Hypoxia's Function in Cancer

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Abstract

Hypoxia is defined as the inadequate supply of oxygen to the tissue that can occur due to a multitude of causes and is called by various names such as hypoxemic, anemic, ischemic, diffusional, and cytotoxic hypoxia. Cancer-induced hypoxia is an interplay of ischemic, diffusional, and anemic hypoxia, and plays an important role as a prognosticator of the disease and also as a target for treatment modalities. The major mediator of hypoxia in tumor cells is hypoxia-inducible factor (HIF), which is a heterodimeric protein that is upregulated in hypoxic conditions. The consequences of HIF action are the activation and upregulation of several enzymes, transporters, and factors that modulate the neoplastic cell's metabolic functions that result in functional responses to the hypoxic stressor, which resists apoptosis/necrosis, in addition to modifying and refashioning the local microenvironment to suit the neoplastic cell's survival. Metastasis—one of the most feared outcomes of neoplasm—has almost all of its steps upregulated or controlled by hypoxia and HIF. Hypoxia is also responsible for drug resistance to various chemotherapeutic agents by different mechanisms. This makes it harder to treat neoplasms that are susceptible to these drugs. Therefore, treatment modalities acting by blocking HIF, in addition to the standard chemotherapeutics, target the neoplasm from all aspects, making it more comprehensive and more effective.

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Hypoxia · HIF · Cancer · Tumor · Chemotherapy · Tumor metastasis · Chemoresistance

2.1 Introduction

Hypoxia refers to the inadequate oxygenation of tissues. Put simply, the amount of oxygen reaching the tissues is less than what the ideal range of oxygen supply should be. This leads to derangement in the tissue's local microenvironment, leading to adaptive functional alterations and, if persistent, apoptosis (McKeown 2014). At a biochemical level, hypoxia is evident in the form of oxygen-deficient electron transport. There are 4 major mechanisms for the development of hypoxia:

1. Hypoxemic hypoxia: decrease in arterial oxygen partial pressure secondary to causes like poor oxygenation because of pulmonary diseases, poor oxygen tension in the environment (high altitudes), etc.
2. Anemic hypoxia: decrease in the ability of blood to carry oxygen due to decreased oxygen-carrying capacity.
3. Ischemic hypoxia: decrease in the perfusion of the tissues.
4. Diffusional hypoxia: decrease in the diffusion of oxygen to the cells in the tissue due to alterations in the diffusion membrane, turbulence of flow within the microvasculature, etc.
5. Cytotoxic hypoxia: alteration in the cellular metabolism that leads to difficulty in the utilization of oxygen within the cell, and causes include cellular intoxication due to cyanide poisoning.

Cancer-induced hypoxia is a combination of acute (ischemic), chronic (diffusional), and systemic forms (anemic) of hypoxia (Sebestyén et al. 2021) (Fig. 2.1).

The "gold standard" for the measurement of O_2 partial pressures in the tumor microenvironment is by using an intratumor polarographic microsensor technique. The critical O_2 partial pressure in tumors, below which the detrimental changes

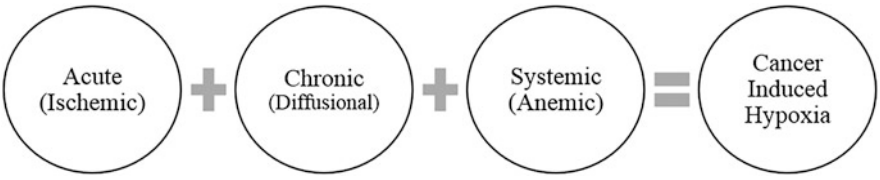

Fig. 2.1 The determinants of cancer-induced hypoxia: cancer-induced hypoxia is not a single entity; rather, it arises from a complex interaction of different types of hypoxias. Acute/ischemic hypoxia is due to inadequate vascular perfusion to the tumor. Chronic hypoxia occurs due to limitations in diffusion and changes in the diffusion permeability. Systemic hypoxia occurs as a response to the anemic state the patients with a tumor present with

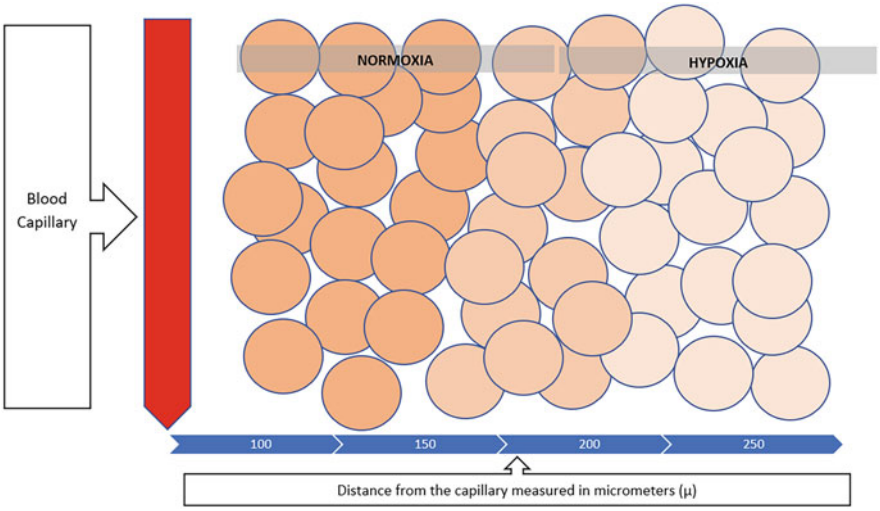

Fig. 2.2 Depiction of Folkman's theory. For a 250 μ radius of capillaries, 1 mm^3 of tissue can survive without the growth of new vessels. In a neoplastic proliferation of cells, the growth exceeds well beyond 3 mm^3 and can thus result in a state of *acute hypoxia,* which, due to the failure of resolution, can lead to necrosis, but is prevented in a tumor by mechanisms like faulty angiogenesis triggered by hypoxia

associated with hypoxic changes and derangement have been observed, was found to be 8–10 mmHg.

