Antiangiogenic therapy: Creating a unique "window" of opportunity

Antiangiogenic therapy for solid tumors clearly destroys tumor vasculature and reduces tumor growth. As an unexpected bonus, drugs that neutralize VEGF signaling generate a "normalization window" for tumor vasculature. This occurs via the recruitment of pericytes to the tumor vasculature, an effect associated with the transient stabilization of vessels and improved oxygen delivery to hypoxic zones. The normalization process is mediated by angiopoietin-1 and matrix metalloproteinases and creates a window of opportunity for improved sensitivity to ionizing radiation and the delivery of chemotherapeutic drugs.

The rationale for antiangiogenic therapy in cancer is multifaceted. Growing tumors produce angiogenic factors to recruit host vasculature or circulating vascular progenitor cells to provide the substrate for the synthesis of new blood vessels. These angiogenic vessels provide a conduit for blood flow to deliver nutrients and oxygen to meet the metabolic demands of the growing neoplasm. In turn, as the tumor enlarges, necrotic and hypoxic zones in tumors are created due to the immature nature of tumor vasculature. In certain regions of tumors, blood vessels contain the proper repertoire of endothelial-lined, pericyte-enwrapped vessels that may provide nutritive blood flow, while in other areas, the vessels are highly permeable, ectatic, and lack stabilizing pericytes. These immature vessels do not provide nutritive flow and create a hypoxic environment with high intratumoral hydrostatic pressures, thus reducing the sensitivity of tumor cells to radiotherapy as well as impeding the delivery of chemotherapeutic drugs (Jain, 2003).

Clearly, antiangiogenic agents reduce the vascular density of tumors and ultimately tumor burden (Kerbel and Folkman, 2002). There are data suggesting that antiangiogeneic agents may further increase hypoxia in the remaining tumor cells by choking off the blood supply to tumors, thus perpetuating the cycle of hypoxia, tumor growth, and continued angiogenesis. In murine models of solid and metastatic tumors, antiangiogenic agents greatly eliminate tumor blood vessels and tumor burden. However, in human cancers, antiangiogenic agents need to be combined with other therapies to show a significant clinical outcome. There are many potential reasons for the lack of a robust effect of monotherapy, including dosing regimens of the antiangiogenic drugs, but to date, combination therapy is the standard of care. Analysis of how antiangiogenic agents reduce vessel density shows that these drugs reduce vascular permeability, destroy "less mature" vessels, and increase the recruitment of pericytes to stabilize other vessels in tumors. This apparent stabilization has been termed the normalization window, defined as a period of time where tumor blood flow and oxygenation transiently increases, thus providing an opportunity to better deliver chemotherapeutic agents and radiation therapy (Jain, 2001). After continued treatment beyond the normalization window, pericyte-replete vessels are also destroyed. The molecular basis for normalization is not known, but the paper by Winkler et al. (2004) in this issue of *Cancer Cell* sheds insights into the mechanisms of pericyte recruitment after inhibition of angiogenesis with an antibody that blocks VEGF receptor 2, DC101.

In order to gain insights into the kinetics of the normalization window, the authors compared the doubling time of U87 glioma tumors implanted into a cranial window model in mice. Mice received either ionizing radiation or treatment with

