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Multifaceted control of DNA repair pathways by the hypoxic tumor microenvironment

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ABSTRACT

Hypoxia, as a pervasive feature in the microenvironment of solid tumors, plays a significant role in cancer progression, metastasis, and ultimately clinical outcome. One key cellular consequence of hypoxic stress is the regulation of DNA repair pathways, which contributes to the genomic instability and mutator phenotype observed in human cancers. Tumor hypoxia can vary in severity and duration, ranging from acute fluctuating hypoxia arising from temporary blockages in the immature microvasculature, to chronic moderate hypoxia due to sparse vasculature, to complete anoxia at distances more than 150 μM from the nearest blood vessel. Paralleling the intra-tumor heterogeneity of hypoxia, the effects of hypoxia on DNA repair occur through diverse mechanisms. Acutely, hypoxia activates DNA damage signaling pathways, primarily *via* post-translational modifications. On a longer timescale, hypoxia leads to transcriptional and/or translational downregulation of most DNA repair pathways including DNA double-strand break repair, mismatch repair, and nucleotide excision repair. Furthermore, extended hypoxia can lead to long-term persistent silencing of certain DNA repair genes, including *BRCA1* and *MLH1*, revealing a mechanism by which tumor suppressor genes can be inactivated. The discoveries of the hypoxic modulation of DNA repair pathways have highlighted many potential ways to target susceptibilities of hypoxic cancer cells. In this review, we will discuss the multifaceted hypoxic control of DNA repair at the transcriptional, post-transcriptional, and epigenetic levels, and we will offer perspective on the future of its clinical implications.

1. Introduction

Cancer cells in solid tumors grow within a complex microenvironment, consisting of associated stromal and immune inflammatory cells, diverse extracellular signaling molecules, and the altered conditions of low oxygen, low pH, and low nutrient levels [1,2]. These attributes of the microenvironment collectively enable the growth and proliferation of neoplastic cells. Hypoxia, or low oxygen content, is particularly prevalent in the tumor microenvironment. It arises as rapid cell proliferation outpaces the development of sufficient organized vasculature, leading to regions of tumors with sparse vessels as well as to structural and functional abnormalities of the new microvasculature [3]. Hypoxic or anoxic regions are found in 50–60% of locally advanced solid tumors and have been demonstrated in a wide range of malignancies, including breast, cervical, head and neck, prostate, rectal, pancreatic, lung, brain, and soft tissue cancers (reviewed in [4–6]). Though hypoxia is pervasive within tumors, it is temporally and spatially heterogeneous. Transient disruptions in perfusion can induce acute hypoxia on a timescale of minutes to hours while oxygen diffusion limitations can lead to more chronic hypoxia over hours to days [7]. In addition, cycles of hypoxia and reoxygenation can occur due to dynamic changes in microvessel perfusion and lead to the generation of reactive oxygen species [7].

In solid human cancers, the presence and level of hypoxia generally correlate with features of aggressive tumors, but also appear to have independent clinical significance (reviewed in [4,5]). Direct oxygen level measurements in tumors have shown hypoxia to be an

Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; BER, base excision repair; DSB, double-strand break; H3K4me2, histone H3 lysine 4 dimethylation; H3K4me3, histone H3 lysine 4 trimethylation; H3K9me2, histone H3 lysine 9 dimethylation; H3K9me3, histone H3 lysine 9 trimethylation; HIF, hypoxia-inducible factor; HDAC, histone deacetylase; HR, homologous recombination; HRE, hypoxia response element; JHDM, Jumonji homology domain; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, non-homologous end joining; PARP, poly(ADP-ribose) polymerase; PTM, post-translational modification; TLS, translesion synthesis; TSA, trichostatin A.

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independent negative prognostic factor for disease-free and overall survival in cervical cancer, head and neck cancer, and soft tissue sarcomas [4]. Endogenous and exogenous markers of hypoxia also show prognostic significance for poorer patient outcome in many tumor types [4,5]. In addition, hypoxic gene expression signatures serve as adverse prognostic markers in breast cancer, head and neck cancer, and glioblastoma [5]. Poorer outcomes in patients with hypoxic compared to normoxic tumors are associated with both progressive locoregional disease and increased risk of metastasis [4,8].

Lack of oxygen increases cellular resistance to radiotherapy *via* a decrease in fixation of free radical DNA damage as well as resistance to some chemotherapy due to poor drug delivery or restrained cell proliferation [6]. However, cellular adaptions induced by hypoxia also directly promote tumor progression and metastasis through changes in gene expression, genomic changes, and clonal selection [6]. The hypoxia-inducible factors (HIFs), heterodimeric transcription factors consisting of a hypoxia-regulated α-subunit and a constitutively expressed β-subunit, mediate many of the cellular effects of hypoxia [9,10]. Hypoxia-induced stabilization of the most broadly expressed α-subunit, HIF-1α, leads to direct transcriptional activation by the HIF-1α/β dimer of genes involved in cell growth, migration, energy metabolism, and angiogenic signaling. Additional signaling pathways, including the mTOR, NF-κB, and unfolded protein response pathways, are affected by hypoxia and mediate additional changes in transcription and translation [11,12].

One key cellular event induced by hypoxia that contributes to tumor progression is genetic instability, itself an enabling feature of cancer (reviewed in [13]). The tumor microenvironment, and hypoxia in particular, have been shown to lead to both large-scale chromosomal aberrations and small-scale DNA mutations. Cells grown as tumors *in vivo* acquire increased levels of genomic rearrangements and higher levels of point mutations and small deletions in reporter genes compared with cells grown in cell culture [14–18]. *In vitro*, hypoxic stress leads to similar genomic rearrangements, comparable elevations in mutation frequency, DNA over-replication with gene amplification, and fragile site induction [16,18–23]. Importantly, *in vitro* hypoxic exposure of fibrosarcoma and melanoma cells not only generated genomic instability, but also led to increased metastatic efficiency in mice [20]. The current evidence thus strongly supports a link between hypoxia, genomic instability, and tumorigenesis.

