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Role of Hypoxia in the Hallmarks of Human Cancer

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ABSTRACT

Hypoxia has been recognized as one of the fundamentally important features of solid tumors and plays a critical role in various cellular and physiologic events, including cell proliferation, survival, angiogenesis, immunosurveillance, metabolism, as well as tumor invasion and metastasis. These responses to hypoxia are at least partially orchestrated by activation of the hypoxia-inducible factors (HIFs). HIF-1 is a key regulator of the response of mammalian cells to oxygen deprivation and plays critical roles in the adaptation of tumor cells to a hypoxic microenvironment. Hypoxia and overexpression of HIF-1 have been associated with radiation therapy and chemotherapy resistance, an increased risk of invasion and metastasis, and a poor clinical prognosis of solid tumors. The discovery of HIF-1 signaling has led to a rapidly increasing understanding of the complex mechanisms involved in tumor hypoxia and has helped greatly in screening novel anticancer agents. In this review, we will first introduce the cellular responses to hypoxia and HIF-1 signaling pathway in hypoxia, and then summarize the multifaceted role of hypoxia in the hallmarks of human cancers. J. Cell. Biochem. 107: 1053–1062, 2009.

KEY WORDS: HYPOXIA; HYPOXIA INDUCIBLE FACTOR; CANCER; MICROENVIRONMENT; METASTASIS; METABOLISM

Tumorigenesis in human is a multistep process that involves the sequential acquisition of a number of genetic, epigenetic, or somatic alterations as a result of increasing genomic instability caused by defects in cell cycle checkpoint controls [Hanahan and Weinberg, 2000]. These alternations enable cancer cells to acquire characteristics different from normal cells: resistance to growth inhibitory factors, proliferation in the absence of exogenous growth factors, evasion of apoptosis, limitless replication potential via the reactivation of telomerase, abnormal angiogenesis, evasion of destruction by the immune system, invasion and metastasis [Hanahan and Weinberg, 2000; Bao et al., 2004]. In addition to the genetic, epigenetic, or somatic changes that occur in cancer, the tumor microenvironment is now considered to be a critical factor in malignancy progression and metastasis, and it influences the response to conventional anti-tumor therapies [Hanahan and Weinberg, 2000; Roskelley and Bissell, 2002]. As one of the most pervasive microenvironmental stresses and common features of solid tumors, hypoxia plays an important but complex role in mediating or regulating each of these hallmarks in the progression of human tumors from microinvasive to metastatic cancers in vivo (Fig. 1). Herein, we focus on the role of hypoxia, especially the hypoxia-inducible factor-1 (HIF-1) pathway, on the hallmarks of human cancers.

HYPOXIA AND THE HIF-1 SIGNALING PATHWAY

THE CELLULAR RESPONSES TO HYPOXIA

Mammalian cells undertake a variety of responses to maintain oxygen homeostasis, a precise balance between the need for oxygen as an energy substrate for oxidative phosphorylation and other essential metabolic reactions and the inherent risk of oxidative damage to cellular macromolecules. Oxygen is only able to diffuse 100–180 μm from the end of the nearest capillary to cells before it is completely metabolized [Powis and Kirkpatrick, 2004]. However, rapidly growing tumors quickly outstrip the vascular supply and, thus, result in a poorly vascularized microenvironment characterized by hypoxia, low pH, and nutrient starvation [Pouyssegur et al., 2006; Denko, 2008]. Therefore, intratumoral hypoxia occurs when cells are located greater than this distance from a functional blood vessel for adequate diffusion of oxygen as a result of rapid tumor cell proliferation and abnormal blood vessels [Semenza, 2007a; Bertout et al., 2008]. To survive and grow in this hypoxic microenvironment, tumor cells co-opt adaptive mechanisms to switch to a glycolytic metabolism, promote proliferation, become resistant to apoptosis, obtain unlimited replication potential and genomic instability, evade immune attack, induce angiogenesis, and migrate to less hypoxic areas of the body (Fig. 1).