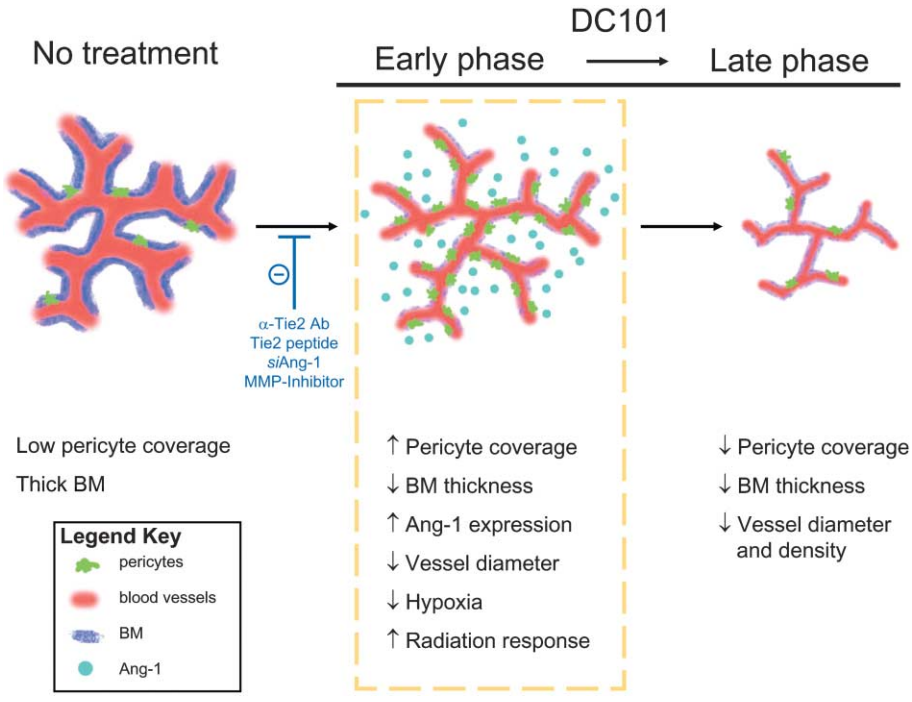

Figure 1. Schematic diagram depicting the mechanism of vascular normalization by DC101

Untreated tumor vasculature is characterized by dilated and tortuous vessels, thickened basement membrane (BM), and little pericyte coverage. DC101 treatment rapidly elicits an early phase of vascular normalization (marked by the box) in which there is an increase in pericyte recruitment mediated by the Ang-1, since a Tie-2 blocking Ab (α-Tie 2), a peptide antagonist for Tie 2 or siRNA treatment against Ang-1, effectively reduces the pericyte coverage. There is a concomitant decrease in blood vessel diameter with no change in vessel density, a decrease in BM thickness, and decrease in tumor hypoxia. The changes in BM thickness are due to increases in collagenase activity, as MMP inhibitor can block this response. The changes mediated by DC101 in tumor extracellular matrix and vasculature open a window in which tumor oxygenation increases temporarily, thus allowing for effective cytotoxic killing of tumor cells by ionizing radiation. During the later phase of DC101 treatment, there is a relapse of tumor hypoxia and gradual loss of pericyte coverage as blood vessel density continues to drop.

DC101 or combinations of the two. Remarkably, the authors show a synergistic effect of combination therapy in delaying tumor doubling time by more than 21 days only in a restricted window of treatment time, namely radiation therapy given on days 4–6 after the start of DC101 treatment. Upon closer examination, there was a marked decrease in tumor hypoxia during DC101 treatment beginning on day 2 and lasting through day 5. The ability of DC101 to increase tumor oxygenation (i.e., decrease the hypoxic tumor fraction) supports the idea that anti-VEGF therapy redistributes blood flow to hypoxic zones, thereby providing oxygen for radiation-induced DNA damage and cell death (Figure 1).

To further probe the potential mechanisms to explain how VEGFR2 blockade reduces tumor hypoxia, the authors show that DC101 mediates its effect by increasing pericyte recruitment to tumor blood vessels, thereby stabilizing or normalizing the once leaky, dilated tumor vasculature. This normalization process has been previously described by a reduction in abnormal, leaky tumor vessels (lacking stabilizing support cells or pericytes) and an increase in the number of anatomically patent vessels (enveloped by pericytes). Treatment with DC101 dramatically increases the number of pericyte-covered vessels detected, supporting previous studies using either VEGFR inhibitors or neutralizing antibodies (Inai et al., 2004; Tong et al., 2004). At first glance, these data are reminiscent of data showing that genetic withdrawal of VEGF can lead to preferential pruning of non-pericyte-covered vessels (Abramovitch et al., 1999; Benjamin et al., 1999). To distinguish whether the observed increases in pericyte coverage are due to vessel pruning or actual recruitment of pericytes, this paper elegantly documents the power of serial imaging of living blood vessels in tumors and shows that neutralization of VEGF signaling with DC101 increases pericyte recruitment without changing overall tumor vascular density. The increase in pericyte coverage is maintained until day 5, when vessel density significantly drops, partly due to an increase in vascular cell apoptosis. After continued therapy (8 days), pericyte-covered vessels decrease in number concomitant with reductions in vascular density, indicating that pericyte-covered vessels are still subject to regression, similarly observed during VEGF withdrawal (Baluk et al., 2004).

