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Dynamic expression of *R-spondin* family genes in mouse development

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Abstract

R-spondins (Rspo) are a recently discovered secretory protein family with four members in human and mouse. We and others demonstrated that R-spondins can activate canonical Wnt signaling and β-catenin-dependent gene expression. Our study further demonstrated that R-spondins are novel ligands for the Frizzled8 and LRP6 (LDL-receptor-related protein 6) receptors. To gain insight into their biological functions, the RNA expression pattern of the mouse *R-spondin* family genes was analyzed during mouse development. Our study shows that *R-spondin* gene transcripts are widely expressed with distinct patterns in mouse at different developmental stages.

Keywords: R-spondin; Wnt; Frizzled; LRP5/6; β-catenin; Mouse embryogenesis; Gene expression

1. Results and discussion

The *R-spondin* (*Rspo*) family genes, which encode cysteine-rich secretory proteins containing a thrombospondin type 1 repeat, were recently isolated in human, mouse, and *Xenopus* (Kamata et al., 2004; Kazanskaya et al., 2004; Kim et al., 2005; Nam et al., 2006). Four members of the *Rspo* gene family exist in both human and mouse (Kazanskaya et al., 2004; Nam et al., 2006). Only two members are identified in *Xenopus* (Kazanskaya et al., 2004), and several ESTs (expressed sequence tags) from other vertebrates are identified in the Genbank database, while no homologous genes are identified in the *Drosophila melanogaster* and *Caenorhabditis elegans* genome. Several studies show that Rspos can activate the canonical Wnt signaling pathway at the receptor level and induce β-catenin/TCF (T cell factor)-dependent gene activation (Kazanskaya et al., 2004; Kim et al., 2005; Nam et al., 2006). The signaling activity of Rspo mimics that of canonical Wnt proteins, therefore suggesting that Rspo family proteins are a novel class of signaling ligands that induce canonical Wnt signaling. Indeed, our recent biochemical study demonstrates that the Rspo protein can directly interact with the extracellular domain of the Frizzled8 and LRP6 receptors (Nam et al., 2006), respectively, confirming that the Rspo family of proteins are novel ligands for Wnt signaling.

Despite recent progress in R-spondin research, the expression pattern of *Rspo* genes during mouse embryonic and fetal development has not been determined in detail; however, limited expression data were provided by Kazanskaya et al. (2004). To gain further insight into the biological roles of the *Rspo* genes during mouse development, we systematically determined the RNA expression pattern of four *Rspo* genes in mouse embryo/fetuses.

1.1. RT-PCR analysis of R-spondin gene expression in mouse embryogenesis

By using RT-PCR analysis, we determined the presence of *Rspo* transcripts in mouse embryos at different developmental stages (Fig. 1A). While significant expression of the *Rspo3* gene was detected at E7, less expression of *Rspo2* and *Rspo4* RNA expression was observed at the same stage. *Rspo1* expression was undetectable. Expression of all *Rspo* genes including *Rspo1* and *Rspo2* genes was

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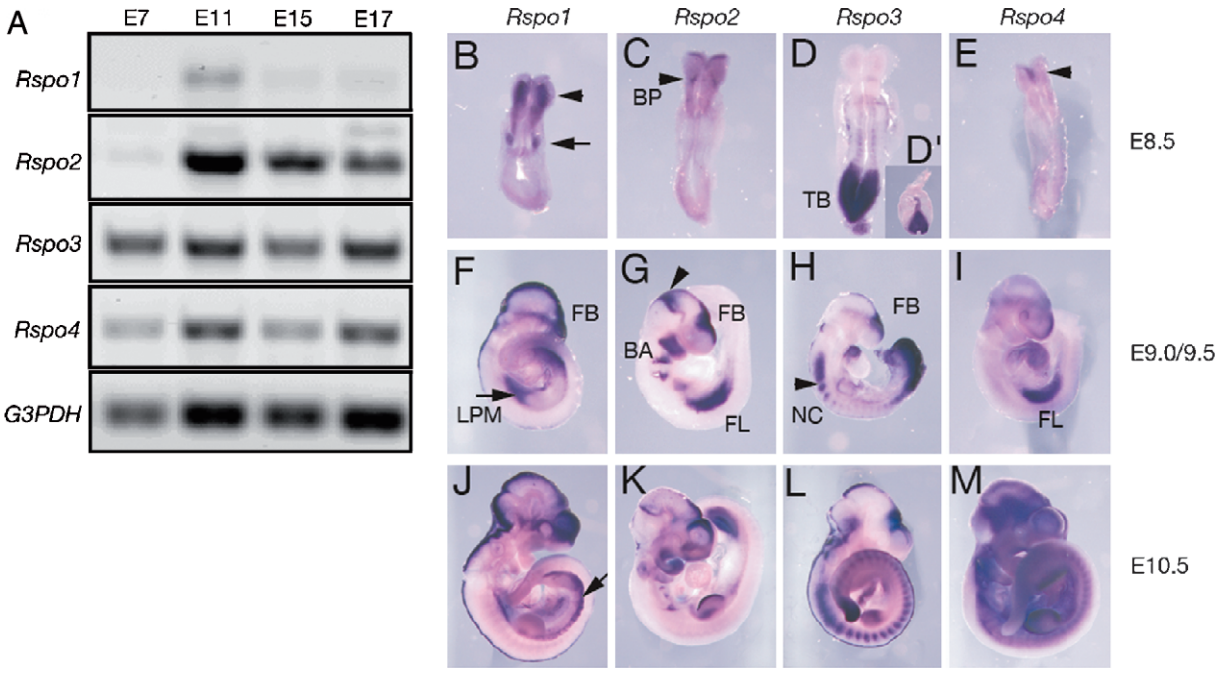

