

Tumor Hypoxia Imaging

□□ *Commentary on Rajendran et al., p. 5435*

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In this issue of *Clinical Cancer Research*, Rajendran et al. (1) have assessed the value of pretherapy fluoromisonidazole (FMISO) positron emission tomography (PET) imaging, an indicator of tissue hypoxia, in predicting survival of 73 patients with head and neck cancers. In this study, the FMISO imaging results were not used to guide therapeutic management and, thus, had no influence on the survival results. The images of 58 (79%) patients indicated significant hypoxia based on a PET image intensity threshold criterion established by the experienced University of Washington group. A univariate analysis showed a significant correlation between survival and two measured variables: the maximum tumor/blood ratio (T/B_{\max}) at 4.5 hours after FMISO administration ($P = 0.002$) and the hypoxic tumor volume ($P = 0.04$). The presence of node metastases was also correlated with survival ($P = 0.01$). A similar univariate analysis of a subset of 52 patients who had a 2'-fluoro-2'-deoxyglucose (FDG) PET scan did not yield a correlation between FDG standardized uptake value and survival ($P = 0.14$).

This result is surprising because in an earlier publication, Rajendran et al. (2) showed good voxel-by-voxel correlation between FDG and FMISO uptake in head and neck cancer (0.62), but not in breast cancer (0.47), glioblastoma multiforme (0.38), or soft tissue sarcoma (0.32). Interestingly, the investigators report that no variable was predictive of survival in a multivariate analysis that included the FDG maximum standard uptake value, whereas both nodal status and T/B_{\max} (or HV) were highly predictive when the FDG maximum standard uptake value was removed from the model. Could the biology behind these observations shed some light on these results?

The Role of O₂ and the O₂ Molecular Sensing and Regulating Mechanisms in Tumor Biology and Metastases

Hypoxia or oxygen deprivation is a key factor in tumor progression and resistance to therapy due to its effect on various metabolic, molecular-genetic, pathophysiologic adaptive processes including neoangiogenesis. Tumor hypoxia is a

spatially and temporally heterogeneous phenomenon, resulting from the combined effect of many factors, including tumor type and volume, disease site (specific organ or tissue), regional microvessel density, blood flow, oxygen diffusion and consumption rates, etc. The most important regulatory factor of the hypoxia-signaling pathway activity in cells is hypoxia-inducible transcription factor 1 (HIF-1; Fig. 1). HIF-1 mediates adaptive responses to reduced O₂ availability. HIF-1 is a heterodimeric protein consisting of an oxygen-regulated α -subunit and a stable β -subunit (3). HIF-1 α undergoes rapid turnover in the presence of oxygen (half-life is <5 minutes), being degraded by the ubiquitin-proteasome pathway through the interaction with the von Hippel-Lindau (VHL) protein (4). VHL recognition of the HIF-1 α subunit is dependent on the hydroxylation of conserved proline residues within HIF-1 α , which occurs only when oxygen is available (5, 6).

Recent studies indicate that many common tumor-specific genetic alterations also lead to increased HIF-1 α expression and/or activity. Thus, genetic and physiologic alterations within tumors may act synergistically to increase HIF-1 transcriptional activity and, consequently, to putative development of invasive and metastatic properties that define the aggressive cancer phenotype (7). For example, p53 loss-of-function leads to an increase in HIF-1 and vascular endothelial growth factor expression. HIF-1 expression increases the stability of p53 (8), whereas p53 decreases the stability of HIF-1 in a murine double minute-2-dependent manner (9). The phosphatidylinositol 3-kinase/AKT pathway also influences HIF-1 α levels through transcriptional regulation (in contrast to the proteasome degradation pathway) via the downstream effector mammalian target of rapamycin (10). HIF-1 α post-translational modifications such as phosphorylation, hydroxylation, and stabilization cause the up-regulation of many HIF-1 α inducible genes [e.g., vascular endothelial growth factor (VEGF), erythropoietin (EPO), and glucose transporters (GLUT)] and key glycolytic enzymes, including hexokinase (11), even under normoxic conditions. The up-regulation of these tissue factors and enzymes contributes to a more aggressive tumor phenotype.

