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The Role of Hypoxia in Tumor Progression, Metastasis, and Effect on Tumour Microenvironment

Zoleykha Feyzollahi Mirak [1,2], **Seyed Bahram Behzad** [2,3]

[1]Cellular developmental biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[2]Plasma medicine (plas-med) interest group- Universal Scientific Education & Research Network (USERN), Tehran, Iran
[3]Department of Cellular & Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Correspondence to: Behzad S.B. (E-mail: behzadbahram12@gmail.com)

Abstract

The origins of the mesenchymal cells participating in tissue repair and pathological processes, notably tissue fibrosis, tumor invasiveness, and metastasis, are poorly understood. However, emerging evidence suggests that epithelial- mesenchymal transitions (EMTs) represent one important source of these cells. As we discuss here, processes similar to the EMTs associated with embryo implantation, embryogenesis, and organ development are appropriated and subverted by chronically inflamed tissues and neoplasias. The identification of the signaling pathways that lead to activation of EMT programs during these disease processes is providing new insights into the plasticity of cellular phenotypes and possible therapeutic interventions. Hypoxia is an important phenomenon in the tumor microenvironment. Hypoxic tumors are more aggressive and resistant to anti-neoplastic treatments. HIF-1a plays a major role in the response of tumors to hypoxia, and it is mainly responsible for the "angiogenic switch". HIF-1a contributes to tumor aggressiveness, invasiveness and resistance to radiotherapy and chemotherapy. Targeting HIF-1a is an attractive strategy, with the potential for disrupting multiple pathways crucial for tumor growth. We review recent findings on the potential efficacy of small molecules to downregulate HIF-1a. These promising drugs inhibit HIF-1a synthesis or transcriptional activity by blocking a variety of steps in several different signaling pathways. Blocking HIF-1a activity should not only downregulate tumor angiogenesis, but also interfere with glycolytic metabolism and tumor cell growth. This strategy could also improve the efficiency of established tumor therapies.

Keywords: Hypoxia, Metastasis, Tumor microenvironment, HIF-1

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1. Introduction

In the development of a cancer, the transformation of epithelial cells into a neoplastic and progressively invasive tumor occurs though the acquisition of several procancer characteristics that can take years or decades to develop. The particular stages of transformation have been established and a general consensus exists about the properties of a successful malignancy. While many therapeutics have been developed to combat these properties, these therapies are not universally successful, and their efficacy depends on the type and site of the primary tumor, its degree of vascularization, the proliferative compartment of the tumor, and in particular, the tumor microenvironment. The latter is

the key support system of a cancer, and is an important source of critical protumorigenic factors that facilitate growth, invasion, angiogenesis, and metastatic ability. Our focus here is to examine how the reliance of tumors on their microenvironments for development and preservation of key cellular functions is now recognized not only as a major contributor to cancer aggression and treatment resistance but also as a potential target for novel therapeutic intervention strategies.

1.1. The extracellular matrix

The extracellular matrix is comprised of various cell types and secreted proteins that help maintain the organization of higher-order cellular structures. In addition to containing various cell types, the matrix is deposited as a mix of such proteins as collagens, fibronectin, laminins, hyaluronan, plasminogen, proteases, and numerous others, which collectively form an inflexible scaffold to which cells attach [1]. In addition, other secreted cellular proteins such as cytokines and extracellular matrix remodeling proteins normally reside in the extracellular matrix [2]. These proteins are released when the matrix is degraded, and upon their release become activated due to proteases and other activating enzymes present in the extracellular environment, further contributing to the regulation of extracellular matrix turnover [3].

Within tissues, cells are surrounded by a meshwork of proteins and proteoglycans collectively called the extracellular matrix (ECM), which compartmentalizes tissues. The ECM is divided into two distinct layers: (i) the basement membrane, which is composed of sheet-like layers of ECM and lies under epithelial cells segregating tissues into functionally distinct regions; and (ii) the interstitial matrix, which exists within intercellular space [4]. The ECM serves multiple functions that are critical for embryonic development and wound repair. These functions include providing tissues with shape and flexibility and acting as a cushion to absorb external pressure. The ECM also serves as a base for cell anchorage, which mediates cell polarity, intracellular signaling, and assists in migration [5]. The key to the ECM's function lies in its unique composition and structure. The ECM is constructed in a specific pattern that is critical to its ability to carry out these functions and we will discuss later in this chapter how alterations in the expression level or arrangement of proteins within the ECM can be used to manipulate its function [6].

