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Mechanosensitive β-catenin signaling regulates lymphatic vascular development

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Keywords: FOXC2; lymphovenous valves; lymphatic valves; Wnt/β-catenin signaling

Abbreviation: LVVs, lymphovenus valves; LVs, lymphatic valves; OSS, oscillatory shear stress; ECs, endothelial cells

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Abstract

The Wnt/β-catenin signaling is an evolutionarily conserved pathway that plays a pivotal role in embryonic development and adult homeostasis. However, we have limited information about the involvement of Wnt/β-catenin signaling in the lymphatic vascular system that regulates fluid homeostasis by absorbing interstitial fluid and returning it to blood circulation. In this recent publication we report that canonical Wnt/β-catenin signaling is highly active and critical for the formation of lymphovenus valves (LVVs) and lymphatic valves (LVs). β-catenin directly associates with the regulatory elements of the lymphedema-associated transcription factor FOXC2 and activates its expression in an oscillatory shear stress (OSS)-dependent manner. The phenotype of β-catenin null embryos was rescued by FOXC2 overexpression. These results suggest that Wnt/β-catenin signaling is a mechanotransducer that links fluid force with lymphatic vascular development.

Text

Wnt/β-catenin signaling is evolutionarily conserved among a variety of species and regulates diverse biological processes such as cell proliferation, apoptosis, cell polarity, cell differentiation, and migration (H. Clevers & R. Nusse, *Cell,* 2012 Jun 8;149(6):1192-205. doi: 10.1016). Not surprisingly, Wnt/β-catenin signaling in one way or another influences the development of nearly every organ in the animal body. In addition, aberrant Wnt/β-catenin signaling is associated with a variety of diseases including cancer, osteoporosis, diabetes, and Alzheimer's disease.

Wnt/β-catenin signaling is regulated at multiple levels (B.T. MacDonald *et al., Dev Cell*, 2009 Jul;17(1):9-26. doi: 10.1016). Wnt ligands, receptors, co-receptors and mediators are regulated at both transcriptional and post-translational levels. In the absence of Wnt ligands β-catenin, which is the centerpiece of the Wnt/β-catenin signaling, is cytoplasmic and membrane bound. β-catenin associates with cadherins and is an integral part of the adherin junctions that mediate cell-cell and cell-matrix interaction. β-catenin translocates to the nucleus when Wnt ligands bind to the Frizzled/Lrp receptor/coreceptor complex. Inside the nucleus β-catenin associates with the TCF/LEF family of transcription factors to activate their target genes.

Due to its membrane localization, interaction with adherens junctions and its critical role in Wnt/β-catenin signaling it has been long suspected that β-catenin might be a mechanotransducer (B.M. Gumbiner, *Cell*, 1996 Feb 9;84(3):345-57). β-catenin might sense cell-extrinsic physical forces and translate them into cell-intrinsic signals. Our recent identification of a role for β-catenin in lymphatic vascular morphogenesis might be a strong evidence in favor of this “mechanotransduction” model.

Lymphatic vascular system regulates fluid homeostasis by absorbing interstitial fluid and returning it back to blood circulation (H. Chen *et al, Microvasc Res,* 2014 Nov;96:16-22. doi: 10.1016). Lymphatic vasculature is also critical for

absorbing digested lipids from the intestine and for immune cell trafficking. The fluid within the lymphatic vessels is commonly known as the lymph. Genetic disorders or surgical and radiological damage to lymphatic vessels leads to lymphedema, a disease characterized by the swelling of tissues. Lymphedema patients are prone to infections and inflammations. There is currently no cure for lymphedema and only palliative treatments like massages are available. Understanding the mechanisms that regulate lymphatic vascular development might provide an opportunity to treat lymphedema.

Lymphatic endothelial cells (LECs) that originate predominantly from embryonic veins undergo stepwise morphogenesis to form the lymphatic vasculature. In the mature lymphatic vasculature lymphatic capillaries absorb the interstitial fluid and drain it into the collecting lymphatic vessels. Lymphatic valves (LVs) within the collecting vessels regulate the unidirectional flow of the fluid. Finally, lymph is returned to blood circulation at the junction of jugular and subclavian veins via two pairs of lymphovenus valves (LVVs). As LECs are constantly exposed to oscillatory lymph flow, lymphatic vascular development and homeostasis it is speculated to be force regulated. Specifically, oscillatory shear stress (OSS) is known to promote the expression of lymphedema associated transcription factor FOXC2. However, how OSS is sensed and translated into FOXC2 expression was unknown.

In this study we report that canonical Wnt/β-catenin signaling is highly active and critical for LVVs and LVs formation. LEC-specific deletion of β-catenin in mouse embryos resulted in severe lymphedema. Further analysis revealed that the lymphatic vessels were defective and LVVs and LVs were absent. These phenotypes were reminiscent of *Foxc2*$^{-/-}$ embryos. Consistently, FOXC2 expression was defective in the LECs of mice lacking β-catenin. And, ectopic expression of FOXC2 partially yet dramatically rescued the lymphatic vascular phenotypes of mice lacking β-catenin. Further, β-catenin directly associates with the regulatory elements of FOXC2 in LECs. Importantly, OSS activated the expression of β-catenin and FOXC2 in LECs. Inhibition of Wnt/β-catenin signaling with small molecules prevented the activation of FOXC2 expression by OSS. These results strongly suggest that Wnt/β-catenin signaling is mechanically activated by fluid flow in LECs (**Figure 1**).

