

The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy

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Abstract: Hypoxia is a non-physiological level of oxygen tension, a phenomenon common in a majority of malignant tumors. Tumor-hypoxia leads to advanced but dysfunctional vascularization and acquisition of epithelial-to-mesenchymal transition phenotype resulting in cell mobility and metastasis. Hypoxia alters cancer cell metabolism and contributes to therapy resistance by inducing cell quiescence. Hypoxia stimulates a complex cell signaling network in cancer cells, including the HIF, PI3K, MAPK, and NFκB pathways, which interact with each other causing positive and negative feedback loops and enhancing or diminishing hypoxic effects. This review provides background knowledge on the role of tumor hypoxia and the role of the HIF cell signaling involved in tumor blood vessel formation, metastasis, and development of the resistance to therapy. Better understanding of the role of hypoxia in cancer progression will open new windows for the discovery of new therapeutics targeting hypoxic tumor cells and hypoxic microenvironment.

Keywords: hypoxia, cancer, metastasis, angiogenesis, treatment resistance

Introduction

Twenty-five percent of deaths in the United States are caused by cancer, and the number of incidences increases due to population growth, prolonged life expectancy, and an abundance of risk factors including smoking, a lack of activity, and obesity.¹ A common feature of most tumors is a low level of oxygen, called hypoxia, the severity of which varies between tumor types (Table 1). In intensively proliferating and expanding tumor tissue, oxygen demand is surpassed by oxygen supply, and the distance between cells and the existing vasculature increases, hampering oxygen diffusion and creating even more hypoxic milieu.^{2,3} It is generally accepted that the oxygen level in hypoxic tumor tissues is poorer than the oxygenation of the respective normal tissues and on average it is between 1%–2% O₂ and below (Table 1). However, tumor oxygen level depends on the initial oxygenation of the tissue, the size and stage of the tumor, the method of oxygen measurement, and in which part of the heterogenic tumor tissue the measurement was performed (Table 1).⁴⁻⁷ Tissue normoxia, also known as physoxia, is the oxygenation in healthy tissues, which varies widely between the organs due to diversified blood vessel network and metabolic activity. Oxygen concentration in humans ranges between approximately 9.5% O₂ in the renal cortex⁷ to 4.6% O₂ in the brain with neurons extremely sensitive to hypoxia.⁸⁻¹⁰ These oxygen values are far from the experimental in vitro conditions. The oxygen concentration commonly used in the laboratory setting is 20.9% O₂, which means that cell culture is performed in hyperoxic rather than physoxic conditions of respective organs. In order to better understand principles of oxygenation

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Table 1 Comparison of the oxygenation in organs and respective tumors

Tissue/organ	Physoxia (median % O ₂)	Reference	Cancer	Hypoxia (median % O ₂)	Reference
Brain	4.6	8,9	Brain tumor	1.7	6,122
Breast	8.5	6	Breast cancer	1.5	6,123
Cervix (nullipara)	5.5	4,6	Cervical cancer	1.2	4,6
Kidney cortex	9.5	7	Renal cancer	1.3	124
Liver	4.0–7.3	125,126	Liver cancer	0.8	125,126
Lung	5.6	127	Non-small-cell lung cancer	2.2	127
Pancreas	7.5	128	Pancreatic tumor	0.3	128,129
Rectal mucosa	3.9	130	Rectal carcinoma	1.8	130

in vitro and in vivo, basic knowledge of the physics of gases is required for newcomers in the hypoxia research field which has been neatly described in a recently published review.¹¹

Cancer cells respond differently to decreased oxygenation leading to cell death or cell survival which partially depends on the time of exposure to hypoxia. The discrepancy and lack of consistency in experimental oncology regarding the definition of acute versus chronic hypoxia often with different biological consequences was thoroughly reviewed.^{12,13} In general, it is accepted that acute hypoxia is an abrupt and brief exposure to short-term hypoxia which occurs when blood vessel occlusion lasts for at least several minutes.¹⁴ It is reversible and often leads to oxygen fluctuations called cycling hypoxia. In acute hypoxia in vitro, cells are usually exposed to continuous hypoxia between a few minutes and up to 72 hours.¹² Short-term hypoxia allows cells to survive in these adverse conditions by activating autophagy, an apoptotic and metabolic adaptation of cells. Autophagy is achieved by decreasing oxidative metabolism.^{15,16} On the contrary, others have shown that cycling hypoxia led to increased reactive oxygen species (ROS) production, what contributed to tumor cell survival and progression.^{17,18} Moreover, both short- and long-term hypoxia was shown to increase radio-resistance of cancer cells both in vitro^{17,19} and in vivo.^{17,20} In addition, acute hypoxia was associated with more aggressive tumor phenotype through induction of spontaneous metastasis.^{12,21,22}