The ECM also affects cellular activity by serving as a reservoir for proteins required for proper tissue function and repair. This includes a plethora of growth factors and proteases. Growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor β (TGFβ) are involved intricately in development and continued expression of these factors is required to maintain tissue homeostasis during adulthood. These pleiotropic molecules have been shown to robustly affect proliferation, survival, and migration in numerous cell types. Once growth factors are secreted from cells, they often become embedded within the ECM and require ECM degradation by proteases such as elastase to release the active protein allowing it to interact with surrounding cells and transduce downstream signaling. For instance, the ECM serves as a VEGF 'sink.' High levels of VEGF are found incorporated within the ECM lattice shielded from cellular contact. Matrix metalloproteinases (MMPs), a family of proteases, which degrade structural proteins within the ECM, liberate VEGF from the ECM allowing it to bind to receptors on the cell surface and activate downstream pathways [7,8]. Also, TGFβ is deposited within the ECM in a latent form, which requires proteolytic processing to generate active TGFβ [9]. The latent form binds to microfibrils of elastic fibers, which prevents cleavage by shielding the protein from proteases. Breakdown of microfibrils by elastase releases latent TGFβ freeing it for cleavage into its active form [10].

1.2. Role of the tumor microenvironment on the Development of cancer

The tumor microenvironment is a growing target for consideration of cancer therapeutics due to its varied influence on the cells and on the physical aspects of

chemotherapeutic delivery [11]. Several drawbacks to traditional chemotherapies that do not account for the microenvironment are: the tumor vasculature, which is highly disordered and leaky; tumor core hypoxia, which confers radiation resistance on tumor cells in this state; cells furthest from blood vessels become growth-arrested, preventing efficacious chemotherapeutic inhibition of proliferating cells; and the upregulation of acid transporter and other transporter proteins, which efficiently excrete chemotherapeutics from cancer cells and hinder successful cancer treatment [12].

1.3. Hypoxia, Anerobic Metabolism and Interstitial Hypertension

The biologic behavior of cancer cells is determined not only by the intrinsic characteristics of those cells but also by the local microenvironment in which they exist [13]. The structure, organization, and function of the vasculature and interstitium is abnormal in most solid, malignant tumors compared to normal tissues and contributes to a hostile metabolic milieu characterized by hypoxia, anaerobic metabolism, low pH, and high interstitial fluid pressure (IFP). These features of the microenvironment have all been associated to varying degrees with more aggressive tumor behavior and impaired response to radiotherapy or chemotherapy [14,15,16]. New drugs that target the microenvironment directly, or aspects of tumor biology that indirectly contribute to microenvironmental dysfunction, are now being tested in the clinic with the aim of improving response to conventional treatments and overall patient outcome [17,18].

1.4. Hypoxia

Hypoxia develops in tumors when the metabolic demand for oxygen exceeds availability. This is thought to begin early in tumor development as oxygen consumption by the growing tumor mass outstrips the delivery capacity of the vasculature. Tumor hypoxia can broadly be classified as either acute or chronic according to the temporal characteristics and underlying pathophysiology [19]. Acute and chronic hypoxia coexist in most tumors, although the relative balance between the two and the contributions of each to the overall hypoxic state is not known in most circumstances. There is mounting evidence to indicate that acute and chronic hypoxia may influence tumor behavior and response to treatment in different ways [16,17]. Oxygen supply and consumption are tightly controlled and closely balanced in most normal tissues. However, in tumors, supply and consumption are often decoupled due to loss of normal physiologic regulation and changes in molecular signaling that provide selective growth and survival advantages. Oxygen levels below 10–15mmHg lead to activation of the hypoxia-inducible factors 1 and 2 (HIF1 and HIF2), which influence the expression of over 100 genes involved in angiogenesis, metabolism, pH regulation, proliferation, metastasis formation, and a range of other molecular and cellular processes [18]. Vascular endothelial growth factor (VEGF) produced by tumor and stromal cells stimulates new vessel formation via endothelial cell proliferation, migration, and survival [20].

Platelet derived growth factor (PDGF) promotes maturation and stability of new vessels by enhancing pericyte recruitment and interaction with endothelial cells. Although angiogenesis is upregulated in most tumors, the controls that regulate this process under normal physiologic conditions are lost, resulting in a vascular network that is structurally and functionally abnormal and inefficient at delivering oxygen and other nutrients [21].

Oxygen consumption by cancer cells is also an important determinant of tumor hypoxia. Oxygen consumption rates in tumors typically are intermediate in range between normal tissues with low and high metabolic activities [22]. However, there may be substantial spatial and temporal variability within individual tumors and from one tumor to the next. Biomechanical models have suggested that an increase in oxygen consumption may have a much more profound effect on the development of hypoxia than a similar reduction in oxygen delivery under some anatomic and physiologic conditions [23]. The cumulative result of imbalances among the many factors influencing oxygen supply and consumption in tumors is temporal and spatial variability in oxygen concentration. At any point in time, there is a continuum of oxygen concentrations in most tumors

that varies from anoxia at one extreme to very high levels typical of normal tissues at the other. The activation or suppression of cellular and metabolic processes, the induction of genes involved in adaptation to hypoxia, and tumor response to radiotherapy or chemotherapy depend on these oxygenation patterns in a dynamic and interactive manner [23,24].