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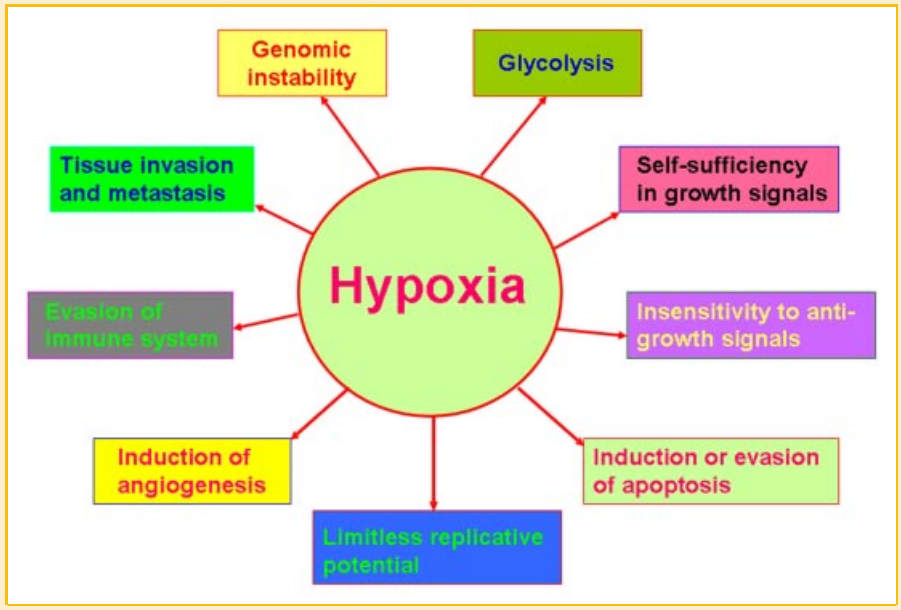

Fig. 1. The role of hypoxia in the hallmarks of human cancer. Under hypoxic microenvironments, cancer cells co-opt adaptive mechanisms to switch to a glycolytic metabolism, promote proliferation, induce or evade apoptosis, obtain unlimited replication potential and genomic instability, evade immune attack, induce angiogenesis, and invade and metastasize. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

HIF-1 SIGNALING PATHWAY IN HYPOXIA

Hypoxia-inducible factors (HIFs) play an essential role in the maintenance of oxygen homeostasis in metazoan organisms and primarily mediate adaptive responses to reduced oxygen levels [Semenza, 2007b; Kaelin and Ratcliffe, 2008].

HIFs are composed of two subunits, HIF-α and HIF-β (also known as ARNT). Mammalian HIF-α subunits are encoded by three genes: *HIF-1α*, *HIF-2α*, and *HIF-3α*. HIF-1α is ubiquitously expressed, whereas HIF-2α and HIF-3α exhibit a more restricted tissue distribution. HIF-1β is constitutively expressed and is largely insensitive to changes in oxygen levels, whereas the levels of all three HIF-α subunits are acutely regulated by hypoxia. HIF-1, a heterodimer of HIF-1α and HIF-1β, is overexpressed in various human cancers and has been recognized as one of the master regulators of oxygen homeostasis and mediates a wide variety of responses to hypoxia in tumorigenesis [Zhong et al., 1999; Bertout et al., 2008]. Current reports have shown that hypoxia activates HIFs by regulating two major switches that converge on α subunits. The first molecular switch regulates the overall cellular level of HIF-α while the second one controls transcriptional activities of HIF-α [for review see Kaluz et al., 2008]. There are two types of oxygen sensors, prolyl hydroxylase (PHD) proteins and factor inhibiting HIF-1 (FIH), to ensure full repression of the HIF pathway in well-oxygenated cells by regulating both the destruction and inactivation of HIF-α subunits [Jaakkola et al., 2001; Lando et al., 2002; Pouyssegur et al., 2006; Lisy and Peet, 2008]. In the presence of O_2, HIF-α is earmarked for degradation by hydroxylation which is catalyzed by PHDs, and then interact with the ubiquitin E3-containing ligase von Hippel–Lindau (pVHL). This interaction causes HIF-α to be ubiquitylated and then the polyubiquitylated HIF-α is targeted to the proteasome for degradation [Ohh et al., 2000; Kaelin and Ratcliffe, 2008; Kaluz et al., 2008]. However, increases in the HIF-α isoform under hypoxia are largely due to the regulation of protein degradation. In the absence of oxygen, the oxygen sensor proteins PHDs and FIH are inactive because of the lack of available oxygen. HIF-α is stable and translocates to the nucleus and heterodimerizes with constitutively expressed HIF-β. The heterodimer then interacts with cofactors and binds to the hypoxia-response elements (HREs) in the promoters or enhancers of hypoxia-responsive genes which are crucial to mediate systemic hypoxia responses [Bertout et al., 2008; Chandel and Simon, 2008; Simon and Keith, 2008] (Fig. 2).

ROLE OF HYPOXIA IN THE HALLMARKS OF CANCER

RESISTANCE TO ANTI-PROLIFERATION SIGNALS AND INDEPENDENCE FROM EXOGENOUS GROWTH FACTOR SIGNALS

It is well established that cell proliferation is strictly regulated by the concerted action of both pro-proliferative and anti-proliferative signals. On the other hand, cells also show a remarkable regulatory flexibility that allows them to thrive under different external microenvironments. Cells launch various signaling pathways in response to external microenvironment alterations and in order to coordinate cell growth with stress responses [Lopez-Maury et al., 2008]. To continue growing under hypoxic conditions, cancer cells adapt to the absence of exogenous mitogenic growth signals and become resistant to the anti-proliferative signals [Harris, 2002].

