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Review Article

R-spondin signaling as a pivotal regulator of tissue development and homeostasis

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ABSTRACT

R-spondins (Rspos) are cysteine-rich secreted glycoproteins which control a variety of cellular functions and are essential for embryonic development and tissue homeostasis. R-spondins (Rspo1 to 4) have high structural similarity and share 60% sequence homology. It has been shown that their cysteine-rich furin-like (FU) domain and the thrombospondin (TSP) type I repeat domain are essential for initiating downstream signaling cascades and therefore for their biological functions. Although numerous studies have unveiled their pivotal role as critical developmental regulators, the most important finding is that Rspos synergize Wnt signaling. Recent studies have identified novel receptors for Rspos, the Lgr receptors, closely related orphans of the leucin-rich repeat containing G protein-coupled receptors, and proposed that Rspos potentiate canonical Wnt signaling via these receptors. Given that Wnt signaling is one of the most important developmental signaling pathways that controls cell fate decisions and tissue development, growth and homeostasis, Rspos may function as key players for these processes as well as potential therapeutic targets. Here, I recapitulate the Wnt signaling and then outline the biological role of Rspos in tissue development and homeostasis and explore the possibility that Rspos may be used as therapeutic targets.

1. Introduction

Tissue homeostasis is a highly regulated biological process which maintains the right body composition and its dysregulation often leads to pathological conditions. For instance, dysregulation in developmental stage induces clearly a disadvantage in biological organization. Bone is a tightly regulated tissue which contributes to the mechanical support of the body. Bone homeostasis is mainly maintained by 3 players; (1) bone-resorbing osteoclasts which are originated from hematopoietic stem cells (HSCs), (2) bone-forming osteoblasts which derive from bone marrow stromal cells (BMSCs) and; (3) osteocytes which are differentiated from osteoblasts. Osteoclasts differentiation is critically regulated by receptor activator of NF-κB ligand (RANKL) and its decoy receptor osteoprotegerin (OPG), which are secreted by osteoblasts [1–3] and probably also by osteocytes [4,5]. Dysregulation of bone homeostasis causes pathological conditions such as osteoporosis, which in turn leads to increasing risk of fracture. Fractures further lead to mobility limitation, which ultimately leads to the so-called 'locomotive syndrome' [6] and severely affect quality of life and are often associated with mortality. Thus, understanding the mechanisms by which tissue homeostasis is regulated is of the most significance.

R-spondins (Rspos) were first discovered as proteins belonging to the thrombospondin (TSP) family [7,8] and then identified as direct activators of Wnt/β-catenin signaling [9]. In the last decades, numerous studies have demonstrated the importance of Wnt signaling and its regulators, including Rspos, in the maintenance of tissue homeostasis. Wnt signaling is well known to regulate cell proliferation and function as well as tissue development, growth and maintenance including bone [10]. Recent findings have expanded our understanding of Wnt signaling and its regulatory functions, its critical regulation by Rspos in tissue development and maintenance and its use as a potential therapeutic target. In this review, I summarize recent findings related to Rspos and Wnt signaling to explore their biological role and function in tissue homeostasis.

