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Tissue, cellular and sub-cellular localization of the Vangl2 protein during embryonic development: Effect of the *Lp* mutation

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Abstract

Loop-tail (*Lp*) mice show a very severe neural tube defect, craniorachischisis, which is caused by mis-sense mutations in the *Vangl2* gene. The membrane protein Vangl2 belongs to a highly conserved group of proteins that regulate planar polarity in certain epithelia, and that are also important for convergent extension movements during gastrulation and neurulation. A specific anti-Vangl2 antiserum was produced and used to examine the tissue, cell type, and sub-cellular localization of Vangl2 during embryogenesis. Vangl2 protein is expressed at high levels in the neural tube and shows a dynamic expression profile during neurulation. After neural tube closure, robust Vangl2 staining is detected in several neural and neurosensory tissues, including cerebral cortex, dorsal root ganglia, olfactory epithelium, retina, mechanosensory hair cells of the cochlea, and optic nerve. Vangl2 is also expressed during organogenesis in a number of tubular epithelia, including the bronchial tree, intestinal crypt/villus axis, and renal tubular segments derived from ureteric bud and from metanephric mesenchyme. Examination of Vangl2 localization in the neural tubes and cochleas of the normal and *Lp*/*Lp* embryos shows disruption of normal membrane localization of Vangl2 in independent alleles at *Lp* (*Lp*, Lp^{m1Jus}) as well as overall decrease in the expression level.

Keywords: Neural tube defetcs; Vangl2; Planar cell polarity; Looptail mouse model; Protein expression pattern; Tubulogenesis; Organogenesis; Neural tube closure

1. Results and discussion

Neural tube defects, NTDs, are amongst the most frequent and debilitating congenital abnormalities in humans, occurring in 1 per 1000 live births. The etiology of NTDs is complex and involves both genetic and environmental factors (Frey and Hauser, 2003). The molecular and cellular mechanisms regulating neural tube closure are extremely complex and poorly understood. However, the recent characterization of NTD mutants in vertebrates and invertebrates has identified genes, proteins and signaling pathways that are highly conserved during evolution and that play a critical role in neural tube formation. One such gene, *Vangl2*, is mutated in the mouse mutant *looptail* (*Lp*) (Kibar et al., 2001a; Kibar et al., 2001b; Murdoch et al., 2001). While *Lp*/+ heterozygotes show a characteristic kinked or "looped" tail, *Lp*/*Lp* homozygotes are not viable and display a very severe NTD (craniorachishisis) with failure of the neural tube to close from the midbrain-hindbrain region to the most caudal extremity of the embryo (Strong and Hollander, 1949).

Originally identified in *Drosophila* (*Van Gogh*/*Strabismus*) in a genetic screen for defects in planar cell polarity (Taylor et al., 1998; Wolff and Rubin, 1998), mammalian *Vangl2* belongs to the so-called core planar cell polarity (PCP) gene family (reviewed in Adler, 2002; Mlodzik, 2002). These genes are critical for establishment of planar

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(as opposed to basal-apical) polarity of cells within epithelial sheets (Gubb and Garcia-Bellido, 1982; Nübler-Jung et al., 1987). PCP is visible and has been best studied in the orientation of the eye unit structure (eight photoreceptor cells of the ommatidium) and the uniform polarity of hair (trichome) on wing cells. In vertebrates, PCP is noticeable in a number of structures, including the appearance of body hair, the uniform orientation of stereo-ciliary bundles on hair cells of the cochlea, and polarization of cilia in the oviduct (Guo et al., 2004; Montcouquiol et al., 2003; Chailley et al., 1987). In addition to Vangl2, the PCP family includes the plasma membrane receptor Frizzled (Fz), cytoplasmic proteins Dishevelled (Dsh/Dvl) and Prickle (Pk), and the atypical cadherins Flamingo (Fml) and Diego (Klingensmith et al., 1994; Theisen et al., 1994; Chae et al., 1999; Gubb et al., 1999; Usui et al., 1999; Feiguin et al., 2001; Jenny et al., 2005). During establishment of PCP in *Drosophila* wing epithelia, PCP proteins become asymmetrically re-distributed within each cell: the Vang-Pk complex assumes an apical–proximal position while Fz-Dsh accumulates at the apical–distal side (Axelrod, 2001; Tree et al., 2002; Bastock et al., 2003). The specific asymmetric localization of the PCP proteins appears to propagate a positional signal required for PCP (Fanto and McNeill, 2004).

