“Contextual” Synthetic Lethality and/or Loss of Heterozygosity: Tumor Hypoxia and Modification of DNA Repair

Norman Chan¹²³ and Robert G. Bristow¹²³

Abstract
Hypoxia exists in every solid tumor and is associated with poor prognosis because of both local and systemic therapeutic resistance. Recent studies have focused on the interaction between tumor cell genetics and the dynamic state of oxygenation and metabolism. Hypoxia generates aggressive tumor cell phenotypes in part owing to ongoing genetic instability and a “mutator” phenotype. The latter may be due to suppression of DNA mismatch repair (MMR), nucleotide excision repair (NER), and double-strand break (DSB) repair. We propose a theoretical model in which hypoxia-mediated defects in DNA repair can lead to "contextual loss of heterozygosity" and drive oncogenesis. Additionally, hypoxia-mediated repair defects can be specifically targeted by DNA damaging agents and/or "contextual synthetic lethality" to kill repair-deficient cells and preserve the therapeutic ratio. These proposed concepts support the interrogation of solid tumors to document repair defects in both oxic and hypoxic tumor subregions as a conduit to novel clinical trials within the context of personalized medicine.

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The microenvironment of solid tumors differs greatly from that of normal tissues as it can contain regions of hypoxia (a decreased level of oxygen), increased interstitial fluid pressure, and decreased pH and nutrient delivery (1, 2). Hypoxia is associated with both local and systemic therapy resistance, and decreased disease-free survival has been observed in many human cancers (3–11). Importantly, hypoxia is an adverse prognostic factor in cancers treated with either radiotherapy or surgery. Hence hypoxia is not only a determinant of local radio- or chemoresistance, but also tumor progression and systemic metastasis. The latter may be due to altered transcription and translation of metastatic genes, but could also be secondary to clonal selection of a "mutator" phenotype (12, 13). This unstable phenotype may result from hypoxia-mediated suppression of DNA mismatch repair (MMR), nucleotide excision repair (NER), and double-strand break (DSB) repair [whether by homologous recombination (HR) or nonhomologous end-joining (NHEJ)]. Suppression of DNA repair in oxic and hypoxic cells may, therefore, have profound consequences, given that residual or misrepaired DNA breaks can be highly carcinogenic and generate chromosomal alterations in oncogenes and tumor suppressor genes during tumor progression (14, 15).

This report critically reviews preclinical and clinical studies that link tumor hypoxia and DNA repair pathways as drivers of genetic instability and tumor progression. We also highlight recent work in which these DNA repair defects in aggressive cancer cells can be exploited with novel therapeutic approaches.

Models of Hypoxia and Resistance to Radiotherapy and Chemotherapy

The abnormal vasculature of tumors resulting from unregulated angiogenesis is probably the most important contributor to the development of both chronic and acute hypoxia in the majority of solid tumors (reviewed in ref. 1). Tumor blood vessels are often chaotic, leaky, unevenly distributed, and generally of poor quality. Chronic hypoxia, or potentially anoxia (a complete lack of oxygen), develops in solid tumors because of abnormally long intravascular erythrocyte transit times. Together with the irregular distribution of tumor blood vessels and limited diffusion of oxygen through the tumor interstitium, this leads to hypoxia at distances greater than 150 μm from the blood vessels. Acute hypoxia or anoxia arises because of transient changes in blood flow and can be due to temporary occlusions of blood vessels, possibly aggravated by elevated interstitial fluid pressure and altered hypoxic vasodilation (1, 2, 16, 17). This opening...
and closing of tumor blood vessels can expose tumor cells to cycles of hypoxia and reoxygenation (termed “cycling” hypoxia).

For decades, tumor biologists have studied the association between tumor oxygenation and response to radiotherapy and chemotherapy (reviewed in ref. 18). The relative level of oxygen at the time of irradiation determines the efficacy of radiotherapy, by limiting the type and number of lethal DNA lesions (e.g., DNA DSBs). Radiation-mediated free radicals result from ionizations in, or very close to, the DNA and create DNA lesions that are oxygen dependent (1). At partial pressures of oxygen (pO2) below 10 mmHg, tumor cells can acquire “radiobiologic” hypoxia, whereby anoxic cells are up to three times more radioresistant than oxic cells [the oxygen enhancement ratio (OER); ref. 1]. The OER is calculated as the ratio of doses needed to achieve the same biological effect (cell death) under hypoxic and oxic conditions, and can be as high as 3.0 for most tissues (1, 19–21). The success of fractionated radiotherapy is, therefore, partially attributed to reoxygenation of hypoxic regions over multiple treatments.