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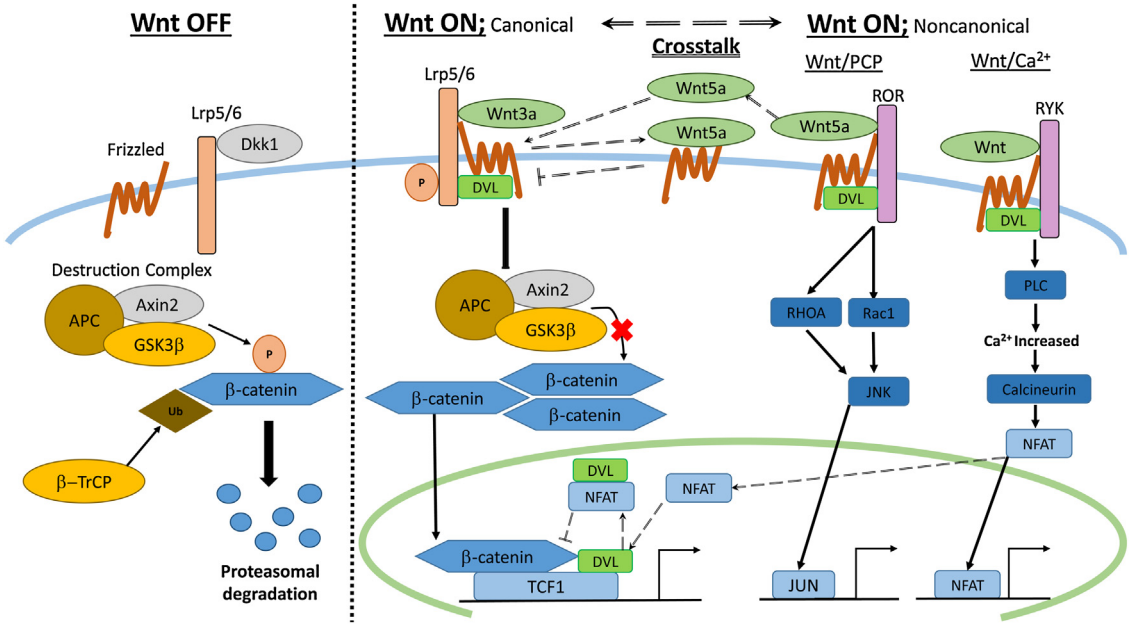

Fig. 1. Schematic diagram of Wnt signaling regulation. When the Wnt signaling is off, so-called destruction complex phosphorylate β-catenin followed by βTrCP-induced ubiquitination and its proteasomal degradation. When the Wnt signaling is on, the Wnt/receptor complex initiates downstream signaling via (1) non-phosphorylated β-catenin and transcription factor TCF1 (canonical signaling), (2) JNK (noncanonical Wnt/PCP signaling) or (3) NFAT (noncanonical Wnt/Ca^{2+} signaling). Both Wnt5a and NFAT can suppress canonical Wnt signaling (dashed arrows). Wnt5a competes with Wnt3a for binding to Frizzled to inhibit the initiation of canonical signaling, while NFAT interacts with nuclear DVL competitively against β-catenin to downregulate downstream gene transcription.

2. Wnt signaling and tissue homeostasis

Wnt signaling consists of 2 pathways; β-catenin-dependent "canonical" signaling and β-catenin-independent noncanonical signaling (Fig. 1, solid arrows). In brief, when Wnt ligand is apart from its receptor Lrp5/6 and co-receptor Frizzled, canonical Wnt/β-catenin signaling is "off" due to proteasomal degradation of β-catenin, which is phosphorylated by a so-called destruction complex which includes Axin2, APC, CK1 and GSK3β. When a Wnt ligand such as Wnt3a binds to Lrp5/6 and Frizzled and the canonical Wnt signaling is "on", Lrp5/6 are phosphorylated leading to stabilization of cytosolic β-catenin, translocation into the nucleus and subsequent activation of downstream target genes by binding to transcription factor TCF1. Canonical Wnt/β-catenin signaling is mainly involved in cell proliferation and differentiation and in particular, positively affects osteoblast differentiation and activities [10,11]. Noncanonical Wnt signaling is subdivided into 2 main pathways; Wnt/PCP signaling and Wnt/Ca^{2+} signaling. Noncanonical Wnt/PCP signaling is initiated through noncanonical Wnt ligand such as Wnt11 or Wnt5a binding to Frizzled and co-receptor such as receptor tyrosine kinase-like orphan receptor (Ror), followed by the activation of small GTPase RAC1, RHOA and Jun-N-terminal kinase (JNK). Noncanonical Wnt/PCP signaling is mainly involved in regulating cell polarity during cell movement [11–13]. An important finding for bone homeostasis is that the Wnt5a-Ror2 pathway functions as a critical regulator for osteoclastogenesis [14]. The noncanonical Wnt/Ca^{2+} signaling pathway is also initiated by noncanonical Wnt ligand binding to Frizzled and co-receptor such as RYK, which in turn induces activation of phospholipase C (PLC), followed by increasing in intracellular calcium concentration. This event activates calcineurin and nuclear factor of activated T cells (NFAT), which in turn translocates into nucleus and regulates downstream target genes. Noncanonical Wnt/Ca^{2+} signaling is involved in cancer [15,16], maintenance of HSCs [17] and osteoblastogenesis [18,19].