1.5. Hypoxia and the DNA Damage Response

Regions of hypoxia are present in all solid tumors and can occur at early or late stages of tumor development. Levels of hypoxia range from near 0% pO2 (anoxia) to 8% [25]. Elegant studies using direct oxygen tension measures in numerous tumor types demonstrated a correlation between the level of hypoxia and prognosis, with lower oxygen levels associated with poorer prognosis[26]. We have shown previously that severe hypoxia induces a robust DNA damage response (DDR). Although, interestingly this seems to occur in the absence of DNA damage detectable by either comet assay or the formation of p53 binding protein 1 (53BP1) foci [27,28]. In contrast, reoxygenation events, which occur as a consequence of irregular perfusion of the tumor, induce significant levels of DNA damage in a reactive oxygen species (ROS)-dependent manner[29]. Failure to repair these lesions due to the loss of either repair pathways and/or p53 can then lead to increased genomic instability and tumor progression[30]. Because of their intrinsic connection, hypoxia and reoxygenation can be considered as two facets of the same stress. The focus of this chapter is the DDR induced by the tumor microenvironment and specifically conditions of low oxygen, hypoxia. First, we will discuss how hypoxia and reoxygenation can promote DDR induction and signaling [29]. Then we will explore the role of hypoxia in deregulating DNA repair and how this can potentially be exploited for novel therapeutic strategies. We will conclude by highlighting the impact of low oxygen on the proposed role of the DDR as a barrier to tumorigenesis [31]. From their study it was concluded that a selection pressure to lose p53 activity occurred as a result of the hypoxic tumor microenvironment. The induction of p53 in hypoxic conditions was, in part, attributed to the observation that the levels of human homolog of mouse double minute 2 (hMDM2) decrease during exposure to hypoxia [27]. One of the principal roles of hMDM2 is to keep p53 in check by targeting it for proteosomal degradation [32]. In the absence of hMDM2, p53 accumulates or stabilizes. This finding raised a pertinent question and gave perhaps the first hint that hypoxia-induced p53 was not behaving as might be expected. *Mdm2* is a target of p53, clearly containing p53 response elements and responding to increased levels of p53 and yet in the presence of hypoxia-induced p53, levels of hMDM2 fall [33]. In fact, further work showed that this was a widespread phenomenon; genes expected to be induced in response to hypoxia as a result of being characterized p53-targets were not induced. This led to the hypothesis and subsequent evidence to support it that, in response to hypoxia, p53 with trans repressive rather than trans activating capabilities is induced [34]. Figure-1 shows a schematic representation of the DNA repair pathways and examples of how they are deregulated by hypoxia [23].

1.6. Hypoxia-Inducible Factor 1 (HIF-1) Mediated Adaptive Responses in the Tumor

In order to maintain tissue homeostasis, it is necessary to maintain a tight control over the rate of cell division and cell loss. A stable number of cells in tissues also requires a stable blood supply to perfuse it adequately. This delicate balance is disturbed in tumors [27]. By acquiring mutations, cancer cells escape regulatory mechanisms and proliferate uncontrollably. As the tumor mass enlarges and outgrows adjacent vasculature, the delivery of oxygen and nutrients is unable to meet the demand of the tissue [22]. Therefore areas that are poorly perfused suffer from low oxygen tension (hypoxia), low glucose (hypoglycemia), and increased waste products (acidosis) [23]. The rate limiting ‘‘nutrient’’ is oxygen, as this is consumed most rapidly by the tissue as it is being delivered. Studies of tumor architecture revealed more than half a century ago that hypoxic regions existed in human tumors [36]. The hypoxic regions neighbored necrotic areas that were localized at a great distance from the