Hypoxia induces a shift from aerobic to anaerobic glycolysis (Embden-Meyerhof pathway), which is associated with increased glucose utilization in both normal and malignant cells (12). During the adaptive response to hypoxia, the expression of genes encoding key glycolytic enzymes and glucose transporters is increased (13). Paradoxically, tumor cells growing under conditions of normal oxygen tension can also show an elevated glycolytic rate, known as the Warburg effect (14). Namely, an increased expression of glycolytic enzymes and glucose transporters can be induced by activation of one of several oncogenic signaling pathways (refs. 15, 16; Fig. 1).

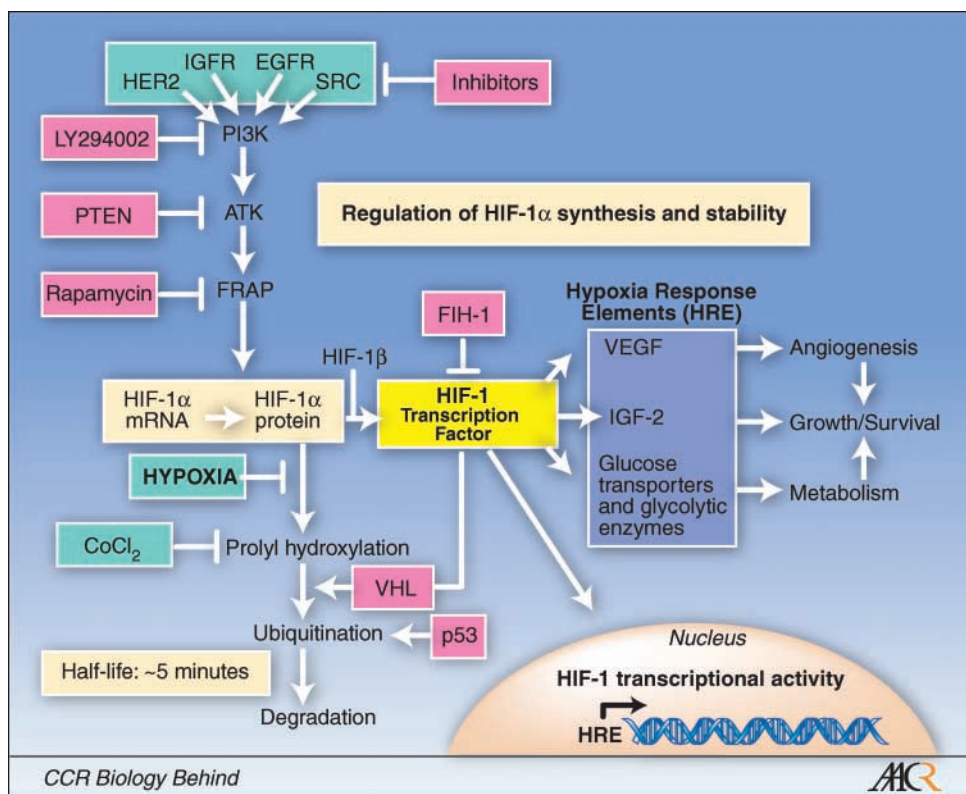
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Received 3/2/06; accepted 3/7/06.

Grant support: Grants R01 CA84596 and P01 CA59017 (C. Ling) and grants R01 CA83066, R01 CA102673, P50 CA86438, and R21 CA093501 (R. Blasberg).

