Internal and Emergency Medicine (2020) 15:911–915
https://doi.org/10.1007/s11739-020-02417-w

IM-POINT OF VIEW

Practical implications of the 2019 Nobel Prize in Physiology or Medicine: from molecular adaptation to hypoxia to novel anti-anemic drugs in the clinic

Fabiana Busti[1] · **Giacomo Marchi**[1] · **Alice Vianello**[1] · **Domenico Girelli**[1]

Received: 24 April 2020 / Accepted: 22 June 2020 / Published online: 2 July 2020

Abstract
The 2019 Nobel Prize for Medicine or Physiology was assigned to three prestigious physician-scientists, Gregg L. Semenza, William G. Kaelin, and Peter J. Ratcliffe, who clarified the molecular mechanisms of hypoxia adaptation. This viewpoint traces their fundamental findings, which have paved the way for the development of innovative drugs for a wide range of common diseases, including cancer and anemia.

Keywords Hypoxia · HIF · Erythropoiesis · Iron homeostasis · PHD inhibitors · Nobel Prize

In 2019, the Nobel Prize in Medicine or Physiology was jointly awarded to three physician-scientists, Gregg L. Semenza (Johns Hopkins University, Baltimore, MD, USA; pediatric geneticist), William G. Kaelin Jr. (Harvard University, Cambridge, MA, USA; internist and oncologist) and Peter J. Ratcliffe (University of Oxford, Oxford, United Kingdom; nephrologist) for their elegant studies that independently contributed to understand "how cells sense and adapt to oxygen availability". Of note, in 2016, they had already received the Albert Lasker Medical Research Award, sometimes referred to as the "America's Nobel".

Oxygen (O_2) serves as a key substrate in many essential cellular functions, particularly ATP generation in mitochondria. Humans have developed multiple hypoxia-response strategies [1], including increased red blood cell (RBCs) production, angiogenesis (both improving O_2 delivery), and metabolic reprogramming of cells (optimizing O_2 consumption and utilization). The ability to maintain O_2 homeostasis has allowed humans to survive in strongly inhospitable habitat, such as high altitudes (e.g., Tibetan populations), where O_2 levels are extremely low. In 1938, the Nobel Assembly at Karolinska Institutet had already awarded another researcher interested in adaptation to O_2 changes, Corneille Heymans, who first described the "carotid bodies" as the sensory organs able to rapidly modulate breathing and blood pressure according to arterial O_2 levels.

Semenza, Kaelin, and Ratcliffe have elucidated the molecular mechanisms underlying cell adaptation to hypoxia, a phenomenon that had remained elusive for a long time. It is involved in several physiological conditions, such as embryonic development, response to exercise, ventilatory acclimatization to high altitude, and even immune defenses. On the other hand, its dysregulation may be associated with common diseases, such as anemia, cancer, ischemic disorders, retinal neovascularization, sleep apnea, pulmonary hypertension, and transplant rejection [1].

Pioneer studies by Semenza's group in the 1980s pointed out the mechanisms regulating the O_2-dependent kidney production of erythropoietin (EPO), which in turn modulates erythroid precursor survival, proliferation, and differentiation. They highlighted that EPO expression was regulated at transcriptional level through a hypoxia-responsive element (HRE) in the 3′- flanking region of the *EPO* gene. In 1992, the same group biochemically purified the protein complex of transcriptional factors binding HRE, defined as hypoxia-inducible factors (HIFs) [2], whose coding genes were subsequently cloned in 1995 [3]. HIFs are heterodimers consisting of an O_2-regulated HIF-α subunit, and a constitutively expressed HIF-β subunit [1], also known as the aryl hydrocarbon nuclear translocator (ARNT) protein. Three different isoforms of HIFs-alpha have been identified in

✉ Fabiana Busti
fabiana.busti@univr.it

[1] Department of Medicine, Section of Internal Medicine, EuroBloodNet Referral Center for Iron Metabolism Disorders, Azienda Ospedaliera Universitaria Integrata Verona, University of Verona, 37134 Verona, Italy

mammals: (i) HIF1-α, ubiquitously expressed; (ii) HIF2-α, restricted to specific cell types such as vascular endothelial cells, renal interstitial cells, enterocytes, hepatocytes, cardiomyocytes, and astrocytes; and (iii) HIF3-α, less characterized and whose role remains to be explored. Besides EPO, several hundred genes in all cell types are known to be activated by HIFs binding to HRE sequences, including those controlling angiogenesis (e.g., vascular endothelial growth factor, VEGF), iron absorption (e.g., divalent metal transporter-1, DMT-1), inflammation, mitochondria bioenergetics, cell proliferation, and macromolecules metabolism. Under hypoxic conditions, the HIF1-α subunits are protected from degradation and accumulate in the nucleus, where they dimerize with the HIF1-β subunits. The complex binds the HRE sequences, modulating transcription of hypoxia-regulated genes [1] (Fig. 1).

