Novel roles of HIF-PHIs in chronic kidney disease: the link between iron metabolism, kidney function, and FGF23

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Abstract

Hanudel et al. investigated the effects of hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) on iron metabolism in a chronic kidney disease (CKD) mouse model and showed that vadadustat, an HIF-PHI, exerted beneficial effects on anemia and iron disorders independently of erythroferrone. Vadadustat also inhibited the progression of CKD and the CKD-associated increase of plasma fibroblast growth factor 23 in CKD mice. This study provides new insights into the action of HIF-PIHs in CKD.

Keywords: HIF-PHI, CKD, anemia, iron, erythroferrone, FGF23

Anemia is the most common complication of chronic kidney disease (CKD). Both erythropoietin (EPO) and iron are essential for erythropoiesis. Iron is an essential part of hemoglobin involved in oxygen binding and transport, and EPO is required for the survival, proliferation and differentiation of erythroid precursor cells. CKD-associated anemia is induced by multiple factors; the main causes are EPO deficiency and absolute or functional iron deficiency due to increased hepcidin levels.[1] Both erythropoiesis and iron homeostasis are disturbed in CKD, and erythropoiesis-stimulating agents (ESAs) and iron supplements are commonly used for the management of anemia in CKD worldwide.

Hepcidin, a hepatocyte-derived hormone, is a key regulator of body iron homeostasis. More specifically, hepcidin reduces iron efflux from intracellular iron by binding to ferroportin (FPN), a cellular iron exporter, and induces its internalization and degradation. Increased hepcidin levels may impair the mobilization of stored iron and iron absorption from the small intestine owing to degradation of FPN, thereby disturbing of iron metabolism in patients with CKD. Hepcidin production is homeostatically regulated by iron levels. Anemia-induced renal hypoxia augments EPO production, and increased erythropoiesis reduces serum and tissue iron concentration owing to a greater demand for iron to produce new red blood cells, thereby suppressing hepcidin expression. However, hepcidin suppression in response to anemia was not observed in a mouse model with impaired erythrocyte development, suggesting a role of an unknown factor that suppresses hepcidin derived from erythrocytes.

Subsequently erythroferrone (ERFE), derived from erythroblasts in the bone marrow and spleen, was recently identified as a new mediator that suppresses hepcidin

production.[2] Both endogenous and exogenous EPO enhance the production and secretion of ERFE from erythroblasts, and increased levels of circulating ERFE act directly on hepatocytes to suppress hepcidin production, thereby increasing the availability of stored iron from macrophages and hepatocytes as well as dietary iron absorption from enterocytes. Thus, ERFE is a new player in the link between erythropoiesis and iron metabolism, and it may modulate iron homeostasis in CKD as well by inhibiting liver hepcidin production. Several studies have shown the association between ERFE and iron metabolism markers, such as hepcidin, ferritin, or serum iron, in patients with CKD[3]; however, to date, the role of ERFE in the anemia of CKD remains poorly characterized.

Hypoxia-inducible factor (HIF) is a key transcription regulator of erythropoiesis that acts via EPO production. HIF-α forms a heterodimer with HIF-β to activate target gene transcription. Under hypoxic conditions, HIF-prolyl hydroxylation is reduced, and HIF is no longer degraded, thereby activating the target genes through translocation to the nucleus. Under normoxic conditions, HIF is targeted for rapid degradation via hydroxylation of specific proline residues by the von Hippel-Lindau (VHL) tumor suppressor protein, which acts as a substrate of E3 ubiquitin ligase. Prolyl hydroxylase domain (PHD)-mediated proteasomal degradation of HIFs by the pVHL-E3 ubiquitin ligase also involves HIF-α prolyl-4-hydroxylation by oxygen- and iron-dependent PHD dioxygenase. Therefore, pharmacological activation of the HIF pathway, and the subsequent increase in endogenous EPO production, constituted a therapeutic target for treatment of anemia in CKD. In addition to ESA, HIF-PHD inhibitors (HIF-PHIs) have been developed, and several PHD-PHIs have been recently approved in

several countries for the treatment of CKD-associated anemia.

