Opening Windows into Tumors

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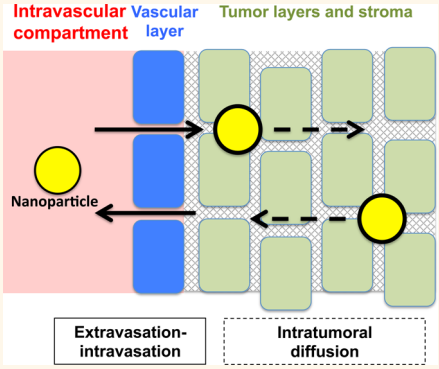
ABSTRACT Delivery of nanoparticles to tumors is limited by vascular permeability and intratumoral diffusion. In this issue of *ACS Nano*, Jiang *et al.* show that the manipulation of tumor physiology using antiangiogenic therapy can improve the tumor penetration of quantum dots with 20 and 40 nm hydrodynamic diameters. This Perspective describes the problems, challenges, and perspectives of using antiangiogenic therapy in combination with nanometer-sized drugs and contrast agents in preclinical and clinical studies.



Dramatic progress has been made in the synthesis and characterization of engineered nanoparticles for imaging and treatment of cancers. The delivery of these nanoparticles to tumors and metastasis is still plagued by poor penetration, diffusion, and retention in the tumor.[1,2] Recent studies have shown that the "active" targeting of nanoparticles to specific ligands on tumor cells does not dramatically increase the net delivery to tumors,[3,4] likely because the endothelial barrier and intratumoral diffusion are the main rate-limiting steps. There is a need to alter the tumor microenvironment to increase and maximize the total dose of drug-containing nanoparticles accumulating in the tumor, and to deliver the drug to the majority of cancer cells.

Currently, the predominant view in the field of nanomedicine is that tumor physiology is unique and can be exploited for targeting of nanoparticles. Angiogenic neovasculature in tumors grown in mice and potentially in some human tumors is leaky and permeable. The classic work of McDonald and colleagues described remarkable openings (pores) of irregular sizes between tumor endothelial cells.[5] These pores enable nanoparticles to extravasate into the tumor matrix. This is defined as the enhanced permeability and retention effect (EPR). Maeda *et al.* exploited this transport principle to accumulate relatively large proteins and dextrans in tumors.[6] Unfortunately, the EPR effect seems to be heterogeneous and more limited to fast-growing mouse tumors than to slowly growing human tumors.[7]

> In the present issue of ACS Nano, Jiang *et al.* describe how antiangiogenic therapy improves tumor accumulation of larger quantum dots.

Once nanoparticles enter the tumor, the tumor vascular permeability and transvascular transport is limited and is related to the physicochemical properties of particles. Researchers are starting to focus on biological strategies to address these transport limitations by altering signaling mechanisms. Vascular endothelial growth factors (VEGFs) increase vascular permeability during angiogenesis *via* activation of VEGF[8] and neuropilins NRP receptors.[9] Interestingly, Ruoslahti's lab discovered that iRGD peptides bind to integrin on tumor endothelial cells, are cleaved, and have high affinities to neuropilin-1. The use of iRGD as a tumor priming agent led to increased penetration of small molecules, nanoparticles, and proteins in different tumor animal models.[12] Another approach is to use

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bradykinin. Bradykinin receptors are elevated in many solid tumors,[10] and their inhibition by specific antagonists can decrease the leakage in tumor vasculature.[11] Maeda and colleagues demonstrated that bradykinin, nitric oxide, and prostaglandin synthesis promote the EPR effect in tumors.[11] Unfortunately, these strategies only transiently increase tumor permeability and also cause systemic side effects.

