achieve antitumor effects while minimizing feedback activation of the parallel pathway? (2) Do our preclinical systems, where drugs are often tested in vitro for short periods of time, provide a reliable measure of antitumor effect when feedback activation occurs on this timescale?

Along with the recent genetic evidence from tumors acquiring resistance to BYL719 (Juric et al., 2014), the findings of Schwartz et al. (2015) and Costa et al. (2015) underscore the fact that tumors can and will maintain PI3K activation through a variety of mechanisms in the face of pharmacologic inhibitors. Our ability to devise the right combinations of PI3K isoform inhibitors, or to select the right subgroup of patients who can benefit from these agents without suffering intolerable toxicities, will determine the ultimate clinical impact of this class of drugs.

AUTHOR CONTRIBUTIONS

All authors contributed to the concept, writing, and approval of manuscript.

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Vascular-Promoting Therapy Reduced Tumor Growth and Progression by Improving Chemotherapy Efficacy

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In this issue of *Cancer Cell*, Wong and colleagues describe a novel approach of increasing the number of functional blood vessels in tumors using a low-dose therapy regimen of Cilengtide and Verapamil. This method enhanced Gemcitabine delivery, uptake, and metabolism within tumor cells to reduce tumor growth and progression.

The development of new blood vessels from preexisting vessels is a multifaceted process known as angiogenesis and a well-established "hallmark of cancer". The wealth of proangiogenic molecules produced by tumor and stroma cells induces angiogenesis, remodelling of the vasculature, and recruitment of many types of lymphoid and myeloid cells as well as endothelial progenitors. The tumor vasculature thus differs markedly from normal vessels, is a key route for metastasis, and is essential for nutrient and metabolite exchange.

The concept of controlling metastatic tumors by targeting tumor blood supply was first proposed in the early 1970s, and, since then, many drugs targeting blood vessels have been developed (Figure 1); most specifically inhibit vascular endothelial growth factor (VEGF) A function, a major proangiogenic molecule in cancer (Bridges and Harris, 2011). They have, in several tumor types, a major therapeutic role, in others a more ancillary effect. Antiangiogenic therapy has increased progression-free survival of patients with many cancers, but resistance results in antiangiogenic therapy having little impact on overall survival.

During antiangiogenic treatment (Figure 1A), a temporary window of opportunity occurs when therapy re-balances the pro- and antiangiogenic signals to the point that vessels become more "natural" with improved blood flow in tumor regions previously poorly perfused being observed, referred to as tumor vasculature normalization (Jain, 2014). Increased chemotherapy delivery to the tumor occurs during this short time frame with improved drug uptake and reduced side effects observed (Batchelor et al., 2013). However, the duration of vascular normalization is time- and dose-dependent, and the onset varies between patients.

Another mechanism targeting tumor vasculature is the development of vascular-disrupting agents that induce endothelial cell death by disrupting their cytoskeleton and adhesion to matrix and activating local coagulation (Figure 1B). Thus vascular-disrupting agents result