Resistance of hypoxic cells to chemotherapy is caused by a number of factors including: (i) decreased drug action in the absence of O2 (as is the case for bleomycin and etoposide); (ii) decreased effect of cell-cycle dependent agents in poorly proliferating hypoxic cells; (iii) altered pH gradients (altered activity of alkylating agents and antimetabolites); (iv) induction of gene amplification (e.g., methotrexate resistance); and (v) overall decreased drug diffusion and delivery to cells distant from functional vasculature.

However, these classic concepts of tumor resistance have recently been made more complex on the basis of data from functional studies using HR-proficient and -deficient cells (22, 23). Prolonged chronic hypoxia can lead to decreased expression of HR genes (Fig. 1), which decreases the radioresistance of these cells compared with acutely hypoxic cells and is quantified by a decreased OER (22). Furthermore, chronically hypoxic cells that are HR deficient

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**Fig. 1.** Hypoxia decreases DNA-repair protein expression in vitro and in vivo. A. Western blot of RKO colorectal cancer cells showing decreased expression of the HR DSB repair protein RAD51, and the DNA MMR protein MSH2, under hypoxic conditions in vitro (e.g., 72 hours exposure at 0.2% O2). Hypoxia can also stabilize the p53 protein as shown. HIF-1α is shown as a positive control for hypoxia. B. RKO xenograft costained in situ for hypoxia (EF5, green) and RAD51 (red). Line intensity profile across the EF5-avid gradient shows inverse association between the hypoxic marker EF5 and RAD51 in vivo. Scale bar represents 100 μm.
and reoxygenated before oxic irradiation are more radiosensitive than oxic HR-proficient cells. These studies confirmed a previous report that the OER was reduced in isogenic cell lines that were deficient in HR (23).

Thus, whereas acutely anoxic tumor cells may be highly resistant to ionizing radiation, chronically hypoxic tumor subregions may contain cells with differential radio- and chemosensitivity, which together determine the overall sensitivity of the tumor to cancer treatment (Fig. 2).

Genetic instability can also arise in anoxic and hypoxic cells (24–26). In response to anoxia, cell-cycle checkpoint-proficient cells activate ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) DNA-damage kinase–mediated intra–S-phase arrest of DNA replication. When reoxygenated, cells can generate re-active oxygen species (ROS) and DNA breaks, which lead to a CHK2-dependent, G2 arrest and attempted repair of ROS-mediated DNA damage (27–30). In contrast, diffusion-limited, chronically hypoxic cells may slowly adapt to increasingly low oxygen conditions and bypass these checkpoints. These proliferating hypoxic cells may then be prone to DNA replicative errors (13). Therefore, tumor hypoxia can be both spatially and temporally heterogeneous with dynamic gradients of oxygenation, and lead to differential biology with respect to signaling and repair of DNA damage. Models of hypoxia-mediated aggression should take into account the effects of hypoxia on DNA repair as this could alter the sensitivity of tumor cells to current cancer treatments, or provide novel treatment strategies through synthetic lethality; these concepts are discussed below.