Importantly, noncanonical Wnt signaling can inhibit canonical Wnt signaling via both extracellular and intracellular mechanisms (Fig. 1, dashed arrows). Indeed, it has been reported that Wnt5a, binds to Frizzled2 to induce noncanonical Wnt signaling and compete with the canonical Wnt ligand Wnt3a, thus suppressing Wnt/β-catenin signaling [20]. Other studies have shown that NFAT interacts directly with the Wnt signaling adaptor protein, Dishevelled (Dvl) in a Ca^{2+}-dependent manner [21]. Since Dvl in the nucleus binds to β-catenin leading to stabilization of β-catenin [22], noncanonical Wnt signaling-induced NFAT acts as an inhibitor for canonical Wnt signaling. Further investigations are needed to explore the biological role of this crosstalk between the canonical and noncanonical Wnt cascades.

3. R-spondin signaling and tissue homeostasis

3.1. Structure and molecular mechanism of R-spondin

R-spondins (roof plate-specific spondins, Rspos), are cysteine-rich secreted glycoproteins which control a variety of cellular and tissue functions [23]. In mammals, Four R-spondins (Rspo1 to 4) show high structural similarity and 60% sequence homology [24]. They all contain four distinct domains: a putative signal peptide domain, a cysteine-rich furin-like (FU) domain, a thrombospondin (TSP) type I repeat domain and a basic amino acid-rich (BR) domain [9]. The FU domains are essential to amplify the Wnt ligand-dependent activation of canonical Wnt signaling [9,25,26]. After the identification of Lgr4/5/6, previously thought to be orphan receptors, as mediators of Wnt and Rspo signaling [27–30], crystal structure analysis confirmed that one of FU domains of the Rspos binds to Lgr receptors [31–33]. The other FU domain binds to the cell-surface transmembrane E3 ubiquitin ligase Znrf3/Rnf43 [34,35], which antagonizes Wnt signaling by ubiquitinating Frizzled receptors followed by endocytosis of Wnt receptor complex [36,37]. In this context, the Rspo-Lgr complex binds to Znrf3/Rnf43

