

Keeping the engine primed: HIF factors as key regulators of cardiac metabolism and angiogenesis during ischemia

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Abstract Myocardial ischemia, the most common cause of cardiac hypoxia in clinical medicine, occurs when oxygen delivery cannot meet myocardial metabolic requirements in the heart. This deficiency can result from either a reduced supply of oxygen (decreased coronary bloodflow) or an increased myocardial demand for oxygen (increased wall stress or afterload). Patients with stable coronary artery disease as well as patients experiencing acute myocardial infarction can experience episodes of severe ischemia. Although hypoxia is an obligatory component, it is not the sole environmental stress experienced by the ischemic heart. Reperfusion after ischemia is associated with increased oxidative stress as the heart reverts to aerobic respiration and thereby generates toxic levels of reactive oxygen species (ROS). During mild ischemia, mitochondrial function is partially compromised and substrate preferences adapt to sustain adequate ATP generation. With severe ischemia, mitochondrial function is markedly compromised and anaerobic metabolism must provide energy no matter what the cost in generation of toxic ROS adducts. Ischemia produces a variety of environmental stresses that impair cardiovascular function. As a result, multiple signaling pathways are activated in mammalian cells during

ischemia/reperfusion injury in an attempt to minimize cellular injury and maintain cardiac output. Amongst the transcriptional regulators activated are members of the hypoxia inducible factor (HIF) transcription factor family. HIF factors regulate a variety of genes that affect a myriad of cellular processes including metabolism, angiogenesis, cell survival, and oxygen delivery, all of which are important in the heart. In this review, we will focus on the metabolic and angiogenic aspects of HIF biology as they relate to the heart during ischemia. We will review the metabolic requirements of the heart under normal as well as hypoxic conditions, the effects of preconditioning and its regulation as it pertains to HIF biology, the apparent roles of HIF-1 and HIF-2 in intermediary metabolism, and translational applications of HIF-1 and HIF-2 biology to cardiac angiogenesis. Increased understanding of the role of HIFs in cardiac ischemia will ultimately influence clinical cardiovascular practice.

Keywords HIF · Hypoxia · Heart · Ischemia · Angiogenesis · Metabolism

Myocardial fuel choice and oxygen supply

A remarkable feature of the heart, one of the most metabolically active organs in the body, is its ability to use a wide variety of fuel substrates in aerobic respiration for energy production. Oxygen, delivered in blood through the coronary arteries, is efficiently extracted by the heart for generation of reducing equivalents by mitochondria, focal points for fatty acid or pyruvate oxidation, and for ketone body utilization [1]. Fuel choice in the adult heart is not homogenous under all conditions, but instead is highly dependent upon substrate availability as well as tissue oxygenation [2]. The adult heart preferentially uses fatty

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acids during times of adequate oxygen availability [2, 3]. This preference changes to glucose utilization via pyruvate oxidation in mild ischemia, if glucose is available, and to glycolysis during severe ischemia.

Aerobic respiration is an efficient means for generating energy, but it comes at a price. Reactive oxygen species (ROS) are generated under physiological conditions with the primary source being the mitochondrial electron transport chain. If left unchecked, elevated ROS lead to cumulative cellular damage induced by oxidative stress. ROS levels are markedly elevated during ischemia/reperfusion injury and perhaps during ischemia or hypoxia itself [4]. Following reperfusion, a naive heart experiences a profound increase in oxidative stress [5] and reduced aerobic energy production [6]. The mitochondria are both the major source [7] and the most affected subcellular component of ROS generated during ischemia/reperfusion [8, 9]. However, ROS also have a role in normal physiological processes, including cell growth and differentiation, vascular tone, and cardiac growth, due to signal transduction pathways activated by ROS [10, 11]. Hence, ROS homeostasis is essential for proper functioning of cardiac myocytes and other cells types in the basal as well as stressed state.

Ischemia/reperfusion and preconditioning

A protective response by the heart and other organs to sublethal levels of hypoxia or ischemia, known as preconditioning, results in the induction of adaptive physiological processes. This process is relevant not only to coronary artery patients experiencing repetitive ischemia due to coronary artery vasospasm or subocclusions, but also to diabetic patients with microvascular heart disease as well as to hypertensive and cardiomyopathic heart disease patients with increased myocardial wall stress and consequently greater oxygen demand. Ischemic preconditioning has an immediate as well as a delayed component [12, 13]. The immediate preconditioning response occurs within minutes to hours [14] and is mediated by ion channels as well as by signaling molecules produced during ischemia [15]. The delayed preconditioning response, a transcriptional response lasting from hours to days, is conferred by *de novo* synthesized cellular factors [16].

