COMMENTARY

Ironing out pulmonary arterial hypertension

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Iron and oxygen are essential to aerobic organisms, and their biologic functions are intertwined. For example, iron as a component of hemoglobin is required for oxygen transport to tissues, which is critical for energy generation. However, excess iron in combination with oxygen can generate toxic free radicals that damage cellular components. It is therefore not surprising that iron and oxygen homeostasis are both tightly controlled and closely interwoven (reviewed in refs. 1 and 2). Emerging literature suggests an intriguing link between iron and oxygen in the regulation of pulmonary vascular function. Hypoxia is well described to cause pulmonary vascular constriction as a mechanism to match blood flow with ventilation. Studies have also demonstrated that systemic iron deficiency exaggerates, whereas iron supplementation ameliorates, the pulmonary vasoconstriction response to hypoxia (3–5). Moreover, iron deficiency is highly prevalent in patients with pulmonary arterial hypertension (PAH) (6–9), a heterogeneous disease characterized by elevated pulmonary artery pressure with concomitant elevated pulmonary vascular resistance that can be idiopathic, heritable, or associated with drugs, infections, connective tissue disease, congenital heart disease, or other causes (reviewed in ref. 10). If left untreated, PAH leads to progressive right heart failure and can ultimately be fatal. Iron deficiency in PAH patients is associated with greater disease severity and worsening survival, and intriguingly, iron supplementation has been shown to improve exercise capacity and quality of life in small clinical studies (6, 8, 9, 11, 12). However, it remains unclear how systemic iron deficiency impacts pulmonary vascular function and influences PAH. A study in PNAS by Lakhal-Littleton et al. (13) provides mechanistic insights into local iron regulation in the lung vasculature and how iron deficiency specifically in pulmonary artery smooth muscle cells (PASMCs) may contribute to the pathogenesis of PAH.

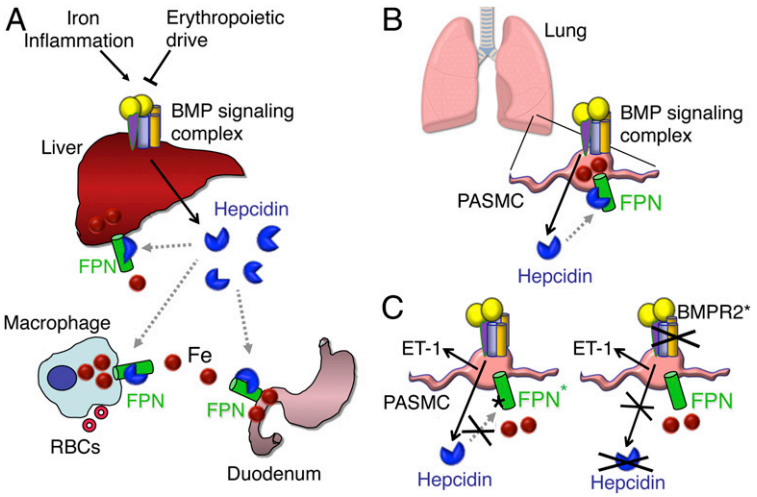

Fig. 1. Schematic diagram depicting the role of the hepcidin/ferroportin axis in systemic iron homeostasis and local iron homeostasis in PASMCs. (*A*) Systemic iron homeostasis. The liver produces hepcidin in response to stimulatory signals from iron and inflammation and to inhibitory signals from erythropoietic drive via the BMP signaling pathway to govern systemic iron homeostasis. Hepcidin binding induces ferroportin (FPN) degradation on duodenal enterocytes, iron-recycling macrophages, and hepatocytes to block iron (Fe) release into the bloodstream from dietary sources and body stores. (*B* and *C*) Proposed model by Lakhal-Littleton et al. (13) for local iron homeostasis in PASMCs (*B*) and how abnormalities in the PASMC hepcidin/ferroportin axis lead to PAH (*C*). (*B*) PASMCs produce hepcidin in response to BMP signals. Hepcidin has an autocrine effect to degrade ferroportin, block iron release, and maintain adequate intracellular iron levels. (*C*) Mutations in ferroportin that block hepcidin binding (*Left*) or mutations in BMP receptor type 2 (BMPR2) associated with PAH that block hepcidin production (*Right*) lead to unregulated ferroportin activity and intracellular iron deficiency in PASMCs, resulting in increased production of the vasoconstrictor ET-1.

