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Hypoxia-Mediated Biological Control

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ABSTRACT

When oxygen demand is greater than oxygen supply, cells need to rapidly adjust their metabolism in order for the tissue to survive. Oxygen sensing by an organism influences a host of processes including growth, development, metabolism, pH homeostasis, and angiogenesis. Hypoxia also contributes to a wide number of human diseases including vascular disease, inflammatory conditions and cancer. Recently, major advances have been made in understanding the response of cells and tissues to hypoxia with the goal of providing mechanistic insight and novel therapeutic targets. In this article we review both the normal biological effects of hypoxia as well as the alterations that occur in specific disease conditions with an emphasis on the cell signaling and gene transcription mechanisms that underlie the changes associated with chronic hypoxia. Comparisons of studies in the fields of cardiac ischemia and tumor angiogenesis reveal the complexities within the microenvironment that control responses to hypoxia. It is clear that more interaction between researchers in these fields will improve the development of therapies that either promote or prevent hypoxic responses. J. Cell. Biochem. 112: 735–744, 2011.

KEY WORDS: HYPOXIA; HIF; CARDIOVASCULAR PRECONDITIONING; ANGIOGENESIS

Hypoxia, the reduction of normal oxygen tension, results from a multitude of causes that lead to decreased oxygen delivery or availability. The pO_2 levels across the body range from 150 mm Hg in the upper airway (21% O_2 humidified) to as low as 5 mm Hg (~1% O_2) in the retina [Taylor, 2008]. The precise level of oxygen tension considered to be hypoxic differs between organs but most tissues trigger a hypoxia response below venous pO_2 (40 mm Hg or ~6% O_2) [Simon and Keith, 2008]. Maintaining oxygen homeostasis is complex and involves coordinated efforts between hormonal, autocrine and neuronal mechanisms. Not surprisingly, an imbalance of these factors frequently results in disease with hypoxia as the driving force for the pathologic progression.

The adaptations necessary for survival in hypoxic conditions depend on the level of regulation. The response to global hypoxia, such as what occurs with high altitude exposure, modifies the physiology of the entire organism by increasing respiration rate for example; whereas, localized hypoxia, such as at the site of an injury, modifies local cell signaling with confined effects on blood vessel recruitment. Global oxygen is sensed by specialized chemoreceptor cells in the carotid and neuroepithelial bodies. Contributions from mitochondrial production of H^+ and direct membrane effects lead to a reduction in outward potassium current in these cells during hypoxia. The resulting membrane depolarization, calcium entry, and neurotransmitter release immediately alters the physiology of the organism stimulating an increase in respiratory and heart rates [Lahiri et al., 2006]. Responses to chronic hypoxia, on the other hand, may take hours to occur and generally result in widespread changes in gene expression. In general, there are two adaptation choices in chronic hypoxia: reduction of oxygen consumption by switching to anaerobic metabolism and decreasing energy usage (i.e., reduced proliferation) or, alternatively, increasing oxygen delivery to cells via increased erythropoiesis and blood vessel formation [Mole and Ratcliffe, 2008].

CELLULAR O_2 SENSING AND GENE REGULATION BY HYPOXIA

The identification of a hypoxia-inducible transcription factor resulted from efforts to understand the mechanism behind one of the more salient effects of chronic hypoxia, the increased production of the red blood cell (RBC) maturation factor, erythropoietin (EPO). Using DNA–protein interactions at the 3′ enhancer region of the EPO gene, a protein complex was discovered that bound only during hypoxic conditions. Binding of this protein complex, later named hypoxia-inducible factor-1 (HIF-1), resulted in increased transcription of EPO mRNA and subsequent increases in EPO production [Semenza et al., 1991]. Through further studies, it became apparent that oxygen sensing was not limited to specific chemoreceptor cells, but that most cells were capable of responding to changes in oxygen tension and that several proteins coordinate the control of hypoxia-mediated gene transcription [Maxwell et al., 1993].

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HYPOXIA-INDUCIBLE FACTOR

The cellular response to hypoxia is a multi-step process, and many of the transcriptional responses are orchestrated by HIFs. These transcription factors are heterodimers composed of an α and β subunit and are members of the basic helix-loop-helix (bHLH) proteins of the PER-ARNT-SIM (PAS) DNA binding protein family [Wang et al., 1995]. HIF-β, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is not oxygen-responsive itself, but is necessary for formation of the functional HIF complex. The HIF-α/β heterodimers bind to target genes on their hypoxic-response elements (HREs), a DNA binding motif in the promoter or enhancer region of the target gene with the core nucleotide sequence 5′-RCGTG-3′ [Semenza and Wang, 1992]. HIF binding to HREs results in transcriptional upregulation of target genes that mediate multiple adaptations to hypoxia that are described below.

