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PTEN Connection in HHT2

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The hierarchical network of arteries, veins and capillaries of our cardiovascular system is laid out during development, and further expanded and matured postnatally. Precise regulation of endothelial proliferation and behavior is needed during development and throughout life to maintain proper architecture of the vasculature. In this issue, Alsina-Sanchis and colleagues report that phosphatase and tensin homolog (PTEN) connects bone morphogenic protein-9 (BMP9) activation of activin-receptor-like kinase-1 (ALK1) to phosphatidylinositol 3-kinase (PI3K) signaling in endothelial cells and implicate PI3K-stimulated endothelial proliferation in arteriovenous malformation (AVM) in hereditary hemorrhagic telangiectasia-2 (HHT2)[1].

HHT is a rare autosomal dominant disorder that affects approximately 1/5000 people. HHT1 is caused by loss of function of one allele of a TGFβ co-receptor called endoglin while HHT2 is caused by loss of function of ALK1, a TGFβ family type I receptor known phosphorylate SMADs 1/5/8. In both HHT1 and HHT2, vascular overgrowth and malformations called telangiectasias occur focally throughout the body. The telangiectasias appear to arise from abnormal connections between enlarged post-capillary venules and arterioles, which results in fragile vessels prone to bleeding[2].

Previous studies had revealed ALK1 suppresses endothelial proliferation and that lack of Alk1 in mice leads to vascular overgrowth and malformation[3, 4], but precisely how AlK1 controls vascular growth and morphogenesis was unknown.

Alsina-Sanchis, Vinals and colleagues set out to solve this mystery. They observed retinal vascular development in *Alk1*+/− mice, manipulated BMP-9/ALK1 signaling in cultured human umbilical vein endothelial cells (HUVECs), analyzed HHT-2 patient-derived tissue specimens and went back to mice to test what they had learned. *Alk1*+/− mice showed increased endothelial proliferation and widened venous and arterial retinal vessels by post-natal day 9. In vitro, BMP9 decreased VEGF-stimulated HUVEC proliferation and pAKT and pERK levels. Pre-treatment experiments showed that BMP9 must precede VEGF-A addition by at least 2 hours to see these reductions, a clue that perhaps biosynthesis or stabilization of a regulator(s) might be needed for the BMP9 anti-angiogenic effects.

PTEN dephosphorylates phosphatidylinositol (3,4,5) P3 (PIP3) to phosphatidylinositol (4,5) P2 (PIP2), reversing the enzymatic action of PI3K, a potent brake on PI3K-AKT-mTOR

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signaling. Since mice with endothelial deletion of PTEN phenocopy vascular defects in *Alk1*+/− mice[5], the authors speculated that PTEN might be involved in ALK1 function. Indeed, they found PTEN mRNA, protein and activity all increased in BMP9 treated HUVECs. siRNA knockdown PTEN substantially increased pAKT and rendered BMP9 unable to block VEGF-A proliferation. Taken together, the in vitro experiments show PTEN is needed to suppress pAKT and for BMP9 to inhibit endothelial proliferation.

With this discovery in hand, the investigators scoured public data bases on HHT2 tissue specimens, which revealed overexpression of genes related to the PI3K/AKT pathway. Immunostaining confirmed the data: endothelial proliferation and markers of PI3K activation (pAKT, pNDGR1 and pS6) were increased in HHT2 tissue sections. Significant correlation was found between pNDGR1 positivity and the severity of nosebleeds experienced by HHT2 patients from which tissue was obtained, linking PI3K activation to a common feature of HHT2. The authors returned to the Alk1+/− mice to assess PI3K contribution to the vascular hyperplasia in the post-natal retina. Endogenous PI3K activity was reduced genetically by replacement of one allele with a kinase-dead form of the PI3K catalytic subunit and PI3K was inhibited pharmacologically with a pan-PI3K inhibitor. In both experiments, retinal vessel hyperplasia at post-natal day 7, measured by vessel width, was reduced to wild-type levels. This in vivo data indicates PI3K inhibition is sufficient to reverse the Alk1+/− phenotype, and thus may be a strategy to treat HHT2.

In summary, data from mouse models, in vitro experiments and patient samples has revealed a critical link between ALK1 and PI3K that is needed to properly regulate endothelial proliferation and vessel morphogenesis in vivo; in vitro experiments show PTEN is the link. Extrapolating to HHT2, *ALK1* haploinsufficiency should reduce the ability of the ALK1 ligand, BMP9, to increase PTEN sufficiently to dampen down PI3K-pAKT signaling in pro-angiogenic settings. Further experiments are needed to determine if PTEN is indeed deficient or reduced in HHT2.

The reliance on the retina as the vascular test-bed is an important limitation of this study as it relates to HHT2. The still-developing postnatal retinal vessels may not fully reflect the vascular microenvironments where telangiectasias and AVMs typically develop in HHT patients. Telangiectasias occur on the face and oral and nasal mucosal membranes; endangering AVMs can form in the lungs, liver, gastrointestinal tract, and brain. HHT2 patients are more likely to have AVMs in the liver while HHT1 patients are more likely to have AVMs in the lung and brain. Clearly the vascular bed plays a role in the pathogenesis of HHT. Another aspect is that HHT vascular lesions do not occur everywhere in the body despite haploinsufficiency in every cell. It would be of interest to learn if inherently low PTEN levels in subsets of endothelial cells might contribute to localized AVM formation.

