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## Review Article

## Dental regenerative therapy targeting sphingosine-1-phosphate (S1P) signaling pathway in endodontics

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## ABSTRACT

The establishment of regenerative therapy in endodontics targeting the dentin-pulp complex, cementum, periodontal ligament tissue, and alveolar bone will provide valuable information to preserve teeth. It is well known that the application of stem cells such as induced pluripotent stem cells, embryonic stem cells, and somatic stem cells is effective in regenerative medicine. There are many somatic stem cells in teeth and periodontal tissues including dental pulp stem cells (DPSCs), stem cells from the apical papilla, and periodontal ligament stem cells. Particularly, several studies have reported the regeneration of clinical pulp tissue and alveolar bone by DPSCs transplantation. However, further scientific issues for practical implementation remain to be addressed. Sphingosine-1-phosphate (S1P) acts as a bioactive signaling molecule that has multiple biological functions including cellular differentiation, and has been shown to be responsible for bone resorption and formation. Here we discuss a strategy for bone regeneration and a possibility for regenerative endodontics targeting S1P signaling pathway as one of approaches for induction of regeneration by improving the regenerative capacity of endogenous cells.

*Scientific field of dental science:* Endodontology

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## 1. Introduction

Since 1989, Japan has pioneered the “8020 campaign” with the aim of preserving at least 20 teeth at the age of 80 years. However, based on a survey in 2016 of the state of dental disease in Japanese population, more than 50% of individuals have achieved 8020, 6 years before the target [1]. In the current era of super-aging society with a lifespan of almost 100 years, 80 years of age is considered a passage point. With this trend of increasing life span in Japan, there is greater emphasis on preserving teeth and their supporting alveolar bone and maintenance of masticatory functions.

Endodontics is a treatment method for dental pulp disease, which is secondary to caries and apical periodontitis, and is caused by bacterial stimulation via the root canal. In endodontic disease where the supportive alveolar bone is lost extensively by the radicular cyst, i.e., if alveolar bone regeneration after the infectious source removal can be guaranteed, we can expect to preserve more teeth. This is likely to contribute to the improvement of patient's

quality of life (QoL). Regenerative therapy can largely be divided into two strategies: cell transplantation with stem cells and the improvement of regenerative ability of cells *in vivo*. Recent progress on stem cell transplantation indicates research being conducted on clinical application of induced pluripotent stem (iPS) cells, embryonic stem (ES) cells, and somatic stem cells, in addition to, studies on quality control and safety assurance of cells and tissue processing but unresolved issues in terms of cost still remain. Current approaches targeting the application of signaling molecules and scaffolds aimed at improving the regenerative capacity of endogenous cells are useful for solving many of these problems.

As one of the novel strategies in this field, an approach using sphingosine-1-phosphate (S1P) molecule and S1P signaling pathway *in vivo* has been reported [2,3]. S1P is abundantly present in the body, especially in the blood, and is made from phospholipids of cell membranes of various cells, as well as, vascular cells and bone cells. Similar to prostaglandins, S1P acts as a signaling molecule via 5 different receptors, S1PR1 to S1PR5 [4] that controls cell differentiation, proliferation and migration via receptors, and is called a lipid mediator [2–4]. Novel drug treatment strategy targeting S1PR1 [5] was clinically applied to multiple sclerosis, an autoimmune disease, based on the mechanisms of lymphocyte

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migration mediated by S1PR1 and its release from lymphoid tissues [6–8]. In recent years, considerable progress has been made to elucidate the role of S1P signaling pathway in bone tissues. Currently, research efforts are underway to study the following mechanisms: osteoclast differentiation inhibitory effect by osteoclast progenitor cell migration control via S1PR1 [9]; osteoblast differentiation promoting effect [10–13] and bone formation [11–14] via S1PR1, S1PR2, and S1PR3; and osteoporosis treatment through S1PR2 regulation by osteoclasts and osteoblasts [15–18]. Additionally, S1P regulates both the promotion of osteoblastogenesis and the inhibition of adipogenesis in C3H10T1/2 cells, which are functionally similar to mesenchymal stem cells [19,20].

In this review, the research on bone regeneration targeting the S1P signaling pathway, which the authors are also involved in, and the future development of regenerative endodontics are discussed.

## 2. Role of S1P signaling pathway *in vivo*

S1P is generated when sphingosine, which is a constituent of cell membranes and is biosynthesized via ceramide from sphingomyelin, is phosphorylated by sphingosine kinase 1 (SphK1) and 2 (SphK2). Then, S1P is exported extracellularly via ATP-binding cassette (ABC) transporters and S1P transporter spinster homolog 2 (SPNS2). Sphingosine was initially thought to function as an intracellular messenger molecule, but, during the 1990s, S1P was reported as a ligand of a G protein-coupled receptor with seven transmembrane domains identified as the endothelial differentiation gene (EDG) [21]. Hence, the EDG receptor was known as the S1P receptor. Until date, 5 types of G protein-coupled receptors (S1PR1–S1PR5) [4] have been identified, and they are known to mainly activate members of the  $G_i$ ,  $G_q$ , and  $G_{12/13}$  family proteins for signal transduction (Fig. 1) [22–24]. Of these, S1PR1, S1PR2, and S1PR3 are widely expressed in almost all organs and tissues in this order [25–27], but expression of S1PR4 and S1PR5 are limited to specific cells such as lymphocytes and nerve cells [28]. As shown in Fig. 1, the S1P signaling pathway activates a unique signaling pathway downstream from the receptor and exerts physiological functions. There are two known degradation pathways of S1P: S1P lyase (SPL)-mediated degradation into phosphoethanolamine and palmitoaldehyde [29]; and S1P phosphohydrolase that dephosphorylates S1P to produce sphingosine [3]. However, under normal conditions, the balance between S1P production and degradation is regulated to a certain level, and it is thought that various disorders are caused when this balance is lost. Therefore, enzymes involved in the production and degradation of S1P might become targets for drug discovery in the future.

S1P signaling pathways are reported to be associated with many diseases. Some of the identified diseases so far consist of angiogenesis involving receptors S1PR1, S1PR3 [30], tumor angiogenesis involving S1PR1 [31], and anti-angiogenesis involving S1PR2 [32]. As described above, S1P is abundantly present in blood and thus is closely associated with angiogenesis, and S1PR1 knockout mice are embryonically lethal with impaired angiogenesis [26]. In addition, double knockout mice with SphK1 and SphK2 are embryonically lethal due to abnormal development of the vascular system and nervous system [33]. In diabetes, there is an increase in S1P concentration in animal models of type 1 diabetes and improvement in glucose tolerance by S1P-improved pancreatic  $\beta$  cell function [34], an increase in S1P concentration in type 2 diabetes and a report of improved insulin resistance [35]. As such, S1P is thought to be involved in maintaining homeostasis of glucose metabolism. In prostate cancer, SphK1 is overexpressed in tumor tissue [36], and the metabolism of SphK1/S1P is associated with resistance to chemotherapy of prostate cancer cells [37,38] and radiation ther-

apy [39]. Therefore, it has been suggested that SphK1/S1P signaling pathways may be targets for the treatment of prostate cancer.