Enduring changes in blood flow and low oxygen availability resulting in chronic hypoxia are especially pronounced in larger tumors and contribute to long-term cellular changes. In experimental settings, chronic conditions are considered when the cells are incubated in hypoxia between a few hours and as long as several weeks.¹² Longer exposure to hypoxia is associated with high frequency of DNA breaks, accumulation of DNA replication errors since hypoxia hampers DNA repair systems including homologous recombination and mismatch repair, potentially leading to genetic instability and mutagenesis.^{23–25} Of

note, acute hypoxia also leads to genomic instability due to delayed DNA damage response and rapid p53-dependent apoptosis.²⁶ It was suggested that cells lacking functional p53 are more susceptible to genomic instability and potentially tumorigenesis if they experience reoxygenation after acute exposure to hypoxia.²⁶

Nonetheless, cycling hypoxia represents the situation of oxygenation in tumor tissues. Oxygen fluctuation occurs at irregular intervals in cancer with sporadic reoxygenation periods due to dysfunctional tumor vascularity and heterogeneous blood supply.^{27,28} Undoubtedly, both chronic and acute hypoxic regions in tumors directly affect clinical responses to therapy by influencing tumor growth, ability to metastasize, and resistance to cell death.

Signaling pathways related to tumor hypoxia

Hypoxia induces a number of complex intracellular signaling pathways such as the major hypoxia-inducible factor (HIF) pathway. Other hypoxia-associated pathways include PI3K/AKT/mTOR,^{29,30} MAPK also known as ERK pathways,^{31–33} and the NFκB.³⁴ These pathways are involved in cell proliferation, survival, apoptosis, metabolism, migration, and inflammation.

PI3K/AKT/mTOR, MAPK, and NFκB signaling pathways are also stimulated in a hypoxia-independent manner by a number of factors such as cytokines, chemokines, and growth factors which bind to receptor tyrosine kinases, G protein-coupled receptors, toll-like receptors (TLR), and alarmins receptors on the cell surface, which eventually may also lead to HIF-1α activation (Figure 1). In addition, in cancer cells epigenetic changes and acquired mutations of the pathways' members and overactivation/overstimulation of receptors cause uncontrollable cancer cell growth.³⁵ Targeting non-HIF pathways provides a promising target for anti-neoplastic therapy and each pathway is a vast topic on its own. More information regarding the role of the non-HIF pathways in cancer can be found elsewhere. This review will