Fig. 1. Expression of *R-spondin* genes during early mouse embryonic development. (A) RT-PCR analysis of *R-spondin* (*Rspo*) genes in E7-17 mouse embryos. Expression of *G3PDH* gene was used as a control. (B–M) Whole-mount *in situ* hybridization of *Rspo1* (B, F, and J), *Rspo2* (C, G, and K), *Rspo3* (D, D′, H, and L), and *Rspo4* (E, I, and M) in mouse embryos at the stages of E8.5 (B–E), E9.0 (H), E9.5 (F,G, and I), and E10.5 (J–M). Abbreviations: BP, branchial pouch; FL, forelimb; FB, forebrain; HL, hindlimb; LPM, lateral plate mesoderm; NC, neural crest; PS, primitive streak.

dramatically increased by E11. *Rspo1* expression was significantly reduced in the E15 and E17 fetuses. *Rspo2* expression was also reduced at later stages. In contrast, *Rspo3* and *Rspo4* expression was maintained consistently from E11 to E17. Clearly, this result demonstrated that the expression of *Rspo* genes was dynamic and developmentally regulated.

1.2. Expression of R-spondin genes in mouse embryos between E7.5 and E10.5

To determine the embryonic domains in which each member of the *Rspo* genes is expressed, we performed whole-mount *in situ* hybridization analysis in mouse embryos aged between E7.5 and E10.5, using antisense RNA probes specific to each of the *Rspo* genes. Consistent with the RT-PCR analysis result in the E7 embryo RNA sample (Fig. 1A), among four *Rspo* genes examined, only the *Rspo3* gene was strongly expressed in the primitive streak and allantois in mouse embryos at E7.5 stage (Fig. 1D). Although expression of both *Rspo2* and *4* genes was detected in the E7 embryonic RNA sample by RT-PCR analysis (Fig. 1A), we failed to detect any significant specific expression of these genes by whole-mount *in situ* hybridization (data not shown).

At E8.5, *Rspo1* expression was detected in the mesenchymal cells of the presumptive diencephalon and mesencephalon region (arrowhead in Fig. 1B). In the trunk region, *Rspo1* expression was seen in a small patch of lateral plate mesodermal cells proximal to the intermediate mesoderm at the level of the rostral somites (arrow in Fig. 1B). Weak but significant *Rspo2* expression was detected in the anterior of prospective midbrain and first branchial pouch in the E8.5 embryos (arrowhead in Fig. 1C). Strong expression of the *Rspo3* gene was evident in the primitive streak, somites, and allantois (Fig. 1D). Weak *Rspo4* gene expression was detected in the groove of neural fold in the E8.5 embryos (arrowhead in Fig. 1E).

Between E9.0 and E10.5, a complex expression pattern of *Rspo* genes was detected throughout the whole embryo. Significant *Rspo1* expression was observed in the forebrain and the dorsal neural tube (roof plate) along the body axis (Fig. 1F and J). Sections of the stained embryos further revealed that both head mesenchymal cells and neuroepithelial cells within the forebrain region expressed the *Rspo1* gene as indicated (Fig. 2A). Mesenchymal cells surrounding the optic placode of E10.5 embryos were also positive for *Rspo1* expression (Fig. 2I). As detected in the E8.5 embryos, the *Rspo1* gene was continuously expressed in the medial part of lateral plate mesoderm adjacent to the intermediate mesoderm along the body axis (arrows in Figs. 1F, J, and 2E).

Rspo2 expression was also detected in the forebrain, but only in the neuroepithelial cells of the dorsal neural tube and the midbrain/hindbrain junction (Figs. 1G, K, and 2B) at E9.5/10.5. Interestingly, dorsal midline expression of *Rspo2* within the developing neural tube extended to the rostral hindbrain region and was lacking in most of the hindbrain and body trunk (arrowhead in Fig. 1G). Additional *Rspo2* expression was detected in the branchial arches in