



A Secreted Protein in the Wnt Signaling Pathway-R-Spondin3: A Mini-Review



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Abstract

R-spondins (Roof plate-specific Spondins, RSPOs) are secreted proteins, which act as activators of Wnt/ β -catenin signaling and play roles in cell proliferation and differentiation, embryonic development, vasculogenesis as well as many human diseases. R-spondin3 (RSPO3) is a member of RSPOs family, containing two adjacent cysteine-rich furin-like domains, which act as an agonist in Wnt signaling. In this mini-review, we discuss and classify recent progress in understanding the protein functions of RSPO3 in embryonic development, vasculogenesis, and its role in cancers.

Keywords: RSPO3; Wnt signaling; Embryonic development; Vasculogenesis; Cancers

Introduction

The Wnt signaling pathway is one of the key signaling pathways which controls cell proliferation and differentiation, muscle development, bone formation, morphogenesis during embryo genesis and in adults [1,2]. The R-spondins (Roof plate-specific Spondins, RSPOs) is a recently reported protein family including four secreted proteins (RSPO1-4). RSPOs contain two furin-like cysteine-rich domains, a thrombospondin Type 1 repeat and a C-terminal region enriched with basic charged amino acids [3]. Leucine-rich repeat-containing G-protein coupled receptors (LGR 4-6) and ZNRF3/RNF43 are considered obligate high-affinity receptors of RSPOs [4]. They can potently enhance Wnt signals by amplifying target cell sensitivity to Wnt ligands through increasing Wnt receptor levels [5,6] (Figure 1A). RSPO3 is a member of the RSPO family, in vertebrates, which can activate the Wnt signaling and play important roles in embryonic patterning, vasculature remodeling and tumorigenesis [7-10].

RSPOs-mediated Wnt signaling activation. The β -catenin signaling is initiated by association of Wnt with Fizzled and LRP5/6 receptors, it subsequently activates Dishevelled to recruit the Axin complex (GSK-3 β , CK1 γ , Axin, APC) to the receptor. When RSPOs bind to LGR4/5/6 and RNF43/ZNRF3 receptors, the complex is internalized by endocytosis and induced ubiquitination and degradation of RNF43/ZNRF3 to stabilize Wnt receptors and enhance Wnt response, β -catenin becomes stabilized and enters the nucleus. B, Domains of RSPO3. LGR4/5/6, Leucine-rich repeat-

containing G-protein coupled receptors 4/5/6; RNF43, Ring finger protein 43; ZNRF3, Zinc and ring finger 3; LRP5/6, Low-density lipoprotein receptor-related protein 5/6; HSPG, Heparan sulphate proteo glycans; DVL, Dishevelled; GSK-3 β , Glycogen synthase kinase-3 β ; CK1 γ , Casein kinase 1; TCF, T-cell factor; FU, Furin-like domains; N-gly, N-glycosylation; TSP-1, Thrombospondin-1; BR, Basic residues.

RSPO3 and its Roles

RSPO3, also known as cysteine-rich and single thrombospondin domain containing-1, is a 31 kDa secreted protein, which shares about 40% amino acid (aa) identity with the other three RSPO family members [11,12]. Each RSPO family member has a distinct expression pattern to potentiate Wnt/ β -catenin signaling. RSPO3 contains two adjacent cysteine-rich furin-like domains (aa 35-135) with one potential N-glycosylation site (aa 36), followed by a thrombospondin (TSP-1) motif (aa 147-207) and a region rich in basic residues (aa 211-269) (Figure 1B). Only the furin-like domains are needed for β -catenin stabilization [12]. Within aa 21-209, human RSPO3 shares 93%, 92%, 97%, 96% and 92% aa identity with mouse, rat, equine, bovine and canine RSPO3, respectively [13], so they have similar function in vertebrate development.

It is reported that RSPO3 is highly expressed in vascularized tissues such as coronary stems, the allantois and the primitive streak and controls vascular morphogenesis in zebrafish, Xenopus, mouse and human [14,15]. Embryonic lethality in Rspo3-deficient

mice is caused due to vascular defects in placenta [7,16]. Inducible deletion of endothelial RSPO3 resulted in perturbed developmental and tumor vascular remodeling with reduced micro vessel density [8]. Tissue-specific ablation of RSPO3 in the heart with the *Islet1-Cre* line leads to defective secondary heart field development [17]. RSPO3 is a crucial regulator of coronary artery formation in the developing heart [18], but also shown to activate the non-canonical

Wnt/calcium pathway in the endothelial compartment of the postnatal retina and lungs to promote blood vessel maintenance [8]. Moreover, RSPO3 can protect tissues against mesenteric ischemia/reperfusion by tightening endothelial cell junctions and improving vascular integrity [19]. Thus, RSPO3 plays an important role in angiogenesis and vessel sprouting.

