Vascular Changes in Cancer Treatment—Seeing Is Believing

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Understanding the effects of novel cancer drugs on the tumor microenvironment will be crucial for their successful use in patients. In this issue of *Cancer Research*, Ghosh and colleagues discuss the effect of two promising heme-targeting agents on the vascular network in mouse models of lung cancer, utilizing emerging optoacoustic imaging approaches. The findings demonstrating significant, dynamic modulation of tumor hypoxia with the heme-targeting drug treatments create important opportunities for image-guided combination therapy.

See related article by Ghosh et al., p. 3542

The importance of the tumor microenvironment in modulating disease progression and response to treatment is widely accepted in the cancer community. Blood vessels serve to carry oxygen into the tissue, affecting both proliferation and sensitivity of cells to chemo- and radiotherapy. It is, however, only with the continuing development of methods, allowing accurate insight into the tumor microenvironment and its dynamic landscape, that this knowledge can be translated to the clinic.

In this issue of *Cancer Research*, Ghosh and colleagues (1) present a new class of drugs showing significant impact on the tumor microenvironment and complex effects on hypoxia. The two compounds tested, HSP2 and CycT, were previously shown by the authors to suppress the growth of non–small cell lung cancer xenografts through reducing the production, uptake, and function of heme (2, 3), an iron-containing molecule important in multiple cellular processes. The anticancer properties of the drugs were attributed to disruption of ATP generation process. The reports support a growing body of evidence identifying the importance of heme in cancer and the potential of its targeting in therapy (4). Detailed investigation of the interaction between the heme-targeting compounds and the tumor microenvironment is therefore, very timely.

For dynamic *in vivo* characterization of the tumor vasculature in response to the treatment, Ghosh and colleagues (1) turned to optoacoustic imaging. Combining the principles of optical and ultrasound imaging, the emerging imaging modality utilizes pulsed light illumination to stimulate generation of ultrasound in the body (the "photoacoustic effect"). This approach enables interrogation of the optical properties of the tissue, at depths much exceeding those available to all-optical imaging methods such as diffuse optical tomography or microscopic approaches. Owing to the particularly strong optical absorption of hemoglobin, the protein responsible for oxygen transport capabilities of blood, optoacoustic imaging was adopted as a promising method to offer insight into the structure and function of vascular networks, particularly relevant in cancer.

In the study by Ghosh and colleagues (1), the new heme-targeting agents are hypothesized to affect the vascular network and oxygen transport in the tumor, highly relevant to their action *in vivo*. Dynamic optoacoustic measurements of the abundance and oxygenation of the blood circulating in the tumors reveal a profound effect, painting a complex picture of relationships between the therapy, cancer cells, and tumor microenvironment. Targeting of heme is shown to affect the oxygen balance on both sides of the supply–demand equation, reducing cellular oxygen consumption while modulating its supply through remodeling the vasculature. The precise *in vivo* insight is supported by detailed IHC analysis of hypoxia, vasculature, and proliferation markers, with added validation of the *ex vivo* findings in a more clinically relevant orthotopic lung model.

Beyond important biological findings, the work by Ghosh and colleagues (1) presents an encouraging development in optoacoustic imaging, demonstrating successful application of a novel technique. "Oxygen-enhanced optoacoustic tomography" (OE-OT; ref. 5) relies on optoacoustic measurements of change in local blood oxygenation when breathing gas is switched from room air to pure oxygen to infer information about the functional capabilities of the tumor blood vessels. Described and validated before for established antivascular agents (6), the approach is confirmed in this study to offer sensitive insight into modulation of tumor vasculature by novel therapeutic agents.

The measurements revealed a decreased blood volume and improved oxygenation in the treated tumors compared with controls, suggesting a less extensive, but more functional vascular network. IHC analysis using pimonidazole and carbonic anhydrase IX confirmed these findings, showing decreased tumor hypoxia following treatment, consistent with previously reported OE-OT results (6). The observed pattern of response to the treatment is in line with optoacoustic characterization of a clinically approved antiangiogenic agent, trebananib, carried out by Bohndiek and colleagues (7) in a mouse model of ovarian cancer, suggesting a similar mechanism of action for the novel compounds, and curious cross-talk between the heme flux and vascularization.

The measured decrease in blood volume coupled with increased oxygenation is consistent with the phenomenon of "vascular normalization." First described by Jain (8), the theory postulates that the initial disruption of the fast growing and chaotic tumor blood vessel network by treatment with antiangiogenic agents later leads

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to its restructuring, ultimately improving tumor blood supply and oxygenation, which is linked to increased treatment response and decreased tumor aggressiveness. Although not universally confirmed for all cancers, vascular normalization has a profound implication for the use of antiangiogenic drugs in clinical practice as, depending on the timing and dose, they may be used to either starve the tumor of oxygen or improve oxygenation, sensitizing it to further treatment. This complex, dynamically changing effect on the tumor is likely responsible for the mixed results in clinical use of antiangiogenic intervention to leverage the cytotoxic effect of chemotherapy.

The approach assumed by Ghosh and colleagues (1) has the potential to overcome this challenge. Sampling the effect of the proposed drugs on the vasculature over time using OE-OT can help detect the dynamic changes in the vasculature and identify the optimal window of opportunity for the treatment, for example, to ensure sufficient functional vascularization and oxygen availability to maximize effect of concurrent cytotoxic therapy. Considering the additional effect on decreased oxygen consumption, as confirmed by the authors by *in vitro* and *ex vivo* methods, the heme-targeting drugs show significant promise for tumor reoxygenation.

In the era of precision and personalized therapy, the ability to time treatments efficiently, based on the in-depth understanding of the effect of each drug on the tumor, will be of particular importance. This is apparent from the growing popularity and success of the adaptive therapy paradigm (9), aimed to delay evolution of aggressive, resistant phenotypes through treatment time optimization. Modulators of the tumor microenvironment, in combination with functional imaging methods to accurately follow their effect, such as described by Ghosh and colleagues (1), may be used in a similar fashion, maintaining an ecosystem that favors a more benign disease.

Because of constraints on depth of penetration and other technical limitations, the technology is unlikely to be used clinically in patients with lung cancer anytime soon. However, the presented approach for temporal characterization of treatment response dynamics can be reproduced in malignancies for which optoacoustic imaging is clinically available, such as breast, or otherwise feasible, such as head and neck or prostate cancers, to better characterize effects of clinical and preclinical treatments on tumor vasculature. In addition, the preclinical implications of this work are equally important. While more research is urgently required to understand the mechanism of action of the compounds on tumor vasculature and oxygen transport, the study introduces the heme-targeting agents as potentially powerful modulators of tumor oxygen environment, with promising applications in combination therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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