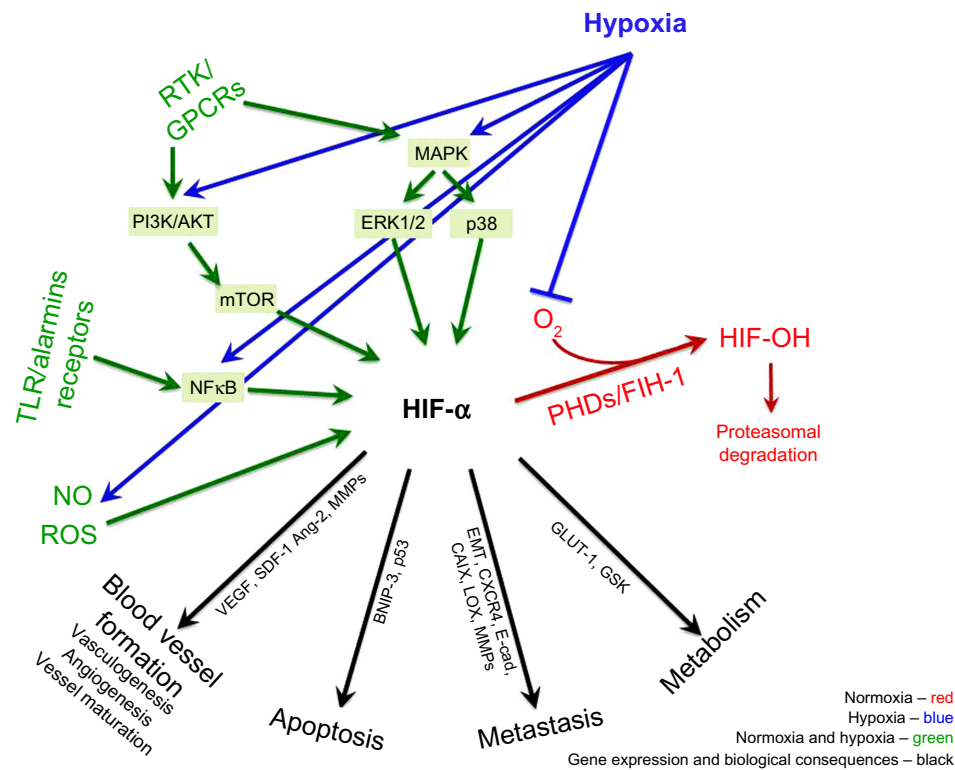


Figure 1 Regulation of HIF in normoxic and hypoxic conditions.

Notes: HIF- α , a transcription factor, can be regulated by both hypoxic and non-hypoxic factors. In normoxia, HIF- α subunits are hydroxylated by oxygen sensors, including PHD and FIH-1 enzymes, causing polyubiquitination and proteasomal degradation of hydroxylated HIF- α subunits (red arrows). PHD and FIH-1's activity is oxygen-dependent (red arrows); in hypoxia (blue arrows) these enzymes lose their activity due to decreased oxygenation, resulting in HIF- α protein stabilization, accumulation, and translocation into the nucleus resulting in gene transcription and biological consequences (black arrows). HIF is also modulated in a hypoxia-independent manner in response to nitric oxide (NO), reactive oxygen species (ROS), cytokines, lipopolysaccharides, and growth factors through receptor tyrosine kinases (RTK), G protein-coupled receptors (GPCRs), toll-like receptors (TLR) and alarmins receptors. The non-hypoxic HIF regulation is mediated by a number of different signaling pathways including NF κ B, PI3K/AKT/mTOR, and MAPK/ERK (green arrows). These pathways, as well as ROS production, are additionally regulated by hypoxia, which results in multiple levels of HIF- α stimulation, both hypoxic and normoxic. As a result, HIF accumulation and activation alters blood vessel formation, apoptosis, metastasis, and metabolism via a number of genes including VEGF, SDF-1, Ang-2, MMPs, BNIP-3, p53, epithelial-to-mesenchymal transition (EMT), E-cad, CXCR4, LOX, CAIX, GLUT-1, and GSK (black arrows).

mainly concentrate on the HIF pathway and its involvement in tumor progression.

HIF pathway

Cellular adaptation to hypoxia is primarily mediated by a family of transcriptional regulators, HIF, which was identified 2 decades ago.³⁶ The hypoxic induction and protein stabilization of HIF- α subunits (HIF-1 α , HIF-2 α , and HIF-3 α) is regulated by oxygen sensors, including PHD and FIH-1 enzymes. PHDs and FIH-1 are upstream of HIF- α and their activity is oxygen-dependent.³⁷ In oxygenated cells, HIF- α subunits are hydroxylated by PHDs and FIH-1, which facilitate the tagging of HIF- α -OH by pVHL, polyubiquitination and protein degradation of HIF- α by the proteasome.³⁸ When the oxygen level drops, the PHD enzymes lose their activity, the hydroxylation of the HIF- α subunit is inhibited and the degradation halted. The non-hydroxylated, stabilized HIF- α subunits translocate to the nucleus where they dimerize with constitutively expressed HIF- β subunit,

bind to DNA and initiate gene transcription of the adaptive pathways (Figure 1).³⁷

Apart from hypoxia, the HIF pathway is modulated in a hypoxia-independent manner. HIF- α stabilization and activity is regulated by epigenetic changes and mutations, which lead to a loss of tumor-suppressor functions (ING4, p53, PTEN, VHL) and a gain of oncogene functions (Ras, Raf, Src, mTOR, and Myc).^{39–41} Hypoxia-independent HIF- α regulation occurs in response to cytokines, lipopolysaccharides, and growth factors, mediated by PI3K/AKT/mTOR,^{29,30} MAPK,⁴¹ and NF κ B pathways.^{42,43} In addition, mitochondrial ROS^{44,45} and nitric oxide (NO)⁴⁶ were shown to up- or downregulate HIF-1 α accumulation (Figure 1).