An unexpected breakthrough in the comprehension of the hypoxia adaptation mechanisms came from the oncologist William G. Kaelin. He was studying Von-Hippel Lindau (VHL) syndrome, a rare inherited disorder characterized by an increased susceptibility to angiogenic tumors (especially renal cell carcinoma, and hemangioblastomas), occasionally detected in subjects with secondary polycythemia. The VHL disease is caused by inactivating mutations of the tumor suppressor gene *VHL*, in addition to a second mutation in the tumor tissue. Kaelin's group discovered that mutated VHL cells accumulated HIF1-α and exhibited abnormal overexpression of hypoxia-regulated genes, such as VEGF [4].

In the early 2000s, Kaelin and Ratcliffe independently provided the missing connecting piece, demonstrating that degradation of the HIF1-α subunits derives from the interaction with the VHL protein. Under normoxic conditions, HIF1-α is hydroxylated at one or two prolyl residues (proline 402 and proline 564) by O_2-dependent prolyl hydroxylase domain enzymes (PHD). A key role in this sense is played by PHD2, while the role of other analogues (PHD1 and PHD3) is less established. PDH2 hydroxylation facilitates the VHL protein binding, which in turn recruits an E3 ubiquitin-protein ligase complex labeling HIF-α subunits for proteasomal degradation [4–6] (Fig. 1). However, nowadays, it is clear that the regulation of HIFs is much more complicated than initially believed since their activity and stability

