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Hypoxia inducible factor (HIF) in the Tumor Microenvironment: Friend or foe?

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Abstract

Hypoxia acts as an important regulator of physiological and pathological processes. Hypoxia inducible factors (HIFs) are the central players involved in the cellular adaptation to hypoxia and are regulated by oxygen sensing EGLN prolyl hydroxylases. Hypoxia affects many aspects of cellular growth through both redox effects and through the stabilization of HIFs. The HIF isoforms likely have differential effects on tumor growth via alteration of metabolism, growth, and self-renewal and are likely highly context-dependent. In some tumors such as renal cell carcinoma, the EGLN/HIF axis appears to drive tumorigenesis, while in many others HIF1 and HIF2 may actually have a tumor suppressive role. An emerging role of HIF biology are its effects on the tumor microenvironment. The EGLN/HIF axis plays a key role in regulating the function of the various components of the tumor microenvironment, which include cancer-associated fibroblasts, endothelial cells, immune cells, and the extracellular matrix (ECM). Here, we discuss hypoxia and the diverse roles of HIFs in the setting of tumorigenesis and the maintenance of the tumor microenvironment as well as possible future directions of the field.

Keywords

Hypoxia; HIF; tumor microenvironment; cellular homeostasis; mouse model

Overview of the Importance of Hypoxia and HIF to Cellular Homeostasis

The cellular response to hypoxia, or low oxygen levels, is an evolutionarily conserved program driven by the transcription factors known as the hypoxia inducible factors (HIFs). Although we often associate hypoxia with ischemic pathophysiologic states such as stroke or cardiovascular disease, our cells must also deal with constant fluctuations in oxygen on a daily basis (Pugh and Ratcliffe, 2003). This is a normal part of cellular and tissue homeostasis. This is exemplified during embryogenesis, when hypoxia is an intermittent consequence of developing prior to established blood vessels, and nutrients and oxygen must

Compliance and ethics

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diffuse to their target cells. The importance of hypoxia and the cellular response to it was further demonstrated in knockouts of the HIF proteins that were shown to be embryonic lethal due to cardiac and vascular malformations in HIF-1a knockout mice (Kotch et al., 1999) and divergent phenotypes in HIF-2α mice depending on the genetic background of the mice (Patel and Simon, 2008). More recent studies have shown that the hypoxia response pathway regulates critical roles in the liver (Nath and Szabo, 2012), osteoblastic niche cells (Rankin et al., 2012), intestine (Colgan and Taylor, 2010), and the heart (Giordano, 2005; Nakada et al., 2017).

In hypoxic conditions, HIF is rapidly stabilized. When bound to its cognate hypoxia response elements (HREs) within enhancer elements, HIF transcription factors increase the transcription of its target genes. These downstream effectors of HIF teleologically aid in diverse biological processes that aid in survival during hypoxia. For instance, hypoxic cells often shift their metabolism to glycolysis (Semenza et al., 1994), and reduce mitochondrial numbers through mitophagy (Semenza, 2007; Zhang et al., 2007) and decreased biogenesis(LaGory et al., 2015) thus reducing dependence on oxidative metabolism. Furthermore, HIF enhances hematopoiesis (Goldberg et al., 1988; Goldberg et al., 1987), angiogenesis (Shweiki et al., 1992), tissue remodeling (Graham et al., 1999), epithelial permeability (Synnestvedt et al., 2002) and vascular tone (Bodi et al., 1995), which may increase oxygen delivery. Growth is delayed by cycle arrest through the induction of cell cycle inhibitors p21 (Goda et al., 2003) and p27 (Hackenbeck et al., 2009) (Figure 1). Not surprisingly, this powerful coordinated program of gene expression promotes survival in harsh environments. However, this hypoxia program also helps tissues to heal. The preconditioning of tissues against ischemia/hypoxia strengthens and protects them against tremendous physiological insults such as cardiac ischemia (Olenchock et al., 2016), stroke (Karuppagounder and Ratan, 2012; Ratan et al., 2004; Ratan et al., 2007; Reischl et al., 2014), and even lethal radiation damage (Taniguchi et al., 2014).

