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Hypoxia-mediated regulation of macrophage functions in pathophysiology

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REVIEW

Abstract

Oxygen availability affects cell differentiation, survival and function, with profound consequences on tissue homeostasis, inflammation and immunity. A gradient of oxygen levels is present in most organs of the body as well as in virtually every site of inflammation, damaged or pathological tissue. As a consequence, infiltrating leukocytes, macrophages in particular, are equipped with the capacity to shift their metabolism to anaerobic glycolysis, to generate ATP and induce the expression of factors that increase the supply of oxygen and nutrients. Strikingly, low oxygen conditions (hypoxia) and inflammatory signals share selected transcriptional events, including the activation of members of both the hypoxia-inducible factor and nuclear factor κB families, which may converge to activate specific cell programs. In the pathological response to hypoxia, cancer in particular, macrophages act as orchestrators of disease evolution and their number can be used as a prognostic marker. Here we review mechanisms of macrophage adaptation to hypoxia, their role in disease as well as new perspectives for their therapeutic targeting.

Keywords: HIF, NF-κB, tumor

Introduction

Oxygen concentration in the atmosphere is normally 21%, corresponding to 159 mmHg. Within our tissues, oxygen levels span from 150 mmHg to 20–70 mmHg (2.5–9% oxygen), whereas markedly lower levels (<1% oxygen) have been described in wounds and necrotic tissue sites. The condition of reduced oxygen tension is defined as hypoxia. Immune functions rely on energy availability, which, in a normoxic environment, is assured by the mitochondria-dependent oxidative phosphorylation pathway, leading to production of ATP (1). Hypoxia characterizes virtually every site of inflammation, damaged or pathological tissue, thus forcing infiltrating leukocytes, macrophages in particular, to move against oxygen gradients and to undergo a metabolic switching towards activation of anaerobic pathways, to maintain their energy requirements (1).

Macrophages play a central role in inflammation and host defense (2) and are characterized by considerable diversity and plasticity (3, 4). In tissues, mononuclear phagocytes respond to environmental cues (e.g. microbial products, damaged cells, activated lymphocytes) with the acquisition of distinct functional phenotypes, differentially affecting disease onset and progression (3). In response to various signals, macrophages may undergo classical M1 activation [stimulated by Toll-like receptor (TLR) ligands and IFN-γ] or alternative M2 activation (stimulated by IL-4/IL-13); these states mirror the T_h1 versus T_h2 polarization of T cells (3). Several lines of evidence show that hypoxia strongly affects macrophage functions and indicate that immune responses and energy metabolism are inter-related events (3, 5). Hence, there is a need to address how the quality of the immune response is affected by changes in oxygen availability.

Various physiological and pathological conditions (e.g. inflammation, wound healing, atherosclerosis, tumors, ischemia) are characterized by low oxygen tension (1). Hypoxic regions of solid tumors are often characterized by high accumulation of macrophages, which contribute to tumor angiogenesis and development (6). This trophic action of tumor hypoxia on tumor-associated macrophages (TAMs) is clinically relevant, as a high TAM number is considered a negative prognostic marker in several human malignancies, including Hodgkin's disease, glioma, colangiocarcinoma and breast carcinoma (3, 7). New efforts are delineating the mechanisms driving macrophage adaptation to low

oxygen levels. As a result of these efforts, the hypoxia-inducible factor (HIF) and nuclear factor κB (NF-κB) families of transcription factors have been identified as 'master regulators' of the cellular response to hypoxia (1, 8), though other factors [e.g. activator protein 1 (AP-1), early growth response protein 1 (Egr-1)] have been implicated in shaping the hypoxic phenotype of these cells (9).

Here we review evidence suggesting that different oxygen levels play a determinant role in driving macrophage differentiation and functions and that the selective control of their transcriptional apparatus may be a promising strategy to pilot their functions in disease.

Transcriptional regulation in hypoxia

Hypoxia is perceived, at a cellular level, through oxygen sensor relays that operate inside the cell and can lead to the activation of transcriptional activators. The HIF and NF-κB families of transcriptional factors have been identified as crucial regulators of this metabolic adaptation (10, 11).

