Minireview

Hypoxic tumor microenvironment: Implications for cancer therapy

Sukanya Roy[1], Subhashree Kumaravel[1], Ankith Sharma[1], Camille L Duran[2], Kayla J Bayless[2] and Sanjukta Chakraborty[1]

[1]Department of Medical Physiology, Texas A&M Health Science Center, College of Medicine, Bryan, TX 77807, USA; [2]Department of Molecular & Cellular Medicine, Texas A&M Health Science Center, Bryan, TX 77807, USA
Corresponding author: Sanjukta Chakraborty. Email: schakraborty@tamu.edu

Impact statement

Hypoxia contributes to tumor aggressiveness and promotes growth of many solid tumors that are often resistant to conventional therapies. In order to achieve successful therapeutic strategies targeting different cancer types, it is necessary to understand the molecular mechanisms and signaling pathways that are induced by hypoxia. Aberrant tumor vasculature and alterations in cellular metabolism and drug resistance due to hypoxia further confound this problem. This review focuses on the implications of hypoxia in an inflammatory TME and its impact on the signaling and metabolic pathways regulating growth and progression of cancer, along with changes in lymphangiogenic and angiogenic mechanisms. Finally, the overarching role of hypoxia in mediating therapeutic resistance in cancers is discussed.

Abstract

Hypoxia or low oxygen concentration in tumor microenvironment has widespread effects ranging from altered angiogenesis and lymphangiogenesis, tumor metabolism, growth, and therapeutic resistance in different cancer types. A large number of these effects are mediated by the transcription factor hypoxia inducible factor 1α (HIF-1α) which is activated by hypoxia. HIF1α induces glycolytic genes and reduces mitochondrial respiration rate in hypoxic tumoral regions through modulation of various cells in tumor microenvironment like cancer-associated fibroblasts. Immune evasion driven by HIF-1α further contributes to enhanced survival of cancer cells. By altering drug target expression, metabolic regulation, and oxygen consumption, hypoxia leads to enhanced growth and survival of cancer cells. Tumor cells in hypoxic conditions thus attain aggressive phenotypes and become resistant to chemo- and radio- therapies resulting in higher mortality. While a number of new therapeutic strategies have succeeded in targeting hypoxia, a significant improvement of these needs a more detailed understanding of the various effects and molecular mechanisms regulated by hypoxia and its effects on modulation of the tumor vasculature. This review focuses on the chief hypoxia-driven molecular mechanisms and their impact on therapeutic resistance in tumors that drive an aggressive phenotype.

Keywords: Hypoxia, cancer, HIF1α, tumor microenvironment, inflammation, angiogenesis, lymphangiogenesis, chemoresistance, radioresistance

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Introduction

Physiological oxygen concentration in tissues or normoxia is hampered during cancer progression that gives rise to a low oxygen concentration in the TME known as hypoxia. The specific oxygen concentration during tumor development differs depending on the tumor tissue type and is on average 1–2% or even less.[1] Hypoxia can arise due to an abnormal rate of cell division, development of solid tumor masses that disrupt blood vessels from penetrating and hence a resulting drop in oxygen diffusion.[2] Intratumoral hypoxia is characterized by formation of irregular vascular networks and regions within the solid tumor that have areas of low oxygen.[3] Hypoxia can be classified as acute hypoxia where cells are exposed to low oxygen concentration for a brief period of a few minutes and chronic hypoxia typically seen in large tumors where low oxygen concentrations are prolonged in TME.[4] Acute as well as chronic hypoxia can lead to a number of cellular changes that ultimately contribute to metastasis and tumor-associated chemoresistance or radioresistance.[5] These changes range from a metabolic shift towards glycolysis for faster ATP synthesis, induction of anti-apoptotic and pro-tumorigenic signaling pathways, and decreased drug penetration. In addition to chronic and acute, a third kind of hypoxia known as intermittent or cycling hypoxia has also been seen in certain regions of different solid tumors.[6] Cyclic hypoxia, defined by periodic sequence of hypoxia and

reoxygenation, (H-R cycles) is involved in upregulating angiogenesis, metastasis, immune evasion, and increased resistance to therapy in cancer.[6] The differing ramifications of a hypoxic TME on the growth, spread, and therapeutic resistance of cancer cells are profound and make understanding the different facets crucial.[7] This review aims to summarize the role of hypoxia in shaping cancer progression and therapeutic tolerance focusing on specific regulated signaling pathways and drug resistance mechanisms.

Hypoxia and cancer progression: Role of hypoxia inducible factor

The various components of the TME such as cancer-associated fibroblasts (CAFs), stromal cells, lymphatic and blood vessels, and immune cells undergo pro-tumorigenic adaptation predominantly mediated by hypoxia inducible factor (HIF) when exposed to hypoxia. HIF proteins are a family of transcription factors that consist of oxygen sensitive members such as HIF-1, HIF-2, and HIF-3 of which HIF-1α is the most widely characterized and studied.[2] The HIF-1 and HIF-2 primarily play a role as transcriptional activators and hence have both unique and overlapping target genes, while the function of HIF-3 is a little less clearly understood.[8] The transcriptional complex of HIF-1 consisting of HIF-1ß and HIF-1α is constitutively synthesized in normoxic conditions.[5] HIF-1α under normoxia is hydroxylated at proline residues 402 and 577 and marked for ubiquitination by a prolyl hydroxylases (PHDs). Subsequently, HIF-1α interacts with the tumor suppressor von Hippel Lindau protein (pVHL) and finally is degraded by 26S proteasome. Under hypoxia, the activity of PHDs which require O_2 as a cofactor is reduced and HIF-1α is stabilized in the cytoplasm. Mitochondrial O_2 sensors also stabilize HIF-1α by increasing the production of mitochondrial reactive oxygen species (mtROS) which helps in HIF-1α nuclear translocation. Post nuclear translocation HIF-1α forms a heterodimer with aryl hydrocarbon nuclear receptor (ARNT) and transactivates genes which have hypoxia responsive elements (HREs) in their promoter and enhancer regions. HIF2α acts in a similar manner and shares overall 48% amino acid identity.[9] Low O_2 conditions or hypoxia stabilize HIF-1α by taking advantage of the low availability of O_2, an essential PHD substrate needed for PHD-mediated hydroxylation.[10,11]

