

Blood flow boosts BMP signaling to keep vessels in shape

Claudio A. Franco¹ and Holger Gerhardt^{2,3,4,5}

¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal

²Max-Delbrück Center for Molecular Medicine, 13125 Berlin, Germany

³Department of Oncology, Vascular Patterning Laboratory, Vesalius Research Center, 3000 Leuven, Belgium

⁴German Center for Cardiovascular Research, 13347 Berlin, Germany

⁵Berlin Institute of Health, 10117 Berlin, Germany

Bone morphogenic proteins (BMPs) and blood flow regulate vascular remodeling and homeostasis. In this issue, Baeyens et al. (2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201603106>) show that blood flow sensitizes endothelial cells to BMP9 signaling by triggering Alk1/ENG complexing to suppress cell proliferation and to recruit mural cells, thereby establishing endothelial quiescence.

How physical forces and signaling events interact in the process of organogenesis and how they integrate to maintain tissue homeostasis is one of the most complex and fascinating questions in biology. The physical influence of blood flow and pressure on the endothelial lining and supporting mural cells of all blood vessels plays a prominent role in forming and maintaining vascular patterning and function. Without blood flow, blood vessels only form a primitive network that does not acquire the hierarchical branching pattern of arteries, capillaries, and veins that ensure efficient tissue supply. Recent studies highlight a multitude of effects of blood flow–mediated shear stress on endothelial cell shape, alignment, gene regulation, and cell–cell signaling events, jointly contributing to proper vascular function. Deregulation of these endothelial adaptations in the adult can cause, or contribute to, severe vascular dysfunction. Although we have learned about many effects, genes, and signaling pathways that are directly or indirectly influenced by flow, and many potential regulators, we still lack fundamental insight into the mechanisms that integrate flow sensing and endothelial responses. To date, we do not know precisely how endothelial cells sense the direction and magnitude of flow or how quantitative flow changes translate into qualitative changes in endothelial cell behavior, such as the inhibition or stimulation of proliferation and recruitment of supporting mural cells. In this issue, Baeyens et al. provide new mechanistic insight into the interaction between blood flow and bone morphogenic protein (BMP) signaling in the vascular disease hereditary hemorrhagic telangiectasia (HHT; Baeyens et al., 2016).

HHT is an inherited genetic disorder that affects blood vessels leading to arteriovenous malformations (AVMs) and bleedings (Peacock et al., 2016). The most common genetic mutations associated with HHT are linked to decreased BMP

pathway activity, with heterozygous mutations in the transmembrane proteins endoglin (ENG), activin A receptor–like kinase 1 (ACVRL1/ALK1), and cytosolic sterile α motif domain containing 4A (SMAD4) accounting for almost 85–90% of known familial cases (Peacock et al., 2016). *Acvrl1*- or *Eng*-null mice die at early/mid gestation, with grossly defective vasculature (Arthur et al., 2000; Oh et al., 2000). Inducible homozygous deletion of *Alk1* and *Eng* in adult mice gives rise to AVMs with poor smooth muscle coverage and hemorrhages, with a stronger incidence in *Alk1*-deficient mice (Park et al., 2008; Mahmoud et al., 2010). Biochemical studies showed that BMP9 and BMP10 activate ALK1, inducing phosphorylation of the intracellular signal-transducing proteins SMAD1, SMAD5, and SMAD8. ENG works as a coreceptor for ALK1, significantly enhancing BMP activity. ALK1/ENG downstream signaling leads to nuclear translocation of activated SMADs that act as transcription factors. ALK1/ENG-dependent gene expression regulates a large range of biological activities, including cell proliferation, migration, and differentiation (Peacock et al., 2016).

Baeyens et al. (2016) observe that AVM formation in retinas of newborn *Alk1*-deficient mice occurs primarily in areas of higher blood flow, similar to previous observations in the adult mouse (Park et al., 2009). Interestingly, other recent publications have also linked blood flow, ALK1, and AVMs. Beth Roman's group demonstrated that increases in endothelial cell number in AVMs in *Alk1* mutant zebrafish are dependent on flow (Corti et al., 2011). Furthermore, the Roman group proposed that *Alk1* expression is regulated by blood flow and that flow activates *Alk1*-dependent phosphorylation of SMADs downstream of heart-derived BMP10, which is carried by the blood stream (Laux et al., 2013). Likewise, Zhou et al. (2012) also demonstrated that blood oscillatory shear stress in the context of disturbed flow induces phosphorylation of SMAD1 and SMAD5 in endothelial cells in vitro and in vivo. However, this SMAD activation was pro-proliferative and BMP independent (Zhou et al., 2012).