Fingolimod (FTY720), an agonist of S1PR1 and S1PR3, reduces the number of lymphocytes in the peripheral blood, especially T lymphocytes, and exerts an immunosuppressive effect [5]. In this way, as a multiple sclerosis drug for oral administration originating from Japan, it has been clinically applied in the United States in 2010 and in Japan in 2011 [6–8]. Thus, in the S1P signaling pathway and therapeutic applications, many agonists and inhibitors targeting the receptor have been developed.

## 3. Function of S1P signaling pathway in bone tissue

Homeostasis of bone tissue is maintained by the balance between bone formation and resorption. Since it has been newly discovered that the physiological function of S1P is to regulate the migration of osteoclast precursor cells and suppress bone resorption [9], the role of S1P in bone tissue and bone remodeling including osteoclast/osteoblast differentiation has been widely explored. From previous research reports, we discuss the function of S1P and S1P signaling pathway on osteoclast, osteoblast, and application for osteoporosis.

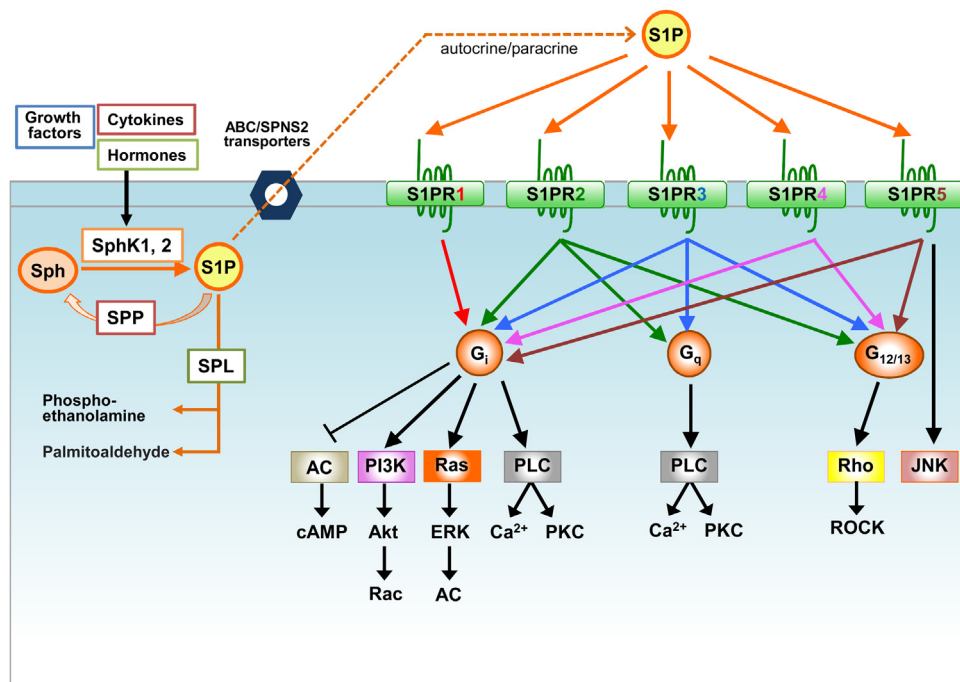
### 3.1. Osteoclasts

In relation to bone tissue and S1P, it was known that S1P plays an important role in the coupling between osteoblasts and osteoclasts [40]. In 2009, it was reported that S1PR1 was expressed in osteoclast precursor cells, and that progenitor cells return to blood by cell migration, resulting in the inhibition of maturation to osteoclasts [9]. In addition, administration of FTY720 suppressed bone resorption in a mouse osteoporosis model, and in mice lacking S1PR1, osteoclast precursor cells adhered to the bone surface, increasing osteoclast formation and bone resorption [41,42]. This suggests that decreased serum S1P levels and deletion of S1PR1 are associated with bone resorption and osteoporosis. Furthermore, in patients with rheumatoid arthritis (RA), high levels of S1P and SphK1 were expressed [41,42], and Nuclear factor- $\kappa$ B (NF- $\kappa$ B)-mediated Fas/S1PR signal crosstalk was involved in RA pathology with bone destruction of the temporomandibular joint. SN50 that targets nuclear translocation of p50 NF- $\kappa$ B, suppresses RANKL-induced osteoclastogenesis. These suggest that NF- $\kappa$ B-mediated Fas/S1PR signaling could also be a therapeutic target for RA [43,44].

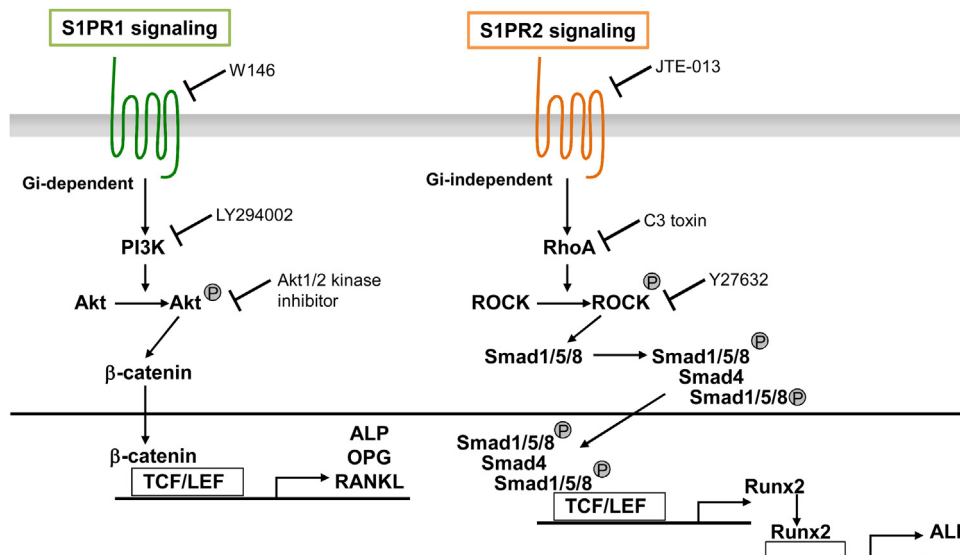
On the other hand, S1PR2 expressed in osteoclast precursor cells is known to cause negative chemotaxis (chemorepulsion) with respect to the concentration gradient of S1P. It has a function of migrating osteoclast precursor cells from blood with high S1P concentration to a bone surface with a low concentration [15]. Active vitamin D ( $1\alpha, 25\text{-(OH)}_2$  vitamin D<sub>3</sub>) preparations as a drug for osteoporosis suppresses S1PR2-mediated chemorepulsion in osteoclast precursor cells. As a result, osteoclast precursor cells migrate from the bone surface to blood vessels, and then the inhibition of bone resorption is occurred [16]. Therefore, S1PR2 is clinically important for not only bone resorption but also bone formation.