HIF switch in hypoxia

Cancer cells shift their dependence between HIF1α and HIF2α based on the level of oxygen concentration available to them. While HIF2α is more stable at higher oxygen concentration and is active in chronic hypoxia, stable and higher levels of HIF1α are observed during acute hypoxia.[12,13] These differences in stabilization patterns arise partly from the ease of hydroxylating HIF2α by PHD and asparagynyl hydroxylases (FIH-1) during acutely low oxygen levels.[14] Multiple studies point to the temporal regulation of HIF1α or HIF2α levels during hypoxia. This switch from HIF-1 to HIF-2 and HIF-3 signaling is required in order to adapt the endothelium to prolonged hypoxia.[8] HIF1α levels are usually highest at 4–8 h after onset of hypoxia after which they decrease and diminish at 18–24 h, while HIF2α levels stabilize much later around 24–72 h of hypoxia.[13,15–17] In addition to the ease of hydroxylation of HIF1α, ubiquitination by heat shock protein (HSP)-70 complex and ensuing degradation under chronic hypoxia takes place exclusively with HIF1α.[18,19] miR-429 has been seen to regulate the switch from HIF1α to HIF3α during chronic hypoxia in endothelial cells indicating a potential involvement of a variety of cell type-specific molecular factors that govern hypoxia-mediated effects.[20]

Cyclic hypoxia and its impact on HIFs

Cyclic or intermittent hypoxia has recently come under increasing scrutiny because of the high levels of HIF1α found in cancer cells during periodic hypoxia.[21,22] Stable and enhanced HIF1α levels of cyclic hypoxia have been attributed to multiple mechanisms such as the post-translational modification of HIF1α or high phosphorylated HIF1α levels during intermittent hypoxia takes place possibly through PKA activation in HAMEC-1 and EAhy926 endothelial cells. This heightened level of HIF1α also leads to increased migration and angiogenesis by endothelial cells.[15,21,23] The effects of cyclic hypoxia in induction of angiogenesis and migration become important while considering therapeutic resistance of cancer cells. Verduzco *et al.*[24] have shown that exposure to intermittent hypoxia leads to cancer cells having low levels of p53 and E-cadherin and led to increased survival, metastasis, and drug resistance to etoposide. The genes induced by HIFs activate signaling cascades ultimately contributing towards cancer growth, angiogenesis, lymphangiogenesis, metastasis, and resistance to therapeutic intervention.[1,9,25–27] Signaling pathways regulated by HIF-1α and consequently hypoxia can be distinguished under the following major areas:

Role of HIF-1α in mitochondrial biogenesis

HIF-1α preferentially shifts dependence of cellular metabolism from mitochondrial respiration to glycolysis. As shown in Figure 1, HIFα proteins play a major role in mitochondrial biogenesis by regulating expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC 1α), that is activated by PGC1β, in turn activated by pro-oncogenic transcription factor MYC.[28,29] Activated HIF-1α induces MYC-associated factor X(MAX)-interacting protein 1 (MAXI1) expression that represses MYC activity by binding to MYC target genes' (such as PGC1α/β) promoter sites. Additionally, Forkhead-box protein O3a (FOXO3a), activated by hypoxia transmits this inhibitory effect of HIF-1α on MYC by reducing MYC structural stability and upregulating expression of miRNA which interferes with MYC translation.[29,30] In cells expressing HIF-2α an opposite effect of hypoxia on MYC activity is observed, HIF2α binds and stabilizes MAX-MYC heterodimer needed to exert downstream effects of MYC. The consequence of HIF2α regulation is thus the pro-tumorigenic regulation of cell cycle proteins.[31,32] However, the inhibitory effect on PGC1α/β and in extension on mitochondrial biogenesis is primarily mediated by HIF-1α. Hypoxia drives reactive oxygen