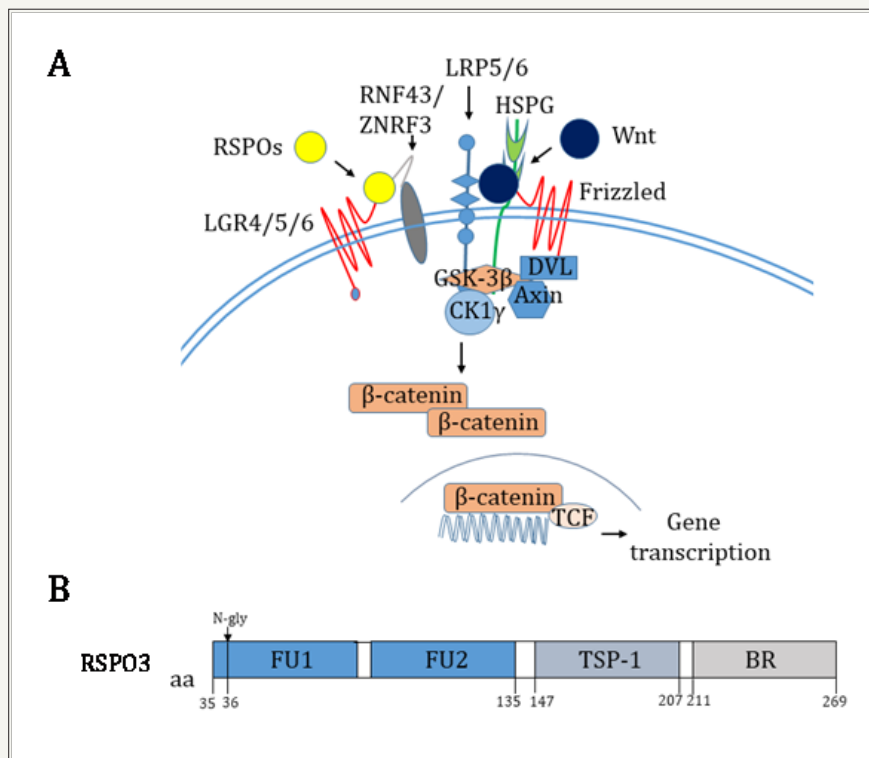


Figure 1: Overview of RSPOs activating Wnt signaling and RSPO3 domains.

RSPO3 can induce and synergize with Wnt ligands to maintain homeostasis in normal tissues by Wnt pathway. It has been shown that Wnt signaling is markedly activated in several cancers [20]. Recurrent RSPO3 translocation was identified in 8.0% of colon cancer. There are two configurations of the fusion transcripts, known as PTPRK (exon1)-RSPO3 (exon2) and PTPRK (exon7)-RSPO3 (exon2) [21,22]. Indeed, high frequencies of PTPRK-RSPO3 fusions (31%) and RNF43 mutations (24%) have been reported in colorectal traditional serrated adenomas [23]. Interestingly, RSPO3 fusions are found in prostate cancers, lung cancer and Schwannoma [24,25], and it is identified as sites of integration for mouse mammary tumor virus induced mammary tumors [26,27]. Furthermore, involvement of RSPO3 in growth of pre-existing tumors was recently shown in RSPO3-fusion-positive xenograft models, where anti-RSPO3 treatment inhibited the tumor growth [22,23]. RSPO3 is an oncogenic driver, rapidly causing intestinal cancer and extensive crypt hyperplasia, concomitantly stimulating stem cells and supportive niche cells [10]. Therefore, identification of RSPO3 translocations in cancer provides an attractive target for Wnt pathway inhibition using antibody approaches and an intuitive

predictive biomarker for RSPO3 antibodies. These might thus serve as predictive biomarkers to identify Wnt-dependent tumors.

Conclusion

In summary, RSPO3 not only contributes to the embryonic development, but also promotes angiogenesis and vessel sprouting. It also has been recognized as an oncogene to actively drive tumor genesis. Therefore, RSPO3 may be as a useful candidate target for underlying the etiology of some human diseases even in cancers.

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