Due to the diversified character of tumors including hypoxic and inflammatory phenotype, signaling pathways are activated simultaneously and they frequently share a number of target genes. HIF-1 α and NF κ B together regulate over 1,000 genes, and thus control malignant and metastatic phenotype of cancer cells since they both: i) enhance cell

survival via a number of growth factors and inhibition of proapoptotic pathways, ii) contribute to tumor neovascularization via VEGF, VEGF receptors, COX-2, iNOS, iii) regulate cell detachment via downregulation of adhesion molecules such as cadherins, and iv) induce cell migration and invasion through matrix degrading enzymes.⁴³ The HIF and NFkB pathways are controlled by a negative feedback loop mechanism and also intersect via alarmins. Tissue damage and necrosis, which can be also induced by hypoxia, increases the presence of alarmins, the endogenous markers for damage, which are recognized by receptor for advanced glycation endproducts (RAGE) and some of TLRs. In addition, the expression of RAGE receptor is also upregulated by HIF-1 α . In turn, alarmin receptors strongly activate NFkB and proinflammatory gene expression. Moreover, the basal HIF-1 α mRNA expression is regulated by NFkB in non-hypoxic conditions since HIF-1 α promoter was shown to be responsive to certain NFkB subunits.³⁹

The HIF pathway is required during physiological processes and is implicated in cancer biology by regulating hundreds of genes.^{47–49} This master regulator facilitates tumor growth by promoting angiogenesis via VEGF and SDF-1,^{50,51} metabolism via regulation of GLUT-1, GLUT-3, and glycolytic enzymes,^{52–54} and regulating cell apoptosis and cell survival via BNIP-3,⁵⁵ p53,^{56,57} TGF- β , and bFGF.³ Moreover, HIF- α contributes to cancer metastasis by altering cancer cell adhesion and motility through regulation of epithelial-to-mesenchymal transition (EMT) and E-cad, ZEB1, -2 and TCF3 expression,⁵⁸ as well as migration and invasion abilities through CXCR4,⁵⁹ CAIX,⁶⁰ LOX,⁶¹ MMP-2, and MMP-9.^{47,62,63}

The role of hypoxia in progression and metastasis in cancer

Pathological hypoxia is a common microenvironment factor in tumors that facilitates cell survival and propagation of the tumor. Key cellular responses to hypoxia triggered by overexpression of HIF-1 α and HIF-2 α subunits and their downstream targets increase blood vessel formation, aggressiveness, metastasis, and resistance to treatment.

Blood vessel formation

Blood vessels create a network of tubes and capillaries which nourish the entire body with oxygen and nutrients. Thus, the way they are formed and function is crucial in embryogenesis and physiology. Blood vessels consists of endothelial cells (ECs) which create a tight barrier between the blood and tissue, and interact with ECM. In embryogenesis, blood vessels are formed de novo by vasculogenesis involving

bone marrow-derived endothelial progenitor cells (EPCs)⁶⁴ followed by angiogenesis, a process where new blood vessels are created from pre-existing vasculature.⁶⁵ Lastly, the vessels undergo maturation which includes physical interaction with smooth muscle cells and pericytes. Abnormal angiogenesis is a feature of pathological conditions including tumor progression, where hyperproliferating cancer cells surpass their blood supply and become hypoxic. Hypoxia induces the imbalance between pro- and anti-angiogenic factors' production, which leads to enhanced, rapid and chaotic blood vessel formation. Hypoxia and potent transcription factors HIF-1 α and HIF-2 α have been shown to be involved in all steps of blood vessel formation.^{36,64,65} i) Hypoxia and HIF- α subunits contribute to the EPCs' recruitment from the bone marrow and induction of their differentiation into ECs by regulation of VEGF, a primary regulator of vasculogenesis. This is also mediated through stimulation of pro-angiogenic molecule production such as VEGF-R2 (Flk-1), members of the FGF family and PDGF, important in the primitive vascular network formation.^{64,66} ii) Hypoxia and HIF- α are also involved in the angiogenesis process by inducing enzymes' expression (ie, MMPs) in order to sprout and split the pre-existing vessels. In turn, neovessels allows ECs to migrate in response to chemoattractants across ECM. Additionally hypoxia induces ECs' proliferation by regulation of VEGF-R1 (Flt-1), Ang-1 and Ang-2 expression.⁶⁷ iii) Finally, hypoxia and HIF- α support vessel maturation via induction of Ang-1, PDGF, and TGF- β to recruit supporting cells such as smooth muscle cells and pericytes creating mature and stable blood vessels.⁶⁷

