O_2 Sensing: Only Skin Deep?

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The transcription factor HIF-1 mediates adaptive responses to hypoxia, and its activity is negatively regulated by O_2-dependent binding of the von Hippel-Lindau (VHL) protein. In this issue, Boutin et al. (2008) use conditional knockout mice to demonstrate that sensing of O_2 by keratinocytes in the epidermis leads to alterations in cutaneous blood flow that affect the production of the hormone erythropoietin, thereby modulating red blood cell production and the O_2-carrying capacity of blood.

The physiology of modern day mammals has been shaped by natural selection over the past several billion years, during which life on Earth has thrived in an O_2-rich environment. Metazoan species evolved a key protein, the transcription factor hypoxia-inducible factor 1 (HIF-1), to regulate O_2 delivery to tissues. Decreased O_2 in the blood of mammals is sensed by the carotid body, which is located at the bifurcation of the common carotid artery and activates the cardiovascular and respiratory centers in the central nervous system to increase ventilation and cardiac output (Kline et al., 2002). A reduction in O_2 is also sensed by cells in the liver and kidney, which respond by producing erythropoietin (EPO), the hormone that controls red blood cell production and, thus, the O_2-carrying capacity of blood (Stockmann and Fandrey, 2006). HIF-1, originally discovered as the factor that mediates hypoxia-induced transcription of the human *EPO* gene (Semenza and Wang, 1992), is also required for O_2 sensing by the carotid body (Kline et al., 2002). HIF-1 is composed of two subunits: constitutively expressed HIF-1β and O_2-regulated HIF-1α (Wang et al., 1995). Database searches led to the identification of HIF-2α, which is also regulated by O_2, dimerizes with HIF-1β, and regulates an overlapping but distinct battery of target genes (Elvidge et al., 2006; Patel and Simon, 2008). Under normoxic conditions, HIF-1α and HIF-2α are subjected to hydroxylation on proline residues (402 and 564 in human HIF-1α). The modification is required for the binding of the von Hippel-Lindau (VHL) tumor suppressor protein, the recognition component of an E3 ubiquitin-protein ligase that targets HIF-1α for proteasomal degradation (Kaelin, 2005; Schofield and Ratcliffe, 2005). Under hypoxic conditions, hydroxylation is inhibited and the VHL protein does not bind to HIF-1, leading to the accumulation of HIF-1α and HIF-2α.

Homozygous deletion in the mouse germline of the *Hif-1α* or *Vhl* locus results in embryonic lethality. Using Cre-lox technology to generate conditional knockout mice at these loci in specific cell types and at specific time points in postnatal life has provided insights into complex homeostatic mechanisms, which may not have been possible using other approaches. That has certainly proven to be the case with the deletion of these genes in mouse epidermal cells (keratinocytes) as Randy Johnson and colleagues report in this issue (Boutin et al., 2008). They show that deletion of the *Vhl* gene in mouse keratinocytes results in increased EPO levels in plasma and development of polycythemia, a condition in which too many red blood cells are produced and blood viscosity increases. Interestingly, humans with an inherited form of polycythemia are homozygous for a germline missense mutation that reduces, but does not eliminate, VHL activity (Ang et al., 2002). But given that EPO is not expressed in the skin, how does loss of the VHL protein in mouse keratinocytes result in increased production of EPO and red blood cells?

The skin is perfused by capillaries that are located in the dermis (Figure 1). Regulation of cutaneous blood flow is an important component of the homeostatic mechanism that maintains the human core body temperature at 37°C despite wide fluctuations in environmental temperature. Vasodilation of dermal capillaries increases heat loss, whereas vasoconstriction prevents it. As Boutin et al. (2008) now show, deletion of VHL in mouse keratinocytes causes a marked increase in cutaneous blood flow and dysregulated expression of HIF-1α and especially HIF-2α. This results in the expression of HIF-1-regulated genes, including *Nos2*, which encodes inducible nitric oxide synthase. The expression of Nos2 leads to increased generation of nitric oxide (NO), a potent vasodilator. Cutaneous vasodilation, that is, more blood flow to the skin, results in less blood flow to other organs. In the liver, blood flow is sufficiently reduced to result in tissue hypoxia, a condition that induces expression of the *Epo* gene. Consistent with this mechanism for increased EPO production, Boutin et al. did not observe elevated EPO expression in the keratinocyte-specific *Vhl* knockout mice that also lacked either *HIF-2α* (in keratinocytes) or *NOS2* (in all cells). Remarkably, transdermal administration of nitroglycerin (an NO donor) to wild-type mice boosted plasma levels of EPO, whereas systemic treatment did not. Thus, the homeostatic mechanism for maintaining the O_2-carrying capacity of blood can be disrupted by the dysregulation

of cutaneous blood flow (Figure 1). But do these intriguing observations have any relevance for normal mammalian physiology?