OXYGEN-DEPENDENT REGULATION OF HIF-1α

Much of our knowledge about the regulation of HIFs is through the study of the oxygen-sensitive HIF subunit, HIF-1α. There is some evidence that HIF-1α is regulated at transcriptional and translational levels, but because HIF-1α protein is degraded in normoxia, its primary response to hypoxia is through protein stabilization (Fig. 1) [Wang et al., 1995; Lisy and Peet, 2008]. Under normoxic conditions, HIF-1α is hydroxylated within its oxygen-dependent degradation domain (proline residues 402 and 564) by oxygen-dependent prolyl hydroxylase domain proteins (PHDs) [Epstein et al., 2001]. Hydroxylated HIF-1α subunits have an increased binding affinity for the von Hippel–Lindau (vHL) protein, a component of the E3 ubiquitin ligase complex and are subsequently subjected to ubiquitin-dependent proteasomal degradation [Maxwell et al., 1999; Cockman et al., 2000]. Acetylation of HIF-1α by arrest defective-1 protein (ARD1) may also be necessary for complete recognition by vHL [Jeong et al., 2002]. The loss of HIF-1α protein through ubiquitin-mediated mechanisms occurs for most cultured cell models exposed to O_2 concentrations above 5% and stabilization of HIF-1α protein increases relative to the extent of hypoxia.

Aside from protein stabilization, normoxic modifications of HIF-1α can also affect its transcriptional activity. Hydroxylation on asparagine 803 in the C-terminal transactivation domain of HIF-1α is catalyzed in normoxia by factor inhibiting HIF-1 (FIH). This hydroxylation results in the loss of HIF-1α interactions with the transcriptional co-activators CREB binding protein (CBP) and P300 [Mahon et al., 2001; Elkins et al., 2003]. Because these interactions are necessary for HIF to assemble properly at the HRE, hydroxylation abrogates HIF-mediated transcription. Several reports have also shown effects of sumoylation, phosphorylation, and nitrosylation on the stability and transcriptional activity of HIF-1α, suggesting that there may be additional undiscovered pathways that regulate HIF-1α stability and function [Mylonis et al., 2006; Berta et al., 2007; Li et al., 2007].

Because hydroxylation of HIF-1α by PHDs is the limiting step for its degradation, these enzymes are essential for the regulation of the HIF-1α protein levels. There are four mammalian PHDs and each contributes to the hydroxylation of HIF-1α relative to its intracellular abundance [Appelhoff et al., 2004]. These enzymes require oxygen and 2-oxoglutarate as co-substrates and iron and ascorbic acid as cofactors. The affinity of the PHDs for oxygen is low relative to normal intracellular oxygen concentrations (K_m 10-fold higher than normal tissue O_2 concentration) [Hirsila et al., 2003]. As such, oxygen is typically the rate-limiting step for HIF-1α hydroxylation and resultant degradation. Similar to the PHDs, FIH is also a 2-oxoglutarate dependent dioxygenase whose enzyme activity has an absolute requirement for oxygen [Mahon et al., 2001]. In hypoxia, neither PHDs nor FIH are functional, resulting in stabilization of HIF-α that then translocates into the nucleus, dimerizes and transcriptionally activates target genes.

Another layer of oxygen-dependent HIF-1α regulation resides at the level of mitochondrial function. Multiple studies have demonstrated that an operating electron transport chain (ETC) is necessary for stabilization of HIF-1α in hypoxia [Chandel et al., 1998; Taylor, 2008]. Cytochrome oxidase, complex IV of the ETC, has an extremely low K_m for oxygen (<1 μM); it is therefore not surprising that the mitochondria should also have a role in the oxygen-dependent regulation of the cell [Rolfe and Brown, 1997]. Current data suggest two mechanisms for interactions between mitochondria, PHDs and oxygen sensing. First, oxidative stress, as modeled by addition of hydrogen peroxide, stabilizes HIF-1α in

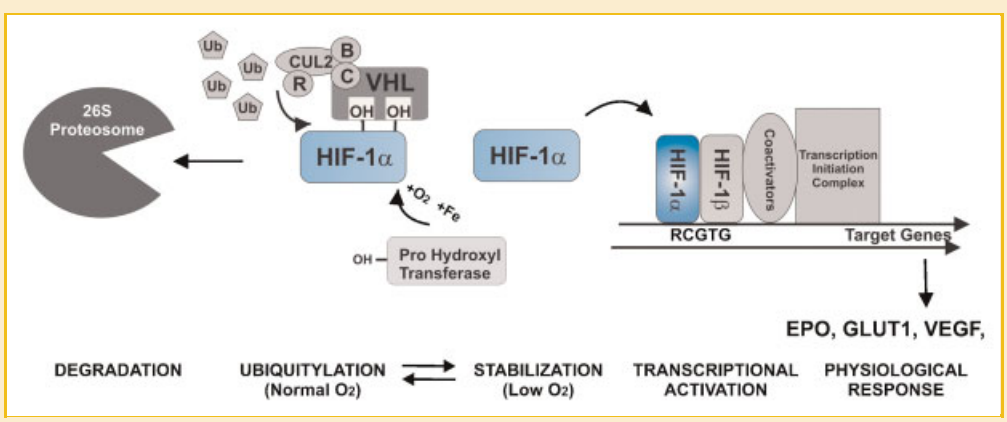

Fig. 1. Model for hypoxia-mediated stabilization of HIF-1α.

