The heart forms early in development and delivers oxygenated blood to the rest of the embryo. After birth, the heart requires kilograms of ATP each day to support contractility for the circulation. Cardiac metabolism is omnivorous, utilizing multiple substrates and metabolic pathways to produce this energy. Cardiac development, metabolic tuning, and the response to ischemia are all regulated in part by the hypoxia-inducible factors (HIFs), central components of essential signaling pathways that respond to hypoxia. Here we review the actions of HIF1, HIF2, and HIF3 in the heart, from their roles in development and metabolism to their activity in regeneration and preconditioning strategies. We also discuss recent work on the role of HIFs in atherosclerosis, the precipitating cause of myocardial ischemia and the leading cause of death in the developed world.
HIF in the heart: development, metabolism, ischemia, and atherosclerosis

Andrew Kekūpa’a Knutson, Allison L. Williams, William A. Boisvert, and Ralph V. Shohet

Center for Cardiovascular Research, John A. Burns School of Medicine, University of Hawai‘i, Honolulu, Hawai‘i, USA.

The heart forms early in development and delivers oxygenated blood to the rest of the embryo. After birth, the heart requires kilograms of ATP each day to support contractility for the circulation. Cardiac metabolism is omnivorous, utilizing multiple substrates and metabolic pathways to produce this energy. Cardiac development, metabolic tuning, and the response to ischemia are all regulated in part by the hypoxia-inducible factors (HIFs), central components of essential signaling pathways that respond to hypoxia. Here we review the actions of HIF1, HIF2, and HIF3 in the heart, from their roles in development and metabolism to their activity in regeneration and preconditioning strategies. We also discuss recent work on the role of HIFs in atherosclerosis, the precipitating cause of myocardial ischemia and the leading cause of death in the developed world.

Hypoxia-inducible factor 1 (HIF1) is the central actor of an ancient, highly conserved pathway that responds to low-oxygen conditions. This transcription factor is composed of two subunits: constitutively expressed HIF1β and oxygen-sensitive HIF1α (1). In normoxic conditions, the HIF prolyl hydroxylases (PHDs) and asparaginyl hydroxylase (Factor Inhibiting HIF or FIH, respectively) modify residues on HIF1α that target the protein for degradation and prevent its transcriptional activity (2, 3). During hypoxia, these posttranslational modifications are limited, allowing HIF1α to enter the nucleus, dimerize with HIF1β, and bind to genomic hypoxia response elements to promote transcription. Another HIF isoform, HIF2α, senses oxygen and plays a more restricted, albeit important role, especially in the vasculature (4). Less is known about the final member, HIF3α; analysis of this isoform has been complicated by multiple variants encoded from the Hif3a locus (i.e., NEPAS and IPAS). Additionally, full-length HIF3α lacks a C-terminal transactivation domain but still dimerizes with HIF1β, thereby acting as a negative regulator of HIF1 and HIF2 activity (5).

The heart depends on oxidative metabolism to produce the large amounts of ATP required to sustain contractility. It is exquisitely sensitive to hypoxia, and as a result, cardiac ischemia is the most common cause of death in the developed world. Beyond systemic roles in oxygen delivery, through erythropoiesis and angiogenesis, HIF-regulated pathways help to protect the heart against hypoxia, tuning the balance of metabolic pathways to provide ATP and activating cell-survival pathways. HIF has other important roles in the heart, directing embryonic development and response to limited oxygen delivery caused by coronary disease. It even plays a role in the progression of atherosclerotic arterial disease that underlies ischemia in many vascular beds, especially the heart. This Review examines what we have learned about the pleiotropic role of HIF in the heart, touching especially on its participation in cardiac development, metabolic response to stress, and atherosclerosis.

The HIF pathway in heart development

The heart is the first organ to form in the embryo, and its development is necessary for maturation of a working vasculature and formation of other organs. Numerous factors are responsible for the specification and maturation of cardiac cells, including transcription factors (e.g., NKX2.5, TBX, GATA4, MESP1), morphogens (e.g., BMP4, FGF, TGF-β), and epigenetic mechanisms (e.g., DNA methylation) (6, 7). Together, these pathways give rise to numerous heart cell types and lineages by initiating specific transcriptional programs (8). Studies over the past 25 years have shown important roles for oxygen and the HIF pathway during embryonic development, particularly in heart morphogenesis, with a still-improving mechanistic understanding (9–11).