Interesting questions still remain. We still do not know whether β-catenin directly senses fluid flow at LEC membrane or if additional intermediate sensors are involved. We cannot completely rule out the contribution of β-catenin in adherens junctions during lymphatic vascular morphogenesis. And, we also do not know if any Wnt-ligands are involved in this process. Further dissection of this powerful signaling pathway using genetic and molecular tools will be needed to address these questions and test the “mechanotransduction” model. Importantly, we hope that the numerous small molecule agonists and antagonists of Wnt/β-catenin signaling might provide an opportunity to treat lymphedema in the not so distant future.

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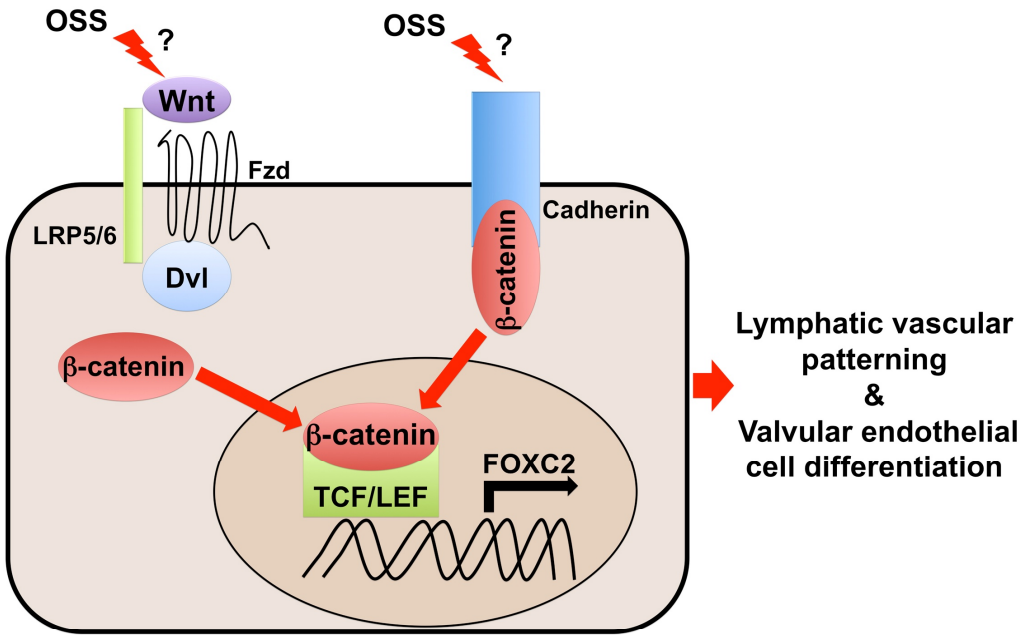
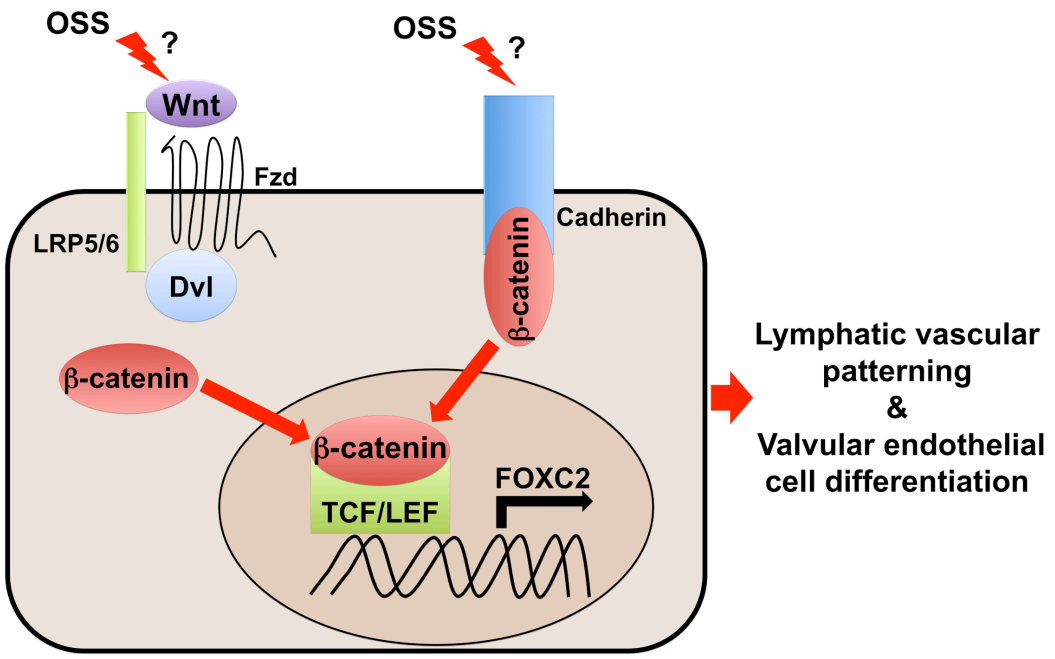


Figure Legend

Figure 1. A Model for the mechanosensitive β-catenin signaling during lymphatic vascular development. OSS may trigger β-catenin activation either in Wnt ligands dependent manner or in Wnt ligands independent manner.



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