
To the Editor:

I read with great interest the article by Evans et al. (1), showing that with increasing WHO grade human glioma exhibits tumor hypoxia of increasing severity as determined by analysis of the injected hypoxia marker EF5 on immunohistochemistry. At the same time, the intensity of EF5 staining in the overall group was significantly associated with the time to tumor recurrence. The authors suggest that hypoxia is associated with tumor aggression and that specific targeting of hypoxic human glioma cells may provide a therapeutic benefit.

Published data on the oxygenation of human glioma are limited partially due to the difficulty of performing and interpreting data obtained from needle electrode experiments. Although glioma has been shown to be less well oxygenated than surrounding brain tissue, previous reports have failed, similar to the needle electrode measurements described by Evans et al. (1), to show a difference in oxygenation between grade 3 anaplastic astrocytoma and grade 4 glioblastoma multiforme (2). However, because the presence of necrosis in histopathologic sections of glioblastoma is characteristic of this entity (3), one would assume increased severity of hypoxia in grade 4 tumors, e.g., due to poor oxygenation in the “perinecrotic” tumor regions. Previous studies of the hypoxia-associated protein hypoxia-inducible factor-1α, which is considered an endogenous marker of tumor hypoxia, have shown that regions of hypoxia are consistently present in perinecrotic areas of glioblastoma (4, 5). Hypoxia-inducible factor-1α immunoreactivity scores in one series were significantly higher in glioblastoma than in low-grade astrocytoma (4), corresponding to the EF5 data of Evans et al. (1). Interestingly, although high hypoxia-inducible factor-1α expression has been shown to be associated with poor prognosis in several tumor entities (reviewed in ref. 6), no such correlation has yet been reported for glioblastoma.

I would like to address two issues concerning the clinical significance of the data presented by Evans et al. (1).

First, the detection of different oxygenation levels in gliomas of different WHO grade may be very valuable in the investigation of processes leading to the malignant progression of these tumors. However, for the clinician, the finding that oxygenation of grade 3 tumors is worse than in grade 2 and better than in grade 4 is not as impressive. In other words, if tumor oxygenation as measured by EF5 binding is merely a surrogate of tumor grade, then the additional information is limited. It would, however, be of great interest to determine if prognosis, e.g., measured as time to recurrence or overall survival, within the entity of glioblastoma multiforme is associated with EF5 binding; although patient numbers are still limited, the data shown by Evans et al.’s Fig. 3 seem to suggest that (1). Such an association would be a novel finding as, to my knowledge, a prognostic role of tumor oxygenation in glioblastoma detected by either needle electrode or endogenous or injectable marker has not yet been documented.

Second, I do not share the optimistic view of Evans et al. (1) regarding the possibility to therapeutically target hypoxic tumor cells in malignant glioma. Despite the inclusion of the surrounding edema and generous margins used in radiotherapy of malignant glioma, recurrences are localized almost exclusively completely inside the planning target volume receiving the full dose of 54 to 60 Gy (7). Assuming the presence of hypoxic, radiation-resistant tumor cells as one reason for treatment resistance, escalation of radiation dose is a logical consequence. Unfortunately, both intensity-modulated radiation therapy to a total dose of 90 Gy (8) and, very recently, integration of a radiosurgery boost with single doses of 15 to 24 Gy (9), implying quite severe dose escalation, were completely ineffective in prolonging survival or even shifting the localization of recurrence from central to marginal in glioblastoma patients. Two of the most promising strategies to overcome or exploit tumor hypoxia, showing some effectiveness in other tumor entities (10, 11), have also been ineffective in glioblastoma multiforme: In a phase I/II trial using full or partial ARCON (accelerated radiotherapy, carbogen, nicotine- amide) protocols, the median survival in the treatment arms was between 9.7 and 11.1 months (12). Similarly, a phase II trial investigating the addition of the hypoxic cytotoxin tirapazamine to conventional radiotherapy to 60 Gy showed median survival times of 10.8 and 9.5 months for two dose levels of the drug and no significant effect in prognostic subgroups (13).

In summary, detection of EF5 binding may, in the future, be used to determine differences in oxygenation not only between but also within established prognostic subgroups, such as WHO grade groups that might establish a prognostic role of tumor hypoxia within these entities. Deriving a therapeutic benefit from this information, however, remains difficult.

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References
In Response:

The authors would like to thank Dr. Vodermark for his comments on our recent publication, "Hypoxia is Important in the Biology and Aggression of Human Glial Brain Tumors" (1). In his letter, he poses several issues concerning the clinical significance of our data. The first issue relates to the possibility that EF5 binding is merely a surrogate of tumor grade. One of our goals is to determine whether hypoxia can be added to standard pathological data in order to provide better prognostic information. This applies not only to patients with glioblastoma multiforme but also for patients with lower grade tumors. These studies are ongoing under the auspices of a funded NIH clinical trial. From a statistical standpoint, the comment is absolutely correct. From a biological standpoint, however, it seems quite critical to understand tumor micro-environmental factors that are associated with the designation of high grade. This knowledge would underlie any attempt to effectively modify therapy.

