Molecular Pathways: A Novel Approach to Targeting Hypoxia and Improving Radiotherapy Efficacy via Reduction in Oxygen Demand

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Abstract

Tumor hypoxia presents a unique therapeutic challenge in the treatment of solid malignancies. Its presence has been established to be a poor prognostic factor in multiple cancer types, and past hypoxia-directed approaches have yielded generally disappointing results. Previous approaches have centered on either increasing oxygen delivery or administering agents that preferentially radiosensitize or kill hypoxic cells. However, a novel and potentially more effective method may be to increase therapeutic benefit by decreasing tumor oxygen consumption via agents such as metformin or nelfinavir in a patient population that is enriched for tumor hypoxia. This promising approach is currently being investigated in clinical trials and the subject of this article.

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the strategies currently under investigation to address the issue of tumor hypoxia and of the biologic rationale underlying novel therapeutic strategies to alter tumor oxygenation as a means to improve the efficacy of radiotherapy.

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Background

Hypoxia/anoxia is a well-characterized component of the solid tumor microenvironment. In their comprehensive review of studies in the literature examining tumor hypoxia using polarographic needle electrode systems, Vaupel and colleagues (1) found that the overall median pO2 levels in malignant brain tumors and cancers of the uterine cervix, head and neck, and breast were 10-mm Hg with the hypoxic fraction (percentage of tumor with pO2 < 2.5 mm Hg) approximately 20% to 30%. In general there is not a correlation between tumor diameter and median pO2 or hypoxic fraction. For many tumors, there is spatial heterogeneity of hypoxia, i.e. there is no characteristic topologic distribution of pO2 within tumors (periphery vs. center). For example, Evans and colleagues (2) showed that there was substantial intra- and intertumoral hypoxic heterogeneity within human grade 4 glial neoplasms. The majority of cells within these tumors had levels of hypoxia that were mild to moderate (defined as 10%–0.5% pO2) rather than severe (approximately 0.1% pO2).

Even if only a minority of cells within a tumor are hypoxic, this can have a negative effect on outcome (3). Hypoxia is associated with chemoresistance, increased genomic instability, and the propensity for invasion and metastasis (4). Hypoxic cells are more resistant to radiotherapy because O2 must be present for optimal fixation of DNA damage induced by ionizing radiation (5). Hypoxic cells are relatively resistant to radiotherapy, requiring 2.5 to 3 times the radiation dose as normoxic cells to result in the same level of cell killing (5). Gray and colleagues (6) found this to be the case in a landmark study examining a wide range
of cells and tissues. Furthermore, these authors showed that it was the presence of oxygen during the actual time of irradiation that resulted in sensitivity. A free radical is the primary product induced by ionizing radiation that leads to DNA damage/lethality. When oxygen, which is highly electron affinic, is present, it reacts rapidly with the free radical, hence “fixing” the damage. Without oxygen, this free radical damage can be reversed by hydrogen donation from nonprotein sulfhydryls in the cell. For these multiple reasons, hypoxia correlates with poor prognosis in a variety of cancers, including carcinoma of the cervix (7), head and neck cancer (8, 9), and sarcomas (10). In patients with head and neck squamous cell carcinomas (HNSCC) treated with definitive radiotherapy, hypoxia has been shown to adversely affect not only local/regional control but also survival (3).

