

Benefits and Risks of Manipulating the HIF Hydroxylase Pathway in Ischemic Heart Disease

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2.1 Introduction

Ischemic heart disease is a major cause of morbidity and mortality in the Western world. It occurs when oxygen delivery cannot meet the metabolic needs of the heart, as observed in patients with stable coronary artery disease as well as those experiencing acute myocardial infarction. Although conditions leading to myocardial injury have been well studied, and physical means of revascularization by stenting or coronary bypass surgery are well developed, there remains a need to define treatments that limit damage in the acute phase or promote revascularization by medical means. In particular, mechanisms that preserve cellular function during ischemia remain poorly understood.

Experimental models of myocardial ischemia in rodents have demonstrated that prior exposure to sublethal cycles of ischemia-reperfusion (I/R) protects tissues such as the heart from subsequent ischemia. There is compelling evidence that this ischemic preconditioning (IPC) is, at least in part, conferred through hypoxic activation of the transcription factor: hypoxia-inducible factor (HIF). HIF is a master regulator of oxygen homeostasis that induces the expression of hundreds of genes in response to hypoxia, including those that stimulate glycolysis, angiogenesis, and erythropoiesis. These changes help the organism adapt to oxygen deprivation at both the cellular and tissue levels. Pharmacological modulators of HIF are consequently being pursued as therapeutic targets for myocardial (as well as more general tissue) ischemia.

HIF is an α/β heterodimeric transcription factor, whose α subunit is regulated through posttranslational modification by HIF prolyl hydroxylases (PHDs, *prolyl hydroxylase domain*): PHD1, 2 and 3 (reviewed in Kaelin and Ratcliffe [1]).

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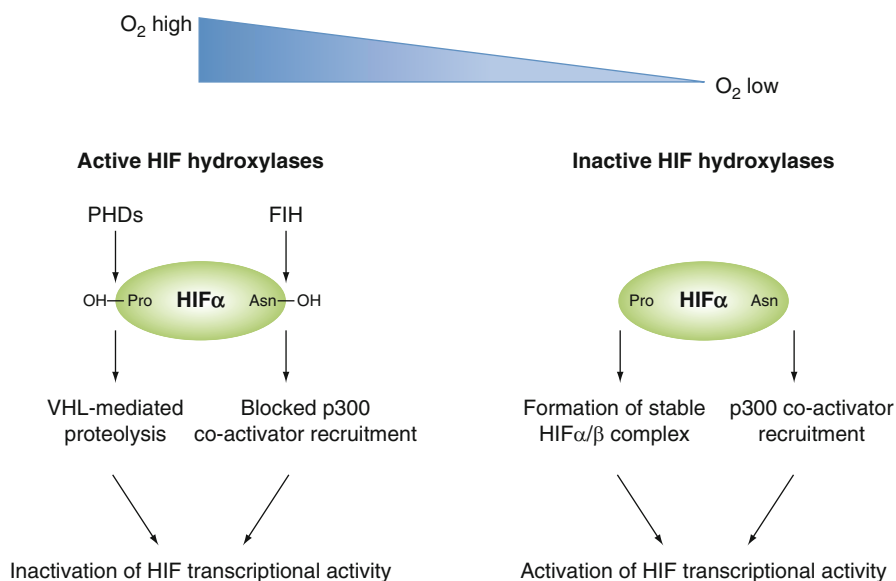


Fig. 2.1 Dual regulation of HIF- α subunits by prolyl and asparaginyl hydroxylation. In the presence of oxygen, active HIF prolyl hydroxylases (*PHDs*), as well as factor inhibiting HIF (*FIH*), downregulate and inactivate HIF α subunits. PHDs hydroxylate prolyl residues to promote von Hippel–Lindau tumor suppressor (*VHL*)-dependent proteolysis of HIF α subunits. FIH, on the other hand, hydroxylates an asparaginyl residue, which blocks p300 co-activator recruitment from activating HIF α -subunit transcriptional activity. In hypoxia, HIF hydroxylases (*PHDs* and *FIH*) are inactive and these processes are suppressed, which allows the formation of a transcriptionally active HIF complex