Each HIF is a heterodimer composed of the constitutively expressed β subunit and an α subunit whose stability depends on the oxygen level (10). In mammals, three closely related transcription factor complexes exist: HIF-1, HIF-2 and HIF-3. Under normoxic conditions, the α subunit is hydroxylated by prolyl hydroxylases (PHDs), recognized by the protein product of the von-Hippel–Lindau (VHL) tumor-suppressor gene, ubiquitinated and degraded by the proteasome. Under hypoxic condition, PHDs are not active and consequently HIF-α is not degraded but can translocate to the nucleus, and can dimerize with the β subunit. The heterodimeric transcription factor induces the transcription of genes mediating cellular adaptation to a low oxygen environment (10). HIFs can also be stabilized and exert their activity under normoxic conditions in response to bacterial products, cytokines, inflammatory mediators and stress (12, 13).

HIF over-expression correlates with increased patient mortality in several cancer types (14). HIFs directly activate the expression of several pro-angiogenic factors, including vascular endothelial growth factors (VEGFs), VEGF receptors (VEGFRs), plasminogen activator inhibitor 1, angiopoietins, platelet-derived growth factor B, the surface receptor tunica internal endothelial kinase 2 (Tie-2) and the metalloproteinases matrix metalloproteinase 2 (MMP-2) and MMP-9 (15). In addition, HIFs mediate various cellular and physiologic events, including cell proliferation, survival and metabolism, glycolysis, immunosurveillance as well as tumor invasion and metastasis (10).

The transcriptional profile of hypoxic macrophages clearly demonstrated that HIF-1 and HIF-2 are important factors regulating macrophage functions in hypoxia, through activation of a distinct set of genes mediating unique biological functions (16). HIF-1 has been associated with anti-microbial and effector functions in myeloid cells and has been implicated in the pathophysiology of sepsis (17, 18). Conditional deletion experiments have shown that HIF-1α-deficient cells are unable to mount anti-bacterial and inflammatory responses, effects that involve the reduced expression of inducible nitric oxide synthase (iNOS) and the decreased production of ATP by glycolysis (18, 19). HIF-2 expression has been detected in TAMs, and may mediate expression of angiogenic factors and favor tumor growth (16, 20).

The distinct roles of HIF-1 and HIF-2 in the regulation of myeloid cell functions are, however, still poorly understood. A recent report described that HIF-2 drives macrophage production of soluble VEGFR-1 (sVEGFR-1), whereas HIF-1 drives macrophage production of VEGF. This study suggests that hypoxia can stimulate the production of anti-angiogenic molecules from mononuclear phagocytes in a granulocyte/macrophage colony-stimulating factor (GM-CSF)-rich environment and proposes specific and independent roles for HIF-1 and HIF-2 in hypoxic macrophages (21).

Hypoxia has long been shown to stimulate NF-κB-mediated signaling (8), and several pieces of evidence indicate that both HIF and NF-κB proteins are redox-sensitive proteins regulated by the same oxygen sensors. In macrophages, the increased production of reactive oxygen species due to hypoxia promotes NF-κB activation and the synergy between hypoxia and LPS (22). In contrast, inhibition of PHDs suppressed the LPS-induced expression of tumor necrosis factor α (TNF-α) in macrophages, through NF-κB inhibition (23). The IκB kinase (IKK) multisubunit complex, containing catalytic subunits termed IKKα, -β and -γ, phosphorylates and targets the NF-κB inhibitor IκB for degradation, thus promoting NF-κB activation (24). It has been shown that hypoxia leads to NF-κB activation through decreased PHD-dependent hydroxylation of IKKβ (25), whereas ablation of IKKβ impairs HIF-1α accumulation in hypoxia, suggesting that NF-κB transcriptionally regulates HIF-1α (11). A physical interaction between IKKγ [which is also known as NF-κB essential modulator (NEMO)] and HIF-2α enhances HIF-2α transcriptional activity (26). The role of additional transcription factors in hypoxia, including AP-1 and Egr-1, was recently reviewed by other authors (9). More recently, hypoxia was also shown to be able to inhibit the expression of pro-inflammatory cytokines in macrophages, by inducing the glucocorticoid-induced leucine zipper (GILZ) factor (27).