At a systemic level, iron is provided by absorption from the diet, by recycling from aged red blood cells, and by release from body stores. Iron entry into the bloodstream from all of these sites is controlled by the iron exporter ferroportin whose expression and function is governed by the iron hormone hepcidin (reviewed in ref. 14) (Fig. 1*A*). Mechanistically, hepcidin binding directly inhibits ferroportin's iron export capacity and induces ferroportin internalization and

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Author contributions: J.L.B. wrote the paper.

Conflict of interest statement: J.L.B. has ownership interest in Ferrumax Pharmaceuticals and has received consulting fees from Keryx Biopharmaceuticals and Disc Medicine.

See companion article on page 13122.

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Published online June 6, 2019.

www.pnas.org/cgi/doi/10.1073/pnas.1908298116

degradation. Circulating hepcidin predominantly originates from hepatocytes, where its production is regulated by a number of systemic cues to titrate iron availability to body needs (Fig. 1*A*). For example, iron loading induces hepatic hepcidin expression, thereby down-regulating ferroportin to prevent iron overload. Hepatic hepcidin is also induced by inflammation to limit iron availability to microbial pathogens. In contrast, hepatic hepcidin is inhibited by anemia and hypoxia to boost the iron supply for erythropoiesis.

Recent evidence has demonstrated that ferroportin also plays a critical role in cellular iron homeostasis in tissues with no known role in systemic iron homeostasis. For example, a conditional knockout of ferroportin in cardiomyocytes led to fatal cardiac iron overload in mice (15). Intriguingly, there is also emerging evidence that extrahepatic hepcidin production can regulate ferroportin expression in an autocrine fashion to exert local control of iron homeostasis in some tissues. Indeed, a conditional knockout of hepcidin or a conditional knockin of a hepcidin-resistant ferroportin specifically in cardiomyocytes resulted in cardiomyocyte iron deficiency and fatal cardiac metabolic and contractile dysfunction despite normal systemic iron homeostasis (16).

In their PNAS study, Lakhal-Littleton et al. (13) now extend these findings to PASMCs. They demonstrate that an inducible, conditional knockin of a hepcidin-resistant ferroportin in smooth-muscle cells of mice leads to a marked up-regulation of ferroportin in PASMCs, but not in other vasculature beds examined. This results in iron deficiency specifically in PASMCs with otherwise normal systemic iron homeostasis parameters. In vitro assays confirmed that isolated PASMCs from these mice are indeed resistant to ferroportin down-regulation by exogenously added hepcidin. Moreover, isolated PASMCs from wild-type mice and humans produce and secrete hepcidin, and small interfering RNA knockdown of endogenous hepcidin production increases endogenous ferroportin levels and reduces cellular iron content in PASMC cultures. Together, these data confirm an important functional role for ferroportin in controlling cellular iron content in PASMCs in vivo. These data also suggest that hepcidin production in PASMCs may exert a cell-autonomous effect to regulate ferroportin for local iron control (Fig. 1*B*), which would be the second such example of local iron homeostasis regulation by the hepcidin/ferroportin axis; however, further studies are needed to confirm a functional role for PASMC hepcidin production in vivo.