However, in CKD, the effects of HIF-PHIs on iron metabolism via the EPO-ERFE-hepcidin axis remains unclear. Regarding this issue, Hanudel et al. have recently report in this issue that vadadustat, an HIF-PHI, ameliorates CKD-associated anemia independently of ERFE.[4] In that study, a mouse model of adenine-induced CKD with ERFE gene knockout (ERFE KO) was exposed to vadadustat treatment and results were compared with CKD wild-type (WT) mice. Vadadustat ameliorated microcytic hypochromic anemia in both WT and ERFE KO mice with CKD to the same degree. Regarding the parameters of iron metabolism, vadadustat augmented hepatic EPO mRNA expression and serum levels of EPO in both WT and ERFE KO mice with CKD. Increased hepcidin mRNA expression in the liver of ERFE KO mice with CKD was reduced by vadadustat treatment, and no difference was noted in hepatic mRNA expression of hepcidin among non-CKD, CKD, and vadadustat-treated CKD in WT mice, respectively. In contrast, serum hepcidin levels was increased in CKD mice as compared to non-CKD mice, and vadadustat diminished the increase in serum hepcidin levels in both WT and ERFE KO mice with CKD. Vadadustat also increased the expression of FPN at both mRNA and protein levels in the duodenum. Moreover, the increased hepatic and splenic iron levels, as well as the reduced serum iron levels, were improved by vadadustat in both WT and ERFE KO mice with CKD. In addition to EPO production via HIF activation, vadadustat corrected iron dysregulation by ameliorating the impairment of dietary iron absorption and stored iron mobilization through the hepcidin-FPN axis in CKD. Surprisingly, same vadadustat action was also observed in ERFE KO mice with CKD,

providing new insights into the effect of vadadustat on iron metabolism.

As described above, EPO-mediated ERFE production in erythroblasts is necessary for suppressing hepcidin production. However, vadadustat suppressed CKD-induced hepcidin production in both WT and ERFE KO mice, indicating that vadadustat inhibits hepcidin in an ERFE-independent manner. Serum EPO levels correlated with ERFE mRNA expression in the bone marrow and spleen of non-CKD and CKD WT mice; however, no significant differences in serum ERFE levels were observed among non-CKD, CKD, and vadadustat-treated CKD WT mice. No difference in serum ERFE levels was seen between CKD patients and non-CKD patients, and it was significantly increased in patients with dialysis CKD.[3] However, to date, no clinical studies have been conducted to examine the effect of HIF-PHIs on serum ERFE levels in CKD.

The mechanism of action of vadadustat on the link between the hepcidin-FPN pathway and iron metabolism was investigated. Vadadustat-mediated HIF stabilization might directly or indirectly suppress hepcidin expression via transcriptional regulation or dependency of EPO. Furthermore, vadadustat reduced the increased hepatic iron content in CKD, lowering hepcidin production. However, the mechanism of action of vadadustat has remained unresolved, and further studies are necessary to address the role of vadadustat in hepcidin regulation in CKD. Moreover, HIF-2α promotes iron absorption by transcriptionally regulating the expression of duodenal iron importers (duodenal cytochrome B [Dcytb] and divalent metal transporter 1 [DMT1]) and exporter (FPN) because these iron transporters serve as target genes of HIF-2α.[5] Therefore, vadadustat-induced HIF activation might directly ameliorate the status of reduced iron absorption by

increasing the expression of iron transporters in the duodenum.

In the present study, vadadustat suppressed fibroblast growth factor 23 (FGF23) and improved kidney function in mice with CKD. Molidustat, another HIF-PHI, also suppressed the increase in plasma intact FGF23 levels and attenuated kidney disease progression in adenine-induced CKD mice.[6] Thus, HIF-PHIs might be promising therapeutic candidates owing to their additional effects on improving CKD-mineral and bone disorder (CKD-MBD) and CKD progression. HIF activation is thought to exert beneficial effects on CKD by increasing EPO production or ameliorating anemia; however, the precise mechanism is still unclear. Additionally, Yu et al. reported that the PHD inhibitor L-mimosine exerted dual action on CKD progression in a rat model of subtotal nephrectomy. Mid-term administration of L-mimosine inhibited renal fibrosis, and macrophage infiltration and CKD progression; however, the long-term administration of L-mimosine worsened all these parameters.[7] Therefore, the protective effect of HIF activation against CKD progression might depend on the timing of HIF-PHI administration. In contrast, a recent study reported that in patients with CKD at stage 3-5 receiving either roxadustat or placebo, there was no significant between-group difference in progression of CKD, as measured by the rate of change in eGFR over time.[8]

FGF23 is mainly produced in osteocytes to regulate phosphate homeostasis. Plasma levels of FGF23 are elevated in patients with CKD, which is an independent risk factor for end-stage renal disease and cardiovascular mortality. HIF activation, as well as EPO and iron deficiency, can increase FGF23 mRNA transcription with increased post-transcriptional cleavage.[9] Vadadustat increased plasma total and intact FGF23 levels in

non-CKD mice. In contrast, in CKD mice, vadadustat diminished the elevated plasma levels of total and intact FGF23, which is inconsistent with previous findings. According to the authors, vadadustat reduced plasma FGF23 levels in the CKD model owing to the amelioration of kidney function and impaired iron utilization. However, the mechanism by which HIF-PHIs affect FGF23 regulation in CKD still needs to be clarified.