### 3.2. Osteoblasts

Regarding the role of S1P in osteoblasts, promotion of chemotaxis [40], production of interleukin-6 [45], and suppression of apoptosis via phosphatidylinositol 3-kinase (PI3K)/Akt signaling [46] had been observed. However, its effects on osteoblast differentiation and its mechanism had not been clarified. Since osteoblasts express a large amount of S1PR1 among S1P receptors, we identified an increase in osteoprotegerin (osteoclast suppressor; OPG) expression and alkaline phosphatase (ALP) activity



**Fig. 1.** Sphingosine-1-phosphate (S1P) production and S1P/S1PR signaling pathways. Phosphorylation of sphingosine (Sph) by sphingosine kinase (SphK) activated by extracellular signals leads to production of S1P. S1P lyase (SPL) is the enzyme responsible for degrading S1P. Extracellular S1P released via transporters acts as an autocrine or paracrine cue and activates or inhibits a variety of signaling pathways through G-protein-coupled receptors termed S1PR1-S1PR5, regulating cellular differentiation, migration, and growth. (SPP = S1P phosphatase, AC = adenylate cyclase, PLC = phospholipase C, PKC = protein kinase C, JNK = Jun amino terminal kinase)



**Fig. 2.** Schematic of the mechanisms of osteoblast differentiation by S1PR1 and S1PR2 signaling based on our previous reports [10,11]. Gi-dependent S1PR1 signaling induces PI3K activity, and increases Akt phosphorylation, thereby inducing the translocation of β-catenin to nuclear, resulting in the increase of OPG expression and ALP activity. On the other hand, Gi-independent S1PR2 signaling induces RhoA/ROCK, and increases Smad1/5/8 phosphorylation, resulting in the translocation of Smad4, thereby promoting Runx2 and ALP expression. (W146 = selective S1PR1 antagonist, LY294002 = PI3K inhibitor, JTE-013 = selective S1PR2 antagonist, C3 toxin = RhoA inhibitor, Y27632 = ROCK inhibitor)

by S1PR1/Akt/β-catenin signal activation (Fig. 2) [10]. However, the precise mechanism of osteoblast differentiation by PI3K/Akt activation is still unknown. The involvement of other signaling cannot be ignored. Indeed, ALP expression is generally regulated by Wnt and bone morphogenetic protein (BMP)/Smads signaling pathway [47,48]. Authors also reported the presence of T-cell factor/lymphoid enhancer factor (TCF/LEF) binding site of the ALP gene promoter region that regulates ALP expression in osteoblast-like cells [49]. On the other hand, S1P increased not only OPG

expression but also RANKL expression [9]. The OPG/RANKL ratio increased in a S1P concentration-dependent manner, suggesting that S1P suppresses osteoclast maturation. Recently, the RANKL reverse signaling pathway in osteoblasts was revealed [50]. In this pathway, RANKL acts as a receptor molecule for RANK released by osteoclasts, and activates PI3K/Akt/mTORC1, leading to increase of runt-related transcription factor 2 (Runx2) expression, which is an essential osteoblast-related gene regulator, and thereby promoting bone formation. S1P-induced RANKL expression in osteoblasts may

influence this RANKL reverse signaling pathway and promote bone formation.

Furthermore, we also found that a substantial amount of S1PR2 expression during osteoblast differentiation, and S1P-induced ALP expression and calcification were suppressed by both siS1PR1 and siS1PR2 [11]. Therefore, the authors proposed a novel mechanism for promoting osteoblast differentiation through S1PR2 that is different from the Gi-dependent S1PR1/Akt/ $\beta$ -catenin signaling. We found an increase in Runx2 expression that promotes ALP expression [48], due to S1PR2/RhoA/ROCK/Smad signal activation independent of S1PR1 signaling [11] (Fig. 2). In addition, active vitamin D has been reported to promote Smad1/5/8 phosphorylation [51,52]. Therefore, vitamin D preparations may synergistically activate S1PR2/Smad1/5/8 signaling pathway in osteoblasts, in addition to S1PR2-mediated chemorepulsion in osteoclast precursor cells, suggesting that the S1P-mediated osteogenic effects may be further promoted by their combined use.

To clarify the osteogenic effect of S1PR1 and S1PR2 signaling pathway *in vivo*, we investigated the tibial bone properties in mice by intraperitoneal administration of FTY720 and CYM-5520; a pharmacological S1PR2 agonist, respectively. Our findings indicate a possible involvement of S1PR1- and S1PR2-enhanced signaling pathway in bone formation *in vivo* [11]. Furthermore, the osteogenic and angiogenic effects of S1P and FTY720 in a rat fracture model were also identified [14]. It is expected that this will lead to the development of new treatment methods for bone formation. In addition, S1PR2 agonist-induced bone volume was attenuated by ROCK inhibitor as well as S1PR2 inhibitor, consistent with *in vitro* observation [11]. Our findings are consistent with previous publications; osteoblast differentiation is regulated by RhoA/ROCK signaling pathway [53]. By contrast, recent study showed that the activation of the RhoA/ROCK pathway promotes stress fiber formation and reduces chondrocyte and osteoblast differentiation [54]. Additionally, ROCK inhibitor activates BMP signaling and thereby induces osteoblast differentiation [55]. The reason for this discrepancy is currently unknown, but we observed that S1P did not affect BMP-2 production in osteoblast-like cells [11]. On the other hand, Sato et al. reported that osteoblast differentiation induced by BMP-2 is facilitated by the activation of extracellular signal-regulated kinase (ERK) via S1PR1 and S1PR2 [12]. Our study showed that Smad1/5/8 phosphorylation is promoted by S1PR2 signaling pathway without affecting BMP production [11], but the main stream may be the BMPs/Smads signaling cascades [48]. Although the molecular mechanism by which S1PR2 signaling pathway affects RhoA/ROCK and BMPs/Smads signaling pathway remains to be fixed, our findings indicate a possible involvement of S1PR2 signaling in bone formation.

Regarding the effect of the S1PR2 signaling pathway on bone formation, the latest findings regarding the relationship between SPL, an enzyme that degrades S1P, and S1PR2 signaling pathway have shown the possibility of application to the treatment of osteoporosis. When the S1P level was increased in mice by SPL deletion and pharmacological inhibition with 4-deoxyprodoxin, bone mass and bone strength were increased, while adipose tissue was decreased [17]. This study also demonstrated that S1PR2-mediated signaling via novel two different mechanisms such as p38/glycogen synthase-3 $\beta$  (GSK-3 $\beta$ )/ $\beta$ -catenin and Wnt5a/low-density lipoprotein receptor-related protein 5 (LRP5) pathways increases OPG expression, resulting in the inhibition of osteoclastogenesis (Fig. 3A). Particularly, S1PR2-induced Wnt5a and LRP5 were diminished by S1PR2 inhibitor and absent in S1PR2-deficient osteoblasts. In addition, Wnt inhibitory factor and Wnt5a inhibitor also suppressed OPG expression. Further, this signaling potentially promotes osteoblastogenesis instead of suppressing adipogenesis by down-regulating PPAR $\gamma$  in osteoblast and adipocyte precursors (Fig. 3B). Although it is consistent with our reports that S1P

signaling pathway regulates both the promotion of osteoblastogenesis and the inhibition of adipogenesis in mesenchymal stem cells [19,20], up-regulation of OPG might be influenced following the commitment of osteoblastic differentiation or maturation of osteoblasts.