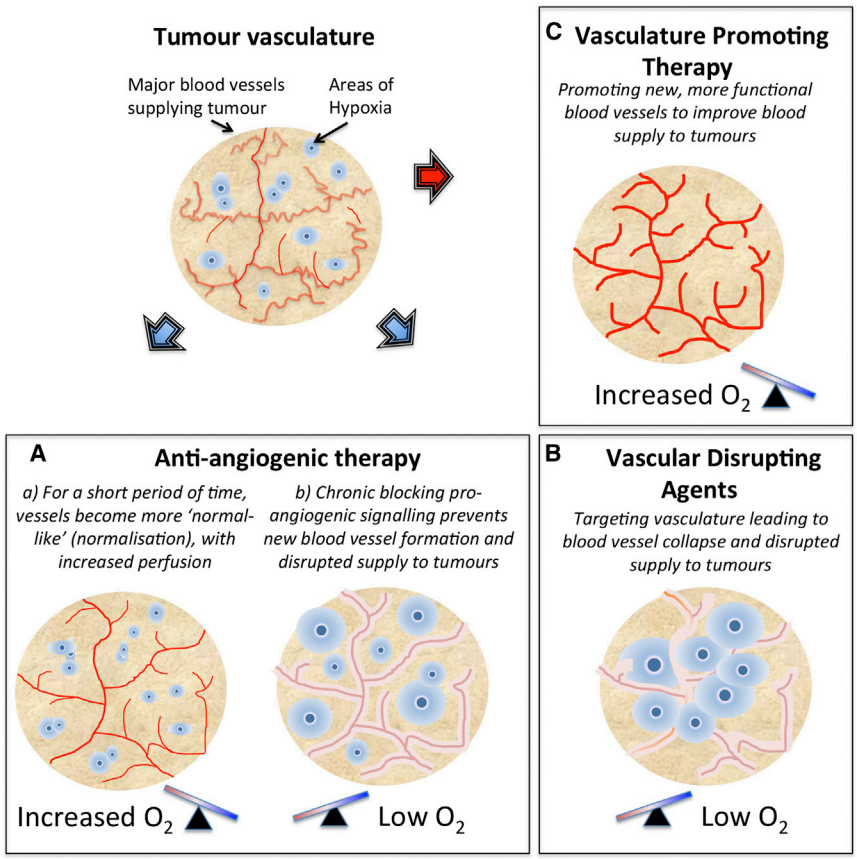

Figure 1. Tumor Growth Is Dependent on Nutrient and Oxygen Supply

New blood vessels are formed in a process known as angiogenesis. However, tumor vasculature is abnormal due to the high abundance of proangiogenic molecules. Different approaches have been taken to target the tumor blood supply to ultimately block tumor growth and progression.

(A) Antiangiogenic therapy has been developed to specifically block the actions of proangiogenic molecules. (a) As a result of removing the chronic presence of proangiogenic molecules, the vasculature becomes more "normal like" with increased blood flow to regions of the tumor. (b) However, disrupted blood flow to regions of the tumor occurs as therapy continues, generating hypoxic regions within the tumor.

(B) Vascular disrupting agents target existing blood vessel cells, leading to cell death, and disrupting the structure and stability of blood vessels and ultimately decreasing blood flow and increasing hypoxic zones within the tumor. Necrosis is more central, and peripheral blood vessels from normal tissues can maintain a vascular rim.

(C) In contrast, vascular promoting therapy promotes new and more functional blood vessels, improving blood supply and perfusion of the tumor and reducing hypoxia within tumors.

in vasculature collapse and starvation of oxygen and nutrients from the tumor. However, toxicity needs to be reduced from agents currently under development before further pursuit.

Many factors contribute to increased tumor aggression and therapy resistance following blood vessel-targeting treatment (Bridges and Harris, 2011). Proangiogenic molecules are mainly released by tumor cells found within low-oxygen (hypoxic) regions within tumors (Semenza, 2014). The newly-formed tumor blood vessels form a chaotic, leaky, and poorly functional vasculature, reinforcing a hypoxic environment within the tumors. Tumor antiangiogenic therapy increases hypoxia further (Franco et al., 2006). Hypoxia is, however, associated with poor patient prognosis and resistance to chemotherapy (Rebucci and Michiels, 2013). Increased hypoxia promotes tumor selection of more aggressive tumor cells better adapted to survive and proliferate under stressful oxygen-deficient growing conditions.

The general nature of the proangiogenic environment within the tumor results in leaky blood vessels with poor blood flow in multiple areas in the tumor. This results in reduced chemotherapy delivery and efficacy with more tumor cells being "shielded" from exposure to treatment. Areas of necrosis and intermittent hypoxia further compound the problem of drug delivery, and the reduction of vessels' angiogenesis following therapy can further restrict chemotherapy delivery (Saggar et al., 2013).