Macrophage differentiation and functions in physiological and pathological hypoxia

Hypoxia forces cells to shift their metabolism to anaerobic glycolysis to generate ATP and induces the expression of factors that increase the supply of oxygen and nutrients (10), with important consequences for macrophage differentiation and activation (Fig. 1).

Hypoxia plays a key role in hematopoiesis. *In vitro*, hematopoietic stem and progenitor cells (HSPCs) maintain their undifferentiated phenotype and expand more extensively in hypoxic than in normoxic conditions (28). Furthermore, functional HIFs are essential to the development and survival of the hematopoietic system, as mouse embryonic stem cells lacking the ARNT (aryl hydrocarbon receptor nuclear translocator) gene, which codes for HIF-β, have impaired ability to generate HSCs *in vitro* (29). Several lines of evidence suggest that stem cells are localized in hypoxic regions and that stem cell niches would be locally hypoxic. The hypoxic endosteal HSC niche is characterized by constitutive expression of HIF-1α (30). Interestingly, mobilization of HSPCs by administration of granulocyte CSF (G-CSF) or cyclophosphamide drives an

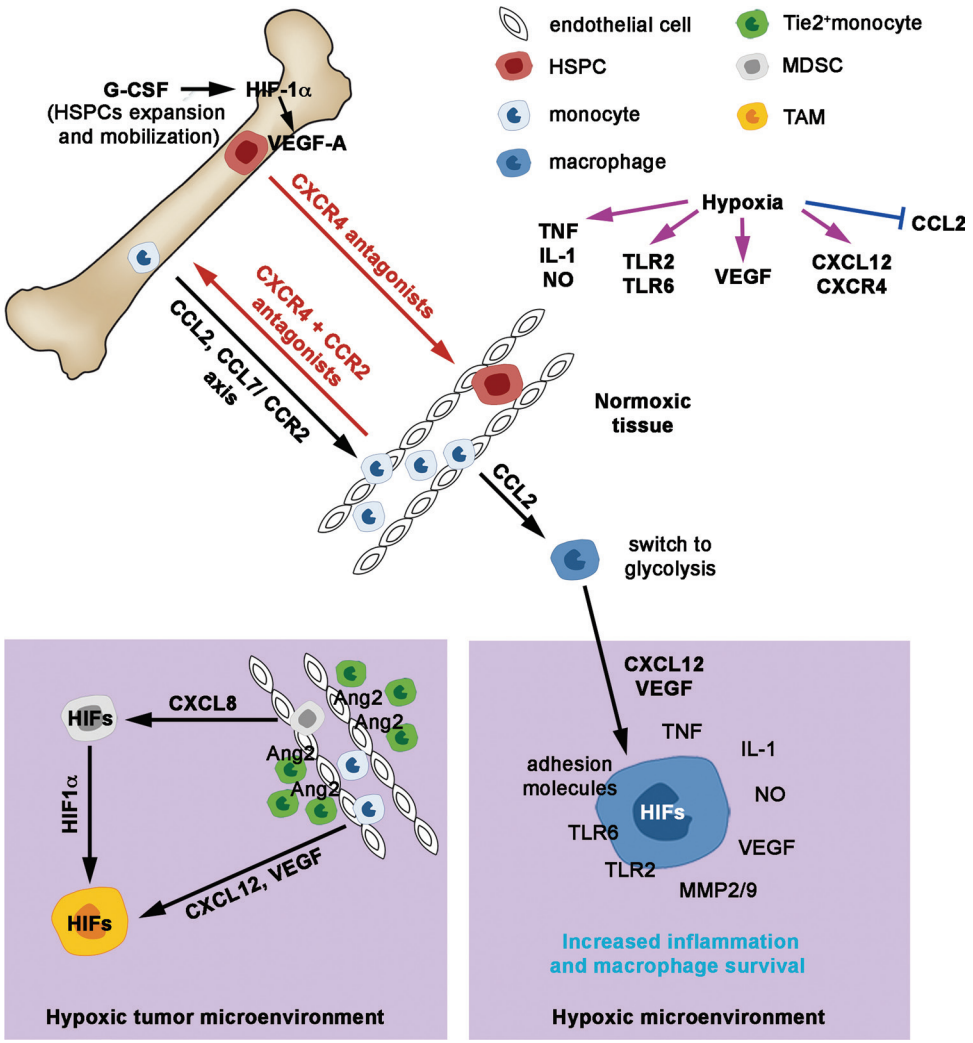

Fig. 1. Pathways of myeloid cell recruitment and functions in hypoxia. HIFs contribute to the mobilization and differentiation of myeloid cell precursors (HSPCs) and to their accumulation in inflammatory sites. In hypoxic areas, HIFs promote the transcription of several genes involved in macrophage-mediated amplification of the inflammatory response, angiogenesis and tissue remodeling. In BM, G-CSF expands HSPCs; the expansion of progenitors depletes the microenvironment of oxygen, leading to local hypoxia, stabilization of HIF-1α and increased transcription and accumulation of VEGF-A. Recruitment of HSPCs to tissues is mainly controlled by the CXCL12–CXCR4 axis, and blockade of CXCR4 prevents progenitor cells homing to the sites of injury. The CCL2, CCL7/CCR2 axis mediates the exit of monocytes from the BM, whereas the return of blood monocytes to the BM can be induced by concomitant treatment with CCR2 and CXCR4 antagonists. Hypoxia activates a multistep migration program of monocytes/macrophages, by inhibiting CCL2 expression and increasing expression of CXCL12 and VEGFs, which promote macrophage infiltration. Similarly, hypoxic tumor vessels express Ang2 and promote accumulation of angiogenic Tie-2⁺ monocytes, thus supporting tumor angiogenesis and progression. The angiogenic chemokine CXCL8 is strongly induced by hypoxia and may contribute to accumulation of MDSCs (102, 103). Finally, hypoxia-induced HIF-1α accumulation promotes differentiation of MDSCs into TAMs.