However, in tumors, neovessels are often abnormal, immature, and leaky. They are either insufficient or excessive depending on the tumor type.⁶⁵ Neovasculogenesis maintains blood flow to the growing tumor tissue that expands rapidly, providing nutrients and oxygen for thriving cancer cells; however, more cells means more demand causing even more hypoxia. Again, hypoxia in turn stimulates angiogenesis to ameliorate hypoxic condition, closing the vicious circle. As a consequence the tumor tissue ends up being highly hypoxic with excessive but dysfunctional vasculature.⁶⁸

Folkman was the first to propose anti-angiogenic therapy to treat cancer in 1971.⁶⁹ A successful use of monoclonal antibody against VEGF (bevacizumab) approved for treatment of metastatic colorectal cancer⁷⁰ followed by multiple solid tumors, has stimulated development of other anti-angiogenic therapies. However, long-term exposure to these agents revealed not only reducing tumor growth, but also more malignant and invasive cancer phenotype increasing metastasis.⁷¹ Long-term exposure to anti-angiogenic agents reduce

tumor; however, at the same time induce more aggressive and metastatic tumor phenotype.^{71,72}

Metastasis

Enhanced angiogenesis is associated with metastasis since permeable and heterogeneous vasculature facilitates the extravasation, circulation, and relocation of tumor cells of tumor cells to new and unaffected tissues escaping the hostile hypoxic environment.⁶⁸ Tumor oxygenation is a critical factor of cancer progression and the overexpression of HIF- α subunits in tumors and their metastases is associated with the aggressiveness of a majority of human cancers and correlates with poor overall survival.^{49,73,74}

It was demonstrated previously that hypoxic cells are more aggressive and invasive with better ability to metastasize. For instance, multiple myeloma cancer cells cultured in hypoxic conditions *in vitro* and injected into mice were able to spread to the new bone marrow faster than the cells cultured in normoxic conditions.^{63,75} Also, exposing an orthotopic

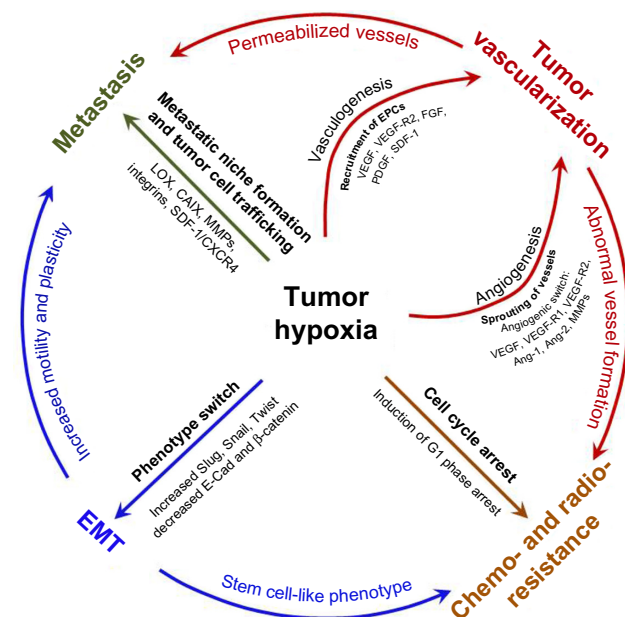


Figure 2 Hypoxia as a driving force of tumor progression and metastasis.