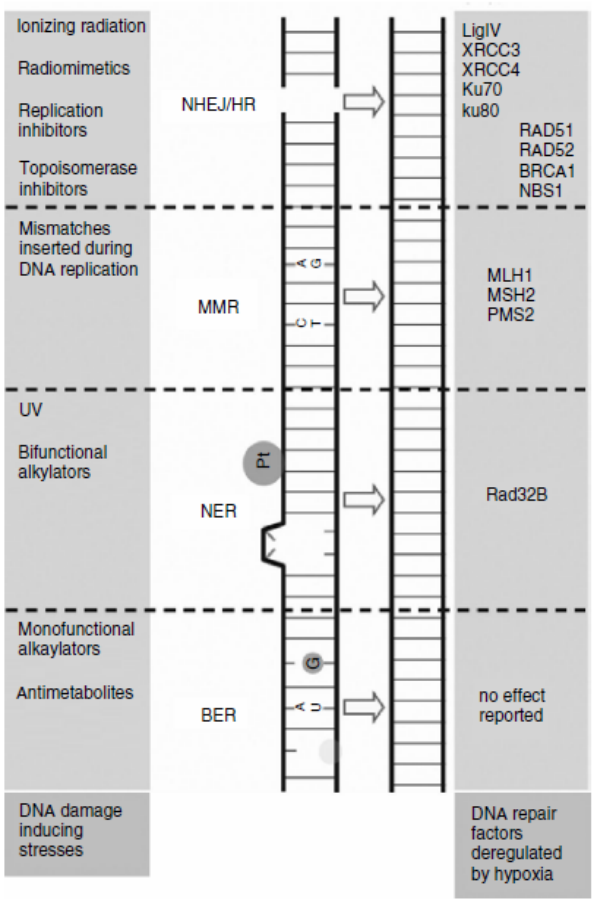

Figure1. DNA damage is induced over 10 000 times a day and is a consequence of both endogenous and exogenous stress (shown on the left hand side of the diagram). A number of different lesions can be formed and these are repaired by distinct cellular pathways. In hypoxia components of these pathways are deregulated (some examples are shown on the right hand side). This deregulation can compromise repair of DNA damage upon reoxygenation and potentially lead to increased genomic instability.

nearest blood vessel. Tumor cells located at 70–120 μm from a blood vessel are inadequately supplied with oxygen, because portion of the oxygen becomes metabolized by the cells closer to the blood vessel [37]. Hypoxia caused by oxygen diffusion limitations is termed chronic hypoxia. Cells located more than 150–180 μm from blood vessels are anoxic, and die by either apoptosis or necrosis [22]. Acute fluctuations in blood flow result in perfusion-limited hypoxia, which is a consequence of transient inhomogeneities in the microcirculation caused by abnormal tumor vessels [17]. Oxygen concentrations in human tumors are therefore highly heterogeneous with many regions at much lower values than the normal tissues from which they arose [37].

Hypoxia is a condition generally unfavorable to cell growth, with more severe hypoxia being more infavorable. Therefore, the cell triggers a cascade of adaptive physiological responses all aimed at eliminating hypoxic stress and facilitating cellular survival. These adaptive responses are designed to bring the oxygen demand of the tissue back in agreement with the oxygen supplied by the vasculature [38,39]. Re-establishing demand to meet supply by definition relieves hypoxia, and is accomplished by a combination of decreasing demand and increasing supply[40]. One major mechanism by which cells reduce their demand for oxygen and energy is to reduce macromolecular synthesis. At moderately severe hypoxia (<0.5% oxygen), cells have been shown to reduce proliferation (DNA and lipid

synthesis), reduce RNA synthesis [41], and decrease protein translation[42]. In the background of this overall reduction in macromolecular synthesis, there are a group of proteins that are actively synthesized that mediate the adaptive response. The transcription factors nuclear factor (NF)-κB, activator protein-1 (AP-1), early growth response protein-1, activating transcription factor-3/4 as well as others have been found to be induced to some degree by hypoxia [43]. However, the transcription factor with the most robust and specific response to hypoxia is the hypoxia-inducible factor 1, or HIF-1. This factor and its downstream target genes will be the focus of this chapter. These adaptive physiological changes are thought to allow for cellular survival. However, these molecular changes inadvertently contribute to the biologic observation that hypoxic tumors have a fundamentally worse clinical outcome [44].

1.7. Oxygen-independent HIF signaling

In addition to intratumor hypoxia, oncogene activation and loss of tumor suppressors may lead to induction of HIF in an oxygen-independent manner. Increased HIF-1 transcriptional activity can be mediated by the activation of the Ras oncogene and the subsequent mitogen-activated protein kinase (MAPK) pathway. Eight serine residues that may serve as putative consensus targets for MAPK family exist within HIF-1α. One mechanism to explain the increased transcriptional activity suggests that HIF-1β binds preferentially to the phosphorylated form of HIF-1 [45]. The phosphorylation of Thr796 also enhances the transcriptional response in hypoxia and also prevents the hydroxylation of Asn803 by FIH-1 [46]. In addition, activation of the phosphoinositide 3-kinase (PI3K) pathway (for example, by activation of the HER2/neu oncogene or loss of the phosphatease and tensin homolog tumor suppressor) can result in increased translation of HIF-1α through the Akt protein kinase-dependent activation of mammalian target of rapamycin (mTOR) [42,47]. HIF signaling is also promoted by decreasing HIF-1α ubiquitination and thus increasing its accumulation, such as in renal cell carcinoma with VHL tumor suppressor loss of function[48]. The loss of VHL function is caused by mutations that render VHL defective in respect to binding to elongin C or HIF-1α [49].