The cells in the tissue meet their oxygen requirements by diffusion. In neoplastic tumor, there is uncontrolled, unregulated cell growth and proliferation. This results in the development of a situation wherein lots of neoplastic cells move from the source of oxygen. According to Folkman's theory at a distance of more than 200–250 μ radius of the capillaries (Sebestyén et al. 2021), the cells are affected by hypoxia (Höckel and Vaupel 2001) (Fig. 2.2).

Exposure to hypoxic environments results in functional abnormalities, which leads to the activation of the adaptive functions of the cell, and if the stressor of hypoxia is chronic, may lead to apoptosis/necrosis. At the same time, this stressor leads to the selection of more resistant and tolerant tumor cells and their preferential growth (Höckel and Vaupel 2001; Jing et al. 2019; Emami Nejad et al. 2021). Hypoxia increases glycolysis within the neoplastic cell and angiogenesis by promoting the activation and release of growth factors (like VEGF) and other survival responses. It also promotes invasion of the neoplastic cells into the surrounding tissue, promoting metastasis by the activation of applicable gene expressions through hypoxia-inducible factors (HIFs) (Lu and Kang 2010).

One of the causes of cancer hypoxia is inadequate and incongruous intratumoral vascularization along with the compression of the existing ones because of unregulated proliferation. This refers to the haphazard angiogenesis in response to the growth factors released by the growing neoplastic cells that fail to adequately perfuse

the tissue. This, along with systemic hypoxia of the patient (often because of anemia), leads to a unique form of genetic reprogramming by hypoxia-induced transcription factors (HIFs). Another cause is the constitutive activation of oncogene-driven signaling pathways that activate hypoxia signaling independent of oxygen supply (pseudohypoxia). Thus, cancer-induced hypoxia and the adaptation mechanisms are two of the major causes of therapy resistance (Sebestyén et al. 2021).

Cancer hypoxia is considered one of the important hallmarks of any neoplastic process. The process of a rapidly proliferating tumor requires an equally rapidly developing vasculature to support the growth and metabolic processes mentioned above. This means that the tumor cells far (around 200–250 μ) from the vasculature will develop features of hypoxia and thus activate the heterodimeric protein HIF.

Hypoxia in the local tumor environment is associated with poorer outcomes in established cancers and is also associated with higher rates of recurrences. Hence, several targets have also been developed to combat the hypoxic response and its implications as discussed later.

Hypoxia can thus be considered an independent prognostic marker for cancer mortality. Hypoxia and hypoxia-inducible factors (HIFs) regulate various characteristic features of neoplasia:

1. Genetic instability.
2. Dedifferentiation.
3. Metabolic alterations.
4. Neovascularization.
5. Invasion–metastasis cascade.
6. Drug resistance.

2.2 Importance of HIF in Cancer Therapy

Hypoxic cells are considered resistant to many routinely used chemotherapeutics, and this can be partially associated with the selection of cells that have lost sensitivity to p53-mediated apoptosis (which is one of the ways by which alkylating agents act) in addition to many other mechanisms of resistance.

Hypoxia is, thus, an important entity (Fig. 2.3) that is being targeted to develop newer modalities as adjuvants to chemotherapeutics and also as the mainstay treatment. Combining drugs that act under hypoxic conditions [hypoxia-activated prodrugs (HAPs)] or act by inhibiting the working of HIF directly or indirectly by acting on its downstream pathways with standard radiotherapeutic modalities and chemotherapeutics has been shown to eliminate the most malignant cells, with maximum mutations, only causing a limited increase in systemic toxicity.

Therefore, targeting hypoxia and its associated features either directly or indirectly by inhibiting the downstream signaling pathways can be an efficient way to overcome chemotolerance/chemoresistance and supplement the activity of the