Notes: Hypoxia stimulates tumor i) vasculogenesis through endothelial progenitor cells' mobilization from the bone marrow to the tumor site by VEGF, VEGF-R2, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and stromal-derived growth factor-1 (SDF-1) and ii) angiogenesis by sprouting of the pre-existing vessels caused by increased production of VEGF, VEGF-R1, VEGF-R2, Ang-1, Ang-2, and MMPs. New blood vessels facilitate cancer cells leaving the primary tumor site, which is enhanced by increased expression of lysyl oxidase (LOX), carbonic anhydrase IX (CAIX), MMPs, integrins, and CXCR4. Hypoxic cancer cells also undergo epithelial-to-mesenchymal transition (EMT) acquiring plastic and mobile phenotype by increasing transcription factors such as Slug, Snail, and Twist and decreasing expression of adhesion molecules such as β -catenin and E-cadherin (E-cad). Chemo- and radio-resistance of patients is caused by EMT-related stemness of cancer cells and hypoxia-induced cell cycle arrest in G1 phase. Hindered drug diffusion due to anomalous vasculature is another mechanism of chemoresistance.

mouse model of cervical carcinoma to a dozen cycles of 10 minutes 7% O₂, which was followed by 10 minutes of air exposure daily, increased the number of lymph node metastases.⁷⁶ Similar observations were recorded in mice bearing sarcoma tumors, where exposure to acute hypoxia augmented the lung metastases.⁷⁷

Mechanistically, hypoxia was shown to influence invasive and migratory behavior of cancer cells via EMT, a trans-differentiation of cells in order to acquire plastic and mobile abilities, a process which alters their gene expression prior to migration.⁷⁸ EMT is physiologically active during embryogenesis and tissue regeneration, as well as in cancerogenesis in many types of solid tumors⁷⁹ and hematologic malignancies.⁶³ Hypoxia-induced EMT is characterized by a decrease in epithelial-associated gene expression, such as E-cad, β -catenin⁸⁰ and an increase in mesenchymal-like gene expression, such as N-cad,⁸¹ vimentin, SMA,⁸² and CXCR4.^{63,75} EMT is promoted by a master regulator TGF- β , also increased by hypoxia, which activates downstream transcription factors such as Smads, Snail, Slug, and Twist, and inhibits expression of E-cad.^{63,83} Interestingly, radio- and chemoresistance was also shown to be associated with EMT phenotype; expression of Snail and Slug antagonizes p53-mediated apoptosis and promotes resistance to radiation and chemotherapeutic agents such as paclitaxel and cisplatin in ovarian cancer cells.⁸⁴

Moreover, HIF-1 α was shown to be expressed in 90% of human gastric cancer biopsies at the front edge of the invading tumor compared to HIF-1 α negative normal tissues.⁷⁴ HIF inhibition significantly reduced the metastasis of gastric cancer cells *in vivo*, and HIF deficient cells were less motile, invasive, and adhesive *in vitro*.⁷⁴ High involvement of the main hypoxic regulator, HIF- α , in all steps of metastasis led to many trials of inhibiting this molecule to diminish cancer cell trafficking thus reducing metastasis. Inhibition of HIF-1 activity using antisense oligonucleotide (EZN-2968) gave effective results and a safe toxicity in a Phase I clinical trial in metastatic, advanced solid malignancy.⁸⁵ Targeting hypoxic cells with a pro-drug, activated only in a hypoxic environment, is one of the newest and highly promising strategies to reduce metastasis, currently undergoing phase I/II clinical trials in multiple myeloma, a model of a process of metastasis.⁸⁶

Apart from targeting HIF- α molecules, another strategy to inhibit metastasis is to target genes downstream of HIF- α . For instance, CAIX is a hypoxia-inducible enzyme widely present in tumors; it is crucial in regulating intra- and extracellular pH, thus CAIX promotes survival and invasion of

cancer cells.⁸⁷ It was demonstrated that, inhibition of CAIX decreased tumor growth and metastasis in pre-clinical breast tumor models.⁸⁷ LOX is another protein elevated in hypoxic human tumor cells (such as breast, and head and neck cancer) and is HIF- α -dependent.⁶¹ LOX secreted by tumor cells accumulates at new sites and mediates bone marrow-derived cell recruitment which forms a “pre-metastatic niche” for future metastasis.⁸⁸ Inhibition of LOX reduced hypoxia-induced recruitment and metastasis in the breast cancer mouse model.⁸⁸ Another target candidate induced by hypoxia in cancer cells is CXCR4 involved in cell trafficking.^{89,90} It was demonstrated that metastatic tumor cells expressing high levels of CXCR4 home to tissues rich in its ligand, SDF-1,^{63,91} and inhibitors of CXCR4/SDF-1 axis resulted in disruption of the metastatic process (Figure 2).⁹⁰