The protective cellular processes induced by delayed preconditioning have been well studied and are aimed at optimizing oxygen delivery, intermediary metabolism, and preserving mitochondrial function. However, less is known about the regulatory network that confers delayed preconditioning [17]. With reperfusion or reoxygenation, as occurs during preconditioning, ROS levels increase and redox-sensitive transcription factors are activated. Being redox-

sensitive transcription factors, HIF members are poised to exert significant control over the protective cellular response generated during delayed preconditioning. One protective response induced during the initial phase of delayed preconditioning is a shift from aerobic to glycolytic metabolism. For the severely ischemic heart, this is beneficial in the short-term, but is insufficient to meet its long-term needs.

To meet the long-term challenges during prolonged ischemia, the heart induces several protective effects aimed at restoring aerobic respiration. These effects include increased angiogenesis in an attempt to restore blood delivery to the ischemic heart as well as augmentation of mitochondrial respiration and mitochondrial biogenesis to maximize energy production from the available oxygen content [12]. Perhaps in anticipation of successfully restoring oxygen delivery to the hypoxic tissue, the ischemic heart also induces expression of endogenous cytoprotective cellular factors including antioxidant enzymes (AOE) that eliminate ROS in the mitochondria and other subcellular compartments [18]. Hence, a key target for preconditioning is the mitochondria [19], a central organelle with multiple biological roles in addition to energy generation [20]. Regulatory factors implicated in mitochondrial biogenesis have been identified [21]. As will be discussed, HIF members have been implicated in regulating the angiogenic and metabolic response to chronic ischemia. By contrast, the transcriptional regulation of the endogenous cytoprotective response remains poorly understood, although it is plausible that HIF members are likewise involved in regulation of this protective response.

Preconditioning and transcriptional regulation of intermediary metabolism

Changes in cardiac fuel substrate preference during ischemia have been delineated, but our knowledge of how substrate utilization is determined at the transcriptional level during chronic ischemia is sparse. The most well-studied regulator of cardiac intermediary metabolism is PPAR α , the transcriptional factor controlling expression of proteins involved in fatty acid uptake, transport, derivation, and oxidation [22]. PPAR α signaling is impaired during hypoxia in neonatal cardiomyocytes as well as in hearts [23, 24], consistent with the observation that substrate preference shifts from fatty acid to glucose utilization during myocardial ischemia [25]. With respect to other mitochondrial substrates, our understanding of the regulation of factors involved in ketone body utilization is limited, despite its use during nutrient stress. Control of glucose use during oxygen availability is also not understood. By contrast, the transcriptional regulator of anaerobic

glycolytic metabolism has been identified as HIF-1, a heterodimer comprised of HIF-1 alpha (HIF-1 α) conferring biological specificity and HIF-1 beta (HIF-1 β) required for biological activity [26]. HIF members are transcriptional regulators of genes involved in hypoxic and other stress responses (Fig. 1) [27, 28]. With completion of the human genome project, a second HIF alpha member closely related to HIF-1 α in composition and regulation was identified, Endothelial PAS domain protein 1 (*EPAS1*), encoding HIF-2 α [29]. Although a wealth of data has been generated about HIF-1 over the past decade, the founding member of the HIF family, less is known about HIF-2. Nevertheless, distinct and, in some cases, complementary roles are emerging with respect to HIF-1 and HIF-2.

Biological roles for HIF-1 and HIF-2 in intermediary metabolism

The role of HIF-1 appears to be the most important in the early phase after exposure to stress where a transition from aerobic to anaerobic metabolism is essential for cell survival. HIF-1 controls expression of cellular factors involved in glucose metabolism which allows cells to shift from aerobic metabolism to anaerobic glycolysis [30], a role analogous to PPAR α and its control of fatty acid metabolism. Elimination of HIF-1 α results in a muted glycolytic response to hypoxia [30], whereas the loss of HIF-2 α has no effect on glycolysis [31], consistent with a selective role for HIF-1 α in control of glycolytic enzyme gene expression. HIF-1 also affects mitochondrial-dependent metabolism by at least three mechanisms. First, HIF-1 induces