Biochemically, HIF is a heterodimer consisting of one of three alpha (HIF-1α, HIF-2α, HIF-3α) subunits bound to the aryl hydrocarbon nuclear translocator (ARNT), also known as HIF-1β (reviewed in (Huang and Bunn, 2003)). Both HIFα and β are members of the basic helix-loop-helix *Per/Arnt/Sim* (bHLH-PAS) family of transcription factors (Figure 2). Both HIF-1α and HIF-2α contain O_2-dependent degradation (ODD) domain with two different proline sites for hydroxylation in the presence of oxygen. Moreover, HIF-1α and HIF-2α contain both N-terminal transactivation domain (N-TAD) and C-terminal transactivation (C-TAD) while HIF-1β contains only one TAD at the C terminus. In comparison, HIF-3α lacks the C-TAD domain but instead contains the leucine zipper (LZIP) domain in its longest variant. HIF-3α also only contains one proline site in the ODD domain (Heikkila et al., 2011). In the C-TAD domain of HIF-1α and HIF-2α, there is a conserved asparagine residue, which can be hydroxylated by an Fe(II) and O2-dependent enzyme, factor inhibiting HIF (FIH), thus blocking the interaction between HIFα and transcriptional co-factors (Lando et al., 2002). While the beta subunit is continually expressed, the alpha subunit is an oxygen-labile protein whose levels fluctuate with the oxygen tension within the cell such that the alpha subunit is stabilized in hypoxia and destabilized in normoxia (Huang et al., 1998; Salceda and Caro, 1997). Under normal atmospheric oxygen tension, oxygen-dependent prolyl hydroxylases use molecular oxygen along with co-factors iron and 2-

oxoglutarate to drive hydroxylation of prolines 402 and 564 of HIF-1α and proline 405 and 531 of HIF-2α (Ivan et al., 2001; Jaakkola et al., 2001). These hydroxyproline moieties then serve as docking sites for the von Hippel Lindau (VHL) protein (Epstein et al., 2001; Hewitson et al., 2003; McNeill et al., 2002) (Figure 3). The VHL protein is the recognition component of an E3 ubiquitin ligase complex that targets HIF for degradation by the 26S proteasome (Kamura et al., 1999; Kibel et al., 1995; Lonergan et al., 1998; Pause et al., 1997; Pause et al., 1999). Thus, prolyl hydroxylases and the VHL protein complex are two key molecules in regulating the levels of HIF, and the hypoxic response. In hypoxic conditions, both HIF-1α and HIF-2α are stabilized and form a heterodimer with HIF-1β, which together binds to HRE sequences residing in the enhancer element of target genes. HIF-1α and HIF-2α regulate some shared as well as unique target genes (Keith et al., 2011) (Figure 3). Both HIF-1α and HIF-2α regulate *GLUT1* and *VEGF*, while HIF-1α specifically regulates *PGK1* and *LDHA* in RCC and mouse ESCs, and HIF-2α regulates *EPO* and *IRS2* in the liver (Wei et al., 2013).

There are three oxygen-dependent prolyl hydroxylases in mammals, and they are known by two different naming systems, which can be confusing. For instance, in one method of gene naming, these enzymes are called the prolyl hydroxylase domain-containing (PHD) proteins and the HIF-regulating isoforms are appropriately named PHD1, PHD2, and PHD3 (Fraisl et al., 2009). Alternatively, these HIF prolyl hydroxylases are also named after the *C.elegans* orthologue *egg laying defective nine (EGLN)* gene (Kaelin, 2011). However, the gene numbering in the EGLN naming system do not match those in the PHD naming system, which creates significant confusion for those not in the field. For example, *EGLN1* is synonymous with *PHD2,* while *EGLN*2 is equivalent to *PHD1* (Bruick and McKnight, 2001; Jaakkola et al., 2001). Thankfully, *EGLN3* simply equates with *PHD3.* While both naming systems exist in the literature, we have adopted the EGLN nomenclature for several practical reasons. In addition to the confusion brought about by the differences in isoform numbering, PHD proteins share naming similarities to the plant homeodomain (PHD) zinc fingers which are found in many chromatin-modifying proteins (Sanchez and Zhou, 2011). Moreover, a simple literature search for PHD proteins will often return a very large list of papers that includes anyone with a Ph.D. degree. Thus, while both EGLN and PHD designations are correct, we strongly feel that EGLN may be a better way to discuss this important area of biology.

In terms of function, *Egln1* appears to be the dominant isoform, since its germline knockout is embryonic lethal (Minamishima et al., 2008). Whole body knockouts of *Egln2* and *Egln3*, on the other hand, were viable and displayed relatively mild phenotypes (Takeda et al., 2008). *Egln1* (Chan et al., 2009) and *Egln3* (Stiehl et al., 2006) are inducible by hypoxia at the mRNA level and are reliable markers of hypoxia. There does appear to be redundancy in the regulation of HIF by the EGLN isoforms, but studies have suggested that the Egln1 primarily regulates HIF1 (Chan and Giaccia, 2010), while Egln3 regulates HIF2 stability (Bishop et al., 2008). Moreover, there is a growing literature that the Egln proteins have HIF-independent functions that could possibly be even more critical than their roles in regulating HIF stability (Garvalov et al., 2014; Henze et al., 2014).