The loss of function of a group of mitochondrial tumor suppressors also leads to increased HIF signaling by inhibiting PHD-mediated hydroxylation of HIF-1α. As has already been mentioned, PHDs are 2-OG-dependent dioxygenases, which catalyze the conversion of a prolyl residue, molecular oxygen, and 2-OG to hydroxyprolyl, carbon dioxide, and succinate using ferrous iron as cofactor [50]. Yet succinate is not only a product of PHDs in the cytosol, but also a substrate for succinate dehydrogenase (SDH) in the mitochondria [51]. SDH is a tricarboxylic acid (TCA) cycle enzyme that converts succinate to fumarate. SDH dysfunction in cells raises the levels of succinate, which then accumulates in the mitochondrial matrix and leaks out into the cytosol [52]. The accumulated succinate, by feedback inhibition of PHDs, leads to HIF-α stabilization, and activation of the HIF complex. A similar situation arises in fumarate hydratase (FH) deficiency. FH is the following TCA cycle enzyme after SDH that converts fumarate to malate. Fumarate is not a product of PHDs but it is chemically similar to succinate. Like succinate, it accumulates in FH-deficient cells and inhibits PHD activity in the cytosol [52]. Although SDH and FH are housekeeping genes with key bioenergetic roles, mutations in these genes predispose patients to cancer [51]. SDH possesses two catalytic subunits (A, B), which are anchored in the inner mitochondrial membrane by subunits C and D. Heterozygous germline mutations in subunits B, C, or D of SDH lead to the development of paraganglioma or pheochromocytoma [53]. SDH subunit A mutation carriers are unlikely to develop hereditary paraganglioma (HPGL) because two types of genes exist for this subunit (paraganglia express both), and one can stand in for the other[54]. Unlike SDH, FH is a homotetrameric enzyme. Heterozygous germline FH mutations have been implicated in hereditary leiomyomatosis renal cell carcinoma, which is associated with skin and uterine leiomyomata (smooth muscle tumors) and papillary renal cancer [55].

1.8. HIF target genes

Hypoxia inducible factor is central to physiological and pathological processes involved in adaptation to

decreased oxygen availability. About 70 direct HIF target genes have been identified, and expression of dozens more genes are either directly or indirectly regulated by HIF [55]. The evolutionary pressure to regulate these genes by hypoxia is to allow the organism or tissues (and tumors, too) to survive and reestablish a normoxic state. The adaptive mechanism can be through decreasing demand for oxygen and increasing supply in the hypoxic tissue[56]. Evolutionarily, this was probably most important in wound healing, where vascular/tissue damage led to regions of hypoxia that would eventually heal and return to normoxia. In the tumor, the normoxic state is not reached, and the adaptive changes continue indefinitely, leading to much of the 'hypoxic tumor phenotype [55]. HIF target genes are involved in angiogenesis, erythropoiesis, iron metabolism, vascular tone regulation (all of which increase oxygen delivery), in glucose uptake, and glycolysis (for energy production and availability of biosynthetic substrates), in pH regulation (to neutralize acidic intracellular pH that results from anaerobic metabolism), as well as in other yet unidentified processes[57] (Figure-2).