Radiation and drug resistance

Resistance of cancer cells to treatment-induced apoptosis is one of the biggest obstacles in cancer therapy.⁹² A vast number of cancer patients relapse and suffer from recurring tumors as a result of micro-residual disease, the resistant subpopulation of cancer cells, leading to local recurrence and/or metastasis.⁹³ Tumor hypoxia develops due to uncontrollable cell proliferation, altered metabolism, and abnormal tumor blood vessels resulting in reduced transport of oxygen and nutrients.¹³ Hypoxia is one of the main features of solid tumors and was shown to correlate with poor prognosis of cancer patients.⁷³ While hypoxia is lethal for many cells, a subpopulation of tumor cells is able to not only adapt to hypoxic conditions but also become resistant to chemo- and radiotherapy. The role of hypoxia in the phenomenon of therapy resistance has been acknowledged for at least 60 years.^{94,95}

Hypoxia confers treatment resistance of cancer cells by regulating processes such as i) inducing cell cycle arrest (quiescence), a state of reduced cell proliferation which protects the cells from external stress,^{96,97} ii) inhibiting apoptosis and senescence of cells, iii) controlling autophagy, p53, and mitochondrial activity.^{94,97} Apart from cellular adaptations influenced by hypoxia, lowered oxygenation of the tumor tissue confers chemoresistance by affecting iv) drug delivery and cellular uptake through associated acidity and drug efflux pump expression such as P-gp,⁹⁸ as well as by the v) lack of oxygen required for the cytotoxicity of a number of chemotherapeutics.⁹⁹

One of the most important parameters of radio-resistance is oxygenation, the state of cell cycle, and the nature of radiation, whether it is gamma, X-ray, neutron radiation, or linear energy transfer including alpha and beta particles.^{94,96}

When oxygen is abundant, the normoxic cells are sensitive to radiation, due to “oxygen fixation” which happens when available molecules of oxygen react with free radicals in DNA generated by ionizing radiation leading to irreversible DNA damage. Whereas, cells irradiated in hypoxic conditions are resistant to death, due to decreased production of DNA radicals (which can be restored “chemical restitution”) caused by reduced generation of ROS and decreased DNA damage.⁹⁵ The cell cycle phase determines the radiosensitivity of the tumors. It was shown that cells exposed to ionizing radiation are the least sensitive at the end of S phase, less sensitive in the G1 phase, and the most radiosensitive in the G2/M phase where DNA repair mechanisms are the most prone to malfunction.¹⁰⁰ Hence, anticancer treatments, both radio- and chemotherapy, preferentially target the bulk of rapidly proliferating tumor cells. On the other hand, the cells which are most resistant to treatment are quiescent, low-proliferating, stem-cell-like cell fractions residing in the most hypoxic region.^{82,84,97,101} For instance, it was demonstrated that glioblastoma cancer stem cells (CD133⁺) showed higher DNA repair and decreased apoptosis in response to irradiation, compared to non-stem-cell-like (CD133⁻) population.^{94,96,102}

Hypoxia causes slow-proliferating stem-cell-like phenotype of cells, decreases senescence, creates chaotic and malfunctioning blood vessels, and augments metastasis, which all together further induces therapy resistance.⁹⁶ Currently, assessment of tumor oxygenation and HIF expression pattern helps determine tumor chemo- and radio-sensitivity.^{94,103} It was reported that head-and-neck cancer samples with high expression of HIF-1 α and HIF-2 α were more resistant to chemotherapy (carboplatin) compared to biopsies with low HIF- α expression which were chemo-sensitive.¹⁰⁴ Patients with oropharyngeal cancer demonstrating high expression of HIF-1 α had a lower chance to achieve complete remission after irradiation.¹⁰³ In addition, irradiation was shown to induce HIF-1 activity, leading to production of angiogenic molecules such as VEGF which protects ECs from irradiation-induced apoptosis.¹⁰⁵ Therefore HIF-1 represents a valid predictive marker and therapeutic target for manipulation, in combination with chemotherapeutics and radiotherapy, in order to sensitize the cells to treatments.⁷³