The Role of HIF in Tumorigenesis

Since hypoxia is important to normal tissues, many have postulated that low oxygen levels contribute to cancer growth and treatment resistance. These assertions have come from an abundant literature that have shown that cancer cells cultured *in vitro* take on many undesirable traits when placed into hypoxia culture, such as resistance to chemotherapy (Samanta et al., 2014; Warfel and El-Deiry, 2014) and radiation (Harada et al., 2012; Moeller et al., 2004; Zhong et al., 2015), increased proliferation and migration (Chen et al., 2015; Gordan et al., 2007; Liao and Johnson, 2007; Semenza, 2010). However, when in vivo experiments were performed to confirm this phenotype, the experimental results were initially counterintuitive. Rather than being a potent oncoprotein, HIF appears to chiefly function as a tumor suppressor *in vivo*. For instance, the deletion of HIF2α in lung cancer (Jiang et al., 2009; Mazumdar et al., 2010) or sarcoma models (Nakazawa et al., 2016) demonstrated accelerated tumor growth, suggesting a baseline tumor suppressive effect. This is mediated by the HIF2-dependent suppression of the *Scgb3a1(Hin-1)* gene in the lung cancer model (Jiang et al., 2009; Mazumdar et al., 2010), and by altered mTORC1 signaling in sarcoma models (Nakazawa et al., 2016). A similar phenotype was observed in mouse models of pancreatic cancer, where HIF1α deletion accelerated tumor growth, also suggesting a basal role in tumor suppression. The mechanism of this tumor suppression by HIF1 is unclear, but may involve the differential activation of immune cells, notably CD45+ immune cell infiltration, and B lymphocytes (Lee et al., 2016). It is notable that one experiment did suggest oncogenic properties of HIF2. When a nondegradable variant of HIF-2α (proline-to-alanine substitutions within the ODD domain) was conditionally overexpressed in a mutant Kras (Kras G12D) model of lung cancer, there was increased tumor burden and decreased survival compared with mice expressing only $Kras^{G12D}$ (Kim et al., 2009). This result appears to be at odds with the previously mentioned reference, however, it should be noted that overexpression of HIF2 in normoxia is not physiologic and may instead reflect off-target biology.

To some extent, the lack of oncogenic properties of HIF should not be surprising since patients with Von Hippel-Lindau (VHL) disease exhibit only a limited range of cancers. The loss of VHL results in widespread stabilization of HIF in many tissues in these patients, but they only get a limited range of lesions including hemangioblastomas of the central nervous system, retinal angiomas, endolymphatic sac tumors, epididymis or broad ligament cystadenomas, renal cysts and renal cell carcinomas, pheochromocytomas, pancreatic cysts and pancreatic neuroendocrine tumors (Lonser et al., 2003), while clear cell renal carcinoma (ccRCC) and hemangioblastomas are the main causes of death (Maher et al., 1990; Neumann et al., 1992). This is in contradistinction to genetic disorders like Li-Fraumeni which have dysregulated p53 function and are prone to many types of sarcomas and aggressive adenocarcinomas. In addition, inactivation of the VHL gene in embryonic stem cells does not promote teratocarcinoma growth (Mack et al., 2003). It should be made clear, however, that HIF is likely responsible for a narrow range of cancers with the most striking being the aforementioned VHL mutant ccRCC driven by HIF-2α (Kondo et al., 2002). Interestingly, HIF-2α inhibitors have been designed and appear to have a specific activity in

this disease (Chen et al., 2016; Cho et al., 2016), but its applicability to other cancers are not clear.

Stromal HIF as a regulator of Cancer Growth

Since the in vivo studies linking HIF and tumorigenesis are relatively weak, there are likely other ways that hypoxia and HIF affect tumor growth. We now understand that the tumor microenvironment plays a strong role in accelerating (Hanahan and Weinberg, 2011; Whiteside, 2008), or possibly constraining tumor growth (Rhim et al., 2014). The role of HIF in the microenvironment is not completely understood. The two-way dialogue between cancer cells and stroma is essential for tumor development and is strongly influenced by hypoxia. The tumor stroma is a fairly ill-defined term for any non-cancerous cell found within a tumor and can be divided into three main categories: cells of mesenchymal origin, cells of hematopoietic origin, and non-cellular components (Pattabiraman and Weinberg, 2014). Cells of mesenchymal origin include fibroblasts, myofibroblasts, endothelial cells, adipocytes, and mesenchymal stem cells. Cells of hematopoietic origin consist of cells of the lymphoid lineage, including T cells, B cells, and natural killer (NK) cells, and those of the myeloid lineage, including macrophage, neutrophils and myeloid-derived suppressor cells (MDSCs). Non-cellular component is mostly extracellular matrix (ECM), which includes proteins, glycoproteins and proteoglycans. Here, we will focus on the cancer-associated fibroblast, endothelial cells, and immune cells.

Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) contains two distinct cell types: cells that are similar to fibroblasts that support normal epithelial tissues and myofibroblasts which are rare in healthy epithelial tissues (Hanahan and Weinberg, 2011). A major feature of activated stromal cells is the formation of contractile stress fibers and expression of α-smooth muscle actin (SMA). Cancer-associated fibroblast secretes a variety of extracellular matrix components, resulting in desmoplastic stroma. Many epithelial tumors exhibit a prominent desmoplastic reaction, including breast, prostate and ovarian cancers. Perhaps the most exaggerated stromal reaction can be found in pancreatic ductal adenocarcinoma (PDAC), which displays extensive desmoplasia that increases intratumoral pressure and blocks intratumoral oxygen transport, rendering the tumors hypoxic (Incio et al., 2016). Moreover, the lack of blood flow from this desmoplastic reaction has been postulated to impede chemotherapy delivery (Koay et al., 2014) and lead to worsened outcomes (Olive et al., 2009). Of note, CAFs within PDAC are often positive for α–SMA-expressing activated pancreatic stellate cells (PSCs), and can account for as much as 80% of the tumor volume (Erkan et al., 2012). Despite the known association of hypoxia, CAF activation, and HIF stabilization within many aggressive solid tumors, the function of HIF within the microenvironment is unclear. One prominent study demonstrated that HIF-1α, but not HIF-2α, activation in human breast cancer stromal cells promotes a shift toward aerobic glycolysis and tumor growth via the paracrine production of lactate that feeds cancer cells (Chiavarina et al., 2012). However, this study merely used in vitro cell culture and in vivo xenograft model, which may not fully recapitulate the in vivo microenvironment, as another

study using syngeneic tumor models found that deletion of fibroblast HIF-1α accelerates tumor growth (Kim et al., 2012).

Other similar studies in head and neck CAFs and vulvar CAFs reveal hypoxia or loss of *EGLN1* leads to inactivation of CAFs and reduced tumor metastasis (Madsen et al., 2015). But again this study was conducted in in vitro setting. In a spontaneous mammary gland tumor study, global *Egln1* haplodeficiency leads to decreased tumor stiffness and metastasis without affecting primary tumor growth, which is due to reduced activation of CAFs (Kuchnio et al., 2015). However, confounding developmental, paracrine, or endocrine events from global *Egln1* deletion cannot be completely ruled out. Compartment-specific ablation of *Egln1* or *Hif1a* using a dual recombinase system (Moding et al., 2015) may be a method to overcome this potential methodological deficiency.

Endothelial cells

Tumor vessels, comprised mainly of endothelial cells, mediate tumor perfusion and also secrete regulatory paracrine factors. Unlike normal blood vessels, tumor vessels have abnormal organization, structure, and function. Intratumoral hypoxia is due to inadequate oxygen supply and disorganized network of vasculature. Mounting evidence suggests that hypoxia and HIF play central roles in regulating tumor endothelial cells. HIF-1α and HIF-2α have complementary functions in physiological and pathological angiogenesis. For instance, the loss of HIF-1α in endothelial cells inhibits blood vessel growth and tumor size by decreasing VEGF expression (Tang et al., 2004), whereas HIF-2α null endothelial cells exhibit high vessel density and branching but poor perfusion and low pericyte coverage through delta-like ligand 4/Notch (DII4/Notch) pathway and angiopoietin 2 (Ang2)-mediated pathway. Thus endothelial HIF2α null mice have reduced tumor growth (Skuli et al., 2009; Skuli et al., 2012). HIF also controls small molecule mediators of vascular response other than angiogenic factors, such as nitric oxide (NO), which affects the overall vascular tension of tumor vessels. The loss of endothelial HIF-1α reduces NO synthesis and impairs tumor cell migration through endothelial layers which is a gatekeeper to metastatic cell intravasation, while endothelial HIF-2α appears to play an opposing role (Branco-Price et al., 2012).

