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Molecular targeting of hypoxia in radiotherapy☆

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ABSTRACT

Hypoxia (low O_2) is an essential microenvironmental driver of phenotypic diversity in human solid cancers. Hypoxic cancer cells hijack evolutionarily conserved, O_2- sensitive pathways eliciting molecular adaptations that impact responses to radiotherapy, tumor recurrence and patient survival. In this review, we summarize the radiobiological, genetic, epigenetic and metabolic mechanisms orchestrating oncogenic responses to hypoxia. In addition, we outline emerging hypoxia- targeting strategies that hold promise for individualized cancer therapy in the context of radiotherapy and drug delivery.

Contents

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1. The hypoxic tumor microenvironment

One of the distinguishing features of cancer cells is their insensitivity to microenvironmental signals, resulting in sustained proliferation and decreased cell death secondary to accumulation of driver mutations and epigenetic changes. [1] This very nature of the oncogenic process establishes a strong negative selective pressure, leading to cellular adaptations creating a heterogeneous tumoral microenvironment, wherein competing clonal populations of cancer cells generate gradients of nutrients, pH, metabolites and ultimately, low O_2 (hypoxia). [2,3] Clinical studies show that at least half of all locally advanced solid cancers contain hypoxic regions; moreover, intratumoral hypoxia is statistically associated to poor prognosis after controlling for tumor stage, histological grade, and lymph node status. [4,5]

In this review, we summarize recent advances on the molecular mechanisms of tumoral hypoxia and its effects upon the response to radiotherapy in human cancers. We outline the main molecular pathways involved in the pathobiology of hypoxic cancer cells whilst emphasizing the emergence of hypoxia as a microenvironmental driver of phenotypic diversity in solid cancers. We also highlight recent developments on targeted hypoxic therapies and delivery methods aimed to selectively eliminate hypoxic cancer cells, impede recurrence and improve patient survival.

2. Pathobiology of hypoxia in human cancers

Hypoxia results from the imbalance between O_2 availability and consumption by cancer and stromal cells; moreover, hypoxic tumoral regions are not static, but rather dynamically evolving as a function of cellular growth, angio(vasculo)genesis and radio- or chemotherapy. [6–10] The tridimensional distribution of intratumoral hypoxic areas is determined by the distance to the nearest perfused capillary, composition of the *interstitium* and metabolic O_2 consumption rates (JO_2) of cancer and stromal cells. The interaction among these factors leads to *diffusion-limiting* or chronic hypoxic gradients whereby O_2 levels vary at the cellular level at a relatively slow rate (hours to days). [11] Additionally, variations in perfusion due to the disorganized nature of tumoral blood vessels [12,13] (*i.e.*, immature endothelial architecture, [14] wide intercellular spaces and lack of pericytic coverage [15]) causes rapid O_2 fluctuations (minutes to hours) between hypoxia or anoxia and reoxygenation, known as *perfusion-limiting* or acute hypoxia. These two types of hypoxia present significant spatio-temporal overlap and impact the interactions between cancerous, stromal and immune host cells thus explaining the wide range of intratumoral O_2 levels measured within each tumor and among patients. [16,17] From this perspective, it is not be surprising that the molecular mechanisms underlying the hypoxic cellular response have evolved variable sensitivities to hypoxic severity and duration.

3. Tumor hypoxia predicts poor clinical outcome

Comparisons between normal and cancerous tissue O_2 levels indicate that independently of origin, most solid human cancers are hypoxic (Table 1). Direct measurements of tumor hypoxia using Clark-type electrodes, often detect tumoral regions where O_2 is <5 mmHg (<0.7%). [16] Indeed, the tumoral O_2 in carcinomas of the breast, [18] *cervix*, [8, 17,19] brain, [20,21] head and neck, [17,22,23] lung, [24] prostate [25] and sarcomas [7,26] ranges between 5.3 and 14 mmHg (0.7% – 1.8%). In contrast, normal tissue O_2 measurements lie between 30 and 52 mmHg (3.9% – 6.8%) whereas arterial O_2 levels lie between 75 and 100 mmHg (9.9% – 13.2%).

Severe hypoxia (<5 mmHg or <0.7%) measured through O_2- sensitive electrodes predicts failure in locoregional control, decreased disease-free and overall survival after radiotherapy in squamous head and neck carcinomas. [17,26,27] In prostate cancers, the median tumoral O_2 was 0.9%; whereas the hypoxic fraction below 10 mmHg (HP_{10}, equivalent to <1.1% O_2) represented 63% of measurements. In addition, the same study showed that hypoxia correlates with early biochemical failure (rise in circulating prostate- specific antigen) and predicts local recurrence after radiotherapy. [28] Fyles *et al.* found that an increased pre-radiotherapy hypoxic fraction (HP_5, equivalent to O_2 <0.5%) correlated with poor disease-free survival in patients with *cervix* cancer (1.2 years median follow-up). [19] Moreover, a recent metanalysis of head and neck carcinomas (n = 10,108; 86 trials) showed that either hyperbaric O_2 or pharmacological radiosensitizers enhance locoregional control and overall survival after radiotherapy. [29]

Further development of isotope-labelled nitroimidazole probes targeting hypoxic tumor cells (reviewed in [30]) has allowed noninvasive, semiquantitative measurements of hypoxia through positron emission tomography (PET). The most common probes are ^{18}F-fluoromisonidazole (^{18}F-FMISO), ^{18}F-[2-(2-nitroimidazol-1[H]-yl)-N-(3,3,3-trifluoropropyl)acetamide] (^{18}F-EF3) and ^{18}F-fluoroazomycin arabinoside (FAZA). [31–33] Studies using ^{18}F-FMISO in lung adenocarcinomas, [34] sarcomas [35] or head and neck carcinomas [36] detected changes in tumoral O_2 levels during radiotherapy and chemotherapy that correlated with poor therapeutic responses albeit with significant inter-individual variability. Studies in animal models and patients show comparable results for ^{18}F-EF3 (or ^{18}F-EF5, a similar molecule) [32,37] and FAZA. [31,38] Nitroimidazoles can also be used to detect hypoxic regions in resected tumors through immunohistochemistry whereas at high doses, serve as chemical radiosensitizers in the preclinical and clinical setting (reviewed in [39,40]). A third approach to measure intratumoral hypoxia is the immunohistochemical detection of endogenous markers such as hypoxia- inducible factor (HIF) -1α (and -2α), [41,42] glucose transporters (GLUT-1 or -3) [43,44], vascular endothelial growth factor (VEGF)-A [45–47] and carbonic anhydrase (CA)-9 [41,48], which also correlate to radiotherapy responses.

4. Oxygen as a radiosensitizer

The first description of O_2 as a radiosensitizer in cancer cells dates back to the observations by Crabtree and Cramer, [49] followed by Gray *et al.*, who demonstrated that improving O_2 delivery sensitized human cancer cells to ionizing radiation. [50–52] These discoveries led to the use of hyperoxia as a therapeutic tool to increase the efficacy of tumor radiotherapy, in parallel to studies aimed at understanding the mechanism of action explaining the properties of O_2 as a radiosensitizer (reviewed in [29]). The “oxygen fixation hypothesis” provided a mechanism explaining the enhancement of cancer cell death upon radiotherapy in non-hypoxic cells. It postulates that radiation- induced DNA