In mice, heart development begins at embryonic day 7.5 (E7.5) with formation of the cardiac crescent and is largely complete by E15 (Figure 1A). During this time, localized regions of hypoxia are observed throughout the embryo, and the stabilization of HIFs tracks with these areas (12). The earliest evidence of nuclear HIF1α in the developing heart is at E8.5 (13), although it is likely stabilized even earlier in newly formed cardiac precursors. By E9.5, HIF1α is found in the myocardium of the outflow tract and the nascent ventricular and atrial chambers (14, 15). From E10.5 to E12.5, HIF1α becomes restricted to the compact myocardium but is absent in the forming trabeculae, endocardium, and epicardium. By E14.5, once septation and chamber formation have occurred, HIF1 is no longer detectable in most of the heart but persists in the interventricular septum (15). Little has been published regarding HIF2α protein in the embryonic heart; however, HIF2α mRNA is detected in cardiac tissues at E9 (16). HIF3α does not appear to be expressed in the embryonic heart, although a splicing variant of HIF3α, called Nepas, is found in the late embryonic and early postnatal heart (17).
HIF-regulated transcription is necessary for important cardiac developmental events including septation (14, 18) and trabeculation (15). Embryos lacking Hif1a arrest at E10 with small hearts and a hypocellular myocardium (19–21). The range of cardiac abnormalities with Hif1a deletion is broad and is influenced by the specific allele and mouse genetic background (ref. 22 and Table 1). Homozygous deletion of the Arnt gene, encoding HIF1β, also results in embryonic lethality at E10, with placental defects that may contribute to heart deformities (23, 24). Mice lacking Hif2a (also known as Epas1) either arrest in embryogenesis or survive to adulthood with multiple organ pathologies, again depending on the allele used and the mouse background (25–27). Hif3a deletion results in right ventricle hypertrophy, although this may arise from defects in lung remodeling, with the major player being NEPAS rather than full-length HIF3α (17). Stabilization of HIF1, through deletion of Phd2, can also have deleterious effects on heart development (28). As with Hif1b mutations, these heart defects may be partly due to defects in placenta development and subsequent oxygen deprivation of the embryo. CITED2, a negative transcriptional regulator of HIF1α, is also important for cardiac development (29, 30).

The confounding effects of deleting HIF genes in the entire embryo can be avoided by the use of tissue-specific conditional knockouts (Table 2). The earliest conditional knockout of Hif1a deletes Hif1a in the embryonic mesoderm at E6.5 using Mesp1-Cre. Only half of Hif1a fl/ΔMesp1-Cre+ embryos survived to E17.5, and heart abnormalities were observed in a third of surviving animals (31). In the same study, using an Nkx2.5-Cre line, which deletes Hif1a at E7.5 in cardiac precursors, a majority of Hif1a fl/ΔNkx2.5-Cre+ animals survived to E17.5, with only 14% presenting heart abnormalities. However, another study using a different Nkx2.5-Cre line found that most Hif1a fl/ΔNkx2.5-Cre+ mice died between E14.5 and E17.5, and almost all had ventricular abnormalities, including incomplete septation (14). This study also deleted Hif1a using other Cre lines, including Myh6-Cre in cardiomyocytes at E12, Wt1-Cre in epicardial cells at E9.5, and Tie2-Cre in endocardial cells at E9.5, and found that these mice all survive to birth. Experiments with conditional deletion of Hif1b in early cardiac lineages are sparse; only one study deleted this gene in adult cardiomyocytes (32). Further research into this factor could be partic-

---

**Table 1. Phenotypes of mice null for HIF pathway genes**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Phenotype</th>
<th>Mouse background</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hif1a</td>
<td>Hif1atmRsjo</td>
<td>Lethal by E10; heart defects; myocardial thinning</td>
<td>C57BL/6 × 129</td>
</tr>
<tr>
<td></td>
<td>Hif1atm1Jhu</td>
<td>Lethal by E10; heart defects; myocardial thinning</td>
<td>129/SvJ</td>
</tr>
<tr>
<td></td>
<td>Hif1atm1Pec</td>
<td>Lethal by E10; cardia bifida</td>
<td>129/SvJ × Swiss</td>
</tr>
<tr>
<td>Amt (i.e., Hif1b)</td>
<td>Amttm1Rcm</td>
<td>Lethal between E9.5 and E10.5 placental and heart defects</td>
<td>129/5v</td>
</tr>
<tr>
<td></td>
<td>Amttm1Rcm</td>
<td>Lethal between E9.5 and E10.5 placental defects</td>
<td>C57BL/6 × 129</td>
</tr>
<tr>
<td>Epas1 (i.e., Hif2a)</td>
<td>EpasPtmRcm</td>
<td>Lethal by E16.5</td>
<td>C57BL/6 × 129/5v</td>
</tr>
<tr>
<td></td>
<td>EpasPtmRcm</td>
<td>Lethal in utero</td>
<td>129 (isogenic)</td>
</tr>
<tr>
<td></td>
<td>EpasPtmRcm</td>
<td>Lethal in utero</td>
<td>C57BL/6 (congenic)</td>
</tr>
<tr>
<td></td>
<td>EpasPtmRcm</td>
<td>¼ survival; cardiac hypertrophy</td>
<td>C57BL/6 × 129</td>
</tr>
<tr>
<td></td>
<td>EpasPtmRcm</td>
<td>Lethal between E9.5 and E12.5</td>
<td>129/Sv</td>
</tr>
<tr>
<td>Hif3a</td>
<td>Hif3atmRcm</td>
<td>Viable; abnormal lung development; right ventricle hypertrophy</td>
<td>C57BL/6</td>
</tr>
<tr>
<td>Egln1 (i.e., Phd2)</td>
<td>EglnPtmRcm</td>
<td>Lethal between E12 and E14; placental and heart defects</td>
<td>C57BL/6 × 129/56</td>
</tr>
</tbody>
</table>
A master transcription factor, Nkx2.5, is known to be essential for heart development (14). Loss of Nkx2.5 in mice results in heart abnormalities, including septal defects and myocardium thinning, which can be attributed to the downregulation of genes critical for cardiac development (15, 16). These findings highlight the importance of Nkx2.5 in regulating early cardiac differentiation and the potential role of HIF1α in modulating these processes.

