The R-spondin protein family

Wim BM de Lau1, Berend Snel2 and Hans C Clevers*1

Abstract
The four vertebrate R-spondin proteins are secreted agonists of the canonical Wnt/β-catenin signaling pathway. These proteins are approximately 35 kDa, and are characterized by two amino-terminal furin-like repeats, which are necessary for potentiation of Wnt signal potency, and a thrombospondin domain situated more towards the carboxyl terminus that can bind matrix glycosaminoglycans and/or proteoglycans. Although R-spondins are unable to initiate Wnt signaling, they can potently enhance responses to low-dose Wnt proteins. In humans, rare disruptions of the gene encoding R-spondin1 cause a syndrome of XX sex reversal (phenotypic male), palmoplantar keratosis (a thickening of the palms and soles caused by excess keratin formation) and predisposition to squamous cell carcinoma of the skin. Mutations in the gene encoding R-spondin4 cause anhidrosis (absence or hypoplasia of nails on fingers and toes). Recently, leucine-rich repeat-containing G-protein-coupled receptor (Lgr)4, Lgr5 and Lgr6, three closely related orphans of the leucine-rich repeat family of G-protein-coupled receptors, have been identified as receptors for R-spondins. Lgr5 and Lgr6 are markers for adult stem cells. Because R-spondins are potent stimulators of adult stem cell proliferation in vivo and in vitro, these findings might guide the therapeutic use of R-spondins in regenerative medicine.

Keywords Adult stem cells, canonical Wnt signaling, furin-like repeat, Lgr5, R-spondin, thrombospondin domain.

Gene organization and evolutionary history
The R-spondins are members of a superfamily of thrombospondin type 1 repeat (TSR-1)-containing proteins. The prototype member (discovered in 1971) was isolated from platelets that had been stimulated with thrombin, and was therefore designated ‘thrombin-sensitive protein’ [1]. The TSR-1 repeat (also known as properdin repeat) was then characterized in the thrombospondin proteins (TSPs), in which it is repeated three times [2]. TSP1 and TSP2 are secreted multimeric matricellular proteins that, in addition to the TSP repeat, share homology in an amino-terminal globular region, von Willebrand factor domain, type II repeats (epidermal growth factor (EGF)-like), type III repeats (calcium binding) and the carboxy-terminal region. These modular proteins act by bringing together cytokines, growth factors, membrane receptors and extracellular proteases. Several proteins involved in the complement pathway (properdin, C6, C7, C8A, C8B, C9) and extracellular matrix proteins, such as mindin, F-spondin and SCO-spondin, contain one or more TSR-1 repeats.

The prefix R in the R-spondin subfamily of TSR-1-containing proteins derives from the expression of the gene encoding murine R-spondin1. This gene is transiently expressed in the neural tube at 10 and 12 days post-conception, in the boundary region between the roof plate and neuroepithelium, hence its name R(oof plate specific)-spondin [3]. In addition to the presence of the TSR-1 domain, all four R-spondin members are characterized by the presence of a carboxy-terminal region with positively charged amino acids and, importantly, two furin-like cysteine-rich repeats near the amino terminus of the mature protein. Furin repeats (first seen in the endoprotease furin) are also present in receptors for growth factors such as EGF, insulin, hepatocyte growth factor (HGF) and neurotrophic factors. The R-spondin family was discovered over a 4-year period. R-spondin3 was discovered in 2002 [4], whereas descriptions of R-spondin1 [3] and R-spondin2 followed in 2004 [5]. Finally, R-spondin4 was characterized in 2006 [6].

R-spondin homologs (defined by two Fu domains followed by a TSP1 domain) are present in all vertebrates, in primitive chordates such as the lancelet Branchiostoma floridae, in the hemichordate acorn worm Saccoglossus kowalevskii and in the echinodermate sea urchin Strongylocentrotus purpuratus (Figure 1). No homologs with an R-spondin domain composition are found in
invertebrate model organisms such as *Drosophila* or *Caenorhabditis*, or any other primitive animal. Given this phylogenetic distribution, an R-spondin-like gene was likely to have been present in the deuterostome ancestor and, given its absence outside the deuterostome clade, also originated there. An evolutionary tree of these sequences rooted on the primitive deuterostomes clearly shows the two successive genome duplications that generated present day R-spondin diversity in vertebrate species such as fish and mammals (Figure 1).