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doi:10.1158/1078-0432.CCR-06-0517

Fig. 1. Regulation of HIF-1 α synthesis and stability. Two processes that affect HIF-1 transcriptional activity are (i) signaling through the phosphatidylinositol 3-kinase (PI3K)/AKT/FKBP12 rapamycin-associated protein (mammalian target of rapamycin; FRAP) pathway, which influences the rate of HIF-1 α synthesis, and (ii) endogenous oxygen levels (and the functional status of certain proteins, including VHL and p53), which influence the rate of HIF-1 α degradation. Green boxes, positive, up-regulating factors that increase HIF-1 α production and/or stability. Red boxes, negative, down-regulating factors (including specific drugs) that decrease HIF-1 α production and/or stability. HIF-1 (blue box) is a heterodimeric protein consisting of an oxygen-regulated α -subunit and a stable, constitutively expressed β -subunit. HIF-1 is an important regulatory protein because it affects the transcriptional activity of many genes by binding to hypoxia response elements (HRE) in their upstream regulatory regions, and because these genes control angiogenesis, metabolism, and tumor growth/survival. (Modified from Trends in Molecular Medicine, vol. 8, G.L. Semenza, HIF-1 and Tumor Progression, S62-S67, ©2002, with permission from Elsevier).



Different Technologies for Identifying and Measuring Tissue Hypoxia and the Biological Basis for Imaging Hypoxia with Different Biomarkers/Probes

Given the importance of hypoxia in cancer progression and therapy, there has been a growing impetus to develop noninvasive imaging methods to detect and assess tumor hypoxia. In the absence of an imaging technique to directly determine the partial oxygen pressure (pO_2) in tissue, current noninvasive methods must be corroborated. Validation experiments are done by comparisons with direct pO_2 probe measurement (considered the "gold standard") or by immunohistochemical techniques, which provide detailed micro-distribution data of relative (not absolute) pO_2 level. Physical measurement of pO_2 levels using polarographic oxygen electrodes has been shown to be of prognostic value (17–19). These devices provide a direct measure of pO_2 at each location point and, when controlled by a computer-driven stepper motor, can typically achieve one reading every 10 seconds. This method is invasive, however, and provides selected pO_2 data along a series of individual sampling tracks, with data reliability being subject to the adequacy of the tumor sampling. Immunohistochemical methods are based on antibody detection of exogenous hypoxia markers, such as pimonidazole (20) or 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide (EF5; refs. 21, 22), which are injected into the patient before surgical resection of tumor tissue. Immunohistochemical methods yield microscopic information on hypoxia in relation to tumor histology. Such data require the acquisition of tissue specimens by invasive time-consuming immunohistochemical techniques,

however, and provide only relative pO_2 information. In addition, only a small number of sections can be realistically stained per patient. Immunohistochemical methods are therefore inherently limited and subject to sampling errors. These limitations have spurred enthusiasm for the development of noninvasive imaging methods. Imaging provides the opportunity to monitor the magnitude and three-dimensional pattern of tumor hypoxia within patients sequentially over time during therapy. Nevertheless, any new noninvasive imaging methods should be subject to validation against direct pO_2 probe measurement as well as immunohistochemistry to substantiate concordance with the *in vivo* microdistribution of the probe within the tumor.

Endogenous molecular markers of tumor oxygenation have been suggested and studied. For cervical cancer patients, HIF-1 might represent a reliable intrinsic marker for tumor hypoxia and prognosis (23). GLUT-1 has also been shown to be an endogenous marker of hypoxia for oral squamous cell carcinoma and rectal carcinoma (24, 25). Another much cited endogenous marker of tumor oxygenation is carbonic anhydrase 9 (26). It has been shown to be a prognostic indicator in cervical cancer (27) and in invasive breast cancer studies (28). None of these markers can be universally used across many tumor types, however, because they are likely to be cell type specific and are not reliable for the reasons discussed above.