In a hypoxic microenvironment, cancer cells can grow more independent of exogenous mitogenic growth signals than normal cells by overexpressing the mitogenic growth factors themselves or constitutively activating the downstream pathways of these growth factors. Hypoxia can induce cancer cells to express various growth

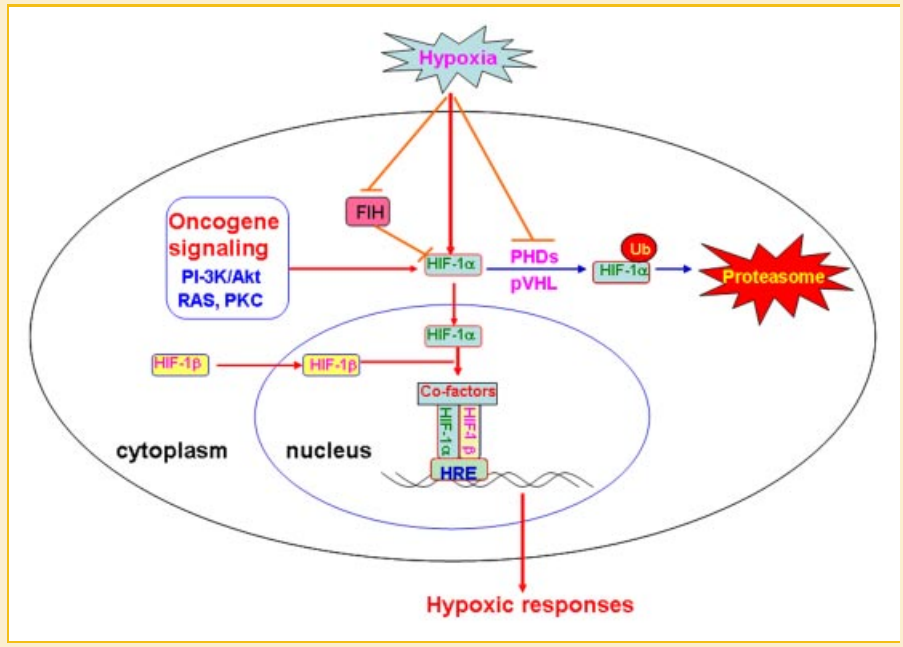

Fig. 2. Hypoxia signaling through HIF-1 pathways in human cancer. Two oxygen sensors, prolyl hydroxylase domain (PHD) proteins and factor inhibiting HIF-1 (FIH), ensure full inhibition of HIF-1α in well-oxygenated cells by regulating the destruction and inactivation of HIF-1α, respectively. Under hypoxic conditions, the oxygen sensor proteins PHDs and FIH are inactive because of the lack of available oxygen, and HIF-1α protein hydroxylation and pVHL association are decreased. Hypoxic cells redirect HIF-1α away from ubiquitylation and being targeting to the proteasome for degradation toward signaling to the nucleus. After translocation to the nucleus, HIF-1α heterodimerizes with the constitutively expressed HIF-1β subunit, and the heterodimer interacts with cofactors and binds to DNA at hypoxia-response elements (HRE) in the promoters or enhancers of numerous hypoxia-responsive genes. HIF-1-mediated transcriptional regulation is synergistically enhanced by cofactors such as p300/CBP, SRC-1, and Ref-1. Hypoxia-inducible genes regulate various biological processes, including cell proliferation, apoptosis, angiogenesis, metabolism, and metastasis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

factors such as EGF, insulin, IGF-1, IGF-2, and PDGF, which are known to promote cell proliferation [Maxwell et al., 2001; Powis and Kirkpatrick, 2004]. p42/p44 MAPK has been reported to phosphorylate HIF-1α and activate the transcription of HIF-1-response genes to regulate cell proliferation [Richard et al., 1999]. Moreover, hypoxia-induced HIF-1α activates transcription of vascular endothelial growth factor (VEGF) and VEGFR1 to promote endothelial cell proliferation and blood vessel formation [Harris, 2002].

In addition to taking advantage of various hypoxia-induced growth-promoting signaling, cancer cells also modify growth-inhibitory signaling events. For example, tumor suppressor PTEN can inhibit the PI-3K/Akt pathway and regulate cell growth and survival. PTEN is deleted or mutated in some human cancers. Restoration of wild-type PTEN in glioblastoma cell lines lacking functional PTEN ablates hypoxic induction of HIF-1-regulated genes. Thus, PTEN mutations may promote tumor growth by synergistically promoting HIFs-mediated responses [Zundel et al., 2000; Harris, 2002].