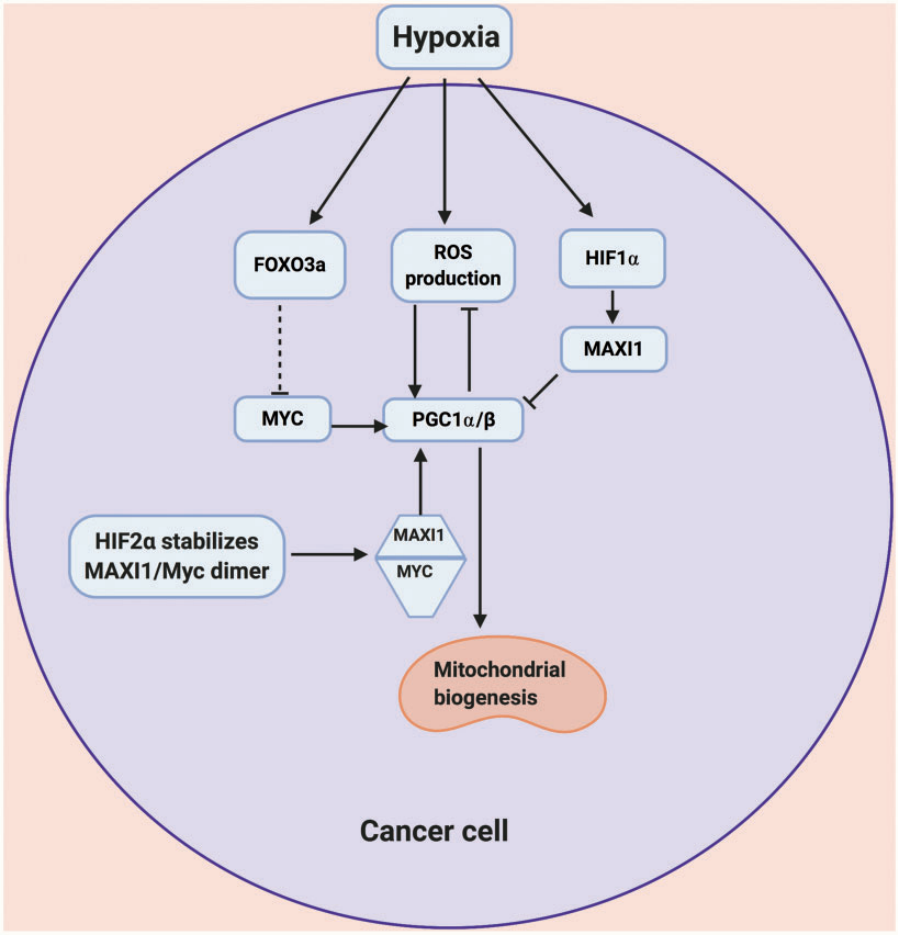

Figure 1. Hypoxia-mediated mechanisms of mitochondrial biogenesis in tumor cell. Hypoxia activates FOXO3a gene transcription which in turn destabilizes structural integrity of Myc; ROS production is upregulated under hypoxia which stimulates PGC1α/β enzymatic activity that ultimately removes ROS thus facilitating cancer cell survival; Hypoxia also activates MAXI1 which interacts with PGC1α/β and inhibits mitochondrial biogenesis by PGC1α/β. In cancer cells that express HIF2α, the MAXI1/MYC dimer is stabilized that leads to higher mitochondrial biogenesis. FOXO3a: Forkhead box protein O3; ROS: reactive oxygen species; HIF1/2α: hypoxia inducible factor 1/2α; MYC: myelocytomatosis gene; PGC1α/β: peroxisome proliferator-activated receptor gamma coactivator 1-alpha/beta; MAXI1: myc-associated factor X interactor 1.

species (ROS) production in cancer cells which triggers antioxidant enzyme activity through PGC1α/β expression that ultimately contributes to cancer cell survival by ROS removal.[33]

Role of HIF-1α in cellular metabolism

Apart from the role of HIFα proteins in controlling mitochondrial biogenesis, the principal role of hypoxia during tumorigenesis and cancer progression is regulation of cellular metabolism. HIF-1α activates transcription of key glycolytic genes such as glucose transporter1 (GLUT1), GLUT3, phosphoglycerate kinase 1 (PGK), and hexokinase (HK)-1/2. Additionally, lactate dehydrogenase A (LDHA) responsible for NAD^+ replenishment and monocarboxylate transporter 4 (MCT4), the transporter responsible for lactate efflux, are also induced by HIF-1α. This HIF-1α-mediated regulation of glycolytic genes ultimately results in a shift towards dependence of cancer cell metabolism on glycolysis.[34,35] In addition to inducing glycolytic gene expression, HIF-1α also reduces the rate of mitochondrial respiration in hypoxic cancer cells. Pyruvate dehydrogenase, a critical enzyme belonging to the pyruvate dehydrogenase complex (PDC) converts pyruvate to acetyl-CoA that then enters tricarboxylic acid cycle (TCA cycle) in mitochondria. Pyruvate dehydrogenase kinase 1 (PDK1) phosphorylates and inactivates pyruvate dehydrogenase and as a result pyruvate is unavailable for mitochondrial respiration. PDK1 is induced by HIF-1α with the net effect of cancer cells utilizing glycolytic ATP. An additional advantage of reducing mitochondrial dependence is the generation of lower levels of mtROS.[36,37] It has been shown that HIF-1α also reduces mtROS by inducing two proteins: LONP1, a protease that degrades cytochrome c oxidase subunit 4 isoform 1 (COX 4–1) and COX4-2, a more efficient form of COX4-1.[38] These studies show that hypoxia increases glycolysis and reduces mitochondrial respiration and ROS production in tumor cells.

Impact of hypoxia on the tumor microenvironment

Tumor cells along with the local non-malignant cells constitute the TME. It has been well understood now that the

interactions of the neighboring stromal cells in the microenvironment are critical for the progression of tumor cells. The TME is primarily comprised of the chemical microenvironment that includes the pH, nitric oxide, and metabolites (like glucose) and the cellular microenvironment that involves tumor cells, stromal cells, and the extracellular matrix (ECM).[7] Here, we mainly focus on the influence of hypoxia on stromal cells and ECM and their effects on tumor progression. One of the major cells that constitute the TME are the CAFs.[39] CAFs are spindle shaped cells that are actively involved in secreting ECM proteins and are abundantly present in connective tissues.[40] During cancer development, majority of CAFs are derived from a mesoderm-derived precursor cell. However, CAFs also originate from trans-differentiation of pericytes, from endothelial cells through endothelial-mesenchymal transition, and from epithelial cells by epithelial-mesenchymal transitions.[41] CAFs have also been derived from normal fibroblasts during chemotherapy as a result of the created hypoxic environment.[42] CAFs in the TME are large spindle shaped cells that express α-smooth muscle actin and are stiffer than normal fibroblasts.[2,43]. However, there are additional biomarkers like vimentin (VIM), fibroblast activation protein (FAP), and fibroblast-specific protein-1 (FSP-1) that are used to identify CAFs.[44,45] Hypoxia is one of the main driver events that alters the functioning of CAFs in TME and defines its role in tumor progression. CAFs affect both tumor development and progression in various ways both by directly regulating tumor growth factors, EMT mechanisms, and chemokines. Hypoxic conditions increase the expression of the angiogenic factors angiopoietin and vascular endothelial growth factor (VEGF) in CAFs as well as regulate the expression of the chemokine CXCL3 in activated myofibroblasts.[25] However, CAFs also provide critical metabolites and undergo metabolic reprogramming to support glycolysis.[46,47] HIF-1α also mediates metabolic reprograming in CAFs by upregulating the expression of MCT4 and subsequently downregulating caveolin-1 in order to supply energy to the growing tumor cells.[48] Tumor cells switch their metabolic state between glycolysis and oxidative phosphorylation through metabolic interplay with CAFs, and exhibit the Warburg effect under hypoxia and reverse Warburg effect under normoxia.[49] This metabolic interplay is critical as it further promotes tumor growth and metastasis.[49] Tumor cell-derived ROS decreases the expression of caveolin-1 in CAFs which further stabilizes HIF-1α.[50,51] This metabolic symbiosis however does not occur equally in all tumor types and accounts for tumor heterogeneity in the same tumor and thus significantly affects therapeutic strategies and chemoresistance.[51]