The mammalian R-spondins have a similar five-exon gene organization and protein domain structure. The human family members share a pair-wise amino acid similarity of 40% to 60% (Figure 2). The amino-terminal hydrophobic signal peptide ensures that secretion is encoded by the first exon, whereas the two cysteine-rich furin-repeat domains are encoded by exons 2 and 3, and a single TSP1 domain is encoded by exon 4. Exon 5 encodes a region in the protein that is solely characterized by its high density of basic amino acids.

**Characteristic structural features**

The four R-spondin proteins share a common domain architecture. An amino-terminal endoplasmic reticulum signal peptide ensures entry into the secretory pathway. The processed mature protein has two cysteine-rich furin-like repeats at the amino terminus. The central TSR-1 domain is followed by a region with a high number of basic amino acids at the carboxyl terminus (Figure 2). The two furin-like repeats near the amino terminus are related to a domain seen in the subtilisin-like proprotein convertase family member furin. Although the function of this domain in furin is unknown, its prevalence in a number of important receptors for growth factors, such as EGF, insulin, HGF and neurotrophic factors, suggests it makes a significant functional contribution.

Mass spectrometry approaches have provided some insight into the molecular structure of the furin domains in R-spondins [7]. That study by Li *et al.* recorded the pattern of disulfide bonds between the 15 available cysteine residues present in these domains. In a purified peptide containing both furin-like repeats of R-spo2, they determined the free and interconnected cysteine residues. In total, five free cysteine residues were found: three in...
furin repeat 1 and two in furin repeat 2. All interconnected cysteine residues appeared to be separated by only two or three intervening amino acids. No crystallographic study of furin-like repeats in R-spondins is yet available. However, such analyses have been performed for the EGF receptor and insulin growth factor receptor 1 [8,9]. These revealed the existence of three pairs of linked cysteine residues in furin-like repeat 1 that successively bridge 5, 8 and 18 intervening residues. No unbound cysteine residues remained. It is unclear whether these divergent outcomes reflect consequences of the techniques used or structural differences underlying the specific roles of these domains in the proteins studied.

The second domain that is common to all four R-spondins is a TSR-1 domain. The human genome harbors 41 proteins that contain TSR-1 domains. The number of the TSR-1 domains in these proteins varies from 1 to 18. All of the TSRs occur either in secreted proteins or in the extracellular portion of transmembrane proteins. The TSR-1 domain in R-spondin may have a role related to glycosaminoglycan (GAG)/proteoglycan binding. Several observations supporting such a role have been made in other TSR-1-domain-containing proteins. Multiple amino acid sequence alignments of TSRs show that a typical TSR domain consists of 60 amino acids, of which 12 are highly conserved [10,11]. X-ray crystallography of the TSR-1s of human TSP1 led to the discovery of the CWR layer, an architecture composed of three antiparallel strands. Strand A assumes a rippled conformation, whereas strands B and C assume regular β-sheets. The side chains of the tryptophan residues in the A strand make up two W-layers. Two arginine residues in the B strand comprise the R-layers. The alternate stacking of the cationic guanidinium groups of the arginine residues with the aromatic side chains of the tryptophan residues provide vital stabilization in the structure of this small domain. Additional solidity derives from the C-layers, disulfide bonds capping the amino-terminal and carboxy-terminal ends of the strands. The exposed tryptophan residues and arginine residues define the front face of the domain and are likely to contact the negatively charged repeating disaccharide units of GAGs and proteoglycans. Moreover, the disaccharide units in GAGs span approximately 9 Å, enabling two units to fit into the recognition groove of the TSR-1 [12]. The three-dimensional structure of R-spondins is not yet available, but molecular modeling techniques have also predicted a GAG-binding site for the TSR of R-spondin 4 [13]. A recently reported binding of R-spondin3 to the transmembrane proteoglycan syndecan-4 is consistent with these findings [14].
It will be of interest to determine the GAG-binding specificity of the four R-spondin TSR-1s and to translate this knowledge into functional models.