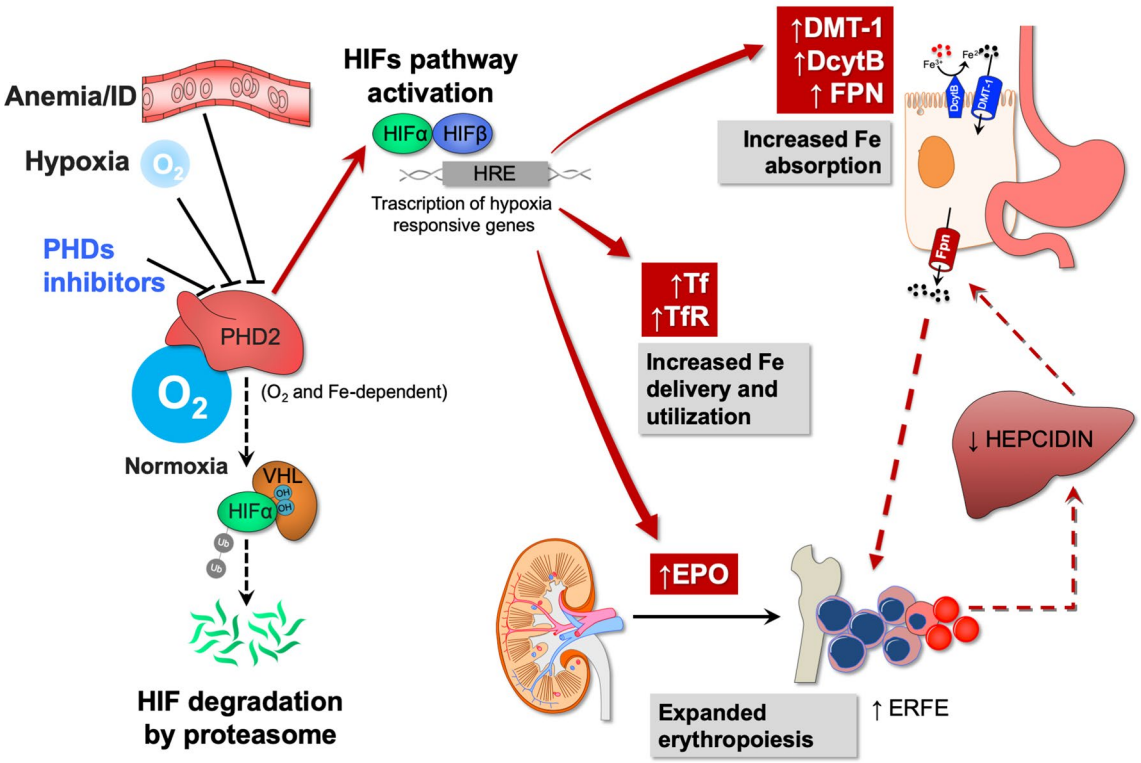

Fig. 1 Hypoxia coordinately increases erythropoiesis and iron absorption. Under normoxic conditions, PHD2 hydroxylates the HIF-alpha subunit, which is bound by VHL protein and degraded by the proteasome. In the presence of low O_2-availability, anemia, or iron deficiency (ID) HIF pathway is activated and leads to transcription of hypoxia-responsive genes. This results in EPO-mediated expansion of erythropoiesis and coordinately increases intestinal iron absorption through stimulation of DMT-1, DyctB, and ferroportin (FPN) gene expression. FPN activity is also increased by liver hepcidin suppression mediated by erythroferrone, which is produced by the expanding erythroblasts after EPO-stimulation. Finally, HIF pathway stimulates transferrin (Tf) and transferrin receptor (TfR) synthesis, which further improves iron transport and utilization by cells. Altogether, this circle (red arrows) explain how PHD inhibitors (in blue) act as effective anti-anemic drugs, at least in CKD anemia

are influenced by many other different proteins, in either O_2-dependent or O_2-independent manner.

The relevance of HIF pathway for erythropoiesis and iron metabolism

As mentioned above, increased RBC production is one of the major hypoxia adaptation mechanisms, which in turn improves the O_2-carrying capacity to tissues. EPO release occurs through an O_2-sensing system located in the renal inner cortex and outer medulla, where specialized cells respond to low O_2-availability [7].

A physiological increase in erythropoiesis is observed in subjects exposed to low environmental O_2 levels (e.g., those living at high altitudes), while a pathological increase may occur in subjects affected by chronic respiratory diseases causing hypoxia (e.g., obstructive sleep apnea syndrome) or with congenital mutations disrupting O_2-sensing systems, such as Chuvash polycythemia.

Erythropoiesis needs large amounts of iron (~ 20–25 mg daily), mostly derived from recycling spleen macrophages which eliminate senescent RBCs. Iron-restricted erythropoiesis, either due to absolute or functional iron deficiency (ID), leads to progressive RBC alterations (microcytosis and hypochromia) eventually causing anemia [8]. Systemic iron homeostasis is regulated by hepcidin, a 25-amino acid peptide mainly produced by the liver in response to multiple stimuli, particularly increased body iron stores and inflammation [9]. Hepcidin interacts with its receptor, i.e. ferroportin, the only known cellular iron exporter in mammals, inducing its internalization and lysosomal degradation. As a result, iron duodenal absorption and recycling by macrophages are tuned down lowering circulating iron levels. Hepcidin deficiency or suppression due to inherited or acquired conditions (i.e. hemochromatosis or expanded/ineffective erythropoiesis) may lead to iron accumulation of variable degree. By contrast, hepcidin excess (e.g., during acute or chronic inflammatory disorders) causes iron sequestration within cells, a condition also known as functional ID [8].

Of note, HIF2 pathway is critical in both erythropoiesis and iron homeostasis [10], as illustrated in Fig. 1.

In the enterocytes, HIF2 transcriptionally activates the iron transporter DMT-1, the ferric reductase DcytB (both localized at the apical cell membrane) and the iron exporter ferroportin (at the basal membrane) [7], coordinately leading to the up-regulation of intestinal iron absorption. This process represents an essential adaptive response to ID. At molecular level, ID decreases the activity of PHDs that are oxygen- and iron-dependent enzymes. Thus, the HIF2-α subunit is no longer hydroxylated, but stabilized, and induces the transcription of the iron absorption genes [11]. On the bone marrow-liver side, HIF2-induced EPO leads to increased production of erythroferrone (ERFE) by erythroid precursor, which in turn represses liver hepcidin [12]. Although the mechanism involved in the ERFE-mediated hepcidin suppression has not yet fully clarified, a recent study suggested a direct interaction of ERFE with the bone morphogenetic proteins (BMPs, especially BMP6) that inhibit the hepatic BMP/SMAD signaling, critical for hepcidin control [13].