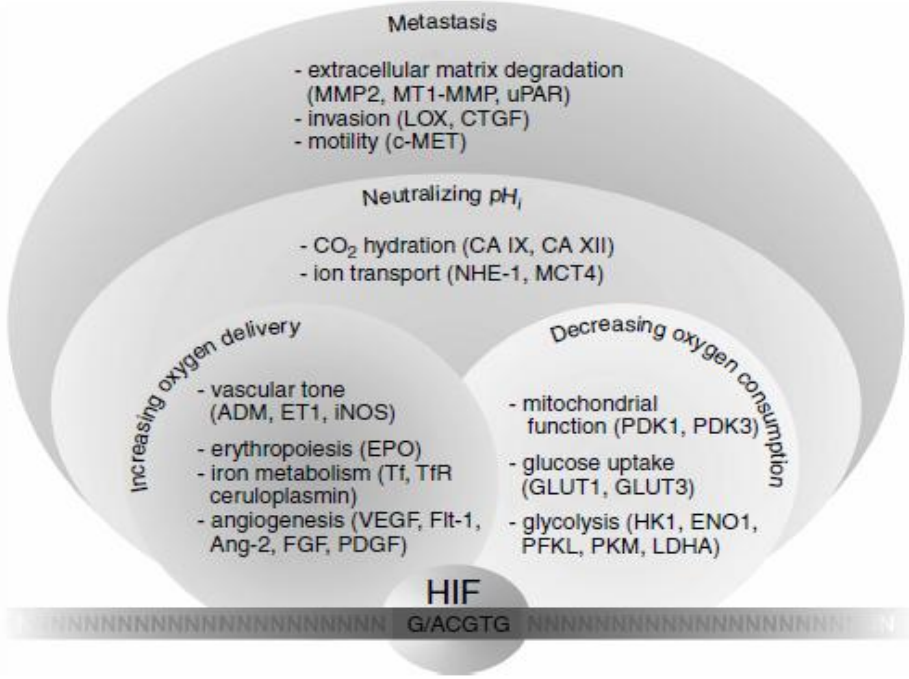

Figure2. Examples of hypoxia-inducible genes and the pathways they contribute to. major group is involved in increasing oxygen delivery by controlling the vascular tone (ADM adrenomedullin; ET1, endothelin-1; iNOS, inducible nitric oxide synthase), erythropoiesis (EPO, erythropoietin), iron metabolism (Tf, transferrin; TfR, transferrin receptor; ceruloplasmin), and angiogenesis (VEGF, vascular endothelial growth factor; Flt-1, VEGF receptor; Ang-2, angiopoietin 2; FGF, fibroblast growth factor; PDGF, platelet derived growth factor). Hypoxic cells also adapt by expressing genes involved in decreasing oxygen consumption by decreasing mitochondrial function (PDK1 and 3, pyruvate dehydrogenase kinase 1 and 3), increasing glucose uptake (GLUT1 and 3, glucose transporter 1 and 3) and glycolysis (HK1, hexokinase 1; ENO1, enolase 1; PFKL, phosphofructokinase L; PKM, pyruvate kinase M; LDHA, lactate dehydrogenase A). Because of inefficient tumor vasculature and glycolytic metabolism, hypoxic cells also express genes involved in neutralizing pHi by CO2 hydration (CA IX and XII, carbonic anhydrase IX and XII) or ion transport (NHE-1, Na+/H+ exchanger; MCT4, monocarboxylate transporter 4). All of the previously mentioned genes finally contribute to metastasis, along with hypoxia-regulated genes involved in extracellular matrix degradation (MMP2, matrix metalloproteinase 2; MT1-MMP, membrane type 1 MMP; uPAR, urokinase plasminogen activator receptor), invasion (LOX, lysyl oxidase; CTGF, connective tissue growth factor), and motility (c-Met, HGF receptor tyrosine kinase).

1.9. Hypoxia and metastasis

One mechanism by which a cell could respond (or adapt) to a hypoxic environment would be to move to a more well-oxygenated one. In order to migrate to a secondary site, cells from a primary tumor need to first overcome cell-to-cell adhesion contacts, break through the ECM, and penetrate into the circulatory system. Once there, they must overcome anchorage-dependent growth (anoikis) and be able to escape host immunity, and again penetrate from the vessels to a location, where they could implant and proliferate [58]. Cell migration therefore depends on the migratory machinery located intracellularly and on the interaction of the cell with the ECM [59,60]. Many of the hypoxia-regulated processes described above can positively contribute to these metastatic activities. Standard neutral intracellular pH combined with low extracellular pH promote an aggressive phenotype. Cellular pH regulators enhance the invasiveness of cancer cells and even promote directional cell migration by their linkage to the cytoskeleton and their ion-translocating activity [61,62]. For example, the hypoxia-inducible NHE1 accumulates at the leading edge of migrating cells, where in the thin, mitochondria-free glycolytic lamellipodium (actin-rich structure that protrudes into the ECM) it helps shuttle protons out of a site of their massive local production. NHE1 together with AE 2 also regulate local osmotic swelling supporting the leading edge outgrowth due to their function in transporting Na^+ and Cl^- ions, respectively [59]. CAs constitute another portion of pH-regulating cellular components. Apart from pH regulation, which is a common function of all active CAs, CA IX is unique because it is also capable of actively functioning in cell deadhesion. CA IX is localized on the cell surface, especially in the regions of cell-to-cell contacts, and overlaps with E-cadherin in adhering lateral membranes [59]. E-cadherin and β-catenin are central cell adhesion molecules that generate tight intercellular contacts linked to the actin cytoskeleton. CA IX decreases the level of cytoskeleton-linked E-cadherin by competitively binding to β-catenin, and thus destabilizes cell contacts. Hypoxia has been also shown to downregulate E-cadherin by increasing the expression of its transcriptional repressors Snail and SIP [61].

A very potent activator of cell motility is the hepatocyte growth factor (HGF), a stromal-cell derived cytokine that signals through binding to the HGF receptor tyrosine kinase encoded by the Met proto-oncogene. MET (HGF receptor tyrosine kinase) signaling has been shown to alter the expression, topographical localization and activity of cadherins, integrins, and MMPs, supporting invasion through the stroma [63]. Hypoxia has been shown to induce the MET gene and enhance HGF-MET signaling [64]. Apart from increasing angiogenesis and lymphangiogenesis, which provide routes for tumor dissemination, hypoxic upregulation of VEGF also further enhances metastatic spread by directly increasing vascular permeability [63].