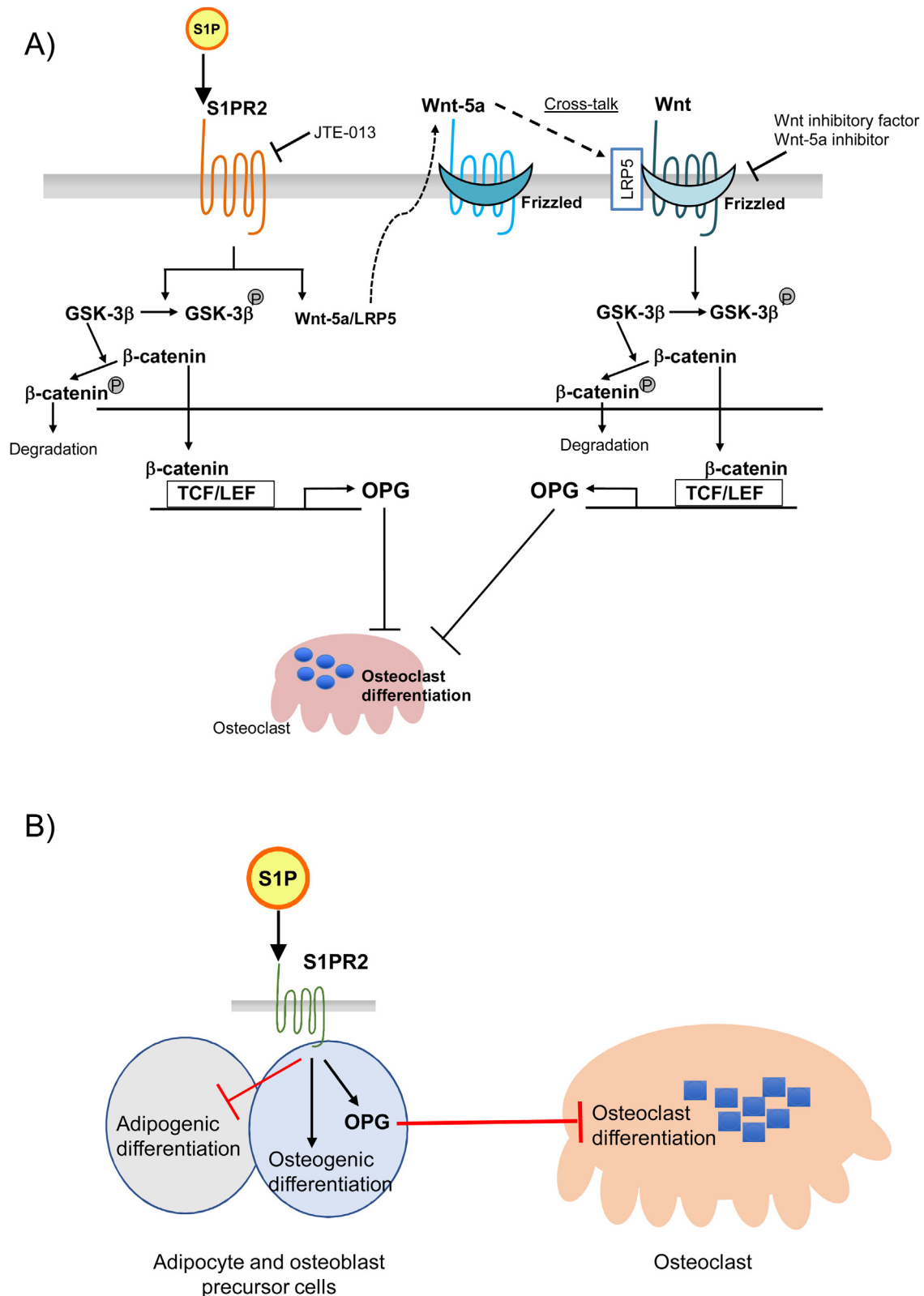
In addition, S1P concentration in human serum was positively correlated with bone formation markers and serum calcium, but not with bone resorption markers [17]. The current treatments for osteoporosis can be divided into the following: bone catabolism that inhibits bone resorption by osteoclasts to suppress bone metabolism; and bone anabolism that promotes bone formation by osteoblasts and activates metabolism. However, there is no drug that can be applied to all patients with osteoporosis. As a novel therapeutic drug, it needs to be effective even in cases where the risk of bone fracture still persists despite administering bisphosphonates and human type monoclonal antibody preparation, a molecular targeted therapy that targets RANKL. Inhibition of SPL showed a similar increase in bone mass to that of intermittent administration of parathyroid hormone, and its strength was superior. That is, it was suggested that the inhibition of SPL might exert a high bone anabolism effect and prevent the deterioration of bone quality.

Currently, clinical trials of the SPL inhibitor LX2931 have been initiated in RA. However, inhibition of S1P degradation increases the S1P concentration, resulting in the enhancement of all S1P receptor signals. As such, it has been pointed out that lymphocytic cytopenia may occur as a side effect [18]. Meanwhile, when S1PR2 agonist (CYM-5520) was applied to a mouse osteoporosis model by ovariectomy, lymphopenia as seen in LX2931 was not observed, and a comparative increase in bone mass and osteoblast number as LX2931 was observed [18]. From the above, it was suggested that the bone anabolic effect of the drug targeting S1PR2 would be a useful treatment method for osteoporosis-related diseases.

In addition, it has become clear that the osteoblasts themselves can produce S1P. While prostate cancer is known to be prone to bone metastasis, S1P produced from osteoblasts acts as a paracrine to promote the growth of prostate cancer cells via the S1PR1 signal and an autocrine to differentiate through S1PR3/Runx2 pathway [13]. Therefore, it may be involved in the aforementioned resistance of S1P to chemotherapy of prostate cancer cells [37,38] or radiation therapy [39].

#### 4. Current state of S1P in endodontics and challenges for regenerative therapy

In recent years, the S1P receptor has received attention in endodontics. In 2015, it was revealed that S1PR1 was expressed in lesions of rat apical periodontitis, and it was positively correlated with the expression of receptor activator of NF- $\kappa$ B ligand (RANKL) with an effect of promoting osteoclast differentiation [56]. S1PR1 may be involved in bone resorption or expression of inflammatory factors. In 2018, suppression of RANKL expression and bone resorption in apical lesions by intragastric administration of FTY720 was reported [57]. Since intracellular S1PR expression level changes in response to various extracellular stimuli under pathological conditions such as inflammation, it is possible that S1PR1 and S1PR2 signals act complementarily for bone level maintenance. In the future, it will be necessary to compare the dynamics of intracellular S1P receptors in cells present around the apex under physiological conditions and inflammatory conditions such as apical periodontitis. On the other hand, although there is no report on the relationship between S1PR2 and apical periodontitis, S1PR2 deficient mice was shown to have osteopenia associated with a decrease in OPG [17]. Given this finding, similar to S1PR1, it is possible that S1PR2 signals may act as a coupling factor between osteoclasts and osteoblasts.



