Location, Location, Location-Makes All the Difference for Hypoxia in Lung Tumors

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Hypoxia is a clinically important component of the tumor microenvironment because it adversely affects progression, metastasis, response to chemoradiation therapy, and overall patient survival. Here, we describe how different animal tumor models of lung cancer can yield surprisingly different hypoxic profiles. Clin Cancer Res; 16(19); 4685–7. ©2010 AACR.

In this issue of Clinical Cancer Research, Graves and colleagues examine hypoxia in tumors grown in various types of mouse models (1). Hypoxia has long been known to be an important determinant of radiation sensitivity, but more recently it has also been recognized as a factor that can mediate tumor aggressiveness, including the likelihood to metastasize (2). Hence, studying tumor hypoxia is important, but it requires an appropriate in vivo model. Historically, human xenografts have been grown subcutaneously in mice because of the ease with which it can be done. However, evidence is accumulating that growing the same tumor in an orthotopic location may more faithfully recapitulate the real-life situation. For example, Blouw and colleagues found hypoxia-inducible transcription factor 1 alpha (HIF-1α)–deficient transformed astrocytes grew poorly in the subcutaneous environment, but grew faster and penetrated the brain more rapidly and extensively when grown in the brain (3). Camphausen and colleagues studied gene expression of glioblastoma tumors grown intracerebrally or subcutaneously. They found a great disparity in gene expression profiles between intracerebral and subcutaneously grown glioma tumors (4).

Graves and colleagues chose to investigate hypoxia in several models (shown in Fig. 1A). They grew A549 human lung adenocarcinoma cells either subcutaneously (heterotopic) or in the lung base (orthotopic). Also, they used two different transgenic mouse models in which lung cancers arise spontaneously, one using adenovirally expressed Cre recombinase to activate expression of mutant K-Ras, and another using doxycycline to inducibly express Myc. They then used micro-computed tomography (microCT) scanning to follow the course of these tumors, and also assessed their metabolic activity with fluoroexoxyglucose (18F) positron emission tomography (18F-FDG PET) scanning. For hypoxia detection, Graves and colleagues used both noninvasive imaging with the tracer FAZA (5) and immunohistochemistry with the hypoxia-sensitive marker pimonidazole (Fig. 1B; ref. 6). Their findings were striking. Tumors were visible in all four models by microCT scanning, and in all of these cases the tumors showed high FDG uptake, indicating high metabolic activity. However, appreciable uptake of FAZA was only found in the heterotopic A549 model, consistent with the presence of hypoxia. This finding was validated by immunohistochemical analysis for pimonidazole, with strong binding seen in the heterotopic tumor and minimal binding in the orthotopic or spontaneous models.

As an independent validation of the presence of hypoxia, the authors also assessed the amount of DNA damage induced by the hypoxia-selective cytotoxin PR-104 by quantifying staining for the DNA damage marker γH2AX. In vitro, A549 and the two spontaneous mouse cancer lines showed no increase in γH2AX staining (relative to untreated cells), when exposed to PR-104 under normoxia. However, increased γH2AX staining was seen upon exposure to the drug with increasing hypoxia, which was expected because the drug should only elicit DNA damage under hypoxic conditions. In the in vivo setting, all three cell lines showed a dramatic increase in γH2AX staining with PR-104 administration compared with controls when grown subcutaneously. However, the increase was much less pronounced in γH2AX staining with PR-104 staining in the orthotopic A549 tumors or in the spontaneous Myc- and mutant K-Ras–induced tumors. Taken together, these data support the notion that the presence of hypoxia in lung cancers is strongly influenced by the site and, thereby, the microenvironment in which they are grown.

What are the biological and clinical implications of these findings? The most obvious is that for lung cancer, at least, caution is needed in interpreting oxygenation data from subcutaneous models, because a discrepancy can exist between the amount of hypoxia detected in the same tumor grown heterotopically or orthotopically. The reason for this discrepancy is currently unknown. One hypothesis is that the vasculature in orthotopic tumors is more functional and perfuses better than the vasculature...
in subcutaneous xenografts. However, studying this hypothesis was beyond the scope of the article and not addressed by the authors. Another possibility is that in spontaneous and orthotopic lung tumors, lung cancer cells have greater access to ambient oxygen via the alveoli, allowing for improved oxygenation. The authors point out that this improved oxygenation could be the case for A549 tumors, which grew as diffuse microscopic clusters when implanted in the lung. However, the K-ras– and Myc-induced tumors grew as 1-mL focal masses, making this explanation less likely for these tumors. This finding brings up another unanswered question. Do tumors at other orthotopic sites show a similar discordance in the degree of hypoxia when compared with the identical tumor grown subcutaneously? Is this degree of hypoxia a general phenomenon or is there something specific to lung cancers that makes them less hypoxic? The authors point out that their own data with the polarographic Eppendorf needle electrode (considered by many in the field to be the “gold standard” for in vivo hypoxia measurements; Fig. 1B) with human head and neck tumors show them to be more hypoxic than human lung cancers. Likewise, Eppendorf and EF5 data suggest that glioblastomas are among the most hypoxic human tumors known, with severe hypoxia (0.1% O₂) often present (7). Hence, it is tempting to speculate that head and neck or glioblastoma xenografts grown subcutaneously in mice would exhibit a similar degree of hypoxia as tumors grown orthotopically.

The authors measured the hypoxia in the lung cancers of 24 patients using the Eppendorf electrode. They used different thresholds, either the percentage of measurements <2.5 mm Hg (HF2.5) or the percentage of measurements <10 mm Hg (HF10), and found that 38.1% of patients had an HF2.5 >20%, and 47.6% had an HF10 >20%. In contrast to other sites such as head and neck cancer and cancer of the cervix, few studies have directly measured the PO₂ of lung cancers, which has important clinical significance. Although the level of hypoxia may not be overall as low in lung cancer as it is in some other cancers, clearly a substantial number of lung cancers do have areas where the O₂ level is as low as 10 mm or even 2.5 mm Hg. It is well known that hypoxia is associated with resistance to radiation, primarily owing to the lack of DNA-damaging free radical formation in the absence of O₂ (8).
control in lung cancer remains poor, even with doses of radiation as high as 60 to 70 Gy (9). Does the occurrence of hypoxia in a subset of lung cancers lead to resistance to therapy and failure to be locally controlled? This question remains open; however, data using CuATSM to image hypoxia suggest that the presence of hypoxia in lung cancers may correlate with outcome following treatment with chemoradiation (10). In the future, CuATSM and other methods of detecting hypoxia in solid tumors will be important tools in identifying patients with high hypoxic fractions that might benefit the most from hypoxia-targeting therapies such as hypoxic cytotoxins (e.g., tirapazamine) and hypoxia-modifying modalities (e.g., nicotinamide and carbogen breathing).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

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