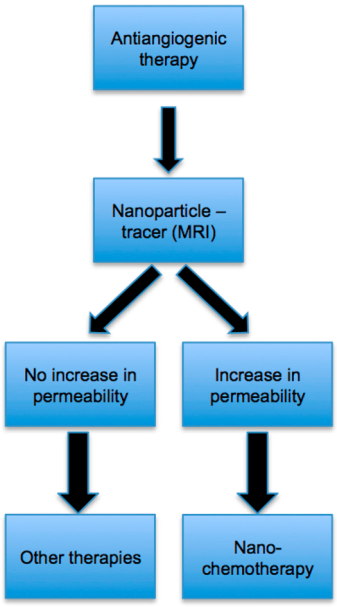
Another method to alter nanoparticle transport is by using antiangiogenic compounds that block VEGF receptor signaling. This alters the tumor interstitial pressure and improves perfusion. The tumor becomes less porous and has more regular vasculature and better pericyte coverage. This approach was proposed by Dr. Rakesh Jain 10 years ago and is called vascular normalization.[13] This tumor priming strategy increases the accumulation of chemotherapy in tumors depending on the treatment stage. This antiangiogenic treatment strategy also led to an increase tumor accumulation of 12 nm quantum dots in mice.[14] In the present issue of *ACS Nano*, Jiang *et al.* showed that the penetration depth of larger quantum dots (QDs) can be achieved using antiangiogenic therapy.[15] The authors developed mice with MCaP0008 breast adenocarcinoma xenografts and treated them with an antibody against VEGFR receptor 2 (VEGFR2). VEGFR2 is the main marker of tumor neovasculature, and anti-VEGFR2 IgG has been shown to block angiogenesis in preclinical models[16] and to improve survival of cancer patients.[17] They observed a significant reduction of blood vessel volume and vessel length, and an increase in pericyte coverage using multiphoton intravital microscopy.[15] The authors used quantum dots coated with 2 or 10 kDa PEG with a final hydrodynamic diameter of 20 or 40 nm, respectively, and demonstrated significant enhancements in accumulation of both nanoparticle sizes in tumors. However, once nanoparticles were extravasated, the smaller QDs showed better distributions in the tumor than the larger. This result is consistent with earlier publications.[18] Compared to the results of the Jain lab, which showed a benefit for 12 nm particles and no benefit for 125 nm particles, Jiang *et al.* showed a benefit for 40 nm particles following anti-VEGFR2 treatment, suggesting that antiangiogenesis treatments can improve the penetration of larger nanoparticles more than was previously thought. The improved extravasation of larger nanoparticles can also improve the amount of drug delivered to the tumor due to higher loading capacities of larger nanoparticles. The main question is whether these results will be applicable to different nanoparticles, tumor types, and antiangiogenic treatments. Theoretical data suggest that larger nanoparticles are less amenable to improved tumor penetration,[19] and there is a complex interplay between physiological makeup of tumors and physicochemical properties of nanoparticles that often dictates penetration efficiency.[20]

The feasibility of priming a tumor for improving tumor accumulation and penetration of nanoparticles using anti-VEGR2 for human patients remains questionable. Antiangiogenesis drugs are effective in some cancers and notoriously ineffective against others. The anti-VEGF antibody Avastin (bevacizumab) does not prolong survival in metastatic breast cancer,[21] but was effective in combination with chemotherapy in metastatic colorectal cancer.[22] VEGFR2 signaling inhibitor Sutent (sunitinib) was not efficacious in metastatic breast cancer,[23] but showed survival benefits in pancreatic neuroendocrine cancers[24] (interestingly, a recent report suggests that sunitinib improves tumor penetration of viral particles in mouse models[25]). Moreover, there is a concern that sunitinib might cause lymph node metastases in preclinical studies,[26] which is yet to be shown in the clinical setting. Recent clinical trials with Eli Lilly's anti-VEGFR2 antibody ramucirumab showed promising effects in several cancers, and the drug CYRAMZA was approved last year for non-small cell lung cancer and also as a single agent for advanced or metastatic gastric or gastroesophageal junction adenocarcinoma.[27] Several clinical trials tested a combination of bevacizumab and the nanoparticulate drugs liposomal doxorubicin and albumin-paclitaxel,[28,29] but the results are difficult to interpret. A recent study by Rakesh Jain demonstrated that bevacizumab actually caused a decrease in tumor perfusion and permeability, and these effects inversely correlated with overall survival using cisplatin and Abraxane (nanoparticulate paclitaxel).[30] Clearly, too much angiogenic treatment can cause a decrease in tumor perfusion, so there is a lot to be understood about the dosage and duration of antiangiogenic treatments needed to promote vascular normalization and to improve accumulation of nanoparticulate drugs in patients.

