Free Radical Biology and Medicine ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Hypoxia and free radicals: Role in tumor progression and the use of engineering-based platforms to address these relationships

Abigail Hielscher [a,b,c,d,*], Sharon Gerecht [b,c,d,**]

[a] Department of Biomedical Sciences, Georgia Philadelphia College of Osteopathic Medicine, Suwanee, GA 30024, USA
[b] Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[c] Johns Hopkins Physical Sciences–Oncology Center, Johns Hopkins University, Baltimore, MD 21218, USA
[d] Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, USA

ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form
11 August 2014
Accepted 15 September 2014

Keywords:
ROS
Hypoxia
HIF
Tumorigenesis
Angiogenesis
Metastasis

ABSTRACT

Hypoxia is a feature of all solid tumors, contributing to tumor progression and therapy resistance. Through stabilization of the hypoxia-inducible factor 1 alpha (HIF-1α), hypoxia activates the transcription of a number of genes that sustain tumor progression. Since the seminal discovery of HIF-1α as a hypoxia-responsive master regulator of numerous genes and transcription factors, several groups have reported a novel mechanism whereby hypoxia mediates stabilization of HIF-1α. This process occurs as a result of hypoxia-generated reactive oxygen species (ROS), which, in turn, stabilize the expression of HIF-1α. As a result, a number of genes regulating tumor growth are expressed, fueling ongoing tumor progression. In this review, we outline a role for hypoxia in generating ROS and additionally define the mechanisms contributing to ROS-induced stabilization of HIF-1α.We further explore how ROS-induced HIF-1α stabilization contributes to tumor growth, angiogenesis, metastasis, and therapy response. We discuss a future outlook, describing novel therapeutic approaches for attenuating ROS production while considering how these strategies should be carefully selected when combining with chemotherapeutic agents. As engineering-based approaches have been more frequently utilized to address biological questions, we discuss opportunities whereby engineering techniques may be employed to better understand the physical and biochemical factors controlling ROS expression. It is anticipated that an improved understanding of the mechanisms responsible for the hypoxia/ROS/HIF-1α axis in tumor progression will yield the development of better targeted therapies.

Contents

Abbreviations: CAF, carcinoma associated fibroblasts; CAM, chick chorioallantoic membrane; DPI, diphenylene; EC, endothelial cell; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; HIF, hypoxia inducible factor; HMVEC, human microvascular endothelial cells; ISCU, iron sulfur scaffolding protein; PDK1, pyruvate dehydrogenase kinase 1; PHD, prolyl 4 hydroxylase domain; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau

* Corresponding author at: Department of Biomedical Sciences, Georgia Philadelphia College of Osteopathic Medicine Biomedical Sciences #139, Suwanee, GA 30024. Fax: +1 678 225 7479.
** Corresponding author at: Department of Chemical and Biomolecular Engineering, Maryland Hall #116, Baltimore, MD 21218. fax: +1 410 516 5510.
E-mail addresses: abigailhi@pcom.edu (A. Hielscher), gerecht@jhu.edu (S. Gerecht).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.09.015
0891-5849/

Introduction

Energy is indispensable for life and is produced as ATP in the mitochondria, a process referred to as oxidative phosphorylation. During metabolism, fuels such as glucose are broken down into pyruvate which passes into the mitochondrial matrix. Here, pyruvate is further metabolized and oxidized to carbon dioxide and high energy electrons in the form of the electron carriers NADH and FADH2. These electrons enter the electron transport chain in the inner mitochondrial membrane, which comprises 5 (I–V) respiratory complexes. Here, electrons are transferred between complexes, generating a proton gradient across the inner mitochondrial membrane. The energy from this electrochemical gradient is used to make ATP from ADP and inorganic phosphate. At the end, oxygen serves as the final electron acceptor, which is subsequently reduced to water. In cases where oxygen is incompletely reduced, reactive oxygen species (ROS) are formed.