These non-heme Fe(II) and 2-oxoglutarate-dependent dioxygenase PHD enzymes are now widely regarded as cellular oxygen sensors that transduce the oxygen status to the cell via posttranslational hydroxylation of HIF α . In the presence of oxygen, PHD hydroxylates two proline residues within a central degradation domain in HIF-1 α and -2 α . This promotes their binding to von Hippel–Lindau tumor suppressor (*VHL*) E3 ubiquitin ligase, leading to proteasomal degradation. A second point of regulation involves asparaginyl hydroxylation by another non-heme Fe(II) and 2-oxoglutarate-dependent dioxygenase termed FIH (factor inhibiting HIF). During hypoxia, reduced PHD and FIH activity allows HIF α subunits to escape proteolysis and assemble into an active α/β heterodimer that induces a broad range of target genes (Fig. 2.1).

A substantial body of work indicates that despite this dual control system, activation of HIF can be achieved through inhibition of the PHD/*VHL* degradation pathway alone. Indeed, several PHD inhibitory drugs are in development to test whether pharmacological modulation of the HIF hydroxylase system to activate HIF protects from subsequent ischemic insult. This type of intervention may have effects in the short term through enhanced cellular metabolism (for example, stimulation of glycolysis, glucose metabolism, and reduced mitochondrial oxygen consumption)

as well as in the medium to longer term through increased perfusion (for example, by stimulation of angiogenesis), giving potential applications both in the acute phase as well as in chronic ischemic heart disease.

The safety of long-term PHD inhibition/HIF activation, however, remains unclear. Given the ubiquitous distribution of the HIF hydroxylase system and wide range of processes affected by HIF, it seems unlikely that all consequences of HIF activation will be beneficial to treating myocardial ischemia; some may even impinge normal physiological function in the heart or other tissues. We consider in this review evidence relating to the benefits and risks of manipulating the HIF hydroxylase system as a therapeutic means of treating myocardial ischemia.

2.2 Benefits

2.2.1 Genetic Manipulation of HIF-1 α

Evidence for the essential role of HIF-1 α in IPC was obtained from transgenic mouse models, wherein haploinsufficiency of *HIF-1 α* is sufficient to ablate the protective effect conferred by IPC on myocardial infarction [2, 3]. This result is similarly present in mice treated with intraventricular infusion of *HIF-1 α* siRNA [4].

In agreement with this, overexpression of HIF-1 α in the myocardium of mice attenuates infarct size and improves cardiac function several weeks (but not 24 h) after coronary artery occlusion [5]. This delayed protective effect is thought to be conferred, at least in part, through increased capillary density in the infarct and peri-infarct zones via transcriptional activation of pro-angiogenic HIF target genes such as vascular endothelial growth factor (VEGF) and angiopoietin-2. Together with the predicted vasodilation from HIF-mediated stimulation of inducible nitric oxide synthase, these changes are postulated to help restore delivery of blood to the heart. It should be noted that the overexpressed HIF-1 α in these mice would be subject to normoxic degradation, thus limiting upregulation of the pathway in the cells that are best oxygenated. The long-term effects of more complete HIF-1 α activation from blockage of the degradation pathway, therefore, cannot be readily deduced from this study.

Further, overexpression of a stable form of HIF-1 α in the epidermis of mice has been shown to induce hypervascularity (in line with the predicted induction of pro-angiogenic HIF target genes) [6]. Interestingly, in contrast to transgenic mice overexpressing myocardial VEGF, in which rapid stimulation of dysregulated angiogenesis leads to fragile and immature vessel formation [7, 8], HIF-1 α overexpression induces blood vessel formation without any leakage or inflammation. Most probably this is because of multiple, coordinated actions on the angiogenic process. It is also possible that effects of HIF activation at sites remote from the site of ischemia may have protective actions (for instance, by increasing circulating endothelial progenitors). This might conceivably assist perfusion of distant tissues and may underlie remote ischemic preconditioning effects, whereby IPC of, for example, the kidney can result in cardioprotection [9].

