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Overview

Hypoxia in solid tumors: biological responses to hypoxia and implications on therapy and prognosis

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Abbreviations:
VHL = von Hippel-Lindau
HIF = hypoxia-inducible factor
HRE = hypoxia-response elements
mTOR = mammalian target of rapamycin
PI3K = phosphatidylinositol 3-kinase
Akt = protein kinase B
Tan IIA = tanshinone IIA compound
VEGF = vascular endothelial growth factor
VEGFR = vascular endothelial growth factor receptor
PET = positron emission tomography
LDH = lactate dehydrogenase
MCT = monocarboxylate transporter
miRs = micro RNA molecules

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Abstract

Tumor development, promotion and ability to spread depend greatly on tumor microenvironment. Rapid growth accompanied by inadequate angiogenesis is the reason why most solid tumors contain hypoxic regions. Activation of hypoxia signaling pathways stimulates neoangiogenesis, alters tumor metabolism, promotes a more aggressive tumor behavior and significantly affects its responsiveness to therapy. Growing amount of evidence suggest that hypoxia induces transcription of tumor promoting genes leading to increased tumor cell proliferation and metastatic potential. Improved understanding of molecular pathways will enable establishment of useful prognostic and predictive factors, along with more effective treatment options.

INTRODUCTION

In addition to being an essential molecule for oxidative phosphorylation in mitochondria, oxygen also functions as an important signaling molecule and regulates a wide range of biological processes, including erythropoiesis, angiogenesis, and cellular differentiation *(1)*. Hypoxic regions are common characteristic of majority of solid neoplasms, resulting from discordance between high metabolic needs of rapid growing malignant tissues and oxygen supply through structurally and functionally impaired microvasculature. Recognition of tumor hypoxia and its clinical significance was hampered for a long time by a lack of standardized methods for routine measurement of intra-tumor partial oxygen pressure *(2, 3)*. In the past decade, considerable progress has been made in the field of tumor oxygenation and its impact on tumor promotion, and metastatic potential, therapeutic response and prognosis in patients with solid malignant neoplasms *(4)*.

Research shows relative resistance of tumorous tissue against ionizing radiation, chemotherapy and other non-surgical treatment modalities *(5)*. Hypoxia is believed to be a key microenvironment factor that induces tumor metastasis. It amplifies adaptations that enable tumor cell survival (anaerobic shift, neovascularization, resistance to apoptosis) and, on the other hand stimulates tumor aggressiveness (gene instability, invasion, metastasis, dedifferentiation) *(6)*.

METABOLIC CHANGES OF TUMOR CELLS IN HYPOXIA

Tumor microenvironment is metabolically heterogeneous; cells close to the functioning microvasculature are well oxygenated and dependant

on oxidative metabolism, whereby distant cells are poorly oxygenated and glycolysis-dependant. Adaptive changes in cell metabolism of tumor cells involve shift from oxidative phosphorylation to glycolysis, increased glycogen synthesis; switch from glucose to glutamine as a major substrate for fatty acid synthesis *(1)*.

Cellular response to hypoxia is modulated by a family of ubiquitous transcription factors known as hypoxia inducible factors (HIF), comprising HIF-1a, HIF-2a and HIF-3a (Figure 1). Metabolic reprogramming is coordinated on a transcription level of hypoxia inducible factor 1 (HIF-1), considered to be a main regulator of oxygen supply and demand *(7)* (Figure 1).

In hypoxic cells HIF-1 increases expression of glucose transporters, glycolytic enzymes (e.g. LDH-A, MCT-4) that stimulate glucose uptake, conversion to lactate and lactate efflux from the cell. On the other hand, well oxygenated cells express MCT-1 and LDH-B, that mediate lactate transport intracellularly and its conversion into pyruvate needed for oxidative metabolism *(8)*.

Role of hypoxia inducible factor (HIF-1)

HIF-1 is a heterodimer comprised of HIF-1a and HIF-1b subunits. HIF-1a expression is regulated by oxygen and degrades swiftly in normoxic conditions. On the other hand, hypoxia stabilizes HIF-1a and results in its accumulation. HIF-1b is constitutively expressed. However, in certain conditions of adequate oxygenation, HIF-1a expression can increase. HIF-1 in tumor cells can be activated by the loss of function of tumor suppressor genes (e.g. VHL) and/or oncogene gain of function with resulting increase in activity of e.g. PI3K/AKT/mTOR signal pathways *(9)*.