**Localization and function**

Extensive functional analysis of the R-spondin proteins, using Wnt reporter assays in 293T cells, uncovered a link with the canonical Wnt/β-catenin pathway [5] (Figure 3). The latter plays a central role in cellular proliferation, differentiation and stem cell maintenance. Activity is initiated when secreted proteins of the Wnt family bind to Frizzled (Fzd) receptors and the low-density lipoprotein receptor related protein 5 or 6 (LRP5/6) co-receptors. At this level, the pathway is controlled by a series of extracellular antagonists (Figure 3). R-spondins uniquely synergize with Wnt proteins. Accordingly, R-spondin activation showed sensitivity to the presence of the extracellular Wnt inhibitor Dickkopf-1 (DKK1) and no synergy could be induced by overexpression of any of the known intracellular components of the pathway. Protein
domain analysis showed that furin repeats are essential and sufficient to mediate the Wnt-potentiating effect of the R-spondins [5,7,15]. The first in vivo experiments documenting this Wnt potentiating phenomenon were performed in early frog embryos [5]. Depletion of R-spondin2 in one blastomere at the eight-cell stage resulted in disorganized somites and a reduction in myotomes at the injected site. Depletion at the gastrula stage resulted in a failure to transcriptionally activate the myoD and myf5 genes, later leading to impaired muscle development. Manipulation of Wnt activity at this developmental stage, in chick and mammals, strikingly phenocopies these effects [16,17]. Canonical Wnt pathway potentiation by R-spondins has also been seen in experimentally induced tumors. A sustained high level of Wnt activity in the tumor was explained by the finding that mammary tumor virus integration sites were seen in both genes for Wnt family members and the gene for R-spondin2 [18].

R-spondins operate during embryogenesis

Wnt signaling is important in almost every fate decision during embryonic development throughout the animal kingdom [19]. The knowledge obtained of the Wnt-enhancing ability of R-spondins together with their dynamic expression patterns in embryonic tissues (Additional File 1) predicts pleiotropic roles for R-spondins during embryogenesis.

R-spondin 1: sex phenotype reversal

R-spondin controls the most fundamental difference between individuals: their sex phenotype (Figure 4). The phenotypic sex of the embryo depends on gonadal sex determination. XY male to female sex reversal is relatively frequent, whereas XX male sex reversal is rare and usually caused by translocation of the sex-determining region Y (SRY) gene. Mutations in RSPO1 (encoding R-spondin1) lead to an extremely rare human syndrome that combines SRY-independent XX male sex reversal with palmoplantar hyperkeratosis (PPK; an abnormal thickening of the palms and soles), and a predisposition to squamous cell carcinoma (SCC) of the skin. Parma et al. [20] described an Italian family with 11 46,XX individuals in two sibships. All affected individuals were phenotypically male. The seven genetic females did not show signs of the PPK/SCC phenotype or sexual ambiguity. Parma et al. postulated that homozygosity for a single mutational event causes both PPK and SCC in XY and XX individuals, and sex reversal in XX individuals. A genetic analysis of this family, complemented with an individual from a family with an independent mutation, proved the presence of mutations in RSPO1. Two types of mutations appeared to result in an absence of functional protein. PPK and SCC could be explained by fibroblast-derived R-spondin1 stimulation of keratinocytes, leading to a reduced level of β-catenin in the affected keratinocytes [20]. The sex reversal appeared to be caused by a failure to mount high R-spondin1 levels in the gonads of affected individuals. This increase in R-spondin production, normally at embryonic day (E)18.5, occurs only in XX gonads and is required to promote oocyte differentiation. A later analysis of Rspos1−/− mice [21] confirmed that an absence of R-spondin1 at the gonadal differentiation stage leads to partial sex-reversed phenotypes. Similar phenomena are also seen in Wnt4−/− mice, probably because of the action of R-spondin1 upstream of Wnt4 [22]. In summary, the Wnt4/R-spondin1 axis is operational in bipotential gonads of XX individuals, driving ovarian development. In XY individuals, the HMG-box-containing transcription factor SRY induces transcription of the SOX9 gene, another member of this HMG box family. This transcription factor then activates the program for testis development. The activation of Wnt4/R-spondin1 in XX gonads not only drives ovarian differentiation, but also suppresses the fibroblast growth factor (FGF)9-stabilization of SOX9 production. In the absence of strong Wnt signaling, the resulting SOX9 production is sufficient to drive at least partial testis development (Figure 4) [23].