Pdk1 encoding pyruvate dehydrogenase kinase 1 [32, 33], a negative regulator of the pyruvate dehydrogenase complex (PDH). PDH is the nexus for pyruvate entry into the mitochondrial oxidation pathway. Thus, increases in *Pdk1* levels results in inhibition of PDH activity, reduced entry of pyruvate into mitochondria, and a subsequent reduction of mitochondrial respiration. Second, HIF-1 inhibits mitochondrial respiration by altering composition of the cytochrome oxidase complex [34]. HIF-1 induces expression of COX4-2 and LON, with the net result being more efficient mitochondrial respiration during times of oxygen deficit. Third, HIF-1 acts in conjunction with c-myc to induce expression of HKII [35], a glycolytic enzyme that shifts glucose away from mitochondrial utilization and also has antioxidant effects [36].

HIF-2 appears to regulate processes that are important in more long-term adaptive or anticipatory responses. The latter include the desired, but potentially perilous, transition back to an oxygenated state. HIF-2 functions to maintain mitochondrial homeostasis by regulating production of cellular factors that alleviate oxidative stress [37, 38]. HIF-2 α null mice exhibit multiple organ pathology resembling a mitochondrial disease state as well as abnormal liver mitochondrial function [37, 38]. The biochemical basis for the HIF-2 α null phenotype in liver involves increased oxidative stress due to reduced expression of antioxidant enzyme (AOE) genes including *Sod2* [38], encoding an essential modulator of mitochondrial oxidative stress [39, 40], and *Frataxin* [37], encoding the oxidative stress-regulated chaperone protein for mitochondrial aconitase. Thus, whereas HIF-1 is a pathway regulator of genes encoding glycolytic enzymes, HIF-2 regulates production of factors important for maintaining mitochondrial homeostasis. Whether these findings for HIF-2 α in the liver hold true for the heart is unknown.

Biological roles for HIF-1 and HIF-2 in cardiac angiogenesis

Hypoxia and HIF factors have been implicated as master regulators of cardiovascular development [41, 42]. Although HIF-1 and HIF-2 may have overlapping transcriptional regulation of proangiogenic genes, transgenic mouse studies support specific activities for HIF-1 and HIF-2 in vascular development that includes stage-, cell-, and tissue-specific roles. Global HIF-1 α knockout mice have abnormal vascular as well as cardiac development [43, 44]. In comparison, global HIF-2 α knockout embryos have more subtle vascular defects [45]. A dominant negative form of HIF-1 α that interferes with both HIF-1 and HIF-2 signaling impairs vascular development in the embryo when expressed in vascular endothelial cells [46]. Cardiomyocyte-

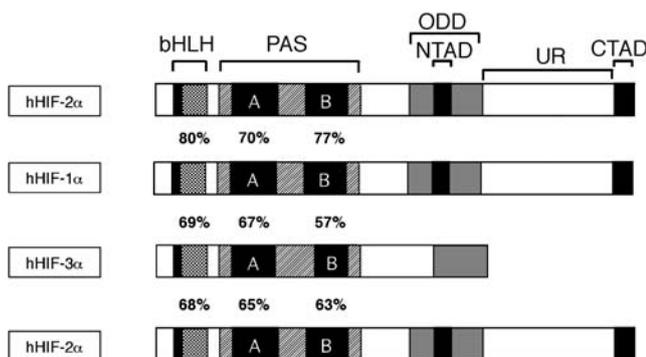


Fig. 1 Alignment of HIF-1 α , HIF-2 α , and HIF-3 α . Homology (percent identity) comparisons of the conserved basic helix-loop-helix (*bHLH*) and PAS-A,B repeats located within the PAS domain (*PAS*). Also indicated are the conserved amino-terminal activation domain (*NTAD*) located within the oxygen-dependent degradation domain (*ODD*) and the carboxy-terminal activation domain (*CTAD*) region. HIF-1 α and HIF-2 α have similar structural and regulatory features whereas spliced variants of HIF-3 α contain none or one (not shown) of the activation domains. The unique regions (*UR*) may confer specific determinants of HIF-1 α and HIF-2 α activation

specific HIF-1 α knockout mice have lower capillary densities and reduced expression of proangiogenic factors [47]; it has not been reported whether endothelial cell-specific HIF-1 α knockout mice have any cardiac vascular phenotype [48]. Restoration of HIF-2 α expression in endothelial cells of global HIF-2 α knockout mice does not fully rescue the HIF-2 α null vascular phenotype [49], suggesting that HIF-2 α expression in other cell types may be required for normal vascular development.