Recent studies have focused on the role of HIFs in cardiac development, especially in the context of congenital heart disease (CHD). CHD is the most common birth defect, occurring in 1 in 100 live births (45, 46). Approximately 10% of CHD arises from occasional autosomal dominant or inherited autosomal recessive mutations, and many genetic translocations, copy number variations, and point mutations have been identified over the past 20 years (47, 48). The remaining CHD cases are thought to arise from a combination of both genetic and environmental factors (49). Given the importance of the HIF axis in heart development, oxygen levels in utero and polymorphisms in HIF pathway genes may play a critical role in the development of CHD.

Numerous cell types contribute to the developing heart, including cardiomyocytes, fibroblasts, endothelial cells, and immune cells (37). The majority of work done so far has focused on HIF in cardiomyocytes, so it will be of interest to understand HIF’s role in other cell types that are likely to have distinct hypoxic responses. For example, coronary vasculature development is dependent on HIF, and HIF dysregulation may contribute to clinically important coronary malformations (38, 39). Recent evidence has also shown HIF’s involvement in sympathetic innervation of the heart (40). The transcriptional response to hypoxia likely integrates multiple factors independent of HIF, including chromatin factors that can sense oxygen and could be major effectors of transcriptional events found in the hypoxic embryonic heart (41, 42).

Still, the role for HIFs in launching cardiac developmental gene programs, possibly at the single-cell level, is necessary to identify direct and indirect effects of HIF activity. Numerous cell types contribute to the developing heart, including cardiomyocytes, fibroblasts, endothelial cells, and immune cells (37). The majority of work done so far has focused on HIF in cardiomyocytes, so it will be of interest to understand HIF’s role in other cell types that are likely to have distinct hypoxic responses. For example, coronary vasculature development is dependent on HIF, and HIF dysregulation may contribute to clinically important coronary malformations (38, 39). Recent evidence has also shown HIF’s involvement in sympathetic innervation of the heart (40). The transcriptional response to hypoxia likely integrates multiple factors independent of HIF, including chromatin factors that can sense oxygen and could be major effectors of transcriptional events found in the hypoxic embryonic heart (41, 42). Also, the relationship between HIF and noncoding RNAs is likely to be an additional axis influencing heart development (43, 44).

**HIF’s role in congenital heart disease**

Congenital heart disease (CHD) is the most common birth defect, occurring in 1 in 100 live births (45, 46). Approximately 10% of CHD arises from de novo autosomal dominant or inherited autosomal recessive mutations, and many genetic translocations, copy number variations, and point mutations have been identified over the past 20 years (47, 48). The remaining CHD cases are thought to arise from a combination of both genetic and environmental factors (49). Given the importance of the HIF axis in heart development, oxygen levels in utero and polymorphisms in HIF pathway genes may play a critical role in the development of CHD.
genes can contribute to CHD. Insights into this may lie in genetic population studies of groups that live at high altitudes, exposed to chronic hypoxia. Remarkably, CHD prevalence has been reported to be ten times higher in these groups than in groups living at sea level (50). Certain genetic polymorphisms, such as those found in Hif2a, that have allowed adaptation to high altitude have also been implicated in contributing to abnormal heart development (26, 51). Efforts to model in utero hypoxia have revealed important insights into the potential contributors and events leading to CHD. In one study, exposure to hypoxia in mouse embryos lacking a single copy of either of the essential cardiac development genes Tbx1 and Nkx2.5 led to an increase in heart malformations and embryonic death (52). This genetic sensitization, combined with abnormal oxygen levels, highlights the combinatorial effects of genetics and environment on heart development. The epigenetic mechanisms linking in utero hypoxia to cardiovascular disease later in life, and possibly across generations, are a current research focus, although the roles that HIFs play are not yet clear (reviewed in ref. 53).

**HIF and cardiac regeneration**

Mature mammalian cardiomyocytes have very low proliferative capacity and are not replenished in damaged adult hearts to any substantial degree. Repopulating damaged areas of the heart with functional cardiomyocytes is a long-sought goal of regenerative medicine and may benefit from refinement of induced pluripotent stem cell techniques. Links between cellular stemness, hypoxia, and HIF activity have been established (54). Many adult stem cells reside in hypoxic niches, an environment that is thought to keep cells in an undifferentiated state, limiting oxidative DNA damage and promoting glycolysis as a main energy source (55). Additionally, genes encoding pluripotency factors (e.g., Oct4) are direct targets of the HIF pathway, specifically HIF2α, supporting the hypothesis that the HIF axis is a direct regulator of pluripotency. However, recent experiments have cast doubt on the presence of resident stem cells in the mammalian heart (56). Instead, a tiny population of adult cardiomyocytes may retain the ability to reenter the cell cycle under extreme circumstances, such as hypoxia (57).