R-spondin 2: necessary for development of limbs, lungs and hair follicles

Limb buds in the early embryo show production of R-spondin2 and 3, while lung buds exclusively produce R-spondin2. The matching requirement of R-spondin2 for lung and limb development was unveiled in a mouse insertion mutant, termed ‘footless’ (Rspos2−/−), and in animals homozygous for a targeted inactivation of the Rspo2 gene [24–26]. The reported overlapping phenotypes in limb development are explained by an absence of functional R-spondin2 protein in the apical ectodermal ridge (AER). The resulting impaired Wnt signal leads to defective expression of the important AER maintenance factors FGF4 and FGF8. The lung defects seen in Rspos2−/− mice are associated with reduced branching of bronchioles. However, this developmental defect can be rescued by culturing ex vivo explants in R-spondin2-conditioned medium. Several additional observations imply involvement of canonical Wnt signaling. First, the effects seen are exacerbated if Rspo2−/− mice are intercrossed with Lrp6 mutant mice [27]. Second, mating of ‘footless’ mice with Wnt reporter mice detected a significant drop in Wnt activity at the distal tips of the branching epithelium. A corresponding reduction of expression of the Wnt target gene Irx3, required for branching, further explained the phenotype [28]. A study investigating the genes responsible for coat features in domestic dogs showed that R-spondin2 is also involved in the Wnt-driven development of the hair follicle [29].
An insertion event in the 3' UTR of this gene appeared to affect mRNA stability in dogs with ‘furnishings’ (extra fur around the mouth and eyes), and they show a threefold increase in transcript expression.

R-spondin3: placenta development
Development of the mouse placenta starts at E8.5 with a fusion between the chorion and allantois, two extra-embryonic tissues. Subsequently, chorioallantoic branching occurs, resulting in a functional labyrinth enabling exchange of gases, nutrients and waste products between embryonic and maternal blood vessels. An insufficient penetration of fetal blood vessels in the labyrinthine zone is seen in Wnt2 and Frzd5 knockout mice [30,31]. In studies analyzing the signals for vasculogenesis and angiogenesis, the targeted disruption of the Rspo3 gene leads to severe vascular defects, especially in the placenta [32,33]. R-spondin3 production is detectable at the chorioallantoic interface. The chorioallantoic fusion appears normal in the absence of R-spondin3. However, fetal blood vessels present in the labyrinth do not properly align with the maternal blood sinus, causing death of the animals around day E10. The same phenomena were reported for Wnt/Fzd mutated animals, implying that R-spondin acts upstream of Wnt.

R-spondin 4: nail development
Anonychia is a mild disorder, defined as the absence of fingernails and toenails. It is mostly seen in autosomal-dominant inherited syndromes. Isolated anonychia shows an autosomal-recessive inheritance. Recently, homozygous and compound heterozygous mutations in the gene lead...
encoding R-spondin4 were found in affected individuals [34-37]. The various genetic alterations all predicted severely impaired synthesis of functional R-spondin4 protein. A study monitoring the effects of these mutations, using R-spondin2 as a template, showed that at least two of these, C78Y and C113R, led to a defect in secretion [7]. The Q65R substitution did not affect secretion, but drastically reduced R-spondin2 activity. Involvement of the Wnt pathway in nail development was recently also deduced in patients that combine anonychia with brachydactyly (shortness of fingers and toes) [38]. The SOX9 transcription factor is essential for the normal development of the terminal phalanges, and associated ‘Anlagen’ like nails [39]. It must initially be induced, but silenced at later stages. Downregulation of SOX9, and subsequent inhibition of chondrogenesis, is mediated by canonical Wnt signaling. Mutual antagonistic activity between SOX9 production and canonical Wnt activity has been deduced from the analysis of gonad differentiation [23]. The phenomena seen in these anonychia/brachydactyly patients seem to be explained by an imbalance between these forces due to duplications of regulatory sequences 5’ of the gene encoding SOX9 [38]. Several reports imply involvement of Wnt7a in this process of terminal phalange differentiation [40,41]. A likely role for R-spondin, as also seen in gonad differentiation, has not been addressed so far.