**Fig. 3.** Model for the regulation of osteoblast/osteoclast differentiation and osteoblast/adipocyte precursor cells by S1PR2 signaling through OPG induction. A) The two different mechanisms involved in OPG induction through S1PR2/GSK-3β/β-catenin and Wnt5a signaling pathways, indicating the inhibition of osteoclast differentiation [17]. B) S1PR2 signaling inhibits adipogenesis but induces osteoblastogenesis through OPG expression in precursor cells [17], resulting in the inhibition of osteoclast differentiation.

Currently, in anticipation of the clinical application of S1P to alveolar bone regeneration in endodontics, we have begun preparation and analysis of a surgical endodontic animal model assuming

a wide range of bone defects. Among five kinds of S1P receptors, S1P increased both S1PR1 and S1PR2 mRNA levels in osteoblast-like cells, suggesting that S1P-induced S1PR1 and S1PR2 signaling



pathway plays a critical role in the osteoblast differentiation. Therefore, selective agonists of S1PR1 and S1PR2 might be useful for clinical application. As mentioned above, the bone anabolic effect of selective S1PR2 agonist [18] would be a useful treatment method for osteoporosis. In multiple sclerosis, in addition to FTY720, selective S1PR1 agonists or S1P receptor drugs without serious adverse events have been developed, and a number of clinical trials are undergoing [8]. The rat lower mandibular first molar was performed pulpectomy and root canal-filled, followed by apex resection and fossa cavity formation, resulting in an apical resection/alveolar bone defect model. By mixing an S1P receptor agonist and a base material that gels while having a sustained release effect and placing it in the alveolar bone defect site, we expect functional bone regeneration [58] from the coexistence of stem cells and vascular endothelial cells in the surgical site. In this case, we can expect that blood clots formed in the surgical site could also act as a scaffold. Considering the local application of S1P receptor agonists to bone tissue compared to BMP-2, local administration of BMP-2 *in vivo* is often used at a concentration of about 1.0 µg/mL [59]. However, FTY720, an analogue of S1P, is used at a lower concentration than BMP-2 at about 1.0 pg/mL [60]. S1P may be more useful compared to BMP-2, because it can be applied at a lower concentration, and since it is clinically applied to multiple sclerosis, there are likely fewer barriers to dental application.

In recent years, the idea of regenerative endodontics has been advocated [61]. In particular, pulp revascularization therapy, which was reported for the first time in 2001 by Iwaya et al. [62], has attracted much attention. The goal of this method is to improve the regenerative capacity by activating stem cells from the apical papilla (SCAPs) present in the apical portion [63,64]. This strategy induces root extension and apical closure of permanent tooth with incomplete root and necrotic pulp. Surgical method to undergo revascularization therapy consists of the following steps: chemical cleaning is followed by removal, cleaning, and disinfection of the necrotic pulp, and then intentional bleeding of the apical portion is performed using instruments for root canal treatment. Hard tissue is formed by the differentiation of stem cells that use blood clots as a scaffold [65,66]. Clinically, the American Association of Endodontics has proposed a 3-step goal to monitor the success of pulp revascularization therapy [67]. The initial step is the elimination of clinical symptoms and restoration of the alveolar bone, followed by increase of the root canal wall thickness and/or extension of the roots of teeth, and finally pulp vitalization response. However, the hard tissue formed in the apical portion by this method is not made up of dentin, but of bone or cementum [61,68]. This leads to excessive calcification in the root canal, osseous ankylosis, and inhibition of vitalization and functions of the vascular reconstruction tissue [69]. Based on the mechanism of tooth development, it is believed that dentin and pulp neural progenitor cells are formed by SCAPs from dental papillae, while periodontal ligament, cementum, and alveolar bone are formed by periodontal ligament stem cells (PDLSCs) [70] from dental follicles. Therefore, stem cells involved in revascularization are believed to be SCAPs. However, since there are PDLSCs, bone marrow stem cells (BMSCs) [71], and dental follicle stem cells (DFSCs) [72] (if there is presence of vital pulp remaining) around the apex, a probable cause of ectopic hard tissue formation would be osteoblast differentiation by the stem cells around these apical roots penetrating into the root canal. On the other hand, dentin sialophosphoprotein (DSPP), which is essential for dentin formation, has been reported the involvement of bone formation based on the analysis of bone phenotype in DSPP-deficient mice [73]. Also, based on studies involving cases where osseous ankylosis occurred by tooth replantation, the pulp was replaced by bone tissue. There are reports that in these cases, osteoclasts appeared in the root canal [74]. In the future, studies are required to verify the

induction mechanism of normal dentin-pulp complex regeneration at the molecular level, and to construct the means of induction. In addition, it is necessary to clarify the mechanism of DSPP gene expression as a key factor in the regulation of dentin and bone formation.

There are no reports on the S1P signal and regenerative endodontics including odontoblast differentiation. The authors revealed that S1P signaling pathway regulates both the promotion of osteoblastogenesis and the inhibition of adipogenesis in C3H10T1/2 cells, which are functionally similar to mesenchymal stem cells [19,20]. Although the roles of S1P signaling in odontoblast differentiation remains to be unclear, we observed that the S1P receptor is expressed in rat odontoblast-like cells (unpublished data). Therefore, we have currently begun to investigate the possibility that S1P may be involved in the activation of SCAPs and induction of odontoblast differentiation in dental pulp revascularization therapy.

On the other hand, several studies have reported the use of DPSCs for pulp regeneration by cell transplantation [75]. The existence of DPSCs was reported around 20 years ago [76], and has since been verified by a number of researchers [77–79]. DPSCs present in undifferentiated mesenchymal dental pulp tissues have the ability to differentiate into various cells, such as nerves, fat, bone, and cartilage [76,77]. In a systematic review by Yamada et al. [80] assessing all the clinical trials using stem cells derived from dental tissue, 20 studies were included in the review containing randomized controlled trials, case series, and case reports between 2000 and 2018. Among these 20 studies, 10 clinical trials used DPSCs. Among these, two clinical trials were on dental pulp regeneration [81,82], and four studies were conducted on the regeneration of alveolar bone [83–86]. These studies have confirmed the regeneration of clinical pulp and alveolar bone by cell transplantation. However, there are scientific issues present for practical implementation for clinical purposes, especially for cells involved in tissue processing. Since the administered cells are alive, it is difficult to remove or inactivate them even if they are combined with the pathogens. It is also necessary to be able to provide an efficient and stable supply of administered cells while ensuring efficacy, safety and quality. In addition, it is necessary to develop a highly sensitive method for the detection of undifferentiated cells and to prevent tumor formation, since in the case of iPSC cells and ES cells, there is a need to pay sufficient attention to the risk of cancerization of cells themselves [87]. In order to attain during clinical application of research and development in the medical field, it is also necessary to promote the research of regulatory science, which is defined as the science to appropriately and swiftly predict, evaluate, and determine the quality, effectiveness, and safety based on scientific knowledge [88]. In addition, the installation of cell culture facilities is expensive. Therefore, regenerative therapy of dental pulp using stem cells in the current status is a likely option, but clinical application to attain this requires further examination.