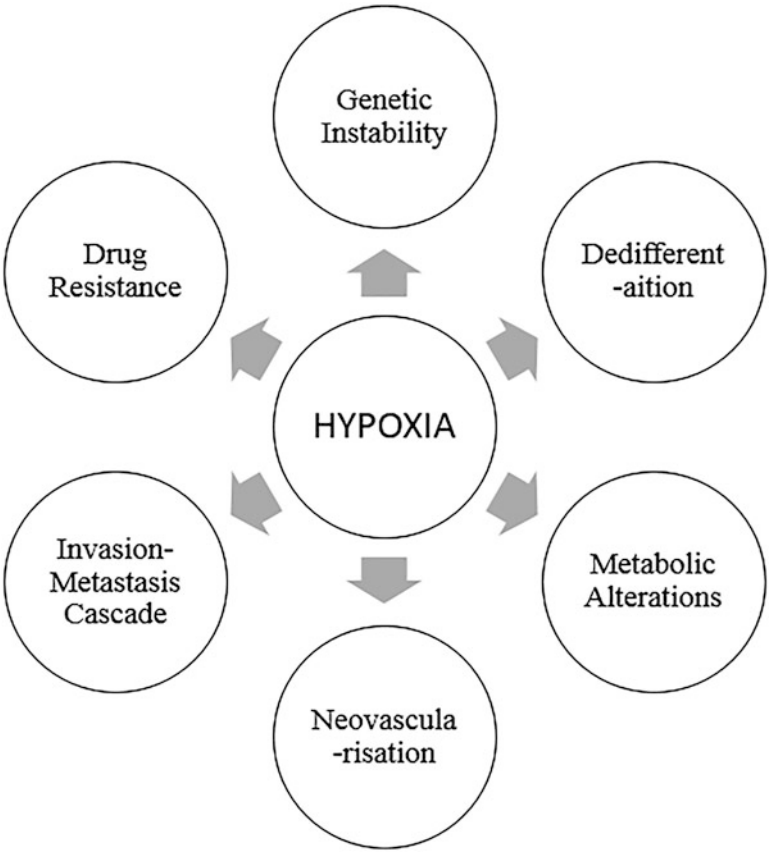

Fig. 2.3 Hypoxia and its consequences. Hypoxia, acting by the induction of hypoxia-inducible factors, regulates and affects the tumor microenvironment and has the abovementioned effects. It promotes the selection of more resistant cells with more neoplastic potential and its survival and metastasis. Hypoxia promotes the sustenance of the tumor microenvironment by promoting vascular angiogenesis and metabolic alterations. The neoplastic cell is more susceptible to the invasion–metastasis cascade because of the HIF-induced metabolic alterations and activation of factors (TWIST) that promote metastasis. Additionally, the tumor may become resistant to the drugs being used for the treatment, a phenomenon called chemoresistance

chemotherapeutics. Researchers are trying to obtain the ideal HIF1 inhibitor that can be successfully used for clinical application.

2.3 Hypoxia-Inducible Factor (HIF)

Hypoxia-inducible factor (HIF) is responsible for the development of all changes that help the neoplastic cell survive in the hypoxic microenvironment of a solid tumor. However, it also plays a key role in the maintenance of oxygen homeostasis

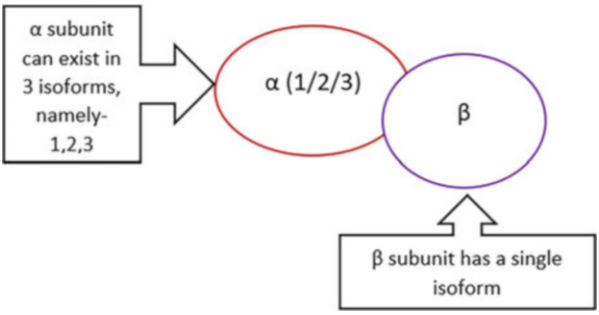

Fig. 2.4 Hypoxia-inducible factor-a heterodimeric protein composed of an alpha (α) and a beta (β) subunit. The alpha subunit can exist in three different isoforms giving specific actions to the protein. The alpha subunit is used for the nomenclature of HIF into HIF-1, HIF-2, and HIF-3. The clinically relevant isoforms are HIF-1 and HIF-2. HIF-3 has a more regulatory effect and promotes gene expression in addition to inhibiting HIF-1 and HIF-2

under physiological conditions. This protein is found in almost all human tissues. It is imperative to understand that it is a physiological response that is exploited and misused by the neoplastic cells to promote their metabolic activities and survival that ultimately makes it responsible for malignancies.

As a response to the decrease in O_2 tension due to the aforementioned mechanisms, the hypoxia-inducible factor (HIF) is activated to mediate the primary adaption at a transcriptional level in neoplastic cells (Huang et al. 2014). HIF is a heterodimeric protein with an alpha (α) subunit and a beta (β) subunit. The alpha subunits have three isoforms: HIF-1α, HIF-2α, and HIF-3α (Fig. 2.4).

HIF-1α and HIF-2α, and their overexpression, are linked with metastasis and unfavorable clinical outcomes (Lu and Kang 2010). The increase in the expression of HIF can be seen in various solid tumors (Simiantonaki et al. 2008) such as carcinoma of the

1. Oral cavity.
2. Breast.
3. Stomach.
4. Prostate.
5. Endometrium.
6. Cervix.

The α subunit is oxygen-labile, that is, hypoxia stabilizes and promotes the activity of the alpha subunit and normoxic states destabilize the subunit's activity, whereas the β subunit has a constitutive action (Fig. 2.5).

HIF-1 (HIF-1α/β) and HIF-2 (HIF-2α/β) have some common targets and some specific targets of action. The common factors include the activation of the genes coding for vascular endothelium-derived growth factor (*VEGF)* and glucose transporter- 1 (*GLUT1*), which promote angiogenesis and glucose uptake, respectively (Figs. 2.6 and 2.7). HIF-1 has a specific action for the activation and upregulation of

Fig. 2.5 The depiction of the oxygen lability of the alpha subunit of the HIF heterodimeric protein. The α subunit is stabilized by inhibition of post-translational hydroxylation of the α subunit and is promoted by the absence of oxygen, whereas the β subunit has a more constitutive action, independent of the oxygen states in the microenvironment

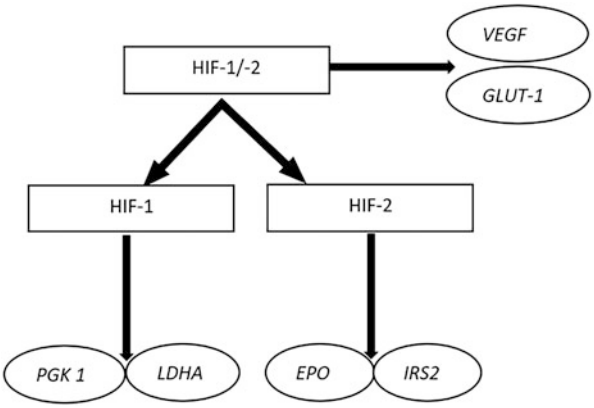

Fig. 2.6 Flowchart describing the varied actions of the HIF heterodimeric protein. HIF-1 and HIF-2 differ in their α subunit isoform, which imparts specific properties and results in differential activation of genes. Both HIF-1 and HIF-2 activate the genes encoding vascular endothelium-derived growth factor (*VEGF*) and glucose transporter-1 (*GLUT1*). HIF-1 selectively activates the genes encoding the glycolytic pathway enzymes: phosphoglycerate kinase-1 (*PGK1*) and lactate dehydrogenase A (*LDHA*), whereas HIF-2 selectively activates the genes encoding erythropoietin (*EPO*) and insulin receptor substrate-2 (*IRS2*)

glycolytic pathway enzymes (*PGK1, LDHA*), whereas HIF-2 acts on the genes coding for *EPO* and *IRS2*. HIF-1 is also implicated in the upregulation of enzymes like carbonic anhydrase-9 and -12, hexose kinase 2 (HK2), and transport proteins like monocarboxylate transporter 1 (MCT1).

