The mouse Secreted frizzled-related protein 5 gene is expressed in the anterior visceral endoderm and foregut endoderm during early post-implantation development

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Abstract

The anterior visceral endoderm (AVE) plays an important role in anterior–posterior axis formation in the mouse. The AVE functions in part by expressing secreted factors that antagonize growth factor signaling in the proximal epiblast. Here we report that the Secreted frizzled-related protein 5 (Sfrp5) gene, which encodes a secreted factor that can antagonize Wnt signaling, is expressed in the AVE and foregut endoderm during early mouse development. At embryonic day (E) 5.5, Sfrp5 is expressed in the visceral endoderm at the distal tip region of the embryo and at E6.5 in the AVE opposite the primitive streak. In Lim1 embryos, which lack anterior neural tissue and sometimes form a secondary body axis, Sfrp5-expressing cells fail to move towards the anterior and remain at the distal tip of E6.5 embryos. When compared with Dkk1, which encodes another secreted Wnt antagonist molecule present in the visceral endoderm, Sfrp5 and Dkk1 expression overlap but Sfrp5 is expressed more broadly in the AVE. Between E7.5 and 8, Sfrp5 is expressed in the foregut endoderm underlying the cardiac mesoderm. At E8.5, Sfrp5 is expressed in the ventral foregut endoderm that gives rise to the liver. Additional domains of Sfrp5 expression occur in the dorsal neural tube and in the forebrain anterior to the optic placode. These findings identify a gene encoding a secreted Wnt antagonist that is expressed in the extraembryonic visceral endoderm and anterior definitive endoderm during axis formation and organogenesis in the mouse.

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1. Results and discussion

The visceral endoderm is an extraembryonic tissue that initially surrounds the epiblast of the egg cylinder stage mouse embryo (Rossant, 1986). Lineage analysis experiments indicate that a group of visceral endoderm cells located at the distal tip of the embryo at E5.5 move to the future anterior region of the embryo and come to lie diametrically opposed to the newly formed primitive streak at E6.5 (Thomas et al., 1998). This group of cells is called the anterior visceral endoderm (AVE). The AVE is believed to be a source of factors that block TGFβ-related and Wnt signals in the proximal epiblast and prevent mesoderm formation on the anterior side of the embryo (Beddington and Robertson, 1999). Recently it has been shown that Cerl and Lefty1, which encode secreted factors that antagonize nodal signaling and are specifically expressed in the AVE, are required to prevent ectopic primitive streak formation in the mouse (Perea-Gomez et al., 2002). As inhibition of Wnt signaling on the future anterior side of the embryo is also thought to be important for proper axis formation, we surveyed genes encoding secreted Wnt antagonists for expression in the AVE. These studies have identified the Sfrp5 gene. Sfrp5 is a member of the Secreted frizzled-related protein (Sfrp) gene family. In vertebrates, there are at least eight family members and they encode secreted molecules with homology to the cysteine-rich ligand binding domain of frizzled receptors (Jones and Jomary, 2002). Cell culture studies and gain of function experiments using Xenopus embryos indicate that Sfrp proteins can bind Wnt molecules extracellularly and interfere with Wnt signaling (Leyns et al., 1997; Wang et al., 1997; Rattner et al., 1997; Chang et al., 1999).
We analyzed Sfrp5 expression in mouse embryos between embryonic day E5.5 and 8.5 by whole mount in situ hybridization. At E5.5, Sfrp5 was expressed in the visceral endoderm at the distal tip of the embryo (Fig. 1A). This expression pattern closely resembles the expression pattern of the AVE-specific genes Hex and Cerl (Thomas et al., 1998; Stanley et al., 2000). Consistent with Sfrp5 being expressed in the distal visceral endoderm cells that move towards the presumptive anterior region of the embryo, we observed that at E6.5 in mid-primitive streak stage embryos, Sfrp5 was expressed in the AVE opposite the primitive streak (Fig. 1B and C). To further characterize these visceral endoderm cells, we examined the expression of Sfrp5 in Lim1−/− embryos. Lim1-deficient embryos lack anterior head structures and a proportion of the mutant embryos develop a secondary body axis (Shawlot and Behringer, 1995). Chimera analysis indicates that Lim1 is required in the visceral endoderm and primitive streak-derived cells for anterior neural development (Shawlot et al., 1999). In E6.5 Lim1−/− embryos, we observed that Sfrp5 was expressed in a thickened region of the visceral endoderm located at the distal tip of the egg cylinder (Fig. 1D). This finding suggests that the AVE does not move properly in Lim1 mutant embryos and remains at the distal tip of the egg cylinder.