Another important component of the endothelial niche is the pericyte, which lines the outside of vascular endothelial cells and is required for a functional vasculature. Tumor-associated vasculature has fewer supporting pericyte cells. Pericyte depletion in early-stage non-hypoxic tumors suppresses neovascularization, tumor growth, and lung metastasis (Cooke et al., 2012). However, in hypoxic tumors, pericyte targeting increases intratumoral hypoxia and lung metastasis through Ang2-mediated pathway (Keskin et al., 2015).

Immune cells

Virtually all adult solid tumors contain infiltrating immune cells including both myeloid cells and tumor infiltrating lymphoid cells (TILs). Myeloid cells include subsets of granulocytes, dendritic cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). TILs can be divided into two subsets of cells with opposing functions: anti-tumor effector T cells which include CD4+ and CD8+ T cells, and

immunosuppressive regulatory T (Treg) cells. HIF induces host immune function, affecting myeloid-derived suppressor cells differentiation and function, regulating lymphocyte development, and driving T cell differentiation and cytotoxicity. HIF-1α is widely expressed in most innate and adaptive immune populations.

Many solid tumors show low oxygen density and abundant macrophage infiltration. Deletion of HIF-1α in macrophages in a progressive murine model of breast cancer results in reduced tumor growth by suppressing tumor-infiltrating T cells (Doedens et al., 2010). Hypoxia significantly increases the expression of PD-L1 on macrophages, dendritic cells and tumor cells through a HIF-1α, but not HIF-2α dependent pathway. Thus blockade of PD-L1 along with inhibition of HIF-1α may provide a novel approach for cancer immunotherapy (Noman et al., 2014). Moreover, hypoxia-induced Semaphorin 3A (Sema3A) contributes to recruitment of macrophages through neuropilin 1 (Nrp1)-plexin signaling (Casazza et al., 2013). Myeloid-derived suppressor cells are another cell source in the tumor microenvironment that can dampen antitumor immunity. Activation of HIF-1α is essential for myeloid cell infiltration and activation in vivo, independent of the function of VEGF (Cramer et al., 2003). Hypoxia via HIF-1α alters the function of MDSC and redirects their differentiation toward tumor-associated macrophages (Corzo et al., 2010). Similarly, mice lacking HIF-2α in myeloid cells displayed reduced tumor associated macrophage (TAM) infiltration in different carcinoma models, which is associated with reduced tumor cell proliferation and progression (Imtiyaz et al., 2010).

T lymphocytes are highly migratory adaptive immune cells that encounter a wide range of oxygen tensions, which are essential for T cell differentiation and function. Naive CD4+ T cells acquire specialized effector functions in response to different cytokines in the microenvironment, which includes Th1 cells, Th2 cells, and Th17 cells. It has been shown that tumor hypoxia promotes the recruitment of Treg cells by releasing chemokines, which in turn, promotes tumor tolerance and angiogenesis (Facciabene et al., 2011). However, there is also contradictory finding suggest otherwise, showing that HIF-1α promotes differentiation of CD4+ Th17 cells while preventing Treg differentiation (Dang et al., 2011). Enhanced HIF activity, due to loss of VHL, modulates the adaptive immune response of CD8+ T cells to persistent infection (Doedens et al., 2013), and maintains the stability and suppressive function of Foxp3(+) T cells and IFN-γ production (Lee et al., 2015). EGLN proteins in T cells suppress pulmonary inflammation, maintain immune tolerance, and may thus create an immunologically permissive environment for tumor colonization in the lung (Clever et al., 2016).

HIF in the metastatic niche

As mentioned previously, hypoxia promotes cell motility. This is interpreted as a model of metastatic progression. Several intriguing mediators of HIF and hypoxia have been postulated. Tumors are stiffer than the surrounding normal tissue, which is induced by ECM deposition and remodeling by resident fibroblasts, and by increased contractility of the epithelium (Frantz et al., 2010). Emerging data indicate the strong link between hypoxia and the composition and the organization of the ECM. It has been shown that HIF-1 directly regulates collagen deposition by activating collagen prolyl hydroxylases, which in turn

promotes invasion and metastasis of hypoxic breast cancer cells (Gilkes et al., 2013). Collagen crosslinking is initiated in the extracellular space by the lysyl oxidase (LOX) family of secreted enzymes. LOX is postulated to be secreted directly from hypoxic breast cancer cells to act directly on the extracellular matrix, as well as directly on osteoblasts and osteoclasts, which enhance tumor cell colonization (Cox et al., 2015). In clear cell renal cell carcinoma (ccRCC), stabilized HIF-1 and HIF-2 directly activate the expression of protein kinase GAS6/AXL by binding to the hypoxia-response element in the AXL proximal promoter. GAS6/AXL promotes cellular invasion and metastasis through activating the SRC proto-oncogene (Rankin et al., 2014; Zhou et al., 2016). Recent data also suggest that the HIF/AXL axis may also contribute to response to immunotherapy and radiation (Aguilera et al., 2016).