2.4 Implications of HIF Activation in Tumors

It is established that solid tumor progression and recurrence are closely related to HIF, which is activated under hypoxic conditions. As discussed above, hypoxia stabilizes the alpha subunit by inhibiting the post-translational hydroxylation of the alpha subunit, which leads to heterodimerization and binding to hypoxia response

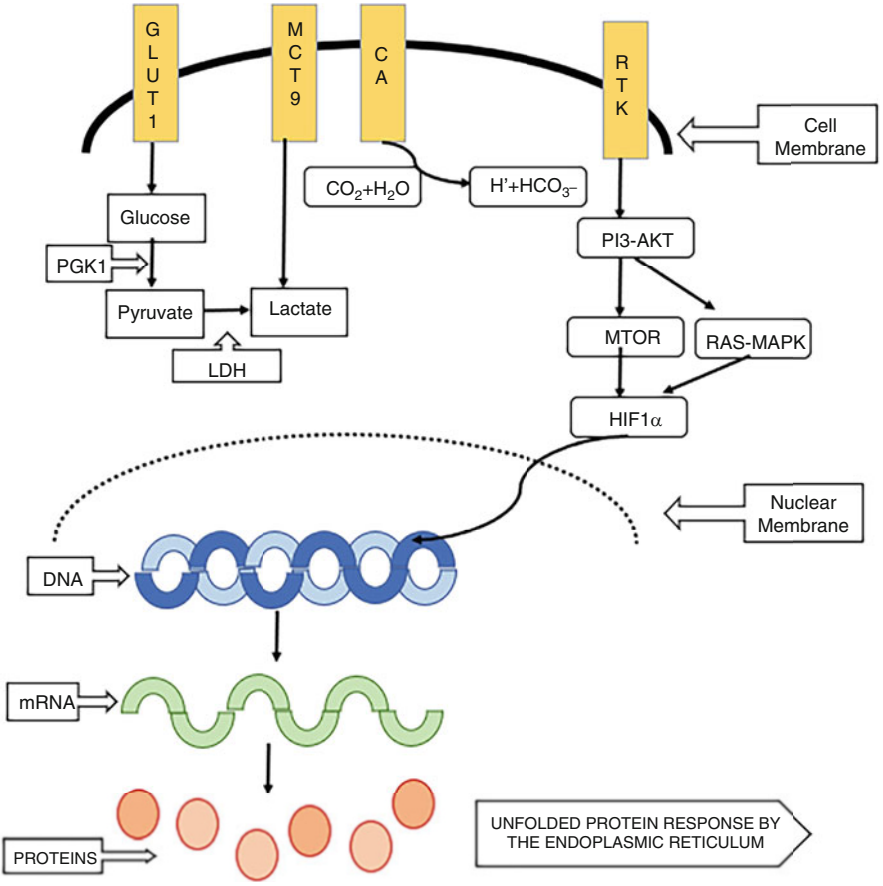

Fig. 2.7 A schematic representation of the potential targets of chemotherapeutics. GLUT1 is upregulated by HIF-1, which causes the entry of lots of glucose into the cells, further promoting the phenomenon of "aerobic glycolysis" or the Warburg phenomenon. This results in rapid conversion to pyruvate by PGK1, which produces ATP production that combats hypoxic free radical and ROS insults. MCT also facilitates the transport of lactate into the cells. Carbonic anhydrase also favors the promotion of a local acidic environment. RTK via the PI3-AKT pathway activates and promotes further HIF1 upregulation by the RAS-MAPK pathway and mTOR pathway. The other level at which it can be regularized is at the genetic level by preventing the unwinding of A, the transcription of the DNA segments to mRNA, or the translation of mRNA into proteins. GLUT1, glucose transporter; MCT, monocarboxylate transporter; PGK1, phosphoglycerate kinase-1; CA, carbonic anhydrase; RTK, receptor tyrosine kinase

elements (HRE) in target genes. This results in the activation of genes that oppose the hypoxic stress and counter the hypoxic insults with metabolic and functional changes at a genetic and biochemical level that is evident in the tumor microenvironment as the effects of HIF.

The implications of HIF activation in tumors can thus be classified as (Sebestyén et al. 2021)

1. Tumor angiogenesis.
2. Metabolic derangement.
3. Altered tumor immune response.

2.4.1 Tumor Angiogenesis

As discussed above, Folkman's theory states that cells beyond the radius of 200–250 μ develop hypoxia, and any tissue that grows beyond 2–3 mm^3 requires new blood vessels. It has been shown that HIF-1 is a major regulatory factor of angiogenesis (Kelly et al. 2003). The upregulation of angiogenesis is one of the characteristic features of hypoxia. This is due to the transcriptional activation of genes encoding platelet-derived growth factor-β (PDGF-β), vascular endothelial-derived growth factor (VEGF), and angiopoietin-2 (ANG-2), and others like fibroblast growth factor (FGF), tumor necrosis factor-α (TNFα), and transforming growth factor-β (TGFβ). They can be activated by both HIF-1 and -2. This leads to endothelial proliferation and, thus, angiogenesis.