Another major player in TME regulation is the ECM, that maintains cell dynamics and regulates the cellular microenvironment.[52] It has been observed that during the initial stages of cancer development, CAFs and other stromal cells secrete factors that modify ECM composition and dynamics.[53] The altered ECM then maintains the behavior of stromal cells, thereby influencing the development and maintenance of TME.[52] Hypoxia affects ECM through fibrosis, regulating collagen deposition and by mediating ECM degradation.[54] HIF-1α-mediated fibrosis enhances cell invasion and migration by increased ECM deposition.[55–57] In adipose tissue, kidney, and liver cells, it has been observed that HIF-1α also influences ECM remodeling.[57–59] ECM stiffness is regulated by hypoxia-mediated collagen deposition. Specifically, HIFs regulate prolyl 4-hydroxylase subunit alpha (P4HA) and procollagen-lysine 2-oxyglutarate 5-dioxygenase (PLOD) enzyme family genes which play a vital role in collagen synthesis.[59] P4HA2 plays a role in breast cancer progression and enhanced expression of PLOD2 by HIF-1α in primary tumors is implicated in sarcoma metastasis and increased mortality in breast cancers.[60–62] PLOD2 also promotes alignment of cancer cells along collagen fibers which in turn enhances invasion and metastasis to lungs and lymph nodes.[63] Apart from P4HA and PLOD genes, HIF also influences collagen deposition through upregulation of lysyl oxidase (LOX) enzymes that regulate collagen crosslinking in the ECM.[64] HIF-increased LOX expression has been associated with breast and head and neck cancer metastasis.[65] LOX expression inhibits E-cadherin and promotes epithelial-mesenchymal transition (EMT), which drives invasion and metastasis.[66] Finally, hypoxia regulates ECM by promoting ECM degradation via expression of matrix metalloproteinases like MMP2, MMP9, and MMP15 and thus significantly influences the tumor microenvironment.[2]

Hypoxia-mediated regulation of cancer immune evasion

Hypoxia can also negatively regulate the antitumor immune responses in cancer and some of the key mechanisms by which it mediates immune evasion in cancers are illustrated in Figure 2. Among the target genes whose expression is regulated by HIF-1α is Cluster of differentiation 47 (CD47). CD47, a membrane protein is responsible for regulating a number of cellular responses such as cell migration, T cell activation, cytokine secretion, and metastasis.[67] Zhang et al. showed that breast cancer cells were protected from bone marrow-derived macrophage-mediated phagocytosis by expressing a stem cell phenotype. They also showed that HIF-1α activates CD47 transcription in these breast cancer cells.[68] CD47 controls immune evasion through CD47-signal regulatory proteinα (SIRPα) axis where SIRPα is expressed on TAMs (tumor-associated macrophages) and myeloid-derived suppressor cells (MDSCs).[69,70] Hypoxia has been shown to stimulate VEGF secretion by M2 macrophages in the TME.[71] TAMs residing in hypoxic areas of TME, achieve a M2-inducing phenotype through increased secretion of molecules like TGFβ.[72] Hypoxic tumor microenvironment has been shown to promote anti-tumor immunity through increased expression of the master regulator of T regulatory cell differentiation, FoxP3.[73] Increased number of T regulatory cells in the tumor microenvironment suppresses CD8+ T cell-mediated cytotoxic killing of tumor cells.[74] Programmed death ligand 1 (PDL1) expressed on the surface of cancer cells binds to its receptor PD1 on the surface of CD8+ T cells and inactivates CD8+ T cell antitumor response. HIF-1α-mediated PDL1 expression was recorded on tumor cells, dendritic cells, as well as macrophages.[75,76]

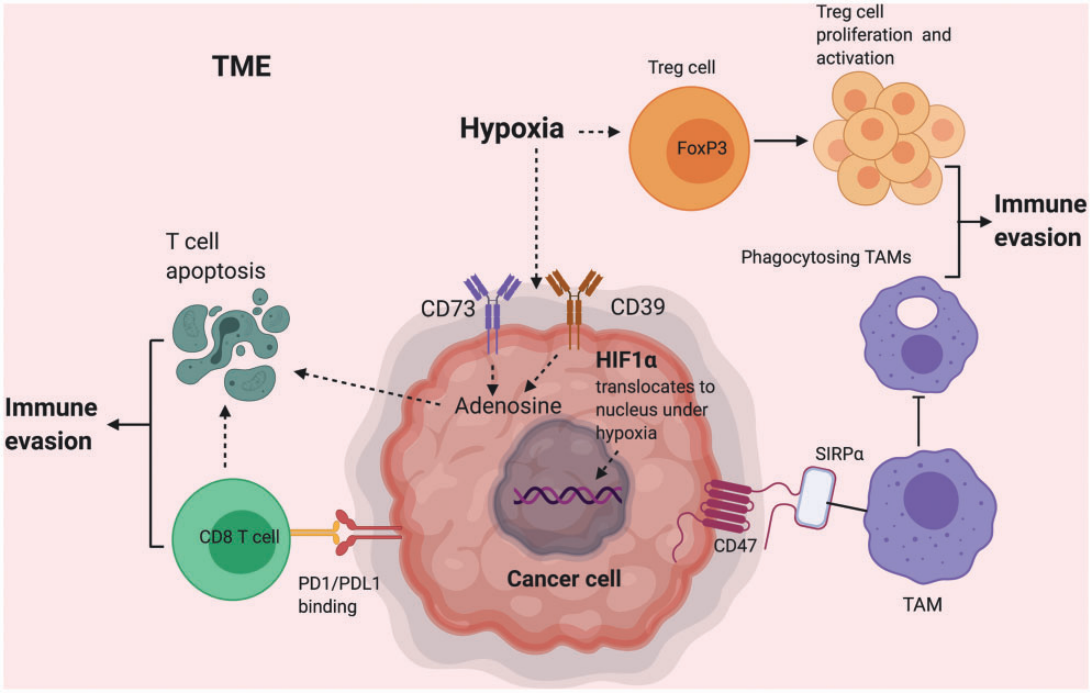

Figure 2. Hypoxia-driven tumor immune evasion pathways. Hypoxia induces CD73 and CD39 gene expression in cancer cell. CD39 converts ATP/ADP to AMP and CD73 converts AMP to adenosine which leads to T cell apoptosis. Under hypoxia, HIF1α translocates to the nucleus in the tumor cell and induces expression of CD47. CD47 then engages with SIRPα on TAMs and inhibits TAM-based tumor phagocytosis. Under hypoxia, cancer cells also express PDL1 which binds to PD1 on CD8+ T cell leading to CD8+ T cell apoptosis. In a hypoxic TME, induced FoxP3 expression leads to T regulatory cell proliferation that then inhibits cytotoxic T cell death. TME: tumor microenvironment; Treg cell: T regulatory cell; FoxP3: forkhead box protein P3; TAMs: tumor-associated macrophages; SIRPα: signal regulatory protein alpha; CD47: cluster of differentiation 47; CD73: cluster of differentiation 73; CD39: cluster of differentiation 39; PD1: programmed death 1(receptor); PDL1: programmed death ligand 1; HIF1α: hypoxia inducible factor 1α; CD8 T cell: cluster of differentiation 8 expressing T cell. Figure was created with Biorender.com.