1.10. Influence of Hypoxia on Metastatic Spread

Metastasis is a process by which tumor cells establish new growths at sites in the body distinct from the primary tumor. This is a major cause of treatment failure and death in cancer patients. The development of metastasis is complex, requiring multiple individual stages to successfully establish a tumor at a secondary site[1]. Tissue structure and function is intimately connected to and controlled by tumor cell–cell and cell–extracellular matrix (ECM) interactions. The metastatic process involves multiple changes at a molecular level that disrupt and modify these interactions [63,1]. These include signaling through cell adhesion molecules (CAMs), such as integrins and cadherins, and tissue remodeling through the action of proteinases, such as plasmin or metalloproteinases (MMPs), as well as apoptotic machinery, chemokines, and growth factors, all of which act together to control processes such as proliferation, survival/apoptosis, migration, and invasion [65]. These interactions are dysregulated in the tumor microenvironment and there is significant heterogeneity in these different interactions and functions between tumor types. The microenvironment of cells in tumors involves both local interactions with surrounding cells and ECM, and exposure to pathophysiological conditions, such as low oxygen tension (hypoxia), low glucose concentrations, high lactate concentrations, low extracellular pH (acidity), and high interstitial fluid pressure (IFP), all of which can vary between different

tumor regions [66]. The expression of many of the genes involved in the various metastatic processes can be affected by exposure to conditions induced by the pathophysiologic environment of tumors, particularly hypoxia. Hypoxia-inducible factors-1 and 2 (HIF-1/2) are key regulators of gene expression under hypoxic conditions and immune histochemical analysis has found overexpression of HIF-1α in many human cancers and their metastases [1]. High levels of both HIF-1α and HIF-2α have been positively correlated with tumor progression and poor prognosis in patients with a variety of cancers (HIF-1α – brain, non-small cell lung carcinoma, breast, ovarian, uterine, and cervical tumors; HIF-2α – non-small-cell lung cancer, head and neck squamous cell, renal cell carcinoma). The acute (transient) hypoxia that occurs in tumors may induce reactive oxygen species (ROS), which in turn can activate HIF-1, promoting persistent oxidative stress and further amplifying HIF-1 activation, with downstream effects on gene expression [67].

1.11. The tumor microenvironment and metastasis

Regions of low oxygen tension (pO2), or hypoxia, are found in most solid tumors. A proportion of tumor cells are in hypoxic regions beyond the maximum diffusion distance of oxygen from a capillary. These cells may be exposed chronically to low oxygen tensions (chronic hypoxia) for hours to days [40]. Tumor hypoxia can also occur transiently due to the substantial instability in microregional blood flow and tissue oxygenation that can occur in animal and human tumors. These fluctuations are thought to be due to transient occlusion and narrowing of vessels and to arteriolar vasomotion [17]. Also, the abnormal architecture of the vascular system itself may produce variations in red cell flow. High IFP may further exacerbate the situation. This blood flow instability, in the context of an already poorly organized and regulated vascular system, can produce short-term (5–60minutes) fluctuations in oxygenation (acute transient hypoxia) [38]. Thus tumor cells adjacent to vasculature may be exposed to short-term hypoxia; however, the actual distance from blood vessels at which hypoxia occurs likely varies widely in different tumors because of the unstable delivery of oxygen within tumor blood vessels and the variable oxygen consumption of tumor cells [68]. The extent of hypoxic regions is heterogeneous even amongst tumors of identical histopathological type, and does not correlate with standard prognostic factors such as tumor size, stage, and grade. Although the definition of hypoxia depends on the effect being studied and varies between different studies, a pO2 level <10–15mmHg (<2% O2 in the gas phase) is generally considered to be associated with changes in the expression of a number of genes and has also been associated with poor prognosis in a number of clinical studies[69]. It is important to note however that certain hypoxia-responsive genes (e.g., carbonic anhydrase-9 (CA-9), glucose transporter-1 (Glut-1), VEGF, and urokinase plasminogen activator receptor (uPAR)) are turned on (albeit to a reduced extent) at higher levels of oxygen (2–3% O2) than other hypoxia-responsive genes (e.g., cathepsin D, glyceraldehyde phosphate dehydrogenase) that may not change until lower levels of oxygenation (<1.0%) occur [70].

There are cell line specific effects of graded oxygen levels on invasive potential and enhanced metastatic efficiency indicating model specific heterogeneity in response to low oxygen concentrations. Most studies of the effects of hypoxia on expression of metastasis-related genes reported to date have exposed cells to prolonged periods at a fixed level of oxygen rather than fluctuating exposures. There is also a high degree of variability in the level of hypoxia used in different studies, with oxygen concentrations ranging from anoxic (<0.1% O2) to severe hypoxia (~0.2% O2) or levels approaching normoxia (3–5% O2). Furthermore, the actual levels of hypoxia induced in these cultures (which depend critically on factors such as the cell density and the surface area to volume ratio of the media) have not often been measured.

