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Review Article

Vascular Onco-Therapies Targeting Continuous and Intermittent Intra-Tumor Hypoxia

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Abstract

Objective: Solid tumors experience intra-tumor hypoxia once they achieve an increase in size. This is due to an imbalance between tumor oxygenation and the metabolic demand of the tumor as well as the development of chaotic microvasculature of the tumor. The hypoxic condition creates several barriers to the delivery of antitumor drugs to the tumor. Intra-tumor hypoxia alters the tumor microenvironment, accelerating the process of tumor angiogenesis, and culminating in the formation of chaotic tumor vasculature. The abnormal and faulty tumor microvasculature alters the interstitial pressure gradients of the tumor which severely impairs delivering drugs to solid tumors. Rectifying this microenvironment is an important avenue of anticancer research. The normalization of tumor vasculature may lead to an excellent anticancer management. **Study Selection and Data Source:** The present review involves recent studies on anticancer research targeting the hypoxia-signaling cascade in solid tumors. **Results and Conclusion:** The present review covers the cause of intra-tumor hypoxia, the resulting problems of anticancer drug delivery to the tumor, and contemporary research to overcome the problem of drug delivery to hypoxic solid tumors in humans.

Keywords: Chronic and cycling hypoxia, hypoxia-signaling cascade, tumor vasculature

Introduction

Solid tumors are complex structures in which the interdependent relationship between tumor and its oxygenation status modulates tumor development and its metastatic dissemination. Earlier studies have shown that irregular blood flow in tumors is responsible for the development of intra-tumor hypoxia. Intra-tumor hypoxia is known to be one of the tumor microenvironment features favoring tumor cell survival and multidrug resistance. Hypoxia is defined as an imbalance between oxygen supply to the tissue and metabolic oxygen demand of the tissue.[1] The intra-tumor hypoxic microenvironment plays a determining role in tumor growth and anticancer therapy responsiveness. Intra-tumor hypoxia can positively modulate tumor development and induce tumorigenesis, tumor angiogenesis, chemoresistance, and radioresistance.

Complex metazoan life forms depend on energy generated through the oxidative metabolism of glucose generating an electrochemical

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gradient that drives synthesis of adenosine triphosphate (ATP), the energy currency of cell. The highly complex and efficient mechanism depends on the utilization of oxygen as a terminal electron acceptor. As a consequence, metazoan organisms have evolved elaborate cellular metabolic and systemic physiological systems that are designed to maintain oxygen homeostasis. Thus, oxygen homeostasis forms an organizing principle for analyzing metazoan evolutionary radiation, physiology, and pathobiology. Our understanding of the mechanisms by which cells and organisms sense hypoxia has dramatically advanced over the last two decades, principally through the discovery of hypoxia-inducible factor-1 (HIF-1). The HIFs are transcriptional factors regarded to be master regulators of oxygen homeostasis. This review focuses on the role of HIFs as master regulators of oxygen homeostasis and, in particular, on recent advances in understanding their roles in tumor development and the metastatic dissemination of hypoxic tumor cells.

The Hypoxia-Signaling Cascade

The origin of the oxygen homeostatic mechanism is a major evolutionary innovation adopted during metazoan evolution in geologically ancient times.[2,3] Oxygen tension in well-vascularized tissues generally prevails at 20 mmHg,[4] but in cellular hypoxic condition, when this pressure goes below 10 mmHg, hypoxic tissue initiates a chain of cellular signaling events, named the hypoxia-signaling cascade. The signaling cascade involves the activation of the transcription factors: HIFs and the master regulators of oxygen homeostasis. HIFs are an evolutionary ancient protein, the ancientness of which indicates the important role played by it in the primordial world during and after the period of oxygenation.[5] The HIFs have three isoforms: designated HIF1–3.[6] The most ubiquitously expressed and biologically relevant among them is HIF-1, which is a heterodimeric protein consisting of HIF-1α and HIF-1β subunits.[7] HIF-1β is expressed consecutively, whereas the regulation of HIF-1α is determined in an oxygen-dependent manner.[2] In normal oxygen concentrations, HIF-1α is bound by the product of a tumor suppressor gene, von Hippel–Lindau (VHL) protein, the binding of which with HIF-1α induces the ubiquitin-mediated proteasomal degradation of HIF-1α.[8] VHL binding is dependent on the hydroxylation of a specific proline residue in HIF-1α by Prolyl Hydroxylase Domain (PHD), which uses oxygen as a substrate, and thus, its activity is inhibited under cellular hypoxic conditions.[9] When PHD became inactivated, HIF-1α accumulates within cells followed by binding with HIF-1β to form functional HIF complexes. HIF increases the expression of genes involved in angiogenesis, adaptation to hypoxia, invasiveness, and resistance to oxidative stress. The most notable and well-studied effect of HIF-1 is the transcriptional activation of vascular endothelial growth factor (VEGF),[2] which is involved in tumor angiogenesis. The angiogenic switch that represents one of the major events in tumor progression is triggered by hypoxia and HIF-1α by inducing the expression of VEGF[10] [Figure 1].