expansion of hypoxic areas throughout the bone marrow (BM). This is associated with increased levels of both HIF-1α and its target gene VEGF-A (31).

HSPCs travel through peripheral blood and lymph in low numbers during homeostasis, but they are mobilized by inflammation, infection, stress and injury. Mobilization and homing of HSPCs to sites of inflammation is mainly controlled by the CXCR4–CXCL12 (receptor–ligand) axis (32). Importantly, expression of the gene for CXCL12 [also known as stromal derived factor 1 (SDF-1)] is regulated by HIF-1 in endothelial cells, resulting in the expression of the chemokine in ischemic tissue in direct proportion to reduced oxygen tension (33). HIF-1-induced CXCL12 expression increases the adhesion, migration and homing of circulating CXCR4⁺ progenitor cells to ischemic tissues, whereas blockade of CXCL12 in ischemic tissue or blockade of CXCR4 on circulating cells prevents progenitor cell recruitment to sites of injury (33).

The direct regulation of CXCR4 by HIF-1 has been demonstrated by our group. In particular, we observed that hypoxia selectively augments CXCR4 expression through HIF-1 activation in human monocytes, macrophages, endothelial cells and cancer cells (34). The chemokines CCL2 and CCL7 enhance monocyte emigration from the BM, through

the engagement of CCR2 (35). Murine Ly6c$^+$ inflammatory monocytes also express CXCR4, but CXCR4 inhibition only minimally increases circulating monocyte frequencies (while markedly enhancing granulocyte frequencies) (36). Interestingly, concomitant blockade of both CCR2 and CXCR4 appears to promote the return of monocytes from the bloodstream to the BM. As hypoxia was shown to induce the CXCR4–CXCL12 axis (34) while inhibiting the CCR2–CCL2 axis (37), oxygen levels appear to play a complex role in controlling the emigration of monocytes from the BM.

Although no conclusive evidence supports a direct role of hypoxia in macrophage differentiation, it was proposed that certain monocyte/macrophage populations survive better under conditions of low oxygen, thereby contributing to their increased numbers at sites of chronic inflammation and tumors (38). Moreover, in chronic hypoxia, although growth factor availability (CSF-1 and GM-CSF) was shown to be critical for maintaining cell viability, anaerobic glycolysis was considered an effective hypoxia-induced survival strategy (39). It is also proposed that as macrophages differentiate from monocytes they begin to adopt a glycolytic metabolism allowing them to adapt readily when exposed to low oxygen conditions (40).