normoxia, whereas antioxidants reduce HIF-1α stabilization in hypoxia [Gerald et al., 2004; Gao et al., 2007]. The mechanism is likely through altered Fenton chemistry resulting in accumulation of the oxidized Ferric (Fe^{3+}) iron form that cannot be used as a cofactor by the hydroxylases. Second, in hypoxia, all of the available intracellular oxygen is utilized by the ETC to produce the maximal amount of ATP leaving the PHDs with no oxygen co-substrate and therefore inactive [Doege et al., 2005]. Further complicating these findings is the activity of nitric oxide (NO); NO inactivates cytochrome *c* oxidase which results in destabilization of HIF-1α in both normoxia and hypoxia. However, NO also may inhibit the PHDs through chelation of Fe^{2+} which results in stabilization of HIF-1α [Hagen et al., 2003; Wellman et al., 2004; Pan et al., 2007]. The role of NO in HIF regulation is clearly multifaceted and dependent on the specific system that is being studied. Like other homeostatic mechanisms, oxygen-dependent regulation of HIF is a complicated process involving a dynamic balance of interactions at each step in order to maintain homeostasis within the cell.

The majority of information regarding the HIF response to hypoxia is through study of HIF-1α, but other isoforms have more recently been identified. Currently, in mammalian cells there are three structurally similar HIF-α isoforms all of which are tightly regulated by intracellular oxygen tension. HIF-1α and HIF-2α share greater than 70% homology in their DNA binding and transcriptional activation domains, and functional studies have shown that HIF-2α and HIF-3α activate hypoxic response genes via dimerization with a HIF-β subunit and binding to the HRE of a target gene [Tian et al., 1997; Gu et al., 1998]. HIF-1α is ubiquitously expressed while HIF-2α has more limited tissue distribution and is most commonly found in tissues with a regulatory role in oxygen delivery such as endothelial cells, renal fibroblasts, cardiac myocytes, and lung type II pneumocytes [Wiesener et al., 2002]. Additionally, while most transcriptional targets are shared, in vitro and in vivo studies have found selected transcriptional targets that are uniquely regulated by a specific isoform. Notably, glycolytic genes such as lactate dehydrogenase-A and hexokinase-2 are solely regulated by HIF-1α whereas cyclinD1 and Twist1 are shown to be under transcriptional control of HIF-2α [Baba et al., 2003; Hu et al., 2003; Wang et al., 2005; Gort et al., 2008]. The less well-studied human HIF-3α locus produces multiple splice variants. These variants lack specific domains of full length HIF such as the transactivation domain but are regulated similar to the other HIF isoforms. One variant (inhibitory PAS domain protein, IPAS) prevents HIF-1 from interacting with HREs thus inhibiting the upregulation of the target gene [Makino et al., 2001; Maynard et al., 2005].

PHYSIOLOGICAL EFFECTS OF HYPOXIA AND ROLE OF HIF

There are many conditions that require an organism to exert a response to hypoxia. The ability of cells to adapt during transient or chronic hypoxia is essential for survival. A role for HIF in these responses has been well defined in many experimental models. HIF-1α deletion in mice is embryonic lethal with developmental defects in neural tube closure, cardiac development and formation of the vasculature. HIF-2α deletion results in either embryonic demise or short survival followed by death due to respiratory failure or global mitochondrial dysfunction [Ryan et al., 1998; Scortegagna et al., 2003]. The following section explores the cell signaling effects of hypoxia on specific critical cellular functions and the angiogenic response.

METABOLISM

In normoxia, cells convert glucose into pyruvate which then enters the tricarboxylic acid (TCA) cycle and generates electrons which are essential for generation of ATP by oxidative phosphorylation in the ETC. When oxygen is low, cells switch to less efficient anaerobic glycolysis resulting in an increase in the conversion of glucose to lactate (the Pasteur effect). This metabolic switch is a critical adaptation for cells in hypoxic conditions, and HIF controls the expression of many genes required to achieve this new phenotype. The importance of HIF in this process is exemplified by the finding that cells lacking HIF-1α have markedly reduced levels of ATP production in hypoxia [Seagroves et al., 2001]. Metabolic genes regulated by HIF-1 in hypoxia include glycolytic enzymes, glucose transporters (GLUT 1 and 3), hexokinase 1 and 2, phosphoglycerate kinase 1 and lactic dehydrogenase A [Semenza, 2001]. The majority of the genes induced by HIF-1 improve glucose uptake and production of pyruvate for anaerobic glycolysis. However, phosphoglycerate kinase 1 (PDK1) is an essential HIF-1 target as it prevents the conversion of pyruvate to acetyl-CoA. Thus, PDK1 prevents glucose from entering the TCA cycle and downregulates mitochondrial respiration. Additionally, it has been proposed that PDK1 serves to block excessive mitochondrial ROS production. The byproduct of this cycle, pyruvate, is converted to lactate-by-lactate dehydrogenase, another enzyme transcriptionally regulated by HIF-1. The conversion of pyruvate to lactate regenerates NAD^+ for continued anaerobic glycolysis [Kim et al., 2006; Stubbs and Griffiths, 2010].

