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$S1P_1$ Bridges Mechanotransduction and Angiogenesis during Vascular Development

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Mechanisms of stabilization of the vascular plexus and the role of mechanotransduction in this process are not well understood. In this issue of *Developmental Cell*, Jung et al. (2012) and Gaengel et al. (2012) describe the ligand-sensitive and mechanosensitive functions of an important vascular G protein-coupled receptor, $S1P_1$.

Mechanical forces impact almost every area of life. Thus, proper mechanosensing and mechanotransduction are important during development, as well as in adult physiology. During the blastocyst stage of development, cell migrations are governed, in part, by mechanical forces (Orr et al., 2006). Later, substratum rigidity contributes to cell differentiation (Kshitiz et al., 2012). As adults, humans rely deeply on the senses of touch and hearing, both of which are the result of complex suites of mechanotransducers acting in concert. Mechanosensing is also essential in vascular development and physiology. The force of flowing blood contributes to the correct formation of the vascular plexus (Hahn and Schwartz, 2009). Also, blood flow creates frictional drag on endothelial cells (ECs), which is termed "shear stress." In straight regions of the vasculature, shear stress is termed "laminar," where flow rate is high and uniform. This type of blood flow causes cells to divide very slowly and induces atheroprotective signaling in ECs. In branched or highly curved regions of the vasculature, shear stress is termed "disturbed." In these regions, blood flow is highly irregular and induces atherogenic signaling in ECs (Orr et al., 2006).

Much of the study of mechanotransduction in the vasculature has focused on ECs, because they are directly exposed to shear stress. Several primary mechanosensors have been proposed to be at work in the endothelium. These include the complex of PECAM-1, VEGFR2, and VE-cadherin; primary cilia on the luminal surface of cells; the endothelial glycocalyx; and G protein-coupled receptors (GPCRs) (Hahn and Schwartz, 2009). Sphingosine-1-phosphate and its GPCR, Sphingosine-1-phosphate receptor-1 ($S1P_1$), have been identified as regulators of endothelial cell proliferation and vascular morphogenesis (Obinata and Hla, 2012). In this issue of *Developmental Cell*, Gaengel et al. (2012) and Jung et al. (2012) reveal that endothelial $S1P_1$ stabilizes the primary vascular network during development and homeostasis.

Both papers begin by asking about the role of $S1P_1$ in vascular plexus stabilization and sprouting angiogenesis. Using the mouse retina model of angiogenesis, Jung et al. show that overexpression of $S1P_1$ leads to a marked decrease in sprouting of new vessels. Conversely, both groups show that an EC-specific knockout of $S1P_1$ leads to a hypersprouting phenotype, in which the vascular plexus becomes overly dense, resulting in inadequate growth of the vasculature into the retina. Interestingly, the ability of $S1P_1$ to regulate sprouting angiogenesis is independent of Notch signaling, suggesting that the two pathways are probably parallel. The authors also observed that vessels lacking $S1P_1$ were "leakier" than wild-type vessels due to destabilization of VE-cadherin, leading to perturbation in blood flow upon $S1P_1$ loss. This finding prompted Gaengel et al. to examine the interrelated effects of $S1P_1$ on VE-cadherin and VEGF signaling, and it conversely fueled the mechanotransduction investigation undertaken by Jung et al. In ECs, if adherens junctions are forming incorrectly, this can lead not only to poor barrier function but also to poor shear stress signaling as a result of the malformation of the mechanosensory complex comprised of PECAM-1, VE-cadherin, and VEGFR2 (Tzima et al., 2005).

Given the interruption of adherens junction formation, does lack of $S1P_1$ lead to poor mechanoresponsive signaling in endothelial cells? Jung et al. show that indeed this is the case. The downstream signaling pathways activated by shear stress in ECs include the ERK/MAPK pathway and the Akt/eNOS pathway. When $S1P_1$ is chemically antagonized or knocked down in ECs, activation of these pathways is ablated. Importantly, $S1P_1$ mutants that are insensitive to S1P binding are also able to transduce shear stress signals, suggesting that $S1P_1$ can be activated in a ligand-independent manner. This raises the exciting possibility that $S1P_1$ can respond not only to blood-derived S1P but also to biomechanical signals independently of its ligand. This observation is reminiscent of another angiogenic receptor, VEGFR2, which can be activated by its soluble ligand, VEGF, as well as by fluid shear stress in a ligand-independent manner (Jin et al., 2003; Tzima et al., 2005). Jung et al. take this investigation one step further and show that $S1P_1$ localization in the aortic endothelium in vivo is regulated by flow. Sections from the descending aorta, where flow is laminar, show colocalization of $S1P_1$ with VE-cadherin. In contrast, $S1P_1$ is found in endocytic vesicles in the lesser curvature of the aorta, where flow is disturbed. The differential localization of $S1P_1$ in different flow areas, coupled with the requirement of $S1P_1$ for eNOS activation in vivo, provides evidence for the hypothesis that shear stress signaling in vivo requires $S1P_1$.

How could $S1P_1$ be working in the system of shear stress sensing in ECs? The authors suggest that $S1P_1$ could be

important for shear stress signaling through proper maintenance of adherens junction organization. This idea is reinforced by the more detailed analysis of $S1P_1$ effects on VE-cadherin performed by Gaengel et al. and is most likely the mechanism of action. Yeh et al. (2008) show that bradykinin receptor B2, another vascular GPCR, complexes with PECAM-1 via the extracellular domain of PECAM-1. This association leads to PECAM-1 and the G protein Gαq/11 associating and mediating endothelial signaling together (Yeh et al., 2008). It is possible that $S1P_1$ could be acting on adherens junctions in a similar, not-yet-described manner. However, another possibility is that $S1P_1$ induces distinct ligand-sensitive and mechanosensitive signaling in ECs. Recently, Scimia et al. (2012) described the distinct signaling functions of the APJ receptor in cardiomyocytes. APJ is a GPCR whose ligand is apelin, an adipokine. Scimia et al. show that when APJ binds apelin, hypertrophy in cardiac muscle is prevented. When apelin is absent, APJ senses stretch in cardiomyocytes and induces a hypertrophic response (Scimia et al., 2012). Thus, the ligand-dependent and -independent signaling mechanisms of APJ act to balance each other out. Could $S1P_1$ be acting in a similar manner?

From a mechanosensory standpoint, a number of interesting and provocative questions are raised. Is expression of $S1P_1$ regulated by flow? And if so, what are the molecular regulatory mechanisms? Clearly, the authors show that its expression is strongly induced in the flow-positive regions of the retinal vascular network, but is that the case in high-flow areas, such as the aorta and the carotid? Similarly, Jung et al. show differential localization of $S1P_1$ in atheroprone versus atheroprotected regions in the aorta, but this does not necessarily translate to function. These observations beg the question of whether $S1P_1$ is also required for endothelial responses to disturbed or atheroprone shear stress and, ultimately, whether it has a role in atherosclerosis associated with disturbed flow patterns.

The observations that $S1P_1$ expression is induced in flow-positive regions and that induction of $S1P_1$ is associated with cessation of EC sprouting are suggestive of an intriguing relationship between flow-mediated biochemical signals and angiogenic factor signaling. Does $S1P_1$ always act as a dual-function receptor throughout development into maturity, or are its functions separated during a certain developmental window? If $S1P_1$ is always mechanosensitive, how do the ligand binding and the mechanosensitive aspects of its function work to regulate vascular processes? The present studies definitely set the stage for investigating the elusive relationship between mechanotransduction and angiogenesis.

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