Hypoxia is associated with danger, microbial attack or tissue degeneration, resulting in the activation of a protective pro-inflammatory phenotype that depends on pathogen-recognition receptors. A number of pieces of evidence indicate that, in hypoxia, innate immune cells are better equipped to maintain their viability and functions, as compared with adaptive immune cells (i.e. lymphocytes) (4). Mononuclear phagocytes exposed to hypoxia produce higher levels of several pro-inflammatory cytokines (e.g. IL-1, TNF-α), cytotoxic mediators (e.g. NO) and adhesion molecules (41–43) and display enhanced phagocytosis and bacterial killing, because of HIF activation (44, 45).

HIF-1-deficient myeloid cells show profound impairment of cell aggregation, motility, invasiveness and bacterial killing (19). TNF-α and NO production by macrophages is HIF-dependent (18). Low oxygen tension activates macrophages to release pro-inflammatory cytokines and to up-regulate pattern recognition receptors and co-stimulatory molecules (46–48). HIF-responsive elements are present in the genes encoding TLRs, including TLR2 and TLR6, which are pattern recognition receptors that are up-regulated in response to hypoxia (49). A synergistic relationship between HIF and NF-κB contributes to myeloid cell responses against pathogens: macrophages infected with Gram-positive or Gram-negative bacteria reveal a marked defect in HIF-1α expression following deletion of the NF-κB activity regulator IKKβ (11).

The mammalian genome encodes three closely related PHDs: PHD1, PHD2 and PHD3. The various PHD isoforms differ with regard to their tissue distribution, protein structure, cellular localization, protein interactions and hydroxylation of HIF-α isoforms. Although it has been proven that deletion of the three PHD enzymes results in stabilization of HIFs (1, 49, 50), how they individually contribute to HIF regulation is still largely unknown. Recent studies applying genetic loss-of-function approaches indicated that PHDs carry out specific and non-redundant *in vivo* functions. PHD1 has been reported to be involved in mitochondrial energy metabolism of skeletal muscle and liver cells and in the intestinal barrier function (51–53). PHD2 is crucial for placentation (for this reason, Phd2 null mice display an embryonic lethal phenotype) and cardiac development (54, 55). PHD3 has been assigned a physiologic function in the development of the sympatho-adrenal system (56). In pathophysiology, PHD2 has been shown to be relevant for the vasculature of expanding tumors (57), whereas PHD2 and PHD3 are cooperatively involved in the development of hepatic steatosis and dilated cardiomyopathy (58). Recent studies have addressed the role of the three PHDs in macrophages (49). In mouse models of sepsis, mice deficient in PHD3, but not mice deficient in PHD1 or PHD2, show higher mortality than wild-type littermates. PHD3 deficiency enhances LPS-induced M1 polarization of macrophages, with abundant levels of pro-inflammatory cytokines and high phagocytic activity. The pro-inflammatory phenotype is associated with HIF-1α protein stabilization and increased NF-κB activation (59). In contrast, a second article, published at the same time as the sepsis study, reports a preferential expression of PHD3 in human pro-inflammatory M1 macrophages (60). Hypoxia does not influence PHD3 expression in M1 macrophages, whereas it significantly up-regulates this gene in M2 macrophages in an activin-A-dependent manner. *In vivo*, PHD3 is highly expressed within inflammatory environments and in a subset of alveolar macrophages under homeostatic conditions. In TAMs, PHD3 is expressed in a heterogeneous way, but exclusively in cells lacking M2 markers (60). Thus, on the one hand, PHD3 has been shown to suppress innate immunity and increase mice survival during sepsis (59); on the other hand, PHD3 expression has been associated with inflammatory M1 macrophages (60). This discrepancy needs further investigation.