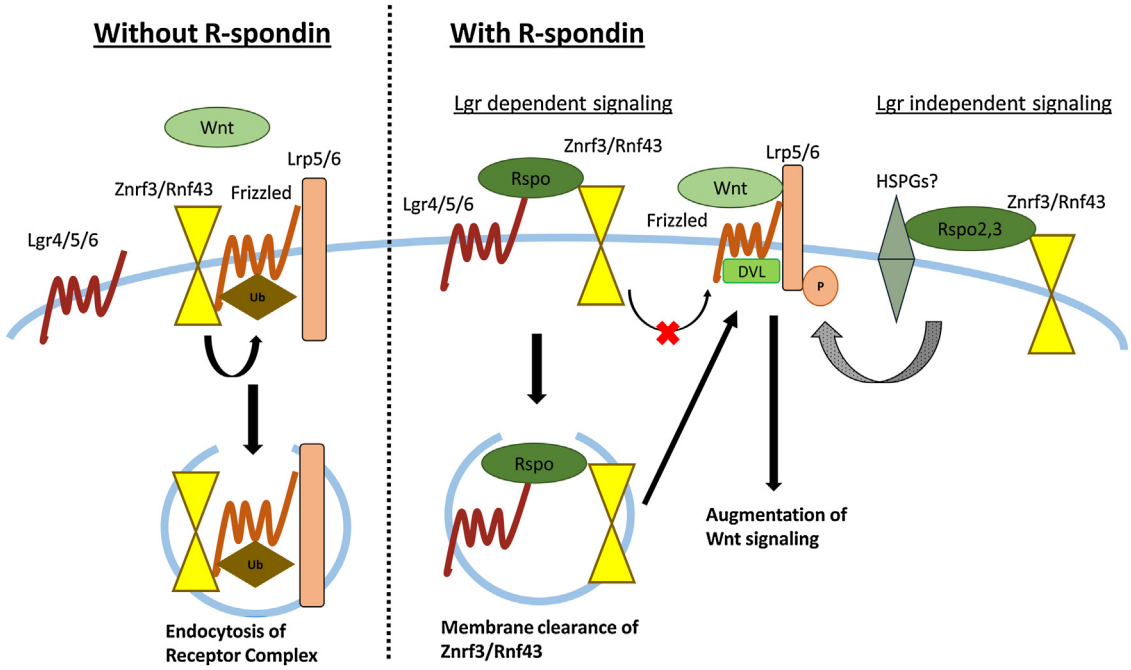

Fig. 2. Schematic diagram of Rspo-induced Wnt signaling augmentation. Without Rspos, Frizzled is ubiquitinated by Znrf3/Rnf43 followed by endocytosis of receptor complex and initiation of Wnt signaling is inhibited. Rspos bind to their receptors Lgr4/5/6 and this complex bind to Znrf3/Rnf43 followed by membrane clearance of these E3 ubiquitin ligases. This allows the receptor complex to initiate downstream signaling, which in turn leads to augmentation of Wnt signaling cascade. Importantly, Rspo2/3 are currently proposed to accelerate Wnt signaling through Lgr-independent signaling.

to block the ubiquitination of Frizzled receptors, leading to augmentation of the Wnt signaling cascade (Fig. 2). Recently, however, it has been reported that Rspo2 and Rspo3 can also amplify canonical Wnt signaling independently of Lgrs, via membrane-bound heparin sulfate proteoglycans (HSPG) [38], previously reported to bind the TSP and BR domains of Rspos [24]. Both these domains were previously considered to be dispensable for amplification of canonical Wnt signaling [9]. Indeed, Rspo3 can bind to Syndecan4, one of the HSPGs, and trigger Wnt/PCP signaling activation but not for canonical Wnt signaling [39]. Later, however, Syndecan4 as well as Syndecan2 were shown to function as inhibitors of canonical Wnt signaling through regulation of Rspo signaling [40,41], while Syndecan1 promotes canonical Wnt signaling through Wnt ligand and Rspo in multiple myeloma cells [42]. These findings clearly suggest the differential role of each Rspo domains and further investigation is needed to identify how and/or whether Lgrs and Syndecans interact with each other to regulate Rspo signaling.

R-spondins are widely expressed in *Xenopus* and during mouse embryogenesis [7,9,43], suggesting a role for Rspos as developmental regulators. Human and mouse genetic studies have demonstrated a biological function for Rspos *in vivo* and have shown that manipulation of distinct Rspos leads to distinct phenotype suggesting unique functionality. Rspos also act as growth factors for organs and tissues including bone, again suggesting the importance of Rspos and Wnt signaling in tissue homeostasis. Here I describe the differential role of each Rspos (Table 1) including their receptor Lgrs.