The principal noninvasive approaches to imaging tumor hypoxia currently include magnetic resonance and radionuclides (PET and single-photon emission computed tomography), but other techniques, such as optical imaging or electron spin resonance, are under investigation. For example, near-IR imaging detects tissue hypoxia as a decrease in local blood pool oxyhemoglobin-deoxyhemoglobin ratio, but with

poor spatial resolution (29). The electron paramagnetic resonance imaging is a newly emerging magnetic resonance imaging technology that can produce images of oxygen levels in normal and tumor tissues (30) and may soon develop into a more widely used method for studies of hypoxia. More conventional magnetic resonance techniques include blood-oxygen-level-dependent (BOLD) imaging, which detects a change in tissue perfusion by the amount of oxygenated blood (31), and have become widely used in functional magnetic resonance imaging applications (32). However, BOLD imaging cannot be used to determine the level of oxygen in tissues or characterize the molecular-genetic changes in tumor cells. Nuclear magnetic resonance spectroscopy can detect increased lactate (a product of anaerobic glycolysis) and decreased ATP levels in ^1H and ^{31}P spectra, respectively, as well as tissue pH, but has poor sensitivity (in millimolar range) and poor spatial resolution ($\sim 1\text{ cm}^3$; ref. 33).

Nuclear imaging approaches exhibit sensitivity that is several orders of magnitude higher than magnetic resonance-based techniques. Although the resolution of modern whole-body PET and single-photon emission computed tomography systems is limited, ranging from 4 to 10 mm, PET can provide quantitative images of a variety of processes involved in hypoxia (34). Using $^{15}\text{O}_2$ inhalation (35), parametric PET images of tissue oxygenation levels, regional oxygen extraction fraction, and metabolic rate can be generated with much higher accuracy than invasive measurements of oxygen tension in tissues (36). PET imaging with $^{15}\text{O}_2$ currently is the "gold standard" for noninvasive imaging of tissue oxygen levels. This approach is not widely used for experimental or clinical imaging because the very short half-life of $^{15}\text{O}_2$ (~ 2 minutes) renders such clinical studies logistically and technically complex as well as expensive.

The first clinical studies to image hypoxia using PET were based on halogenated tracers of 2-nitroimidazoles, such as [^{18}F]FMISO, and were performed by Rasey et al. (37). These compounds become reduced in a hypoxic environment and then covalently bind to intracellular and extracellular molecules (38). Other nitroimidazole compounds have been radiolabeled and studied as potential hypoxia imaging agents. Lehtiö et al. (39) evaluated the use of ^{18}F -fluoro-erythronitroimidazole ([^{18}F]FETNIM) and tested it as a predictor of radiotherapy outcome. They reported that the data of [^{18}F]FETNIM were suggestive but inconclusive. Another 2-nitroimidazole, EF5, has been successfully used as an immunohistochemical marker of hypoxia in surgical trials. PET images have been obtained with [^{18}F]EF5 (40).

Chapman and colleagues developed a variety of compounds in the 2-nitroimidazole family, which contain iodine (e.g., iodoazomycin arabinoside and iodo-azomycin galactopyranosidase). The former has been radiolabeled with ^{123}I and used as a single-photon emission computed tomography tracer in clinical trials with promising results (41, 42). The latter, ^{124}I -azomycin-galactoside, has been studied by microPET in rodent models (43). The properties of these compounds have been compared with other 2-nitroimidazoles (e.g., [^{18}F]FMISO) and reviewed by Chapman et al. (44). Differences between the tracers include the lipid/water partition coefficients, the hypoxia-specific factor, and the $p\text{O}_2$ dependency (or the k -curve) of binding. The optimal hypoxia marker should have a large hypoxia-specific fraction (high sensitivity) and its

k -curve should be similar to the k -curve for radiosensitivity. This is important if the hypoxia marker is to be used for radiotherapy treatment planning, or to assess metastatic potential, or to be used as a prognostic factor of treatment outcome.

