Review

Cancer Research

Oxygen-Enhanced MRI Is a Major Advance in Tumor Hypoxia Imaging

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Introduction and Historical Perspective

It is well established that hypoxia is prevalent in human tumors and is associated with resistance to radiotherapy and chemotherapy (1–3); furthermore, hypoxia contributes to immune checkpoint activation (4–7), higher likelihood for development of metastasis, and worse overall prognosis (8). Despite this knowledge, there is no established method for measuring hypoxia in patients, nor are there FDA-approved means for reducing its impact in the management of patients with cancer.

Literally, dozens of methods have been tried both preclinically and clinically to reduce tumor hypoxia, with varying degrees of success (9). A meta-analysis of over 10,000 patients enrolled in nearly 90 randomized trials over the past 40 years revealed an OR of 0.77 in favor of hypoxia modification strategies (9). Phase III trials have been conducted utilizing radiotherapy with or without (i) increasing oxygen delivery with hyperbaric oxygen (10, 11) or carbogen breathing with nicotinamide (12); (ii) the hypoxic cytotoxin, tirapazamine (13, 14). Evofosfamide, another hypoxic cytotoxin, was found to yield improved survival in a randomized phase II trial when combined with gemcitabine in patients with pancreatic cancer (15). Hypoxic radiosensitizing drugs that mimic the action of oxygen to increase radiosensitivity of hypoxic cells have also been tested in randomized studies (16). Some trials were positive, but not a single method to reduce the impact of tumor hypoxia has been adopted as standard-of-care for any cancer on a worldwide basis. One of the major limitations of all prior trials has been that tumors were not screened for presence of hypoxia prior to patient enrollment. In three trials, imaging or biopsy-based assays were performed, prior to therapy, to identify hypoxic tumors. In all three studies, much better treatment response was observed in those tumors identified as being hypoxic at baseline (12, 16, 17). Unfortunately, however, tumor hypoxia has not been an entry criterion in any hypoxia modification trial reported thus far. Therefore, the prognostic value of hypoxia as a selection marker for hypoxia modification trials is not truly established. Ideally, future clinical trials would incorporate a method that determines whether a tumor is hypoxic before a patient is enrolled in a clinical trial that involves hypoxia modification. This would ensure that the modification is applied only to the cohort that can benefit.

Proof that hypoxic tumor cells could contribute to reduced effectiveness of radiotherapy and chemotherapy was enabled by the development of a polarographic needle (Eppendorf electrode) that could be used clinically. The polarographic sensor was embedded in a needle and movement of the electrode into tissue after insertion under image guidance was computer controlled. Hoeckel was the first to report that hypoxia, as measured with the Eppendorf, was associated with worse survival in patients with cervix cancer (18). To date, over 100 articles have been published using the Eppendorf, involving nearly every solid tumor type (19, 20). In patients with cancers of the head and neck (21, 22), cervix (23, 24), and prostate (25), who received radiotherapy, the presence of hypoxia was independently associated with worse local control. In patients with breast cancer who were treated with neoadjuvant chemotherapy, reduction in hypoxia was associated with paclitaxel treatment (26). Furthermore, hypoxia was associated with greater likelihood for metastasis in patients with soft-tissue sarcomas and cervix cancer (24, 27, 28). Biopsy-based approaches have also been used to identify hypoxic tumors, using "hypoxia marker" drugs such as pimonidazole (12). These invasive methods have been marvelous tools to establish the clinical importance of hypoxia, but they cannot not be incorporated into routine clinical practice because of their invasive nature and sparse tissue sampling, which may not reflect the extent of tumor heterogeneity (29).

Imaging of Hypoxia Is the Solution for Selecting Patients for Clinical Trials

The ideal method for hypoxia detection is imaging. The rationale is 2-fold: (i) the extent of hypoxia varies spatially and (ii) the extent of hypoxia varies temporally (cycling hypoxia). Cycling hypoxia occurs with frequencies ranging from 0.1 to 3 cycles per hour (30, 31). Cycling hypoxia may increase oxidative stress in tumors and indirectly upregulate stress survival pathways, such as HIF1 and NF-κB (32). The impact of cycling hypoxia on treatment response or patient outcome has never been tested, although imaging methods capable of capturing aspects of cycling hypoxia are available.

