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Commentary

Blood vessels have a unified mission to circulate blood throughout the body; however, they have additional diverse and specialized roles in various organs. For example, in the liver, discontinuous sinusoids, which are fenestrated capillaries with intercellular gaps and a fragmented basement membrane, facilitate delivery of macromolecules to highly metabolic hepatocytes. During embryonic development, discontinuous sinusoids also allow circulating hematopoietic progenitor and stem cells to populate the liver and promote blood cell differentiation. In this issue of the *JCI*, Géraud et al. describe an essential role for the transcription factor GATA4 in promoting the development of discontinuous sinusoids. In the absence of liver sinusoidal GATA4, mouse embryos developed hepatic capillaries with upregulated endothelial cell junction proteins and a continuous basement membrane. These features prevented hematopoietic progenitor cells from transmigrating into the developing liver, and *Gata4*-mutant embryos died from subsequent liver hypoplasia and anemia. This study highlights the surprising and extensive transcriptional control GATA4 exercises over specialized liver vascular development and function.

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Capillary diversity

Capillaries are microvessels found throughout the body that serve as intermediate conduits for blood to travel between small arteries (arterioles) and veins (venules). Capillaries can be broadly classified as continuous or discontinuous based on morphological characteristics. Continuous capillaries are defined by dense intercellular junctional proteins, which contribute to tight or adherens junctions, and by the underlying basement membrane, which is produced in part by adjacent pericytes (1). In contrast, discontinuous capillaries have intercellular gaps due to a paucity of junctional proteins and lack an organized basement membrane (2). The distinct properties of continuous and discontinuous capillaries have functional consequences for the surrounding tissue: continuous capillaries are more refractory to the exchange of macromolecules between the blood stream and surrounding tissue, while discontinuous capillaries allow for large molecules and even transmigratory cells to be readily transported between the blood stream and tissue. For example, the tight junctions of continuous brain capillaries prevent circulating toxins from entering the underlying cerebrospinal fluid and brain tissue, while discontinuous liver capillaries facilitate transport of large molecules in the blood to underlying hepatocytes for metabolism (3). Fenestrations, or endothelial cell pores that frequently possess a thin diaphragm across their opening, can be found in both continuous and discontinuous capillaries and serve as an additional selective conduit for exchange of materials across the endothelial cell barrier (2). Importantly, fenestrations and other features of continuous and discontinuous capillaries can be influenced by developmental stage, physiological signals, or toxic insults, underscoring the plasticity of capillary morphology and function (4).

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Liver sinusoidal development and embryonic hematopoiesis

Sinusoids are fenestrated discontinuous capillaries found in the liver, spleen, and bone marrow. These three organs all have hematopoietic roles at various stages of development; therefore, sinusoids are well adapted to facilitate the movement of hematopoietic progenitor or stem cells and mature blood cells between the circulatory system and these tissues. During embryonic development, erythro-myeloid progenitors derived from the yolk sac or definitive hematopoietic stem cells generated in the yolk sac, placenta, umbilical cord, and/or aorta-gonad-mesonephros (AGM) region migrate through the circulatory system and populate the developing fetal liver (5, 6). Within the liver, hematopoietic stem cells proliferate and egress to colonize the spleen, thymus, and bone marrow, which becomes the primary site of hematopoiesis after birth (7). The fetal liver is also a key site of definitive erythropoiesis, and mature red blood cells begin exiting the liver and entering the circulatory system by E12.5 to oxygenate the rapidly growing embryo (8). Monocytes in the fetal liver also give rise to a subset of tissue macrophages that require diaphragmed fenestrations in sinusoidal endothelium in order to exit the fetal liver and populate tissues throughout the body (9). Interestingly, sinusoidal morphology changes during rodent development: by late gestation, junctional proteins are downregulated and fenestrations lose diaphragms and increase in size and number (10). The implications of these alterations in sinusoidal morphology on fetal liver function are poorly understood.