3.2. *Rspo1*

Rspo1 was first identified by the screening for genes specifically expressed in the mouse NSC-19 cell line and interaction with Wnt signaling was unveiled by showing Rspo1 expression in Wnt1 and/or 3 knockout mice [7]. In human, the Rspo1 gene is mutated in individuals with palmoplantar hyperkeratosis and can lead to squamous cell carcinoma of the skin and seminoma [44,45]. Rspo1 was also reported as a sex determinant [44,46] and as a potential therapeutic agent for intestinal epithelium damage [47–51]. Importantly, Rspo1 was also reported to protect from radiation-induced oral mucositis [52], suggesting a role as a promising therapeutic agent for oral mucosal damage.

In bone, Rspo1 was first reported to induce and enhance osteoblast differentiation of mouse C2C12 cells via synergistic effect with Wnt3a [53], a role later confirmed in a human osteoprogenitor cell line [54]. In both cases, Rspo1 synergizes with Wnt3a to enhance canonical Wnt signaling as well as osteogenic markers including alkaline phosphatase activity and osteocalcin expression. Moreover, *in vitro* studies have shown that Rspo1 inhibits osteoclastogenesis by regulating OPG expression by osteoblasts, a mechanism by which Rspo1 protects against inflammatory bone damage from arthritis [55]. Rspo1 administration was also reported to induce an anabolic effect in age-related bone loss mouse models [56]. These findings clearly imply the use of Rspo1 as a potential therapeutic agent against pathological- and aging-related bone loss, although the bone phenotype of Rspo1-deficient mice has not been reported yet.

Of note, excessive activation of Rspo1 signaling induces several adverse events. Tissue microarray of human fibrotic liver samples display excessive Rspo1 expression [57], suggesting a link between Rspo1 and liver fibrosis. In addition, Rspo1 gain-of-function mouse model revealed that Rspo1 activation was sufficient to promote ovarian tumor development [58]. Hence, detailed mechanism of these adverse events should be addressed for better understanding and exploring the potential of therapeutic use of Rspo1.

3.3. *Rspo2*

Rspo2 was the first Rspo to be shown to function as a positive modulator of canonical Wnt signaling [9]. In *Xenopus* embryos, Rspo2 is required for canonical Wnt signaling and for muscle development [9]. In mice, Rspo2 is required for proper limb development [59–61], suggesting the pivotal role of Rspo2 during embryonic

Table 1
An overview of RSPOs function, downstream signal pathways and associated diseases.

RSPOs	Function	Downstream signal pathways	Associated diseases	References
Rspo1	Female sex determination	Wnt4/β-catenin signaling during ovarian development	XX-male sex reversal	[44,46]
	Growth factor for intestinal or oral epithelium	Activation of β-catenin signaling	Gastrointestinal or oral mucositis	[47–52]
	Bone anabolic effect	Enhancement of osteogenic markers and osteoprotegerin expression	Pathological or age-related bone loss	[53–56]
Rspo2	Normal limbs and craniofacial skeletal development	Activation of β-catenin signaling through antagonizing Rnf43/Znrf3	Asymmetric malformation of limbs and craniofacial skeletal defect	[59–66]
	Bone anabolic effect	Activation of β-catenin signaling through Lgr4	Pathological or age-related bone loss	[67,70]
	Inhibition of chondrogenesis	Downregulation of Col2a1 and Sox9 expression	Disarrangement of chondrocyte and ossification of the posterior longitudinal ligament of the spine	[73,74]
Rspo3	Key regulator for vascular stability	(1) Regulation of VEGF expression through β-catenin signaling or (2) controlling Wnt/Ca^{2+} signaling	Placenta abnormality due to vascular defect	[75,76,80]
	Key factor for cardiac development	Regulation of cardiogenic fate markers through β-catenin signaling in embryonic stem cells	Cardiac malformation	[78]
	Determinant for liver zonation	Regulation of zonation marker genes through β-catenin signaling in hepatocytes	Metabolic disorders	[79]
	Regulator for osteoblastogenesis	Enhancement of osteogenic markers through Lgr4	Pathological or age-related bone loss	[85,86]
Rspo4	Normal nail development	Activation of β-catenin signaling (suggested)	Anonychia	[87–89,92]