More recently, Fukuda et al. have shown that HIF also has a role in fine-tuning mitochondrial proteins under conditions of low oxygen. In hypoxia, HIF-1 regulates the COX4 isoform of cytochrome oxidase resulting in a more efficient use of available oxygen within the cell [Fukuda et al., 2007]. The induction of mitochondrial autophagy is another HIF-dependent metabolic adaptation. Zhang et al. [2008] demonstrated both that induction of the autophagy machinery and regulation of mitochondrial mass are HIF dependent. These findings suggest that metabolic adaptations to hypoxia are more complicated than a simple switch to anaerobic glycolysis and the full complexity of this regulation has yet to be characterized.

REGULATION OF PH

Acidification of the microenvironment is another major consequence of the switch to anaerobic glycolysis. Hypoxia-induced acidosis is due to increased lactic acid production, but reduced vascular dispersion of CO_2 also adds to the condition. There are a variety of proteins associated with regulation of pH in cells: Na^+/H^+ exchangers and the vacuolar-type H^+-ATPase actively expel two protons, monocarboxylate transporters move lactic acid bidirectionally, bicarbonate transporters increase cellular alkalinization,

and carbonic anhydrases catalyze the hydration of carbon dioxide to bicarbonate and protons [Chiche et al., 2010]. Although the resulting acidosis is brought about by the hypoxia-induced change in cellular respiration, hypoxia also regulates transcription of genes necessary for pH homeostasis.

Work by Shimoda et al. demonstrated Na^+/H^+ exchangers (NHE) 1 and 6 to be transcriptionally regulated by HIF-1. The increase in expression of these transporters results in an increase in proton efflux from the cells [Shimoda et al., 2006]. Another HIF-1-dependent target is the monocarboxylate transporter (MCT); MCT proteins catalyze the proton-coupled transport of lactate and are highly expressed in glycolytic tissues such as white muscle [Ullah et al., 2006]. Ullah et al. found expression of MCT4 to be induced by hypoxia through interactions on the HRE sites of the promoter sequence regions. One of the best-studied adaptations to hypoxia-induced acidosis is the upregulation of carbonic anhydrase enzymes. Carbonic anhydrases catalyze the conversion of CO_2 to carbonic acid and serve as a link between changes in metabolism and changes in pH. Consequently, carbonic anhydrases IX and XII have been shown to be direct targets of HIF-1 activity [Wykoff et al., 2000]. These enzymes function to acidify the extracellular environment, which in concert with anion exchangers results in maintaining a more alkaline intracellular pH [Chiche et al., 2010]. The combination of these changes allows cells to adapt and survive in a hostile acidic environment.

OXYGEN DELIVERY

Hypoxia induces changes that permit cells and tissues to survive, but hypoxia also initiates system-wide changes in physiology so the organism can sustain viability for longer periods of time. Anemia, high altitudes or infections may all lead to global reductions in the oxygen content of the blood. A major hypoxia-induced adaptation for an organism to survive is to increase oxygen delivery to hypoxic tissues. One method for achieve this is to induce the expression of genes necessary for RBC production and oxygen transport.

EPO, the first gene shown to be regulated by HIF activity, is a hormone that induces production of RBCs in hematopoietic organs such as the bone marrow. EPO is synthesized in the peritubular capillary endothelial cells of the kidney and while its primary action differs depending on the maturity of the developing erythrocyte, the ensuing effect of EPO is increased numbers of mature RBCs [Semenza et al., 1991; Haase, 2010]. Hypoxia also induces the production of other proteins necessary for oxygen transport—primarily those involved in iron metabolism including transferrin, transferring receptor, ceruloplasmin, and hepcidin [Mukhopadhyay et al., 2000; Peyssonnaux et al., 2008]. The result of these adaptations is an increase in circulating RBCs and accordingly, an increase in oxygen delivery to tissues.

ANGIOGENESIS

While the other metabolic adaptations to hypoxia are taking place, another major adaptation, angiogenesis, is also occurring. Angiogenesis, the growth of new blood vessels from preexisting vessels, provides increased blood supply and subsequent oxygen and nutrient delivery to deficient tissues. Hypoxia is one of the most significant contributors to the process as HIF proteins directly regulate the transcription of many factors necessary for stimulating vascular cell migration, notably vascular endothelial growth factor (VEGF) [Forsythe et al., 1996]. Other factors, such as fibroblast growth factor (FGF), placental growth factor (PLGF) and angiopoietin (ANG)-1, do not have known HRE sites, but are believed to be indirectly regulated by HIF in hypoxia [Kelly et al., 2003]. Angiogenesis is necessary for normal development and for transient responses in the adult such as wound healing, inflammation, and pregnancy.