2.2.2 Pharmacological Inhibition and Genetic Manipulation of PHD Enzymes

Small molecule inhibitors of the PHD enzymes potently activate the HIF response both *in vitro* and *in vivo*. Thus, it has been proposed that administration of PHD inhibitors could mimic, at least in part, the protective effects of exposure to hypoxia. Indeed, PHD inhibition likely results in greater HIF activation than the submaximal levels achieved through ischemic insult.

Initial studies using cobalt chloride and the iron chelator desferrioxamine to inhibit PHD enzymes (by displacement of their Fe(II) center or decreasing Fe(II) availability in solution) suggested that PHD inhibition acts similarly to IPC in providing protection against myocardial infarction [10, 11]. However, such inhibitors would be predicted to target other Fe(II)-containing enzymes and likely result in side effects from dysregulation of non-HIF hydroxylase pathways.

Subsequent studies have applied more specific inhibitors of PHD activity, dimethyl-oxalylglycine (DMOG) and FG2216, to rodent models of myocardial ischemia. DMOG is a 2-oxoglutarate analogue that inhibits the 2-oxoglutarate-dependent-dioxygenase family of enzymes (which includes the PHD enzymes); FG2216, on the other hand, is a more selective analogue which is proposed to specifically target the PHD enzymes, making it attractive for therapeutic use. Both DMOG and FG2216 have been reported to minimize tissue damage 24 h to several weeks after myocardial infarction [4, 12–14].

Genetic manipulation of PHD activity has also been shown to protect from myocardial I/R. Although all three isoforms of PHD (1, 2, and 3) can hydroxylate and regulate HIF α *in vitro*, the ubiquitously high level of PHD2 protein across a range of cell lines is thought to account for its dominant role in setting low steady-state levels of HIF in normoxia [15]. In keeping with this, intraventricular infusion with *PHD2*, but not *PHD1* or 3, siRNA reduced post-ischemic infarct area [4, 16, 17]. Similar results were obtained with PHD2 silencing using intramyocardial injection of *PHD2* shRNA [18].

Genetic deletion of *PHD2* (but not *PHD1* or 3) in mice results in embryonic lethality [19]. It has been reported, however, that transgenic mice containing hypomorphic alleles for *PHD2* are viable with no obvious cardiac abnormalities. These mice have improved functional recovery, coronary flow rate, and reduced infarct size following I/R in the isolated mouse heart [20], in agreement with the dominant role of the PHD2 isoform in HIF regulation.

Interestingly, *PHD1* $^{-/-}$ mice, which survive until adulthood with no obvious heart defects, have also been reported to show significant protection from myocardial I/R [21]. Further, this protection against ischemic insult is observed in *PHD1* $^{-/-}$ skeletal muscle [22] and liver [23], indicating that the mechanisms involved are not restricted to the heart. Although the latter phenotypes are thought to involve HIF-dependent pathways, it is curious that the other hallmarks of HIF activation such as polycythemia and angiogenesis are not observed in *PHD1* $^{-/-}$ mice. Indeed, PHD1 has been reported to have HIF-independent functions in regulating cellular proliferation [24] and it is possible that these may contribute to the ischemic protection. Alternatively, it may be

that PHD1 loss induces HIF to a lesser extent than loss of PHD2, such that there is sufficient HIF to provide protection from ischemia without activating erythropoiesis or angiogenesis. Whatever the mechanism, the findings raise the interesting possibility that PHD isoform-specific inhibitors (which have yet to be developed) could provide more targeted drug intervention.

Overall, these studies provide evidence that short-term (or mild chronic) activation of HIF, by either pharmacological inhibition of PHD enzymes or genetic manipulation of PHD/HIF, can be beneficial against myocardial I/R. The protection conferred may occur shortly after HIF induction via changes in cellular metabolism (for example, enhanced glucose uptake and metabolism through activation of HIF target genes such as GLUT-1, pyruvate dehydrogenase kinase, and 6-phosphofructokinase 1) and vasodilation (for example, by induction of nitric oxide synthases). In addition, activation of HIF may confer delayed protection via angiogenesis and vascular remodeling.