This is different from *vasculogenesis*, a process that occurs in the embryonic stage of life, wherein angiogenic precursors from the bone marrow are organized into new, developing tissues. In contrast, angiogenesis refers to the newly formed capillaries that develop by sprouting from preexisting peritumoral capillary networks.

This concept becomes clinically relevant because these angiogenic factors and their receptors are potential targets for chemotherapeutic agents. One instance wherein it has been put into action includes bevacizumab, a monoclonal antibody targeted against VEGF, one of the products of activation of the HIF downstream signaling pathway, and other small-molecular compounds against VEGF-receptors have shown clinical benefits in cases of advanced care.

2.4.2 Metabolic Derangement

One of the hallmarks of neoplasia is the Warburg effect. The Warburg effect, or aerobic glycolysis, is the preferential utilization of the glycolytic pathway rather than oxidative phosphorylation of the intermediate produced even under normoxic conditions. This results in the production of two molecules of ATP for every molecule of glucose utilized in comparison to the 32 molecules of ATP produced for every molecule of glucose utilized. This can be attributed to the overexpression of HIF-1, which is seen in almost 50% of the tumor cells under normoxia. HIF-1 activation leads to the transcriptional activation of genes upregulating GLUT-1-3, pyruvate-dehydrogenase-kinase 1 (PDK1), and pyruvate kinase isoform 2 (PKM2). In addition, this further suppresses the TCA cycle. Activation of the gene encoding PDK1 inactivates the TCA cycle enzyme, pyruvate dehydrogenase (PDH), which catalyzes the conversion of pyruvate to acetyl-CoA.

Rapid production of ATP by the Warburg effect decreases hypoxic reactive oxygen species (ROS) generation and thus protects the neoplastic cells from hypoxia-induced apoptosis. The hypoxia-induced metabolic change redirects glucose metabolites from the mitochondrial oxidative phosphorylation to the cytoplasmic glycolytic pathway to maintain steady ATP production and prevent toxic ROS production (Kim et al. 2006).

The clinical implications are as follows.

Rapid ATP production by "aerobic glycolysis" can increase the resistance to chemotherapeutic drugs like doxorubicin and ara-c (Kim et al. 2006). New drugs like mTOR inhibitors can be developed to downregulate HIF-1-induced metabolic changes. mTOR kinase comes from the family of regulatory proteins that control translational and post-transcriptional modifications of proteins. mTOR hyperactivity is associated with HIF-1 stabilization and, thus, disruption of this pathway can promote the prevention of metabolic derangements and, thus, hypoxia-induced apoptosis (Sebestyén et al. 2021).

2.4.3 Tumor Immune Response

Hypoxia is implicated in tumor resistance. Evasion of the innate immune response elicited by the body against the developing tumors is one of the hallmarks of neoplasia. This evasion is assisted by the hypoxic conditions generated in the tumor's microenvironment. The innate immune response is primarily mediated by two cell types:

1. Natural killer cells (NK- cells).
2. Macrophages.

Macrophage activity is hypoxia-sensitive. There is a shift in the usual antitumoral polarization (M1) under normoxic conditions into the immunosuppressive phenotype (M2) under hypoxic conditions (Díaz-Bulnes et al. 2020). NK cells, on the other hand, retain their tumoricidal activity in hypoxic conditions too (Taylor and Colgan 2017). Moreover, HIF-1-induced hypoxia promotes the immunosuppression activity of suppressor cells derived from the myeloid cell lineage (Vetsika et al. 2019).

The release of VEGF because of HIF-1 further promotes CD4 + T cell differentiation into T-regulatory cells that are immunosuppressive. Cytotoxic CD8 + T cells also undergo immunosuppressive modulation mediated by HIF-1.

2.5 Tumor Metastasis and Hypoxia

Each step of the metastasis process, from the initial epithelial–mesenchymal transition to the final organ-specific colonization, can be regulated by hypoxia, indicating a master regulator role of hypoxia and HIFs in metastasis. Hypoxic conditions promote the invasive potential of tumor cells. HIF-1/-2 activation is associated

with loss of E-cadherin, a component of cellular junctions that protects the cells from undergoing invasion and metastasis. It was observed that antiangiogenic therapy, which was thought to reduce metastasis and invasiveness by blocking neovascularization, rather promoted metastasis and invasiveness in preclinical trials because lack of neovascularization created and further exacerbated hypoxic conditions in the tumor microenvironment.

TWIST1, a regulator of epithelial–mesenchymal transition, is induced in hypoxia, which leads to abnormal EMT. Promotion of the invasion–metastasis cascade occurs partly by the activation of HIF-upregulated proteins responsible for matrix remodeling, like lysyl oxidase (LOX, an extracellular enzyme that covalently modifies collagens to increase focal adhesion kinase activity, cell migration, and metastasis), and metalloproteases; they disrupt cell–cell and cell–matrix (ECM) interactions. Other proteins implicated are cathepsin D, the urokinase-type plasminogen activator receptor.

HIF also activates other genes known to be involved in metastasis and invasion (Table 2.1) such as the c-met proto-oncogene, the chemokine receptor CXCR4, and the autocrine motility factor (AMF).

As discussed previously, cells that survive acidosis and a hypoxic environment not only develop a growth advantage but also become more resistant and invasive by activation of adaptive mechanisms and collection of more genetic mutations with every passing abnormal cell division (Brahimi-Horn et al. 2007; Chan and Giaccia 2007). Multiple approaches to target hypoxia and HIFs may become effective treatment to prevent or reduce metastasis.

