Targeting hypoxia-inducible factor 1 to stimulate tissue vascularization

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Received 31 March 2015
Accepted 6 April 2015
Published Online First 11 January 2016

ABSTRACT

When tissue perfusion is impaired, the resulting reduction in O_2 availability activates hypoxia-inducible factor 1 (HIF-1), which mediates increased transcription of genes encoding multiple angiogenic factors including vascular endothelial growth factor, stromal-derived factor 1, placental growth factor, and angiopoietins, leading to the mobilization of bone marrow-derived angiogenic cells, increased angiogenesis, and arterial remodeling. These HIF-1-dependent responses are impaired by aging or loss of function mutations at the locus encoding the HIF-1α subunit. in mouse models of limb ischemia and lung transplant rejection, the augmentation of HIF-1 activity by gene therapy or chemical inducers was associated with maintenance of tissue perfusion that prevented limb amputation and allograft rejection, respectively. Thus, targeting HIF-1 may be of therapeutic benefit in these clinical contexts and others in which impaired tissue perfusion plays a role in disease pathogenesis.

Ischemic cardiovascular disease due to stenosis of a major artery in the heart (coronary artery disease) or leg (peripheral arterial disease [PAD]) is a major cause of morbidity and mortality in the US population. The frequency and severity of these disorders increase in an age-dependent manner. Maintenance of tissue vascularization is also critical to prevent the rejection of solid organ transplants. Tissue perfusion is tightly regulated to match O_2 supply with O_2 demand, primarily for mitochondrial respiration.

The homeostatic regulation of tissue perfusion is dependent on the activity of hypoxia-inducible factor 1 (HIF-1), which is a transcription factor that is composed of HIF-1α and HIF-1β subunits.[1 2] The HIF-1α subunit accumulates in response to reduced availability in an exponential manner, with dramatic increases at O_2 concentrations below 6% (PO_2 ~40 mm Hg), leading to HIF-1α levels that are half-maximal at 1.5% O_2 (PO_2~10 mm Hg) and maximal at 0.5% O_2.[3] HIF-1α protein is subjected to O_2-dependent hydroxylation, ubiquitination, and proteasomal degradation.[4] The prolyl-4-hydroxylase domain proteins PHD1, PHD2, and PHD3 use O_2 and α-ketoglutarate as substrates to hydroxylate HIF-1α on proline residue 402 or 564, generating succinate and CO_2 as by-products.[5] HIF-1 activity was known to be induced under nonhypoxic conditions by treatment of cells with Co(II) or desferrioxamine,[6] which are believed to exchange with or chelate, respectively, Fe(II) in the catalytic center of the hydroxylases, rendering them inactive; athird class of HIF inducers are α-ketoglutarate analogs, such as dimethyloxalylglycine.[5]

HIF-1 functions as a master regulator of oxygen homeostasis by activating the transcription of genes encoding proteins that either increase O_2 delivery, for example, by stimulating angiogenesis or vascular remodeling, or decrease O_2 consumption, for example, by switching from oxidative to glycolytic metabolism.[7] HIF-1 mediates vascular responses to hypoxia and ischemia by activating transcription of the *VEGF* gene, which encodes vascular endothelial growth factor,[8] as well as genes encoding many other proangiogenic factors, including stromal-derived factor 1 (also known as CXCL12),[9] angiopoietin 1,[10] angiopoietin 2,[11] placental growth factor,[12] platelet-derived growth factor B,[12 13] and stem cell factor (also known as Kit ligand).[10] These factors are secreted from hypoxic cells and bind to cognate receptors on endothelial cells, endothelial progenitor cells, mesenchymal stem cells, and bone marrow-derived angiogenic cells (BMDACs) to stimulate tissue vascularization and thereby increase tissue oxygenation. Many of these factors have been shown to stimulate both angiogenesis and arteriogenesis.[14–16] Control by HIF-1 of a large battery of angiogenic cytokines and growth factors suggests that targeting HIF-1 may be more effective than any individual angiogenic factor. This review will summarize our recent studies demonstrating therapeutic efficacy of this approach in mouse models of PAD and lung transplantation.