INDUCTION OR EVASION OF APOPTOSIS

Tumors grow in an uncontrolled manner through the imbalance of cell proliferation and death. The role of deregulation of apoptosis in tumorigenesis is complex and associated to successive and interdependent genetic and epigenetic events that gradually result in tumor formation [Nelson et al., 2004]. In contrast to normal cells, cancer cells can break the balance between pro- and anti-apoptotic factors to promote cell survival under adverse environmental conditions, such as hypoxic stress, radiation, or chemotherapy [Semenza, 2002; Bao et al., 2004; Blagosklonny, 2004]. The mechanisms of apoptosis regulation under hypoxia are, however, not fully understood.

There is evidence that the mitochondrial permeability transition is a central mechanism in hypoxia-mediated apoptosis. HIF-1α promotes the release of cytochrome *c* from mitochondria into the cytoplasm, and this p53-dependent apoptosis is mediated by Apaf-1 and caspase-9 [Soengas et al., 1999]. This process can be counteracted by members of the Bcl-2 family of anti-apoptotic molecules, such as Bcl-2 itself and Bcl-xL. Evidence has also been provided that Bax, another member of the Bcl-2 family, enhances hypoxia-induced apoptosis. HIF-1 activates BNIP3 and NIX, which induce a mitochondrial-pore permeability transition and cell death via a mechanism that does not involve cytochrome *c* release or caspases [Sowter et al., 2001; Harris, 2002]. In addition, the JNK pathway also contributes to hypoxia-induced apoptosis [Kunz et al., 2001]. Furthermore, only the phosphorylated HIF-1α binds HIF-1β under hypoxia, while the dephosphorylated form of HIF-1α binds p53 and exerts pro-apoptotic effects [Suzuki et al., 2001]. Therefore, the phosphorylation status of HIF-1α under hypoxia is a critical factor for the decision on whether HIF-1 promotes apoptosis. In addition, the effect of HIF-1α on apoptosis is dependent on the cancer type and the presence or absence of genetic alterations that alter the balance between pro- and anti-apoptotic factors [Semenza, 2003].

However, hypoxic exposure can also increase resistance to apoptosis. Due to the lack of effective and adequate blood vessels in the early stages of tumor development, fast growing tumors often become hypoxic. Although hypoxia is toxic to both cancer cells and normal cells, cancer cells can undertake genetic and adaptive changes in response to hypoxia that permit them to be refractory to apoptosis and increase their ability to survive and proliferate compared with normal cells [Harris, 2002; Powis and Kirkpatrick, 2004]. Tumor cells have developed various mechanisms for escaping HIF-1-mediated apoptosis under a hypoxic microenvironment [Dong et al., 2001]. Zhang et al. have reported that, in some rodent cells, hypoxia can up-regulate the expression of the p53-negative regulator MDM2 and increase cells resistance to apoptosis in vivo and promote metastasis [Zhang and Hill, 2004]. Hypoxia can induce the expression of the anti-apoptosis protein IAP-2 or apoptosis repressor with caspase recruitment domain (ARC) [Schmid et al., 2004]. β-Catenin can enhance HIF-1-mediated transcription, thereby promoting cell survival and adaptation to hypoxia in colorectal tumorigenesis [Kaidi et al., 2007]. Moreover, the PI-3K/Akt survival pathway, a critical regulator of cell survival and proliferation, has been shown to be activated in various cell types under hypoxia [Chen et al., 2001; Song et al., 2005; Furuta et al., 2008; Wai and Kuo, 2008; Zeng et al., 2008; Walsh et al., 2009]. Our previous work has revealed that periostin, a secreted protein, can dramatically enhance metastatic growth of colon cancer by both preventing hypoxia-induced apoptosis in cancer cells and augmenting endothelial cell survival via the Akt/PKB pathway [Bao et al., 2004]. Osteopontin (OPN) is another secreted protein that plays an important role in the progression of tumor development [Rangaswami et al., 2006; Wai and Kuo, 2008]. Our recent results suggested that the underlying mechanism of OPN-mediated promotion of tumor development is largely associated with the ability of Akt activation to enhance cell survival under stress [Song et al., 2008a,b]. In this regard, acquired expression of periostin, OPN, and similar types of matricellular proteins may enable tumor cells to thrive in the hypoxic microenvironment [Ouyang et al., 2009; Ruan et al., 2009]. In addition, hypoxia acts as a selective pressure during tumor growth, eliminating cells with wild-type p53 and selectively and clonally expanding cells with mutant or otherwise inactive p53; these results indicate that hypoxia-mediated selection for p53 mutant cells with diminished apoptotic potential in solid tumors may account for the high prevalence of p53 mutations in human cancers [Graeber et al., 1996]. Therefore, hypoxia may provide a physiological selective pressure in tumors for the preferential expansion of variants that have lost their apoptotic potential [Graeber et al., 1996; Bao et al., 2004].