bone development. Several groups have generated Rspo2 mutated mice to identify its role during development. Mice with a transgene insertion resulting in Rspo2 gene disruption ($Rspo2^{Tg}$) exhibit asymmetric malformations of the limbs and are called *Footless,* named after their phenotype [62]. Not only *Footless,* but also Rspo2-deficient mice exhibit hindlimb development defects, lung hypoplasia and branching defects and died immediately after birth due to respiratory failure [59–61,63,64]. These studies support a role for Rspo2 as a critical factor for embryonic development. Rspo2-deficient mice also display craniofacial malformation, characterized by cleft lip, cleft palate and other skeletal defects [63,64]. Detailed analysis revealed that Rspo2 is expressed in branchial arch and contributed to nasal, maxillary and mandibular processes. Attenuated canonical Wnt signaling was observed in $Rspo2^{Tg}$ [61] and Rspo2-deficient mice [59,63], suggesting that Rspo2 regulates embryonic development through canonical Wnt signaling. Furthermore, it was recently reported that Rspo2 serves as a direct antagonistic ligand for Znrf3/Rnf43 without Lgr receptors to regulate human limb development [65]. Rspo2-null zebrafish also displays skeletal malformations, including absence of fin ray skeleton and hypoplasia of the rib [66]. These findings suggest that Rspo2 is a key regulator of musculoskeletal development through Wnt signaling.

In terms of the function of Rspo2 in bone homeostasis, *in vitro* study using the preosteoblastic cell line, MC3T3E1, revealed that Wnt11-induced osteoblast differentiation and mineralization is mediated by Rspo2 signaling [67]. Overexpression of Wnt11 or Rspo2 enhanced BMP2-induced mineralization of MC3T3E1 cells, whereas Rspo2 knockdown completely abolished Wnt11-induced mineralization. Although Wnt11 was reported to activate noncanonical Wnt signaling and repress canonical Wnt signaling [68,69], Wnt11 treatment actually stabilized β-catenin through induction of Rspo2 expression in BMP2-induced mineralization. These data indicate the essential role of Rspo2 in regulating canonical and noncanonical Wnt signaling. Additionally, another group also reported that Rspo2 enhances mineralization of MC3T3E1 cells *in vitro*, as well as potentiates canonical Wnt signaling [70]. These effects were abolished by Lgr4 knockdown. Furthermore, they reported that Rspo2 could inhibit osteoclastogenesis by regulating OPG expression in osteoblasts *in vitro* and that recombinant human Rspo2 treatment rescued ovariectomy-induced bone loss *in vivo*. Other groups also showed that recombinant human Rspo2 treatment significantly decreased Sox9 and Col2a1 mRNA expression, which are essential for chondrocyte differentiation [71,72], as well as increased canonical Wnt signaling in ATDC5 chondrogenic cells [73,74]. The findings that Rspo2-deficient embryos exhibits increased collagen type 2 and Sox9 immunostaining in cartilage at E18.5 [73], indicate that Rspo2 has a pivotal role in chondrocyte differentiation. These studies clearly indicate the association between exogenous Rspo2 and osteo- and chondrogenic differentiation. Further investigation using site-specific deletion of Rspo2 is needed to identify the role of endogenous Rspo2 in bone homeostasis.