HIF-1 MEDIATES ISCHEMIA-INDUCED VASCULAR REMODELING IN A MOUSE MODEL OF CRITICAL LIMB ISCHEMIA

Peripheral arterial disease refers to stenosis of a major conduit artery in the leg that reduces tissue perfusion, leading to ischemia, which is a pathological condition consisting of reduced delivery of oxygen and nutrients as well as reduced removal of toxic metabolites. In the case of complete occlusion of a conduit artery, tissue perfusion distal to the occlusion is entirely dependent upon blood flow through collateral blood vessels. Two types of vascular responses are induced by ischemia: *angiogenesis*, which is the budding of new capillaries

To cite: Semenza GL. *J Investig Med* 2016;**64**:361–363.

from existing vessels; and *arteriogenesis,* which is the remodeling of existing collateral vessels to allow increased blood flow. Of these, arteriogenesis is the process that is essential to increase perfusion distal to the occlusion of a major conduit artery. In 1% to 3% of patients with PAD, the combination of severe stenosis and failure to effectively remodel collateral vessels results in *critical limb ischemia,* the condition in which blood flow is insufficient to maintain tissue viability. More than 50% of patients with critical limb ischemia who are not candidates for endovascular or surgical revascularization procedures to increase perfusion (so-called no-option patients) suffer limb amputation or death within 1 year.[17] Thus, there is an urgent clinical need for novel therapeutic approaches.

Peripheral arterial disease is an age-dependent phenotype, with an overall prevalence of 3% to 10% that increases to 15% to 20% among individuals older than 70 years,[17] which reflects both progressive atherosclerotic disease and an age-dependent impairment of vascular responses to ischemia,[18] including impaired HIF-1 –dependent VEGF expression.[19] Yet most of the preclinical studies testing effects of gene or cell therapies have been performed using young animals (eg, 8-week-old mice), which generally will recover completely from femoral artery ligation, even in the absence of any therapy; in contrast, older mice will sustain permanent tissue damage with severity that increases progressively with age.[10] At every age, tissue damage following femoral artery ligation is greater in *Hif1a*$^{+/-}$ mice, which are heterozygous for a knockout allele at the locus encoding HIF-1α, than in their wild-type littermates. The severity of the disease phenotype is inversely related to the degree of recovery of tissue perfusion following ligation, which in turn is directly related to the expression of HIF-1 α protein and mRNAs encoding angiogenic factors in the ischemic limb.[10] One consequence of the reduced production of angiogenic cytokines was reduced mobilization of BMDACs into the circulation of *Hif1a*$^{+/-}$ mice as compared to wild-type littermates.

Treatment of mice with an intramuscular injection of AdCA5, which is a recombinant adenovirus encoding an engineered form of HIF-1 α that is constitutively active as a result of mutations that render it resistant to O_2-dependent degradation,[12 13] improved the recovery of blood flow and reduced tissue injury in 8-month-old mice.[10] Injection of AdCA5 into the limb was sufficient to induce the mobilization of BMDACs into the circulation, even in the absence of ischemia.

In contrast, AdCA5 treatment was completely ineffective in 17-month-old mice, which showed little recovery of perfusion after femoral artery ligation, resulting in complete limb amputation.[20 21] These results suggested that 8-month- old mice were deficient in the production of angiogenic cytokines that serve as homing signals for the recruitment of BMDACs, which was corrected by AdCA5 injection into the ischemic limb, whereas 17-month-old mice had an additional impairment in the ability of BMDACs to respond to the homing signals.

