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EDITORIAL

The prolyl hydroxylase inhibitor molidustat fails to restore erythropoietin production in the fibrotic kidney

In a recent publication in this journal, Kobayashi et al[1] address the question of whether hypoxia-inducible factor (HIF)-prolyl hydroxylase inhibitors (PHDi) reactivate erythropoietin (Epo) production in myofibroblast-transformed renal Epo-producing cells (REPs). The glycoprotein hormone Epo is the essential regulator of red blood cell formation by inhibiting apoptosis of erythroid progenitor cells. It is induced by hypoxia of any etiology, and its expression is regulated almost exclusively at the transcriptional level by the HIF-2α isoform.[2] In the adult, Epo is predominantly (approx. 90%) produced in the kidneys depending on the renal oxygen supply. The identity, origin, plasticity, and functional characteristics of REPs are still under debate.[3] REPs are unequivocally localized in the deep juxtamedullary cortex and outer medulla under physiological conditions. Originally, REPs were identified as interstitial, capillary-surrounding, fibroblast-like cells expressing CD73 (ecto-5′-nucleotidase). More recent studies have revealed that REPs share characteristics of fibroblasts, pericytes, mesenchymal stem cell-like cells, and neurons, known as telocytes. With regard to the expression of respective cell surface markers, REPs do not seem to represent a homogeneous cell population.

Even though most of the REPs at the corticomedullary boundary possess Epo-producing ability, they do not produce Epo under normoxic conditions. When oxygen supply declines or oxygen demand increases, REPs are recruited along the corticomedullary border and in the renal cortex leading to a substantially increased Epo production in the kidney. However, even under severe hypoxia, REPs represent only a subset of the total interstitial cell population.

In patients with chronic kidney disease (CKD), irrespective of the underlying disease, REPs fail to produce sufficient Epo in response to hypoxia, resulting in renal anemia.[4] The reasons for inadequate Epo production by REPs in CKD are still incompletely understood. Inflammation-induced transdifferentiation of REPs into myofibroblasts, as indicated by an increased expression of the myofibroblast markers desmin or α-smooth muscle actin (αSMA), resulting in loss of Epo-producing ability is presumably involved in the pathophysiology of renal anemia. αSMA-positive myofibroblasts are central players in tissue fibrosis. However, REPs may recover their original physiological properties, including Epo production, after the resolution of fibrogenic inflammatory stimuli.[5] Genetically targeted HIF activation in REPs by deletion of HIF prolyl hydroxylases (PHDs) was able to restore Epo production even in myofibroblast-transformed REPs in a mouse model of unilateral ureteral obstruction (UUO) without affecting the progression of renal fibrosis.[5] In line with these results, pharmacological inhibition of HIF PHDs reactivated Epo production in inactive, αSMA+ REPs and did not recruit dormant REPs in the same CKD mouse model.[6] Furthermore, the PHDi enarodustat suppressed the transformation of cultured renal interstitial fibroblasts into αSMA+ myofibroblasts.[7]

In their recent study, Kobayashi et al[1] used adenine feeding (0.25% adenine-containing diet-fed intermittently for 3 or 5 weeks over a 9-week period) and UUO (for 8 days) to cause kidney damage. Mice with severe adenine-induced nephropathy (AN) developed anemia, and additional phlebotomy revealed relative Epo deficiency in comparison to control mice, ie insufficient Epo levels relative to the degree of hypoxia or anemia. The number of REPs was significantly reduced in mice with severe AN and, importantly, additional phlebotomy-induced anemia did not stimulate Epo production in αSMA+ myofibroblast-transformed REPs. A single injection of the pan-PHDi molidustat significantly increased renal Epo production and recruited REPs both in control and adenine-fed mice. Importantly, molidustat did not activate Epo production in transdifferentiated αSMA+ myofibroblasts of mice with severe AN verifying the results in phlebotomized mice. Following PHDi administration, REPs were predominantly localized at the corticomedullary junction in control mice, whereas their distribution shifted towards the cortex with preserved kidney architecture in mice with severe AN. Here, REPs were found in the proximity of non-injured proximal renal tubules.

See related article: Kobayashi H , Davidoff O , Pujari- Palmer S , Drevin M , Haase VH . EPO synthesis induced by HIF- PHD inhibition is dependent on myofibroblast transdifferentiation and colocalizes with non- injured nephron segments in murine kidney fibrosis. Acta Physiol. (Oxf). e13826.

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By comparing two mouse models of AN with different severity of fibrosis and renal dysfunction, the authors demonstrated higher responsiveness to molidustat treatment for 2 weeks in mice with mild AN in comparison to mice with severe AN, as shown by increased renal Epo mRNA, serum Epo und hematocrit levels. Using lectin adsorption chromatography, they found no significant hepatic Epo production in molidustat-treated mice with both mild and severe AN contributing to serum Epo levels. However, molidustat has the potential to increase hepatic Epo production, as shown before in rats with gentamicin-induced kidney injury.[8] The findings from Kobayashi et al. suggest that in mild CKD PHDi stimulate Epo production in REPs localized in intact non-injured parenchyma of the renal cortex and renal Epo production in αSMA-negative REPs is dependent on the degree of tubulointerstitial fibrosis and residual kidney function. In order to confirm that transdifferentiated REPs can no longer be induced to produce Epo, the PHD-HIF axis was strongly stimulated by the inactivation of *Phd2* in FoxD1-derived renal interstitial cells. Rapidly progressing renal fibrosis by UUO in *Phd2*-targeted mice resulted in a failure of αSMA+ myofibroblast-transformed REPs to synthesize Epo.

Discrepancies between the results from Kobayashi et al[1] and previous studies reporting Epo production in myofibroblast-transformed REPs[5,6] are possibly due to different methodologies. While Souma et al[5] and Dahl et al[6] detected REPs with genetically tagged reporter mice ($Epo^{GFP/wt}$ and $Epo\text{-}Cre^{ERT2}$#1xtdTomato, respectively), Kobayashi et al[1] used RNAscope in situ hybridization for detection of REPs. To stimulate Epo production in the UUO model, Souma et al[5] deleted PHD1-3 with Epo-directed Cre in REPs, whereas Kobayashi et al[1] selectively inactivated PHD2 in FoxD1-lineage REPs. Furthermore, experiments with PHDi were performed in the UUO model using roxadustat by Dahl et al[6] and in the AN model using molidustat by Kobayashi et al[1] Therefore, further studies are needed to clarify the ability of PHDi to reactivate Epo production in myofibroblast-transformed REPs.

The data collected by Kobayashi et al indicate that PHDi is useful for the treatment of renal anemia in patients with mild to moderate CKD and that PHDi treatment should be started early when normal renal parenchyma is largely preserved. In contrast, patients with severe kidney fibrosis might not benefit from the administration of PHDi, particularly in view of potential deleterious effects associated with long-term PHDi treatment (particularly pro-oncogenic, pro-angiogenic, and pro-fibrotic effects as well as pulmonary hypertension). Thus, patients with severe kidney fibrosis might be preferably treated with recombinant Epo, at least as long as safety concerns regarding PHDi remain unanswered.

CONFLICT OF INTEREST

The authors of this editorial do not report any conflict of interest.

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