LIMITLESS REPLICATIVE POTENTIAL

Human somatic cells normally have a finite replicative potential. After a limited number of cell divisions, cells undergo senescence. Cell senescence inhibits the development of cancer by suppressing the proliferation of damaged or stressed cells that are at risk for malignant transformation [Campisi, 2005a]. Senescence-inducing stimuli are potentially oncogenic; therefore, cancer cells have to acquire alterations that allow them to avoid telomere-dependent and oncogene-induced senescence [Hanahan and Weinberg, 2000; Hahn and Weinberg, 2002; Campisi and d'Adda di Fagagna, 2007; Gillies and Gatenby, 2007]. Current reports have revealed that cancer cells can overcome this obstacle, fail to senesce, and then become immortalized [Artandi and DePinho, 2000; Campisi, 2005b; Yaswen and Campisi, 2007]. Immortalization is an essential prerequisite for the formation of a cancer cell. Human cancer cells acquire the property of immortalization through maintenance of telomeres by either up-regulating the telomerase or alternatively lengthening the telomeres [Hanahan and Weinberg, 2000; Hahn and Weinberg, 2002].

Human telomerase (hTERT) has been identified as one of the key effectors in cell senescence. Under a hypoxic environment, telomerase activity in cancer cells increases and promotes cellular immortalization, and the increased telomerase activity is mediated by MAPK activation [Seimiya et al., 1999]. Negative regulation of HIF-1 by PDH2, FIH, HIF-1 siRNA, and the HIF-1 inhibitor YC-1 can elicit massive endometrial cancer cell senescence. hTERT expression is decreased in senescence that is induced by PDH2-mediated HIF-1α down-regulation [Kato et al., 2006]. A recent study has shown that HIF-1α-dependent macrophage migration inhibitory factor (MIF) expression is necessary for hypoxia-induced evasion from cell senescence, and MIF is required for HIF-1α stabilization induced by hypoxia and PHD inhibitors [Winner et al., 2007]. Mouse embryo fibroblasts (MEFs) harboring pVHL inactivation show increased rates of growth arrest and the accumulation of markers associated with senescence [Young et al., 2008]. However, neither hypoxia induction nor treatment with PHD inhibitors is sufficient to induce senescence in wild-type of MEFs [Kim and Sharpless, 2008; Young et al., 2008]. Therefore, the senescence induced by pVHL loss is independent of its ability to downregulate HIF.

INDUCTION OF ANGIOGENESIS

It is well established that local growth and metastasis of a large variety of malignant tumors are dependent on the formation of new blood vessels. To grow beyond 1 to 2 mm in diameter, solid tumors require a blood supply to provide enough nutrients and oxygen. Interestingly, tumor development is associated with both increased microvascular density and an intratumoral hypoxic microenvironment. These paradoxical characteristics arise because the tumor vasculature is structurally and functionally abnormal, resulting in abnormal perfusion that is characterized by marked spatial and temporal heterogeneity.

Tissue hypoxia can induce a number of angiogenic factors that promote angiogenesis, such as VEGF, IL-8, angiogenin, FGF, and PDGF. VEGF plays a major role in physiological blood vessel formation and pathological angiogenesis in tumor growth [Ellis and Hicklin, 2008]. Up-regulation of VEGF and other factors such as angiopoietin 2 (Ang-2) promotes the initiation and progression of angiogenesis [Holash et al., 1999; Dewhirst et al., 2008]. VEGF-A is expressed in most cells, and attracts and guides sprouting neovessels into oxygen-depleted areas in tumor tissues [Pouyssegur et al., 2006]. Hypoxia can promote HIF-1 activity to up-regulate VEGF as a trigger for the initiation of angiogenesis but can also decrease the activity of angiogenic inhibitor thrombospondin to create a pro-angiogenic microenvironment [Laderoute et al., 2000; Dewhirst et al., 2008]. Our previous data [Bao et al., 2004] have shown that the

secreted protein periostin can dramatically enhance the ability of human microvessel endothelial cells to survive under hypoxic conditions, which is compatible with the survival-promoting effect induced by the potent angiogenic factor VEGF. Ang-2 is another key angiogenic factor induced by hypoxia, however, the precise mechanism of regulation of Ang-2 expression in hypoxia remains to be defined [Maisonpierre et al., 1997; Pouyssegur et al., 2006].