For the reasons discussed above, multiple approaches to hypoxia-directed therapy have been used in patients receiving radiotherapy. One strategy to counter hypoxia is to reverse it by increasing oxygen delivery using hyperbaric oxygen (HBO). Although some clinical trials demonstrated a modest benefit in cancers of the head and neck and cervix (11–13), other trials demonstrated no appreciable benefit (14, 15). An alternative approach is the use of hypoxic cell radiosensitizers, specifically nitroimidazoles, such as misonidazole and nimorazole. These drugs are electron affinic, undergoing bioreductive activation under hypoxic conditions leading to the creation of reactive intermediates that can form adducts with target molecules within the cells. For example, misonidazole reacts with intracellular glutathione (GSH) to form covalently bound conjugates. As GSH is an important free radical scavenger, conjugation/depletion of this antioxidant leads to increased DNA damage when cells are exposed to radiation. In this way, nitroimidazoles preferentially radiosensitize hypoxic cells because they do not undergo bioreduction under normoxic conditions. Hence, the use of nitroimidazoles should theoretically increase the therapeutic ratio of radiation. Misonidazole can also be used to image hypoxia by PET scanning when bound to radioactive fluoromisonidazole (18F-MISO), as discussed below. Multiple clinical studies using different nitroimidazoles in various cancer types have been performed, and again show mixed results, with modest benefit (16, 17), no benefit (18, 19), or even worse outcomes (20). Direct, comparative clinical evidence has been provided by Overgaard, who recently published a systematic review and meta-analysis of randomized trials with 4,805 patients with HNSCC treated with radiotherapy and some type of hypoxia-modifying therapy, including HBO, nitroimidazole, or breathing carbogen or normobaric oxygen (21). The conclusion for this analysis was that hypoxic modification of radiotherapy in HNSCC did result in a significantly improved therapeutic benefit, most clearly seen in locoregional control (OR, 0.71; 95% confidence interval, 0.63–0.80; P < 0.001) and disease-specific survival. The improvement in locoregional control translated into an improvement in overall survival for 60% of patients.

Another approach to combat hypoxia is with hypoxic cytotoxins, such as mitomycin C and tirapazamine, which are reduced intracellularly to form an active cytotoxic species in the presence of hypoxia. Trials of mitomycin C in head and neck cancer have shown mixed results, with some reporting improvement in local control (22, 23), whereas others demonstrate no benefit (24, 25). A phase III trial (TROG 02.02, HeadSTART) of tirapazamine for advanced-stage head and neck cancers did not show an overall benefit (26), but a subset analysis demonstrated a trend toward improved locoregional control (92% vs. 81% at 2 years) favoring the tirapazamine arm in p16-negative [with p16 a surrogate for human papillomavirus (HPV)] patients with oropharyngeal cancer (27). The investigators also found that the patients who derived the greatest benefit from the addition of tirapazamine were those with hypoxic tumors as demonstrated on 18F-MISO-PET imaging (28), suggesting that hypoxia-directed therapy can be beneficial, and that upfront patient selection via methods such as imaging is critically important.

The clinical data reviewed above, particularly from the Overgaard meta-analysis, suggest that there may be merit to hypoxia modification in patients treated with radiotherapy, although many individual trials have failed to show a clear improvement in locoregional control or survival (21). Possible explanations for this may be the substantial dose-limiting toxicity associated with some of these agents (such as peripheral neuropathy with misonidazole), thus limiting their dosage in trials or the failure to enrich the study populations for hypoxic tumors, thus diluting the effect of hypoxia modification. The remainder of this review focuses on novel strategies for tackling the hypoxia problem in patients receiving radiotherapy, including drugs that may be better tolerated or imaging techniques that will better identify patients who might benefit from such therapy.

**Clinical–Translational Advances**

An alternative approach to attacking the problem of hypoxia is to address it on the demand side, that is, to decrease O2 consumption. Based on mathematical modeling, Secomb and colleagues (29) predicted that even a 30% decrease in O2 consumption would decrease the hypoxic fraction from 37% to 11%. This approach has not been as well explored as targeting the supply side or using agents that are preferentially toxic to hypoxic cells. Recent reports describe clinically relevant agents that decrease O2 consumption and could lead to improved radiation response, and these results are described below.

In preclinical models, including spheroids, there are published data showing that inhibition of O2 consumption using respiratory inhibitors can lead to increased killing by radiation (30). Similar findings have been made with such drugs as meta-iodobenzyl- guanidine (31) and arsenic trioxide (32). Arsenic trioxide has been postulated to decrease oxygen consumption via inhibition of the electron transport chain (see Fig. 1; ref. 33). However, the toxicity of these agents in vivo has prevented their use in patients. On the other hand, metformin and rosiglitazone, which are used to treat type 2 diabetes, have been also been shown to reduce O2 consumption in vitro by inhibiting complex I in the mitochondrial respiratory chain (34). As shown in Fig. 1, complex 1 is the first complex in the electron transport chain that resides in the mitochondrial inner membrane. In complex 1, two electrons are removed from NADH and transferred to a lipid-soluble carrier, ubiquinone. Complex 1 also translocates four protons across the membrane, thus producing a proton gradient. Zannella and colleagues (35) recently showed that metformin increases oxygenation in vivo within tumor xenografts and improves radiotherapy response by delaying tumor regrowth of xenografts. This in vivo effect was not due to any change in the intrinsic (in vitro) radiation response of the tumor cells induced by metformin, so it was presumed to result from improved oxygenation. The authors went on to analyze clinical data to see whether metformin use during radiotherapy might be associated with better
outcome. Indeed, they found that patients with localized prostate cancer who had been on metformin during their radiation had a reduction in biochemical relapse compared with those who had not been on the drug. Although there is no proof that the improvement in disease outcome was due to changes in oxygenation, these data are consistent with this idea.

