Iron Metabolism and Vascular Remodeling: Novel Insights Provided by Transferrin-1 Receptor Depletion in Mice With Pulmonary Hypertension

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In this issue of the American Journal of Hypertension, Naito *et al.*[1] report novel evidence that heterozygous mice deficient in the transferrin-1 receptor (TfR1) show protection against the development of hypoxia-induced pulmonary hypertension and vascular remodeling associated with this disease process. The depletion of TfR1 by siRNA also attenuated the proliferation of human pulmonary artery smooth muscle cells promoted by culturing with platelet-derived growth factor-BB. These observations provide new insight for identifying how an apparent iron deficiency and/or a disruption in aspects of iron metabolism participate in the development and expression of pulmonary hypertension seen in various forms of the human disease and animal disease models.[2–6]

This group has previously reported evidence for increased TfR1 in the monocrotaline rat model of pulmonary hypertension.[6] It has also been observed that circulating soluble transferrin receptor levels are increased in patients with idiopathic pulmonary arterial hypertension.[2] While most cells are thought to regulate iron uptake though TfR1 expression,[7,8] very little is known about how this system and iron metabolism are functioning in the various cell types that contribute to pulmonary hypertension. Increased TfR1 expression is associated with an iron deficiency in cells, and antibodies for TfR1 have been used for targeting cancer cells.[9] There are distinct similarities between cancer cells and changes in pulmonary arterial smooth muscle cells associated with pulmonary arterial hypertension.[10] Data in the study by Naito *et al.*[1] demonstrate that a deficiency in TfR1 attenuates the growth of pulmonary arterial smooth muscle cells *in vitro* and pulmonary arterial remodeling in the *in vivo* chronic hypoxia mouse model studied. Thus, the TfR1 receptor and/or its influence on cellular iron availability could have an important role in controlling processes contributing to pulmonary arterial smooth muscle remodeling in various forms of pulmonary hypertension.

Several processes important in pulmonary hypertension development could potentially originate from an iron deficiency. The stabilization or increased expression of hypoxia-inducible factor (HIF) is an important aspect of pulmonary hypertension development which could be promoted by an iron deficiency or alterations in cellular iron metabolism.[3] There is evidence for enhanced heme-deficient soluble guanylate cyclase (sGC) activity based on the therapeutic action of a sGC activator,[11] which preferentially binds the heme site of this enzyme when the heme of sGC is oxidized and/or no longer bound to sGC. There is also evidence in pulmonary hypertension for a depletion of subunit 4 of the mitochondrial electron transport chain protein cytochrome oxidase,[12] which is a very sensitive cellular indicator protein for depletion when there is a heme deficiency.[13] Our lab has also detected evidence for the pulmonary hypertension mediator endothelin-1 causing an accumulation of protoporphyrin IX in a manner, which could reflect a deficiency in the availability of iron for the biosynthesis of heme by the mitochondrial ferrochelatase enzyme.[14] The accumulation of protoporphyrin IX is a property of cancer cells which is used for diagnostic detection of these cells.[15] Interestingly, the detection of increased zinc protoporphyrin IX in blood of humans with idiopathic pulmonary hypertension suggests that there is an iron deficiency or impairment of iron utilization for heme biosynthesis in hematopoietic cells.[16] This is because ferrochelatase uses zinc when an adequate amount of mitochondrial iron is not available for heme biosynthesis. Our studies on pulmonary arteries isolated from the chronic hypoxia model for pulmonary hypertension detected increased ferrochelatase activity.[14] While this could result from a HIF-promoted increase in expression of this enzyme, the iron-sulfur cluster dependence of the stability and activity of ferrochelatase[17] suggests that a deficiency of iron availability for these clusters was not detected. Thus,

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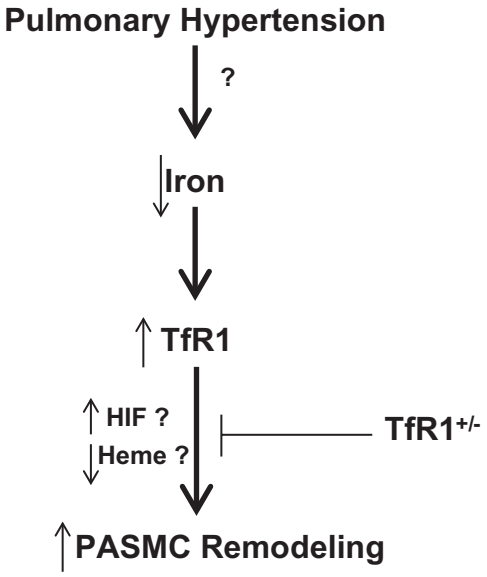

Figure 1. Model hypothesizing how an iron deficiency associated with pulmonary hypertension could promote pulmonary vascular remodeling through increasing the expression of transferrin Receptor 1 (TfR1). While increased hypoxia-inducible factors (HIF) and impaired heme biosynthesis are iron (Fe) regulated processes occurring in pulmonary hypertension, the direct influence of TfR1 expression on these processes, or iron metabolism in pulmonary arterial smooth muscle cells under conditions relevant to pulmonary hypertension have not yet been established.

some of the processes discussed above and others dependent on the availability of iron could contribute to pulmonary hypertension development.

The mechanism through which TfR1 is influencing pulmonary hypertension is not known. Since TfR1 normally functions through increasing cellular iron uptake, its primary action in enabling pulmonary arterial smooth muscle growth might not originate from creating an actual iron deficiency in these cells. While there is evidence that a deficiency in iron bound to iron regulatory proteins functioning to promote the expression of TfR1 and other proteins contributing to intracellular iron transport,[7,8] essentially all aspects of the actual consequences on TfR1 on cellular iron metabolism and processes regulated by iron or the TfR1 relevant to systems influencing pulmonary hypertension development remain to be defined. For example, modulation of a mishandling of iron by TfR1 expression could be a more important factor than modulation of an actual cellular iron deficiency. Therapies delivering iron or creating an iron deficiency clearly influence the progression of pulmonary hypertension.[4–6] However, it is not known if pulmonary hypertension creates an iron deficiency or if an iron deficiency associated with conditions leading to pulmonary hypertension enhances the development of this disease. Diseases that promote the development of pulmonary hypertension such as heart failure and chronic obstructive pulmonary disease show a high incidence of iron deficiency, but, the role of this deficiency in pulmonary hypertension development is not well defined.[18,19] Humans documented to have idiopathic pulmonary arterial hypertension associated with a deficiency in iron appeared to benefit when treated with supplemental intravenous iron.[5] In this study, the effects on skeletal muscle oxygen utilization appear to be most obvious beneficial effect in exercise testing examined.[5] The results of the study by Naito *et al.*[1] suggest that 1 potentially beneficial effect of iron supplementation in pulmonary hypertension associated with an iron deficiency could be suppressing the increased expression of TfR1 and pathophysiological processes stimulated by its increased expression through relationships that are shown in Figure 1.

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DISCLOSURE

M.S.W. is an Inventor on a patent held by New York Medical College for targeting protoporphyrin IX for smooth muscle relaxation. There are no other disclosures or conflicts of interest for the authors.

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