In this issue, Géraud et al. provide important insight into the transcriptional regulation of discontinuous liver sinusoid morphology (11). This group had previously determined that the transcription factor GATA4 is enriched in rat liver sinusoidal endothelial cells (LSECs) compared with rat lung microvascular endothelial cells (LMECs) (12). Géraud and colleagues have now employed transgenic mouse lines to define the function of GATA4 in murine

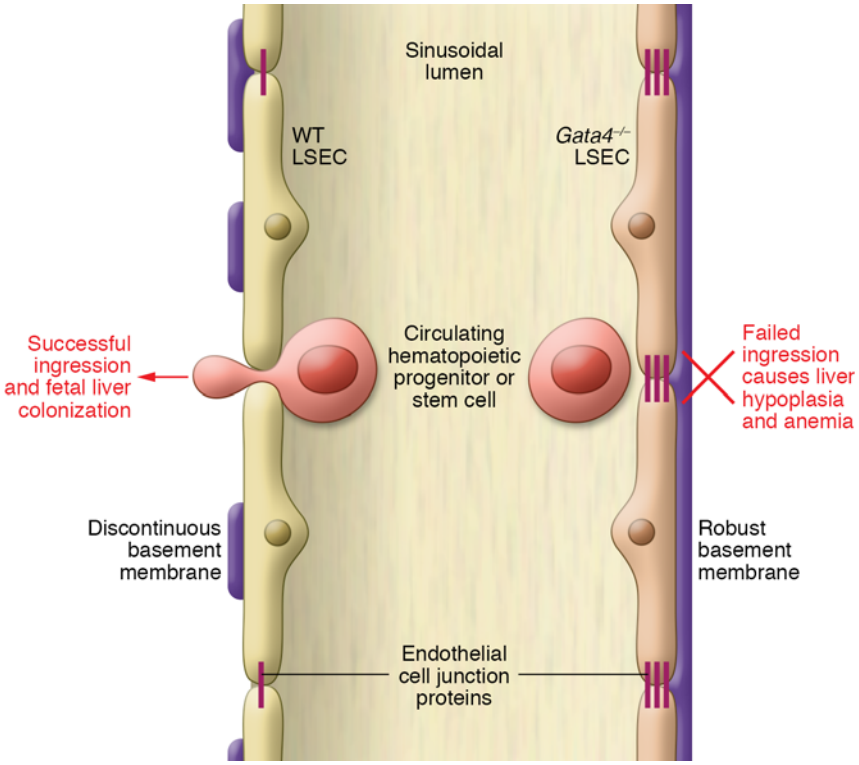

Figure 1. *Gata4* deletion alters liver sinusoid morphology. Genetic deletion of the transcription factor *Gata4* from liver sinusoidal endothelial cells (LSECs) causes upregulation of endothelial cell junction proteins and robust deposition of basement membrane proteins that prevent circulating hematopoietic progenitor or stem cells from colonizing the fetal liver (11). The consequences of this transition from discontinuous sinusoidal to continuous capillary morphology are liver hypoplasia, anemia, and lethality of *Gata4* mutant embryos (11).

LSECs (11). Specifically, they developed a line of mice harboring *Cre* driven by the stabilin 2 (*Stab2*) promoter (*Stab2-Cre*), which is expressed in mature LSECs (11), and exploited an existing *Lyve1-Cre* line (13), which is active in embryonic LSECs (14). Deletion of a floxed *Gata4* allele in either line resulted in mutant embryos with hypoplastic livers, anemia, and prenatal lethality (11). Unlike the liver, other organs were not obviously affected by *Gata4* deletion in either the *Stab2-Cre* or *Lyve1-Cre* line (11). GATA4-deficient livers exhibited microvessels with features of continuous capillaries rather than those of discontinuous sinusoids seen in WT livers (11). In particular, upregulation of endothelial cell junction proteins (CD31 and VE-cadherin) on LSECs was observed by immunostaining, and a more robust basement membrane was detected by electron microscopy and by immunostaining for extracellular matrix components (11). No effect of *Gata4* deletion on LSEC fenestrae was reported, but it would be interesting to know whether this transcription factor affects the number or size of fenestrae or the presence of a diaphragm on the pores. As LSECs are highly endocytic (15), it would also be informative to know whether GATA4 impacts endocytic vesicle density on embryonic LSECs.

Géraud et al. also exploited primary rat LSECs and LMECs to establish a transcriptional profile for discontinuous and continuous capillaries, respectively (11). As rodent LSECs rapidly de-differentiate in culture, precluding knockdown or overexpression studies, Géraud and colleagues manipulated GATA4 expression in human umbilical vein endothelial cells (HUVECs), which are transcriptionally similar to continuous LMECs (11). Upon GATA4 overexpression, HUVECs acquired a transcriptional profile more similar to discontinuous LSECs, indicating that GATA4 can promote a discontinuous LSEC gene program across species and in different endothelial cell types (11).