Translational experiments support potential therapeutic roles for HIF-1 and HIF-2 in cardiac angiogenesis. In cell culture models, HIF-1 α or HIF-2 α over-expression results in increased gene expression of a variety of proangiogenic factors in macrophages [50], cardiomyocytes [51], and vascular endothelial cells [52–55], cell types relevant to the ischemic and infarcted myocardium. Transgenic mice over-expressing HIF-1 α in the heart and subjected to myocardial infarction have increased capillary density in the infarct and peri-infarct zones [56]. Gene delivery of constitutively active HIF-1 α in a rat myocardial infarct model reduces infarct size and increases neoangiogenesis in the peri-infarct zone [57]. Knockout mice lacking the gene encoding the HIF prolyl hydroxylase PHD2, the enzyme targeting HIF α proteins for degradation during normoxia, have elevated levels of HIF-1 α , increased number of mature medium-sized vessels, and enlarged capillaries in the subendocardial region [58]. A potential role for HIF-1 in cardiac vessel formation in human hearts is supported by the finding that a HIF-1 α polymorphism is associated with reduced coronary artery collateral formation, although the mechanism behind this observation remains to be determined [59].

Relevance of HIF factors to cardiac ischemia/reperfusion injury

Environmental conditions present in ischemia/reperfusion are of direct relevance to HIF biology. HIF proteins are activated by oxidative stress [60–63]. HIF proteins are increased in the peri-infarct area after myocardial infarction in rats [64] and humans [65–67] with HIF-2 α expression occurring in remote areas from the infarct [64] suggesting a broader role for HIF-2 in ischemia/reperfusion biology. HIF-2 α has several unique aspects pertinent to oxidative stress and ischemia/reperfusion biology. DNA binding activity of HIF-2 α , but not HIF-1 α , may be differentially regulated by redox conditions [68]. As mentioned previously, HIF-2 α in vivo regulates major AOE and Frataxin gene expression in the liver [37, 38], cellular factors intimately involved in ROS and mitochondrial homeostasis in the heart [69]. More recently, studies with a HIF-2 α knockdown model identified a role for HIF-2 in protection

against ischemia/reperfusion injury in the kidney ascribed to HIF-2 regulation of major AOE gene expression [70], confirming the earlier findings of HIF-2 regulation of AOE gene expression.

Although it has been recognized for a decade that HIF transcription factors are activated by environmental stress, the role of HIF members in cardiac ischemic injury remains unknown. Stabilization of HIF members by proteins that modulate HIF stability [71] or by siRNA directed against PHD2 [72] provides protection against ischemia-induced damage. Over-expression of constitutively active HIF-1 in rat cardiomyocytes confers resistance against ischemia/reperfusion injury in a cell culture model [73]. HIF-1 over-expression in mice reduces infarct size and results in improved cardiac function after myocardial infarction [56]. Yet whether endogenous HIF member(s) confer this protective response during preconditioning is unknown. Mice partially deficient in HIF-1 have increased susceptibility to cardiac ischemia/reperfusion injury after whole animal hypoxia exposure [74], but this has been attributed to impaired induction of renal erythropoietin. Additional research will be needed to identify which HIF member(s) are important in the cardiac preconditioning response and whether HIF factors play an active role locally in the heart.

Conclusion and future perspectives

Cardiovascular diseases are leading causes of morbidity and mortality in the USA and western world. In the USA, acute and chronic treatments of cardiovascular disease states accounts for a substantial portion of USA healthcare expenditures. While conditions leading to myocardial injury have been well studied, endogenous protective responses that preserve cellular and organelle function, particularly mitochondria, during ischemia are poorly understood and thus are not targeted in current therapeutic strategies. The roles for HIF-1 and HIF-2 in myocardial ischemia are currently a largely undefined area of HIF biology. HIF-1 likely regulates glucose utilization in the heart during oxygen deprivation and may play a critical role in regulation of the angiogenic response after myocardial injury. HIF-2 has a complementary role to HIF-1 in regulation of intermediary metabolism in the liver, although its role in the heart remains to be defined, and may also be important in the angiogenic response of the heart. A clearer picture of the roles of HIF-1 and HIF-2 in cardiac biology will emerge with active investigation. If HIF factors prove to be essential factors that orchestrate protective cellular responses to cardiac ischemia, then studies of HIF signaling in the heart may lead to improved prevention or treatment of cardiovascular diseases using focused drug discovery approaches.

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