To answer this question, the authors performed two experiments, one mundane and the other insane. In the mundane experiment, they eliminated the expression of either HIF-1α or HIF-2α in mouse keratinocytes expressing wild-type VHL and analyzed the effect on hypoxia-induced EPO production. One might predict that keratinocyte-specific loss of *HIF-2α* would impair EPO production in response to hypoxia, but surprisingly, loss of *HIF-2α* had no effect. But when mice with a keratinocyte-specific deletion of *HIF-1α* were exposed to hypoxia, the predicted increase in plasma EPO levels was blunted and induction of EPO expression in the kidney was lost. These results suggest that physiological regulation of cutaneous blood flow in response to changes in atmospheric O_2 is mediated by HIF-1α, whereas the pathological dysregulation resulting from VHL loss-of-function is primarily mediated by HIF-2α (Figure 1). Taken together, these results support the conclusion that HIF-1 activity in the skin is important for EPO production by the kidney in response to hypoxia.

To bolster this conclusion, Johnson's colleague Frank Powell performed the insane experiment. He designed what can best be described as a contraption (see supplemental figure in Boutin et al., 2008) to allow the investigators to deliver a different O_2 concentration to the head (and thus the lungs) of mice relative to the body (and thus the skin). Using this contraption, the investigators found that when mice respired under hypoxic conditions but perspired under normoxic conditions, EPO levels were significantly elevated relative to animals whose lungs and skin were exposed to hypoxia. The lack of EPO production in mice whose skin and lungs were exposed to hypoxic conditions suggests that during an acute exposure to environmental hypoxia, blood flow to the skin is reduced, thereby allowing increased blood flow to the vital organs. However, when the skin is exposed to normoxia but the lungs are exposed to hypoxia, the skin no longer senses hypoxia and cutaneous vasoconstriction does not occur. Hence, blood flow to vital organs is reduced (relative to mice in which both skin and respiratory tract were exposed to hypoxia), thereby resulting in greater EPO production. The conclusion that acute environmental hypoxia induces cutaneous vasoconstriction is surprising because, in general, the systemic vasculature dilates in response to acute hypoxia (through production of vasodilators such as NO), whereas the pulmonary vasculature constricts (to shunt blood flow away from areas of the lung that are not being oxygenated). The authors further report that key mediators of pulmonary vasoconstriction, the voltage-gated potassium channel Kv1.5 and the secreted peptide endothelin 1, are also expressed under HIF-1 control in the dermal vasculature in response to hypoxia. Electrophysiological studies are required to further investigate whether smooth muscle cells from dermal vessels show responses to changes in O_2 that are similar to those of pulmonary artery smooth muscle cells and whether Kv1.5 and endothelin 1 are involved. The cutaneous vasoconstrictor response is not maintained (perhaps due to adverse long-term consequences to the skin of reduced cutaneous blood flow), and chronic hypoxia leads to vasodilation of dermal capillaries, perhaps through increased NOS2 production (Figure 1).

Everyone learns in high school biology that frogs can respire through their skin. Does transcutaneous O_2 uptake occur in mice as well? Probably not. Frog epidermis is thin and contains a vast capillary network, whereas mouse epidermis