To test this hypothesis, total bone marrow mononuclear cells were harvested from a donor mouse and cultured for 4 days in the presence of angiogenic growth factors (to induce the BMDAC phenotype) and dimethyloxalylglycine (to induce HIF-1 activity). Recipient mice were subjected to femoral artery ligation followed by intramuscular injection of AdCA5 into the ischemic limb and then 24 hours later, the mice received an intravenous injection of donor-derived BMDACs. The sequential staging allowed time for the HIF-1 –dependent production of angiogenic cytokines that served as the homing signals for subsequent recruitment of the injected BMDACs to the ischemic limb. The combination therapy led to complete limb salvage (no permanent tissue damage), even when both the donor and recipient mice were 17 months old.[21] Induction of HIF-1 activity in BMDACs before administration had 2 important effects. First, HIF-1 activated transcription of the genes encoding β_2 integrins, which mediate adherence of BMDACs to endothelial cells within the ischemic tissue.[20] Second, HIF-1 reprogrammed BMDACs from oxidative to glycolytic metabolism, thereby preadapting the cells to the ischemic microenvironment and increasing their survival after injection.[21] This preclinical model suggests that combined gene therapy and cell therapy using autologous bone marrow cells (subjected to short-term culture in the presence of angiogenic factors and a pharmaceutical inducer of HIF-1 activity) may provide a therapeutic strategy for limb salvage in patients with critical limb ischemia.

HIF-1 IS REQUIRED FOR PRESERVATION OF ALLOGRAFT MICROVASCULATURE IN A MOUSE MODEL OF LUNG TRANSPLANTATION

The 5-year survival after lung transplantation is approximately 50%, which is the poorest outcome among all solid organ allografts. Chronic rejection is associated with airway fibrosis (bronchiolitis obliterans). However, autopsy data indicate that bronchiolitis obliterans is preceded by the loss of airway microvasculature.[22] It is noteworthy that the lung is the only organ for which the main arterial blood supply (bronchial arteries) is not reestablished during transplant surgery.[23] To model this condition in mice, Mark Nicolls and his colleagues at Stanford performed orthotopic tracheal transplantation using donor and recipient mice that differed at the major histocompatibility complex.[24] Perfusion of the allograft was impaired by day 10 after transplantation and partially recovered by day 21; however, when the allograft was from a HIF-1 α conditional knockout (CKO) donor mouse, airway perfusion was severely impaired by day 8 and remained markedly reduced on day 21. The recovery of perfusion in the wild-type allografts was associated with increased expression of angiogenic factors and the recruitment of host Tie2$^+$ BMDACs to the airway, and these processes were markedly impaired in the HIF-1 α^{CKO} allografts.[24]

The fact that the effects of HIF-1 α loss-of-function in the airway were similar to the effects observed in the limb suggested that AdCA5 might have a beneficial effect. Indeed, soaking tracheal allografts (from wild-type donors) in buffer containing AdCA5 before transplantation resulted in a significant increase in the expression of multiple angiogenic factors including placental growth factor, stromal-derived factor 1, and VEGF as well as increased recruitment of host Tie2$^+$ BMDACs to the airway. AdCA5 treatment resulted in only a modest decrease in airway perfusion on day 14 with recovery by day 21, and reduced airway fibrosis at days 14 and 21 after transplantation.[24]

For clinical application, a strategy not involving viral vectors is preferable. Given the availability of small molecule inhibitors of the HIF prolyl hydroxylases, desferrioxamine-lecithin nanoparticles were formulated. Soaking the allografts in a suspension of these nanoparticles immediately before transplantation dramatically increased airway perfusion on days 3 and 10 after transplantation.[25]

From these studies, we conclude that although chronic rejection may be triggered by the immune system, effects on the microvasculature play an essential role in allograft failure and strategies designed to maintain allograft perfusion may reduce the incidence of chronic rejection. Given the parallels with limb ischemia, it is worthwhile to consider whether the age of the allograft donor and recipient may impact on this process and whether combining desferrioxamine nanoparticle treatment of the allograft with autologous BMDAC therapy may provide additional therapeutic benefit in elderly transplant recipients.

Provenance and peer review Commissioned; internally peer reviewed.

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