Modes of HIF-1 protein level increase are diverse. For example, von Hippel-Lindau protein mutation stabilizes HIF-1a protein, while activation of PI3K/AKT/mTOR signal pathway stimulates HIF-1a mRNA translation. Reactive oxygen and nitrogen species inhibit HIF-1a proteasome. Following stabilization of HIF-1a, heterodimer protein activates transcription of numerous genes involved in angiogenesis, proliferation, glycolytic tumor metabolism and regulation of cellular pH *(9)*.

HIF-1 is a mediator of metabolic alterations that govern tumor progression and resistance to treatment. Inhibitors of HIF or metabolic enzymes can impair tumor cell flexibility and render them more susceptible to antineoplastic regimens *(10)*.

HIF family also contributes to transactivation of genes that encode micro RNA molecules (miRs). Micro RNAs

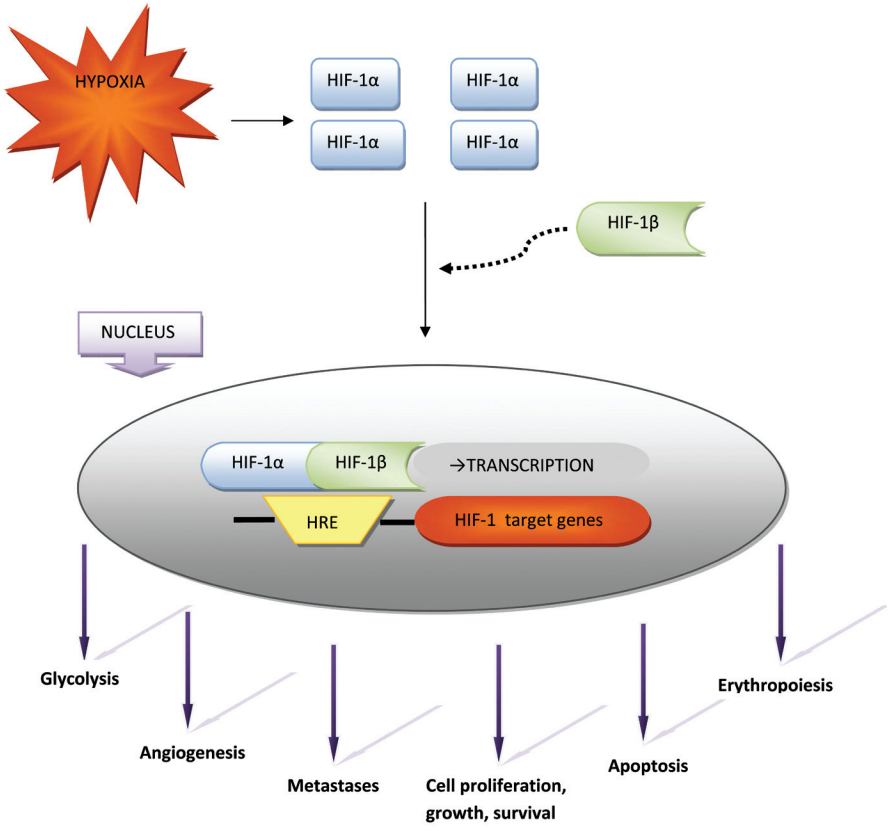

Figure 1. *Hypoxia upregulates various transcription factors. HIF (hypoxia-inducible factor) plays a key role inducing transcription of genes involved in tumor progression, angiogenesis, erythropoiesis, metabolism, apoptosis and tissue remodelling.*

are small regulatory RNA molecules that bind specific messenger RNA (mRNA) sequences, and thereby inhibit translation or stimulate their degradation. Many cell types show increased miR-210 expression typical for a hypoxic response *(11)*.

HYPOXIA AND CANCER METASTASIS

Majority of solid tumors have hypoxic areas. Measurements of tissue oxygenation in breast cancer have shown correlation between large areas of hypoxic tissue and expression of hypoxic markers detected by immunolocalization methods. Staining for CAIX and HIF-1a in breast cancer tissues shows high intensity surrounding necrotic areas. In pre-clinical models, efficient deletion of HIF-1a inhibits primary tumor growth, suppresses lung metastasis and prolongs survival. Loss of HIF-1a inhibits expression of markers of basal breast cancer phenotype, as well as numerous genes involved in epithelial-mesenchymal transition *(12)*.

Expression of numerous genes associated with breast cancer metastasis is increased in hypoxic conditions, correlating with worse outcome *(13, 14)*. Better understanding of molecular pathways involving hypoxia-stimulated breast cancer metastasis could result in potentially beneficial, prognostic as well as therapeutic implications *(15, 16)*.