Based on currently available evidence, there are two different models, the hypoxic crisis model and the acceleration model, that explain the role of hypoxia during initiation or acceleration of angiogenesis [Holash et al., 1999; Cao et al., 2005; Dewhirst et al., 2008]. In the vascular crisis model, hypoxia can induce VEGF to promote the initiation of angiogenesis. However, the acceleration model proposes that hypoxia is not responsible for initiation of angiogenesis and that this initiation is driven by non-hypoxia-mediated mechanisms, such as inducing VEGF by oncogene activation. Once angiogenesis is initiated, cancer cells grow quickly and result in hypoxia. HIF-1 is then increased to accelerate angiogenesis [Dewhirst et al., 2008]. Taken together, hypoxia may promote angiogenesis in tumors in two ways: first, by directly stimulating the expression of a series of angiogenic factors involved in the growth and survival of endothelial cells and second, by inducing a microenvironment favorable for endothelial cells and cancer cell survival, migration, and invasion.

EVASION OF THE IMMUNE SYSTEM

The tumor microenvironment is regulated not only by cancer cells but also by endothelial cells, immune cells, and stromal elements [Shi et al., 2004]. The co-existence of cancer cells and anti-tumor immune cells (''Hellstrom Paradox'') has been a challenging paradox for a long time [Lukashev et al., 2007]. Interestingly, cancer cells undergo various changes and acquire the ability to evade the immunosurveillance. However, the complex mechanisms by which cancer cells evade the immune attack under hypoxic microenvironment are poorly understood. One possibility may be that cancer cells secrete various immunosuppressive factors by HIF-1α-dependent or -independent pathways to protect cancer cells from immune damage.

In addition to cancer cells, immune cells are also often exposed to low oxygen tensions and must adapt to hypoxia to function in tumors and other microenvironments that are located far from the ends of capillaries [Sitkovsky and Lukashev, 2005; Rius et al., 2008]. Monocytes are continually recruited into tumors, differentiate into tumor-associated macrophages, and then accumulate in hypoxic areas. Hypoxia is a critical intratumoral signal that stimulates tumor-associated macrophages to secrete a series of mitogenic factors, proangiogenic cytokines, and immunosuppressive agents by inducing such transcription factors as HIF-1 and HIF-2 [Talks et al., 2000; Lewis and Murdoch, 2005]. Hypoxia also inhibits the ability of macrophages to phagocytose dead or dying cells, present antigens to T cells, and inhibit the anti-tumor effects of macrophages [Murata et al., 2002; Lewis and Murdoch, 2005]. In addition, HIF-1α plays a critical role in the survival of T cells by preventing them from undergoing activation-induced cell death in a hypoxic microenvironment [Makino et al., 2003; Sitkovsky and Lukashev, 2005]. Tumor cells are protected from immune attack in hypoxic and immunosuppressive tumor microenvironments due to the inhibition of anti-tumor T cells. The HIF-1α-mediated anti-inflammatory pathway and hypoxia-induced extracellular adenosine may cooperatively contribute to the tumor-protecting immunosuppressive effects of tumor hypoxia [Sitkovsky and Lukashev, 2005; Lukashev et al., 2007].

These observations support the hypothesis that not only malignant cells but also immune cells adapt to hypoxic microenvironments and promote the expression factors that are necessary to escape immunosurveillance; together, these data suggest that eliminating these signaling from tumor cells enhances the host anti-tumor immune response.

TISSUE INVASION AND METASTASIS

Invasion and metastases are the most common causes of morbidity and mortality induced by cancer. Tumor invasion and metastasis consist of multiple and complicated processes, including cellular disengagement and motility from the local microenvironment, degradation of the surrounding extracellular matrix, and cellular movement, all of which must be successfully completed to permit the outgrowth of metastatic tumors in the new microenvironment [Chambers et al., 2002; Murata et al., 2002; Bao et al., 2004; Ma et al., 2008; Nguyen et al., 2009]. Successful adaptation to varying microenvironmental constraints plays a critical role during tumorigenesis [Joyce and Pollard, 2009]. In the hypoxic environment, tumor cells undertake a series of changes not only to survive and grow in hypoxic microenvironments but also to subsequently expand and promote invasion and metastasis. Clinical studies have shown that tumor hypoxia is one of the important microenvironmental determinants for tumor cell dissemination. Adaptation to hypoxia may represent one of the key events during the transition from in situ to invasive breast cancer [Gatenby et al., 2007]. Another recent work has shown that HIF-1α is a critical regulator of metastasis in a transgenic model of cancer initiation and progression [Liao et al., 2007]. Hypoxic tumor cells are more aggressive, prone to distant metastases, and tolerant to anti-cancer therapy. One report revealed that antisense HIF-1α treatment can inhibit the expression of survivin and β1 integrin, enhance apoptosis in human pancreatic cancer cells, and restrain the invasion and metastasis of pancreatic cancer [Chang et al., 2006]. Hypoxia or HIF-1α overexpression promotes matrigel invasion by HCT116 human colon carcinoma cells, whereas this process is inhibited by HIF-1α siRNA [Krishnamachary et al., 2003]. However, the mechanisms that result in the increased metastatic potential of tumor cells exposed to hypoxia and the exact role of HIF-1α in the metastasis still have not been well defined.