Mechanisms
Receptors
The identification of the membrane component mediating R-spondin signaling has proceeded with trial and error. Contradictory reports proposed that R-spondin bound to Fzd5, LRP5, Kremen receptor and/or Wnts [42-44]. However, three recent reports [45-47] identified Lgr4, Lgr5 and Lgr6 as the receptors of the R-spondin protein family (Figure 3). Each of them can bind all four R-spondins in vitro [48]. RNA interference-mediated deletion of the endogenous Lgr4 in 293T cells resulted in effective removal of the R-spondin-mediated enhancement of Wnt signaling in these cells [48]. A specific rescue occurred by exogenously introducing Lgr4, Lgr5 and Lgr6 [48]. Recently, syndecan-4 was proposed as the receptor for R-spondin3 in the planar cell polarity pathway [14]. An earlier report had claimed a role for R-spondin3 in canonical Wnt signaling [33]. With the current knowledge that the Lgr proteins act as receptors for the furin domains in R-spondins, the R-spondin3/syndecan-4 interaction most likely involves the TSR-1 domain. The Lgr proteins appear to be physically associated with the Fzd/LRP complex. The R-spondin component in Wnt signaling may therefore be mediated by the LRP5/6 Frizzled co-receptors. Of note, R-spondin1 enhances LRP6 phosphorylation [43].

The R-spondin/Lgr axis
A variety of genetic studies were conducted to determine the locations of expression and the physiological roles of Lgr4, Lgr5 and Lgr6 during embryogenesis. Those experiments actually monitored locations of R-spondin-amplified Wnt signaling. Analysis of the Lgr4 receptor, using a variety of genetic models, detected strong expression in cartilage, kidney, adrenal gland, reproductive tracts, the eyes and nervous system cells. The associated phenotypes are diverse and extend over tissues derived from all germinal layers [49-60]. Lgr5, likewise, shows a dynamic and complex expression pattern during embryogenesis [61,62]. Rare Lgr5’ cells are seen in the adult eye, mammary gland, intestinal tract, skin and the reproductive organs [63-65]. Developmental Lgr6 expression is most prominent in the hair placodes, rare cells in the brain, the mammary gland, and the airways of the lungs [62,66].

Importantly, R-spondin/Lgr signaling also operates in several self-renewing adult tissues. The best studied example is the mucosa of the digestive tract, consisting of a stomach, small intestine (Figure 4) and the colon. The first indication that Rspondin1 can act as a growth factor for intestinal epithelial cells, by agonizing canonical Wnt signaling, was found in a transgenic mouse model in which Rspo1 was under the control of the immunoglobulin locus [67]. The essential requirement of Wnt signaling for the physiological maintenance of the stomach cells in these tissues was previously shown in a Tcf4 (T-cell transcription factor 4) ablation experiment and a DKK1 transgenic model [68,69]. The involvement of R-spondin was indirectly uncovered by a Lgr5-driven GFP (green fluorescent protein) knock-in mouse model and a Lgr5/LacZ-driven lineage tracing model [70]. These studies identified the Wnt-target gene LGR5 as a unique marker for the stem cells feeding these tissues [63]. Lgr4 is co-expressed in stem cells, and in addition it is detectable in all other progenitor cells. Isolated Lgr5’ intestinal stem cells can be maintained in vitro and induced to continuously propagate organoids [64,71]. Notably, addition of R-spondin and Wnt constitutes an absolute requirement for these cultures. In mouse intestinal organoids, deletion of these Lgr receptors phenocopies withdrawal of R-spondin. Moreover, absence of Lgr receptors can be compensated by providing cells with the strongest possible Wnt signals. Canonical Wnt/R-spondin signaling is, moreover, implied in establishing the hair follicle cycle and remains crucial for stem cell activity throughout life [63,65,72-76].