The ideal method for measuring hypoxia would (i) be noninvasive, (ii) be amenable to repeated measures, (iii) have high spatial resolution, (iv) be cost-effective, and (v) be directly translatable to human trials. There is a variation in the ability of reported hypoxia imaging methods to meet these performance criteria. See Supplementary Table S1 for a comparison of reported methods.

In this issue of *Cancer Research*, a manuscript from the group of O'Connor validates oxygen-enhanced MRI (OE-MRI) to quantify the spatial variation of hypoxia (33). The principle is based on measurement of R_1, which is sensitive to the oxygen concentration in interstitial fluid and plasma. The approach involves examining the change in R_1 after a challenge with oxygen breathing. The author's hypothesis was that the subvolumes refractory to change in R_1 ($\triangle R_1 = 0$) during oxygen breathing would be hypoxic. They tested the hypothesis by comparing the spatial distribution of $\triangle R_1$ values against the distribution of the hypoxia marker drug

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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pimonidazole in image-registered sections of three tumor lines that varied in extent of hypoxia. Pimonidazole binds to hypoxic cells to form stable protein adducts when oxygen tensions are <10 mm Hg; the adducts are detected by IHC (34, 35). Here, O'Connor found linear correlations between pimonidazole binding and $\triangle R_1$. This type of correlation has also been observed in a brain tumor model (36). O'Connor also demonstrated that correction for perfusion may be necessary for tumors that exhibit extensive necrosis. This was done using dynamic contrast enhanced (DCE)-MRI. Finally, they demonstrated that the hypoxic fraction ($\triangle R_1 = 0$) increased when animals were treated with hydralazine, a vasoactive drug that is well known to increase tumor hypoxia by decreasing perfusion.

The advantage of the OE-MRI method versus other imaging approaches to detect hypoxia is that it is amenable to repeated measures, which enables assessment of cycling hypoxia and response to hypoxia-modifying approaches. Second, MRI has better spatial resolution (Fig. 1) and is less expensive than PET. The inclusion of DCE-MRI to screen for necrosis may be considered a disadvantage of OE-MRI, but both $\triangle R_1$ and DCE-MRI could be incorporated into one imaging session. This method is also potentially superior to blood-oxygen-level dependent (BOLD) MRI, which is based upon measurement of $R_2{}^*$, an MR parameter that is sensitive to the oxygenation state of hemoglobin (37). Aside from the limitations of the BOLD method, as indicated by the authors, hyperoxic gases can be vasoactive. The vasoactive effects can change tumor perfusion and affect hematocrit, which independently affects the BOLD signal (38). As the $\triangle R_1$ method is not dependent upon hemoglobin saturation, it would not be influenced by hemodynamic effects of hyperoxic gas breathing.

The last point to make regarding this article is whether there is a physiologic basis for tumor regions being refractory to hyperoxic gas challenge. First, there are multiple studies demonstrating that subregions of tumors do not increase in pO_2 in response to hyperoxic gas breathing. Falk studied the oxygenation of human tumors with the Eppendorf electrode during air and carbogen breathing, finding a proportion of tumors that were refractory to this challenge (39). Similar results were reported in a rat prostate tumor model using perfluorocarbon magnetic resonance spectroscopy, which can be calibrated to directly measure pO_2 (40). The underlying physiologic mechanism for this lack of response to hyperoxic gas challenge is related to longitudinal oxygen gradients. Longitudinal gradients are defined as the decline in vascular pO_2 moving from arteries to arterioles and finally to capillaries. These gradients exist because oxygen is lost from the blood as it traverses from the lung and into the general circulation. In the hamster cheekpouch, for example, terminal arteriolar pO_2 is near 15 to 20 mm Hg during air breathing (41). In comparison, blood gas pO_2 is near 100 mm Hg during air breathing. Consequently, 80% of oxygen is lost from the vasculature before blood enters the terminal vascular bed. In normal tissues, there is a redundancy in arteriolar supply and microvessels to the extent that the tissues remain sufficiently oxygenated even in the presence of the longitudinal O_2 gradient. Tumors, in contrast, have relatively few arterioles, so the efficiency in maintaining adequate oxygen delivery is significantly compromised. We verified