Several studies have demonstrated that hypoxia, in the absence of reoxygenation, does not induce direct DNA damage [24–26]. Instead, hypoxia-induced genetic instability arises from the impact of hypoxia on DNA damage repair pathways [13]. Numerous mechanisms of DNA repair modulation by hypoxia have been reported, many of which depend upon the type or severity of hypoxia. Acute hypoxic stress rapidly stimulates changes in DNA repair pathways *via* post-translational modifications. On a slightly longer timescale, persistent hypoxia leads to transcriptional and/or translational downregulation of DNA repair proteins. More prolonged moderate hypoxia induces epigenetic regulation of DNA repair genes. Within this review, severe and moderate hypoxia will refer to conditions of ≤0.2% oxygen and 0.5% – 2% oxygen, respectively. In the following sections, we will describe the diverse ways in which hypoxia impacts DNA repair function, classifying them according to post-translational, transcriptional, translational, and epigenetic mechanisms, and we will highlight areas for future research and with potential therapeutic promise.

2. Post-translational control of DNA damage signaling

Post-translational protein modifications (PTMs) allow rapid control of protein functionality in response to cellular events or stressors. These covalent protein modifications, such as phosphorylation, hydroxylation, ubiquitination, or acetylation, can lead to changes in protein enzymatic activity, cellular localization, stability, and interactions with other proteins or DNA. Much of the cellular hypoxic response is initiated by changes in PTMs of HIF [10]. In parallel to HIF signaling, severe hypoxia rapidly induces a wide spectrum of PTMs of proteins involved in DNA damage response signaling and DNA repair, including components of both the ATR–CHK1 and ATM–CHK2 pathways [25,27]. Given the absence of DNA damage under hypoxia, the main stimulus appears to be hypoxia-induced replication stress. Within 6 h of severe hypoxic stress, replication initiation and elongation stall, giving rise to an accumulation of single-stranded DNA and RPA foci [28,29]. It is generally accepted that this S phase arrest, which is independent of checkpoint signaling factors and HIF, is due to the depletion or imbalance of cellular deoxyribonucleotides since certain nucleotide biosynthesis enzymes, including dihydroorotate dehydrogenase and ribonucleotide reductase, require oxygen to function [29,30]. The hypoxic modulation of DNA repair pathways by PTMs serves to coordinate stabilization of replication forks, though it may also induce cell cycle arrest, initiate apoptosis, generate chromatin changes, and affect DNA repair itself (Fig. 1).

The ataxia telangiectasia and Rad3-related (ATR) checkpoint kinase responds to DNA damage that impedes replication fork progression and generates single-stranded DNA [31]. Under hypoxia-induced replication stress, ATR forms nuclear foci and is required for phosphorylation of downstream targets, including CHK1 (S317/S345), H2AX (S139), RAD17 (S645), and NBS1 (S343) [24–26]. Activated CHK1 phosphorylates and inactivates TLK1, a serine/threonine kinase involved in cell cycle progression and processing newly replicated DNA [32]. Phosphorylated H2AX (γH2AX) exhibits pan-nuclear staining under hypoxia but also forms foci that colocalize with RPA foci, likely marking sites of single-stranded DNA [25,27]. In addition, ATR is required for hypoxia-induced monoubiquitination of FANCD2 and FANCI [33]. These PTMs appear critical for stabilizing replication forks, as loss of ATR activity or FANCD2 monoubiquitination leads to DNA damage during hypoxia [28,33]. In addition to promoting stabilization of replication forks, hypoxia-induced ATR also regulates p53. Hypoxia induces ATR-dependent phosphorylation of p53 (S15) and CHK1-dependent MDMX (S367) phosphorylation, which together lead to p53 stabilization, gene transrepression, G1 cell cycle arrest, and promotion of apoptosis [24,34,35].

The ataxia telangiectasia mutated (ATM) checkpoint kinase primarily senses and responds to DNA double-strand breaks (DSBs), which lead to ATM autophosphorylation at serine 1981, foci formation, and phosphorylation of downstream targets [31]. Although hypoxia does not generate DSBs, ATM does undergo autophosphorylation within 6 h of severe hypoxic stress [27,36,37]. However, unlike activation of ATM by DSBs, hypoxic activation of ATM is independent of the MRN (Mre11–Rad50–Nbs1) complex and leads to diffuse localization throughout the nucleus rather than the formation of distinct nuclear foci [27]. Hypoxia-induced ATM is required for phosphorylation of downstream targets, including CHK2 (T68), KAP1 (S824), 53BP1 (S25), and DNA–PKcs (T2609) [27,36–38]. CHK2 in turn is required for phosphorylation of BRCA1 (S988), which is postulated to regulate the choice between error-free and error-prone DSB repair, and p53 (S20) which is required for its stabilization [38,39]. BRCA1, as well as 53BP1, do not form distinct foci under hypoxia, further suggesting that their phosphorylation is not a response to DNA DSBs [27]. Recent evidence by Olcina et al. instead indicates that ATM signaling activation under hypoxia is due to replication stress in the setting of specific chromatin alterations [40]. Hypoxia induces an increase in histone H3 lysine9 trimethylation (H3K9me3), preferentially in the vicinity of replication forks. Increased H3K9me3 is dependent