Finally, HIF1 increases the expression of transferrin and transferrin receptor (TfR1) genes, thus enhancing iron delivery to tissues and utilization by cells [7].

HIFs pathway manipulation in patients with anemia and/or altered iron homeostasis

Considering the implications of HIFs in both erythropoiesis and iron metabolism, stimulation of HIF pathway may represent a promising and effective approach for the treatment of anemia, especially in patients affected by chronic kidney disease (CKD) with impaired EPO production [14]. Another group of patients who may benefit from HIF stimulation could be elderly with "unexplained" anemia, for which multiple mechanisms responsible for both EPO deficiency and blunted response have been postulated [15].

Currently, CKD-related anemia can be effectively corrected by recombinant human EPO (rhEPO), but its administration at doses that normalize hemoglobin levels implies an increased risk of cardiovascular events and mortality [16].

The PHD inhibitors are a novel class of small molecules able to stabilize the HIF-α subunit, thus preventing its degradation. Their main advantages over traditional rhEPO include oral administration, lower costs, and lower immunogenicity. PHD inhibitors not only increase hemoglobin in a dose-related way, but also improve iron uptake and utilization by erythroid progenitors, as suggested by decreased levels of hepcidin, ferritin, and transferrin saturation [17]. These drugs induce persistent production of endogenous EPO at a concentration near the physiological range, at variance with peaks determined by rhEPO administration. Such an effect has been hypothesized to explain the lower rate of cardiovascular adverse events as compared to rhEPO. Interestingly, preclinical evidence also suggested that PHD inhibitors may protect organs from ischemic injury through direct pleiotropic effects [18].

To date, several PHD inhibitors have been registered, with four compounds being more advanced in the clinical programs: roxadustat, vadadustat, daprodustat, and molidustat. Roxadustat has been recently approved in China and is under regulatory review in other countries for the treatment of anemia in CKD dialysis patients. Phase III clinical trials showed that, in these patients, roxadustat was

noninferior to standard therapy (epoetin alfa) [19]. Moreover, in non-dialysis CKD patients, roxadustat was superior to placebo in increasing hemoglobin levels [20]. A critical issue that remains to clarify is whether PHD inhibitors will influence hard endpoints, including mortality.

In spite of their undoubtful benefit in short-term correction of anemia, concerns have been raised on the long-term use of PHD inhibitors, as HIFs are ubiquitously expressed (e.g. in the nervous system) and regulate a broad spectrum of cell functions beyond erythropoiesis. A recent study described an increase of fibroblast growth factor 23 (FGF23) levels in a mouse model treated with PHD inhibitors. High FGF23 levels have been associated with heart dysfunction and reduced bone mineral density in humans [10]. Theoretically, the long-term use of PHD inhibitors may foster latent cancers since HIF activation in tumor hypoxic environment could promote cell proliferation and angiogenesis, a condition that calls for caution, especially in older people. Nevertheless, no data thus far suggest that PHD inhibitors may have favored tumor progression. On the contrary, chronic hypoxia caused by PHD inhibition may inactivate cancer-associated fibroblasts, leading to decreased tumor metastatic spread. Ideally, future compounds able to selectively stimulate HIF2 could reduce undesirable off-target effects.

Negative modulation of HIF2-α may also be of therapeutic potential, as recent works [10] highlighted the role of HIF2-α in stimulating iron hyperabsorption in both hemochromatosis and secondary siderosis. Whether or not selective inhibitors of HIF2-α could hamper iron absorption has yet to be defined. However, such drugs may represent in the future an interesting approach to modulate the hepcidin/ferroportin axis dysfunction underlying most of the iron overload disorders [10].

In conclusion, the fascinating discoveries by Semenza, Kaelin, and Ratcliffe have robustly expanded our knowledge about cell adaptation to hypoxia, which is at the crossroad between erythropoiesis and iron metabolism. Such discoveries are paving the way for new pharmacological solutions able to promote or inhibit HIF signaling, potentially leading to significant improvements of several common disorders including anemias and iron overload disorders.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any study with human and animals by any of the authors.

Informed consent For this type of study, formal consent is not required.