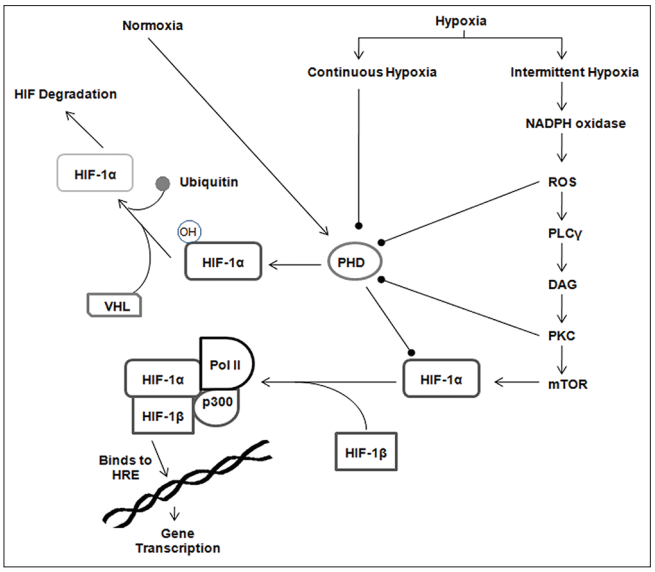

Figure 1: The hypoxia-signaling cascade and its relationship in intra-tumor hypoxia. HIF: Hypoxia-inducible factor, VHL: Von Hippel–Lindau protein, PHD: Prolyl Hydroxylase Domain, mTOR: Mammalian target of rapamycin, PKC: Phospho kinase C, DAG: Diacylglycerol, ROS: Reactive oxygen species, PLC: Phospholipase C, Pol III: RNA Polymerase III, HRE: Hypoxia response element

In solid tumors, hypoxia, or an oxygen tension below physiologic levels, develops as abnormal proliferation outstrips the blood supply. This hypoxic region is involved in the progression of malignancy and results in the development of resistance to radiotherapy.[11] HIF-1α is overexpressed in many different types of human cancers.[12] Its expression is associated with an aggressive phenotype and is a marker for a poor prognosis for many types of tumors, including prostate, oropharyngeal, esophageal, and breast cancers. HIF-1 active or hypoxic cells have been shown to play crucial roles in angiogenesis and radioresistance. This suggests that HIF-1α is a potential target for anticancer therapy.

Continuous Intra-tumor Hypoxia

During acute intra-tumor hypoxia, hydroxylation reactions are inhibited as a result of oxygen deprivation and/or increased mitochondrial production of reactive oxygen species (ROS), which may inhibit hydroxylases by oxidizing a ferrous ion in the catalytic site.[8,13] The loss of hydroxylase activity increases the stability of HIF-1α subunit of HIF allowing HIF-1α and HIF-1β subunits to bind with each other to form stable HIF-1 transcription factor, which will ultimately binds to the HIF-1 recognition sequence, the hypoxia response element (HRE) in target genes.

Intermittent Intra-tumor Hypoxia

Intra-tumor hypoxia may not be constant spatially and temporally. The pattern of tumor oxygenation may be patchy with cycles of oxygenation and cycles of hypoxia, and occurs

in large regions of the tumor, of up to one-fifth of tumor cells, over prolonged periods of time.[14,15] Previous studies have suggested that immature vessels may be more likely to experience cycling hypoxia.[16,17]

Intra-tumor cycling hypoxia results from alterations in the prevalence of erythrocytes in malformed and chaotic tumor microvasculatures.[18] There are many causes of alterations in the erythrocyte flux such as vascular pruning, formation of new vascular connections, and intussusceptions.[19] Among the different causal factors, intussusception is of particular interest and involves splitting of microvessels into smaller parallel vessels over a period of minutes. Since flow resistance is inversely proportional to vessel radius to the fourth power, small changes in microvessel size lead to larger distributions in flow. Vascular remodeling, which may cause changes in flow resistance, is likely responsible for the majority of erythrocyte flux causing cycling hypoxia.