Additionally, basic research is actively being carried out for the application of tissue engineering. Till date, DPSCs have been reported to perform the following functions: promoting differentiation into odontoblasts by cultures, such as, hyaluronic acid [89], polycaprolactone [90], lactic acid and glycolic acid copolymer [91]; regeneration of dentin-pulp compounds (stem cell migration, angiogenesis, and differentiation to odontoblast-like cells) by combination of scaffold materials such as collagen and growth factors such as dentin substrates and BMP [74].

Regarding the effect of S1P on dental pulp, there is a report on S1P migration-promoting effect of pulp stem cells [92] or maintenance of pulp cells by S1P/Akt/ERK signals [93]. However, there are no reports on the S1P signal and regenerative endodontics including pulp-like tissue formation.

## 5. Conclusion

S1P acts as a signaling molecule that controls cell differentiation, proliferation and migration via receptors, therefore, is involved in the activation of the regeneration capacity of cells *in vivo*. We discuss the mechanisms and the functions of S1P signaling pathway in bone tissue for both osteoclasts and osteoblasts that could contribute bone regeneration and the treatment of osteoporosis. In addition, S1PR1 are expressed in lesions of apical periodontitis, and may be involved in bone resorption or expression of inflammatory factors. Taken together, we anticipate the clinical application of S1P to alveolar bone regeneration in endodontics. Furthermore, because S1P enhances osteogenic differentiation of pluripotent stem cells, it is expected that S1P will activate somatic stem cell differentiation potential to generate cells that form hard tissues, leading to a possibility for regenerative endodontics. The establishment of an ultimate treatment strategy for teeth preservation by early recovery of bone, and the lost dentin-pulp structure and functions by S1P signaling pathway will likely aid in the needs of the super-aged society.

## Conflict of interest

The authors state that they have no conflicts of interest.

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## References

- Ministry of Health, Labour and Welfare: Survey of Dental Diseases (2016). <https://www.mhlw.go.jp/toukei/list/62-28.html>.
- Spiegel S, Milstien S. Sphingosine 1-phosphate, a key cell signaling molecule. *J Biol Chem* 2002;277:25851–4.
- Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat Rev Mol Cell Biol* 2003;4:397–407.
- Anliker B, Chun J. Lysophospholipid G protein-coupled receptors. *J Biol Chem* 2004;279:20555–8.
- Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 2002;296:346–9.
- Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, et al. Fingolimod (FTY720): Discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov* 2010;9:883–97.
- Chi H. Sphingosine-1-phosphate and immune regulation: trafficking and beyond. *Trends Pharmacol Sci* 2011;32:16–24.
- Grassi S, Mauri L, Prioni S, Cabitta L, Sonnino S, Prinetti A, et al. Sphingosine 1-Phosphate Receptors and Metabolic Enzymes as Druggable Targets for Brain Diseases. *Front Pharmacol* 2019;10:807.
- Ishii M, Egen JG, Klauschen F, Schellersheim MM, Saeki Y, Vacher J, et al. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* 2009;458:524–8.
- Matsuzaki E, Hiratsuka S, Hamachi T, Takahashi-Yanaga F, Hashimoto Y, et al. Sphingosine-1-phosphate promotes the nuclear translocation of  $\beta$ -catenin and thereby induces osteoprotegerin gene expression in osteoblast-like cell lines. *Bone* 2013;55:315–24.
- Higashi K, Matsuzaki E, Hashimoto Y, Takahashi-Yanaga F, Takano A, et al. Sphingosine-1-phosphate/S1PR2-mediated signaling triggers Smad1/5/8 phosphorylation and thereby induces Runx2 expression in osteoblasts. *Bone* 2016;93:1–11.
- Sato C, Iwasaki T, Kitano S, Tsunemi S, Sano H. Sphingosine-1-phosphate receptor activation enhances BMP-2-induced osteoblast differentiation. *Biochem Biophys Res Commun* 2012;423:200–5.
- Brizuela L, Martin C, Jeannot P, Ader I, Gstalder C, Andrieu G, et al. Osteoblast-derived sphingosine 1-phosphate to induce proliferation and confer resistance to therapeutics to bone metastasis-derived prostate cancer cells. *Mol Oncol* 2014;8:1181–95.
- Petrie Aronin CE, Sefcik LS, Tholpady SS, Tholpady A, Sadik KW, et al. FTY720 promotes local microvascular network formation and regeneration of cranial bone defects. *Tissue Eng Part A* 2010;16:1801–9.
- Ishii M, Kikuta J, Shimizu Y. Chemorepulsion by blood S1P regulates osteoclast precursor mobilization and bone remodeling *in vivo*. *J Exp Med* 2010;207:2793–8.
