Editorial

PTEN (Phosphatase and Tensin Homolog) Connection in Hereditary Hemorrhagic Telangiectasia 2

Joyce Bischoff

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 the proper architecture of the vasculature. In this issue, Alsina-Sanchis et al[1] report that PTEN (phosphatase and tensin homolog) connects BMP-9 (bone morphogenic protein 9) activation of ALK-1 (activin-receptor-like kinase 1) to PI3K (phosphatidylinositol 3-kinase) signaling in endothelial cells and implicate PI3K-stimulated endothelial proliferation in arteriovenous malformation (AVM) in hereditary hemorrhagic telangiectasia 2 (HHT-2).

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HHT is a rare autosomal dominant disorder that affects ≈1/5000 people. HHT-1 is caused by loss of function of one allele of a TGF-β (transforming growth factor-β) coreceptor called endoglin, whereas HHT-2 is caused by loss of function of ALK-1, a TGF-β family type I receptor known phosphorylate SMADs (suppressor of mothers against decapentaplegic) 1/5/8. In both HHT-1 and HHT-2, vascular overgrowth and malformations called telangiectasias occur focally throughout the body. The telangiectasias appear to arise from abnormal connections between enlarged postcapillary venules and arterioles, which results in fragile vessels prone to bleeding.[2]

Previous studies had revealed ALK-1 suppresses endothelial proliferation and that lack of Alk-1 in mice leads to vascular overgrowth and malformation,[3,4] but precisely how ALK-1 controls vascular growth and morphogenesis was unknown.

Alsina-Sanchis et al[1] set out to solve this mystery. They observed retinal vascular development in *Alk1*$^{+/-}$ mice, manipulated BMP-9/ALK-1 signaling in cultured human umbilical vein endothelial cells, 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 postnatal day 9. In vitro, BMP-9 decreased VEGF (vascular endothelial growth factor)-stimulated human umbilical vein endothelial cell proliferation and pAKT (phosphorylated protein kinase B) and pERK (phosphorylated extracellular signal-regulated kinases) levels. Pretreatment experiments showed that BMP-9 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 BMP-9 antiangiogenic effects.

PTEN dephosphorylates phosphatidylinositol (3,4,5) P3 to phosphatidylinositol (4,5) P2, reversing the enzymatic action of PI3K, a potent brake on PI3K-AKT-mTOR (mammalian target of rapamycin) signaling. Because mice with endothelial deletion of PTEN phenocopy vascular defects in *Alk1*$^{+/-}$ mice,[5] the authors speculated that PTEN might be involved in ALK-1 function. Indeed, they found PTEN mRNA, protein and activity all increased in BMP-9–treated human umbilical vein endothelial cells. siRNA knockdown PTEN substantially increased pAKT and rendered BMP-9 unable to block VEGF-A proliferation. Taken together, the in vitro experiments show PTEN is needed to suppress pAKT and for BMP-9 to inhibit endothelial proliferation (Figure).

With this discovery in hand, the investigators scoured public databases on HHT-2 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, pNDGR-1 [phosphorylated N-myc downstream-regulated gene-1], and pS6) were increased in HHT-2 tissue sections. A significant correlation was found between pNDGR-1 positivity and the severity of nosebleeds experienced by HHT-2 patients from which tissue was obtained, linking PI3K activation to a common feature of HHT-2. The authors returned to the *Alk1*$^{+/-}$ mice to assess the PI3K contribution to the vascular hyperplasia in the postnatal 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 postnatal 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 HHT-2.

In summary, data from mouse models, in vitro experiments and patient samples has revealed a critical link between ALK-1 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 HHT-2, *ALK1* haploinsufficiency should reduce the ability of the ALK-1 ligand, BMP-9, to increase PTEN sufficiently to dampen down PI3K-pAKT signaling in proangiogenic settings. Further experiments are needed to determine whether PTEN is indeed deficient or reduced in HHT-2.

The reliance on the retina as the vascular test-bed is an important limitation of this study as it relates to HHT-2. The still-developing postnatal retinal vessels may not fully reflect the vascular microenvironments where telangiectasias and AVMs typically develop in HHT patients. Telangiectasias

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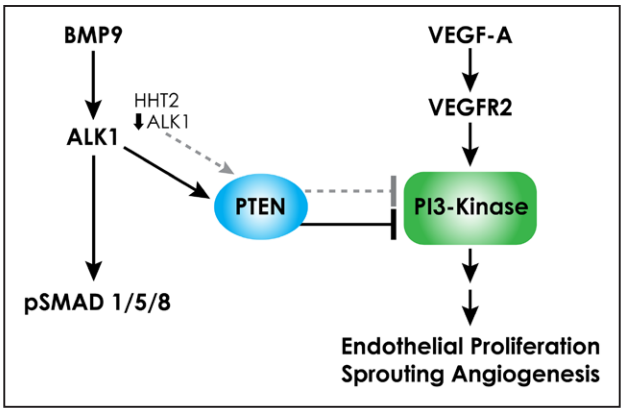

Figure. ALK-1 (activin-receptor-like kinase 1) signaling decreases PI3K (phosphatidylinositol 3-kinase) signaling and endothelial proliferation. In vitro studies show BMP-9 (bone morphogenic protein 9)/ALK-1 increases PTEN (phosphatase and tensin homolog), a phosphatase that reverses the action of PI3K. This implicates PTEN as an endogenous brake to PI3K/pAKT (phosphorylated protein kinase B) signaling, endothelial proliferation, and angiogenesis. In HHT-2 (hereditary hemorrhagic telangiectasia 2), reduced ALK-1 coincides with increased PI3K/AKT signaling (dashed gray lines), which may be caused, as in cultured endothelial cells, by reduced PTEN. VEGF indicates vascular endothelial growth factor; and VEGFR, VEGF receptor.

occur on the face and oral and nasal mucosal membranes; endangering AVMs can form in the lungs, liver, gastrointestinal tract, and brain. HHT-2 patients are more likely to have AVMs in the liver, whereas HHT-1 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 toward identifying molecular drivers of vascular malformations.[6] Increased endothelial PI3K/AKT/mTOR signaling is centerstage in venous malformations,[7–11] lymphatic malformations[12,13] and vascular tumors[14] and now the pathway seems activated in HHT-2 as well. Activating mutations in TIE-2 or mutations downstream in the PI3K catalytic domain drive venous malformations, which can be reversed in animal models with PI3K or mTOR 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 TIE-2 or PI3K overactivate pAKT whereas increased pAKT in HHT-2 seems 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.

Disclosures

None.

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