Overall, the study by Hanudel et al provides new information on the effects of HIF-PHIs in improving the anemia of CKD, which could be useful in current clinical practice. Furthermore, despite its experimental nature, the study provides future directions for research on the action of HIF-PHIs in patients with CKD.

Figure legend

Figure 1. Possible pleiotropic effects of hypoxia-inducible factor (HIF)-prolyl hydroxylase domain (PHD) inhibitors (HIF-PHIs) on iron utilization, chronic kidney disease (CKD) progression, and CKD-associated mineral and bone disorder (MBD) in addition to their well-known enhancement of erythropoiesis through HIF activation.

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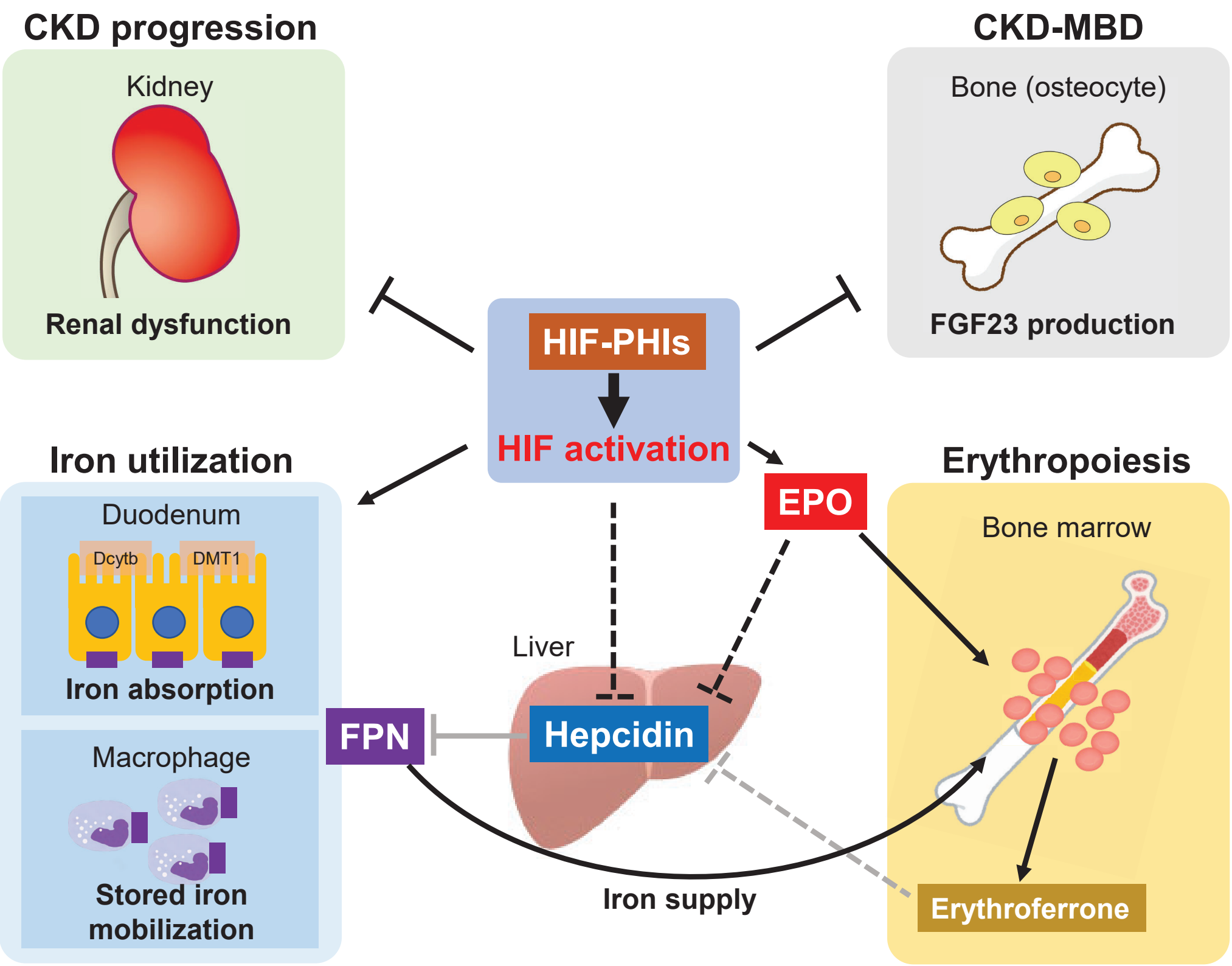
CKD progression
Kidney
Renal dysfunction
CKD-MBD
Bone (osteocyte)
FGF23 production
HIF-PHIs
HIF activation
Iron utilization
Duodenum
Dcytb
DMT1
Iron absorption
Macrophage
Stored iron mobilization
EPO
Erythropoiesis
Bone marrow
Liver
FPN
Hepcidin
Iron supply
Erythroferrone