Long-term HIF activation, for example, through genetic manipulation of the HIF hydroxylase system, however, has potential detrimental effects. These are outlined below.

2.3 Risks

2.3.1 Genetic Manipulation of HIF α

Evidence for the detrimental effects of sustained HIF α activation are obtained from recent studies, whereby overexpression of a stable form of either HIF-1 α or HIF-2 α in cardiomyocytes results in cardiomyopathy [25, 26].

2.3.2 Genetic Manipulation of PHD Enzymes

The effects of chronic PHD inhibitor exposure are largely unknown and existing data derives from *PHD* knockout mice which may not accurately mimic the effects of catalytic inhibition (for example, because of loss of additional non-catalytic effects of the enzyme protein). It is worth noting, however, that supplementation of a certain brand of Canadian beer with cobalt sulfate was identified as a contributing etiological factor in the so-called Quebec beer-drinker's cardiomyopathy (with associated polycythemia) of the late 1960s [27]. This hints at protracted PHD inhibitor usage being potentially detrimental to cardiac function – a possibility that is supported by genetic manipulation of the PHD enzymes in mice.

Widespread, conditional inactivation of *PHD2* in adult mice results in severe polycythemia and hyperactive angiogenesis/angiectasia, in line with the predicted induction of HIF α , pro-angiogenic HIF target genes, and erythropoiesis-promoting HIF target gene erythropoietin. However, these mice also suffer from dilated cardiomyopathy and premature mortality [28–31]. The latter phenotypes may occur either as an indirect consequence of polycythemia and/or as a direct action of *PHD2* loss

in cardiomyocytes. Further studies demonstrate that, in fact, cardiac-specific loss of *PHD2* is sufficient to induce dilated cardiomyopathy and premature mortality in adult mice, which is exacerbated when on a *PHD3*^{−/−} background [25]. Thus, sustained PHD2 inactivation/HIF activation in the heart itself is detrimental to cardiac function and may even play a causal role in the pathogenesis of ischemic cardiomyopathy [25].

Aside from the risks of dysregulated erythropoiesis and angiogenesis, loss of PHD activity in other noncardiac tissues may also pose risks to both cardiovascular and other tissue functions. For instance, *PHD3*^{−/−} mice, though viable and with no obvious cardiac abnormalities, suffer from abnormal sympathoadrenal development that is likely to be the cause of the observed reduced catecholamine secretion and systemic hypotension [32]. In humans, activating mutations in HIF-2 α have been associated with pulmonary hypertension [33]. Systemic administration of PHD inhibitors may therefore result in a range of side effects from HIF activation in tissues other than the heart.

2.3.3 Genetic Manipulation of VHL

As both VHL and PHD negatively regulate HIF, and assuming a lack of divergence in the PHD/HIF/VHL oxygen-sensing pathway, one might predict loss of VHL to phenocopy loss of PHDs (in particular PHD2, given its dominant role in HIF regulation). Indeed, *VHL*^{−/−} mice, like *PHD2*^{−/−} mice, are embryonic lethal due to placental defects [34]. Cardiac-restricted ablation of *VHL* in adult mice leads to dilated cardiomyopathy, lipid accumulation, myocyte loss, fibrosis, and even malignant transformation, in a HIF-1 α -dependent manner [35]. The cardiac phenotype after *VHL* loss is therefore more severe than observed after combined *PHD2/PHD3* inactivation, possibly because of residual PHD1 activity and/or a contribution from PHD and HIF-independent functions of VHL. However, the findings again suggest that long-term, high-level upregulation of HIF pathways is likely to entrain significant side effects.

Overall, genetic studies demonstrate that extensive HIF activation in the heart is potentially deleterious to cardiovascular function. Thus, PHD inhibitors will probably require careful dose titration to achieve the desired risk/benefit profile and/or limitation of the duration of therapy.

2.4 Summary

Current work has defined both benefits and risks associated with the manipulation of the HIF hydroxylase system as a therapeutic means of treating myocardial ischemia.