Fate mapping has suggested a limited number of proliferative adult cardiomyocytes (58). The role of hypoxia and HIFs in this process is now being explored. In one study, adult hypoxic cardiomyocytes were identified by fusing of HIF1α’s oxygen-dependent degradation domain to a tamoxifen-inducible CreERT2 recombinase (57). Introducing this transgene into mice harboring a floxed-TdTomato reporter turns cells red (TdT+) if they are hypoxic at the time of tamoxifen treatment. In 1-month-old mice, a small percentage of cardiomyocytes were hypoxic by this assay. Remarkably, the number of TdT+ cardiomyocytes, albeit small, increased over time without additional tamoxifen treatment, suggesting that a tiny number of cardiomyocytes (0.01%–0.06% of cells) in the adult can reenter the cell cycle. Molecular characterization of these rare cardiomyocytes may reveal what makes these cells special, with hopes to instill these features into nondividing cardiomyocytes.

In contrast to mammals, zebrafish and amphibians efficiently repair damaged hearts through a process of dedifferentiation and proliferation of existing cardiomyocytes (59). Ventricular amputation of zebrafish hearts induces localized hypoxia at the resection site that promotes cardiomyocyte dedifferentiation and proliferaiton (60). Expression of a dominant-negative form of HIF1α in these hearts or exposure to hyperoxia prevented this regeneration. Interestingly, in the more regenerative fish heart, HIF-mediated vascular growth contributes to cardiomyocyte repopulation by serving as a cellular scaffold (61). The neonatal mouse heart is able to regenerate for a few days after birth, and exposing adult mice to hypoxia may also promote cardiac regeneration (62, 63). Recently, moderate hypoxia was shown to affect human cardiomyocyte proliferation through upregulation of YAP1 (64). Current clinical trials are exploring whether hypoxia can ameliorate cardiac damage in humans (65, 66). At this time, the mechanisms underlying cardiomyocyte cell cycle reentry under hypoxic conditions are not fully understood, and the role HIFs play in this process remains undefined.

**HIF and cardiac metabolism**

HIF plays a crucial role in shaping the metabolic response in the heart (Figure 2). The heart synthesizes and consumes approximately 6 kg of ATP every day, largely for contraction and ion flux (67, 68). Cellular metabolism is inextricably linked with cardiac contractility. In the healthy human heart, oxidative phosphorylation generates almost all of the ATP (~95%), with fatty acids utilized as substrate (~70%) (67). Glucose and other carbohydrates provide most of the remainder, with minor contributions from other pathways (67). However, given the crucial, unremitting need for proper cardiac function, the heart is omnivorous and will consume any substrate available to sustain contractility, as seen in the increased reliance on glucose in aging and failing hearts (67). Glycolysis is also increased in other cardiac diseases, and in diabetes there is increased consumption of ketone bodies (67, 69, 70).

While HIF1α promotes glycolysis during hypoxia, it also influences metabolism under normoxic conditions. Deleting HIF1α in cardiomyocytes alone causes decreased ATP, lactate, and phosphocreatine levels, in addition to impaired cardiac contractility (71). Paradoxically, overexpressing myocardial HIF1α can also produce contractile dysfunction, despite increased ATP, glucose metabolism, glycogen stores, and lactate production (72–74). Similarly, cardiac loss of PHD or VHL function, which stabilizes HIF1α (and HIF2α), also leads to upregulation of glycolytic genes and concomitant loss of contractility (75–77). These results suggest that tight control of HIF1α expression is crucial to proper cardiac function and that HIF1α’s effects on metabolism extend beyond glycolysis.

HIF1α also influences mitochondrial metabolism. Reduced HIF activity leads to mitochondrial loss and lipid accumulation, along with decreased oxidative phosphorylation and fatty acid metabolism (72, 73, 76, 77). Reactive oxygen species (ROS) are also generated during hypoxia from disruption of the electron transport chain and a perturbed redox state (78). Detailed work has demonstrated that HIF1α mitigates this toxicity by limiting TCA activity and decreasing mitochondrial mass through upregulation of the mitochondrial proteins PDK1 and BNIP3 (78–81). HIF1 expression and activation can also be regulated by metabolite feedback loops. For example, increased glucose (or GLUT1 expression) limits HIF1α expression through upregulation of ubiquitin degradation pathways (82). Also, in addition to its role in the TCA cycle, succinate is generated as a byproduct of an α-ketoglutarate–dependent PHD hydroxylation reaction and can act as a rheostat to modulate PHD activity and HIF stability (83–86).
targets including Glut1, Epo, and Glut4 (92). HIF3 expression was also found to be upregulated in response to insulin (93). Additional studies have demonstrated that DMOG, desferrioxamine, PHD inhibitors, and other small molecules can also modulate HIF-dependent metabolic processes (83, 94, 95). While HIF1 appears to be the major regulator of cellular metabolism, further study will be necessary to specifically delineate the roles of each of the HIF proteins and regulators in cardiovascular metabolism.