Enormous strides have been made in recent years towards identifying molecular drivers of vascular malformations[6]. Increased endothelial PI3K/AKT/mTOR signaling is center-stage in venous malformations[7–11], lymphatic malformations[12, 13] and vascular tumors[14] and now the pathway appears activated in HHT2 as well. Activating mutations in TIE2 or mutations downstream in the PI3K catalytic domain drive venous malformations, which can be reversed in animal models with PI3K or mTOR [i]inhibitors[8, 10, 11]. Yet major questions

remain: how does increased PI3K/pAKT cause different types of vascular malformation? In venous malformations, activating mutations in TIE2 or PI3K over-activate pAKT whereas increased pAKT in HH2 appears to result from loss of an inhibitor, PTEN. Perhaps upstream regulators titrate the PI3K signaling to regulate endothelial behavior and morphogenesis in nuanced ways. It is clear that such mechanisms must be maintained throughout life as vascular overgrowths and malformations often worsen over a lifetime. As investigators elucidate molecular mechanisms and identify potential drugs to treat vascular malformations such as AVMs, it will be important to develop means for localized delivery to reduce potential harm to unaffected endothelium, as life-long therapy may be necessary for these genetic disorders.

Abbreviations

PTEN	phosphatase and tensin homolog
BMP9	bone morphogenic protein-9
ALK1	activin-receptor-like kinase-1
PI3K	phosphatidylinositol 3-kinase
AVM	arteriovenous malformation
HHT-2	hereditary hemorrhagic telangiectasia-2
HUVEC	human umbilical vein endothelial cells

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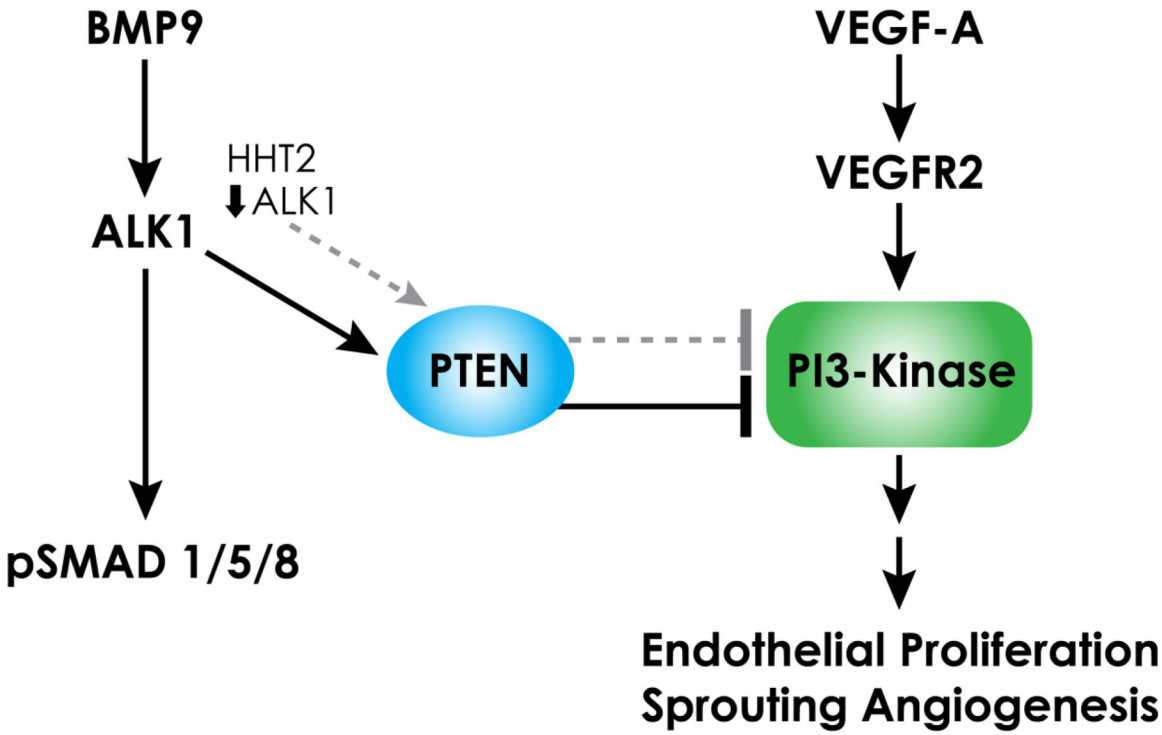

Figure 1.

ALK1 signaling decreases PI3-Kinase signaling and endothelial proliferation. In vitro studies show BMP9/ALK1 increases PTEN, a phosphatase that reverses the action of PI3-kinase. This implicates PTEN as an endogenous brake to PI3K/pAKT signaling, endothelial proliferation and angiogenesis. In HHT2, reduced ALK1 coincides with increased PI3K/AKT signaling (dashed gray lines), which may be caused, as in cultured endothelial cells, by reduced PTEN.