2.6 Tumor Hypoxia and Chemoresistance

Chemotherapy is one of the mainstays for the treatment of cancers. In a significant proportion of cases, tumors are either intrinsically resistant or develop resistance during chemotherapy. Despite hypoxic conditions, the apoptotic processes are inhibited by metabolic compensatory mechanisms, resulting in a reduction in the sensitivity to chemotherapeutic drugs that block cell division. Additionally, in neoplastic cells, hypoxic conditions induce the upregulation of drug transporter proteins, further enhancing the cell's chemoresistance. Furthermore, perfusion hypoxia develops due to dysregulated angiogenesis that impairs the distribution and delivery of chemotherapeutics. Some chemotherapeutics require optimal O_2 concentrations for its cytotoxic effects (Sebestyén et al. 2021). Resistance to chemotherapy can thus be predicted by estimating the expression of HIF1α and its upregulated genes in some types of squamous cancers (Muz et al. 2015).

2.6.1 Hypoxia and Drug Resistance

Initially, in hypoxia, HIF1α induces the upregulation of the MDR1/ABCB1 efflux transporter, which leads to the development of resistance to chemotherapeutic drugs

Table 2.1 Details of the steps of the invasion–metastasis cascade and hypoxia at different levels

Metastasis–invasion cascade		
Stage	Mediators	Action of hypoxia
Epithelial–mesenchymal transition (EMT)	E-cadherin is a functional requirement for cell connection and loss of E cadherin is a hallmark of EMT	Transcription of repressors like SNAIL, TWIST1, TCF3, ZEB1, and ZEB2.
Invasion, extracellular matrix modulation, and cell motility	Basement membrane dysregulation	HIF-1α-dependent upregulation of CTSD[1], uPAR[2], and MMP2[3] via a proteolytic cascade. Hypoxia also upregulates the expression of LOX[4]. Activation of MET by HIF-1- increases cell motility. Induction of AMF[5], a tumor-secreted cytokine by HIF-1 and VEGF under hypoxia to enhance proliferation, migration, and angiogenesis through autocrine or paracrine mechanisms.
Intravasation, circulation, and extravasation	Aggressive, neoplastic cells separate from the tumor and enter the circulation, and travel to the target metastatic site	In addition to promoting angiogenesis and lymphangiogenesis, VEGF induced by hypoxia is also associated with increased microvascular permeability and interstitial fluid pressure (IFP), both of which contribute to an increased chance of intravasation.
Homing	Chemokine receptor CXCR4 plays a key role in metastatic homing of tumor cells to organs expressing a high level of its ligand SDF1	Hypoxia may increase metastatic homing by inducing CXCR4 expression
Proliferation at the metastatic site	Sustenance and growth of secondaries	The secondary sites may develop hypoxic states due to sudden compromise in the vasculature due to metastatic proliferation, but neoplastic cells with already upregulated hypoxia genes due to hypoxic conditions in the primary site have a better chance of survival and establishment of a secondary metastatic site.

like doxorubicin that are its substrates (Sebestyén et al. 2021). Activation of nuclear factor, erythroid 2 like (NrF2) by hypoxia, further activates HIF1α, which upregulates multidrug resistance genes such as *MDR1/ABCB1, MRP1/ABCC1*, and *BCRP/ABCG2,* resulting in resistance to a variety of other chemotherapeutics (Belisario et al. 2020). Mechanisms of resistance and sensitivity to chemotherapeutic agents under hypoxic conditions are shown in Tables 2.2 and 2.3.

Table 2.2 The mechanism of resistance to various treatment modalities

Mode of therapy		Hypoxia	
Mechanism of action/property of drug	Examples	Effect of hypoxia	Mode of resistance to agent
Ionizing radiation		No free radical generation due to lack of O_2 – No DNA oxidation	Failure to induce breaks in the DNA
Antibiotics that induce DNA breaks	Bleomycin		
Cycle selective chemotherapeutic drugs	5-Fluorouracil	Cell cycle arrest in G1/G2 phase	Repair of the cell before progression to the S or M phase
Drugs extensively bound to tumor cells	Taxanes	Distance from the vasculature increases (indirect effect)	Compromised drug exposure
Basic drugs	Doxorubicin	Acidosis in the extracellular environment (indirect effect)	Decreased uptake
Multiple		Resistance to apoptosis	Genetic selection of TP53 mutants
Multiple	Etoposide		Downregulation of BID, BAX (pro-apoptotic proteins)
Multiple	DHFR[1] amplification, methotrexate	Genomic instability	Mutagenesis
DNA methylating agents		Suppression of DNA repair	Downregulation of MMR
ABC transporter substrates	MDR-1, doxorubicin	HIF-1 stabilization	Expression of ABC[2] transporters
Agents that induce DSBs[3]	Etoposide		Downregulation of NHEJ[4]

Table 2.3 Sensitivity to chemotherapeutic agents under hypoxic conditions

Mode of therapy		Hypoxia	
Mechanism of action/ property of drug	Examples	Effect of hypoxia	Mode of sensitivity to agent
PARP inhibitors	Veliparib	Cell cycle arrest in the S phase	The collapse of stalled replication forks
Acidic drugs	Chlorambucil	Acidosis in the extracellular environment (indirect effect)	Increased uptake
Bulky DNA monoalkylating and crosslinking agents	Cisplatin	Suppression of DNA repair	Downregulation of NER[2], HR[3]

2.7 Hypoxia and New Treatment Modalities

From the discussion so far, it is established that hypoxia and the resultant local acidosis are very characteristic of solid neoplastic tumor pathology. In summary, hypoxia plays an important role in the promotion of the invasion–metastasis cascade, the development of chemoresistance, and alteration in the innate immune response the body lodges against the tumor. Treatment modalities that work by using the hypoxic condition for the destruction of the tumor can prove to be a useful paradigm. Using the following strategies with a combination of immunotherapy can be a better therapeutic approach.

