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Hypoxia and Predicting Radiation Response

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The results from many studies indicate that most solid tumors, regardless of site of origin, contain hypoxic regions. Experimental studies have demonstrated that, apart from the well-known protective effect of hypoxia on the radiation response of cells and tissues, hypoxic conditions can also result in modified gene expression patterns, causing (to a greater or lesser extent in different cell populations) genomic instability, increased invasive capacity, higher propensity to metastasize, enhanced stem cell properties, and ability to survive nutrient deprivation. Clinical trials of hypoxia-targeted treatments have demonstrated improved local tumor control and patient survival in a number of tumor sites. However, our improved understanding of the underlying biology of cellular responses to hypoxia, and its potential interactions with the heterogeneous nature of tumor phenotypes, makes it likely that not every tumor that contains regions of hypoxia would necessarily need (or benefit from) such treatments. New more effective treatments are emerging, but it is likely that these treatments would have the biggest clinical effect in situations where tumor hypoxia is a primary driver of cancer behavior. The challenge for the Radiation Oncology community is the development of robust precision cancer medicine strategies for identifying patients with such tumors, in the setting of other etiological, genomic, and host-tumor factors, and treating these patients with the appropriate hypoxia-targeting strategy to reduce the effect of hypoxia on radiation treatment response. In this context, it is important to consider not only the hypoxic state of the tumor at diagnosis but also the changing characteristics of this state during the course of treatment.

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Introduction

Investigation of the role of hypoxia in the radiation response of tumors has a long history, stretching back more than 50 years to the work of Gray and colleagues[1-4] which defined the levels of oxygen required to sensitize cells to radiation treatment. Subsequent work demonstrated that treatment of tumor-bearing mice with hyperbaric oxygen could improve the response of their tumors to radiation treatment. These findings led to studies in patients (with different tumor types) exposed to hyperbaric oxygen during radiation therapy (RT). There were mixed results, but there were some positive outcomes particularly for patients with squamous cell carcinomas. The inherent difficulties in the use of hyperbaric oxygen led to the development of a series of drugs, which acted as radiation sensitizers for hypoxic cells. Similar to hyperbaric oxygen, these drugs, primarily nitroimidazoles, have been extensively tested in clinical studies, again with mixed results but with a generally positive outcome in head and neck squamous cell cancers (HNSCCs) (as detailed later).

The demonstration that tumor hypoxia is dynamic and changes during the course of radiation treatment gave rise to the concept of reoxygenation of surviving hypoxic tumor cells occurring during the course of fractionated treatment—a mechanism that could reduce the negative effects of hypoxia on radiation response. This was consistent with the observations that radiation sensitizers were less efficacious in

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experimental studies when given with extended fractionated treatments vs single doses or a few large fractions.[5] The recognition that nitroimidazoles were bound preferentially in cells at low oxygen levels led to the development of these (and similar) drugs as both hypoxic cell cytotoxins and potential markers of hypoxic cells.[6-9] Meanwhile, the development of a clinically applicable instrument to measure oxygen levels in tissues based on polarographic oxygen electrode technology (Eppendorf oxygen electrode) facilitated a series of clinical studies examining the potential for hypoxia to predict treatment outcome. This work and subsequent studies of the clinical significance of hypoxia in cancer have been reviewed recently.[10,11] An important consideration is that, as hypoxia plays a major role in modifying cellular biology and can have a variable influence on cellular survival as well as disease progression and metastasis formation, it is likely that hypoxia would not be equally important in all patients even when present to a similar extent. This article provides a brief overview of the current knowledge in these various areas and concludes with a discussion of possible strategies for moving forward to exploit the hypoxic status of individual cancers.

Hypoxic Cells in Tumors

The tumor microenvironment is characterized by subregions of nutrient deprivation, low extracellular pH, high interstitial fluid pressure, and hypoxia. Hypoxia in tumors develops because of an imbalance between supply of, and demand for, oxygen and, as such, depends both on the extent of blood perfusion and on the oxygen consumption of the cells in the tumor (both tumor and stromal cells). It is well established that in most tumors the vasculature is poorly constructed and may contain tortuosities, loops, shunts, and blind ends with a resulting heterogeneous blood flow.[12] In consequence, there is inadequate oxygen supply to some regions of the tumor. In normal tissues, the oxygen tension (pO_2) ranges from about 20-80 mm Hg. However, tumors often contain regions where the oxygen concentration can significantly decrease to less than 5 mm Hg,[13,14] levels that can cause increased radioresistance (<1-10 mm Hg).