Storozhuk and colleagues (36) found that the addition of metformin to radiotherapy led to delayed tumor regrowth when given to mice bearing A549 and H1299 lung adenocarcinoma xenografts. Simone and colleagues (37) made similar observations in a mouse model, and in a clinical correlate went onto show a dramatic decrease in local relapse in a subset of patients with stage III non–small cell lung cancer (NSCLC) treated with chemoradiotherapy who were taking metformin for diabetes compared with patients not taking the drug. Similar retrospective data analyses have suggested that metformin use is associated with improved treatment response following chemoradiotherapy in patients treated for esophageal and rectal cancers (38, 39).

Drugs that target the PI3K–Akt pathway also decrease tumor hypoxia (40, 41). In our own work, we have shown that the HIV protease inhibitor nelfinavir can decrease tumor hypoxia (42, 43). Nelfinavir has been shown to inhibit the PI3K–Akt pathway, although it probably does not do so directly (44). How does PI3K–Akt inhibition alter tumor oxygenation? There is some evidence (40, 41, 43) that these drugs may improve blood flow by “normalizing” vascular blood flow within tumors. Hence, these drugs may address the problem on the supply side. However, there is also evidence that they may affect the demand side. Kelly and colleagues (45) have shown that treatment of cells in vitro with inhibitors of the PI3K pathway, including NVP-BEZ235 and NVP-BKM226, both inhibitors of PI3K–mTOR, results in decreased O2 consumption. We have made similar observations using multiple drugs such as the Akt inhibitor GDC-0068 and the dual PI3K–mTOR inhibitors NVP-BG7226 and GDC-0098 (A. Maity; unpublished data). Pharmacologic or genetic inhibition of this pathway decreased the oxygen consumption rate (OCR) in vitro in SQ20B HNSCC cells and other cell lines by 30% to 40%. Inhibition of this pathway also increased phosphorylation of the E1α subunit of the pyruvate dehydrogenase (PDH complex
of Ser293 in SQ20B cells; Fig. 1). This phosphorylation inhibits activity of this critical gatekeeper of mitochondrial respiration, which catalyzes the conversion of pyruvate to acetyl coA, which, in turn, can then enter the Krebs cycle to start oxidative phosphorylation (OXPHOS). Hence, inhibition of the PDH complex would be predicted to decrease OXPHOS and reduce OCR, and offers an explanation for how the PI3K–Akt pathway affects O2 metabolism, although we have not yet determined the exact steps connecting Akt to E1\tau phosphorylation. As further evidence of a causal relationship, introduction of exogenous PDH-E1\tau that contains serine to alanine mutations, which can no longer be regulated by phosphorylation, blunted the decrease in OCR seen with PI3K–mTOR inhibition. We have also shown that nelafitinib decreases in vitro OCR in a variety of cells. Studies are currently under way to determine mechanistically how this drug reduces OCR. Decreasing tumor hypoxia should increase \textit{in vivo} radiation response. In fact, dual PI3K–mTOR inhibitors and nelafitinib have both been shown to delay tumor regrowth following radiation (41, 42, 44).