A new approach that addresses most of these challenges is reported in this issue of *Cancer Cell* (Wong et al., 2015). They have developed "vascular promotion therapy" as a means to improve efficacy, a distinct approach from those of antiangiogenesis and vascular normalization (Figure 1C). The authors used a low-dosage schedule with vasculature-affecting agents Cilengitide and Verapamil in combination with the chemotherapeutic agents Gemcitabine or Cisplatin to target lung and pancreatic ductal adenocarcinoma tumor growth. Cilengitide, a selective inhibitor of α_v integrins, leading to inhibition of the FAK/src/AKT pathway resulting in cell death (apoptosis) in endothelial cells, was originally developed as an antiangiogenic agent. Cilengitide failed in clinical trials for the treatment of glioblastoma when administered in high doses; however, a proangiogenic effect with the enhancement of tumor angiogenesis was observed following low dosing of Cilengitide (Reynolds et al., 2009). Verapamil, a calcium channel blocker, increased vessel dilation and blood flow in tumors by relaxing blood vessel muscles; this has been shown to increase chemotherapeutic efficacy of agents such as Gemcitabine.

The authors initially assessed Cilengitide, Varapamil in combination with Gemcitabine in tumors grown subcutaneously, under the skin, in vivo in various schedules mimicking human dosing regimens. Tumor progression was initially assessed by injecting cancer cells into the tail vein of mice, and tumor cells were allowed to settle and grow in various metatatic locations before treatment. More clinically relevant and complex in vivo models where cancer cells were grown orthotopically in the pancreas, in the site of the primary tumor that the cells were derived from, and a spontaneous, naturally formed, pancreatic cancer model were also used. Imaging techniques, blood-flow, tumor perfusion, and total blood volume within the tumors were employed to fully assess the outcome of therapy on tumor blood supply. Flow cytometry and CT scans

examined markers and drug concentrations in the organs, tumors, and blood levels. Examining changes in marker expression allowed the magnitude of hypoxia to be scored.

The data reported by Wong et al. (2015) indicate that co-administration of Cilengitide and Verapamil increased tumor angiogenesis, yet the vessels that formed were more functional and less leaky, resulting in improved blood flow. The vascular promotion strategy was effective in both highly and poorly vascularized tumors. Crucially, the degree of hypoxia within tumors was reduced following vascular promotion. Tumor growth and progression was reduced, even after cessation of treatment, leading to an extension in survival in in vivo models.

Additionally, delivery and uptake of the drug Gemcitabine improved, with reduced side effects. Gemcitabine uptake into cells is regulated by equilibrative nucleoside transporters (ENT) 1 and 2, and concentrative nucleoside transporter 3 (CNT3) (Farrell et al., 2009). Gemcitabine is metabolized by rate-limiting metabolizing enzymes such as deoxyxytidine kinase (DCK). The authors demonstrate that ENT1 and 2 expression is downregulated in tumor cells under hypoxic conditions and ENT1 and 2 expression was significantly elevated following vascular-promoting therapy. CNT3 was also upregulated following Cilengitide treatment, which mediates the unidirectional flow of the drug into the cells, thereby increasing the efficacy of Gemcitabine. Cilengitide also increased DCK expression. As a consequence of the "vascular promotion" therapy, an increase in Gemcitabine uptake by ENT1 and 2, and a decreased efflux of the drug due to CNT3 led to the enhanced expression and saturation of DCK, thus increasing the potency of Gemcitabine. Wong et al. (2015) also demonstrated that Cisplatin efficacy improved following vascular promotion therapy. However, this was not due to the influence on the uptake of Cisplatin, but rather reflected the improved delivery by blood vessels to tumor regions previously poorly perfused.

This study provides key evidence that vascular promotion therapy can increase chemotherapy delivery to tumors as well as enhance drug uptake and reduce side effects. These data thereby challenge the negative concept that targeting tumor vasculature ultimately leads to aggressive tumors as a consequence of increasing tumor hypoxia, because vascular promotion therapy improves blood flow and reduces tumor growth and progression. Future studies should establish if vascular promoting therapy improves response to radiation, which is dependent on oxygen levels, and also whether it can facilitate other approaches to cancer treatment by improving vascular access, such as monoclonal antibodies and nanoparticles (Neijzen et al., 2014).

Key issues for the clinic relate to the heterogeneity within different tumor types and within a tumor, variation between patients, safety in the presence of vascular disease, and if vascular promoting therapy will be beneficial in metastatic disease and in different organs.

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