Dkk1 encodes a secreted Wnt antagonist that is reported to be expressed in the AVE (Glinka et al., 1998; Pearce and Rossant, 1999). To determine whether Sfrp5 and Dkk1 overlap in their expression in the AVE, we compared the expression patterns of Sfrp5 and Dkk1 in E6.5 mid-primitive streak stage embryos. In agreement with the published results, we observed that Dkk1 was expressed in the AVE in a restricted crescent pattern (Fig. 1E and F). Comparing mid-primitive streak embryos hybridized with Sfrp5 and Dkk1 probes, we observed that the expression patterns of Sfrp5 and Dkk1 in the AVE overlapped, but that the expression pattern of Sfrp5 appeared to be broader than Dkk1 (compare Fig. 1C and F).

A number of genes expressed in the AVE, including HNF3β, Hex and Cerl, are also expressed in the definitive endoderm (Ang et al., 1993; Thomas et al., 1998; Belo et al., 1997; Biben et al., 1998; Shawlot et al., 1998). During gastrulation, the definitive endoderm emerges from the anterior portion of the primitive streak and replaces the visceral endoderm (Lawson and Pedersen, 1987). To determine whether Sfrp5 is expressed in the definitive endoderm, we examined E7.5–8.5 embryos. In early E7.5 embryos, we detected only weak expression of Sfrp5 in the anterior region of the embryo near the embryonic-extraembryonic junction (data not shown). However, in E7.5 embryos that had developed a neural plate, Sfrp5 was expressed in a crescent pattern marking the presumptive foregut pocket and the lateral domain of expression extending beyond the edges of the neural plate (Fig. 2A). At E7.75, as the neural fold developed, Sfrp5 was expressed in a crescent pattern underlying the neural plate (Fig. 2B). At E8, as the foregut pocket developed, Sfrp5 was expressed in the endoderm at the lateral edges of the foregut pocket and in the endoderm underlying the cardiac mesoderm (Fig. 2C–E).
endoderm lying just beneath the heart from which the liver develops (Fig. 2F). Sfrp5 is also expressed in the dorsal portion of the neural tube just caudal to the hindbrain. In embryos that had completed turning, we noted an additional domain of Sfrp5 expression in the forebrain anterior to the optic placode (Fig. 2G).

In summary, we report that the mouse Sfrp5 gene is expressed in the AVE and in the foregut endoderm during early post-implantation development. The expression pattern of Sfrp5 in the AVE and in the foregut endoderm resembles in part the expression pattern of the divergent homeobox gene Hex (Thomas et al., 1998). Hex is required in definitive endodermal tissues in mice for normal forebrain, liver and thyroid formation (Martinez Barbera et al., 2000). Previously, the Sfrp5 gene has been reported to be expressed in the retinal pigment epithelium and the pancreas in human tissues and in the developing liver and gut tissues in Xenopus embryos (Chang et al., 1999; Pilcher and Krieg, 2002). In the chick, the Crescent gene, a member of the Sfrp gene family is expressed in the hypoblast, a tissue that expresses many of the same genes as the mouse AVE and is likely its tissue equivalent (Foley et al., 2000). A Crescent homologue has not been described in the mouse. Our finding that Sfrp5 is expressed in the AVE suggests that Sfrp5 may fulfill the role of Crescent in the mouse.

The other known gene encoding a Wnt antagonist expressed in the AVE is Dkk1. Our studies suggest that Sfrp5 and Dkk1 expression overlap in the AVE, but that Sfrp5 expression is broader. In the chick, Crescent also appears to be more broadly expressed in the hypoblast than Dkk1 (Foley et al., 2000). Dkk1-deficient mice created by gene targeting have anterior neural defects, but chimera analysis indicates that Dkk1 is not required in the visceral endoderm (Mukhopadhyay et al., 2001). A determination of the essential role(s) of Sfrp5 in mammalian development will require the generation of Sfrp5-deficient mice.

2. Experimental procedures

2.1. In situ hybridization

Whole mount in situ hybridization was performed using a Sfrp5 cDNA clone obtained from Genome Systems, Inc. The accession number of the Sfrp5 cDNA clone is AA797570. Whole mount in situ hybridization was performed essentially as described by Wilkinson (1992). The Dkk1 cDNA clone, accession number W79975, was obtained from Genome Systems Inc.
2.2. Mice

Embryos used for whole mount in situ hybridization were obtained by crossing B6SJL/F1 male mice with CD1 female mice. Lim1 mice used in this study were maintained on an outbred background. Experimental procedures involving mice were reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee.

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References


