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Modeling Liver Hereditary Hemorrhagic Telangiectasia with Right Heart Overload to the Core of Liver Sinusoidal Endothelial Cells.

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Abbreviations: *ACVRL1*, *Activin A Receptor Like Type 1*; *ACVR2B*, *Activin A Receptor type 2B*; *AVMs*, *Arteriovenous Malformations*; *BMP9*, *Bone Morphogenetic Protein 9*; *BMP10*, *Bone Morphogenetic Protein 10*; *ENG*, *Endoglin*; *GDF2*, *Growth Differentiation Factor 2*; *HHT*, *Hereditary Hemorrhagic Telangiectasia*; *ID1-3*, *Inhibitor of DNA binding 1-3*; *LSECs*, *Liver Sinusoidal Endothelial Cells*; *PI3K*, *Phosphatidylinositol 3 kinase*; *PTEN*, *Phosphatase and Tensin homolog*; *TNF*, *Tumor Necrosis Factor*.

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Hereditary Hemorrhagic Telangiectasia (HHT)

HHT (a.k.a. Osler-Weber-Rendu Disease) is an inherited autosomal dominant systemic disease characterized by multiple arteriovenous malformations (AVMs) that shunt the capillary beds, leading to bleeding or right heart overload. Its incidence in North America is estimated at 1:10,000, and the most common clinical manifestations are spontaneously recurrent nose bleeds (epistaxis) and telangiectasias, that frequently appear in preadolescence. Telangiectasias are small AVMs found on the lips, buccal and gastrointestinal mucosa, face and fingers. They appear as pinpoint to pinhead-size lesions that may grow with increasing patient age. Large AVMs occur in the lungs, liver or brain and may lead to arteriovenous shunting or sudden and lethal bleeding. Diagnosis of HHT is established with \geq three clinical features: epistaxis, mucocutaneous telangiectasias in characteristic locations, visceral AVMs, and a first-degree relative diagnosed with HHT. Identification of a heterozygous mutation in *Activin A Receptor Like Type 1 (ACVRL1, alias ALK1)*, *Endoglin, (ENG)* or *SMAD4* establishes the diagnosis when clinical features are ill defined. Whereas hemorrhage is frequently the presenting manifestation of brain AVMs, lung and liver AVMs manifest as arteriovenous shunts. Liver AVMs are detected in 40% to 70% of patients, but <10% are symptomatic. Although most of the vascular architecture in HHT patients may be nearly normal in children, nose bleeds appear in over 90% of young adults and dermal and gastrointestinal telangiectasias progressively emerge later in life. Internal organ AVMs may be present at birth, usually reaching their final size in adult life. In the natural history of HHT, one wild-type allele supports the development of a nearly normal circulatory system. However, AVMs progressively develop as a consequence of second inflammatory or angiogenic hits.(1) The natural history of HHT is also influenced by individual genetic background. For example, genetic variants of

ADAM17, which regulate TNF activity, impact the severity of pulmonary HHT.(2) Although symptomatic AVMs are managed medically, liver transplantation is recommended for patients developing high-output heart failure or portal hypertension.(1)

Signaling context of the BMP9/ALK1/SMAD4 and Wnt/ β -catenin pathways in the liver

Genetic mutations causing HHT affect the TGF- β signaling pathway (Figure 1). ALK1 is a type I cell surface receptor for BMP9 (gene symbol: *GDF2*) and BMP10, while ENG is a component of the TGF- β receptor complex that modulates ligand cell surface availability. While pulmonary and brain AVMs are more common in patients with *ENG* mutations; liver AVMs are associated with *ALK1*; and combined juvenile polyposis/HHT harbor SMAD4 mutations.(1)

The main body source of BMP9 and BMP10 are hepatic stellate cells and the heart, respectively. Circulating and ENG-bound BMP9/BMP10 bind to a tetrameric signaling complex formed by two subunits of ALK1 and two subunits of the activin A receptor type 2B (ACVR2B) in LSECs, leading to phosphorylation of SMAD1/5/8, which complexes with SMAD4, translocates to the nucleus and regulates the expression of target genes downstream the BMP response elements, such as inhibitors of DNA binding (ID1-3).