Other clinically useful agents have also been shown to decrease O2 consumption by cells. Using electron paramagnetic resonance (EPR) oximetry, a proven method to obtain direct absolute measurement of oxygen in tissue, Crokart and colleagues (46) found that the \textit{in vivo} administration of commonly used NSAIDs, including diclofenac, indomethacin, and piroxicam, caused a rapid reduction in hypoxia within murine liver tumors and fibrosarcomas. The administration of NSAIDs led to decreased tumor perfusion, so the reduction in hypoxia was not caused by an increase in oxygen supply, but more likely primarily mediated by a decrease in mitochondrial respiration. The administration of NSAIDs led to an augmentation in tumor regrowth delay following a single 18-Gy fraction of radiation. Using EPR oximetry, Danhier and colleagues (47) showed that the chemotherapeutic agent paclitaxel in a micelle formulation (M-PTX) dramatically reduced hypoxia within tumors grown in mice. Additional experiments showed that this change was due to both an increase in blood flow and an inhibition of O2 consumption. This dual effect led to synergistic, functional improvement when M-PTX was delivered with 10-Gy irradiation.

Although these results are very interesting and suggestive that agents that improve tumor oxygenation can lead to improved radiation response, they are hardly definitive. First, these studies have been performed in mouse tumor models, which for obvious reasons may not reflect the situation in patients. Second, interpretation of these results is confounded by the fact that these drugs may also affect intrinsic radiosensitivity. Therefore, their \textit{in vivo} effects when combined with radiation may not be exclusively due to altered tumor oxygenation. For example, metformin, which in the study cited above was not found to alter \textit{in vitro} radiosensitization (35), has been shown by others to impair the repair of DNA damage following radiation \textit{in vitro} (48) and lead to radiosensitization (36).

**Current Clinical Trials**

As discussed above, there is retrospective evidence that patients with prostate, lung, and gastrointestinal cancers taking metformin may have better outcomes following radiotherapy (35, 38, 39). A phase II trial that is now open in the United States (NRG-LL001, NCT02186847) randomizes patients with stage III NSCLC receiving chemoradiotherapy to either receive metformin during radiation or not. A similar study (ALMERA, NCT02115464) is currently recruiting patients in Canada. Metformin is also being investigated in the treatment of prostate cancer. A clinical trial (NCT01864096) is currently being planned at Princess Margaret Cancer Center in Toronto in which nondiabetic patients with early-stage prostate cancer are given metformin as a lead-in before the initiation of definitive radiotherapy. Biopsies will be taken before metformin administration, and then again before the start of radiotherapy, which will allow for assessment of biomarkers reporting on metformin activity and tumor hypoxia (R. Bristow and M. Koritzinsky; personal communication).

Although these studies will give a clearer answer as to whether the use of metformin will improve outcomes when administered with chemoradiotherapy, it is difficult to conclude that the effect is secondary to changes in oxygenation. One way of directly studying this would be to assess hypoxia noninvasively in patients receiving metformin or other drugs that can alter tumor oxygenation. Hypoxia imaging, using such agents as radiolabeled nitroimidazoles, has been available for some time and is being slowly introduced into the clinical setting (49). Therefore, the means currently exist to treat patients with a given agent and determine radiographically whether their tumors become less hypoxic. At our institution, we are using such an approach in an open phase II trial (NCT02207439) using nelafitinib in combination with cisplatin and radiation for locally advanced, HPV-negative, larynx cancer. The patient population for this trial is enriched for hypoxia (tobacco-induced and HPV-negative), with evidence that hypoxia modification is beneficial (27, 28). Patients undergo baseline hypoxia imaging (18F-EF5 PET/CT), receive 2 weeks of a “lead-in” period of nelafitinib, undergo repeat 18F-EF PET/CT, and then are treated with standard platinum-based chemoradiotherapy with nelafitinib. We hypothesize that the patients showing the greatest decrease in tumor hypoxia secondary to nelafitinib, as assessed by EF5-PET/CT scanning, will derive the greatest benefit.

**Conclusions**

Tumor hypoxia remains a significant issue in multiple cancers, with its presence associated with poor clinical outcomes. We believe decreasing oxygen consumption to be a promising method in reversing hypoxia. Identifying, selecting, and enriching populations with hypoxic tumors will be paramount to conduct clinical trials of novel agents that we hope and anticipate will improve outcomes for our patients.

**Authors’ Contributions**

Conception and design: A. Lin, A. Maity
Development of methodology: A. Lin
Writing, review, and/or revision of the manuscript: A. Lin, A. Maity

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