Unsurprisingly, HIF signaling affects the metabolic response to heart disease in myriad ways. Many cardiac pathologies lead to reduced oxygen availability either directly (reduced oxygen delivery) or indirectly (mismatch between demand and supply). Both HIF1 and HIF2 are expressed after acute ischemic injury, and HIF1 has been shown to be protective, at least partly as a result of increased glycolysis and reduced oxidative metabolism (74, 96–100). Chronic ischemia can develop from atherosclerosis, aortic stenosis, hypertrophic cardiomyopathy, and hypertension, all of which can lead to heart failure. Although often described as “non-ischemic cardiomyopathy,” hypertrophy and dilation can also produce stabilization of HIF1 and upregulation of its metabolic pathways (73, 101). Given the reduced ATP production of glycolysis compared with oxidative phosphorylation, long-term HIF1 stabilization may not always be beneficial, as observed in heart failure

Work in cancer cells shows that several additional citric acid and glycolytic metabolites can also regulate PHD activity and HIF stability (84, 85, 87), but additional mechanistic studies will be needed to assess their importance in the heart.

While HIF1 drives the initial response to hypoxia, HIF2 appears to regulate the long-term response to restore oxygen homeostasis. Global loss of HIF2α leads to cardiac hypertrophy, along with impaired mitochondrial metabolism and increased ROS generation due to loss of antioxidant enzymes (25). Cardiomyocyte-specific loss of HIF2α increases ischemic damage (88). HIF2α overexpression in cardiomyocytes also increases transcription of HIF target genes (and produces a dilated cardiomyopathy) (75), indicating a role for HIF2 in chronic hypoxia and mitochondrial maintenance. Additionally, VHL deletion in adipocytes causes cardiac hypertrophy via HIF2-dependent changes in lipid metabolism and inflammatory signaling (89). Interestingly, global loss of FIH, the other HIF hydroxylase, leads to an increased metabolic rate and insulin sensitivity that does not coincide with increases in glycolysis, fatty acid oxidation, or HIF1 target gene expression (90, 91). Rather, FIH appears to regulate mitochondrial ROS production and glycogen storage (91). Limited data exist on HIF3 in the heart, but recent work in cardiomyocytes in vitro indicates it may behave similarly to PHDs by limiting expression of typical HIF1

---

**Figure 2. Effects of HIF on metabolism.** HIF expression or stabilization has many effects on cell metabolism, both directly and indirectly. Under normoxic conditions, HIFα and HIF2α (HIFα) are hydroxylated by PHDs (and FIH) using available oxygen and α-ketoglutarate, which leads to proteosomal degradation. When oxygen tension is low, HIFα translocates to the nucleus, where it binds to DNA with its heterodimeric binding partner HIFβ to initiate transcription of HIF target genes. These transcription products affect all levels of cellular metabolism to reduce oxidative phosphorylation (OXPHOS) in mitochondria and favor glycolysis, from substrate transport to apoptotic signaling molecules (e.g., BNIP3). Some evidence suggests that HIFα may also directly modulate mitochondrial metabolism, through a currently undefined mechanism. Metabolite levels are independently affected by changes in oxygen tension and HIF expression through reduction of PHD activity, which influences concentrations of several citric acid metabolites (e.g., α-ketoglutarate and succinate). Dashed lines indicate transport across the mitochondrial membrane, where these metabolites serve distinct roles in each location. PPP, pentose phosphate pathway.
patients whose hearts utilize glycolysis to a greater degree than those of healthy counterparts (67, 69, 73, 101). Recent work also indicates that HIF-dependent maladaptive pathways, e.g., fructose, can be upregulated under cardiac stress and contribute to cardiac pathology (101). Diabetes, obesity, and insulin resistance can also modify HIF’s metabolic effects through reduced HIF1 expression and glucose uptake as well as increased oxidative stress (86, 102–105). Given the increasing prevalence of diabetes and metabolic disorders, additional study of the effects of metabolic stress on HIF signaling in the heart is needed to develop more effective therapies for these diseases.

**HIF and angiogenesis**

Following the path-breaking work of Judah Folkman (106), the understanding of angiogenesis has burgeoned over the past 30 years. This work initially focused on VEGF and a few other molecules for positive angiogenic treatments, and their antagonists for cancer therapies and age-related macular degeneration. Exciting work has also been done on angiogenesis in noncardiac vascular beds, especially the retina and peripheral extremities; these models form the basis of much of our understanding of clinical angiogenesis and have been well reviewed elsewhere (107–111).

Genetic evidence suggests that modulation of HIF activity in the cardiovascular system could have clinical benefit. Several SNPs at the Hif1a locus are associated with vascular response (112–115). As it became clear that HIF was a principal regulator of angiogenesis, mediating VEGF expression and a host of other molecules, clinical efforts focused on increasing HIF1 expression in peripheral artery disease to induce collateral circulation in the lower extremities. While preclinical and phase I studies produced encouraging results, a larger clinical trial ultimately found no significant functional improvement (109, 112, 116–118). Preclinical work also indicated that viral expression of HIF1 could improve cardiac perfusion after ischemia (119); however, subsequent clinical studies were less encouraging (120, 121).