Mutations in HHT have shown that the BMP9/ALK1 pathway is essential for arresting the cell cycle and activating anabolic pathways leading to endothelial cell maturation, interactions with hepatic stellate cells and development of fenestrae. Thus, in patient samples with ALK1 mutations, Phosphatidylinositol 3 kinase (PI3K) signaling is

upregulated, increasing endothelial cell proliferation. At the opposite, BMP9 blocks LSEC proliferation by inducing PTEN, that downregulates PI3K/AKT and VEGF signaling, thus stabilizing adherence junctions and blocking endothelial cell proliferation.(3) Therefore, VEGF/VEGFR2 signaling is a promising therapeutic target in HHT. Blocking VEGF with bevacizumab reduces epistaxis, gastrointestinal bleeding and improves cardiac function in liver AVMs. In mouse models, inhibition of PI3K with wortmannin decreases AVM.(2)

Also, BMP9/10 cross talk with other pathways, including p38 Mitogen-Activated Protein Kinase (MAPK), Extracellular signal-regulated kinase (ERK) and Wnt/ β -catenin.(4) Wnt and TGFB pathways crosstalk via SMAD4. Wnts are a family of soluble, short-range paracrine ligands that interact with cell surface Frizzled receptors, thereby blocking GSK3 β -dependent phosphorylation of Ser/Thr recognition sequences targeted by the ubiquitin ligase β TRCP; thus, protecting β -catenin and SMAD4 from proteasomal degradation, potentiating both pathways.(5)

Wnt/ β -catenin signaling is a master regulator of functional liver zonation, whereby metabolism(6) and diversity of LSECs(7) are distributed along a porto-central axis exposed to differential gradients of oxygen and gut-derived molecules. Endothelial cells lining the central veins secrete Wnt2 and Wnt9b, activating the expression of β -catenin target genes such as glutamine synthetase (GLUL) within one-to-two layers of pericentral hepatocytes, whereas the rest of the liver expresses the default periportal-type metabolic program revealed by specific markers like glutaminase 2 (GLS2).(6)

Animal Models of Hereditary Hemorrhagic Telangiectasia

HHT has been modeled in mice and zebrafish by deleting components of the BMP9/ALK1 axis. Total loss of ENG or ALK1 leads to embryonic lethality, with enlarged, fragile vessels in yolk sacs. ALK1^{-/-} embryos showed delayed maturation of major blood vessels, with incomplete smooth muscle, large arteriovenous connections and loss of arterial and venous identities. Later generation of heterozygous ALK1^{+/-} mice developed vascular lesions between three to 24 months, but with a relatively low penetrance (< 50%) and few AVMs, which contrasts with the 95% penetrance of the human disease. However, these models reproduced the time course of the human disease, with an onset triggered by second angiogenic or inflammatory hits. Further work showed that inducible biallelic deletion of ALK1 targeted to organ-specific capillary-venous endothelial cells resulted in AVMs that progressively appeared during mouse growth, characterized by Phosphatidylinositol 3 kinase (PI3K) activation and endothelial cell proliferation.(8)

Deletion of ALK1 in liver sinusoidal endothelial cells highlights the importance of BMP9 signaling in sinusoidal cell maturation and identity.

Using *Stab2-Cre^{tg/wt};Wls^{fl/fl}* mice, which deletes a pan Wnt ligand secretion mediator in LSECs, the authors previously demonstrated that angiocrine Wnt signals from LSECs control liver growth and metabolic liver zonation.(7) Stabilin-2 (STAB2) is a transmembrane receptor involved in angiogenesis, lymphocyte homing, cell adhesion, and scavenging of hyaluronic acid, bacteria and glycosylation end products.

