Evaluation of the Effect of Anti-Angiogenic Therapy on Tumor Vasculature in Breast Cancer Mouse Xenograft

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Background: The anti-angiogenic component of combination therapy can result in multiple responses that benefit overall tumor response. It can (i) control tumor growth through the suppression of neovascularization in the tumor, (ii) improve delivery of the cytotoxic component of chemotherapy by normalizing tumor vasculature, and (iii) interfere with vasculogenic activities of these cells by attenuating the recruitment of circulating endothelial progenitor cells to the tumor. However, questions about the effects of anti-angiogenic therapy on tumor physiology and progression need to be addressed, particularly regarding its role in the induction or modulation of tumor hypoxia and the potential mechanisms of tumor resistance to anti-angiogenic therapy.

Objectives: Magnetic resonance imaging (MRI) can characterize tumor vascular parameters non-invasively and longitudinally. The new MRI technique we developed to measure vascular volume (*VV*) and permeability surface area products (*PS*) was used to evaluate the effect of anti-angiogenic therapy on tumor vasculature. Whether the therapy induces tumor hypoxia was assessed through optical imaging using MDA-MB-231 human breast cancer cells engineered to express red fluorescence protein, tdTomato, under hypoxic conditions.

Methods: MDA-MB-231 human breast carcinoma cells were orthotopically inoculated to the mammary fat pad of female SCID mice. Animals were treated with either i) saline, ii) paclitaxel at a dose of 20 mg/kg intraperitoneally every 4th day, iii) pazopanib at a dose of 100 mg/kg orally twice daily, iv) the combination of paclitaxel and pazopanib [1], v) bevacizumab at a dose of 10 mg/kg intraperitoneally once daily, or vi) the combination of paclitaxel and bevacizumab for 10 days. MR images were acquired pre-, 5 and 10 day post-treatment. Vascular parameters, *VV* and *PS*, of the tumors were visualized and quantified using a horizontal bore Bruker Biospec 9.4T MR spectrometer with albumin-GdDTPA conjugate, using a traditional approach [2]. Images were acquired, pre and post contrast of 0.2 ml of albumin-GdDTPA (60 mg/ml). We have also developed a novel method to measure *VV* and *PS*, that is based on a saturation recovery 3D gradient echo imaging with short recovery time of 20 ms and 90-degree flip angle, and this technique is insensitive to water exchange kinetics. Other experimental parameters are: echo time: 1.5 ms; repetition time: 5 ms; number of averages: 4; matrix size $128 \times 64 \times 64$. Tumor vascular volume map measured with this method coregistered with the hypoxia marker is shown in Fig. C Changes in tumor hypoxia were determined through anti-angiogenic therapy experiments on MDA-MB-231 cells that express red fluorescent proteins under the control of the hypoxia response element (HRE), which is activated by hypoxia-inducible factor-1 (HIF-1) signaling in hypoxic tumor cells. Animals were treated with bevacizumab at a dose of 10 mg/kg intraperitoneally every 4th day for 12 days, and tumor hypoxia was monitored non-invasively by a Xenogen optical imaging system. At the end-point, the tumors were excised, cut into 1 mm slices, and imaged using the Xenogen imaging system. MR data were analyzed using in-house software written in the IDL programming environment. Image registration and 3D renderings were performed using Amira visualization software.

Results: The combination of paclitaxel, a chemotherapy drug, and pazopanib, an anti-angiogenic agent, significantly reduced *VV* after 5-day treatment, as characterized by MRI (**Fig. A**). Interestingly, pazopanib or bevacizumab alone, and the combination of paclitaxel and bevacizumab did not induce statistically significant reduction of *VV* and *PS* of the MDA-MB-231. The combination of paclitaxel and pazopanib has a tendency to delay the tumor growth, although no significant tumor growth inhibition was observed in any of treatment groups (**Fig. B**). Anti-angiogenic treatment with bevacizumab induced hypoxia in the tumor after 12-day treatment, as demonstrated by optical imaging (**Figs. C** and **D**).