Tumor cells that lie beyond the diffusion distance for oxygen in respiring tissue (>50-150 μm away from blood vessels) can quickly outstrip blood supply and are exposed to chronically low-oxygen tensions.[15] These diffusion-limited conditions for a duration of hours to days are referred to as "prolonged" or "chronic hypoxia."[16] The cells in these regions may remain hypoxic until they die (due to lack of oxygen or nutrients) or are reoxygenated.[17] Hypoxia can also be transient or "cycling" due to rapid perfusion changes in the tumor vasculature, leading to short periods of acute hypoxia or anoxia (from minutes to hours) followed by reoxygenation[16] (Fig. 1). It has also been suggested that, owing to the potential length of blood vessels in large tumors, hypoxic cells may also occur close to vessels that have flow but in which the oxygen in the blood is depleted.[18] As discussed later, hypoxia experienced by cells can lead to many metabolic changes, thus affecting the proportion of surviving hypoxic cells in the tumor. Hypoxia may also lead to a reduction in levels of DNA repair proteins, potentially reducing DNA repair capacity, and enhancing the possibility of genome instability. This could partially counteract the radioprotection afforded by the hypoxic state.[19]

An important aspect of hypoxia in tumors is that it tends to be very heterogeneous, with some regions of a tumor showing low levels of hypoxia whereas other regions demonstrate much higher levels. This heterogeneity means that a small piece of the tumor, as captured in a single biopsy, may not reflect the true level of hypoxia in that tumor. This mandates caution in interpreting data on hypoxia markers in tumors obtained from single biopsies, particularly in relation to overall tumor response. It also suggests that genomic analyses of tumors (eg, epigenetic or gene expression changes) may vary depending on whether the biopsy is primarily oxic or hypoxic at the time of assay. Therefore, using approaches to assess hypoxic levels in tumors that sample multiple regions (or image all) of the tumor is desirable. Current estimates suggest the need to sample at least 3-4 different regions of the tumor to obtain a reliable estimate of the hypoxic level but, if this is based on tissue arrays of small punch biopsies out of tissue blocks, larger numbers may be required.[10,20]

It is also important to recognize the limitations of different approaches to measure tumor hypoxia. The polarographic technique using an inserted needle (eg, Eppendorf probe) provides a range of oxygen values but measures these in a volume of tissue around the needle tip related to the diffusion distance of oxygen in the tissue. Conversely, assessments based on immunohistologic measures of cellular uptake of extrinsic markers such as nitroimidazoles provide much finer detail and potentially allow assessment of hypoxia in tumor and stromal components of the tumor in situ, but they detect cells that are only at low enough oxygen levels to allow reduction of the molecules to a product that can bind inside the cell. These levels are usually <1-5 mm Hg; thus, only the most hypoxic cells are identified. The expression of hypoxia-inducible factors (HIF)-1 and HIF-2 are increased at higher levels of oxygen (<5-20 mm Hg). Therefore, immunohistologic measurements of HIF-1, HIF-2, or proteins upregulated by HIF, such as expression of CA-9 and Glut-1, can reflect the cellular response to a more moderate level of hypoxia. Measurements of genetic signatures of hypoxia based on changes in gene or protein expression associated with hypoxia would equally tend to reflect these higher oxygen levels but again may be based solely on analysis of a small volume of tumor tissue.

Effect of Hypoxia on Cellular Biology in the Context of Radiation

Tumor hypoxia elicits numerous changes in cell biology that can influence tumor response to RT. Principally this occurs through 2 different mechanisms. The first is by activation of signaling pathways that function to influence adaptation to hypoxia. The second is through changes in cell signaling pathways that influence cell phenotype and intrinsic radiation