It was proposed that the initial steps in metastasis involve epithelial-mesenchymal transition (EMT) [Yang et al., 2004; Polyak and Weinberg, 2009]. The well-polarized, adhesive epithelial cells are converted to non-polarized mesenchymal cells. EMT is a multi-step process that requires the coordination of multiple cellular events, including the disruption of intercellular adhesion mediated by cadherins at adherens junction, the loss of apicobasal polarity, cytoskeletal architecture reorganization, and the degradation of the basement membrane. Both hypoxia and overexpression of HIF-1α have been shown to promote EMT and metastatic phenotypes

[Krishnamachary et al., 2006; Peinado et al., 2007; Yang et al., 2008]. Hypoxia-induced HIFs activation is associated with a concomitant loss of E-cadherin, one of the landmarks of invasion and a crucial feature of EMT [Beavon, 1999]. Hypoxia may attenuate the expression of E-cadherin via activation of the lysyl oxidase (LOX)-Snail pathway to promote tumor invasion and metastasis, indicating that hypoxia-induced LOX and HIFs are important factors that regulate tumor microenvironments to favor metastasis [Imai et al., 2003; Erler et al., 2006; Pouyssegur et al., 2006; Croker and Allan, 2008]. Notch signaling and Wnt pathways are also required to convert the hypoxic stimulus into EMT, increased motility, and invasiveness [Jiang et al., 2007; Sahlgren et al., 2008]. All these data indicate that tumor hypoxia and/or HIF signaling are strongly associated with malignant progression.

GENOMIC INSTABILITY

It is well known that genomic instability is responsible for cellular changes that confer progressive transformation on cancer cells [Hanahan and Weinberg, 2000; Huang et al., 2007; Shen et al., 2008]. Genotoxic stress activates cell cycle checkpoints and delays cell cycle progression to allow for DNA repair [Bao et al., 2001]. Genetic defects in DNA repair mechanisms and cell cycle checkpoints result in increased genomic instability and cancer predisposition. Emerging evidence indicates that hypoxia as a tumor microenvironmental stress can drive genomic instability through increased chromosomal rearrangement, gene amplification, and induction of intrachromosomal fragile sites in hypoxic cells [Coquelle et al., 1998; Bristow and Hill, 2008]. The frequency of point mutations in tumorigenic cells cultured under severe hypoxic treatment is 3.4-fold higher than that observed in normally oxygenated cells [Hockel and Vaupel, 2001]. Current studies have shown that hypoxia can suppress the expression of DNA mismatch repair (MMR) genes and result in increased mutagenesis [Bindra et al., 2005, 2007; Huang et al., 2007]. The loss of MMR renders human colon carcinoma cells hypersensitive to the ability of hypoxia to induce microsatellite instability and generate highly drug-resistant clones in the surviving population [Kondo et al., 2001]. Hypoxia contributes further to genetic instability by HIF-1α dependent transcriptional repression of the mismatch repair genes MSH2 and MSH6 [Koshiji et al., 2005]. In addition, polyploidy is also much more common in hypoxia. Hypoxia can decrease the expression levels of Brca1 and Rad51, which are two important mediators of homologous recombination in mammalian cells [Bindra et al., 2005, 2007]. Hypoxia also regulates cell cycle checkpoints and DNA repair in the prostate epithelium, thereby driving genomic instability and tumor aggression [Chan et al., 2007]. Furthermore, several DNA damage and DNA repair checkpoints proteins, including ataxia-telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), Chk1, Chk2, Brca1, and p53, are activated in response to hypoxic exposure [Hammond et al., 2002, 2003; Gibson et al., 2005, 2006; Bindra et al., 2007].

GLYCOLYSIS IN HYPOXIC CELLS

In addition to the roles of hypoxia and the HIF-1 pathway in directly and indirectly mediating and regulating these above hallmarks of cancer, the adaptive shift from aerobic to anaerobic metabolism can also be regarded as one of the important hallmarks of cancer cells under hypoxia [Gillies and Gatenby, 2007; Gatenby and Gillies, 2008]. The above essential hallmarks of cancer are interwined with an altered cell-intrinsic metabolism, either as a consequence or as a cause [Kroemer and Pouyssegur, 2008].