The normal physiological process of angiogenesis is tightly regulated and typically involves coordinated signaling between a variety of cell types. For example, wounded skin is at a greater risk for infection and experiences decreased perfusion both of which can lead to localized tissue hypoxia. As the wound heals, fibroblasts reorganize to repair the damaged tissue, endothelial cells remodel new vessels that penetrate into the granulation tissue and leukocytes invade to prevent infection. These events act in concert using autocrine and paracrine responses to hypoxia including release of multiple cytokines and growth factors [Frantz et al., 2005; Fong, 2008]. This process is incredibly complex and the development of healthy, functional vasculature requires a delicate balance between angiogenic activators and inhibitors as well as proper functioning of their receptors and downstream targets.

Under hypoxic conditions, angiogenesis is primarily driven by HIF-dependent transcription of VEGF. Hypoxia also translationally regulates VEGF by increasing the stability of the mRNA though HuR protein binding [Levy et al., 1998]. The hypoxic area of tissue creates a VEGF signaling gradient that attracts neighboring endothelial cells and monocytes via receptor–ligand interactions. VEGF has multiple isoforms and multiple receptors. For example, VEGFA, the proangiogenic isoform, interacts with two receptor tyrosine kinases, VEGF receptors 1 and 2 (VEGFR), whereas, VEGFC and VEGFD interact with VEGFR-3 to stimulate lymphogenesis. Interestingly, VEGFR-1 (Flt-1) is directly regulated by HIF-1 signaling while VEGFR-2 (Flk-1) is indirectly upregulated in hypoxia and appears to have a positive feedback loop via VEGF binding as demonstrated by Shen et al. in endothelial cells [Gerber et al., 1997; Shen et al., 1998]. In addition to acting as a chemoattractant, VEGF also induces sprouting angiogenesis by inducing endothelial cell proliferation and tube formation as well as altering the ECM by changing integrin expression and enzyme activity. For example, VEGF increases expression of serine proteases, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). The serine proteases degrade the microenvironment and allow sprouting endothelial cells to migrate into the hypoxic tissue [Pepper et al., 1991; Ferrara et al., 2003].

Hypoxia-driven angiogenic signaling goes beyond VEGF production, which is important as many of these additional targets may become clinically relevant targets. HIF regulates the transcription of other pro-angiogenic genes including angiopoietin 1, angiopoietin 2, matrix metalloproteinases 2 and 9, plasminogen-activator inhibitor-1, and platelet-derived growth factor-β [Rankin and Giaccia, 2008]. Each factor has a specific role in the development of new blood vessels and the precise regulation of these factors is necessary to prevent vasculature that is out of balance. HIF signaling plays a central role in the entire process of angiogenesis—from the early sprouting phenotypes, to the changes

in the ECM and to the stabilization of new mature vessels [Hanze et al., 2007]. Any alterations to HIF function or activity can significantly change the course of hypoxic adaptations and result in disease.

HYPOXIA RESPONSES IN DISEASE

HYPOXIA AND ISCHEMIC HEART DISEASE

The heart requires a high level of metabolism for its function, thus it is extremely sensitive to hypoxia. The most common causes of myocardial hypoxia are ischemia due to reduced coronary blood flow and increased demand due to cardiac stress. Induction of HIF-1α levels is an early response to cardiac ischemia that has been observed in both animal models and in patients [Lee et al., 2000]. The responses of cardiac myocytes to hypoxia parallel those seen in other cell types with additional specific effects on genes that control excitability and calcium handling.

A clear role for HIF-1α in the response and rescue of cardiac myocytes from ischemic damage is evidenced by studies using genetic models that lack HIF-1α function. Although global HIF-1α knockout animals (HIF-1α$^{-/-}$) are embryonic lethal, heterozygous HIF-1α$^{+/-}$ animals are viable and appear to have normal cardiac function. However, when HIF-1α$^{+/-}$ animals are subjected to pressure overload due to transient aortic constriction, they are significantly more likely to develop heart failure and have reduced levels of intracellular store Ca^{2+}. HIF-1α loss in non-cardiac cells including those of the vasculature and kidney certainly contribute to this phenotype. Global HIF-2α knockout animals (HIF-2α$^{-/-}$) have vascular defects that are not rescued by restoring HIF-1α in endothelial cells, suggesting that HIF-2α expression in other cell types is also important and that HIF-1α cannot compensate completely for the loss of HIF-2α function [Duan et al., 2005].

Selective loss of HIF-1α in cardiac myocytes reduces cardiac vascularization and function. In addition to the reduction in expression of genes necessary for angiogenesis and glucose metabolism such as VEGF and GLUT-4, the changes in cardiac function are possibly caused by the observed loss of calcium handling proteins such as the sarco-endoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2) [Huang et al., 2004]. Although SERCA2 is not directly regulated by HIF-1α, it is negatively regulated by oxidant signaling, thus HIF-1α-mediated signaling may indirectly affect the Ca^{2+} dynamics of in cardiac myocytes responding to ischemia [Lounsbury et al., 2000].