Manipulating tumors and metastases is one of the ways to achieve breakthroughs in cancer nanomedicine and drug delivery.

Additionally, there is a need to develop objective biomarkers to assess or to predict efficacy of nanoparticle penetration in tumors in patients. In publications by Jiang *et al.*[15] and Chauhan *et al.*,[14] quantum dots were used, but they are less applicable as imaging tracers in humans for various reasons. Magnetic resonance imaging (MRI) appears to be the most suitable imaging modality, due to its safety and excellent anatomical and spatial resolution and contrast. The best emerging choice for assessing tumor vascular permeability to nanoparticles appears

to be ultrasmall iron oxide nanoparticle Feraheme (ferumoxytol), which is the iron supplement used off label as a transverse relaxivity MRI contrast agent. Ferumoxytol is a long-circulating 20 nm single core carboxymethyl dextran nanoparticle that penetrates tumors *via* the EPR effect and is taken up by tumor-associated macrophages, thereby generating negative contrast (darkening) in T2-weighted images. Although its MRI contrast properties are far from ideal, ferumoxytol has shown promise as a cancer staging agent and angiogenesis marker.[31,32] Several clinical trials are now underway to test Feraheme as a surrogate marker of tumor "leakiness." Thus, BIND-014 (docetaxel nanoparticles for injectable suspension) is being studied in patients with advanced urothelial carcinoma, cervical cancer, cholangiocarcinoma or carcinomas of the biliary tree, and squamous cell carcinoma of the head and neck, and ferumoxytol imaging will also be investigated at U.S. sites (ClinicalTrials.gov, Identifier: NCT02479178). Merrimack Pharmaceuticals will use tumor permeability to ferumoxytol as a biomarker and predictor of liposomal irinotecan (ClinicalTrials.gov, Identifier: NCT01770353). This is an exciting application of a nanoparticle contrast agent to assess tumor "leakiness" to nanosized drugs and to stratify patients based on the EPR effect. In a similar fashion, the effect of the antiangiogenic therapy on tumor leakiness and accumulation of ferumoxytol could be assessed prior to initiating any nanochemotherapy (Scheme 1).



Scheme 1. A proposed workflow for the combination of antiangiogenic (vascular normalization) therapy and nanomedicine. After initiating angiogenic therapy, a nanoparticulate contrast agent is administered to assess the degree of vascular leakiness. The current agent is MRI contrast agent (ferumoxytol), but other modalities such as ultrasound-responsive emulsions and CT contrast (gold) can be used. On the basis of imaging data, a decision is made as to the use of nanochemotherapy or conventional chemotherapy

Outlook and Future Challenges. According to a recent editorial in *Nature Biotechnology*,[33] "... a whole raft of academic nanotechnology research focuses on developing delivery vehicles that will never see reduction to clinical practice. This matters because the hard problems in macromolecule drug transport—crossing the blood-brain barrier, engaging intracellular targets and accessing solid tumors—will be addressed neither by decades-old technology nor delivery vehicles that pose immunogenicity risks and toxicity concerns." The improved accumulation of larger particles following antiangiogenic therapy reported by Jiang *et al.* is a welcome finding. Going one step ahead, it will be important to answer questions as to whether enhanced nanoparticle accumulation following vascular and possibly tumor stroma remodeling is sufficient for therapeutic benefits, both in preclinical models and in humans. Such studies are not straightforward because of the confounding effect of antiangiogenic therapy on tumor growth. Another unanswered question is whether vascular normalization increases nanoparticle delivery performance in metastatic lesions. Lastly, there is an unmet need in reliable imaging tracers that can predict accumulation of actual nanotherapeutics in tumors **prior** to administering the nanoparticles containing chemotherapeutic agents. Manipulating tumors and metastases is one of the ways to achieve breakthroughs in cancer nanomedicine and drug delivery.

Conflict of Interest: The authors declare no competing financial interest.

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