Interactions between Continuous and Intermittent Hypoxia

The overall oxygenation state of a tumor region has profound effects on whether that region will experience cycling hypoxia, so chronic hypoxia and cycling hypoxia are inextricably linked phenomena. For example, poorly perfused areas that are far from microvessels are unlikely to be affected by changes in red cell flux, and the pO_2 in this region is unlikely to change much because it is already quite low. Alternatively, regions that are perfused by an abundance of vessels are equally unlikely to experience large changes in oxygen tension due to changes in flow through only one of the feeding microvessels.

The temporal variations between different reports of cycling hypoxia make the two processes difficult to differentiate. For example, while variations in the order of seconds or minutes seem clearly to be cycling hypoxia, reports of variations in the order of hours to days blur the distinction.[20] It is well known that chronic hypoxia has important physiological consequences for tumors and leads to treatment resistance, but cycling hypoxia appears to have effects that are distinct from those of chronic hypoxia. Other researchers have shown that metastatic frequency is dependent on the degree of tumor hypoxia and that cycling hypoxia increases metastatic frequency over that of chronic hypoxia.[21,22] Tumor-bearing mice subjected to cycling hypoxia have also shown increased expressions of genes associated with metastasis, including CXCR4 and VEGF among others.[23] The effects of cycling hypoxia are not limited to metastasis. Cycling hypoxia appears to influence HIF-1α protein levels and transcriptional activity more than chronic hypoxia.[24] Cycling hypoxia may increase ROS due to repeated hypoxia re-oxygenation cycles, and a recent study involving a transgenic model of breast cancer showed evidence of significant oxidative damage to DNA and lipids caused by cycling hypoxia.[25] Furthermore, it is clear that the microenvironment greatly affects the mammalian target of rapamycin (mTOR) activity, and cycling hypoxia seems to have an opposite effect to chronic hypoxia on mTOR function and interactions with HIF-1.[26] This is significant because mTOR is an integral part of the mTOR complex that is involved in modifying the response to changes in nutritional and energy status and oxidative stress. Hypoxia and oxidative stress both induce the unfolded protein response (UPR), which alters protein expression, metabolism, and cell death in response to stress.[27] It seems likely that cycling hypoxia will affect the UPR, since genes controlled by HIF-1 are often contained in the stress granules formed by the UPR and cycling hypoxia increases oxidative stress.[28] However, further investigations into these changes are needed to better understand the pathophysiological responses to cycling hypoxia. In accordance with the clear effects on the environment of the tumor, hypoxia, and cycling hypoxia have important implications for tumor treatment.[29] In the setting of radiotherapy, oxygen helps to stabilize treatment-induced DNA damage such that the DNA cannot be effectively repaired. Therefore, chronic hypoxia decreases the cytotoxic effects of radiation. Furthermore, the delivery and activity of chemotherapeutic agents is often decreased under hypoxic conditions. In addition to the chemo- and radioresistance conferred on tumor cells by chronic hypoxia, cycling hypoxia is known to cause resistance to radiation therapy. Therefore, understanding these microenvironmental effects is critical in order to design better targeted therapeutic strategies.

Interactions between Intra-tumor Hypoxia and Angiogenesis

The term angiogenic switch refers to the balance of pro- and antiangiogenic factors, leading to the initiation of angiogenesis. Low tissue oxygenation is generally considered to be the predominant weight that tips the scales in favor of angiogenesis, since hypoxia enhances HIF-1 protein levels and activity, directly upregulating VEGF.[30] Other microenvironmental components, such as oncogenes and growth factors, can act via the PI3K-AKT pathway to increase the expression of HIF-1 to the point that it overcomes oxygen-mediated degradation.[31] For primary tumors to grow beyond a few millimeters in diameter, angiogenesis is generally required. In early malignant breast tumors, HIF-1 expression has been correlated with VEGF levels and angiogenesis.[32] This is consistent with a model of HIF-1 causing VEGF production and angiogenesis, but it does not answer the question of whether hypoxia-mediated stabilization of HIF-1 is the principal underlying cause of neovascularization. There was no evidence of vascular stasis before angiogenesis, although angiogenesis was enhanced by HIF-1α upregulation. This led us to propose the acceleration model, suggesting that HIF-1α is not necessary for angiogenic initiation but instead accelerates neovascularization. This would mimic the role of angiogenesis in wound healing.[33] However, more evidence

is needed to better understand the temporal and functional relationships between vessel cooption and angiogenesis. It is not only the onset of angiogenesis that is of interest. Ongoing angiogenesis affects the delivery of oxygen, nutrients, and therapeutic agents to the tumor. A study using a fluorescent reporter for HIF-1 expression clearly showed the presence of some well-oxygenated microvessels in regions with high expressions of HIF-1 as tumor grows.[34] It is possible that ROS and/or NO may stabilize HIF-1 in these regions, leading to VEGF expression in the absence of hypoxia and causing vascular remodeling and angiogenesis.