Important adhesion molecules mediated by hypoxia include the beta-1 integrins. The level of constitutive activity of different beta-1 integrins has been found to correlate with invasive capacity, and the use of monoclonal antibodies to inhibit their expression can block invasion *in vitro*. For example, HIF-1 increased expression of beta-1 integrin in pancreatic cancer cell lines and the use of antisense HIF-1 inhibited its expression and reduced metastases presentation *in vivo* [71,72]. Survivin, which is an antiapoptotic protein,

was also found to be regulated by HIF-1 expression in this study, and the authors postulated that the combined reduction of survivin and beta-1 integrin were responsible for the reduced metastases observed. In a study with human fibrosarcoma cells hypoxic exposure *in vitro* was found to enhance apoptosis resistance and increase lung colonization but this effect was blocked by treatment of the cells with a Ras inhibitor (farnesyl thiosalicylic acid). VEGF-A expression was shown to be inhibited following the treatment but increased HIF-1α expression associated with the hypoxic exposure was not inhibited. These data emphasize that different cell types/lines can respond differently to similar stimuli [73].

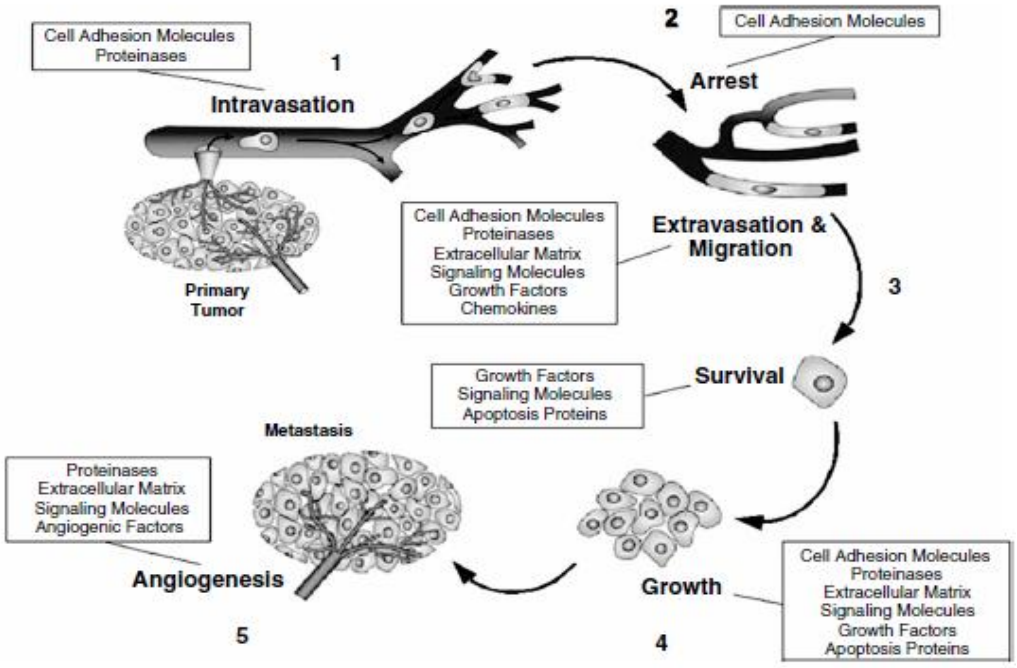

Figure3. The process of metastasis. Schematic diagram showing the stages of metastasis. (labeled 1–5) and the classes of molecules known to be involved at each stage.

2. Conclusions

It is clear that the hypoxia-mediated increase in HIF-1a plays a critical role in both the establishment and progression of many common cancers through the HIF-1- dependent activation of genes that allow cancer cells to survive and metastasize in the hostile hypoxic tumor environment. Additionally, increased HIF-1 activity arises through the activation of oncogenes and/or inactivation of tumor suppressor genes. Increased HIF-1a is correlated with the increased expression of survival factors such as VEGF, aggressive tumor growth, and poor patient prognosis.

The interest in HIF-1a as a cancer drug target stems from associations such as this. A number of agents with anticancer activity have been reported to decrease HIF-1a or HIF-1 transactivating activity in cells. This has been proposed, often on the basis of limited evidence, to contribute to the agents' antitumor activity, for example, through decreased formation of angiogenic factors such as VEGF. However, it is not always clear that HIF-1 inhibition can occur at therapeutically relevant concentrations of the agents. Not infrequently the concentration of the agents required to inhibit HIF-1 is considerably higher than the concentration necessary to inhibit cell growth.

The role of TME in cancer progression is currently attracting impressive interest in the field. Hypoxia is a condition that often occurs at late stages of cancer, and even before that, HIFs can be upregulated due to environmental acidification and the presence of glycolytic metabolites.

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