Frontiers
Now that the Lgr proteins have been established as the receptors for R-spondins, directly funneling into the canonical Wnt pathway through Frizzled and Lrp, several
gaps in our knowledge of R-spondins can be addressed. For example, crystallographic studies of R-spondin and R-spondin/Lgr complexes are required to understand how the interaction-induced information is transferred to the Wnt/Fzd/Lrp signaling unit. The increase in Lrp6 phosphorylation, associated with the presence of R-spondin in the Wnt receptor complex, needs to be understood in greater detail. In particular, the dedicated kinase and the specific substrate for this reaction among the five conserved PPPSPXS motifs in Lrp need to be identified [77]. Another challenge is to determine the exact composition of the operating Wnt receptor complexes. A key question here is whether the Lgr/R-spondin module constitutes a standard feature of canonical Wnt signals in vertebrates or an accessory option. It will also be important to determine to what extent preference in the cooperation between the various components in vivo plays a role. Another challenge will be to find out the specificity and site of synthesis of the R-spondins that control particular biological processes. Because the R-spondins are also stimulators of stem cell development, it is anticipated that future research will use R-spondin-based strategies for the manipulation of adult stem cells in regenerative medicine settings. The first findings, supporting the therapeutic potential of in vivo administered R-spondins, were found in a mouse model for inflammatory bowel diseases similar to Crohn’s disease [78]. Future attempts to replenish disease-damaged epithelial tissue along the gastrointestinal tract, including Barrett’s disease, will likely exploit R-spondin-mediated ex vivo expansion of the epithelia of interest [64,71,79].

### Additional files

Additional file 1: Table 1. Summary of developmentally regulated R-spondin1, 2, 3 and 4 expression.

### Abbreviations

AER, apical ectodermal ridge; DKK1, Dickkopf-1; E, embryonic day; EGF, epidermal growth factor; Fzd, Frizzled; GAG, glycosaminoglycan; HGF, hepatocyte growth factor; Lgr, leucine-rich repeat-containing G-protein-coupled receptor; Lrp, lipoprotein-receptor-related protein; PPK, palmoplantar hyperkeratosis; SCC, squamous cell carcinoma; TSP, thrombospondin protein; TSP-1, thrombospondin type 1 repeat.

### Competing interests

The authors declare that they have no competing interests.

### Acknowledgement

We would like to thank Johan van Es for critically reviewing the manuscript.

### Author details

Hubrecht Institute, Developmental Biology and Stem Cell Research, Royal Netherlands Academy of Arts and Sciences, University Medical Center Utrecht, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands. Theoretical Biology and Bioinformatics, Padualaan 8, 3584 CH, Utrecht, The Netherlands.