Hypoxia, in addition to having direct effects on the survival, differentiation, and activation of key immune effectors also activates an immune metabolism modulating molecule, adenosine. A hypoxic TME upregulates the expression of CD39, an ectonucleoside triphosphate dihydrophosphohydrolase (ENTPD1) which converts ATP/ADP to AMP and CD73 a 5′-ectonucleotidase that finally converts AMP into adenosine.[77,78] Moreover, hypoxia induces increased expression of the cognate receptor of adenosine, A2A adenosine receptor (A2AR).[79] Notably, the adenosine-A2AR binding causes T cell apoptosis, negatively affects T cell activation and effector responses.[80] Thus, hypoxia negatively regulates anti-tumor immune responses by facilitating immune evasion and promoting T cell apoptosis through adenosine production.

Hypoxia and lymphangiogenesis

It has been very clearly demonstrated that lymphatic vessels play a critical role in promoting tumor progression and metastasis. Expression of lymphangiogenic growth factor, VEGF-C, high lymphatic vessel density, and high incidence of lymphatic invasion have been associated with metastasis and reduced survival in cancer patients.[81–84] However, the impact of a low oxygen environment on the lymphatic vasculature and effect on cancer metastasis has not been clearly elucidated although multiple pathways have been implicated in hypoxia-mediated regulation of the lymphatic-cancer crosstalk.[85–87] Tumor-induced lymphangiogenesis is promoted by secretion of growth factors such as VEGF-C, VEGF-D, PDGF-B, IGF1 and -2, FGF2, HGF, and other mediators such as angiopoietin-2, sphingosine-1- phosphate, adrenomedullin, and IL-7 by the cancer cells and other cells within the TME.[85,88–90] Hypoxia has been shown to favor the processes of lymphangiogenesis and metastasis of cancer cells via lymphatic vessels predominantly through activation of HIF-1-α.[91] HIF-1α regulates the expression of growth factors, tumor-promoting pathways and cytokines that are involved in lymphatic endothelial cell (LEC) proliferation, migration, and subsequent lymph node metastasis.[86] HIF-1α levels are shown to correlate with the expression of VEGF-C and peritumoral lymphangiogenesis in breast cancer.[87] Further, HIF-1α expression also correlates with increased VEGF-C and lymphatic vessel density in biopsies from oral squamous cell carcinoma patients.[92] Interestingly, in contrast, Schito et al. have shown that in MDA-MB-231 breast cancer cells, hypoxia decreased the mRNA levels of VEGF-D and did not increase the VEGF-C levels. However, it was shown that hypoxia induces LEC proliferation and migration via HIF-1α→PDGF-B→PDGFRβ signaling.[93] HIF-1α expressed during tumor hypoxia influences peritumoral lymphangiogenesis.[87] Hypoxia increases apelin expression in cancer cells.[94] The apelin/APJ pathway has been implicated in progression of lymphangiogenesis and lymph node metastasis.[95] Hypoxia also increases expression of the β1 integrin subunit, PI3K/Akt and mTOR pathways, mechanisms that have been widely implicated in promoting

lymphangiogenesis and lymph node metastasis.[81] Inhibition of mTOR with rapamycin reduces lymphangiogenesis in primary tumors and prevents spread of cancer cells to the cervical lymph nodes.[98] Hypoxia activates transcription factors, activator-protein-1 (AP-1), and Prox-1 that are closely associated with the lymphatic vasculature.[97] AP-1 activation co-operates with HIF-1α for upregulation of VEGF gene expression in hypoxic conditions.[96] AP-1-inducible genes are triggered in LECs due to hypoxia, including ET-1, MMP9, and c-jun.[97] The AP-1 complex exemplifies a network of transcription factors that coordinately function in lymphangiogenesis and promote tumor development and progression.[96,97] The expression of Prox-1, a transcription factor essential for embryonic lymphangiogenesis, is increased under hypoxia or induction of HIF-1α. Importantly, both Prox-1 and NFκB activate the VEGFR-3 promoter in LECs, that enhances the responsiveness of LECs to VEGFR-3 ligands VEGF-C and VEGF-D, ultimately resulting in robust lymphangiogenesis.[25] The ET-1/ETBR signaling, that activates HIF-1α, induces LEC proliferation and branching morphogenesis concomitantly with activation of p42/44 mitogen-activated protein kinases (MAPK) and Akt pathways. HIF-1α silencing desensitizes VEGF-A and VEGF-C production in response to ET-1 or hypoxia, implicating HIF-1α/VEGF as downstream signaling molecules of ET-1 axis.[46] The ET-1/ETBR axis and hypoxia may thus act through a HIF-1α-dependent mechanism to promote lymphangiogenesis via VEGF family members. Hypoxia has been shown to directly promote the attraction and adhesion of cancer cells to the lymphatic endothelium by inducing CXCR4 levels in cancer cells and its ligand CXCL12 in LECs.[51] Further, hypoxia induces NOTCH signaling that downregulates VEGFR2 in LECs and decreases migration of lymphatic vessels which might be critical for maintaining the balance between lymphangiogenesis and angiogenesis.[53]