Intriguingly, Lakhal-Littleton et al. (13) find that their ferroportin mutant mice developed clinical features of PAH and right heart failure. These findings could be prevented and at least partially reversed by intravenous (i.v.) iron supplementation, consistent with a pathogenic role for iron deficiency specifically in PASMCs in inducing PAH in vivo. The authors also show that expression of the vasoconstrictor endothelin-1 (ET-1), which is known to play a role in the pathogenesis of PAH (10), is increased in the pulmonary artery smooth muscle layer and in isolated PASMCs from their mutant mice, and this increase could be reversed by iron supplementation. ET-1 is also increased in the lungs of wild-type mice fed an iron-deficient diet and in isolated PASMCs treated with an iron chelator, whereas iron lowers ET-1 expression. Moreover, i.v. iron is able to prevent ET-1 induction by hypoxia in normal human volunteers. Importantly, administration of an endothelin subtype-A receptor antagonist prevents and at least partially reverses the findings of PAH and right ventricular failure in their mutant mice. These data suggest that up-regulation of ET-1 is the molecular link between iron deficiency and PAH in their mice (Fig. 1*C*) and that reduction in ET-1 expression may contribute to beneficial effects of iron supplementation reported in human patients with PAH (11, 12). Although Lakhal-Littleton et al. (13) do not investigate how iron deficiency up-regulates ET-1 expression, ET1 was previously demonstrated to be regulated by hypoxia-inducible factors (17), transcription factors that are active under iron-deficient and hypoxic conditions but targeted for degradation by iron- and oxygen-dependent prolyl hydroxylases under iron- and oxygen-sufficient conditions (1, 2).

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Finally, Lakhal-Littleton et al. (13) explore how these findings may be relevant to patients with hereditable PAH, which is most commonly due to mutations in *BMPR2*, encoding bone morphogenetic protein (BMP) receptor type 2 (18). Notably, BMP signaling is a key transcriptional regulator of hepcidin expression in hepatocytes to control systemic iron homeostasis (14) (Fig. 1*A*). Lakhal-Littleton et al. (13) show that endogenous hepcidin expression is also regulated by BMP6 ligand in isolated PASMCs and that expression of both basal and BMP6-induced hepcidin is reduced in PASMCs harboring a *BMPR2* mutation associated with PAH. Moreover, lower hepcidin expression is associated with increased ferroportin and ET-1 expression in *BMPR2* mutant PASMCs compared with control PASMCs, and ET-1 expression can be reduced in *BMPR2* mutant PASMCs by exogenous hepcidin or iron supplementation. Together, these data suggest that one mechanism by which *BMPR2* mutations may cause PAH is by blunting PASMC hepcidin production, thereby increasing ferroportin expression and lowering intracellular iron levels specifically in PASMCs, ultimately causing an increase in ET-1 production (Fig. 1*C*).

There are several reasons why these results are important. First, they expand the growing evidence of a physiological role for the hepcidin/ferroportin axis in governing the local iron economy in addition to their well-described role in systemic iron homeostasis. Second, they provide in vivo evidence of a causal role for and mechanistic insight into how iron deficiency contributes to the pathophysiology of PAH, offering a biologic basis for iron supplementation therapy that has shown benefit in some small clinical studies. Finally, they provide a model for how *BMPR2* mutations may contribute to familial PAH by interfering with the local hepcidin/ferroportin axis and cellular iron homeostasis in PASMCs. However, there are several open questions that require further study. Does PASMC hepcidin regulate ferroportin expression in a cell-autonomous fashion in vivo? Are there other tissues in which the hepcidin/ferroportin axis plays a role in local iron homeostasis? How is local iron regulation by the hepcidin/ferroportin axis influenced by systemic iron homeostasis, and what are the means of crosstalk between these pathways? Are there cell contextual differences in the mechanisms of hepcidin regulation in PASMCs, other extrahepatic tissues, and hepatocytes? Importantly, prospective randomized controlled trials are needed to establish whether iron supplementation is truly beneficial for PAH patients and, if so, which patients may benefit most.

Acknowledgments

J.L.B. is supported by the NIH grant R01-DK087727.

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