Short-term (or mild, chronic) activation of HIF, like IPC, is protective against ischemic insult. Although this has been determined using interventions that precede ischemia, two findings raise the possibility that PHD inhibitors could equally be

applied post-ischemia. First, HIF activation lasts several days following ischemic insult [36]. Second, cycles of I/R applied at the onset of, rather than preceding, ischemia are still able to confer protection (a process known as ischemic post-conditioning [37]). The ability to treat myocardial ischemia by post-event drug intervention would make PHD inhibitors particularly useful in the clinical setting.

Prolonged, excessive HIF activation, on the other hand, phenocopies ischemic cardiomyopathy and is deleterious to cardiovascular function. It may also have detrimental side effects in noncardiac tissues if applied in a systemic manner. Ablation of *PHD1* in mice induces hypoxia tolerance without effect on PHD2-/HIF-regulated pathways such as erythrocytosis. In this regard, a PHD1-specific inhibitor, though not yet available, may be beneficial.

In summary, PHD inhibitors that activate HIF are an attractive therapeutic option for minimizing tissue damage from myocardial ischemia or improving perfusion by medical means. However, care will be required to avoid side effects from uncontrolled activation of hypoxia pathways. This highlights the need for time, dose, tissue, and/or PHD isoform-specific drug interventions in order to minimize the potential deleterious side effects of PHD inhibitors.

References

1. Kaelin Jr WG, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell*. 2008;30:393–402.
2. Cai Z, Manalo DJ, Wei G, et al. Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation*. 2003;108:79–85.
3. Cai Z, Zhong H, Bosch-Marce M, et al. Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1 alpha. *Cardiovasc Res*. 2008;77:463–70.
4. Eckle T, Kohler D, Lehmann R, El Kasmi K, Eltzschig HK. Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. *Circulation*. 2008;118:166–75.
5. Kido M, Du L, Sullivan CC, et al. Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. *J Am Coll Cardiol*. 2005;46:2116–24.
6. Elson DA, Thurston G, Huang LE, et al. Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1alpha. *Genes Dev*. 2001;15:2520–32.
7. Lee RJ, Springer ML, Blanco-Bose WE, Shaw R, Ursell PC, Blau HM. VEGF gene delivery to myocardium: deleterious effects of unregulated expression. *Circulation*. 2000;102:898–901.
8. Carmeliet P. VEGF gene therapy: stimulating angiogenesis or angioma-genesis? *Nat Med*. 2000;6:1102–3.
9. Kant R, Diwan V, Jaggi AS, Singh N, Singh D. Remote renal preconditioning-induced cardioprotection: a key role of hypoxia inducible factor-prolyl 4-hydroxylases. *Mol Cell Biochem*. 2008;312:25–31.
10. Dendorfer A, Heidbreder M, Hellwig-Burgel T, Jöhren O, Qadri F, Dominiak P. Deferoxamine induces prolonged cardiac preconditioning via accumulation of oxygen radicals. *Free Radic Biol Med*. 2005;38:117–24.
11. Xi L, Taher M, Yin C, Salloum F, Kukreja RC. Cobalt chloride induces delayed cardiac preconditioning in mice through selective activation of HIF-1alpha and AP-1 and iNOS signaling. *Am J Physiol Heart Circ Physiol*. 2004;287:H2369–75.