Hypoxia and targeting angiogenesis for cancer therapy

Tumors require nutrients and oxygen from the surrounding tissue to survive. After reaching a certain size,[99] enlarging tumors must obtain their own blood supply to meet increasing metabolic needs. When tumors become hypoxic and continue to grow, HIFs secrete various factors that influence angiogenesis.[100] One of the major angiogenic genes regulated by HIF-1α is the VEGF, a primary regulator of angiogenesis[1,100] and highly induced in several cancers.[1,101] Hypoxic stabilization of HIFα results in expression of angiogenic factors like nitric oxide synthase, adrenomedullin, and interleukin 8.[102–104] Suppression of HIF-1α in endothelial cells alters NO levels, suppresses tumor cell migration, and inhibits tumor metastasis. But loss of HIF-2α was observed to have the opposite effect.[46] Hypoxia also influences angiogenesis by altering the fiber morphology in the ECM.[105] As discussed above, tumors secrete proangiogenic factors, VEGF, basic fibroblast growth factor (bFGF), and placental growth factor (PIGF), that activates neighboring endothelial cells and induces proliferation and migration toward the tumor mass.[106] As tumor angiogenesis serves as a rate limiting step to continued growth, it has emerged as a promising potential therapeutic target.[107] Several drugs targeting VEGF or its receptors have been developed and approved for cancer therapy. While there are some clinical examples of success using anti-angiogenesis drugs in combination with chemotherapeutics,[108] the use of anti-angiogenic drugs as a monotherapy has not significantly improved patient survival rates.[109–110] After treatment, the tumor can upregulate other angiogenic stimulators, such as bFGF, PlGF, TGFβ, and angiopoietins, circumventing the loss of VEGF signaling.[112–114] This acquired resistance is possibly a response to the increase in tumor hypoxia induced by the anti-angiogenic therapy, that affects multiple cellular signaling pathways promoting tumor progression, invasiveness, and metastasis.[112,115–117] Further, the ineffectiveness of the combination therapy may be attributed to the architecture of the tumor vasculature. The tumor vessels are highly tortuous and heterogeneous, with thin-walled, leaky large vessels, devoid of pericyte coverage, and irregularly branched smaller vessels that connect randomly. As a result, oxygen distribution to the tumor cells through these irregular blood vessels causes hypoxic regions within tumor cells. These tumor cells, while adapting to the hypoxic microenvironment, become more aggressive and attain therapeutically resistant tumor phenotypes.[118] Endothelial cells associated with the tumor in many cases have lost polarity, detached from the basement membrane, and contain wide junctions, causing the vessels to be leaky.[119–122] Because the vascular endothelial cells no longer serve as a functional barrier, tumor vessels enable transendothelial tumor cell migration.[120] The irregular vessels and loss of pericyte coverage cause non-uniform blood flow within the tumor which further prevents uniform delivery and uptake of co-administered chemotherapeutic agents and impedes immune cell infiltration.[122] Additionally, tumor hypoxia caused by anti-angiogenic therapies can hinder the efficacy of irradiation and chemotherapies that function through the formation of ROS to kill tumor cells.[123,124] As the tumor vasculature serves as an impediment to the efficacy of chemotherapy, improving vessel function through increasing and normalizing blood flow throughout the tumor, enhancing pericyte coverage, and decreasing vessel permeability, can improve chemotherapeutic delivery throughout the tumor. Further, by reducing tumor hypoxia, clinicians can prevent development of a more aggressive and metastatic phenotype and enhance radiation therapy.[123,125] Intriguingly, anti-angiogenic drugs, such as bevacizumab, when used at low doses can normalize the tumor vasculature. The vessels become less tortuous with improved blood perfusion, decreased interstitial pressure, and increased tumor oxygenation, preventing tumor cell diapedesis due to enhanced endothelial cell barrier function.[123,125–130] In additional studies, tumors implanted in mice heterozygous for PHD2, which acts as an oxygen sensor to promote the HIF-driven hypoxic response, showed increased perfusion and oxygenation. PHD2 haplodeficiency promoted tumor vessel maturity and normalization and prevented tumor cell invasion, diapedesis, and metastasis.[120] These data support that normalization of the tumor vasculature through

modulation of HIF-mediated signaling is likely to benefit treatment of solid tumors.

Hypoxia and tumor therapy outcome

The role of drug resistance in cancer metastasis

Resistance of cancer cells to chemotherapy/radiotherapy can be broadly divided into two classes: intrinsic and acquired resistance. Factors conferring resistance to tumor cells prior to therapy result in development of intrinsic drug resistance, while acquired resistance refers to the development of resistance in cancer cells during the course of drug administration/treatment. Both of these resistance modes give rise to a population of tumor cells that do not respond to chemotherapeutic treatments and continue to proliferate rendering treatment ineffective.[131] A number of factors described below results in drug resistance of cancer cells:

Drug efflux pathways are well-recognized to confer drug resistance in various cancers. Three most extensively studied members of the well-known ATP-binding cassette (ABC) transporter family that regulates the bulk of the chemotherapeutic efflux across the plasma membrane are related to multi-drug resistance (MDR). These three proteins are, MD1(aka P-glycoprotein/ABCB1), MRP1(MDR-associated protein 1/ABCC1), and BCRP (breast cancer resistance protein/ABCG2). These three proteins play critical roles in eliminating a broad range of widely used chemotherapeutic agents. MDR1 overexpression (both before and after chemotherapy) is associated with intrinsic as well as acquired drug resistance in hepatic and renal cancers along with leukemia and lymphoma.[132,133] MDR1 is found to be highly expressed on the surface of epithelial cells forming the lining of excretory organs like colon, renal proximal tubules, bile ductules, etc. compared to other tissues under normal conditions which highlights the role of MDR1 in drug excretion.[134,135] Similarly, BCRP has been correlated with drug resistance in breast cancer and leukemia.[136,137] The role of ABC transporter overexpression in cancer cells is further underlined by the finding that cancer stem cells also show high MDR transporter overexpression levels, particularly for BCRP.[138]

Hypoxia is thus a critical element of the TME contributing to drug resistance. A number of reasons such as low drug bioavailability in hypoxic tumors, HIF-1α-mediated MDR1 overexpression and decreased expression of drug targets such as topoisomerase II have been reported to be responsible for hypoxia-mediated therapeutic resistance in cancer.[139]

Hypoxia and tumor chemoresistance

The most prominent player in hypoxia-induced chemoresistance is HIF-1α gene identified through a number of studies where poor patient prognosis has been correlated with HIF-1α overexpression in a variety of cancer types such as neuroblastoma and non-small-cell lung cancer (NSCLC).[5] A number of HIF-1α regulated genes such as Bcl-2, Glut-1, VEGF, and MDR1 induces chemoresistance.[140] Thus, HIF-1α inhibition-based treatment regimens have been shown to rescue hypoxia-mediated chemoresistance.[141] In neuroblastoma, hypoxia-mediated chemoresistance is seen for drugs such as doxorubicin, cisplatin, etoposide, etc.,[142] while head and neck squamous cancer and gastric cancer acquire resistance towards cisplatin, 5-fluorouracil.[143,144] Different mechanisms have been identified by which this chemoresistance is induced, including upregulation of P-glycoprotein expression, downregulation of topoisomerase, blockade of apoptosis, induction of autophagy, and upregulation of telomerase.