Genetic models that increase HIF-1α function are cardioprotective, including transgenic overexpression of constitutively active HIF-1α and transgenic reduction in expression of the proline hydroxyl transferase enzyme, PHD2. Overexpression of active HIF-1α reduces the extent of infarction following coronary ligation and provides cardioprotection in diabetes-induced cardiac fibrosis [Kido et al., 2005; Xue et al., 2010]. Reduction in HIF-1α degradation by transgenic loss of PHD2 expression results in hearts that are less sensitive to damage caused by ischemia reperfusion [Hyvarinen et al., 2010]. These models show that enhancing HIF-1α function can improve outcome and lead to cardiac protection.

The role for HIF-1α in protection from damage following cardiac ischemia makes it an obvious target for study in preconditioning. Myocardial preconditioning is a protective response to transient hypoxia that includes improved oxygen delivery, more efficient metabolism, and preservation of mitochondrial function. There is an increase in angiogenesis, increased mitochondrial respiration, and elevated production of antioxidant enzymes that prevent ROS damage during reperfusion, all of which are potentially influenced by HIF. A study using in vivo delivery of small interfering RNA (siRNA) showed that siRNA against HIF-1α diminishes the cardioprotective effect of preconditioning, whereas siRNA targeting PHD2 increases HIF-1α levels and improves cardiac protection [Eckle et al., 2008]. Furthermore, the microRNA, miR-199, that targets HIF-1α is selectively downregulated in cardiac myocytes during preconditioning, suggesting another pathway for HIF-1α upregulation during transient ischemic events leading to preconditioning [Rane et al., 2009].

There are several avenues for potential therapies that use the hypoxia signaling mechanisms to improve cardiovascular protection from ischemic injury. Using pro-angiogenic therapies can improve ischemic-induced cardiac protection, but may also have the added benefits of improved microvascular function and reduced hypertension. Always a caution, however, is that the endothelial permeability induced by HIF-1α could promote vascular edema and inflammation, which may cause organ damage. Non-selective targeting of the HIF induction may also have untoward effects on tumor formation described in the next section.

HYPOXIA AND CANCER

Malignant tumors frequently have areas of hypoxic or anoxic tissue as the oxygen consumption in these highly metabolic tissues cannot match the oxygen delivery to these sites. The very adaptations to hypoxia used for cell survival in these harsh conditions may help to select for highly aggressive and increasingly malignant cells. Gene expression changes in hypoxic environments aid in maintaining proliferation, pH regulation and increasing vascularization in such regions. A cycle is established where the rapidly growing tumor repeatedly outgrows its blood supply; the hypoxic response occurs thus maintaining the tissue viability and tumor can continue to grow. Targeting the hypoxic response would decrease tumor cell survival as it could no longer adjust for survival in low oxygen.

Measurements of HIF-1α levels as a marker of hypoxia in tumor tissue are correlated with poorer outcomes and more aggressive disease in the majority of malignancies including breast, ovarian, colorectal and lung cancers (Table I) [Wong et al., 2003; Semenza, 2010]. In cancer cells HIF-1α can be stabilized through standard hypoxia-mediated regulation but also can be stabilized by mutations in regulatory proteins. The stabilization of HIF-1α and subsequent regulation of target genes provides a survival advantage for tumor cells. Thus the rapid growth and resultant hypoxia select for cells with a strong response to hypoxia and the ability to quickly adapt to a changing environment. Work by Liao et al. [2007] has shown that HIF-1α accelerates tumor progression in that HIF-1α$^{MEC-/-}$ tumors grew at a much slower rate than wild type. Because of the intimate association between HIF-1α, hypoxia and tumorigenesis, better understanding of the complex regulation of HIF-α proteins will lead to a more in depth understanding of malignant progression. Furthermore, increased understanding

TABLE I. Increased Expression of HIF-1α and VEGF in Multiple Tumor Types

Tumor type	Outcome	Median pO_2	Refs.
Bladder	Reduced OS	ND	Theodoropoulos et al. [2004]
Breast	Reduced OS and DFS	10 mm Hg	Delli Carpini et al. [2010], Vaupel et al. [2002]
Colorectal	Reduced OS	ND	Baba et al. [2010], Cao et al. [2009]
Cervical	Reduced OS and DFS	4 mm Hg	Birner et al. [2000], Delli Carpini et al. [2010], Lyng et al. [2000]
Endometrial	Reduced OS	ND	Sivridis et al. [2002]
Gastric	Reduced OS	ND	Qiu et al. [2010]
Glioblastoma	Reduced DFS	ND	Irie et al. [2004], Nam et al. [2004]
Hepatocellular	Reduced OS and DFS	ND	Schoenleber et al. [2009], Talks et al. [2000]
Nasopharyngeal	Reduced DFS	ND	Hui et al. [2002]
Osteosarcoma	Reduced OS and DFS	ND	Bajpai et al. [2009], Yang et al. [2007]
Ovarian	Reduced OS	ND	Delli Carpini et al. [2010], Osada et al. [2007]
Renal	Reduced OS	5 mm Hg	Dorevic et al. [2009], Lawrentschuk et al. [2009]

OS, overall survival; DFS, disease-free survival; ND, no data available.

of HIF regulation and function will provide novel targets for future therapies.