Although much focus has been on HIF1, HIF2 also plays an important, distinctive role in angiogenesis. Like HIF1, several HIF2 (Epas1) knockout models displayed vascular defects, although the specific phenotypes differed between genetic backgrounds and from those seen in HIF1 deletion (122). While HIF1 and HIF2 share a number of transcriptional targets to activate angiogenesis, the limited and cell-specific nature of HIF2 expression (brain, heart, lungs, liver, kidneys, intestines, vasculature) suggests it serves a nonredundant role in this process (123). Studies to date indicate that HIF1 is crucial to early steps (sprouting, intussusception), while HIF2 influences later stages of vessel stabilization and maturation (122, 124). Additionally, HIF1 and HIF2 have been reported to have different sensitivities to oxygen tension (with higher HIF1 hydroxylation and turnover at physiological levels of hypoxia) and different temporal expression in the hypoxic endothelium (HIF1 > HIF2 in the first 24 hours, HIF2 > HIF1 after 24 hours), which appears to support their complementary roles in vessel formation and development (124, 125). Limited data on HIF3 suggest it can also modulate angiogenesis, with delayed expression in the endothelium upon hypoxia exposure (similar to HIF2) and some effects on heart, lung, and vascular development (125). While therapeutic modulation of HIF2 in clinical trials has largely been limited to small-molecule inhibitors to limit tumor vascular formation, preclinical study of HIF2 stabilization indicates that it may improve perfusion in ischemic injury (122, 126). For now, more definitive evaluation of HIF modulation for cardiac angiogenesis awaits more specific therapeutics and delivery methods, improved noninvasive evaluation of perfusion, and a deeper understanding of the underlying molecular signaling.

**HIF and preconditioning**

The concept of preconditioning tissue with short intervals of ischemia to limit more severe ischemic injury has been a tantalizingly attractive clinical strategy for decades. Naturally occurring responses to ischemia in the heart suggest the benefits of this approach. Patients with myocardial infarction who had preceding angina or collateral vessels had better recovery than those who did not (112, 127, 128), suggesting that prior exposure to hypoxia and angiogenesis, or perhaps “remodeling” of metabolism, both of which are likely to be HIF-related, may be protective. A number of recent reviews discuss HIF and preconditioning in the heart (10, 112, 129–131). Treatment, whether it is ischemia, hypoxia, or small molecules that activate related pathways, can be delivered either directly to the tissue of interest, or indirectly (often referred to as remote preconditioning). The timing of treatment can also vary, including before (preconditioning), during, or after prolonged ischemic insult (postconditioning). Here we highlight a few recent publications that provide new insight into mechanisms underlying the protective effects of preconditioning.

Both genetic and pharmacological studies suggest that HIF is involved in preconditioning. Many of these protective effects relate to transcriptional activity in known pathways that promote glycolysis and oxygen delivery, or minimize oxidative metabolism, which all fall into the late phase of preconditioning (>24 hours after treatment) (10, 112, 130). However, some evidence suggests that HIF1 is also involved in the early phase of preconditioning (within hours of therapy) before transcription is upregulated, possibly by directly modulating mitochondrial metabolism (78, 132). The advantages of HIF signaling are not limited to cardiomyocytes, as deletion of HIF1 in endothelial cells also abolishes cardioprotection conferred by ischemic preconditioning (112, 133). Interestingly, while HIF is involved in several of these pathways, its activation may not be necessary for some aspects of protection, as regulation of downstream molecules such as adenosine is also beneficial (112, 129–131, 134). These results suggest that in addition to HIF itself, identification of HIF targets involved in preconditioning may produce additional candidates to more specifically modulate cardioprotective pathways.

In addition to direct preconditioning, there has been exciting recent progress in understanding remote preconditioning to protect the heart. These models usually involve temporary ischemia of a remote region prior to cardiac injury, typically a limb, but systemic treatments to alter oxygen availability can also be considered forms of remote conditioning. Numerous studies have found that exposure to ambient hypoxia, administration of hypoxic blood, or small-molecule inhibitors of HIF degradation can confer cardiac protection before ischemic injury (10, 63, 100, 112, 129, 135, 136). While remote preconditioning can provide protection through both humoral and neural pathways, humoral factors...
lesional macrophage foam cell function by rendering the cells more inflammatory and apoptotic while suppressing their capacity to metabolize lipids.

α-proliferation of these cells in the atheroma by upregulating factors such as CD98 and macrophage migration inhibitory factor (MIF). HIF1-α types in atherosclerosis: endothelial cells, macrophages, and smooth muscle cells. Whereas HIF1-α plays multiple roles in the development of atherosclerosis. This sketch shows the major effects of HIF1-α on the three most important cell types in atherosclerosis: endothelial cells, macrophages, and smooth muscle cells. Whereas HIF1-α directly induces vascular endothelial growth factor (VEGF), endothelin-1, and matrix metalloproteinases (MMPs) in endothelial cells to facilitate angiogenesis, its effect on vascular smooth muscle cells is to induce proliferation of these cells in the atheroma by upregulating factors such as CD98 and macrophage migration inhibitory factor (MIF). HIF1-α also regulates lesional macrophage foam cell function by rendering the cells more inflammatory and apoptotic while suppressing their capacity to metabolize lipids.