Some chronic infections, e.g. infection by *Mycobacterium tuberculosis* (Mtb), are characterized by the formation of granulomas that consist predominantly of T cells and macrophages (61). Granulomas in guinea pigs, rabbits, non-human primates and humans, but not mice, are characterized by low levels of oxygen (62). Mtb-infected macrophages sequestered inside the hypoxic environments of the granuloma differentiate into lipid-loaded macrophages that contain triacylglycerol (TAG)-filled lipid droplets that may provide a fatty-acid-rich host environment for Mtb (63). In these hypoxic macrophages, Mtb persists into a dormant state. Local oxygen tension modulates immune responses to control the multiplication of the pathogen. A hypoxia-activated anti-microbial effector mechanism that has been described recently is the vitamin-D-dependent up-regulation of human β defensin 2 (hBD2) in macrophages resulting in the growth inhibition of intracellular Mtb (64).

Lipid accumulation is also characteristic of macrophages present in human atherosclerotic lesions. Hypoxic areas are present in the atheromatous plaque, and lesion progression is associated with the formation of lipid-loaded macrophages, increased local inflammation and angiogenesis. Foam cell formation is promoted by the high expression of HIF-1 in intraplaque macrophages (65).

Hypoxia rises in the synovia of joints of patients with rheumatoid arthritis (RA) (66) and is likely to contribute to RA by promoting inflammation, angiogenesis, cellular infiltration (through induction of chemokines) and cartilage degradation. Many

of these processes are controlled by macrophages. Mice in which HIF-1α was specifically inactivated in macrophages had reduced disease symptoms in the collagen-induced arthritis (CIA) model, with lower infiltration of myeloid cells, decreased paw swelling and decreased disease development (19).

Pulmonary hypertension (PH) is characterized by elevated pulmonary-artery pressure due to vascular wall remodeling and vasoconstriction (67). Hypoxia has been demonstrated to induce an inflammatory response that precedes the development of PH. The finding that the presence of M2 macrophages (68) is associated with proliferation of smooth muscle cells in the pulmonary artery *in vitro* and the development of PH *in vivo* suggests that these macrophages may play a significant role in the etiopathogenesis of PH (67). In agreement with this hypothesis, T_h2 cytokines, which are involved in M2 polarization, and Fizz-1 (found in inflammatory zone 1), an M2 marker, have been implicated in the development of PH (67, 69, 70)

Hypoxia and/or anoxia are the natural consequences of ischemic processes. Macrophages contribute to tissue repair and remodeling during acute and chronic ischemic vascular diseases. Macrophages accumulate in cardiac tissue in myocardial infarction (MI) (71). HIF-1 and HIF-2 are up-regulated in macrophages in damaged tissues where they orchestrate post-infarction remodeling (72). In the central nervous system, both resident macrophages (microglia) and infiltrating macrophages participate to responses to hypoxia (73). The region surrounding the infarct core, known as the ischemic penumbra, is characterized by moderate ischemic conditions. In this setting, HIF is activated and microglia can function in a neuroprotective manner and promote regeneration by releasing growth factors and modulating the immune response (74). The idea of a neuroprotective function for myeloid cells in the acute phase of ischemic stroke was supported by a study using a transgenic mouse model that allows for selective ablation of proliferating microglia/macrophages (75).

A role for PHDs has been described in the modulation of macrophage functions in the setting of hindlimb ischemia (76). PHD2-haplosufficient (*Phd2*$^{+/-}$) mice resist tissue ischemia. Macrophages derived from these mice show a skewing toward an anti-inflammatory, pro-angiogenic program and favor tissue healing and arteriogenesis (remodeling of pre-existing arteriolar connections into true collateral arteries) in ischemic skeletal muscle. Emerging evidence indicates that changes in glucose metabolism and the pentose phosphate pathway influence macrophage polarization (77). In particular, the M2-polarizing signal IL-4 promotes higher oxygen consumption and reduced flux of the glycolytic pathway. In contrast, the M1-polarizing signal LPS results in decreased oxygen consumption and increased glycolysis. These results raise the question of whether metabolic changes in macrophages influence the inflammatory immune response. In addition, it will be important to understand how metabolic disorders (e.g. obesity, diabetes) may affect macrophage adaptation and the inflammatory response in hypoxia.