**Hypoxia and Mismatch Repair**

DNA MMR is responsible for recognizing and repairing erroneous insertion, deletion, and misincorporation of bases that arise during DNA replication (31). Suppression of the MMR pathway by hypoxia has been previously documented with specific down-regulation of the MMR proteins MLH1, MSH2, and MSH6 (Fig. 1), leading to genomic instability (15, 32–34). Several mechanisms for the decreased gene expression have been proposed. Koshiji and colleagues reported that the altered expression of MSH2 was associated with hypoxic up-regulation of hypoxia inducible transcription factor 1α (HIF-1α), which displaced c-MYC from the msh2 promoter in a p53-dependent manner (34). Nakamura and colleagues suggested that down-regulation of the mlh1 gene was repressed by DEC1/2 and decreased binding to E-box-like motifs in the mlh1 promoter region (35). Other work has shown that the repression of MLH1 and MSH2 occurs via a HIF-1α–independent shift in occupancy from activating c-MYC/MAX to repressive MAD1/MAX and MNT/MAX complexes at the proximal promoters of both genes (36).
Based on data from germline or somatic loss of MMR gene expression, MMR-deficient hypoxic cells would be expected to be more sensitive to topoisomerase poisons such as camptothecin and etoposide (37), as well as to certain alkylating agents such as 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (38) and mitomycin C (14, 39). Conversely MMR defects can also impart resistance to many common chemotherapeutic agents, including DNA minor groove binders (40), antimetabolites such as 6-thioguanine (41), certain alkylating agents such as temozolomide (42), and certain platinum compounds such as cisplatin (43). Therefore, the functional effects of hypoxia on MMR gene expression and consequences for tumor cell radiosensitivity and chemosensitivity require further study as this may direct individualized cancer therapy (see Table 1).

**Hypoxia, the Nucleotide Excision Repair, and Fanconi Anemia Pathways**

NER is an important DNA repair pathway responsible for the removal of helix-distorting DNA adducts, including UV-induced cyclobutane pyrimidine dimers and 6-4 photoproducts. Lung cancers harboring increased expression of the NER protein ERCC1 (44, 45) have been reported to be resistant to cisplatin. Conversely, NER-deficient cells are sensitive to cisplatin and alkylators such as mitomycin C and temozolomide. The Fanconi anemia (FA) pathway primarily responds to DNA damage that causes stalling of DNA replication forks during S phase, and FA cells are also more sensitive to DNA cross-linking agents (14).

Very little is known about the effect of hypoxia on NER. Rezvani and colleagues have recently reported that HIF-1α transcriptionally regulates the expression of two NER proteins, XPC and XPD, by binding to the hypoxia-responsive elements within their promoters (46). Additionally, the NER protein RAD23B has been reported to be downregulated under hypoxia through a mechanism involving HIF-1α-dependent activation of miR-373 (47). Two contradicting reports have been published using a host-cell reactivation (NER-dependent repair of a UV-damaged plasmid) assay to measure functional NER. Yuan and colleagues first showed that hypoxia combined with low pH (24 hours × 0% O₂ + pH 6.5) decreased host-cell reactivation of a UV-damaged plasmid encoding the luciferase gene (48). Subsequently, a recent report has shown increased repair of a UV-damaged adenovirus expressing lacZ under conditions of hypoxia (12 to 24 hours × 1% O₂) or hypoxia + low pH (pH 6.5; ref. 49). Kuhnert and colleagues have recently reported that FANC D2-deficient fibroblasts are hypersensitive to radiation under hypoxic conditions; this may explain the discrepancy between the clinical and cellular radiosensitivity of FA patients (50). Further studies are needed to clarify. FA protein expression and function under hypoxic conditions.

**Hypoxia and Double-Strand Break Repair**

One of the most critical DNA lesions requiring repair are DSBs, which are primarily repaired by the HR and NHEJ pathways (51–53). HR is a template-guided repair pathway operating in the S and G2 phases of the cell cycle and results in error-free repair. By contrast, NHEJ can occur throughout the cell cycle without the use of a homologous template and can be precise or imprecise, depending on the structure of the DNA end. Several groups have reported that the expression and function of HR repair proteins, including RAD51, BRCA2, and BRCA1, are compromised under hypoxic conditions (Fig. 1; refs. 12, 22, 54). Given the relationship between HR and the cell cycle, it was an important observation that decreased HR gene expression was independent of p53, HIF-1α, and cell-cycle distribution (12, 22, 54). Data pertaining to the function of the NHEJ pathway are more conflicting, with reports suggesting it is either unchanged (54), or possibly up-regulated (55), by hypoxia.

