Published in final edited form as:

Int J Radiat Oncol Biol Phys. 2007 ; 69(2 Suppl): S56–S58.

Identification and Targeting Hypoxia in Head and Neck Cancer – A Brief Overview of Current Approaches

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Introduction/Abstract

Tumor hypoxia is a common phenomenon in solid tumors. It been shown to adversely affect treatment outcomes in patients with head and neck squamous cell carcinoma (HNSCC).¹ There has been strong interests in targeting hypoxia over the years; however, the clinical effort has been impeded by the failure to accurately identify patients who would most benefit from hypoxia targeted therapy. The negative results of several clinical trials can be attributed to patient heterogeneity and that many studies were underpowered to enroll patients who would benefit from such therapy. This session will briefly address different approaches of assessing tumor hypoxia and discuss some strategies for targeting this microenvironmental factor.

Approaches to Assess Tumor Hypoxia

A commonly accepted approach for assessing hypoxia has been by direct tumor pO₂ measurement with a polarographic electrode (Eppendorf pO₂ Histogram, Hamburg, Germany). Polarographic needle provided the first convincing evidence that hypoxia existed in human solid tumors.² Since then, several studies have confirmed the prognostic significance of electrode-detected tumor hypoxia in HNSCC.¹ In a large multi-institutional study, the fraction of pO₂ values ≤ 2.5 mmHg (HP2.5) was the only factor associated with poor survival on multivariate analysis.¹ However, this is an invasive method as it requires the placement of a needle directly into the tumor for measurements. Other drawbacks of this approach include the high cost of the equipments, tumor inaccessibility, high inter-observer variability, failure to distinguish necrosis from hypoxia, and the lack of spatial information. Therefore, its use has not been widely popularized.

Another method for assessing tumor hypoxia is through the administration of an injectable hypoxia marker such as pimonidazole (1-(2-nitro-1-imidazolyl)-3-N-piperidino-2-propanolol)³, and EF5 (nitroimidazole[2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide]).⁴ These compounds form stable adducts with intracellular macromolecules and this binding is proportionally inhibited as a function of increasing oxygen concentration. Detection of these adducts with antibodies can provide information on the relative oxygenation of tissue at a cellular resolution. This approach is limited by the requirement for exogenous drug administration and additional biopsies for staining. At this time, there is minimal clinical data regarding the prognostic significance of these agents in HNSCC. In a small series of HNSCC, high pretreatment pimonidazole staining correlated with a higher risk of locoregional relapse in patients treated with radiotherapy alone but not in patients treated with radiotherapy plus carbogen and nicotinamide, which were used to modulate tumor hypoxia.⁵

Hypoxia imaging using specialized PET or SPECT tracers is another promising approach. However, these technologies require expensive dedicated equipments for both imaging and tracer generation. This topic is being addressed in details by Rischin et al. in the same issue.

A popular approach for assessing hypoxia is the use of endogenous markers, which are proteins that are induced by hypoxic exposure. Presently, the most widely investigated proteins in HNSCC are hypoxia inducible factor-1 (HIF-1) and carbonic anhydrase IX (CA IX).⁶ A major advantage of endogenous markers is that levels of these proteins can be assessed on archival materials, thereby allowing rapid correlation with treatment outcomes. In addition, molecular markers require neither injection of foreign material nor additional invasive procedures. However, a significant concern to this approach is that these proteins can be regulated by factors other than hypoxia. For example, HIF-1 α expression can be influenced by several non-hypoxic stimuli including nitric oxide, cytokines, trophic stimuli and oncogenes. Therefore, combining several endogenous markers could improve the specificity of such an approach. For example, the combination of HIF-2 α and CA IX was superior to individual marker alone in predicting treatment outcomes in patients treated in the randomized CHART trial (Continuous Hyperfractionated Accelerated Radiotherapy).⁶ Recently, using a combination of genomic and proteomic approaches, we have identified several novel hypoxia regulated proteins including Galectin-1 (Gal-1), Lysyl Oxidase (LOX) and Ephrin A-1. The combination of these markers plus CA IX cytoplasmic and CA IX membrane staining resulted in a hypoxia score that can be used to predict for cancer-specific survival in 101 locally advanced HNSCC patients treated at our institution (Figure 1).