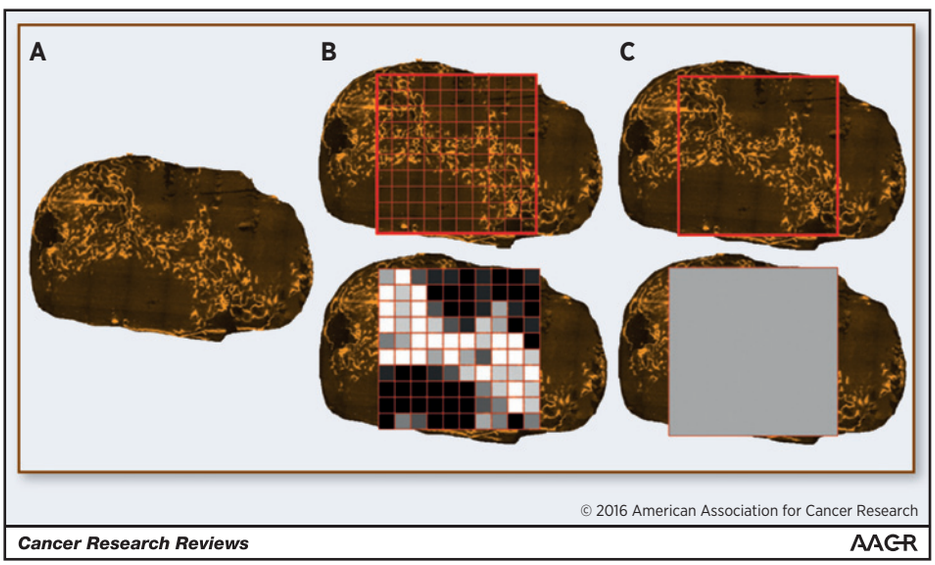

Figure 1.
A, histologic section of a fibrosarcoma grown in a Fischer-344 rat. The tumor is approximately 16 × 12 mm in cross section. This tumor was part of a previous report involving PET imaging of hypoxia; details of histology methods and analysis were described in that report (35). Briefly, the rat was administered with the hypoxia marker drug, EF5, 3 hours prior to sacrifice; orange staining regions indicate EF5 binding in hypoxic regions. The lobes on either side of the prominent hypoxic band represent viable tissue as demonstrated by hematoxylin and eosin stain and perfusion marker (not depicted). B, each small red box represents 1 mm^2 spatial resolution, which is typical of that achieved with MRI (top). The bottom section discretizes intensity of EF5 staining in each box in grayscale. The most intensely stained regions are white, nonstaining regions are in black, and intermediate regions are in gray. With this spatial resolution, the hypoxic band is still clearly visible. C, the red box represents 1 cm^2 spatial resolution, which is more typical of PET and optical methods (top). Bottom, intensity of EF5 staining on the same grayscale as B. Here the color is coded as gray, because the voxel contains a mixture of hypoxic and nonhypoxic regions. These partial volume effects obscure the presence of the hypoxic band and instead report the voxel as representing an intermediate level of hypoxia.

that tumor arterioles are subject to the same longitudinal oxygen gradients as normal tissues (42). Using skin-fold window chambers containing a rat mammary carcinoma, we used polarographic microelectrodes to show that the average pO_2 of tumor-feeding arterioles was 30 mm Hg. When breathing gas was switched to 100% O_2, terminal arteriolar pO_2 only rose by a factor of 2, whereas blood gas pO_2 increased 5-fold (from 100 to 500 mm Hg). This steep in the oxygen gradient occurs because hemoglobin is fully saturated in the lung with air breathing. Therefore, excess O_2 from hyperoxic gases can only be absorbed in plasma, which cannot retain O_2 to the same extent as hemoglobin. Indeed, it was shown that tumor microvessels most distant from feeding arterioles were hypoxic and refractory to breathing either carbogen or oxygen (43).

In summary, the rationale for being able to measure tumor hypoxia remains exceedingly compelling. To maximize the likelihood that patients will benefit from methods to selectively eliminate hypoxic cells, methods to identify patients who have hypoxic tumors are urgently needed. The OE-MRI method described in the article by O'Connor and colleagues, holds promise as a relatively cost-effective method that could be adopted for clinical trials with minimal additional validation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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