TUMOR METABOLISM AND PROGRESSION

In hypoxia tumor cells can grow independently of traditional growth regulations. Hypoxia induces transcription of multiple growth factors shown to promote cell proliferation including transforming growth factor-β, and platelet-derived growth factor [Harris, 2002]. Autocrine overexpression of mitogenic factors allows for minimal regulation of growth signaling. Hypoxia induces anti-apoptotic genes such as Bcl-2 and NF-κB, as well. The combination of these normal adaptations and malignant transformations leads to the survival of highly aggressive tumor cells.

Because of the high number of mutations in tumor cells, alterations in the regulation of hypoxic responses occur in normal oxygen conditions. A classic example of this occurs in renal cell carcinomas (RCC) where vHL is inactivated by both germline and sporadic mutations. The inactivation of vHL results in HIF-1α stabilization and transcriptional activity in normal oxygen conditions. As a result these tumors are highly vascularized and express high levels of VEGF [Baldewijns et al., 2010]. Activation of the PI3K pathway, another mutation commonly found in cancers, is also linked to normoxic stabilization of HIF-1α. The mechanisms are less well characterized but may be through activation of growth factor receptors and mediated by mTOR signaling [Hudson et al., 2002; Rankin and Giaccia, 2008]. For a more complete list of mutations resulting in increased HIF activity see Semenza [2003].

In 1924, Otto Warburg observed that malignant cells metabolize glucose into lactic acid even in the presence of oxygen, hence known as the Warburg effect. Tumor cells can afford to use glycolysis instead of oxidative phosphorylation because of their amplified glucose uptake. The PI3K-AKT pathway has been implicated in this switch [Vander Heiden et al., 2009]. HIF-1α, which is stabilized by AKT, has been shown to regulate transcription of glucose transporters and enzymes in the glycolytic pathway thus normal adaptations to hypoxia provide a survival advantage for malignant cells [Chen et al., 2001]. Malignant cells are able to support glycolysis through both normal adaptations to hypoxia and oncogenic transformation allowing for expression of HIF-1α target genes. Cells that can metabolize larger amounts of glucose can grow and divide faster, and therefore proliferate and thereby expanding the tumor mass. This metabolic switch is dependent on more than just response to hypoxia, but further understanding the hypoxic response may provide a pharmacologic target in this pathway that dramatically reduces tumor cell growth without affecting normal cells which primarily use oxidative phosphorylation to generate ATP.

METASTASIS

Tumor metastasis is the most common cause of morbidity and mortality in cancer and is a multi-step, complicated process. Hypoxia and HIF have integral roles in the development of metastasis. HIF is a major regulator of invasion, epithelial–mesenchymal transition (EMT) and angiogenesis, all of which are needed for establishment of a successful metastasis. Angiogenesis, the normal adaptation to hypoxia, is exploited in cancer in a variety of ways. In order for new vessels to get to hypoxic tissue, endothelial cells need to remodel the extracellular matrix (ECM). Factors are secreted by hypoxic cells, including urokinase-type plasminogen activator receptor (UPAR), matrix metalloproteinase-2 (MMP-2), and plasminogen-activator inhibitor 1 (PAI-1). These and other HIF-dependent factors degrade the ECM as the endothelial or tumor cells invade through the basement membrane. In addition to degrading the ECM, hypoxia also increases the motility of cells in a paracrine manner through secretion of factors such as TGF-α [Finger and Giaccia, 2010]. The increased motility goes hand-in-hand with hypoxia-induced EMT. In order for EMT to occur, cells must lose function of E-cadherin, a major protein of the adherens junctions in epithelial cells. Hypoxia upregulates transcriptional repressors of E-cadherin such as SNAIL, TWIST, TCF, and ZEB1/2 [Imai et al., 2003; Krishnamachary et al., 2006; Yang et al., 2008]. The normal hypoxic response is supportive of a highly aggressive, metastatic tumor cell.

Angiogenesis itself also significantly affects the dissemination of tumor cells; hypoxic tumors need increased blood supply to maintain growth, but the vessels that develop within a tumor are abnormal. They are tortuous, leaky, and disorganized vessels that never fully mature. The angiogenic signaling cascade is deregulated and continually activated via factors secreted from the hypoxic tumor cells. The persistent signal to sprout new blood vessels leads to an imbalance between pro- and anti-angiogenic factors. As a result the vessels are constantly responding to high levels of pro-

angiogenic factors such as VEGF and never have a chance to properly form [Chung et al., 2010]. For example, pericytes, support cells that surround microvessels and are necessary for complete maturation of the vessels, disassociate when a vessel receives a growth signal. As the vessels growing near a tumor do not receive an off signal, pericytes do not reassociate with the vessels and consequently, the vessels formed are leaky and structurally abnormal [Morikawa et al., 2002].

