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Invited Review-pharmacology across disciplines

Hypoxia-inducible factor prolyl 4-hydroxylase inhibition in cardiometabolic diseases

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ABSTRACT

Hypoxia-inducible factor prolyl 4-hydroxylases (HIF-P4Hs, also called PHDs and EglNs) are enzymes that act as cellular oxygen sensors. They are the main downregulators of the hypoxia-inducible factor (HIF). HIF-P4Hs can be targeted with small molecule inhibitors, which stabilize HIF under normoxia and initiate the hypoxia response. Such inhibitors are in phase 2 and 3 clinical trials for the treatment of anemia due to their ability to induce erythropoietin and iron metabolism genes. Recent data suggest that HIF-P4H inhibition has a therapeutic role beyond anemia in cardiac ischemia, obesity and metabolic dysfunction, and atherosclerosis. The molecular level mechanisms involved are HIF stabilization driven changes in gene expression that improve perfusion and endothelial function, reprogram metabolism to promote glucose intake and glycolysis over oxidative metabolism, reduce inflammation and beneficially modify innate immune system. This review discusses the recent findings in detail.

Contents

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1. Hypoxia-inducible factor prolyl 4-hydroxylases (HIF-P4Hs)

1.1. HIF-P4Hs in cellular oxygen sensing

Hypoxia-inducible factor prolyl 4-hydroxylases (HIF-P4Hs, EC 1.14.11.29), also called PHDs and EglNs, are enzymes that act as cellular oxygen sensors [1,2]. In the presence of molecular oxygen, and the other reaction cofactors/-substrates Fe^{2+} and 2-oxoglutarate (α-ketoglutarate), HIF-P4Hs hydroxylate two prolyl residues in the hypoxia-inducible factor (HIF) α subunit, which directs it for proteasomal degradation via von Hippel Lindau protein (pVHL) (Fig. 1) [1]. Ascorbate, which is not a direct cofactor, is needed to support the catalytic reaction. The reaction yields 4-hydroxyproline, succinate and CO_2 (Fig. 1). HIF-P4Hs react slowly with O_2 and their affinity to it is very low, which makes them sensitive to any decline in cellular oxygen tension [3–6]. Therefore, when oxygen availability becomes limiting the catalytic activity of HIF-P4Hs is compromised and HIFα is no longer hydroxylated and it escapes degradation. The stabilized HIFα translocates to the nucleus where it dimerizes with HIFβ (ARNT) and forms an active transcription factor dimer, which binds to hypoxia-responsive elements in numerous genes (Figs. 1 and 2). These include genes that regulate erythropoiesis and iron metabolism, angiogenesis, glucose and lipid metabolism, inflammation, tumorigenesis and metastasis and extracellular matrix homeostasis (Fig. 2). Two paralogues, HIF1α and HIF2α, exist in human. HIF has been reported to have altogether >300 target genes, which initiate the hypoxia response pathway aiming to increase oxygen delivery and restrict its usage. Some target genes are more specific for HIF1α (such as glycolytic genes) whereas HIF2α is the main driver of transcription of for example erythropoietin (EPO) [1,7].

1.2. Consequences of genetic inhibition of HIF-P4Hs

There are three HIF-P4H isoenzymes in human and mice [8–10]. HIF-P4H-2 (PHD2, EglN1) is the most abundant and phylogenetically oldest form, and the major one regulating HIFα stability [11,12]. HIF-P4H-1 (PHD1, EglN2) and HIF-P4H-3 (PHD3, EglN3) have more restricted expression and have been suggested to have additional substrates to HIFα [2,13,14]. In agreement, knock out of *Hif-p4h-2*, but not *Hif-p4h-1* or *Hif-p4h-3*, is embryonic lethal due to placental and cardiac defects, the former causing lethality between E12.5 and E14.5 [15]. Large spectrum conditional knock out of *Hif-p4h-2* causes massive erythrocytosis and angiogenesis, and premature death [16,17]. *Hif-p4h-1*$^{-/-}$ mice have no major defects [15]. Although being otherwise normal, the *Hif-p4h-3*$^{-/-}$ mice have abnormal sympathoadrenal development with reduced adrenal medullary secretory capacity and decreased systemic blood pressure [18]. In human, *HIF-P4H-2* mutations have been characterized in patients with familial erythrocytosis [19]. In Tibetans that are multigenerationally adjusted living at high altitude, HIF-P4H-2 mutations confer sensitivity to oxygen enabling efficient HIFα degradation under hypoxia and prevention against massive erythocytosis [20].