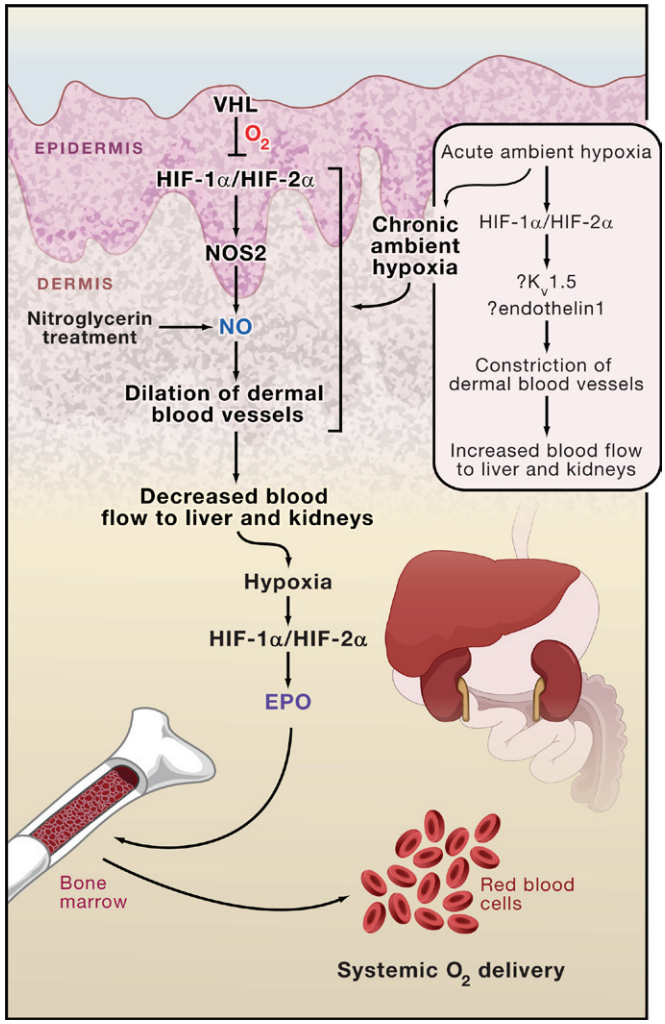

Figure 1. An Epidermal Response to Hypoxia

Loss of the VHL protein in epidermal keratinocytes results in constitutive expression of the transcription factors HIF-1α and HIF-2α, leading to increased expression of *Nos2* and enhanced production of the vasodilator nitric oxide (NO). NO diffuses into dermal capillaries and induces vasodilation, thereby increasing blood flow to the skin and decreasing blood flow to the kidneys and liver. The resultant tissue hypoxia in these organs induces the expression of HIF-1α and HIF-2α, which mediate the production of EPO and its secretion into the blood. In the bone marrow, EPO binds to erythroid progenitor cells and promotes their survival and proliferation, leading to an increase in mature red blood cells that transport O_2 to all tissues in the body. (Inset) In response to acute ambient hypoxia (such as a rapid ascent to high altitude), cutaneous vasoconstriction (perhaps mediated by the potassium channel Kv1.5 and endothelin 1) increases blood flow to vital organs to maintain their oxygenation. However, this response is not sustained and chronic hypoxia leads to cutaneous vasodilation, perhaps through expression of Nos2 and production of NO.

is thicker and is not vascularized, thus resulting in a diffusion distance between air and dermal capillaries that is too great to serve as an efficient means of O_2 uptake.

Finally, does dysregulation of cutaneous blood flow have any effect on body temperature homeostasis? Remarkably, mice lacking VHL in their keratinocytes die from hypothermia when subjected to cold stress due to a failure of cutaneous vasoconstriction (R. Johnson et al., personal communication). Considering the complex homeostatic mechanisms that are subserved by the cutaneous vasculature, the study by Boutin et al. elegantly demonstrates that beauty is not the only characteristic that is skin deep!

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A TRIFfic Perspective on Acute Lung Injury

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Acute lung injury (ALI) is a leading cause of death in people infected with H5N1 avian influenza virus or the SARS-coronavirus. Imai et al. (2008) now report that ALI is triggered by the signaling of oxidized phospholipids through Toll-like receptor 4 (TLR4) and the adaptor protein TRIF. These findings provide insight into the molecular pathogenesis of ALI, a condition for which treatment options are currently very limited.

Acute lung injury (ALI) affects more than 200,000 people in the US each year, with approximately 75,000 deaths, making it an important cause of morbidity, mortality, and health care expenditure (Rubenfeld et al., 2005). Bacterial and viral infections are important risk factors for ALI, but aspiration of gastric contents, major trauma, and repeated transfusions are additional risks. ALI is also a leading cause of death in people infected with H5N1 avian influenza virus or the coronavirus that causes SARS (severe acute respiratory syndrome). In this issue, Imai et al. (2008) report surprising insights from murine studies that provide a new perspective on the mechanisms contributing to ALI in humans.

The alveolar membrane of the lungs is the largest surface area of the body that is in continuous contact with the outside environment, and a complex set of defenses have evolved to protect it against inhaled particulates and microbes. The alveolar wall is a delicate structure, consisting of a thin alveolar epithelial layer, a basement membrane composed of collagens, glycoproteins, and glycosaminoglycans, and a thin endothelial cell layer. Surfactant phospholipids and associated proteins lining the alveolar surface are critical in reducing surface tension in alveolar fluid, so that alveoli do not collapse at low lung volumes. Cells called type II pneumocytes in the alveolar walls produce surfactant and actively transport sodium ions from the lumen to the interstitium, facilitating passive water movement from the alveoli to the interstitium and lymphatics in order to keep the airspaces dry. Acute damage to epithelial or endothelial cells in the alveolar membrane causes the clinical syndrome of ALI, in which the alveolar spaces fill with proteinaceous exudates, producing severe alterations in gas exchange, critical hypoxemia, and death in the absence of aggressive medical care. The hallmark findings of ALI include acute neutrophilic inflammation and an array of proinflammatory cytokines in the lungs, suggesting that activation of innate immunity is an initial event, whether or not overt infection is present. Activation of innate immune pathways combined with the physical stresses created by mechanical ventilation cause a synergistic increase in lung injury, but the mechanisms underlying ALI are not clear (Dos Santos and Slutsky, 2006).

In order to identify susceptibility factors for lung injury, Imai and colleagues screened several strains of mice using a