An initial model of hypoxia-induced transcriptional repression of HR genes was proposed by Bindra and colleagues (12, 56), who showed that the hypoxic down-regulation of RAD51 and BRCA1 is associated with a switch from E2F-based transcriptional activation to that of repression. However, RNA and protein expression of HR genes can be discordant under hypoxia (54). Another model invokes translational repression as the basis for decreased HR protein expression (22, 57). Under hypoxia, this translational suppression is controlled through at least two distinct pathways; first, by protein kinase-like endoplasmic reticulum kinase (PERK)-mediated phosphorylation of eIF2α, which is required for the recruitment of aminoacylated tRNA, and second, by disruption of the

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**Table 1. Summary of known hypoxia-induced DNA repair defects and anticancer agents with potential for increased efficacy**

<table>
<thead>
<tr>
<th>Hypoxia-mediated defect in DNA repair</th>
<th>Chemotherapeutic agents with potential for increased efficacy</th>
<th>Class of agent showing synthetic lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (e.g., RAD51, RAD54, BRCA1, BRCA2, XRCC3)</td>
<td>Alkylators, topoisomerase inhibitors, ionizing radiation</td>
<td>PARP inhibitors</td>
</tr>
<tr>
<td>MMR (e.g., MLH1, MSH2, MSH6)</td>
<td>Alkylators, topoisomerase inhibitors</td>
<td>DNA polymerase inhibitors</td>
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</tbody>
</table>
mRNA cap-binding complex, eIF4F (58). However, specific studies comparing the exact role of transcription and translation in mediating differential protein expression within the MMR, NER, FA, and DSB repair pathways in hypoxic versus oxic cells have not yet been reported. Additionally, HIF-1α–dependent activation of miR-210 has been shown to down-regulate the RAD52 HR protein (47).

HR-defective cells are known to be more sensitive to mitomycin C and cisplatin, and suggest that hypoxia would drive a similar sensitivity if HR function were compromised. Indeed, similar to the sensitization of HR-defective hypoxic cells to ionizing radiation, Chan and colleagues also observed sensitization to cisplatin and mitomycin C, but not taxanes (Fig. 2C; ref. 22). Some studies suggest that these tumor cells with HR defects may also be more sensitive to etoposide (59). Additionally, HR-defective cells are more sensitive to inhibition of poly-ADP ribose polymerase 1 (PARP1) because of synthetic lethality (60–62), and this special case in relation to hypoxic cells is discussed in detail below.

Targeting Hypoxia-Induced Repair Defects: “Contextual Synthetic Lethality”

Synthetic lethality is the concept that mutation in two genes leads to death, whereas mutation of either alone is compatible with viability (63). Cells with defects in the HR pathways can be preferentially sensitized to inhibitors of the single-strand break (SSB) repair protein PARP1 (50, 60, 61, 64–68). Indeed, tumor cells exposed to chronic hypoxia leading to an HR defect have increased sensitivity to these agents (Fig. 2C; ref. 62). The use of PARP inhibitors to target hypoxic tumor cells is an example of “contextual synthetic lethality,” in which a hypoxia-induced repair defect is targeted by inhibiting or disrupting the backup pathway. This approach has significant therapeutic potential as highly potent and selective PARP inhibitors have already shown clinical effectiveness in treating BRCA-deficient tumors (64). It, therefore, seems reasonable to take advantage of deficiencies in DNA repair to kill hypoxic cells that could acquire a repair-deficient and mutator phenotype. This approach would still preserve the therapeutic ratio because very few normal tissues contain hypoxic cells.

A caveat to this approach is the requirement for proliferation, as PARP inhibitors mediate their toxicity by inducing collapsed replication forks (61). It has been previously shown that tumor cells can have hypoxia-mediated decreases in DNA-repair protein expression at moderate levels of hypoxia that still allow for proliferation (22). Therefore, hypoxic tumor cells at an intermediate distance from the blood vessels would theoretically still be sensitive to this approach. This hypothesis is testable using bromodeoxyuridine staining to detect proliferating cells, EF5 staining to detect hypoxic cells, RAD51 staining to detect HR-deficient cells, and γH2AX staining to detect DNA damage and/or cell death.