Implications in Cancer Biology and Therapy

Hypoxia alters the trajectory of cancer and makes tumors more difficult to treat. This is not surprising since the lack of oxygen directly antagonizes chemotherapy and ionizing radiation in damaging DNA. However, we must be careful to assign correlation and causation between effects caused by lack of oxygen and those promoted by HIF (Kaelin, 2017). Hypoxia is often associated with larger and more unfavorable tumors, and HIF is stabilized in hypoxia, but it does not necessarily follow that HIF plays a causative role in the pathophysiology of solid tumors. In fact, many recent studies have shown the opposite: that the EGLN/HIF axis acts as a tumor suppressor in preclinical models and in selected human data.

The actions of HIF within stromal cells are unknown and are ripe for further study. We summarize findings of HIF function from many different studies in Figure 4. What is clear is the hypoxic adaptations of tumors also dramatically alter the metabolism and function of stromal cells. (Figure 4). We currently do not know if altering EGLN/HIF expression within the tumor stroma has any functional consequences and further study is needed. However, this problem is methodologically challenging, since there is currently no system that can allow abrogation of HIF function in the stroma without also altering it in the tumor (conventional Cre-lox methodology). Orthotopic transplants into genetically altered mice have confounding developmental issues. Thus, given these methodological constraints, a new system may be needed.

The use of a dual recombinase system might allow for manipulation of the tumor genetics and the stroma separately. We and others (Schonhuber et al., 2014) have developed a system that initiates pancreatic tumorigenesis in a Flp-dependent fashion using a Pdx1-Flp along with a coordinated activation of Kras (G12D) oncogene (FSF-Kras) and a p53 allele (*Trp53*$^{FRT/FRT}$). We can create multi-allelic mice with the capacity to alter the EGLN proteins, HIF proteins, or other key regulators within stromal cells independent of the tumor genetics. This allows complete spatial and temporal control of gene deletion in the stroma or tumor tissues (Figure 4). This same system could afford temporal deletion of genes within the same tumor. Thus, we believe that this powerful system could be used to interrogate each stromal component in a definitive fashion (Figure 5).

The function of HIF in the tumor and its stroma is of critical importance not only from a basic biology standpoint, but also because we now have the capability to target the function of the HIF transcription factors. For instance, a small molecular inhibitor PT2399 specifically binds to the HIF-2α PAS B domain and causes tumor regression in ccRCC. It is important to understand the possible off-target effects of this drug if it were to be used in other cancer types that do not depend on HIF2 for its growth. Moreover, does HIF1 expressed in CAFs have an effect on stromal formation or treatment resistance? The answers to these questions should be fully understood before pursuing other HIF antagonists for the clinic.

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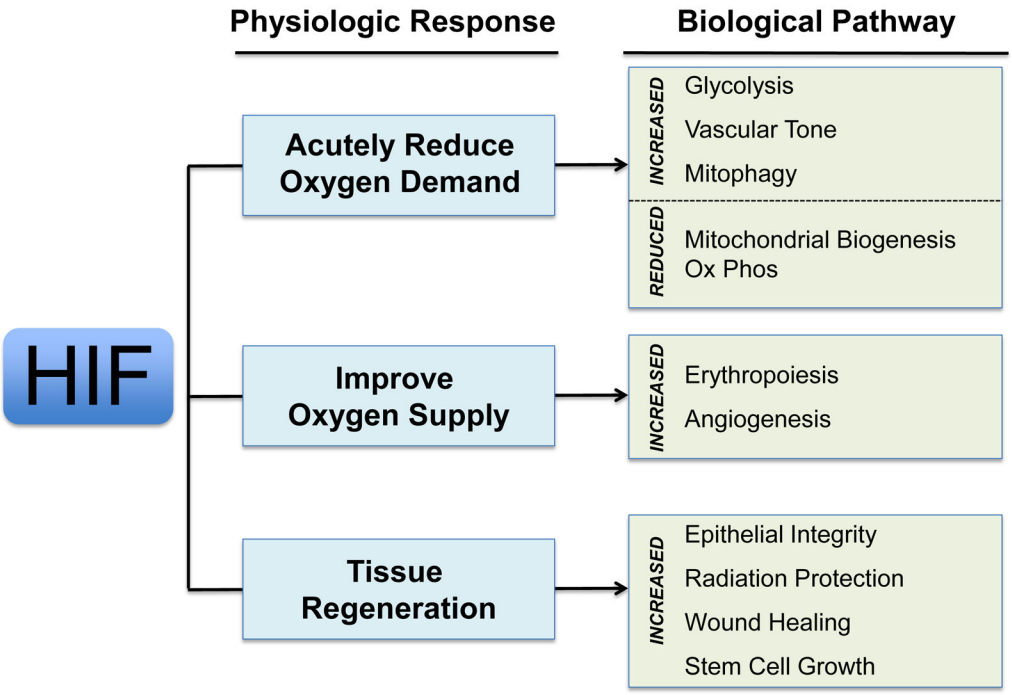