Induction of P-glycoprotein expression. Under hypoxic conditions, HIF-1α binds to the promoter of MDR1 gene and induces overexpression of membrane transporter P-gp that then mediates chemoresistance. P-gp is an important efflux transporter belonging to ABC family of transporters which transports hydrophobic/amphiphilic drugs out of the cell when overexpressed in chemoresistant cells thus causing increased cell proliferation.[26] A large number of chemotherapeutic drugs have been identified as substrates of P-gp all of whom are amphiphilic in nature.[134,145] Since low solubility of P-gp substrates has been identified, treatment strategies such as the usage of liposome nanoparticle mediated drug delivery have been suggested to prevent P-gp-mediated drug efflux and increase intracellular drug availability.[146]

Altered drug target expression. Resistance to known topoisomerase II inhibitors such as etoposide has been observed in breast cancer cells as a response to hypoxia in the TME. Such resistance is believed to be caused by HIF-1α-mediated low nuclear levels of topo IIα.[147]

Hypoxia-mediated anti-apoptosis. Mitochondria-based cell apoptosis through Bcl family proteins Bax and Bad are direct targets of hypoxia-mediated microRNA, miR-26a overexpression. In a study published in 2018, miR26a overexpression by HIF-1α in hypoxic conditions was identified which was inversely related to Bax and Bad expression leading to resistance to the drug temozolamide.[148]

Autophagy induction and metabolic regulation. HIF-1α caused protective autophagy in cancer cells exposed to fenretinide leading to drug resistance by HeLa cells. Inhibition of HIF-1α led to enhanced cancer cell death by fenretinide.[149] Colorectal cancer cells with high ATP levels were found to be resistant to 5-fluorouracil and oxaliplatin which points to the metabolic regulation by HIF-1α. HIF-1α is known to upregulate PDK1 protein which reduces mitochondrial respiration by phosphorylating and inactivating pyruvate dehydrogenase (PDH). As a result of this, hypoxic cancer cells become dependent on glycolysis as a fast and cheap source of ATP which is thus produced in high levels.[27] Inhibiting glycolysis in colorectal cancer cells resensitized them to 5-fluoruracil and cisplatin treatment.[37]

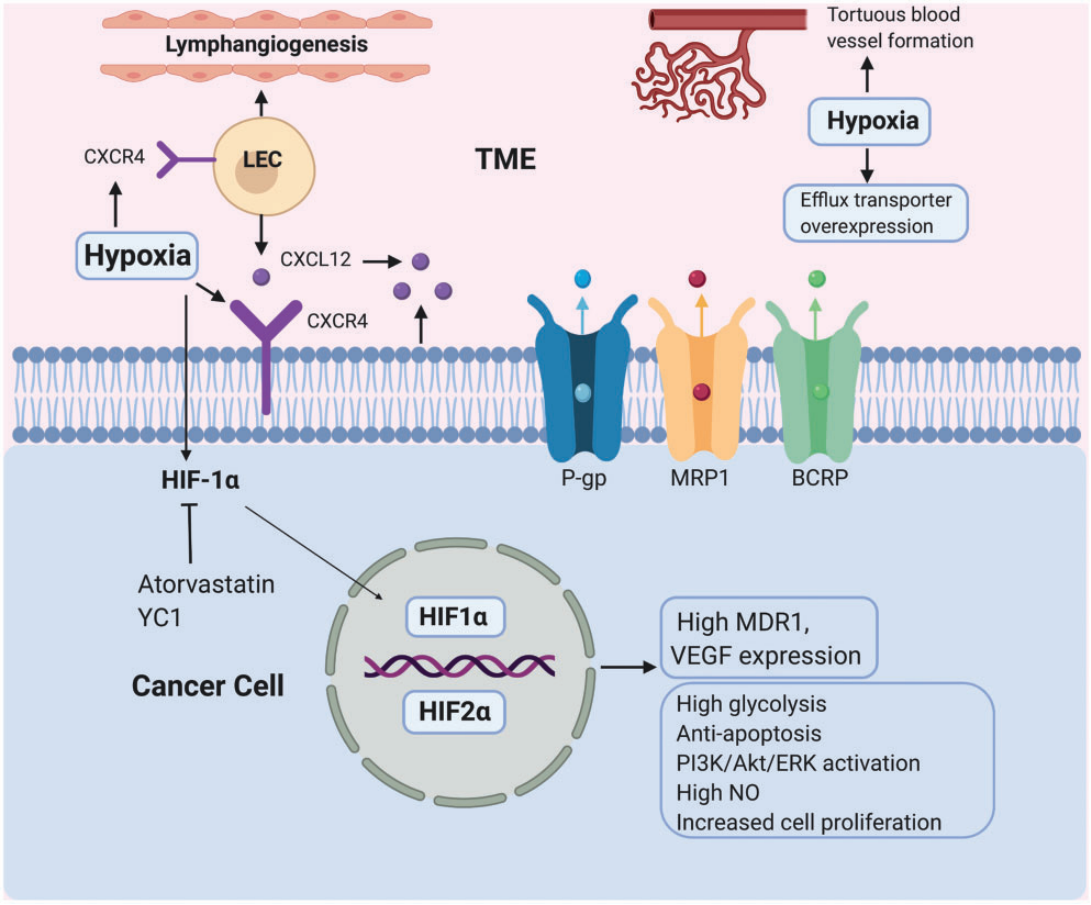

Figure 3. Hypoxia-associated tumor microenvironment of a cancer cell and related effects: Hypoxia causes nuclear translocation of HIF-1α which binds at various sites in the DNA and upregulates expression of MDR1, VEGF, NO, etc. PI3K/Akt/ERK signaling is activated, drug availability of topoisomerase II is reduced, intracellular ATP levels are upregulated due to high rate of glycolysis. CXCR4 and CXCL12 expression is upregulated in presence of hypoxic conditions from both cancer cell and LECs. Tortuous abnormal blood vessel growth further contributes to the alterations in the TME under hypoxia. High efflux transporter (P-gp, MRP1, BCRP) expression leads to drug efflux from cancer cell. Figure was created with Biorender.com.