In contrast, Baeyens et al. (2016) demonstrate that blood flow sensitizes endothelial cells toward BMP9, changing its EC₅₀ from 60 pg/ml in no-flow conditions to 3.5 pg/ml when exposed to 1.2 Pa of shear stress. Interestingly, ALK1 and

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Correspondence to Holger Gerhardt: holger.gerhardt@mdc-berlin.de



ENG appear to have different roles during BMP9 flow signaling. Whereas ALK1 is required for any BMP9 signaling, ENG seems to be particularly critical for flow-dependent sensitization to low BMP9 concentrations. This finding fits well with the inducible mouse models of HHT and may explain why Alk1 deletion has a stronger impact in AVM formation than deletion of Eng. Mechanistically, Baeyens et al. (2016) propose that blood flow enables a physical interaction between ALK1 and ENG, which is responsible for the increased sensitivity to BMP9. Indeed, the authors were able to coimmunoprecipitate ALK1 and ENG when endothelial cells are exposed to flow but not in the presence of BMP9 alone. Collectively, the results by Baeyens et al. (2016) argue that enhanced BMP signaling is the key mediator for endothelial quiescence in response to flow (Fig. 1). Knockdown of either ALK1 or ENG reversed two known but critical effects of shear stress on endothelial cells: suppression of proliferation and mural cell recruitment (Baeyens et al., 2016). Intriguingly, in mice where ALK1 was randomly deleted in a subpopulation of labeled endothelial cells, only the ALK1-deficient cells proliferated more and showed decreased mural cell coverage. Given that endothelial cell proliferation and reduced recruitment of mural cells have previously been suggested to drive AVM formation, the newly identified convergence of blood flow and BMP signaling on these processes provides a molecular mechanism for the development of HHT lesions when ALK1 or ENG are defective.

Like any good research, the current work by Baeyens et al. (2016) not only provides new insight but also raises further questions. How shear stress triggers the formation of a complex between Alk1 and ENG, and whether this interaction itself functions as a real rheostat for SMAD phosphorylation, remains unclear. Moreover, it is not clear why this interaction activates mostly SMAD1, and SMAD5 to a lesser extent, but not SMAD8. Whether SMAD1 is functionally involved also remains untested, although the genetic contribution to HHT by SMAD4 mutations clearly suggests that a SMAD complex should be part of the mechanism. Given the multiple levels of cell-intrinsic dynamic regulation of SMAD shuttling and transcriptional activity, future work is needed to unravel whether flow and BMP signaling provide a system of reinforcement of a tonic (continuous and sustained) stimulus or whether, like in other signaling circuits, oscillatory patterns and temporal effects become important. Is there an upstream flow sensor such as Piezo1 and are the calcium signals known to be activated by flow required for Alk1/ENG complexing? It would be interesting to test the potential contribution of the transmembrane protein 100 as it has been shown to be involved in BMP9-ALK1 signaling and AVM formation (Somekawa et al., 2012) and to regulate calcium influx (Weng et al., 2015), a known mediator of flow signaling, in cells. Another open question is the link between BMP and Notch signaling in AVM formation. Both pathways have now been demonstrated to regulate AVM onset and both regulate common endothelial behaviors, such as cell migration and proliferation (Murphy et al., 2008). Notch and BMP signaling cross talk at different levels and can be synergistic or antagonistic depending on the cellular context. SMADs and Notch intracellular domain can physically interact to promote expression of the Notch-dependent target genes HES1 and HEY1/2, which can modulate endothelial fate (Itoh et al., 2004).

In addition to these open questions directly related to the biochemical pathway involved, the cell biological and morphogenic mechanism of AVM or other vascular malformations also