Published: 22 March 2012

### References

1. Baeniger NL, Brodie GN, Majerus PW: A thrombin-sensitive protein of human platelet membranes. Proc Natl Acad Sci U S A 1971, 68:240-243.
2. Lawler J, Hynes RO: The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologies with several different proteins. J Cell Biol 1986, 103:1635-1648.
3. Kamata T, Katsube K, Michikawa M, Yamada M, Takada S, Mizuawa H: R-spondin, a novel gene with thrombospondin type 1 domain, was expressed in the dorsal neural tube and affected in Wnts mutants. Biochim Biophys Acta 2004, 1676:51-62.
4. Chen JZ, Wang S, Tang R, Yang QS, Zhao E, Chao Y, Ying K, Xie Y, Mao YM: Cloning and identification of a cDNA that encodes a novel human protein with thrombospondin type I repeat domain, HPTWST. Mol Biol Rep 2002, 29:287-292.
5. Kazanskiy O, Glinka A, del Barco Barrantes I, Stannen P, Niehrs C, Wu W: R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for Xenopus myogenes. Dev Cell 2004, 7:25-34.
6. Kim KA, Zhao J, Andamanni S, Kakitani M, Oshima M, Binnerts ME, Abo A, Tomizuka K, Funk WD: R-Spondin proteins: a novel link to beta-catenin activation. Cell Cycle 2006, 5:23-26.
7. Li SJ, Yen TY, Endo Y, Kluzinski M, Baljinnyam B, Macher B, Callahan R, Rubin JS: Loss-of-function point mutations and two-furin domain derivatives provide insights about R-spondin2 structure and function. Cell Signal 2009, 21:916-925.
8. Ogino H, Ishitani R, Nureki O, Fuku K, Yamana Y, Kim JH, Saito K, Sakamoto A, Inoue M, Shirouzu M, Yokoyama S: Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. Cell 2002, 110:775-787.
9. Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Ellerman TC, Cosgrove LJ, Ward CW: Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. Nature 1996, 394:395-399.
10. Guo NH, Kutzsch HC, Negre E, Vogel T, Blake DA, Roberts DD: Heparin- and sulfatide-binding peptides from the type I repeats of human R-spondin promote melanoma cell adhesion. Proc Natl Acad Sci U S A 1992, 89:3040-3044.
11. Guo NH, Kutzsch HC, Negre E, Zabrenetsky VS, Roberts DD: Heparin-binding peptides from the type I repeats of thrombospondin. Structural requirements for heparin binding and promotion of melanoma cell adhesion and chemotaxis. J Biol Chem 1992, 267:19349-19355.
12. Tan K, Duquette M, Liu JH, Dong Y, Zhang R, Joachimak A, Lawler J, Wang JH: Crystal structure of the TSP-1 type 1 repeats: a novel layered fold and its biological implication. J Cell Biol 2002, 159:373-382.
13. Ayadi L: Molecular modelling of the TSP domain of R-spondin 4. Bioinformatics 2008, 3:119-123.
14. Ohkawara B, Glinka A, Niehrs C: Rspos3 binds syndecan 4 and induces Wnt/PCP signaling via clathrin-mediated endocytosis to promote morphogenesis. Dev Cell 2010, 3:303-314.
15. Kim KA, Wagle M, Tran K, Zhan X, Dixon MA, Liu S, Gros D, Karver W, Yonkovich S, Tomasovic N, Binnerts M, Abo A: R-Spondin family members regulate the Wnt pathway by a common mechanism. Mol Biol Cell 2008, 19:3586-3596.
16. Cossu G, Borello U: Wnt signaling and the activation of myogenesis in mammals. EMBO J 1999, 18:667-670.
17. Borycki AG, Emerson CP Jr: Study of skeletal myogenesis in cultures of unsegmented paraxial mesoderm. Methods Mol Biol 2003, 179:351-357.
18. Lowther W, Wiley K, Smith GH, Callahan R: A new common integration site, Int2, for the mouse mammary tumor virus in mouse mammary tumors identifies a gene whose product has furin-like and thrombospondin-like sequences. J Virol 2005, 79:10093-10096.
19. Nuuse R: Wnt signaling in disease and development. Cell Res 2005, 15:28-32.
20. Parma P, Ridi O, Vidal V, Chaboissier MC, Dellambra E, Valenti M, Guerra L, Schedli A, Camerino G: R-spondin1 is essential in sex determination, skin differentiation and malignancy. Nat Genet 2006, 38:1304-1309.
21. Tomizuka K, Kizukihiko K, Ikada R, Sugawara Y, Iba Y, Kojima A, Yoshimoto A, Yamawaki K, Amagai M, Inoue A, Oshima T, Kakitani M: R-spondin1 plays an essential role in ovarian development through positively regulating Wnt-4 signaling. Hum Mol Genet 2008, 17:1278-1291.
22. Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP: Female development in mammals is regulated by Wnt-4 signalling. Nature 1999, 397:405-409.
23. Kashimada K, Koopman P: Sry: the master switch in mammalian sex determination. Development 2010, 137:3891-3893.
null
Korving J, Abo A, Peters PJ, Wright N, Poulsom R, Clevers H: Lgr5(+) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 2010, 6:25-36.

66. Jakó V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H: LGR5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet 2008, 40:1291-1299.