Another class of hypoxia markers relies on the reduction of a chelated metal and its selective deposition in hypoxic tissue [e.g., ^{60}Cu - or ^{64}Cu -labeled diacetyl-bis(N^4 -methylthiosemicarbazone) (ATSM); ref. 45]. Cu-ATSM reflects hypoxia-induced changes in tissue redox status and the activity of microsomal bioreductive enzymes (NADH-cytochrome b_5 reductase and NADPH-cytochrome $P450$ reductase), which play a major role in the retention of Cu-ATSM in tumors (46). The hypoxic specific fraction of this agent is lower than for the 2-nitroimidazoles, and their oxygen dependency of binding varies significantly between different tumor cell lines (47). Nevertheless, clinical studies at Washington University (St. Louis, MO) have shown an inverse correlation between the level of ^{60}Cu -ATSM uptake in tumor and clinical outcome data in patients with non-small-cell lung cancer and cervical carcinoma (48).

Determination of the optimum hypoxia tracer is an ongoing debate, and the answer will depend not only on the sensitivity of a tracer to hypoxia but also on tumor location relative to the pattern of radiotracer distribution in the body and the route of metabolite excretion (i.e., nonspecific background radioactivity).

FDG PET as a Surrogate/Biomarker of Hypoxia—Comparison with FMISO

Increased glycolysis reflects increased glucose transport and hexokinase activity, and this increase in glucose use can be imaged using a radiolabeled analogue of glucose [^{18}F]2-fluorodeoxyglucose ([^{18}F]FDG) and PET. This imaging strategy has been recognized for nearly three decades and was initially applied to malignant brain tumors by DiChiro et al. (49). Only small-bore (head-only) PET tomographs were available at that time, until the introduction of commercial whole-body PET scanners in the early 1990s. Now, whole-body [^{18}F]FDG PET imaging is routinely and widely used in the clinic for tumor diagnosis and staging of the extent of disease (50), as well as for monitoring the efficacy of anticancer therapies (51).

The regulation of glucose use in cells is complex and reflects the sum of multiple inputs at various levels involving different metabolic pathways. For example, increased glycolysis can be a response to an increase in cellular energy and substrate requirements, to an increase in cell proliferation and synthesis rates, and to the activation of specific oncogenic pathways (e.g., phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin) that can occur in the presence of adequate oxygen (Warburg effect). In addition to these inputs that increase HIF-1 α levels through enhanced translation and transcription, the mutation and functional inactivation of specific proteins (VHL and p53) results in the stabilization and a reduced degradation rate of the HIF-1 α protein. The effects of tissue hypoxia, which are also largely mediated through the stabilization of HIF-1 α , represents one of severe factors that must be considered. Although there is a convergence of inputs on HIF-1 α and thereby on the HIF-1 transcription factor, there has not always been a consistent pattern observed between local glucose use and hypoxia as monitored by the various methods described above. Therefore, it should not be surprising that the literature comparing [^{18}F]FDG and [^{18}F]FMISO PET imaging or

comparing [^{14}C]FDG and [^{18}F]FMISO autoradiography with immunohistochemical (pimonidazole) staining of tissue sections has provided mixed, and sometimes discordant, results (1, 52–56). The consistent discrepancies that have been observed between FDG and FMISO uptake indicate that regional hypoxia and glycolysis do not always correlate.

The body of evidence in the literature suggests that the identification of hypoxia in tumors of patients is best pursued through noninvasive imaging by the use of validated hypoxia markers, such as [^{18}F]FMISO and PET. Although [^{18}F]FMISO may not be the optimal marker (other hypoxia markers continue to

be developed), [^{18}F]FMISO provides a validated and reliable index of tissue hypoxia for individualized cancer treatment. Inclusion of [^{18}F]FDG PET in the pretreatment and posttreatment assessment of tumors provides an additional functional assessment that measures a different biological variable(s). Although the study of Rajendran et al. (1) in this issue of *Clinical Cancer Research* suggests that [^{18}F]FMISO imaging is better than [^{18}F]FDG for predicting survival in head and neck cancer, there are differences of opinion and this issue may depend, in part, on the cutoff criteria for “high” versus “low” [^{18}F]FMISO tumor values.

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