The most important strategies/targets to use the hypoxic conditions and HIF signaling for the treatment of the tumor include

1. Hypoxia-activated prodrugs (HAPs).
2. HIF-1α expression.
3. HIF-1 transcription.
4. HIF-1 target gene products.
5. Tyrosine kinase receptors.
6. RAS-MAPK signaling.
7. mTOR,
8. Unfolded protein response (UPR).

2.8 Hypoxia-Activated Prodrugs

Five different chemical groups (nitro groups, quinones, aromatic N-oxides, aliphatic N-oxides, and transition metals) have the potential to be metabolized by enzymatic reduction under hypoxic conditions, and thus provide the basis for the development of bioreductive prodrugs or hypoxia-activated prodrugs for exploiting tumor hypoxia. These are drugs that gain activation under hypoxic conditions, thereby limiting the action of the drugs beyond the site at which the action is required, ensuring high selectivity.

Tirapazamine is 50–200 times more toxic to hypoxic than normoxic cells, and is selectively activated by reductases in hypoxic cells inducing DNA damage due to breaks in base pairs, leading to cell death (PubChem [Internet] 2004); however, clinical trials are not very promising (DiSilvestro et al. 2014). The most recent use has been in the form of a phase 1 trial as an intra-arterial embolization with hypoxia-activated tirapazamine for unresectable hepatocellular cancer (b).

The prodrug apaziquone (E09), a mitomycin C derivative, showed positive preclinical trials but did not prove effective in clinical trials. Its use in vesicular cancer as a phase 2 clinical trial showed promising effects. (c) Phase II clinical trials for another prodrug TH302 in combination with sunitin have shown favorable results against metastatic neuroendocrine pancreatic cancer. (d) Other prodrugs have been included in Table 2.4 discussing the target, clinical trial, and the type of cancer it was studied for.

Table 2.4 A brief description of the various drugs acting at different steps, their mechanism of action, and their current clinical status

Drug details			Clinical trial (latest)		
Strategy/target	Class	Name	Phase	Status	Type of carcinoma under study
HAP	Aromatic N-oxide	Tirapazamine	2	Active, not recruiting	Hepatocellular carcinoma
	Quinone	Apaziquone	3	Terminated	Bladder cancer
	Nitro	TH302	2	Terminated	Metastatic neuroendocrine pancreatic cancer
	Nitro	PR104	2	Completed	Advanced hepatocellular cancer
	Aliphatic N-oxide	AQ4N	2	Unknown	Glioblastoma multiforme
	Nitro	Caricotamide and tretazicar	2	Terminated	Advanced hepatocellular cancer
HIF-1α expression	Synthetic RNA oligonucleotide	EZN-2968	1	Completes	Liver metastases
	Topoisomerase inhibitors	Topotecan (along with melphalan)	3	Ongoing	Retinoblastoma
	HSP90 inhibitor- benzoquinone ansamycin antibiotics	Geldanamycin, 17-AAG (tanespimycin)	1	Completed	Unspecified adult solid tumor, protocol specific
HIF-1 transcription	Dithiodiketopiperazines	Chetomin	–	–	–
	DNA intercalators	Echinomycin	–	–	–
HIF-1 target gene products	GLUT1	Phloretin	–	–	–
		Fasentin	–	–	–
	MCT1	α-Cyano-4-hydroxycinnamate	–	–	–
	CA-9, CA-12	Aryl sulfonamides	–	–	–
Tyrosine kinase receptor	Monoclonal antibody- anti-VEGF	Bevacizumab	1/2/3	Ongoing	Several different malignancies
RAS-MAPK	BRAF- ATP kinase competitive inhibitor	Sorafenib	2	Ongoing	Pancreatic cancer
mTOR		Rapamycin, everolimus	1/2/3	Ongoing	Several different malignancies

(continued)

Table 2.4 (continued)

Drug details			Clinical trial (latest)		
Strategy/target	Class	Name	Phase	Status	Type of carcinoma under study
	mTORC1 allosteric binders of rapamycin-binding domain				
UPR	Proteasome inhibitor	Bortezomib	1/2/3	Ongoing	Several different malignancies
	HSP90 inhibitor- benzoquinone ansamycin antibiotics	Geldanamycin, 17-AAG (tanespimycin)	1	Completed	Unspecified adult solid tumor, protocol specific
	IRE1	Salicylaledehydes	–	–	–
	SERCA	Celecoxib	–	–	–

2.9 HIF-1α Expression

HIF-1α expression can be inhibited by using the anti-sense mRNA oligonucleotide developed against the mRNA coding sequence for HIF-1α (drug EZN-2968) that prevents its translation into proteins in a dose-dependent fashion (Jing et al. 2019). Drugs like topotecan and irinotecan, which are topoisomerase inhibitors, inhibit HIF1α translation and reduce the expression of the same (Bertozzi et al. 2014).

Heat shock proteins (HSPs) act as chaperones in the cell, for instance, by guarding against the transport of proteins to proteasomes. Inhibitors to these proteins like geldanamycin promote proteasomal degradation of HIF1α under hypoxic conditions. GA analogs like 17-AAG (tanespimycin) and 17-DMAG (alvespimycin) and EC154 are under evaluation in clinical trials. Ubiquitin acts as an important tagging protein that in turn acts as a marker for protein degradation. Alteration in this function is implicated in several cancers. Deubiquitinase (DUBs) can be used to combat ubiquitylation. DUBs undergo reciprocal regulation by hypoxia, and, thus, this can act as a good target for treatment modalities (Mennerich et al. 2019).

2.10 HIF-1 Transcription

The HIF transcription is dependent on several co-activators like p300/CBP. Using these as targets for inhibiting transcription of HIF can thus aid in enhancing the efficacy of other chemotherapeutics. Dithiodiketopiperazines like chetomin, a metabolite derived from fungi, inhibit the binding of HIF with its co-activator (p300) and show antitumor effects. Other drugs like DNA intercalators also inhibit the unwinding of DNA strands and thus prevent transcription of HIF-1.