While much preclinical work has focused on preconditioning, this may not be a realistic option for most at-risk patients. Postconditioning could provide clinical benefit, especially if it could be applied at the time of coronary intervention. Studies have suggested that ischemic postconditioning can limit ischemic damage in the heart, but others indicate this treatment may not offer long-term benefits (129, 131, 137, 138). Pharmacological modulation through PHD inhibitors and/or HIF antagonists could allow more precise targeting and temporal regulation of HIF signaling to enhance its protective effects in future studies of postconditioning.

Since the first demonstration of ischemic preconditioning of the heart (139), substantial effort has gone into elucidating the mechanisms of this protection for clinical application. Unfortunately, inconsistency in the timing and delivery of preconditioning therapies in both preclinical and clinical studies makes it difficult to compare results and discern the ideal window for treatment (137, 140). These issues, combined with difficulties inherent in excluding confounding factors such as age, comorbidities, and patient presentation, limit the conclusions that can be drawn from work thus far (137, 140). Meaningful progress on new HIF-based therapies will only be possible when better preclinical models are combined with carefully controlled clinical trials and relevant endpoints.

The role of HIF in atherosclerosis

Atherosclerosis of the coronary arteries, with subsequent thrombotic occlusion, is the principal cause of myocardial infarction. Among the numerous factors contributing to atherosclerosis, the two most important are dyslipidemia and inflammation. It is generally accepted that the macrophage is the most important inflammatory cell in the etiology of atherosclerosis owing to its ability to take up and process lipids and its contribution to inflammation in the plaque. Vascular smooth muscle cells (VSMCs) also play an important role, as their proliferation and migration toward the neointima form the fibrous cap that protects the inflammatory contents of the plaque from contacting the blood and initiating a thrombus. Endothelial cells (ECs) overlying the artery play a key role in regulating the influx of lipoprotein particles and inflammatory cells into the intimal space where atherosclerosis occurs.

Hypoxia plays an important role in atherogenesis (141). Over years, buildup of extracellular matrix and lipid material leads to formation of hypoxic areas in the plaque. This is especially true of macrophage-rich areas (142), and VSMCs and ECs also both respond to hypoxia by expressing HIF. Whereas direct evidence of HIF1-α expression is seen in both carotid and femoral endarterectomy from patients (143), strongly indicating its participation in atherogenesis, little is known about HIF2-α’s role. Many HIF1-regulated genes are expressed in the plaque region, including VEGF and endothelin-1 (144). While HIF1 influences macrophage functions known to mediate atherogenesis, it also regulates other innate immune cells such as neutrophils (145–147) and dendritic cells (148–150) as well as adaptive T and B cells (151–156), in which HIF1 controls cellular functions such as survival, migration, and cytokine production. Although the effects of HIF expression can be wide-ranging, its effects in the three most important cell types of atherosclerosis — macrophages, VSMCs, and ECs — are of particular interest (Figure 3).

Macrophages. Macrophages contribute to atherogenesis through multiple pathways, and hypoxia affects many of these (157, 158). Atherosclerotic plaque is hypoxic. Under these hypoxic conditions, lipid accumulation is increased, the ability of macrophages to efflux cholesterol is compromised, and HIF1-α also directly suppresses the expression of ABCA1, an important transport protein that facilitates the excretion of cholesterol out of lipid-loaded macrophages (159–161). Interestingly, ApoA1, the key apolipoprotein that acts as a cholesterol acceptor in reverse cholesterol transport, was recently found to be expressed by macrophages, and its expression is also inhibited by HIF1-α (160). Studies using HIF1-α-deficient myeloid cells have revealed that HIF1-α has a pleiotropic role in macrophage function, including its ability to regulate glucose metabolism,
chemotaxis, and apoptosis (162). It is possible that the increase in apoptosis is due to accumulation of unesterified cholesterol in macrophages under hypoxic conditions (163). In vivo studies with LDL receptor-null mice transplanted with bone marrow isolated from HIF1α-null mice showed that HIF1α deficiency in myeloid cells led to a 72% reduction in atherosclerosis in these mice (162).

HIF1α is intimately involved in the inflammatory response of macrophages. Activating macrophages with agents such as LPS or IFN-γ prompts these cells to become proinflammatory through HIF1α activation, eventually leading to production of cytokines like TNF-α and IL-6 as well as inducible NO synthase (iNOS) (164). This is due, in part, to the activation of macrophages leading to the accumulation of succinate, which acts to stabilize HIF1α and boost the transcription of proinflammatory genes (165). Moreover, deficiency of HIF1α in macrophages leads the cells to adopt an antiinflammatory M2 polarization phenotype (166). HIF2α also appears to regulate M2 polarization in macrophages (167). Whereas LPS or IFN-γ increases the production of HIF1α, they strongly suppress HIF2α expression. On the other hand, M2-inducing agents such as IL-4 and IL-13 enhance the production of HIF2α.