- Kikuta J, Kawamura S, Okiji F. Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibody-resorptive action of active vitamin D. *Proc Natl Acad Sci U S A* 2013;110:7009–13.
- Weske S, Vaidya M, Reese A, von Wnuck Lipinski K, et al. Targeting sphingosine-1-phosphate lyase as an anabolic therapy for bone loss. *Nat Med* 2018;24:667–78.
- Weske S, Vaidya M, von Wnuck Lipinski K, Keul P, et al. Agonist-induced activation of the S1P receptor 2 constitutes a novel therapy for the treatment of osteoporosis in mice. *Bone* 2019;125:1–7.
- Hashimoto Y, Matsuzaki E, Higashi K, Takahashi-Yanaga F, Takano A, et al. Sphingosine-1-phosphate inhibits differentiation of C3H10T1/2 cells into adipocyte. *Mol Cell Biochem* 2015;401:39–47.
- Hashimoto Y, Kobayashi M, Matsuzaki E, Higashi K, Takahashi-Yanaga F, et al. Sphingosine-1-phosphate-enhanced Wnt5a promotes osteogenic differentiation in C3H10T1/2 cells. *Cell Biol Int* 2016;40:1129–36.
- Lee MJ, Van Brocklyn JR, Thangada S, Liu CH, Hand AR, et al. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science* 1998;279:1552–5.
- Futerman AH, Hannun YA. The complex life of simple sphingolipids. *EMBO Rep* 2004;5:777–82.
- Spiegel S, Cu villier O, Edsall LC, Kohama T, Menzelev R, Olah Z, et al. Sphingosine-1-phosphate in cell growth and cell death. *Ann N Y Acad Sci* 1998;845:11–8.
- Rosen H, Goetzl EJ. Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. *Nat Rev Immunol* 2005;5:560–70.
- Takuwa Y. Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors. *Biochim Biophys Acta* 2003;1582:112–20.
- Takuwa Y, Takuwa N, Sugimoto N. The Edg family G protein-coupled receptors for lysophospholipids: their signaling properties and biological activities. *J Biochem* 2002;131:767–71.
- Takuwa N, Du W, Kaneko E, Okamoto Y, Yoshioka K, Takuwa Y. Tumor-suppressive sphingosine-1-phosphate receptor-2 counteracting tumor-promoting sphingosine-1-phosphate receptor-1 and sphingosine kinase 1-Jekyll Hidden behind Hyde. *Am J Cancer Res* 2011;1:460–81.
- Im DS, Ungar AR, Lynch KR. Characterization of a zebrafish (Danio rerio) sphingosine 1-receptor expressed in the embryonic brain. *Biochem Biophys Res Commun* 2000;279:139–43.
- Kunkel GT, Maceyka M, Milstien S, Spiegel S. Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nat Rev Drug Discov* 2013;12:688–702.
- Waeber C, Biondeau N, Salomone S. Vascular sphingosine-1-phosphate S1P1 and S1P3 receptors. *Drug News Perspect* 2004;17:365–82.
- Visentin B, Velicki JA, Sibbald BJ, Cavalli AL, Moreno KM, Matteo RG, et al. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages. *Cancer Cell* 2006;9:225–38.
- Du W, Takuwa N, Yoshioka K, Okamoto Y, Gonda K, Sugihara K, et al. S1P(2), the G protein-coupled receptor for sphingosine-1-phosphate, negatively regulates tumor angiogenesis and tumor growth *in vivo* in mice. *Cancer Res* 2010;70:772–81.
- Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol* 2005;25:11113–21.
- Fox TE, Bewley MG, Unrath KA, Pedersen MM, Anderson RE, Jung DY, et al. Circulating sphingolipid biomarkers in models of type 1 diabetes. *J Lipid Res* 2011;52:509–17.
- Fayyaz S, Japtok L, Kluser B. Divergent role of sphingosine-1-phosphate on insulin resistance. *Cell Physiol Biochem* 2014;34:134–47.
- Brizuela L, Dayon A, Doumerc N, Ader I, Golzio M, Izard JC, et al. The sphingosine kinase-1 survival pathway is a molecular target for the tumor-suppressive tea and wine polyphenols in prostate cancer. *FASEB J* 2010;24:3882–94.
- Pchejetski D, Golzio M, Bonhoure E, Calvet C, Doumerc N, Garcia V, et al. Sphingosine kinase-1 as a chemotherapy sensor in prostate adenocarcinoma cell and mouse models. *Cancer Res* 2005;65:11667–75.
- Pchejetski D, Doumerc N, Golzio M, Naymark M, Teissié J, Kohama T, et al. Chemosensitizing effects of sphingosine kinase-1 inhibition in prostate cancer cell and animal models. *Mol Cancer Ther* 2008;7:1836–45.
- Nava VE, Cu villier O, Edsall LC, Kimura K, Milstien S, Gelmann EP, et al. Sphingosine enhances apoptosis of radiation-resistant prostate cancer cells. *Cancer Res* 2000;60:4468–74.
- Ryu J, Kim HJ, Chang EJ, Huang H, Banno Y, Kim HJ. Sphingosine-1-phosphate as a regulator of osteoclast differentiation and osteoclast-osteoblast coupling. *EMBO J* 2006;25:5840–51.
- Kikuta J, Iwai K, Saeki Y, Ishii M. S1P-targeted therapy for elderly rheumatoid arthritis patients with osteoporosis. *Rheumatol Int* 2011;31:967–9.
- Boyce BF. Advances in osteoclast biology reveal potential new drug targets and new roles for osteoclasts. *J Bone Miner Res* 2013;28:711–22.
- Hutami IR, Izawa T, Mino-Oka A, Shinohara T, Mori H, Iwasa A, et al. Fas/S1P1 crosstalk via NF- $\kappa$ B activation in osteoclasts controls subchondral bone remodeling in murine TMJ arthritis. *Biochem Biophys Res Commun* 2017;490:1274–81.