2.11 HIF-1 Target Gene Products

HIF1-regulated facultative glucose transporter is a potential target for chemotherapeutic drugs. Elevated GLUT1 levels have been outlined in a variety of tumor types and have been demonstrated to be a negative prognostic indicator. Many experimental GLUT1 inhibitors, such as phloretin, act indirectly, but fasentin targets GLUT1 directly.

The lactate transporter monocarboxylate transporter 1 (MCT1) has been viewed as a potential target for eliminating hypoxic cells by glucose starvation. The metabolic derangement observed is the Warburg effect – the shift to glycolysis – and it is facilitated by an increase in the generation of pyruvate (PGK2- upregulated by HIF-1) and its conversion to lactate by lactate dehydrogenase A (LDHA-upregulated by HIF-1).

Aerobic tumor cells expressing MCT1transporter can use lactate as a preferred substrate for respiration, and inhibition of MCT1 by α-cyano-4-hydroxycinnamate increases glucose consumption in vitro. The proposed model is that the stimulation

of glucose consumption in aerobic tumor cells compromises glucose penetration into hypoxic regions, leading to the selective death of hypoxic cells in tumors.

MCT4 is upregulated in an HIF1α-dependent manner, and there is an increase in the expression of MCT4 in tumor cells. MCT4 export of lactate and H^+ prevents intracellular acidification and assists in the remodeling of the extracellular environment, but specific inhibitors of MCT4 are yet to be reported.

Carbonic anhydrases are metalloenzymes that catalyze the reversible reaction of carbon dioxide to carbonic acid. The expression of CA9 and CA12 is controlled by HIF1, and CA9 is also regulated through the UPR. Silencing both CA9 and CA12 results in marked inhibition of the growth of LS174 human colon carcinoma cell xenograft tumors. Extensive drug development efforts have identified a range of compounds with varying selectivity for CA9 and CA12; several compounds inhibited tumor growth and metastasis selectively in CA9-positive tumor models.

2.12 Drugs Targeting Hypoxic Signaling Tyrosine Kinase Receptors, RAS-MAPK Pathway, and mTOR Pathway

Targeting the actions of HIF by either direct inhibition or by indirectly targeting the downstream pathways activated/upregulated by HIF can act as suitable therapeutic modalities. VEGF, as discussed previously, has significant effects on the neoplastic cell's physiological functions. Monoclonal antibodies developed against VEGF (bevacizumab) and VEGF-receptors have been proven to be clinically beneficial, especially in advanced cases. mTOR inhibition can decrease the levels of HIF1α and HIF2α by modulating the translation of IF mRNA, which is under the control of the PI3k/AKT/mTOR pathway. Similarly, the RAS-MAPK pathway can also be inhibited and can help downregulate the HIF-1 expression.

2.13 UPR Targets

The role of unfolded protein response (UPR) in oxygen sensing and hypoxic cell survival has provided many potential molecular targets for combating hypoxic cells. Severe hypoxia leads to an increase in the levels of unfolded proteins in the endoplasmic reticulum (ER), leading to the induction of the unfolded protein response (UPR). The UPR is mediated by three signaling pathways:

1. PERK–eukaryotic translation initiation factor-2A (eIF2A)-activating transcription factor 4 (ATF4) pathway.
2. Inositol-requiring enzyme-1 (IRE1)–X-box binding protein-1 (XBP1) pathway.
3. ATF6 pathway.

These pathways activate responses to suppress protein synthesis, stimulate protein degradation in the ER, and activate apoptosis and autophagy to resolve ER stress. Two drug strategies are being pursued to kill hypoxic cells selectively through

UPR targets. One approach seeks to inhibit the UPR by targeting PERK, ATF4, and IRE1. A second approach seeks to heighten the ER stress to overload the UPR on the assumption that the UPR has reached its maximum capacity in hypoxic cells. Evidence that the ER stressors thapsigargin and bortezomib elicit hypoxia-selective cytotoxicity in vitro supports this approach.

2.14 Conclusion and Future Aspects

Hypoxia is one of the key players in neoplasm and metastatic development. Hypoxic conditions in the tumor trigger a response from the neoplastic cells, which make it more resistant. It allows the selection of those neoplastic cells with upregulated genetic expressions that in turn allow the neoplastic cell's proliferation and sustenance. These include modified metabolic pathways, release of growth factors promoting angiogenesis, and modifying the local cellular interaction to promote metastasis. The major pathway by which hypoxia acts is by hypoxia-inducible factor (HIF), which acts at a genetic level by selectively upregulating enzymes and factors. This has major implications such as major metabolic derangements, altered tumor immune response, and neovascularization in a disorderly fashion. Metabolic derangements allow the neoplastic cells to generate energy with low oxygen availability and create metabolites that allow the hypoxic cells counter the reactive oxygen species and other harmful metabolites. Alterations in the tumor immune response result in the evasion of these proliferating cells from under the scanner of the immune cells in circulation and tissue either by deactivating/blunting their responses or by preventing their activation. The invasion–metastasis cascade is also positively upregulated under hypoxic conditions by various mechanisms, which includes activation of transcription factor repressors like TWIST and upregulation of proteins implicated in metastasis. Hypoxia has a negative impact on drugs routinely used for chemotherapy by various mechanisms, and to overcome this, development of drugs that inhibit the hypoxic response is essential. Several drugs have been used to target the pathways at different levels, and their results are promising. While some drugs are already being used, the potential use of other drugs warrants our attention as a mainstay as well as an adjuvant chemotherapeutic agent.

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