. Future investigations should be directed to address the following questions: How can we efficiently and reproducibly isolate and maintain dental stem cells in culture? How can effectively and safely expand the isolated stem cells? What are the exact mechanisms underlying BMPs or Wnts' functions in regulating dental stem cell proliferation and differentiation? Can Wnt-BMP cross-talk be further exploited in order to establish potent and synergistic biofactors for dental regeneration? What are the biocompatible scaffold materials that can be used for biofactor-programmed stem cell therapies for regenerative dentistry? We may expect to get some satisfactory answers to these questions in next 5—10 years.

Conflicts of interest

The authors declare no conflicts of interest.

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