Recently, deficiency in the MMR proteins MSH2 and MLH1 were shown to be synthetically lethal with disruption of the DNA polymerases POLB and POLG, respectively (69). Both of these MMR proteins are known to be down-regulated by hypoxia and therefore inhibition of POLB or POLG may show contextual synthetic lethality with hypoxia. At the moment, clinically useful inhibitors of POLB or POLG are not yet available, but given the strong inhibition of MSH2 and MLH1 by hypoxia, this is a concept that warrants further study. A final example is the observation that the FA pathway can be compromised under hypoxic conditions (50), and FA defective cells are more sensitive to ATM inhibitors (70). Table 1 summarizes known hypoxia-induced DNA repair defects and agents that may potentially have synthetic lethality or increased efficacy under hypoxic conditions.
Hypoxia and “Contextual Loss of Heterozygosity”

We propose that tumor hypoxia may drive malignant progression, and possibly carcinogenesis, through a model of "contextual loss of heterozygosity" (LOH) for DNA repair genes. Instead of an inactivating mutation, contextual LOH could occur by hypoxia-mediated loss of expression and function of one allele of a DNA repair gene, in which the other allele is already inactivated by genetic deletion, mutation, or hypermethylation (Fig. 3). If the gene in question is a tumor suppressor gene involved in DNA-damage checkpoint control (e.g., ATM, ATR, Rb, p53, or MDM2) or a critical DNA repair protein (PARP1, DNA-PKcs, BRCA1 or BRCA2), malignant transformation or progression may result. In fact, we have documented monoallelic losses for a number of DSB and SSB repair genes in prostate cancer, a tumor in which hypoxia is known to be a negative predictive factor (3, 71, 72). This model could also be tested for colorectal cancer, in which regions of hypoxia have been documented in normal mucosa, benign adenoma, and carcinomas (73). Germline mutations in MLH1 or MSH2, two genes known to be suppressed by hypoxia, are linked to hereditary nonpolyposis colorectal cancer (74). Furthermore, accumulation of K-ras mutations (a common alteration in colorectal cancer) has been correlated to hypoxia-induced decreases in MSH2 expression (15). Thus, it is conceivable that colorectal cells with only one normal allele of mlh1 or msh2 could have further reduced functional protein expression under hypoxic conditions. This situation could ultimately drive genetic instability, carcinogenesis, and tumor progression. A similar biology could also underlie hypoxic modification of NER status and UV- or carcinogen-induced skin cancers (75–78). This hypothesis will require testing of the effect of hypoxia on carcinogenesis and tumor progression using isogenic models, which are wild-type, heterozygous, or homozygous null for DNA-repair gene expression and function.

Conclusions

A prerequisite for the use of novel therapies or predictors of outcome based on these preclinical studies is the ability to predict the fraction of repair-deficient hypoxic cells in solid tumors. One strategy using xenografts could involve using a serial injection of two different hypoxic markers, such as pimonidazole and EF5 in combination with markers of proliferation (e.g., bromodeoxyuridine), and blood vessels, as described by Ljungkvist and colleagues (79). Intratumoral regions that are matched for the two hypoxic markers are chronically hypoxic, and those mismatched for staining are acutely hypoxic. Simultaneous staining for DNA repair proteins (e.g., RAD51) would allow correlation of hypoxic states to DNA-repair protein expression. The relative repair of DNA-DSBs could then be tracked as a function of acute and chronic hypoxia following DNA damage and be correlated to tumor radio- and chemoresponse. If proven, this concept will be clinically feasible when innovative, noninvasive imaging techniques are developed to track both acute and chronic hypoxia during treatment, to allow effective intervention with novel therapies including the use of synthetically lethal approaches.

To this end, noninvasive techniques for imaging tumor hypoxia are being developed, including the use of radio-labeled 2-nitroimidazoles imaged with positron emission tomography (PET; 18F-FMISO, 18F-F-AZA, 18F-EF5, and 60Cu-AITSM), or single photon emission computed tomography (SPECT; 123I-AZA) to achieve linically useful signal-to-noise ratios (80). Additionally, functional computed tomography (CT) and blood oxygen-level dependent (BOLD) magnetic resonance imaging (MRI) are being developed to provide information about the tumor microenvironment (perfusion, vascular permeability, extracellular volume, and hypoxia) as well as detailed anatomic information (81–83).

In summary, the current literature has shown that hypoxic tumor cells can have suppression of the HR, NER, and MMR pathways. However, the impact of hypoxia on the NHEJ and base-excision repair pathways still requires additional study. Further understanding of the contextual synthetic lethality to these and other DNA damage signaling and repair pathways could define new approaches to chemoprevention and selection of the best agents to individualize cancer therapy.

Disclosure of Potential Conflicts of Interest

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