Finally, Géraud et al. reported that the emergence of continuous capillary features in *Gata4* mutant livers preceded liver hypoplasia and anemia (11). Erythro-myeloid progenitor cells that typically colonize the fetal liver from the circulation around E10.5 were markedly reduced in livers but elevated in the blood of *Gata4* mutants at E11.25 (11). Hematopoietic stem cell populations followed a similar aberrant localization pattern in the liver and blood of E13.25 *Gata4* mutants (11). These progenitor and hematopoietic stem cells from mutant embryos appeared to have no intrinsic defects, as these populations expanded and differentiated in transplantation experiments and in vitro (11). Instead, Géraud and colleagues propose that the continuous capillaries that aberrantly displace discontinuous sinusoids in *Gata4* mutant livers prevent progenitor and hematopoietic stem cells from colonizing the mutant liver, where they would subsequently undergo expansion and differentiation (Figure 1).

Outstanding questions

Because the capillarization process (i.e., transformation from discontinuous sinusoids to continuous capillaries) described in these *Gata4* mutant embryos encompasses both upregulation of endothelial cell junction proteins and deposition of basement membrane, one might question whether one or both of these phenotypes is essential for blocking fetal liver colonization. Murine embryos in which endothelial cell junctions are artificially strengthened by genetically replacing VE-cadherin with a VE-cadherin–α-catenin fusion construct have phenotypes that are strikingly similar to those of the *Gata4* mutants described by Géraud et al., including hypoplastic livers, anemia, and failed transmigration of hematopoietic stem and progenitor cells from the circulation into the liver

(16). Therefore, strong and/or upregulated endothelial cell junctions are clearly a sufficient impediment for fetal liver colonization. Interestingly, embryos in which *Gata4* is deleted from embryonic hepatic mesenchymal cells develop hyperactive stellate cells and produce excessive extracellular matrix around the hepatic vasculature before dying at E13.5 with hypoplastic livers and anemia (17). Although the Delgado et al. study did not assess fetal liver colonization by hematopoietic stem and progenitor cells, the reported phenotypes suggest that excessive sinusoidal basement membrane may also be sufficient to block this process. Nevertheless, it is not clear why hematopoietic stem and progenitor cells preferentially colonize the bone marrow and spleen later in development, despite an increase in open pore fenestrae, a downregulation of endothelial cell junctional proteins, and a persistently discontinuous basement membrane that would presumably favor continued transmigration through the hepatic sinusoids (10).

Another interesting question is whether GATA4 is important for the maintenance of discontinuous liver sinusoids once they are established, as Géraud and colleagues demonstrated that GATA4 is expressed in adult murine, rat, and human LSECs (11). An inducible *Stab2-Cre* or *Lyve1-Cre* line would help address the temporal influence of GATA4 over discontinuous hepatic sinusoidal characteristics. Such lines could also clarify whether GATA4 and discontinuous sinusoids are required for differentiated blood cells to egress from the fetal liver and reenter the circulation. Macrophage precursors require diaphragms on LSEC fenestrae for this egression process (9), but a robust basement membrane may serve as a critical impediment, even in the presence of diaphragmed fenestrae. An inducible *Stab2-Cre* line might also help clarify whether GATA4 promotes discontinuous sinusoidal development in other organs, such as the bone marrow and spleen, which undergo colonization by hematopoietic stem cells later in development (7). Géraud and colleagues report that GATA4 is not expressed on STAB2-positive endothelial cells in adult bone marrow and spleen (11), but whether it contributes to the initial development of those sinusoids is an open question. If GATA4 does not contribute to discontinuous sinusoidal development in these hematopoietic organs, identifying other transcription factors that act in lieu of GATA4 will clarify the organ-specific derivation of sinusoidal vessels.

Capillarization of hepatic sinusoids is associated with advanced liver fibrosis (18); therefore, the question of whether GATA4 transcriptionally maintains discontinuous hepatic sinusoid morphology has important clinical implications. Human patients with cirrhosis and advanced liver fibrosis have diminished hepatic GATA4 protein levels compared with patients with healthy livers or early-stage disease (17), although it in unclear whether GATA4 levels are altered specifically in the LSECs of these patients. Would upregulation of GATA4 in LSECs of cirrhotic livers reverse the capillarization process and associated fibrosis? Genetic rodent models could be designed to address this question. In vivo vascular GATA4 overexpression would also validate and provide functional context for the finding by Géraud et al. that cultured endothelial cell lines with continuous characteristics (HUVECs and bEnd3 cells) assume a transcriptional profile more consistent with discontinuous LSECs when GATA4 is overexpressed in vitro (11). Altogether, the new evidence provided by Géraud and colleagues about the importance of GATA4 in establishing discontinuous hepatic sinusoidal morphology during mid-gestation raises interesting follow-up questions about the extent to which GATA4 exercises temporal and spatial influence over discontinuous capillary morphology throughout the lifetime of an organism.

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