- [44] Hutami IR, Tanaka E, Izawa T. Crosstalk between Fas and S1P1 signaling via NF- $\kappa$ B in osteoclasts controls bone destruction in the TMJ due to rheumatoid arthritis. *Jpn Dent Sci Rev* 2019;55:12–9.
- [45] Kozawa O, Tokuda H, Matsuno H, Uematsu T. Activation of mitogen-activated protein kinase is involved in sphingosine 1-phosphate-stimulated interleukin-6 synthesis in osteoblasts. *FEBS Lett* 1997;418:149–51.
- [46] Grey A, Chen Q, Callon K, Xu X, Reid IR, Cornish J. The phospholipids sphingosine-1-phosphate and lysophosphatidic acid prevent apoptosis in osteoblastic cells via a signaling pathway involving G(i) proteins and phosphatidylinositol-3 kinase. *Endocrinology* 2002;143:4755–63.
- [47] Rawadi G, Vayssiere B, Dunn F, Baron R, Roman-Roman S. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. *J Bone Miner Res* 2003;18:1842–53.
- [48] Lee KS, Kim HJ, Li QL. Runx2 is a common target of transforming growth factor  $\beta$ 1 and bone morphogenetic protein 2, and cooperation between Runx2 and Smad5 induced osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. *Mol Cell Biol* 2000;20:8783–92.
- [49] Matsuzaki E, Takahashi-Yanaga F, Miwa Y, Hirata M, Watanabe Y, et al. Differentiation-inducing factor-1 alters canonical Wnt signaling and suppresses alkaline phosphatase expression in osteoblast-like cell lines. *J Bone Miner Res* 2006;21:1307–16.
- [50] Ikebuchi Y, Aoki S, Honma M, Hayashi M, Sugamori Y, Khan M, et al. Coupling of bone resorption and formation by RANKL reverse signalling. *Nature* 2018;561:195–200.
- [51] Xiao HQ, Shi W, Liu SX. Podocyte injury is suppressed by 1, 25-dihydroxyvitamin D<sub>3</sub> via modulation of transforming growth factor- $\beta$ 1/bone morphogenetic protein-7 signaling in puromycin aminonucleoside nephropathy rats. *Clin Exp Pharmacol Physiol* 2009;36:682–9.
- [52] Zou M, Yu J, Nie G. 1, 25-dihydroxyvitamin D<sub>3</sub> decreases Adriamycin-induced podocyte apoptosis and loss. *Int J Med Sci* 2010;7:290–9.
- [53] Khatiwala CB, Kim PD, Peyton SR, Putnam AJ. ECM compliance regulates osteogenesis by influencing MAPK signaling downstream of RhoA and ROCK. *J Bone Miner Res* 2009;24:886–98.
- [54] Strzelecka-Kiliszek A, Mebarek S, Roszkowska M, Buchet R, Magne D, Pikula S. Functions of Rho family of small GTPases and Rho-associated coiled-coil kinases in bone cells during differentiation and mineralization. *Biochim Biophys Acta Gen Subj* 2017;1861:1009–23.
- [55] Yoshikawa H, Nakase T. Stimulation of ectopic bone formation in response to BMP-2 by Rho kinase inhibitor. *Clin Orthop Relat Res* 2009;467:3087–95.
- [56] Xiao L, Zhu L, Yang S, Lei D, Xiao Y, Peng B. Different correlation of sphingosine-1-phosphate receptor 1 with receptor activator of nuclear factor kappa B ligand and regulatory T cells in rat periapical lesions. *J Endod* 2015;41:479–86.
- [57] Xiao L, Zhou Y, Zhu L, Yang S, Huang R, Shi W, et al. SPHK1-S1PR1-RANKL axis regulates the interactions between macrophages and BMSCs in inflammatory bone loss. *J Bone Miner Res* 2018;33:1090–104.
- [58] Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* 2014;507:323–8.
- [59] Kato A, Miyaji H, Ishizuka R. Combination of root surface modification with BMP-2 and collagen hydrogel scaffold implantation for periodontal healing in beagle dogs. *Open Dent J* 2015;9:52–9.
- [60] Yin Z, Fan L, Wei L. FTY720 protects cardiac microvessels of diabetes: a critical role of S1P1/3 in diabetic heart disease. *PLoS One* 2012;7:e42900.
- [61] Diogenes A, Simon S, Law AS. Regenerative endodontics. In: Haegreaves KM, Berman LH, editors. *Cohen's pathways of the pulp*. 11th ed. St. Louis: Elsevier; 2016. p. 447–73.
- [62] Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent Traumatol* 2001;17:185–7.
- [63] Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008;34:166–71.
- [64] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One* 2006;1:e79.
- [65] Saoud TM, Sigurdsson A, Rosenberg PA, Lin LM, Ricucci D. Treatment of a large cystlike inflammatory periapical lesion associated with mature necrotic teeth using regenerative endodontic therapy. *J Endod* 2014;40:2081–6.
- [66] Paryani K, Kim SG. Regenerative endodontic treatment of permanent teeth after completion of root development: a report of 2 cases. *J Endod* 2013;39:929–34.
- [67] American Association of Endodontics: Regenerative Endodontics, <https://www.aae.org/specialty/clinical-resources/regenerative-endodontics/>.
- [68] Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. *J Endod* 2007;33:377–90.
- [69] Song M, Cao Y, Shin SJ, Shon WJ, Chugai N, Kim RH, et al. Revascularization-associated intracanal calcification: Assessment of prevalence and contributing factors. *J Endod* 2017;43:2025–33.
- [70] Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004;364:149–55.
- [71] Murakami M, Hayashi Y, Iohara K, Osako Y, Hirose Y, Nakashima M. Tropic effects and regenerative potential of mobilized mesenchymal stem cells from bone marrow and adipose tissue as alternative cell sources for pulp/dentin regeneration. *Cell Transplant* 2015;24:1753–65.
- [72] Yao S, Pan F, Prcic V, Wise GE. Differentiation of stem cells in the dental follicle. *J Dent Res* 2008;87:767–71.
- [73] Tsukamoto-Tanaka H, Ikegame M, Takagi R, Harada H, Ohshima H. Histochemical and immunocytochemical study on hard tissue formation in dental pulp during the healing process after tooth replantation in rat molars. *Cell Tissue Res* 2006;325:219–29.
- [74] Bakhtiar H, Mazidi SA, Mohammadi Asl S, Ellini MR, et al. The role of stem cell therapy in regeneration of dental-pulp complex: a systematic review. *Prog Biomater* 2018, <http://dx.doi.org/10.1007/s40204-018-0100-7>.
- [75] Hosoya T, Morito A, Yamamoto J. Current overview and future prospects on dental pulp regeneration –A literature review of revascularization and tissue engineering-. *Jpn J Conserv Dent* 2019;62:177–89 (in Japanese).
- [76] Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000;97:13625–30.
- [77] Hilkens P, Gervois P, Fanton Y, Vanormelingen J, Martens W, Struys T, et al. Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. *Cell Tissue Res* 2013;353:65–78.
- [78] Kawashima N. Characterisation of Dental Pulp Stem Cells: A New Horizon for Tissue Regeneration? *Arch Oral Biol* 2012;57:1439–58.
- [79] Morito A, Kida Y, Suzuki K, Inoue K, Kuroda N, Gomi K, et al. Effects of basic fibroblast growth factor on the development of the stem cell properties of human dental pulp cells. *Arch Histol Cytol* 2009;72:51–64.
- [80] Yamada Y, Nakamura-Yamada S, Konoki R, Baba S. Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine. *Stem Cell Res Ther* 2020;11:175.
- [81] Xuan K, Li B, Guo H, Sun W, Kou X, He X, et al. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Sci Transl Med* 2018;10:455.
- [82] Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Arijii Y, et al. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem Cell Res Ther* 2017;8:61.
- [83] Aimetti M, Ferrarotti F, Cricenti L, Mariani GM, Romano F. Autologous dental pulp stem cells in periodontal regeneration: a case report. *Int J Periodontics Restorative Dent* 2014;34(Suppl 3):s27–33.
- [84] Ferrarotti F, Romano F, Gamba MN, Quirico A, Giraudi M, Audagna M, et al. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: a randomized controlled clinical trial. *J Clin Periodontol* 2018;45:841–50.
- [85] Hernández-Monjaraz B, Santiago-Osorio E, Ledesma-Martínez E, et al. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: a case report. *J Int Med Res* 2018;46:2983–93.
- [86] Aimetti M, Ferrarotti F, Gamba MN, Giraudi M, Romano F. Regenerative treatment of periodontal intrabony defects using dental pulp stem cells: 1-year follow-up case series. *Int J Periodontics Restorative Dent* 2018;38:51–8.
- [87] Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and safety issues of stem cell-based therapy. *Int J Med Sci* 2018;15:36–45.
- [88] Tominaga T, Asahina Y, Uyama Y, Kondo T. Regulatory science as a bridge between science and society. *Clin Pharmacol Ther* 2011;90:29–31.
- [89] Almeida LDF, Babo PS, Silva CR, Rodrigues MT, Hebling J, Reis RL, et al. Hyaluronic acid hydrogels incorporating platelet lysate enhance human pulp cell proliferation and differentiation. *J Mater Sci Mater Med* 2018;29:88.
- [90] Louvrier A, Euvrard E, Nicod L, Rolin G, Gindraux F, Pazart L, et al. Odontoblastic differentiation of dental pulp stem cells from healthy and carious teeth on an original PCL-based 3D scaffold. *Int Endod J* 2018;51(Suppl 4):e252–63.
- [91] Gangolli RA, Devlin SM, Gerstenhaber JA, Lelkes PI, Yang M. A bilayered poly (lactic-co-glycolic acid) scaffold provides differential cues for the differentiation of dental pulp stem cells. *Tissue Eng Part A* 2019;25:224–33.
- [92] Howard C, Murray PE, Namerow KN. Dental pulp stem cell migration. *J Endod* 2010;36:1963–6.
- [93] Pan HY, Yang H, Shao MY, Xu J, Zhang P, Cheng R, et al. Sphingosine-1-phosphate mediates AKT/ERK maintenance of dental pulp stem cell homeostasis. *Int Endod J* 2015;48:460–8.