Vertebrate orthologs of fly PCP genes have been cloned (Kibar et al., 2001a; Park and Moon, 2002; Carreira-Barbosa et al., 2003; Goto and Keller, 2002; Wallingford et al., 2000; Curtin et al., 2003; Hamblet et al., 2002; Wang et al., 2006a) and have been shown to regulate convergent extension (CE) movements in zebrafish and *Xenopus* embryos (Wallingford and Harland, 2002; Torban et al., 2004a). During gastrulation, dorsal mesenchymal cells and neuroepithelial cells polarize by forming medial–lateral protrusions, which enable cells to move specifically toward the midline; cells interdigitate with each other, causing lengthening of the tissues in the anterior–posterior direction with concomitant thinning in the medial–lateral direction. CE contributes significantly to neural tube closure and is regulated by a vertebrate counterpart of fly PCP signaling referred to as the non-canonical Wnt pathway (Fanto and McNeill, 2004). Similar to *Lp* mice, homozygous mutants of *Celsr1* (mammalian ortholog of *Flamingo*) (Curtin et al., 2003) and double *Fz3*/*Fz6* or *Dvl1*/*Dvl2* mutants (Wang et al., 2006a; Hamblet et al., 2002) exhibit craniorachischisis. Alterations in the organization of ciliary bundles of cochlear hair cells are also detected in each of these mutants (Curtin et al., 2003; Wang et al., 2006a; Wang et al., 2006b).

Although an open neural tube (craniorachischisis) and cochlea disorganization are the most evident defects in *Lp* mice, a number of additional developmental abnormalities have been noted, including defects in the heart (Henderson et al., 2001), alterations in optic nerve trajectories (Rachel et al., 2000), minor cerebral abnormalities (Wilson, 1982; Kibar et al., 2001b), open eyelids and malfunction of the reproductive system (Strong and Hollander, 1949). mRNA expression studies have previously detected *Vangl2* mRNA in the neural tube (Kibar et al., 2001a; Tissir and Goffinet, 2006). However a detailed analysis of the cellular and subcellular distribution of the Vangl2 protein in embryonic tissues has yet to be reported. In this report, we investigated the expression of Vangl2 protein in developing mouse embryos. This was carried out to identify tissues and cell types in which the protein may play a role in PCP or CE, and that may be disrupted by the *Lp* mutation. To understand the mechanisms contributing to developmental defects seen in *Lp* mice, we compared Vangl2 protein distribution in neural tube and cochlea of normal mice and of both known alleles at the *Looptail* mutation (*Lp* and Lp^{m1Jus}).

1.1. Detection of Vangl2 protein by immunoblotting

To study Vangl2 expression *in vivo*, we raised polyclonal rabbit antisera against a fusion protein consisting of the predicted N-terminal cytoplasmic portion of the Vangl2 (13–64 amino acids) fused in-frame to GST. This segment was chosen because it is not conserved in Vangl1 (~40% similarity), the second Van Gogh-like gene, and yields specific antiserum when used as immunogen (Torban et al., 2004b). Immunoblotting experiments with this antiserum using mouse tissues from E13.5 embryos (Fig. 1A) shows the presence of a single band of the expected ~65 kDa size in the head (lane 2) but not in the trunk of the same embryo (lane 1). Its electrophoretic mobility is similar to that of Vangl2 protein expressed in HEK293 cells transfected with full-length *Vangl2* cDNA and used as a positive control (Fig. 1A, lane 5). Neither neocortex nor striatum from adult mice had detectable levels of Vangl2 protein (Fig. 1A, lanes 3 and 4). The Vangl2 protein was enriched in membrane fractions (Fig. 1B, lanes 1 and 3) compared to total cell lysates (Fig. 1B, lanes 2 and 4).

1.2. Vangl2 expression in developing neural tube

Craniorachischisis is the most noticeable defect in the *Lp* mouse. Apparently, it arises from a failure of neuroepithelial cells to undergo convergent extension and, perhaps, as

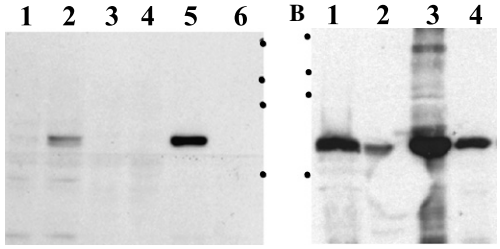

Fig. 1. Detection of Vangl2 protein in mouse embryonic and adult tissues. (A) Expression of Vangl2 in fetal tissues: lane 1, E13.5 trunk; lane 2, E13.5 head; lane 3, adult cortex; lane 4, adult striatum; lane5, HEK293 cells expressing a transfected *Vangl2* cDNA construct used as positive control; lane 6, HEK293 cells transfected with control pCB6 vector. (B) Vangl2 protein is enriched in membrane fractions: lane1, membrane fraction from E13.5 fetal brain; lane2, total E13.5 fetal brain lysate; lane3, membrane fraction from *Vangl2* transfected HEK293 cells; lane 4, whole cell lysate of the *Vangl2* transfected HEK293 cells.