Tracking Normalization of Brain Tumor Vasculature by Magnetic Imaging and Proangiogenic Biomarkers

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Clinical assessment of the response to antiangiogenic therapy has been cumbersome. A study in this issue of *Cancer Cell* demonstrates that a combination of magnetic resonance imaging (MRI) for quantification of normalized vessels with measurements of circulating levels of proangiogenic factors, including FGF2, SDF1, and viable circulating endothelial cells, provides an effective means to evaluate the response of recurrent glioblastoma to a prototypical pan-VEGF receptor tyrosine kinase inhibitor, AZD2171.

Glioblastoma (GBM) is one of the most frequent malignant brain tumors in adults and has a poor response to chemotherapy and radiation. Treatment of newly diagnosed GBMs with conventional chemotherapeutic agents, such as temozolomide and radiation, results in partial response and a median survival of 12–14 months. Moreover, conventional therapy of recurrent GBM is associated with poor outcomes, with less than 10% of patients having progression-free survival at 6 months. Recently, it has been shown that coexpression of a constitutively active variant of EGFR, EGFRvIII, and PTEN confers sensitivity of GBM to EGFR tyrosine-kinase inhibitors (Mellinghoff et al., 2005). Methylation of the 0^6-methylguanine-DNA transferase gene promoter also has been shown to correlate with favorable treatment response to temozolomide (Hegi et al., 2005). However, despite all of these interventions, all GBM patients relapse and succumb to tumor progression.

GBMs are known to be driven by hypoxia and VEGF-A overexpression. Moreover, the vasculature of GBMs is atypical, disorganized, and leaky, resulting in the generation of vasogenic brain edema. Therefore, defining the mechanism by which antiangiogenic intervention may target GBM vasculature will not only optimize strategies to block growth of this tumor but also diminish the neurological complications associated with brain edema. Clinical trials have been designed to evaluate the role of antiangiogenic agents in the treatment of GBMs, but the application of antiangiogenesis therapy to GBM has been cautious and slow for fear of intracranial hemorrhage.

Using elegant imaging and molecular approaches, Rakesh Jain and colleagues have demonstrated that antiangiogenic agents through selective pruning and maturation of unstable vessels promote the emergence of "normalized," pericyte-coated smaller vasculatures that are efficient conduits for delivering chemotherapeutic agents and oxygen (Jain, 2005). These findings have rekindled the hope that judicious introduction of antiangiogenic agents may circumvent fatal hemorrhagic complications.

In this issue of *Cancer Cell*, Batchelor et al. provide evidence for normalization of GBM blood vessels in patients treated with a pan-VEGF receptor tyrosine kinase inhibitor, AZD2171, in a phase 2 study (Batchelor et al., 2007). The rationale for using AZD2171 was based partially on results showing a decrease in perfusion and vessel density in an in vivo breast cancer model (Miller et al., 2006) . Furthermore, using an orthotopic glioma model, Jain and colleagues had previously identified the optimal window of time to deliver anti-VEGFR2 antibody to achieve a synergistic effect with radiation (Winkler et al., 2004). During the window of normalization, there was improved oxygenation, increased pericyte coverage, and upregulation of angiopoietin-1 leading to a decrease in interstitial pressure and permeability within the tumor (Winkler et al., 2004). In the present study, the window of normalization was quantified using magnetic resonance imaging (MRI) from day 1 to 112 of AZD2171 administration. The authors used MRI gradient echo, spin echo, and contrast enhancement to measure blood volume, relative vessel size, and vascular permeability and concluded that AZD2171 was more effective in pruning large size than small microvessels. Furthermore, using defined magnetic imaging parameters k^{trans} and extracellular-extravascular volume fraction the authors demonstrated that sculpting of the large and leaky vessels was associated with decreased vascular permeability, which persisted much longer than the changes in relative vessel size. These data are in agreement with reduced vasogenic brain edema as detected by MRI in patients with malignant brain tumors treated with bevacizumab, a neutralizing antibody to VEGF-A, together with

chemotherapy (Pope et al., 2006). Reduction of vasogenic brain edema during AZD2171 treatment allowed elimination or reduction of corticosteroids, the chronic use of which is responsible for serious neurological and medical morbidity.

As the MRI measurements of vascular size were done empirically, this study raises several questions. (1) Are small vessels more "normalized" than the large vessels or vice versa? (2) Are the large or small vessels differentially pruned and eliminated during antiangiogenic therapy? (3) Does increased vessel size over time indeed correlate with the magnitude of enhanced tumor vessel permeability? Despite using the most sophisticated MRI technology, there are several factors that limit precise assessment of truly normalized vessels. First, the determination of a "normalization window" by this group has heretofore been made in xenograft models using direct measurements of vessel size, density, and permeability by intravital microscopy (Winkler et al., 2004). In the current paper, the evaluation of glioma vessel normalization relied upon the evaluation of relative vessel size and permeability using MRI, as serial biopsy could not be performed on these patients. Second, MRI determination of vessel size has previously been performed only in animal tumor models using optimal contrast agents and has not been validated in human tumors, and is thus subject to a number of confounding factors (Dennie et al., 1998). For example, use of Gd-DPTA, a relatively small-molecule contrast agent, in this study necessarily relies on "first pass" dynamic measurements of magnetic susceptibility, which would be subject to the leakage of this agent across the blood-tumor barrier and would threaten the accuracy of perfusion estimations, which depend on compartmentalization of the contrast within the vessels (Dennie et al., 1998; Pathak et al., 2001). Vessel size determinations in animal tumors typically have been done with larger-contrast molecules, like MION, which can equilibrate in the blood but are yet to be approved for human use (Dennie et al., 1998; Pathak et al., 2001). Other factors degrading the accuracy of vessel size determination might include vessel tortuosity and disturbances as well as regional variations in brain tumor blood flow (Cha, 2003). Obviously, if vessel size and permeability measurements can be validated and standardized, perhaps with larger-molecular-weight contrast agents, these measurements might become reliable surrogate markers for assessing the response of gliomas to antiangiogenic agents.