3.4. *Rspo3*

Rspo3 is the first member of the Rspo family identified in 2002, and was initially called PWTSR [8]. Lately renamed Rspo3, its significant role in angiogenesis was discovered. Indeed, targeted disruption of Rspo3 in mice resulted in lethality by embryonic day 10.5 due to impaired formation of normal placenta [75] and knockdown of Rspo3 gene in Xenopus embryos induces vascular defects [76]. Knockdown of Rspo3 resulted in downregulation of VEGF, a key contributor of vessel formation, via decreased Wnt signaling supporting a role for Rspo3 in angiogenesis. Specific deletion of Rspo3 in developing limb mesenchyme revealed that limb development is ultimately normal in the mutant adult mice, while slight growth retardation was observed during development [77]. By using Cre transgene driven by Isl1, which marks a subset of undifferentiated cardiac progenitors, Rspo3 was shown to be essential for cardiac development [78]. In addition, Rspo3 also serves as a key determinant for proper function of liver [79]. Similar to Rspo1 and Rspo2, Rspo3 potentiates canonical Wnt signaling [24,25]. Although activation of canonical Wnt signaling by Rspo3 was increased when Syndecan4, one of the member of transmembrane HSPGs, was downregulated [40], Rspo3 can also activate noncanonical Wnt/PCP signaling via its binding to Syndecan4, which promote head cartilage morphogenesis in *Xenopus* [39]. These studies suggest the important role of Rspo3/Syndecan4 axis in the crosstalk between canonical and noncanonical Wnt signaling. Rspo3 also regulates vascular stability through noncanonical Wnt/Ca^{2+} signaling [80]. These findings clearly indicate that Rspo3 functions through both canonical and non-canonical Wnt signaling and that Rspo3 may act as a central regulator for crosstalk between 2 Wnt signaling pathways.

Genome-wide association study (GWAS) revealed the significant association of Rspo3 with hip bone mineral density (BMD) in postmenopausal women [81], increased spine BMD in Icelanders [82] and fracture risk from 3 combined cohorts [83]. Rspo3 gene is also used as a genetic marker for ultrasound-assessed bone mass in young adults population [84], suggesting the relation-

ship with not only aging-related bone mass changes but also bone accrual during early adulthood. Of note, my laboratory have reported that Rspo3 haploinsufficiency in mice unexpectedly leads to higher trabecular bone mass [85]. Recently, *in vitro* study using human adipose-derived stem cells (hASCs) also showed that Rspo3 knockdown by shRNA induced increased osteogenic potential [86]. Rspo3-knockdown-induced enhancement of osteogenic potential in hASCs was diminished by the loss of Lgr4, suggesting the significant role of Rspo3-Lgr4 axis in osteogenesis. Since Rspo3 is thought to act as a potentiator of canonical Wnt signaling and enhanced canonical Wnt signaling is thought to exert a positive effect on bone formation, these studies indicate a complexed role for Rspo3 in osteogenesis.

3.5. *Rspo4*

Rspo4 inactivating mutation was found in anonychia, a human rare autosomal recessive congenital syndrome, characterized by complete or partial absence of fingernails and toenails without significant bone abnormalities [87–89]. However, Rspo4 gene was not mutated in a patient with congenital nail hypoplasia with underlying skeletal defects [90], suggesting the differential regulation of Rspo4 in nail and skeletal development. In mice, Rspo4 expression was localized to developing nail mesenchyme at embryonic day 15.5 [87] and 14.5 [89]. In addition, Rspo4 expression was also detected in the dental mesenchymal cells at embryonic day 14.5 [43] and developing mouse molar tooth at postnatal day 1 and 10 [91]. These observations suggest a potential role of Rspo4 in regulating the interaction between epidermis and mesenchyme during development.

In the anonychia patients, Rspo4 gene is mutated in the FU domain, which, as mentioned above, is essential for potentiating canonical Wnt signaling [87,89,92], and therefore suggest a compromised canonical Wnt signaling in this disease. From a mechanistic point of view, similar to the other Rspos, Rspo4 can potentiate canonical Wnt signaling, but its effect is significantly weaker than that of the other Rspos [25]. This differential activating potential was further confirmed in 2 systems; 10-times more amount of Rspo4 protein is required for haploid human cell line HAP1-7TGP cells [38] and 6 to 100-times more concentrated Rspo4 was shown to be needed for HEK293-STF cells [93] to obtain the same level of activation as other Rspos. Hence, although Rspo4 plays a significant role in nail development, its function and efficacy seems strictly restrained compared to other Rspos.

