

Title

Atypical Cadherin Negotiates a Turn

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Planar Cell Polarity (PCP) signaling is involved in many polarized cell behaviors. In this issue of *Developmental Cell*, Tatin et al. (2013) show that the atypical cadherin *Celsr1* is transiently localized to cellular protrusions in lymphatic endothelial cells and acts to orient valve-forming cells perpendicular to the vessel axis.

In the circulatory system, luminal valves ensure one-way flow of blood and lymph in the heart, veins, and lymphatic vessels. Mouse genetic models and classic observations have provided substantial information about valve morphogenesis in lymphatic vessels (Bazigou and Makinen, 2013; Sabine et al., 2012). Along the longitudinal lymphatic vessel, the earliest sign of valve formation is the local emergence of clustered endothelial cells with elevated expression of two transcription factors, *Prox1* and *Foxc2* (E16.5 in Figure 1A). Cooperation between these two factors leads to delimitation between valve-forming endothelial cells and other cells in the vessel wall. A subset of valve-forming cells then protrudes into the vessel lumen to form a disc-like structure called a valve leaflet, which begins depositing extracellular matrix (E17.5 in Figure 1A). As maturation proceeds, the valve becomes V-shaped (mature valve in Figure 1A).

In this issue of *Developmental Cell*, Tatin et al. (2013) continue to elucidate lymphatic valve morphogenesis by imaging valve formation at single-cell resolution. They report that a dramatic collective behavior of valve-forming cells takes place before the onset of leaflet formation (between E16.5 and E17.5 in Figure 1A). These cells initially adopt an elongated morphology along the longitudinal axis of the vessel before undergoing a 90 degree reorientation while maintaining their highly elongated cell morphology as well as tissue integrity (Figure 1B). This reorientation appears to be driven by active cell migration, and once the valve-forming cells align perpendicular to the longitudinal axis, they migrate into the vessel lumen, initiating leaflet formation (E17.5 in Figure 1A).

This directional cell reorientation relative to the vessel axis prompted the authors to examine the expression of the evolutionarily conserved “core group” members

of the PCP pathway. PCP originally described the asymmetric organization of cells within the plane of the epithelium. Currently, the purview of PCP has expanded to include the various directional behaviors of rearranging cell populations (Goodrich and Strutt, 2011; Gray et al., 2011). One family of the core group includes the seven-pass transmembrane cadherins, named Celsr1-Celsr3 in mammals and Flamingo (Fmi)/Starry night (Stan) in *Drosophila*. Beginning at E16.5, the valve-forming cells express and localize Celsr1 and another core member, Vangl2, in protrusions extending along the longitudinal axis of the vessel. Given the cell-border distributions of the core group members in all epithelia examined to date (Goodrich and Strutt, 2011; Gray et al., 2011), this intracellular localization is unusual. Even more intriguingly, the majority of the Celsr1- and/or Vangl2-rich projections point against the direction of flow (Figure 1B). This polarized Celsr1 expression precedes reorientation of the cell; later during reorientation of the cells, Celsr1 is also recruited to cell-cell contacts (Figure 1B).

How is valve formation affected by the absence of Celsr1? Surprisingly, the effect of Celsr1 loss is highly specific, affecting neither the territory formation of the valve nor the elongation of valve-forming endothelial cells per se before reorientation. Instead, the majority of mutant cells fail to reorient, and either remain aligned parallel to the vessel axis or show randomized orientations.

The presence of Celsr1 in the membrane protrusions raises several intertwined questions. Does the formation of directionally biased Celsr1-rich protrusions depend on Celsr1 itself? If the protrusions are misoriented or their formation is compromised, do the cells rotate properly? How might other PCP members be involved? For example, in the *Drosophila* epidermis, a core PCP pathway member, Disheveled (Dsh), underpins a bias in the directionality of cellular protrusions that secrete ligands, leading to asymmetric signal activation among neighboring cells (Peng et al., 2012).

Regarding a possible physiological function for Celsr1-rich protrusions, the authors imply that they might sense a global cue such as flow through the vessel.

One could imagine this function being similar to that of cilia, a distinct kind of cellular protrusion shown to be able to sense a unidirectional external flow and refine their polarity accordingly (Marshall and Kintner, 2008). Alternatively, the protrusions may concentrate hypothetical receptors for an upstream ligand(s). This might be reminiscent of the preferential localization of Fmi at the endings of dendritic branches of sensory neurons, which is proposed to potentiate contact-mediated avoidance (Matsubara et al., 2011). Regardless of whether the protrusion functions as a flow or chemical-sensing device, an even more puzzling question is how the cells convert the positional information of Celsr1 localization into perpendicular reorientation.