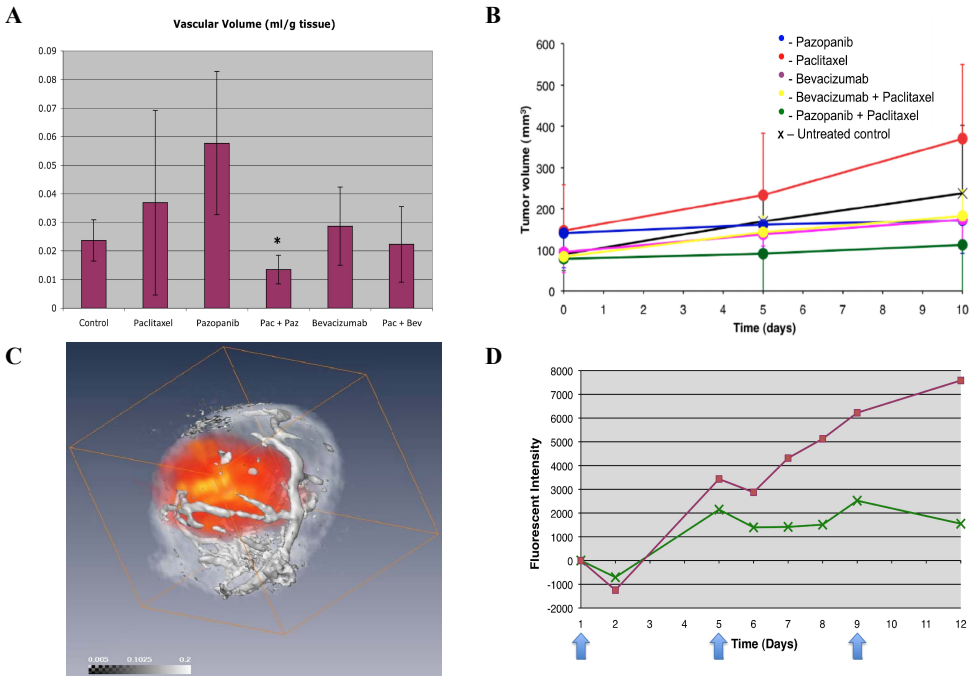

Figure A. Changes in *VV* of MDA-MB-231 tumors after 5-day treatment with different combinations of cytotoxic and anti-angiogenic agents, measured by MRI. **B**. Mean changes in the tumor volume during the course of therapy. **C**. Registration of the *VV* map (gray channel) with a red fluorescence expression (red channel) in hypoxic tumor areas at Day 12 (end point). **D**. Changes in fluorescent intensity from two representative treated tumors (red and green) measured during the course of bevacizumab treatment by non-invasive optical imaging.

Discussion: The reasons for differences in the vascular parameters, as measured by MRI, between the bevacizumab and pazopanib combination therapy groups are not readily apparent. However, hypoxia may play a role in affecting the vasculature to different extent in this particular tumor model. In order to address that we directly measured tumor hypoxia *in vivo* using expression of the fluorescent reporter protein. **Figures C** and **D** indicate that the anti-angiogenic treatment induced hypoxia in the tumor possibly due to a decreased vascular supply. Transient decrease in tumor hypoxia after injection of bevacizumab may be explained by initial normalization of the tumor vasculature. This supports the hypothesis discussed in the earlier report [3]. Since tumor hypoxia causes resistance to chemo- and radiotherapy, the results of this study have important implications for anti-angiogenic therapy.

Conclusion: The combination of paclitaxel and pazopanib decreased *VV* after 5-day treatment, while pazopanib or bevacizumab alone, and the combination of paclitaxel and bevacizumab did not significantly change the vascular parameters in the tumor. Although anti-angiogenic therapy induced a transient decrease in tumor hypoxia after the injection of bevacizumab, it is likely to induce tumor hypoxia post-treatment.

References: [1] Kumar R. et al., *Mol Cancer Ther* **6**, 2012, (2007). [2] Bhujwalla Z.M. et al., *Neoplasia* **3**, 143 (2001). [3] Huang G. and Chen L. *Cancer Biother Radiopharm* **23**(5), 661 (2008).

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