3.6. *Lgr receptors*

Lgr receptors, which belong to G-protein-coupled receptors (GPCRs), contain a large N-terminal extracellular leucine-rich repeat domain that binds glycoprotein hormones [94]. Recent investigations have identified Lgr4/5/6 as receptors for Rspos [27,29,30,95]. All Rspo binds to Lgr4, 5 and 6 with high affinity through their FU domains to regulate canonical and noncanonical Wnt/PCP signaling [29,95]. Interestingly, although Lgr4/5/6 belong to GPCRs, these receptors do not induce typical GPCR signaling activities such as G protein pathway activation or β-arrestin translocation through binding to Rspos [27,29,30], suggesting their unique mechanism.

Lgr receptors were first discovered as markers for stem cells, although Lgr4 is required for maintenance of intestine stem cells [96] and mammary gland stem cell activity [97]. Lgr5 marks intestinal and hair follicle stem cells [98,99] and is upregulated in basal cell carcinoma [100], whereas Lgr6 marks nail stem cells [101], stem cells in the hair follicle [102] and in mouse skin squamous cell carcinoma [103]. Gene ablation studies in mice have further identified the function of each Lgrs. Lgr4-deficient mice exhibited pre- and postnatal lethality [104,105], and display smaller body size. Lgr4-deficient mice display delayed bone formation and reduced bone mass [106]. Moreover, mice carrying global or monocyte-specific knockout of Lgr4 exhibited robust activation of osteoclasts via RANKL signaling and bone loss [107], whereas Lgr4 was down-regulated during *in vitro* osteoclast-like cells fusion [108]. These studies suggest the importance of Lgr4 in bone homeostasis. In fact, a rare nonsense mutation within the human Lgr4 gene was reported to be strongly associated with low BMD and osteoporotic fracture [109]. Of note, Lgr4 also functions as a key regulator of molar tooth development [110]. Lgr5-deficient mice also exhibit neonatal lethality with the condition of ankyloglossia, in which immobilized tongues adherent to the floor of the oral cavity [111]. Immunostaining of Lgr5 revealed its expression in the epithelium of the tongue and mesenchyme of the mandible at E14.5. Lgr5-positive cells have been found in the periodontal ligament [112] and isolated adult human epithelial stem cells from the periodontal ligament expressed pluripotency factors as well as Lgr5 [113]. These studies may contribute the evidence for Lgr5-positive stem cells residing in the periodontal ligament and its potential for using as pluripotent stem cells. While, mice with homozygous deletion of Lgr6 exhibited no significant phenotype, but nail regeneration failure as well as impaired bone regeneration after amputation were observed [101]. However, it is still unclear whether Lgr6-deficient osteoblasts are functionally responsible for the defect in bone regeneration. It has been recently reported that Lgr6 knockdown in BMSCs enhanced osteogenic differentiation and improved fracture healing, while Lgr6 overexpression inhibited it [114]. Those findings suggest that Lgr6 also plays an important role in bone homeostasis.

4. Conclusion

Here I described the Rspo family and their roles in development and tissue homeostasis mainly focusing on bone. It is evident that these secreted proteins have a significant impact by their own or by regulating Wnt signaling activity. However, many questions remained to be elucidated, such as whether Rspos expression is regulated by each other and whether their functions are associated with their Lgr-dependent and Lgr-independent signaling. How Rspos have distinct effects, although functioning via the same axis, is also poorly understood. Moreover, studies to assess whether Rspo signaling may contribute to the therapeutic approach for disease condition are currently under investigation [114–116].

Given that Wnt signaling can be targeted for drug development, understanding how we can manipulate the different players within the Wnt signaling pathways, including Rspos, is a major focus for developing new anabolics for treating bone diseases such as osteoporosis, which in turn leads to further elucidating the tissue homeostasis.

Conflict of interest

Author has nothing to declare.

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