As demonstrated by others, inactivation of HIF-1 α in mouse embryonic fibroblasts increased their susceptibility to carboplatin and etoposide compared to wild-type, both in vitro and in vivo.¹⁰⁶ Similarly, inhibition of HIF-2 α with short hairpin RNA reversed the resistance to doxorubicin and etoposide of human clear cell renal cell cancer cells

(one of the most resistant tumors) by restoring p53.⁵⁷ It was reported that treating tumor-bearing mice with HIF-1 inhibitor (YC-1) induced radiation-induced vessel damage.¹⁰⁵ Similarly, treatment of glioma, squamous and pancreatic cancer cells with the HIF-1 α inhibitor (PX-478) radiosensitized hypoxic cells.¹⁰⁷ Silencing of HIF-1 α with siRNA in mouse embryonic fibroblasts increased susceptibility to irradiation;¹⁰⁶ and also, HIF-2 α inhibition was shown to enhance radiation-induced apoptosis due to HIF-2-mediated increase of p53 activity and accumulation of ROS, thus DNA damage (Figure 2).¹⁰⁸

Tumor microenvironment

The tumor would not thrive without the interaction, cross-talk, and support with the tumor microenvironment including cellular components such as stromal cells, immune cells, ECs as well as non-cellular components including ECM, cytokines, and other mediators.¹⁰⁹ Thus, in hypoxic tumor tissue, not only cancer cells but also the tumor microenvironment is affected by hypoxia-inducible changes.¹¹⁰

Hypoxia was shown to induce metabolic and molecular changes in ECs, increasing expression of pro-angiogenic molecules, blood vessel formation, and thus providing more oxygen and nutrients for tumor cells. Hypoxia also regulates inflammatory mediators and growth factors, which then stimulate platelet, leukocyte, and smooth muscle cell activity. One of the most significant changes is increase in adhesiveness of ECs to neutrophils facilitating NK cell trafficking and local inflammatory reaction.¹¹¹ Depending on the duration of oxygen depletion, hypoxia regulates expression of NO synthase expression contributing to vasoconstriction.¹¹² Since blood vessels nourish tumors, targeting ECs will prevent or reverse tumor growth.^{110,113–115}

Stromal cells, on the other hand, facilitate tumor growth and tumor dissemination mostly by regulating cancer cell adhesion and contributing to cell proliferation and survival. It was shown that hypoxia induces stromal cells to produce a number of factors including Ang-2, ANGPTL-4, PDGF, VEGF, SDF-1, LOX, and SCF (KIT-ligand), influencing ECs and EPCs thus promoting new blood vessel formation and lymphangiogenesis. Also, stromal-derived SDF-1 attracts cancer cells and thus facilitates metastasis.^{115,116}

It was demonstrated that hypoxia leads to immune-resistance and immune-suppression, which help tumor cells to escape from immune surveillance.^{117,118} Some of the immune-suppressive effects include: 1) shedding of immune-recognition molecules by tumor hypoxia, which results in decreased sensitivity to T- and NK-mediated killing;¹¹⁹

2) inhibition of T cells' and dendritic cells' maturation and cytokine production;¹²⁰ 3) and promotion of suppressive cells such as regulatory T cells and tumor-associated macrophages, which block immune effector cells.¹²¹

Therefore, there is an increasing importance of the hypoxic phenotype of stromal and immune cells in the tumor microenvironment providing non-cancer cells as potential novel targets in the fight against the tumor.¹¹⁰

Conclusion

Pathological hypoxia affects both cancer cells and the tumor microenvironment, and plays a pivotal role in the process of cancer progression and dissemination. Hypoxia regulates tumor neovascularization, metabolism, cell survival, and cell death. In addition, hypoxia contributes to EMT-like cancer cell migration and cancer stem-cell-like properties including resistance to treatment, one of the nightmares in the medical field. Each step of the cancer adaptive processes is controlled by hypoxia-activating transcriptional programs involving HIF, NF κ B, PI3K, and MAPK pathways.

Since hypoxia signifies increased tumor progression and aggressiveness hampering patients' survival, direct and indirect methods of measuring hypoxia combined with clinical observations may help to predict patients' outcome as well as identify patients who could benefit from hypoxia/HIF-targeted treatments. Better understanding of hypoxic phenomenon and dissecting out the hypoxia-inducible responses and signaling pathways will grant numerous novel targets in the near future.

Disclosure

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