67. Morris RJ, Liu Y, Marles L, Yang Z, Tembus P, Li S, Lin JS, Sawicki JA, Cotarselis G: Capturing and profiling adult hair follicle stem cells. Nat Biotechnol 2004, 22:411-417.

68. Kim KA, Kakitani M, Zhao J, Oshima T, Tang T, Brenners M, Liu Y, Boyle B, Park E, Emtage P, Funk WD, Tomizuka K: Mitogenic influence of human R-spondin1 on the intestinal epithelium. Science 2005, 309:1256-1259.

69. Pinto D, Clevers H: Wnt control of stem cells and differentiation in the intestinal epithelium. Exp Cell Res 2005, 306:357-363.

70. Korinek V, Barker N, Meier P, van Dorselaer E, Huls G, Peters PJ, Clevers H: Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998, 19:379-383.

71. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, van der Horn K, Batlle E, Saudou E, Darras AP, Haramis AP, van den Born M, Soete G, Pals S, Eilers M, Medema R, Clevers H: The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 2002, 111:241-250.

72. van Genderen C, Okamura RM, Farinas I, Quo RG, Paslov TG, Bruhn L, Grosschedl R: Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev 1994, 8:2691-2703.

73. Lowry WE, Blanpain C, Nowak JA, Gusaich G, Lewis L, Fuchs E: Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. Genes Dev 2005, 19:1596-1611.

74. Alonso L, Fuchs E: Stem cells of the skin epithelium. Proc Natl Acad Sci USA 2003, 100 Suppl 1:11830-11835.

75. Alonso L, Fuchs E: Stem cells in the skin: waste not, Wnt not. Genes Dev 2003, 17:189-1200.

76. Alonso LC, Rosenfield RL: Molecular genetic and endocrine mechanisms of hair growth. Horm Res 2003, 60:1-13.

77. Macdonald BT, Semenov MV, Huang H, He X: Dissecting molecular differences between Wnt Coreceptors LRPS and LRP6. PLoS One 2011, 6:e23537.

78. Zhao J, de Vera J, Narushima S, Beck EX, Palencia S, Shinkawa P, Kim KA, Liu Y, Levy MD, Berg DJ, Abo A, Funk WD: R-spondin1, a novel intestinotrophic mitogen, ameliorates experimental colitis in mice. Gastroenterology 2007, 132:1331-1343.

79. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houtth WJ, Pronk A, Van Gorp J, Sienkema PD, Clevers H: Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. Gastroenterology 2011, 141:1762-1772.

80. Katoh K, Kuma T, Toh H, Miyata T: MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 2005, 33:511-518.

81. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD: Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 2003, 31:3497-3500.

82. Letunic I, Bork P: Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 2007, 23:127-128.

83. Leynis L, Bouwmeester T, Kim SH, Piccolo S, De Robertis EM: Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. Cell 1997, 88:747-756.

84. Hsieh JC, Kodjabachian L, Rebbeurt ML, Rattner A, Smallwood PM, Samos CH, Russe R, David IB, Nathans J: A new secreted protein that binds to Wnt proteins and inhibits their activities. Nature 1999, 398:431-436.

85. Jones SE, Jomary C: secreted Frizzled-related proteins: searching for relationships and patterns. Bioessays 2002, 24:811-820.

86. Kawano Y, Kypta R: Secreted antagonists of the Wnt signalling pathway. J Cell Sci 2003, 116:2627-2634.

87. Niehrs C: Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 2006, 25:7469-7481.

88. Itasaki N, Jones CM, Mercurio S, Rowe A, Domingos PM, Smith JC, Krumlauf R: Wise, a context-dependent activator and inhibitor of Wnt signalling. Development 2003, 130:4295-4305.

89. Carmon KS, Gong X, Lin Q, Thomas A, Liu Q: The R-spondin ligand Frzb is a secreted antagonist of Wnt signaling. Nat Genet 2003, 33:1256-1259.

90. Glinka A, Dolde C, Kirsch N, Huang YL, Kazanskaya O, Ingelfinger D, Boutros M, Crucean MT, Niehrs C, LGR4 and LGR5 are R-spondin receptors mediating Wnt/beta-catenin and Wnt/PCP signalling. EMBO Rep 2011, 12:1055-1061.