Normal tissues and cells in well-oxygenated environments typically rely on highly efficient aerobic metabolism to generate ATP [Kim et al., 2007; Fang et al., 2008; Aragones et al., 2009]. Under hypoxic conditions, however, cancer cells adjust their cellular physiology and metabolism to the new microenvironment and switch their glucose metabolism from the oxygen-dependent oxidative phosphorylation to the oxygen-independent glycolysis. There is abundant evidence that components of the glycolytic pathway are up-regulated in cancer cells. Hypoxic cancer cells use glycolysis as a primary mechanism of ATP production, and HIFs are master regulators of glucose metabolism during hypoxia [Harris, 2002; Semenza, 2003; Brahimi-Horn et al., 2007; Denko, 2008]. Recently, it was revealed that HIF-1 mediates this adaptation to hypoxia in cancer cells by down-regulating tricarboxytic acid (TCA) cycle activity and mitochondrial oxygen consumption through inhibition of pyruvate dehydrogenase [Kim et al., 2006; Papandreou et al., 2006]. HIF-1 can inhibit mitochondrial biogenesis and cellular respiration and increase glycolysis in VHL-deficient renal cell carcinoma by decreased repression of c-Myc activity [Zhang et al., 2007]. HIF-1 and c-Myc collaborate to active hexokinase 2 (HK2) and pyruvate dehydrogenase kinase 1 (PDK1), resulting in increased conversion of glucose to lactic acid [Dang et al., 2008]. Furthermore, mammalian target of rapamycin (mTOR) signaling represents another critical regulator of cellular metabolism and is regarded as a positive regulator of glycolysis as well as of the biosynthesis of amino acids, proteins, lipids, and nucleotides [Wouters and Koritzinsky, 2008]. Therefore, HIFs may collaborate with Myc and mTOR in both synergistic and antagonistic ways to regulate cellular metabolism downstream of some of the most common mutations in cancer [Zhang et al., 2007; DeBerardinis et al., 2008; Wouters and Koritzinsky, 2008]. However, the mystery behind the molecular mechanisms of this metabolic switch under hypoxia is still not well understood.

CONCLUDING REMARKS

Tumorigenesis largely depends on alterations in the heterotypic interactions between incipient cancer cells and their normal neighbors. Virtually all types of human tumors are composed of several distinct cell types, such as cancer cells, immune cells, fibroblasts, and endothelial cells [Hanahan and Weinberg, 2000]. The evolution of a clinically significant invasive cancer from a focus of microinvasion requires not simply tumor mass growth but rather an active collaboration of malignant epithelial cells and normal mesenchymal cells [Gatenby and Gillies, 2008]. Therefore, it is widely accepted that tumors can be regarded as complex tissues in which cancer cells have conscripted and subverted normal cells to serve as active collaborators in their neoplastic process [Hanahan and Weinberg, 2000; Gatenby and Gillies, 2008]. To survive and grow in these complex microenvironments, cancer cells have to

overcome many noxious stimuli, such as hypoxia, that may induce cell death. However, it is not well understood how cancer cells adapt and manipulate pathways to cooperate with other cell types in tumors to promote their own survival and growth under hypoxic microenvironments. Current reports have revealed the microRNA signature of hypoxia in cancer cells, but the mechanisms by which hypoxia regulates the microRNA levels and the role of microRNAs in eliciting hypoxia responses are not well known [Kulshreshtha et al., 2007; Camps et al., 2008; Ivan et al., 2008]. Recently, it has been recognized that cancer stem cells also play critical roles in tumorigenesis and that hypoxia-induced transcription factors may drive tumor growth through the generation or expansion of cancer stem cells [Bao et al., 2006; Keith and Simon, 2007; Rich and Bao, 2007; Croker and Allan, 2008]. However, the role of hypoxia in cancer stem cells in tumorigenesis remains less clear. Likewise, little is known about the precise mechanisms that are responsible for the limitless replication potential and genomic instability characteristics of hypoxia on cancer cells. Another important area will be to establish the molecular basis for how cancer cells switch their metabolism to adapt to hypoxia.

As one of the most pervasive microenvironmental stresses that can impact malignant progression, tumor hypoxia can be found in almost every solid tumor and be now widely recognized as a cause of treatment failure and poor outcome for a wide variety of adult malignancies and, thus, needs to be taken into account when evaluating prognostics and therapeutic options for cancer patients [Lundgren et al., 2007; Le and Courter, 2008]. HIF-1 inhibition may represent a global strategy for targeting the hypoxic tumor microenvironment and there is an extensive effort involved in identifying new more potent and specific HIF-1 inhibitors. However, HIFs-independent pathways may bypass or overcome HIFs inhibition. Therefore, HIF-1 inhibitors may have to be combined with other targeted agents or conventional therapies to integrate hypoxia-targeting methods to get more reasonable results. Focusing research attention on these questions would thus not only be very beneficial for understanding the multifaceted roles of hypoxia on the hallmarks of human cancers but also facilitate the rational design of combination therapies to target hypoxia for cancer treatment.

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