Therapeutic Interventions to Rectify Intra-tumor Hypoxia

Targeting the hypoxia-signaling cascade by inactivating the function of HIF has emerged as an attractive therapeutic strategy in vascular onco-therapy to inhibit tumor progression and metastasis. Previous approaches involve a number of therapeutic strategies to target HIF activation. The genetic deletion of HIF-1 in endothelial cells has been shown to disrupt hypoxia-induced endothelial cell behavior, leading to the inhibition of neovascularization, which ultimately inhibits tumor formation.[35] As an alternative to genetically deleting the HIF gene, suppressing HIF-1 via RNAi could also be used to restrict tumor progression. The RNAi-mediated repression of HIF-1 has been shown to enhance tumor necrosis, thereby resulting in tumor regression,[36] and also to have antitumor effects in various preclinical models including pancreatic cancer, squamous cell carcinoma, and gastric cancer.[37-39] Many anticancer agents could be explored for the inhibition of HIF to normalize tumor angiogenesis to rectify tumor chaotic vasculature, which could then interfere with drug delivery to solid tumors.

Echinomycin, a cycle peptide with antibiotic properties, has been reported to bind to HRE and inhibit DNA binding and the transcriptional activation of HIF-1.[40] Another antibiotic, geldanamycin, is an inhibitor of molecular chaperone heat shock protein 90 (Hsp90) required for HIF-1 protein stability.[41] Geldanamycin has been shown to accelerate HIF-1 degradation and leads to a reduction in HIF-1 transcription activity in kidney and prostate cancer cells.[41] There are other drugs also involved in antiangiogenic therapy by targeting HIF. Several clinical trials have evaluated the efficacy of 17-AAG, an inhibitor of Hsp90, in kidney and breast cancers and shown positive anticancer management possibilities in clinical trials.[42,43]

VEGF has been reported to be highly upregulated in a vast majority of solid tumors, and it is considered to be an important factor in mediating tumor angiogenesis. This has led to the development of antiangiogenesis therapeutic avenues focusing on the development of anti-VEGF drugs (bevacizumab), anti-VEGF receptor (VEGFR) (ramucirumab), and VEGF trap (aflibercept).[44] Human studies have shown that VEGF monotherapy can inhibit tumor growth and affect metastasis in many cancer types.

In addition to monoclonal antibodies, Tyrosine Kinase Inhibitor (TKI) have been developed to inhibit VEGFRs and their downstream targets, resulting in the suppression of endothelial cells leading to the vascular supply of tumors. Many TKIs have shown as potential treatment options, including sorafenib, pazopanib, vandetanib, sunitinib, regorafenib, lenvatinib, and cabozantinib. These multikinase inhibitors have shown improved target affinity when observed clinically.

Another antiangiogenic agent, bortezomib, which is a proteasome inhibitor, has been reported to inhibit tumor adaptation to hypoxia via blocking hypoxic activation of HIF-1 and inducing its target genes, including VEGF and erythropoietin.[45] Previous studies have reported the role of PX-478 in suppressing the constitutive and hypoxia-induced levels of HIF-1 in cancer cells, and it exhibited an antitumor effect in a human tumor xenograft model.[46] Another molecule, NSC-134754, has been reported to inhibit both HIF-1 protein levels and its activity induced by hypoxia and the subsequent induction of HIF-1 target gene expression.[47,48] The aberrant activation of PI3K-AKT-mTOR signaling has also been reported to induce the overexpression of HIF-1 in malignant metastatic tissue. A natural anticancer agent, resveratrol, has been reported to decrease HIF-1 and its target genes by inhibiting PI3K-AKT and mitogen-activated protein kinase activation.[49] The dual PI3K/mTOR inhibitor, NVP-BEZ235, has also been reported to suppress HIF-1 expression and enhance tumor cell apoptosis.[50]

Conclusion

This review provides a glimpse into the tremendous progress that has been made recently in understanding the molecular physiology of the hypoxia-signaling cascade and its role in tumorigenesis and its metastatic dissemination. Because metazoan life on earth has evolved along with oxygenation, it is quite expected that oxygen homeostatic regulation by HIF-1 plays essential roles that broadly span the fields of physiology and medicine. Clinical trials of drugs that inhibit HIF-1 in cancer patients are still ongoing. Finally, the exploration of HIF physiology coupled with rectification of vascular chaos in solid tumors will no doubt shed light on new avenues of antiangiogenic onco-therapy.

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Conflicts of interest

There are no conflicts of interest.

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