In this issue of *Hepatology*,(9) the authors make a seminal contribution to the preclinical modeling of liver HHT, selectively deleting *Alk1* in LSECs, using *Stab2-Cre^{tg/wt}* mice. Despite preservation of the basic vascular liver anatomy, ultrasonography showed increased blood vessel density and contrasted computed tomography

revealed telangiectasias. No signs of portal hypertension were observed, but the hepatic veins were enlarged, with dilation of the vena cava, indicating enhanced liver blood flow and functional arteriovenous shunting, leading to right ventricle volume overload. Accordingly, doppler ultrasonography showed increased blood flow in the post hepatic vena cava, but not in the portal vein. Altogether, the clinicopathological picture corresponds to compensated right heart overload and suggests early-stage HHT, as portocaval shunts lead to overload of the efferent liver blood circuit. Therefore, challenging these mice with inflammatory stimuli could be useful to model the onset and progression of portal hypertension.

The authors combined these studies with immunohistochemical marker detection in mice and patient-derived HHT liver samples, transcriptomics of LSECs and *in vitro* ON/OFF approaches to demonstrate that ALK1-dependent perivenous endothelial cell maturation is essential for secretion of Wnt2, Wnt9b and Rspo3 and, consequently, for the expression of a perivenous metabolic program. Therefore, turning OFF *Alk1* resulted in a substantial loss of the perivenous marker Glul, with a proportional expansion of the periportal markers Hal and Glis2. Mice showed increased Apelin, Pi3k-Akt-dependent angiogenic signaling and LSEC proliferation, but no overt liver fibrosis or sinusoidal capillarization. Despite a mild extracellular matrix thickening of the Disse's space, LSEC fenestrations were preserved.

This model will be instrumental not only for preclinical therapeutic studies, but also to better understand the natural history of liver lesions in HHT. Persistent LSEC proliferation with delayed maturation raises the hypothesis that inflammatory and/or angiogenic challenges could provoke higher-grade AVMs, with further lung and right heart overload. One important point is that these mice start dying by week 35 with a steeply descending curve, and 50% of them die before week 50. This interesting finding

raises further questions: what leads to that abrupt mortality, while 80% of control mice are alive at week 55? How does the thickening of the Disse's space evolve over time, given that the expression of genes linked to angiogenesis and sinusoidal capillarization (*Apln*, *Emcn* and *Cd34*) is upregulated?

Interestingly, *Bmp9*-KO mice generated in the C57BL/6 background develop only subtle lymphatic vessel defects. However, after 10-generation backcross with 129/Ola mice, they develop sinusoidal capillarization, perisinusoidal fibrosis and loss of LSEC fenestrations.(10) Therefore, it may be interesting to manipulate mouse genetic background to model clinical variants of liver HHT upon deletion of *Alk1* in LSECs.

Finally, a recent report on *Alk1* deletion in Kupffer cells revealed that the BMP9/ALK1 pathway is essential for Kupffer cell maturation, homing to periportal liver sinusoids, endocytic ability and protection against systemic bacterial infections, which is in line with the increased susceptibility of HHT patients to *Staphylococcus aureus* infections.(11) This evidence points to the relevance of multicellular interactions in the development of a full-fledged HHT phenotype in humans and forecasts exciting developments coming from multiomics and combined cell targeting opportunities for therapeutic design.