Upregulation of telomerase. A number of studies have shown that HIF-1α causes upregulation of telomerase gene expression by binding to telomerase promoter site and by causing alternative splicing. As a result of this, active telomerase variants are produced and cancer cells are protected from replicative senescence that is also believed to play a role in ultimate cancer cell chemoresistance.[150]

In addition, a number of factors other than HIF-1α have been identified to cause hypoxia-based chemoresistance. Such evidence has come from studies demonstrating that inhibiting HIF-1α only partially reverses chemoresistance.[151] Overexpression of anti-apoptotic members of Bcl-2 family and IAP3 in response to hypoxia is one such example of HIF-1α-independent chemoresistance in cancer cells.[152] This has been clearly demonstrated in studies with different tumor cells, where blocking the activity of HIF-1α did not affect the increased apoptotic susceptibility of the tumors subjected to hypoxia.[152] In addition, nuclear factor kappa-B (NFκB), cyclooxygenase-2 (COX2), STAT3, etc. when inhibited also decrease chemoresistance in hypoxic cancer cells.[153]

Hypoxia and tumor radioresistance

Radiotherapy kills cancer by producing ROS that induces DNA damage in recipient cells. Hypoxia plays a major role and contributes almost three times to radioresistance compared to other factors. This is because the presence of oxygen is critical for radiotherapy-induced generation of ROS. As one of the most electronegative elements inside cells, oxygen respond to ionizing radiation by accepting free radicals generated due to radiotherapy.[154] These free radicals further induce DNA damage by strand breaks and kill cancer cells. Under hypoxia, as cancer cells are deprived of oxygen, free radicals generated from DNA under radiotherapy undergo reduction by molecules containing sulfhydryl (SH) groups that lead to DNA repair.[154] The ability of oxygen to bolster radiotherapy-induced DNA damage is popularly known as oxygen fixation hypothesis.[155] Hypoxic status in cancer types such as cervix carcinoma, sarcoma, head and neck cancer has emerged to be a major prognostic factor for poor radiotherapy results.[155–157] Radiotherapy treatment under hypoxia has shown a number of mechanisms through which ROS adaptation by cancer cells takes place. At the helm of most of these

mechanisms is HIF-1α. HIF-1α transcriptionally activates antioxidant gene expression as well as ROS efflux.[35,158] Hypoxia can also cause increase in ROS during its initial phase (ischemia) which instead of inducing cell death acts a signaling molecules for HIF-1α stabilization.[159] This hypoxic ROS-based HIF-1α activity takes place through PI3K/AKT pathway activation and ERK phosphorylation that ultimately increases HIF-1α expression.[160,161] A number of strategies have been implemented to re-sensitize radioresistant hypoxic tumor cells to radiotherapy by using drug therapy which are briefly summarized as follows:

NO donors. NO homeostasis is critical to prevent hypoxia-mediated therapeutic resistance by cancer cells. Low intracellular NO levels destabilize HIF-1α during hypoxia due to which a number of NO donors such as sodium nitroprusside,[162] nitroglycerin,[163] insulin,[164] spermin nonoate[165] have sensitized hypoxic cancer cells to radiotherapy.

Enzymatic inhibition. Drugs such as diethylmaleate,[166] diethylfumarate[167] (both deplete glutathione which is an antioxidant for ROS), auranofin (inhibits thioredoxin reductase activity),[168] auranofin + Buthionine sulphoximine (thioredoxin reductase inhibition and glutathione depletion)[169] that inhibit the activity of antioxidant enzymes radiosensitize the hypoxic cancer cells by increasing the availability of ROS inside the hypoxic cancer cells.

Metabolic inhibitors. Inhibiting tumor cell metabolism by agents such as dichloroacetate (glycolytic inhibitor),[170] ritonavir (glucose transporter inhibitor)[171] that target the metabolic mechanisms regulated by hypoxic conditions, has been shown to be a potent radiosensitizing technique.

Regulation of oxygen consumption. Drugs like metformin that inhibits mitochondrial complex I as well as glucocorticoids has been shown to decrease oxygen consumption thus increasing oxygen availability for radiotherapy in cancer cells under hypoxia.[172,173]

HIF-1α inhibition. Since HIF-1α is the master regulator for mediating hypoxia-related effects, a number of radiosensitizing agents inhibiting HIF-1α expression, translation and downstream activation have been developed such as atorvastatin,[174] berberine, YC-1 (degrades HIF-1α in addition to inhibiting HIF-1α translation).[175] Gold nanoparticles augmenting ROS production by electron donation have also been shown to be effective in radiosensitization in a variety of tumor types.[176]

Conclusion and future directions

Hypoxia is the key regulator for chemo-/radio resistance in different cancer types that account for increased cancer morbidity. As depicted in Figure 3, a variety of mechanisms both HIF-1 and HIF-2 dependent and independent are adopted by hypoxic cancer cells as a means to bypass therapy and account for tumor heterogeneity that promotes survival in unfavorable conditions. The metabolic interplay between tumor cells and components of a hypoxic TME coupled with activation of lymphangiogenic and angiogenic mechanisms further complicates the response to specific clinical interventions. Thus, the need to identify new drug targets of a hypoxic TME as well as studies combining drug therapy with radiotherapy are needed to better understand and directly target mechanisms contributing to therapeutic resistance of cancer cells. In addition, the specific signaling pathways that are regulated by HIF-1α can be studied to identify prognostic factors under hypoxia which will help to identify radiotherapeutic outcomes. Homeostasis and not a mere absence/presence of molecular species such as NO and ROS have emerged to be important in shaping cellular response to therapy. Thus, in conjunction with identifying and targeting specifically overexpressed downstream targets of HIF-1α targeting antioxidant enzyme production, decreasing oxygen demand, reducing ATP availability by inhibiting glycolysis have emerged to some of the more novel strategies for combating hypoxia-mediated therapeutic resistance. In summary, understanding key mechanisms of hypoxia-based cancer cell growth and progression along with novel therapeutic strategies is required to ameliorate the pro-tumorigenic effects of hypoxia.

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ORCID IDS

Ankith Sharma https://orcid.org/0000-0002-0724-5444
Camille L Duran https://orcid.org/0000-0002-7614-3265
Sanjukta Chakraborty https://orcid.org/0000-0002-4869-3198

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