**Vascular smooth muscle cells.** A key feature of atherosclerosis is the migration and proliferation of VSMCs. Once in the neointima, VSMCs secrete collagen and other structural materials that form the plaque’s fibrous cap. Hypoxia and HIF1α play an important role in VSMC proliferation (168), but the timing of this proliferation has not been determined. It is also not clear exactly what triggers the migration of these cells, although hypoxia is a leading candidate (169). Growth factors such as PDGF and FGF may trigger VSMC mobility (170); whether or not these factors play a role in vivo and the extent to which they are regulated by hypoxia have not been conclusively determined. A recent study has identified CD98 as a potential inducer of VSMC proliferation during intermediate stages of atherosclerosis (171). It is interesting that the heavy chain of CD98, also known as SLC3A2, is a target of HIF1α. Intermittent hypoxia was also found to augment the VSMC proliferation by upregulating inflammatory factors such as IL-6 and epiregulin (172).

MIF is another important cytokine in VSMCs that responds to hypoxia. Stabilization of HIF1α leads to increased MIF production, whereas deletion of HIF1α in SMCs leads to reduced MIF production (173). The increase in proliferation seen in SMCs cultured under hypoxic conditions is abrogated when the cells are treated with MIF siRNA (173). HIF1α can also contribute to the calcification of atherosclerotic plaques by mediating the osteochondrogenic differentiation of VSMCs (174).

**Endothelial cells.** ECs do not normally encounter hypoxia, as they are bathed in a constant flow of blood. Thus, it appears that HIF1α expression in ECs is triggered by other factors such as lipids, cytokines, or reactive oxidative products that may be induced during inflammation. HIF1α expressed by ECs can promote monocyte chemotaxis to the atherosclerosis-prone vessel wall and thereby contribute to the growth of atherosclerotic plaque (175). In hypoxia, when blood flow is interrupted or oxygen-carrying capacity of the blood is diminished, stabilized HIF1α has substantial functional effects in the endothelium. In addition to VEGF, HIF1α upregulates the VEGF receptors VEGFR1 and VEGFR2 on ECs. Atherosclerosis-related changes in endothelial permeability and EC proliferation are affected by VEGF interacting with its receptors (176).

Angiogenesis is a key feature of atherosclerosis. HIF1α mediates the expression of several genes, including endothelin-1 (177), matrix metalloproteinase (178), and VEGF (179), that facilitate angiogenesis in the plaque. Interestingly, while oxidized LDL can promote angiogenesis by inducing HIF1α expression in macrophages (180), native LDL has an antiangiogenic effect by inhibiting HIF expression in ECs (181). One of the most important functions of EC is the production of NO as a powerful vasodilator. HIF1α upregulates iNOS as well as cytochrome c oxidase to increase NO production. This relaxes VSMCs and dilates the blood vessel, increasing oxygen supply to the tissue (182).

There is little doubt that HIF1α is a major player in the pathogenesis of atherosclerosis. However, whether HIF1α acts to exacerbate or inhibit atherosclerosis likely depends on the cell type in which it is expressed as well as the timing of its expression during the course of the disease. A key requirement in considering HIF1α as a therapeutic target for atherosclerosis would be specificity both in the delivery of HIF regulators to the plaque and in targeting of specific cells within the plaque. A greater understanding of the timing and cellular distribution of HIF1α in the plaque is needed to fulfill the promise of modulating the HIF axis in atherosclerosis.

**Summary**

We have reviewed only a portion of the recent work on the HIF axis in the heart. There are exciting new developments in each highlighted area that point to how little we know and how much there is to discover. This includes the potential antagonism of HIF1α and HIF2α and their effects on angiogenesis, fibrosis, and cardiac metabolism (183). Currently, several small molecules that modulate HIF signaling are being assessed in clinical trials (94). One of these compounds, the PHD inhibitor roxadustat, is currently under review in the United States and has already been approved in several countries for anemia associated with chronic kidney disease (184). Other agents that inhibit the action of HIF2α may be useful in pulmonary hypertension and polycythemia (185), and are in clinical trial for treatment of renal cell carcinoma (186). As these enter the clinic, it will be important to determine what effects these treatments may have on patients with heart disease. Perhaps as methods for tissue-specific gene expression and modulation advance, manipulation of HIF activity will become an attractive target in the heart itself. It is easy to imagine a role for such therapies in focused angiogenesis or metabolic modification. The HIF axis is a fertile area of investigation and, increasingly, is a ripe target for clinical manipulation. It will be important to balance the protective and maladaptive effects of HIF signaling to preserve, and improve, cardiac function.

**Acknowledgments**

AKK is supported by a Ruth Kirschstein Postdoctoral Fellowship (F32-HL149319) and a Burroughs Wellcome Fund Postdoctoral Enrichment Program Award. AKK and ALW have been supported by NIH grant T32-HL115505.

Address correspondence to: Ralph V. Shohet, Department of Medicine, John A. Burns School of Medicine, University of Hawaii, BSB 304, 651 Ilalo Street, Honolulu, Hawaii 96713, USA. Phone: 808.692.1469; Email: shohet@hawaii.edu.


98. Kido M, et al. Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progres-