Extracellular adenosine, an endogenous distress signal, is involved in the adaptation to hypoxia (78). In stress situations, such as ischemia or hypoxia, massive ATP degradation increases the local adenosine concentration to a micromolar range. Adenosine binds A_{2A} receptors present on macrophages and inhibits TLR-induced production of pro-inflammatory cytokines and chemokines (78, 79). Following A_{2B} engagement, macrophages produce an anti-inflammatory cytokine (i.e. IL-10) and down-regulate IFN-γ-induced MHC class II and iNOS expression (80, 81). In addition, A_{2B} receptor activation inhibits monocyte CSF (M-CSF)-induced macrophage proliferation (82). Adenosine signaling suppresses macrophage activation, thereby preventing tissue injury after episodes of hypoxia and ischemia.

An additional pathological condition secondary to decreased oxygenated blood supply is pre-eclampsia. Fetoplacental macrophages influence placental development and function through the synthesis and secretion of cytokines and growth factors (83). Overproduction of inflammatory cytokines in response to hypoxia is thought to lead to increased plasma levels and endothelial activation and dysfunction in pre-eclampsia.

Myeloid cells in the hypoxic tumor microenvironment

Tumor hypoxia develops as a result of an imbalance between oxygen supply and consumption in proliferating tumors. When the oxygen consumption rate of a growing tumor is not matched by blood supply, the oxygen partial pressure (pO_2) levels fall and hypoxia arises, thus promoting a process of angiogenesis, the generation of new blood vessels supporting tumor metabolic needs (84).

Several studies provide evidence that the presence of hypoxia within the tumor mass is an independent marker of poor prognosis for patients with various types of cancer, including carcinoma of the cervix, carcinoma of the breast, carcinoma of the head and neck, soft-tissue sarcoma, cutaneous melanoma and prostatic adenocarcinoma (85). Intratumor hypoxia is associated with a malignant phenotype characterized by uncontrolled tumor growth, angiogenesis and increased risk of metastasis (86).

Normal cells can adapt their metabolism to environmental pO_2, whereas tumor cells always favor glycolysis regardless of oxygen availability (the 'Warburg effect') (87). The gene responsible for this 'aerobic glycolysis' is the pyruvate kinase isoenzyme type M2 (M2-PK), an HIF-dependent gene, and the up-regulation of M2-PK is attributable to oncogene-mediated, hypoxia-independent HIF-1 stabilization (88).

Accumulating evidence suggests that tumors require a constant influx of myelomonocytic cells to support their growth. In fact, a high frequency of myeloid cells is associated with poor prognosis (6). Tumor-derived factors sustain myelopoiesis and the accumulation and functional differentiation of myeloid cells, most of which are TAMs. These TAMs are major players in the connection between inflammation and cancer and undertake a number of functions (e.g. promotion of tumor cell proliferation, angiogenesis, incessant matrix turnover, repression of adaptive immunity) that ultimately have an important impact on disease progression (6). TAMs preferentially accumulate in the poorly vascularized regions of tumors and respond to the levels of hypoxia with a transcription program in which mitogenic, pro-invasive, pro-angiogenic and pro-metastatic genes are up-regulated (6). The HIF pathway is essential in the recruitment and activation

of TAMs into solid tumors and contributes in driving both TAM recruitment and pro-tumor functions (89).

We suggested that hypoxia-mediated induction of HIF-1α in TAMs influences the positioning and function of tumor cells, stromal cells and TAMs by selectively up-regulating the expression of the chemokine receptor CXCR4 (34). Furthermore, it has been shown that HIF-1 activation mediates expression of the CXCR4 ligand CXCL12, a chemokine involved in angiogenesis and cancer metastasis (33, 90). TAMs adapt to hypoxia by increased expression of HIF-inducible pro-angiogenic genes, such as VEGF, basic fibroblast growth factor (βFGF) and CXCL8, as well as glycolytic enzymes (91).