Figure 1.
The diverse physiological roles of HIF protein

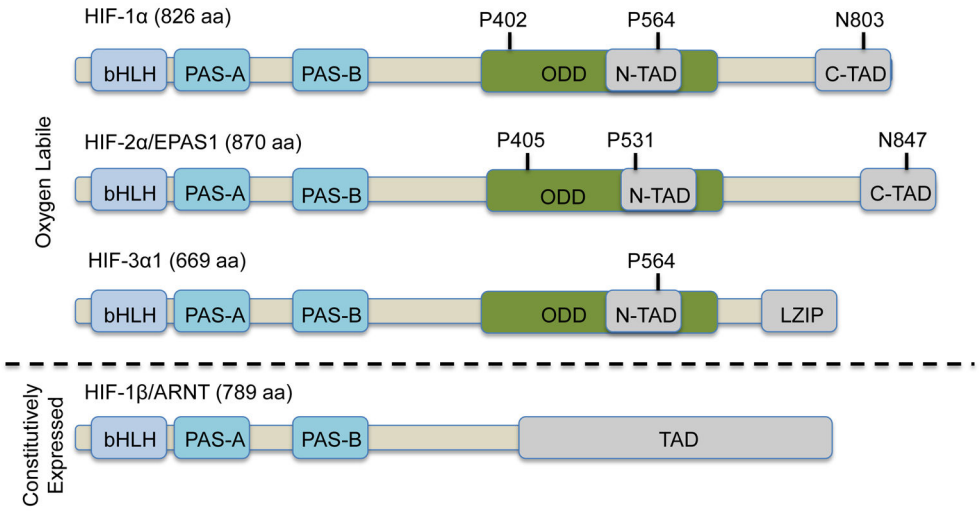

Figure 2.
Components of Human HIF heterodimer.

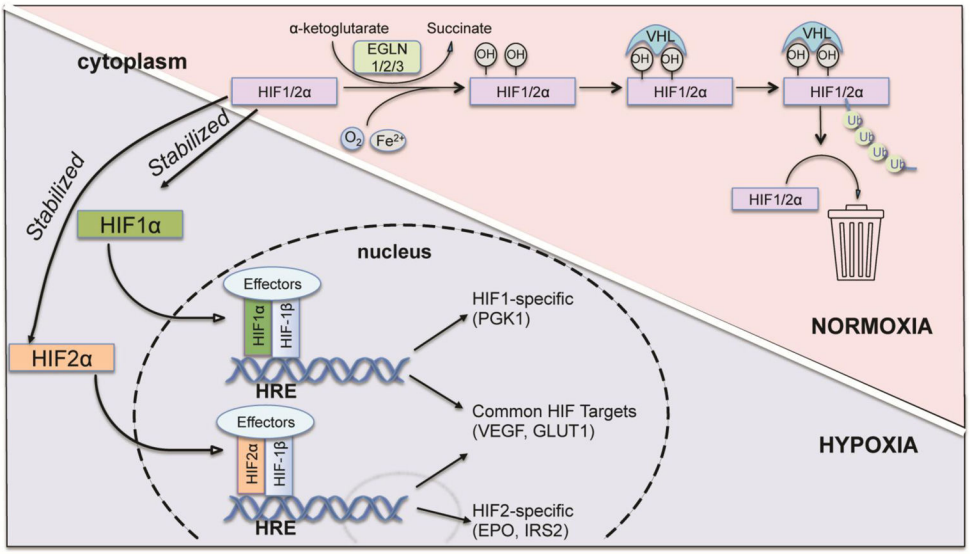

Figure 3.
Oxygen-dependent regulation of HIFα protein.