References

1. Prabhakar NR, Semenza GL (2015) Oxygen sensing and homeostasis. Physiology 30(5):340–348. https://doi.org/10.1152/physiol.00022.2015
2. Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12(12):5447–5454. https://doi.org/10.1128/mcb.12.12.5447
3. Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92(12):5510–5514. https://doi.org/10.1073/pnas.92.12.5510
4. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292(5516):464–468. https://doi.org/10.1126/science.1059817
5. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399(6733):271–275. https://doi.org/10.1038/20459
6. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292(5516):468–472. https://doi.org/10.1126/science.1059796
7. Semenza GL (2009) Involvement of oxygen-sensing pathways in physiologic and pathologic erythropoiesis. Blood 114(10):2015–2019. https://doi.org/10.1182/blood-2009-05-189985
8. Camaschella C, Nai A, Silvestri L (2020) Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. https://doi.org/10.3324/haematol.2019.232124
9. Girelli D, Nemeth E, Swinkels DW (2016) Hepcidin in the diagnosis of iron disorders. Blood 127(23):2809–2813. https://doi.org/10.1182/blood-2015-12-639112
10. Renassia C, Peyssonnaux C (2019) New insights into the links between hypoxia and iron homeostasis. Curr Opin Hematol 26(3):125–130. https://doi.org/10.1097/MOH.0000000000000494
11. Schwartz AJ, Das NK, Ramakrishnan SK, Jain C, Jurkovic MT, Wu J, Nemeth E, Lakhal-Littleton S, Colacino JA, Shah YM (2019) Hepatic hepcidin/intestinal HIF-2alpha axis maintains iron absorption during iron deficiency and overload. J Clin Investig 129(1):336–348. https://doi.org/10.1172/JCI122359
12. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T (2014) Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet 46(7):678–684. https://doi.org/10.1038/ng.2996
13. Arezes J, Foy N, McHugh K, Sawant A, Quinkert D, Terraube V, Brinth A, Tam M, LaVallie ER, Taylor S, Armitage AE, Pasricha SR, Cunningham O, Lambert M, Draper SJ, Jasuja R, Drakesmith H (2018) Erythroferrone inhibits the induction of hepcidin by BMP6. Blood 132(14):1473–1477. https://doi.org/10.1182/blood-2018-06-857995
14. Semenza GL (2019) Pharmacologic targeting of hypoxia-inducible factors. Annu Rev Pharmacol Toxicol 59:379–403. https://doi.org/10.1146/annurev-pharmtox-010818-021637
15. Girelli D, Marchi G, Camaschella C (2018) Anemia in the elderly. HemaSphere 2(3):e40. https://doi.org/10.1097/HS9.0000000000000040

16. Locatelli F, Del Vecchio L, De Nicola L, Minutolo R (2020) Are all erythropoiesis-stimulating agents created equal? Nephrol Dial Transplant. https://doi.org/10.1093/ndt/gfaa034
17. Pergola PE, Spinowitz BS, Hartman CS, Maroni BJ, Haase VH (2016) Vadadustat, a novel oral HIF stabilizer, provides effective anemia treatment in nondialysis-dependent chronic kidney disease. Kidney Int 90(5):1115–1122. https://doi.org/10.1016/j.kint.2016.07.019
18. Schreiber T, Salhofer L, Quinting T, Fandrey J (2019) Things get broken: the hypoxia-inducible factor prolyl hydroxylases in ischemic heart disease. Basic Res Cardiol 114(3):16. https://doi.org/10.1007/s00395-019-0725-2
19. Chen N, Hao C, Liu BC, Lin H, Wang C, Xing C, Liang X, Jiang G, Liu Z, Li X, Zuo L, Luo L, Wang J, Zhao MH, Liu Z, Cai GY, Hao L, Leong R, Wang C, Liu C, Neff T, Szczech L, Yu KP (2019) Roxadustat treatment for anemia in patients undergoing long-term dialysis. N Engl J Med 381(11):1011–1022. https://doi.org/10.1056/NEJMoa1901713
20. Chen N, Hao C, Peng X, Lin H, Yin A, Hao L, Tao Y, Liang X, Liu Z, Xing C, Chen J, Luo L, Zuo L, Liao Y, Liu BC, Leong R, Wang C, Liu C, Neff T, Szczech L, Yu KP (2019) Roxadustat for anemia in patients with kidney disease not receiving dialysis. N Engl J Med 381(11):1001–1010. https://doi.org/10.1056/NEJMoa1813599