Dr. Vodermark's second topic relates to the issue of therapies directed at hypoxic cells in glial neoplasms. Although our article did not specifically address this possibility, it certainly is a major goal of many translational laboratories. One of the obvious applications of our findings would be to direct hypoxia-based therapies. Some of the reported methods for targeting hypoxic cells include intensity-modulated radiation therapy or proton beams, hypoxic cell sensitizers, hypoxia-modifying agents (published examples include RSR13, hyperbaric oxygen, and dodecafluoropentane), hypoxic cytotoxins such as tirapazamine, and hypoxia-activated gene therapy (2–6).

Dr. Vodermark is unenthusiastic about the likelihood of the success of these therapies. There are a small number of studies in the literature, including those mentioned in Dr. Vodermark's letter, where hypoxia-modifying regimes have been applied to patients with glioblastoma multiforme. In some studies, these regimes have not been successful in changing outcome, and in other studies, some clinical effect has been noted. Does this mean that overcoming hypoxia is not a useful therapeutic goal?

It is relevant to revisit the history of radiation oncology's clinical studies of hypoxic cell sensitizers for some perspective. In the 1980s, thousands of patients were treated with the hypoxic cell sensitizer misonidazole and almost all of the individual studies showed no efficacy. A result of these treatment "failures" was that the radiobiology community (incorrectly) questioned whether hypoxia was important in human cancer. We now know that hypoxia is present and clinically relevant in many human cancers. This conclusion was possible because of the development of methods to measure hypoxia in human tumors, specifically the Eppendorf needle electrode (7). These studies indicate that only a percentage of the patients treated in hypoxia sensitizer trials actually had hypoxic tumors, and that many tumors had levels of hypoxia that would not respond to this class of drugs (8). Other potential causes of the "failure" of hypoxic cell sensitizers include the dose-limiting toxicity (9) and statistical issues related to the number of patients treated on each individual trial. Supporting the latter are the results of a meta-analysis of the individual misonidazole trials showing an improvement in outcome in subgroups of patients. These data support the preclinical studies suggesting that misonidazole is a hypoxic cell sensitizer (10).

So what can we take from this history lesson? First, there are major statistical considerations in testing a hypoxia-targeting agent, and the necessary number of patients to study is determined by the heterogeneity of the property (hypoxia) in the population (11). The ability to determine whether a given patient has a hypoxic tumor is critical in testing potential therapies. Secondly, all "anthypoxia" therapies are not the same. For example, certain therapies work better on moderate versus severe hypoxia (12). The quantitative nature of EF5 analysis is unique in being able to assess these issues. Relevant to therapies that modulate hypoxia, e.g., ARCON, there are critical temporal considerations; oxygen must be present at the time of radiation administration. Our ability to measure hypoxia noninvasively using [F-18]-EF5 positron emission tomography imaging could be a major asset in timing radiation to optimize treatments.

We now understand that hypoxia modulates much more than radiation efficacy. Hypoxia affects chemotherapy response (13, 14) and modulates critical biological proteins that determine biologic aggressiveness (15). New chemotherapeutic agents and signal transduction modulators are being developed and used in conjunction with radiotherapy. Important questions as to whether they can access hypoxic regions or whether they induce hypoxia will only be answered through the use of hypoxia-measuring techniques. Finally, the detailed understanding of the presence, patterns, and levels of hypoxia in tumors are critical in our understanding of the molecular mechanisms involved in brain tumor biology. An example is the measurement of HIF-1α in glioblastoma. It is now quite clear that the protein is modified by many factors in addition to oxygen. HIF-1α has been reported to change rapidly with pO2 (17); yet, concerns of its modification during tumor resection and tissue fixation have not been discussed in the literature. HIF-1α induction has been shown to be of prognostic significance in several tumor types, but studies have not been performed to determine whether this is related to, or independent of, hypoxia. Such fascinating and complex possibilities will clearly require accurate assessment of tissue hypoxia at the cellular level, and this is possible only by using agents such as EF5.

In summary, we feel that it is much too early to give up on hypoxia as a therapeutic approach for brain tumors. Now that the clinical measurement of hypoxia is possible, well-planned hypoxia-based regimes can be proposed. Using agents with well-defined mechanisms of action administered only to patients with tumors that have these characteristics will allow the appropriate testing of new treatments. Once again, we thank Dr. Vodermark for his thought-provoking comments. We believe that only with the further development of hypoxia labeling techniques and investigation of hypoxia surrogate markers can these important questions be fully answered.

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References


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