ROS is estimated to occur in 1–2% of the oxygen utilized by mitochondria during oxidative phosphorylation and is therefore considered to be a normal by-product of energy metabolism [1]. Some common forms of ROS produced during oxidative phosphorylation include superoxide anion ($O_2^{\bullet -}$), which itself acts as a ROS or assists in the formation of other ROS such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet -}$). Together, these ROS act as oxidants, stealing electrons from other macromolecules such as nucleic acids, proteins, and lipids. Under homeostatic conditions, cells are well equipped to respond to elevated levels of ROS by activating apoptotic pathways if the level of DNA damage is high [2]. Other mechanisms used by cells to inactivate ROS are endogenous scavenger enzymes (e.g., antioxidants), which convert the ROS into nontoxic products [2]. Increasingly high ROS levels overwhelm the cell's ability to adequately respond. As such, uncontrolled production of ROS poses a significant threat to nucleic acids, proteins, and lipids and, as a result, can lead to a favorable scenario in which oncogenic transformation takes place. As a result, it is not surprising that ROS levels are greater in tumor cells as opposed to nontumor cells [3]. In particular, regions of low oxygen tension, referred to as hypoxia, have been reported to elevate ROS levels associated with tumorigenesis. Given the implications of hypoxia-induced ROS on tumorigenesis, the goal of this review is to elucidate the mechanisms wherein hypoxia induces ROS formation and to further examine how this pathway activates downstream hypoxia targets to promote tumor growth, angiogenesis, metastasis, and therapy responses. We will end by discussing novel engineering-based approaches, which may be utilized to address hypoxia effects on ROS production and tumorigenesis in a platform that better recapitulates the *in vivo* environment. Overall, a better understanding of the mechanisms responsible for ROS-directed tumorigenesis is anticipated to yield the development of novel chemotherapeutic agents designed at interrupting this process.

ROS and tumorigenesis

Several groups have pointed to a role for ROS in tumor progression. For instance, ROS generation was shown to be necessary for anchorage-independent growth of tumors in Kras transgenic mice, a process dependent on the presence of mitochondria [4]. In addition, it was reported that constitutive expression of Rac1, a small GTPase and member of the Rho family, in transgenic mice, promoted the development of Kaposi sarcoma-like tumors, a process in part dependent on ROS-mediated cell proliferation [5]. With regard to metastasis, Ishikawa et al. [6] isolated mitochondria from a mouse tumor cell line that was highly metastatic and transplanted it into a mouse cell line that was poorly metastatic. The authors demonstrated that the mitochondrial DNA from the metastatic tumor cell line induced metastasis and ROS formation from an otherwise nonmetastatic cell line, providing a direct link between mitochondrial dysfunction and ROS production to tumor aggressiveness [6].

Elucidating the origins of ROS in tumors has thus been the subject of extensive research. Although elevated ROS may result from a number of factors including mitochondrial mutations, incomplete reduction of oxygen during respiration, chemical or biological compounds, and poisoning or irradiation [7–9], numerous groups have recently identified a role for hypoxia in ROS production. Moreover, these groups have further demonstrated a role for ROS in stabilization of the hypoxia-responsive subunit, hypoxia inducible factor 1 alpha (HIF-1α), work which is discussed in greater detail below.

Hypoxia and HIF activation

Hypoxia participates in the transcriptional activation of a number of genes, many of which play a prominent role in growth, development, homeostasis, and tumorigenesis. Hypoxia primarily accomplishes these divergent roles through hypoxia inducible factor 1 (HIF-1), a heterodimeric transcription factor which comprises the oxygen responsive HIF-1α subunit and the constitutively active HIF-1β subunit [10]. In the presence of oxygen, HIF-1α is hydroxylated at several prolyl residues by prolyl 4 hydroxylase domain proteins (PHDs) which allow binding of von Hippel-Lindau proteins (pVHLs) [11–13]. VHLs, in turn, recruit E3 ubiquitin ligase which subsequently targets HIF-1α for proteasomal degradation [14,15]. In the presence of low oxygen, however, HIF-1α escapes the above degradation mechanism, translocates to the nucleus, and dimerizes with HIF-1β [10,16,17]. Here, this complex binds to hypoxia response elements in the promoter regions of several target genes, recruiting coactivator proteins and activating gene expression [10,18]. Many of these targets are transcription factors which have been linked to tumor progression, metastasis, and chemotherapy resistance [19].

Given the established roles of ROS, hypoxia, and HIF-1α in tumorigenesis, this review will explore the interconnectedness of the local tumor environment (e.g., hypoxia) and intracellular redox dysfunction and HIF-1α stabilization on tumor progression, angiogenesis, metastasis, and therapy responses. It is important to note that in addition to HIF-1α, ROS-induced HIF-2α stabilization has also been reported. Although an important mechanism potentially regulating tumorigenesis, this review will primarily focus on ROS-regulated HIF-1α pathways in tumor progression given the wide

Please cite this article as: Hielscher, A; Gerecht, S. Hypoxia and free radicals: Role in tumor progression and the use of engineering-based platforms to address these relationships. *Free Radic. Biol. Med.* (2014), http://dx.doi.org/10.1016/j.freeradbiomed.2014.09.015