Another approach for assessing hypoxia is to measure the level of hypoxia-regulated secreted proteins in the blood. Two markers that have been tested in the clinic with mixed results are VEGF and Osteopontin (OPN). Although circulating VEGF levels have been shown to be elevated in cancer patients⁷, the relationship between tumor hypoxia and systemic VEGF levels is unclear. In a pilot study of 54 stage III-IV HNSCC patients, we found OPN to be a hypoxia-regulated protein whose plasma levels correlated with tumor pO₂.⁸ We have also found OPN levels to be an independent prognostic factor for freedom from relapse (FFR) and overall survival (OS) in these patients and in an updated independent patient cohort (Figure 2). Bache et al also found a correlation between OPN tumor staining and tumor pO₂.⁹ These results were confirmed by the Danish Head and Neck Cancer Study Group (DAHANCA) in patients treated with radiotherapy with or without Nimorazole, a hypoxic radiosensitizer. They found that elevated plasma OPN levels correlated with poorer disease-specific survival and that only patients with high OPN levels benefited from Nimorazole.¹⁰

Regardless of which method is used to identify patients with hypoxic tumor, it is critical to select the appropriate patients for hypoxia targeted therapy to ensure treatment success.

Hypoxia Targeted Therapy

It is hypothesized that increase oxygen delivery via modifications of hemoglobin levels can attenuate tumor hypoxia and improve treatment outcomes. Henke et al performed a phase III randomized study of radiation +/- erythropoietin (EPO) in HNSCC. Unfortunately, patients who received EPO had worse locoregional progression-free survival.¹¹ This could be explained partially by the quadratic regression in the correlation between tumor pO₂ and hemoglobin levels: at hemoglobin levels ≤ 14 , increasing hemoglobin levels directly correlates with increasing pO₂; however, at hemoglobin above 14, an inverse correlation is noted with lower pO₂ associated with increasing hemoglobin, presumably from venostasis.¹ More recently, the presence of EPO receptors in tumor cells has also been implicated with worse outcomes in patients receiving EPO on this study.¹²

Increased vascular leakage from immature tumoral vasculatures can result in increased interstitial blood pressure, thereby, worsening tumor hypoxia and impeding effective drug delivery to the tumor. Jain et al. popularized the concept of normalization of tumor vasculature through antiangiogenic therapy such as bevacizumab.¹³ This concept was supported by clinical data in colorectal cancers, where treatment with bevacizumab was shown to reduce tumor interstitial pressure.¹⁴ This concept needs has yet to be tested in HNSCC.

Another promising approach to overcoming tumor hypoxia in HNSCC is the combined use of the nicotinamide vasodilator and carbogen breathing (ARCON) to increase the oxygen partial pressure of tumors. ARCON (Accelerated Radiotherapy with CarbOgen and Nicotinamide) has produced a 3-year local control rate in excess of 80% for advanced stage T3-4 laryngeal and oropharyngeal cancers.¹⁵ Presently, a phase III clinical trial testing the efficacy of ARCON in laryngeal cancers is ongoing in Europe.¹⁵

A promising strategy to exploit tumor hypoxia is through agents that have high selectivity for killing hypoxic cells, the first drug of which is tirapazamine (TPZ or SR4233). In a randomized phase II trial, the combination of TPZ, cisplatin and RT was found to be better than 5FU, cisplatin and RT.¹⁶ In contrast, we found that the addition of TPZ to an aggressive regimen of induction and concurrent cisplatin and 5FU with RT did not result in improved outcomes in a small randomized phase II study.¹⁷ A phase III trial testing the benefit of adding TPZ to concurrent RT and cisplatin has been completed and the results are pending.

TPZ, however, does have several limitations; these include the poor diffusion of TPZ through hypoxic tissue and its requirement of less stringent hypoxia for activation, that can result in normal tissue toxicity in poorly oxygenated organs. There are therefore strong interests in developing novel hypoxic cell cytotoxins with more specific antitumor activity. Dinitrobenzamide mustards (DNBM) are a new and highly potent class of hypoxic cytotoxins discovered by the Auckland University group. These compounds have improved properties over TPZ; including a more stringent requirement for hypoxia for activation and a substantial bystander killing effect. A lead compound of the DNBM family, PR-104 is being studied in details by Dr. J. Martin Brown's laboratory and is presently in phase I-II clinical trials for several solid tumors (personal communication: JM Brown).

Conclusion

After years of efforts, tumor hypoxia continues to represent a therapeutic challenge in HNSCC. Nonetheless, the prospect of reducing its impact is looking brighter with improved ability of detecting and quantifying tumor hypoxia, better understanding of its molecular underpinnings and identification of novel targets for therapeutic exploitation.

Acknowledgements

This work was supported by PHS grant 1 R01 CA118582-01

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Figure 1.

Cancer-specific survival by hypoxia marker score comprised of Galectin-1, Ephrin A1, Lysyl Oxidase, CA IX cytoplasmic and CA IX membrane staining, where a score of 1 was assigned to strong staining for each marker and a score of 0 to negative and weak staining. This has been adjusted for age and hemoglobin levels, 2 other significant factors on univariate analysis.

Figure 2.
Replication of outcome data using the previously defined OPN value of 450 ng/ml in 86 new patients: Overall survival by OPN.