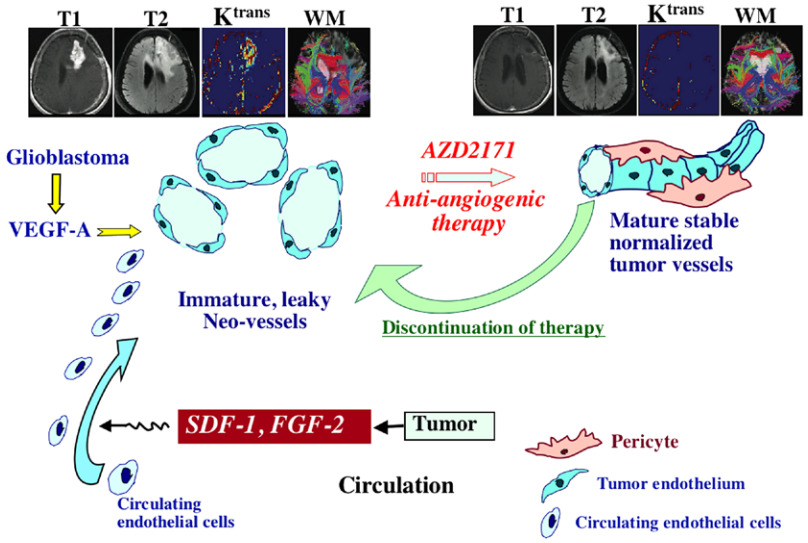

Figure 1. Assessment of Response to Antiangiogenic Therapy with AZD2171 by Utilizing MRI and Biomarkers

ZD2171 targets immature vessels, while favoring the emergence of normalized pericyte coated blood vessels. Recurrence of the tumor is associated with generation of dilated, large, and leaky blood vessels as well as plasma elevation of proangiogenic factors, including FGF2, SDF1, and CECs. Normalization of the blood vessels is associated with a decrease in post-contrast T1-weighted MRI signal (T1), T2-weighted (flair, T2), permeability (K^{trans}), and recovery of white matter integrity as detected by white matter tractography (WM). The MRI photographs were adapted from Batchelor et al. (2007).

Given the limitations enumerated above, the authors took advantage of other surrogate biomarkers of neoangiogenesis, including plasma levels of FGF2, SDF1, and viable circulating endothelial cells (CECs) (Jin et al., 2006; Rafii and Lyden, 2003). The authors showed that progression on treatment with AZD2171 was associated with an increase in CECs, SDF1, and FGF2, while progression after drug interruptions correlated with increases in circulating progenitor cells (CPCs) and plasma FGF2 levels (Figure 1). The increase in plasma levels of SDF1 and FGF2 correlated with the MRI measurements, demonstrating an increase in the relative vessel density and size. Thus, MRI determination of vessel normalization in combination with circulating biomarkers provides for an effective means to assess response to antiangiogenic agents. This pioneering work set the stage for a screening platform for evaluating antiangiogenic agents for the treatment of GBMs.

One other issue that requires further experimentation is that AZD2171 blocks not only VEGFR tyrosine kinase activity in tumor blood vessels but also other tyrosine kinases, including c-Kit, PDGFRα, and PDGFRβ, that are often expressed on the brain tumor cells (Wedge et al., 2005). Therefore, it remains to be determined whether the normalization of the vessels is a direct effect of AZD2171 on endothelial cells and/or an indirect effect on other tyrosine kinases expressed on the tumor cells. Understanding the molecular target of AZD2171 may be critical for diminishing the end-organ toxicity of AZD2171 if this agent will ultimately be used in combination with radiotherapy or chemotherapeutic agents to determine whether there is an impact on survival of patients with GBM. Indeed, in order to capi-

talize on the therapeutic opportunity afforded by a "normalization" window in GBM patients, the addition of chemotherapeutics or radiation will be essential. However, it is encouraging that preliminary analyses indicated an improvement in progression-free survival and overall survival of this group of 16 patients compared to historical controls.

Lastly, one paradox of antiangiogenic therapy leading to vessel normalization is the fact that current antiangiogenic agents, such as AZD2171, seem to eradicate primarily unstable tumor vessels, while selecting for maintenance of stabilized vessels that are believed to efficiently deliver oxygen, nutrients, and chemotherapeutic agents to the tumors. However, delivery of chemotherapeutic agents that are ineffective in treating recurrent GBM or other chemoresistant solid tumors through normalized vessels is unlikely to result in a cure or durable remission; thus, targeting stable tumor vessels may also be necessary. As such, development of effective cytotoxic therapies and/or novel antiangiogenic approaches to target both unstable and normalized tumor vessels to completely deprive the tumor mass of a blood supply is urgently needed to achieve the ultimate goals envisioned for "antiangiogenesis" therapy.

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