The authors addressed the specific role of Celsr1 by complementing the *in vivo* results with examination of primary endothelial cell cultures. These *in vitro* observations suggest that recruitment of Celsr1 to newly established immature junctions leads to a delay in the recruitment of VE-cadherin, thereby inhibiting the formation of stabilized adherens junctions (Figure 1C). This effect fits with an "occupancy-priority" hypothesis, where the arrival of a first protein alters the disposition kinetics or affinity of a second protein. In support of this hypothesis, the authors observe that the tight junction protein Claudin-5 localizes to discontinuous junctions at the borders of valve cells, in contrast to continuous zipper-like junctions between vessel-wall cells (Tatin et al., 2013; Baluk et al., 2007). Further examination of cell junction ultrastructure around the valve territories in developing as well as mature lymphatic vessels, and how they are affected by the absence of Celsr1, will certainly yield more information. It will also be interesting to verify this occupancy-priority hypothesis in the whole organ.

The key players in PCP signaling appear to participate in a growing repertoire of developmental processes. Yet, there must be numerous variations in the specific molecular mechanisms from one system to another, and even among different contexts in the same species (Goodrich and Strutt, 2011). Tatin et al. (2013) present a new take on PCP function in dynamic collective cell behavior in a study that will certainly stimulate further research in both basic and clinical

directions.

Figure legend

Figure 1 Valve morphogenesis in mouse lymphatic vessel

(A and B) During lymph valve development, Prox1^{high} endothelial cells (green) reorient themselves perpendicular to the longitudinal axis and migrate from the lymph vessel into the luminal side to form leaflets that deposit extra cellular matrix (ECM). See details in the text. (B) Celsr1 and another PCP core protein Vangl2 are localized to the membrane protrusions of the Prox1^{high} cells, and are also recruited to cell-cell contacts during reorientation.

(C) In cultured human lymphatic endothelial cells, Celsr1 is localized to the site of cell-cell contact and can recruit Vangl2 and PAR6. Stabilization of an adherence junction (AJ) is inhibited by Celsr1, which suppresses the recruitment of VE-cadherin at the contact site.

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A Mouse mesenteric lymphatic valve development

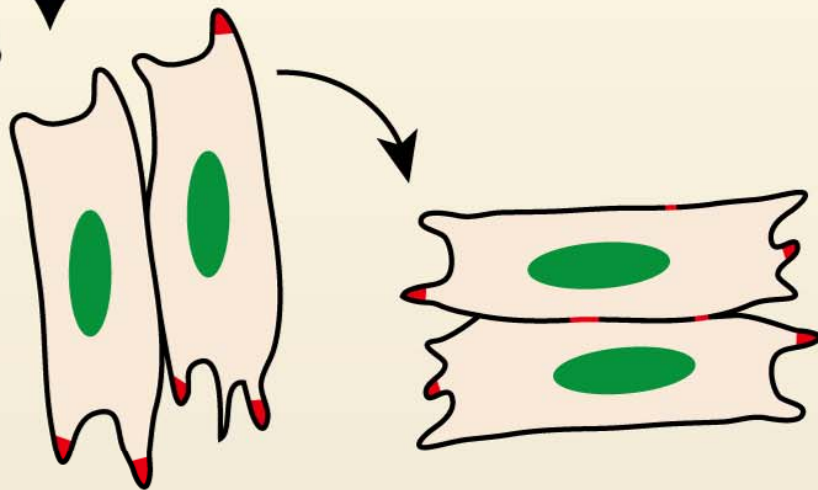
E16.5

E17.5

Mature valve

- Prox1^{high} nuclei
- Lymph flow
- ECM
- Celsr1, Vangl2

B



C Primary cell culture

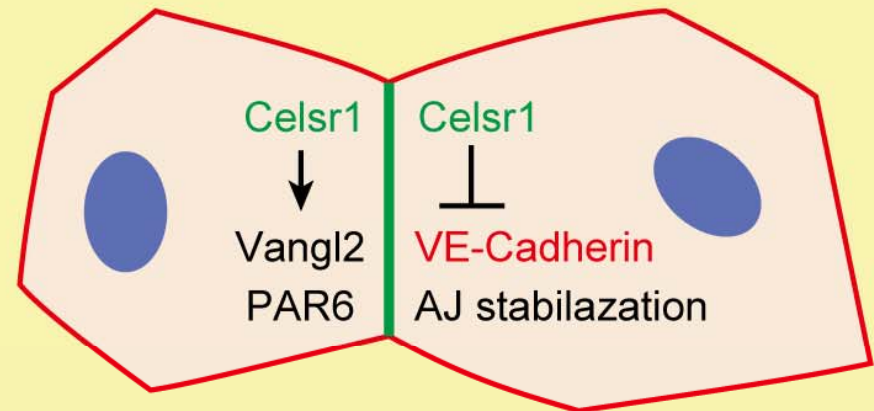


Figure 1