THERAPEUTIC IMPLICATIONS OF TUMOR HYPOXIA

Effect of hypoxia to radiotherapy treatment

HIF-1 protein has an important role in tumor resistance against radiotherapy treatment, either through post-irradiation protection against neoangiogenesis or through amplified anti-oxidative capacity as a component of glycolytic tumor metabolism.

In preclinical trials, blockage of HIF-1a and tumor glucose metabolism affects tumor microenvironment, stimulates metabolic alterations and sensitizes various solid tumors to radiation. Future research should elucidate weather HIF-1 inhibitors and inhibitors of glucose metabolism could prove beneficial in clinical practice. Special attention should be focused on effects of HIF-1 inhibition on tumor glycolytic and redox potential. Optimal timing for introduction of these inhibitors during the treatment course is yet to be established *(17)*.

In order to optimize patients suitable for such treatment, it is necessary to additionally validate imaging techniques for determination of HIF-1 protein expression, changes in glucose metabolism and thereby response to radiotherapy treatment *(18)*. PET imaging of hypoxia can be used to identify patients with hypoxic tumors who could benefit most from additional treatment, either by prescribing hypoxia-modifying drugs or by increasing the radiation dose to the tumor *(19)*.

Hypoxia-mediated resistance to chemotherapy treatment

Hypoxia in solid tumors is associated with development of chemo-resistance. Majority of research focuses on the impact of hypoxia on apoptotic effect of cytotoxic medications, while minority refers to non-apoptotic pathways of chemo-resistance in hypoxia *(20)*. Multiple mechanisms of hypoxia-mediated chemo-resistance are shown in MDA-MB 231 breast cancer cell line. Hypoxic preconditioning of these breast cancer cells results in resistance to multiple categories of chemotherapy medications: anthracyclines, etoposide, mitoxantrone. Acute exposure to these medications results in multiple nuclear and cytoplasmic changes *(15)*.

Cells with higher expression of HIF-1a have better efficacy in double strand DNA repair, increased resistance to carboplatin, etoposide and ionizing radiation in comparison HIF-1a deficient cells. Hypoxia can stimulate chemo-resistance to doxorubicin in experimental models of MCF-7 and HCC1973 cell lines. Likewise, that same resistance of the aforementioned cell lines can be ameliorated through the addition of tanshinone IIA compound (Tan IIA). The assumed mechanism of Tan IIA effect is attributed to reduced expression of HIF-1a *(12)*. In addition to doxorubicin resistance, hypoxia contributes to enhanced epithelial-mesenchymal transition through modulated expression of E-cadherin and vimentin protein levels. Hypoxia reduces E-cadherin expression and increases vimentin expression. Tan IIA returns their expression to a control level *(12)*. These effects need to be confirmed in additional *in vivo* research.

Biological therapy in hypoxic microenvironment

For long it was presumed that anti-angiogenic medications have an important role in cancer management. Recent clinical studies question their effectiveness in breast cancer. Pre-clinical trials suggest that anti-angiogenic medications could even increase invasive and metastatic potential of breast cancer cells. Through stimulation of intra-tumor hypoxia in breast cancer xenografts, sunitinib and bevacizumab lead to proliferation of tumor stem cell population.

Pre-clinical trials indicate that inhibitors of dichloroacetate DCA and HIF inhibitors could enhance currently limited efficacy of anti-VEGF antibody bevacizumab as angiogenesis inhibitor *(21, 22)*.

Melanomas that have the V600E BRAF mutation exhibit increased HIF-1α expression. Vemurafenib targeting mutated BRAF was approved for the treatment of

advanced melanomas. Resistance to vemurafenib is being increasingly recognized. Vemurafenib-resistant melanomas respond to treatment with an increase in HIF-1α levels, which thus serves as one of the mechanism of their resistance *(23).*

CONCLUSION

Hypoxic areas are present in many solid tumors due to inadequate blood supply and deranged vascular architectonics. Tumor cells therefore adapt to hypoxic conditions in order to maintain their own balance of oxygen homeostasis.

Cell adaptation to hypoxia involves well coordinated expression of various groups of genes, many of which are transcriptionally regulated by hypoxia-inducible factor 1 (HIF-1). HIF-1 is a transcription factor made up of HIF-1a and HIF-1b subunits. HIF-1b is constitutively expressed, and control of HIF-1 function is accomplished through oxygen-dependant degradation of the alpha subunit. Active HIF-1 induces expression of many genes, some of which are relevant for tumor progression *(24).*

Prevailing evidence from experimental and clinical trials indicate tight association of tumor cell adaptation to hypoxia with tumor progression as well as contribution to development of resistance to specific oncological treatment modalities (ionizing radiation, cytotoxic medications, targeted biological therapy).

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