Sensitization of endothelial cells to BMP9 by shear stress

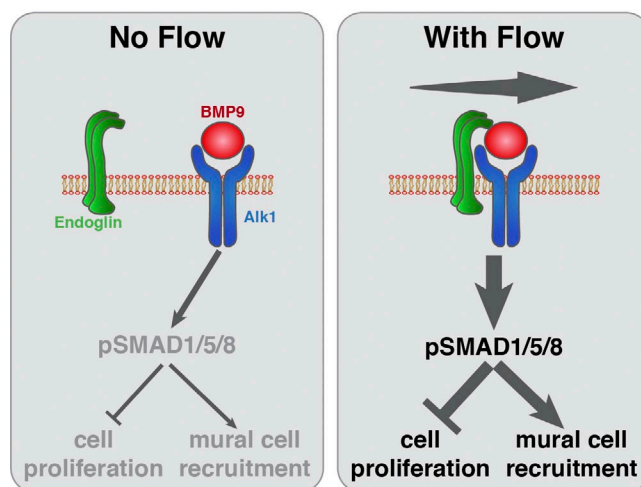


Figure 1. Blood flow promotes ALK1/ENG association to increase BMP9 sensitivity. In the absence of blood flow and at low concentrations of BMP9, ALK1 is not able to phosphorylate its downstream effectors SMAD1/5/8. However, in the presence of blood flow, shear stress promotes the association of ENG with ALK1, which decreases the endothelial EC₅₀ toward BMP9. Thus, blood flow promotes ALK1 tyrosine kinase activity and phosphorylation of SMAD1/5/8 at low concentrations of BMP9. The overall effect of the synergy between blood flow and BMP signaling is the inhibition of cell proliferation and the recruitment of mural cells, promoting vessel stabilization and quiescence. SMADs are likely the key mediators of these effects; however, this relationship is not yet firmly established.

requires further elucidation. Whereas the work by Baeyens et al. (2016) highlights clonal endothelial proliferation as a major effect of deficient BMP signaling, a recent study linked ALK1 activity to endothelial cell migratory behavior in response to flow. Rochon et al. (2016) showed that in zebrafish development, Alk1-positive endothelial cells generally migrate against the direction of blood flow, but Alk1-deficient cells showed an impaired capacity to migrate against the flow direction. Altered endothelial cell migration results in an imbalanced distribution of cells within the network, thereby contributing to formation and enlargement of AVMs. Although the relevant ligand in the zebrafish system appears to be BMP10 and, unlike the mouse work, the AVMs in the study by Rochon et al. (2016) form during vascular development, it is possible that AVMs in general are a result of several altered endothelial behaviors, in addition to proliferation. Recent papers from different groups have demonstrated that endothelial cells exhibit a surprising degree of motility during the process of vessel remodeling. For instance, vessel regression, an essential step in vascular remodeling, is driven by migration of endothelial cells from the regressing vessel segments into neighboring segments (Chen et al., 2012; Franco et al., 2015; Lenard et al., 2015), a process dependent on blood flow (Chen et al., 2012; Franco et al., 2016). Endothelial cells polarize against the flow direction and this provides a directional cue to coordinate endothelial cell movements within the vascular network (Udan et al., 2013; Franco et al., 2016). If and how the mechanisms governing developmental remodeling are different, or similar, to those that control the maintenance of vascular patterning in the adult remains to be seen. It will therefore be critical to investigate the contribution of proliferation and migration in the development of AVMs and HHT, and how

those two cellular behaviors are under the molecular control of ALK1/ENG and flow signaling. It must be anticipated that the identified Alk1/ENG complex will not remain the only signaling hub in the regulation of vascular homeostasis.

From a clinical perspective, understanding the mechanism of HHT is important but remains unsatisfactory as long as we cannot develop effective disease prevention, cure, or at least symptomatic treatment. Changing flow conditions in blood vessels are part of everyday life and HHT patients carry heterozygosity for one of the key BMP signaling components in all cells. Can we learn how to maintain the sensitivity of or resensitize endothelial cells to BMP from this study by Baeyens et al. (2016) of the integration of flow and BMP signaling? Would triggering complex formation between Alk1 and ENG in all vessels be safe? Predicting where the fundamental breakthrough for therapeutic developments will come from is notoriously difficult, but new mechanistic insight, such as the one provided here, represents a promising starting point.

Acknowledgments

The references in this Spotlight are not exhaustive and we apologize to those authors whose work on this topic could not be cited because of space limitations.

C.A. Franco was supported by a Fundação para a Ciência e a Tecnologia investigator (IF/00412/2012), a Fundação para a Ciência e a Tecnologia grant (EXPL/BEX-BCM/2258/2013), the Portugal2020 program (LISBOA-01-0145-FEDER-00739), and a European Research Council starting grant (AXIAL-EC; 679368). H. Gerhardt is supported by European Research Council consolidator grant (REshape; 311719).

The authors declare no competing financial interests.

Submitted: 8 September 2016

Accepted: 12 September 2016

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