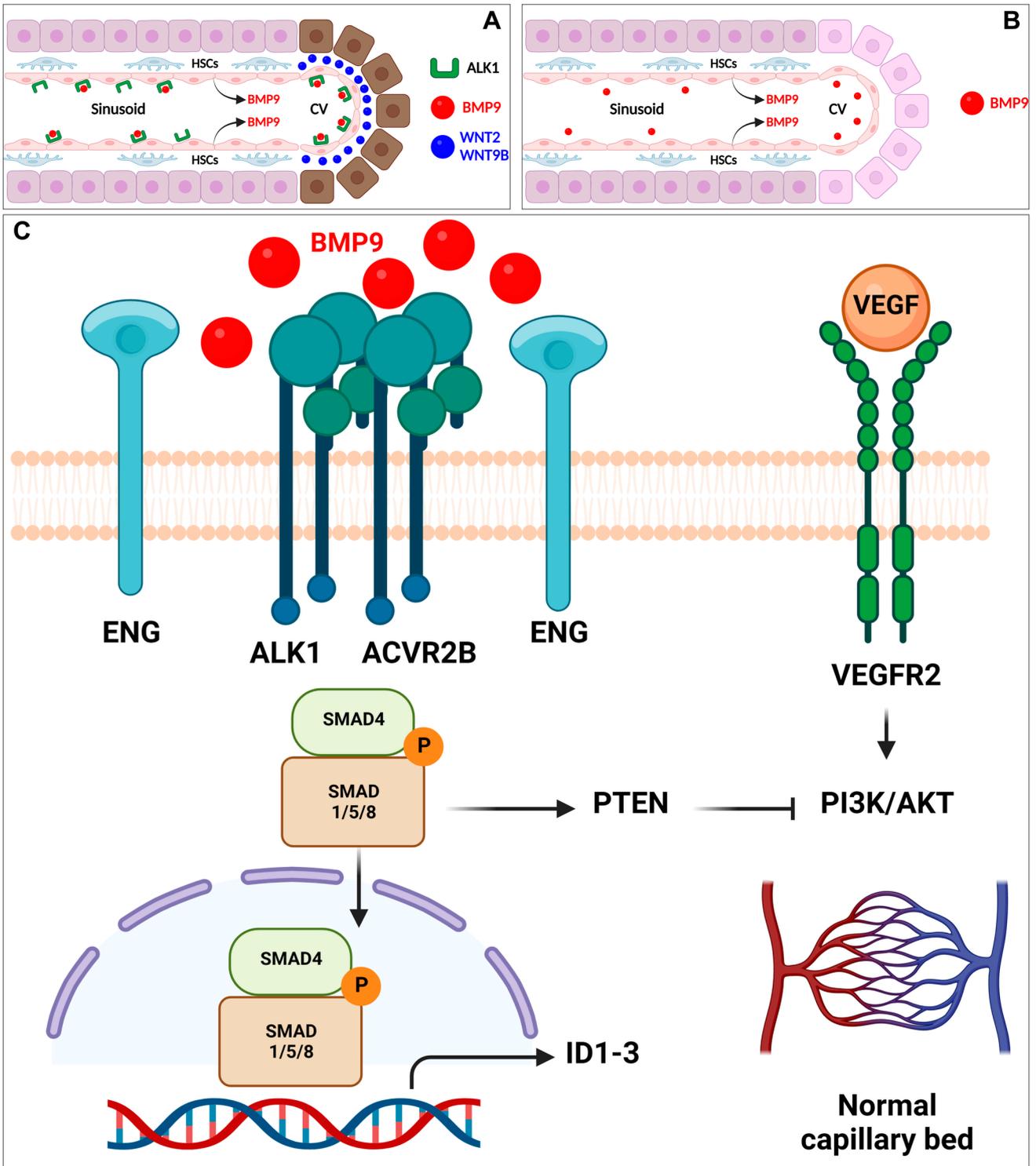
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Figure 1. The control of blood vessel maturation by ALK1 supports the metabolic identity of perivenous hepatocytes. A) In the presence of functional ALK1 receptors, BMP9 secreted by hepatic stellate cells (*HSCs*) controls sinusoidal endothelial cell maturation and hepatocyte homeostasis through secretion of WNT2 and WNT9B. These short-range signals activate β -catenin downstream transcription driving a perivenous metabolic program in pericentral hepatocytes (*brow cells*, proximal to the central vein (CV)). B) When ALK1 is absent, perivenous hepatocytes lose their metabolic identity. C) The BMP9/ALK1-ACVR2B-ENG/SMAD1/5/8-SMAD4 pathway downregulates endothelial cell proliferation and angiogenesis and controls blood vessel maturation through PTEN (Phosphatase and Tensin homolog) and ID1-3 (Inhibitor of DNA binding 1-3). Figure created with BioRender (<https://biorender.com/>).

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Musso, O., Figure 1

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