1.3. Other 2-oxoglutarate-dependent dioxygenases

HIF-P4Hs belong to the enzyme family of 2-oxoglutarate-dependent dioxygenases (2-OGDDs) with about 70 members in human [21]. These enzymes share the same reaction mechanism but have different substrates ranging from proteins to DNA, RNA and fatty acids. The closest relative to HIF-P4Hs among 2-OGDDs is the collagen P4Hs, which using an identical reaction mechanism hydroxylate prolyl residues in procollagen α chains in the endoplasmic reticulum [22]. 4-Hydroxyproline in collagen in needed for the thermal stability of their triplehelical structure [22]. Other 2-OGDDs include a transmembrane P4H (P4H-TM) that also regulates HIFα and erythropoiesis [23], the HIF asparaginyl hydroxylase FIH, collagen lysyl hydroxylases and many epigenetic regulators, such as the TET DNA demethylases and numerous JmjC-domain containing histone demethylases [21].

1.4. HIF-P4H inhibitors

HIF-P4Hs can be inhibited by fumarate and succinate that are naturally occurring intermediates of the Krebs cycle, but which accumulate in cancers deficient of fumarate hydratase (FH) and succinate dehydrogenase (SDH) [24–26]. Being structural analogues of 2-oxoglutarate they inhibit HIF-P4Hs competitively with respect to it (Fig. 1) [26]. Contrary, (*R*)2-hydroxyglutarate, another 2-oxoglutarate analogue that is generated by specific isocitrate dehydrogenase mutant cancers, can support the HIF-P4H reaction (Fig. 1) [27]. HIF-P4Hs can also become inhibited via targeting the iron cofactor. Iron chelators, such as desferrioxamine (DFO), other divalent metals than Fe^{2+} (such as Co^{2+}), nitric oxide and reactive oxygen species (ROS) have also been reported to inhibit the catalytic activity of HIF-P4Hs (Fig. 1) [9,28–31]. In case of triple negative breast cancer, L-cysteine can substitute for ascorbate to support the HIF-P4H catalytic reaction by preventing autooxidation of intramolecular cysteine residues [32].

N-oxalylglycine, and its dimethylated form dimethyloxalylglycine (DMOG) that passes the cell membrane, are broad spectrum 2-OGDD inhibitors that have been widely used *in vitro* and *in vivo*. Though many claim that DMOG is a HIF-P4H inhibitor it inhibits collagen P4Hs and FIH with the nearly same order of magnitude [3]. However, recent kinetic, inhibitory and structural analyses of HIF-P4Hs have enabled the development of more specific small molecule inhibitors for them. Several of these inhibitors are currently in phase 3 and 2 clinical trials for the treatment of anemia due to chronic kidney disease for their ability to induce EPO and iron metabolism genes (Fig. 2) [2]. Many of these orally administered compounds that can be called hypoxiamimetics, target the 2-oxoglutarate cosubstrate (Fig. 1). Additionally, HIF-P4H inhibitors are in preclinical phase evaluation for the treatment of ischemic conditions, peripheral artery disease, tendon injuries and wound healing (clinicaltrials.gov). Recent data by us and others suggest that HIF-P4Hs are intriguing candidates also in cardiac, metabolic and vascular diseases that will be discussed in the next paragraphs.

2. HIF-P4H inhibition in cardiac diseases

2.1. Cardioprotection against acute ischemia by inhibition of HIF-P4Hs

Ischemic heart diseases comprise a heterogeneous group of pathological conditions characterized by insufficient perfusion of the heart. Limitation of blood flow to the myocardium causes hypoxia leading to cell death and cardiac remodeling. Ischemia induces HIF, which in turn activates a wide range of responses aiming to protect cells from hypoxic damage or to promote reoxygenation of the compromised tissue (Fig. 2). All three HIF-P4H isoenzymes are present in the heart, HIF-P4Hs 2 and 3 being the most abundant under basal conditions [33]. The mRNA expression levels of both of these isoforms increase as a response to myocardial ischemia [33]. Over the last ten years different genetic strategies have shown convincing evidence on the role of HIF-P4H-2 inhibition in protecting myocardium against ischemic injury. Eckle and coworkers [34] showed that *i.v.* infusion of siRNA against *Hif-p4h-2* resulted in stabilization of HIF1α and protection during acute cardiac ischemia seen as significant reduction in the size of exper-