Hypoxia strongly affects the accumulation of other myeloid cell populations within the tumor microenvironment, including angiogenic monocytes expressing Tie-2 (92) and the heterogeneous population of immature myeloid cells, called myeloid-derived suppressor cells (MDSCs) (93). Tie-2$^+$ monocytes are mainly clustered in hypoxic areas of solid tumors, in close proximity to nascent tumor vessels, where they are recruited by hypoxia-inducible chemotactic factors, such as the CXCR4 ligand CXCL12 and angiopoietin 2 Ang2 (94). Moreover, hypoxia promotes the HIF-1α-mediated differentiation of MDSCs into TAMs, thus contributing to their intra-tumor accumulation (95). Hypoxia influences lymphocyte functions by enhancing the expression of the suppressive enzymes iNOS and arginase in TAMs and MDSCs (43, 96, 97).

Therapeutic targeting of hypoxic macrophages

HIFs represent a suitable molecular target for cancer therapy, and several HIF inhibitors have been identified so far (1, 98, 99). These include inhibitors of HIF-1α synthesis (digoxin, rapamicyn, topotecan); inhibitors of HIF-1α protein stability [cyclosporine, the guanylate cyclase activator YC-1, the heat-shock protein 90 inhibitor 17-AAG, the histone deacetylase (HDAC) inhibitor LAQ824]; inhibitors of DNA binding (doxorubicin, echinomycin); inhibitors of transactivation (the proteasome inhibitor bortezomib, the anti-fungal agent amphotericin B); and inhibitors of signal transduction [the BCR–ABL (breakpoint cluster region– Abelson tyrosine kinase) inhibitor imatinib, the cyclooxygenase inhibitor ibuprofen, the EGFR inhibitors erlotinib and gefitinib, the HER2 inhibitor trastuzumab] (1).

Despite the generation of this array of inhibitors, very few studies are available that examined their activity on immune cells, as they have been tested mainly on hypoxic cancer cells. Because HIF-1α-deficient myeloid cells have profound impairment in migration and cytotoxicity (19), it is, however, likely that HIF inhibitors also modulate functions of infiltrating leukocytes. How this contributes to the therapeutic effect of the drugs remains still elusive, though.

It is expected that blocking HIF activation in stromal myelomonocytic cells would control the expression of several mitogenic, pro-invasive, pro-angiogenic and pro-metastatic genes (4). As a consequence, the tumor would be deprived of important environmental factors needed for its survival and progression. It was speculated that HIF activation results in divergent effects on innate versus adaptive immunity, leading to an unbalanced immune activation towards the inflammatory phenotype (4). If so, a possible concern related to HIF inhibition is the essential role of phagocytes against infections, as HIF inhibition would interfere with innate immune functions in the event of opportunistic infections. On the other hand, because of a positive effect on the anti-microbial activity of macrophages, HIF agonists could potentially be used alongside conventional antibiotics in localized infections.

Finally, because of their natural ability to accumulate in hypoxic areas, macrophages can be exploited to deliver HIF-regulated therapeutic genes to otherwise inaccessible areas in tumors. Recently a new approach was designed that selectively targets an oncolytic adenovirus to hypoxic areas of prostate tumors, resulting in intratumoral spread and a lasting therapeutic effect (100). Alongside this, interfering with chemotactic pathways, such as VEGF and CXCL12 (34, 38), promoting macrophage recruitment in the hypoxic areas of tumors would provide therapeutic benefits (6). By analogy, inhibition of the hypoxia-inducible gene Ang2 may restrain the recruitment of Tie-2$^+$ proangiogenic monocytes (101). In contrast, activation of the transcriptional activity of HIFs in angiogenic monocytes would benefit vasculature reconstitution, and recent evidence indicates that PHD2 inhibitors may favor tissue healing and arteriogenesis (76).

Conclusions

Hypoxia has a dramatic influence on the phenotype and functions of macrophages. Hypoxic macrophages are involved in several diseases, including infections, chronic inflammation and ischemia. Importantly, the hypoxic microenvironment imposes a metabolic adaptation to macrophages, skewing their functions towards a mitogenic, pro-invasive, pro-angiogenic and pro-metastatic phenotype, thus supporting tumor growth. Elucidation of the molecular pathways underlying macrophage adaptation to hypoxia is expected to provide novel therapeutic strategies. From this perspective, the therapeutic use of selected HIF inhibitors may potentially elicit more sought-after macrophage phenotypes, associated with anti-infective, regulatory and anti-cancer activities.

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