There are several consequences of these poorly formed new vessels that affect both chemotherapy and metastases. One, the immature structure of these vessels does not support the demand of the hypoxic tissue, therefore the cycle of hypoxia continues. Another problem with these vessels is that they alter interstitial pressures, which decreases perfusion and prevents chemotherapeutic drugs from reaching the target tissues. The chronic hypoxia also prevents free radical production by radiotherapy that is key to inducing cell death [Bristow and Hill, 2008]. Quite possibly the most important consequence though is the leakiness of the vessels. The usual tight junctions that form between endothelial cells are not as tightly formed during this pathologic process. These gaps create opportunities for the highly aggressive cells that have already been selected for to intravasate into the vasculature and disseminate throughout the body [Chung et al., 2010]. Prevention of initial hypoxia or inhibition of hypoxia signaling once a tumor is identified would reduce the production of these dysfunctional vessels and decrease the number of escape routes from the primary tumor.

The hypoxic response in tumor cells supports pro-survival and pro-metastasis phenotypes. Thus understanding and characterizing its regulation has become essential to uncovering novel therapeutic targets. A variety of targets have emerged as potential modulators of this process, from the very specific targeted therapies to more general cytotoxic agents found to also have anti-angiogenic properties. It is becoming more apparent that simple inhibition of HIF or VEGF is not potent enough to prevent disease progression. For example the use of bevacizumab, an anti-VEGF monoclonal antibody, demonstrates benefits but only in some tumor types and usually in combination with other therapies. Additionally, as with so many other treatments, resistance can develop and the drug is not without adverse events. Newer agents are being developed all the time to try to attack malignancy from multiple angles and with less toxicity in order to improve outcomes. A large push in current research is towards development of tyrosine kinase receptor inhibitors, but modification of other targets such as notch, CXCR4, angiopoietins are also in clinical trials [Teicher, 2010]. Hopefully, as the significant targets become more evident, specific, beneficial therapies can be created that are targeted directly to an individual tumor.

FUTURE DIRECTIONS

The hypoxic response is a dynamic system encompassing a variety of cellular processes necessary for survival during periods of stress. Abundant evidence supports a role for HIF-1 regulation of gene transcription in both cardiac ischemic responses and tumor survival and angiogenesis. Theoretically angiogenic therapy could be applied for myocardial ischemic preconditioning, postconditioning and to reduce hypertension. None of the larger placebo controlled trials using angiogenic growth factors have revealed positive effects in cardiovascular disease [Simons, 2005; Tongers et al., 2008]. The ineffectiveness could be caused by the inability to selectively target the treatments and the complexities of growth factor timing or combinations to achieve significant benefit. Recent advances in non-invasive imaging of microvascular densities may be useful in future studies to reveal the best regimen with respect to outcomes in hypertension and myocardial conditioning.

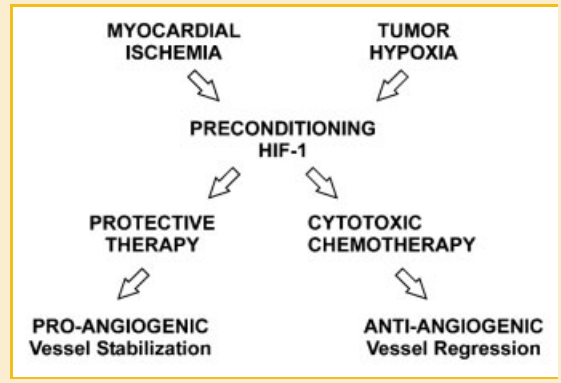

Fig. 2. Diagram representing potential for overlapping therapies in treatments for myocardial ischemia and tumor hypoxia.

Anti-angiogenic therapies have been successfully applied in cancer chemotherapy but have proven less useful or to have adverse outcomes in specific cancer types. In tumors, the complications in effectiveness arise partly due to the need for vascularization to adequately deliver chemotherapy agents. Future studies are needed that explore a two-stage regimen that initially uses angiogenic treatment to enhance drug delivery, followed by anti-angiogenic therapies to provoke vessel regression. This course of therapy may initially render the tumors less likely to activate stress pathways and more vulnerable to the cytotoxic chemotherapy; the following anti-angiogenic treatment would then induces regression of the immature vessels preventing tumor growth and metastases. Thus initial therapies to reduce tumor progression have the potential to overlap with treatments used to protect the heart from ischemic events (Fig. 2). More studies at the cellular level may also reveal new targets for angiogenic or anti-angiogenic therapy and the microRNA findings with respect to hypoxia signaling have opened a new area with great potential for clinical applications. Finally, investigators in the cardiovascular and cancer fields need to keep the communication open across disciplines to more rapidly disseminate information relating to the hypoxia signaling pathways and their effects on individual cell types.

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