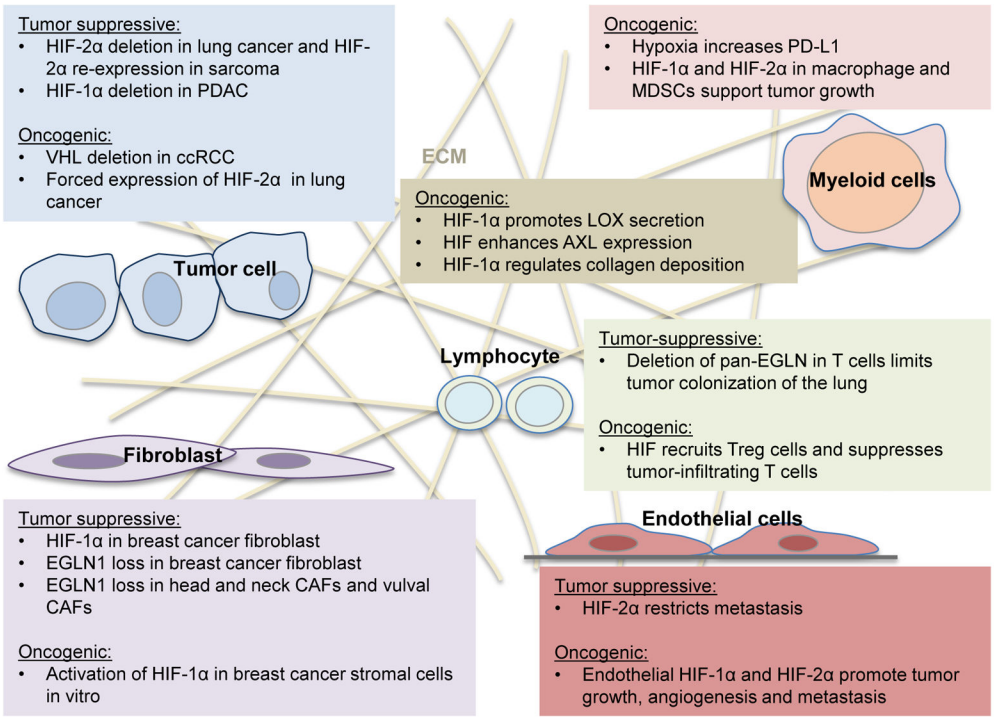

Figure 4.

The oncogenic and tumor suppressive activity of HIF in tumor and stromal cells.

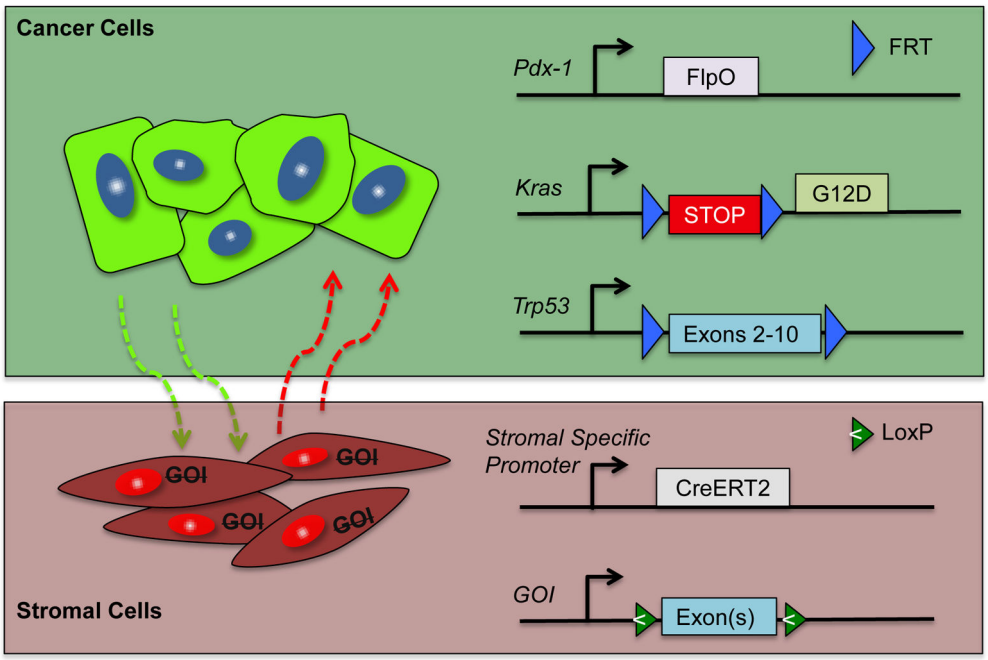

Figure 5.

Dual recombinase system for pancreatic cancer. Flp recombinase directs tumorigenesis in the target tissue by a tissue-specific Flp (in this case, Pdx1-FlpO), which activates *Kras* and knocks out *Trp53*. This leaves stromal components available to be regulated by the Cre/lox system.