12. Zhao HX, Wang XL, Wang YH, et al. Attenuation of myocardial injury by postconditioning: role of hypoxia inducible factor-1alpha. *Basic Res Cardiol*. 2010;105:109–18.
13. Ockaili R, Natarajan R, Salloum F, et al. HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. *Am J Physiol Heart Circ Physiol*. 2005;289:H542–8.
14. Philipp S, Jurgensen JS, Fielitz J, et al. Stabilization of hypoxia inducible factor rather than modulation of collagen metabolism improves cardiac function after acute myocardial infarction in rats. *Eur J Heart Fail*. 2006;8:347–54.
15. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J*. 2003;22:4082–90.
16. Natarajan R, Salloum FN, Fisher BJ, Kukreja RC, Fowler 3rd AA. Hypoxia inducible factor-1 activation by prolyl 4-hydroxylase-2 gene silencing attenuates myocardial ischemia reperfusion injury. *Circ Res*. 2006;98:133–40.
17. Natarajan R, Salloum FN, Fisher BJ, Ownby ED, Kukreja RC, Fowler 3rd AA. Activation of hypoxia-inducible factor-1 via prolyl-4 hydroxylase-2 gene silencing attenuates acute inflammatory responses in postischemic myocardium. *Am J Physiol Heart Circ Physiol*. 2007;293:H1571–80.
18. Huang M, Chan DA, Jia F, et al. Short hairpin RNA interference therapy for ischemic heart disease. *Circulation*. 2008;118:S226–33.
19. Takeda K, Ho VC, Takeda H, Duan LJ, Nagy A, Fong GH. Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. *Mol Cell Biol*. 2006;26:8336–46.
20. Hyvarinen J, Hassinen IE, Sormunen R, et al. Hearts of hypoxia-inducible factor prolyl 4-hydroxylase-2 hypomorphic mice show protection against acute ischemia-reperfusion injury. *J Biol Chem*. 2010;285:13646–57.
21. Adluri RS, Thirunavukkarasu M, Dunna NR, et al. Disruption of HIF-prolyl hydroxylase-1 (PHD-1/-) attenuates ex vivo myocardial ischemia/reperfusion injury through HIF-1alpha transcription factor and its target genes in mice. *Antiox Redox Signal* 2011;15:1789–97.
22. Aragonés J, Schneider M, Van Geyte K, et al. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat Genet*. 2008;40:170–80.
23. Schneider M, Van Geyte K, Fraisl P, et al. Loss or silencing of the PHD1 prolyl hydroxylase protects livers of mice against ischemia/reperfusion injury. *Gastroenterology*. 2010;138:1143–54. e1–2.
24. Zhang Q, Gu J, Li L, et al. Control of cyclin D1 and breast tumorigenesis by the EglN2 prolyl hydroxylase. *Cancer Cell*. 2009;16:413–24.
25. Moslehi J, Minamishima YA, Shi J, et al. Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. *Circulation*. 2010;122:1004–16.
26. Bekereditian R, Walton CB, MacCannell KA, et al. Conditional HIF-1alpha expression produces a reversible cardiomyopathy. *PLoS One*. 2010;5:e11693.
27. Morin Y, Daniel P. Quebec beer-drinkers' cardiomyopathy: etiological considerations. *Can Med Assoc J*. 1967;97:926–8.
28. Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin Jr WG. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. *Blood*. 2008;111:3236–44.
29. Minamishima YA, Moslehi J, Padera RF, Bronson RT, Liao R, Kaelin Jr WG. A feedback loop involving the Phd3 prolyl hydroxylase tunes the mammalian hypoxic response in vivo. *Mol Cell Biol*. 2009;29:5729–41.
30. Takeda K, Aguila HL, Parikh NS, et al. Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. *Blood*. 2008;111:3229–35.
31. Takeda K, Cowan A, Fong GH. Essential role for prolyl hydroxylase domain protein 2 in oxygen homeostasis of the adult vascular system. *Circulation*. 2007;116:774–81.

32. Bishop T, Gallagher D, Pascual A, et al. Abnormal sympathoadrenal development and systemic hypotension in PHD3^{-/-} mice. *Mol Cell Biol.* 2008;28:3386–400.
33. Gale DP, Harten SK, Reid CD, Tuddenham EG, Maxwell PH. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating HIF2 alpha mutation. *Blood.* 2008;112:919–21.
34. Gnarr JR, Ward JM, Porter FD, et al. Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. *Proc Natl Acad Sci USA.* 1997;94:9102–7.
35. Lei L, Mason S, Liu D, et al. Hypoxia-inducible factor-dependent degeneration, failure, and malignant transformation of the heart in the absence of the von Hippel-Lindau protein. *Mol Cell Biol.* 2008;28:3790–803.
36. Willam C, Maxwell PH, Nichols L, et al. HIF prolyl hydroxylases in the rat; organ distribution and changes in expression following hypoxia and coronary artery ligation. *J Mol Cell Cardiol.* 2006;41:68–77.
37. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol.* 2003;285:H579–88.

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