A previous study by Jain's group showed that treatment of MCaIV tumors with DC101 reduced angiopoietin-2 (Ang-2) expression, raising the potential involvement of the angiopoietin family members during normalization (Tong et al., 2004). Interestingly, in the present study, treatment with DC101 upregulates the synthesis of Ang-1 during the phase of pericyte coverage of tumor vessels. This effect appears to be functionally relevant, since treatment of the cancer cells with an antibody that blocks the receptor for Ang-1 and Ang-2 (Tie2) 1, a Tie-2 blocking peptide and siRNA against Ang-1, reduces DC101-stimulated pericyte recruitment. More impressively, DC101-triggered decreases in the hypoxic tumor fraction were abrogated by the Tie-2 blocking peptide. The role of Ang-1 in promoting pericyte recruitment was initially suggested based on analysis of mice deficient in Ang-1. However, the mechanism of how Ang-1 performs the task of pericyte recruitment, as well as how neutralization of VEGF could lead to Ang-1 gene expression, is not known. Presumably, tumor-derived Ang-1 acts on endothelial cell Tie 2 to generate an additional signal or signals to recruit pericytes; however, the nature of signal leading to pericyte recruitment is unknown. Current dogma supports the idea that Ang-1 generally promotes vessel maturation, whereas Ang-2 may serve to antagonize the action of Ang-1 in the absence of VEGF. However, the waters are a bit murky, since the role of Ang-1 in tumor angiogenesis is still under debate. Although many tumors overexpress Ang-1, the regulation of Ang-1 expression by hypoxia, cytokines, and other growth factors has yielded equivocal results (Metheny-Barlow and Li, 2003).

In addition to Ang-1 being an endothelial cell survival factor (Papapetropoulos et al., 1999), it also reduces vascular leakage (Thurston et al., 1999). However, the decrease in vascular permeability during the entire course of DC101 treatment did not quite mirror the pericyte coverage kinetics. The ability of Ang-1 to inhibit permeability was thought to be due to enhancement of cell-to-cell junctions as well as stabilization of blood vessels by promoting mural cell recruitment, yet DC101 blockade in permeability is continually observed even on day 8, when Ang-1 and pericyte coverage are decreased to control levels. This raises interesting questions as to whether other mediators, such as PDGF or the loss of nitric oxide, can affect permeability and promote normalization. Clearly, the mechanisms and mediators of pericyte recruitment in regulating tumor vascular permeability need to be further delineated.

In addition to the changes in pericyte recruitment during DC101 treatment, the authors show a dramatic change in basement membrane (BM) architecture. DC101 reduces BM thickness and increases type IV collagenase activity surrounding the blood vessels. Administration of the Tie2 blocking antibody did not reduce the thinning of BM mediated by DC101, implying that other regulatory mechanisms are likely responsible for the altered extracellular matrix. The data showing a thinning of the BM is in contrast to other studies using VEGFR inhibitors such as AG013736, VEGF-Trap, or VEGF withdrawal. These modalities led to eventual regression of the tumor vasculature (Baluk et al., 2004; Inai et al., 2004) despite the persistent presence of BM, even after complete disappearance of CD31-positive blood vessels. These BM ghosts were suggested to serve as scaffolds for potential revascularization, thus presenting it as another attractive therapeutic target. In addition, Jain's group previously reported BM thickening after DC101 treatment in another tumor model (Tong et al., 2004). The confounding data on VEGFR blockade and BM modification illustrate that disrupting VEGF-VEGF receptor signaling can dynamically alter the BM structure, influencing tumor vasculature and growth.

Regardless of the precise molecular mechanisms of how anti-VEGF therapy synergizes with radiation to reduce tumor size, the paper by Winkler et al. importantly documents the potential mechanisms that govern the generation of a normalization window and provides a rational basis for the combination therapy of antiangiogenic drugs with radiation and perhaps other forms of chemotherapy. Clearly, this work will increase excitement in defining the mechanisms of the normalization window in humans and in harnessing opportunities to improve the sensitivity of tumors to radio- and chemotherapy.

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Chronic versus acute myelogenous leukemia: A question of self-renewal

Leukemia stem cells are defined as transformed hematopoietic stem cells or committed progenitor cells that have amplified or acquired the stem cell capacity for self-renewal, albeit in a poorly regulated fashion. In this issue of *Cancer Cell*, Huntly and colleagues report a striking difference in the ability of two leukemia-associated fusion proteins, MOZ-TIF2 and BCR-ABL, to transform myeloid progenitor populations. This rigorous study supports the idea of a hierarchy among leukemia-associated protooncogenes for their ability to endow committed myeloid progenitors with the self-renewal capacity driving leukemic stem cell propagation, and sheds new light on the pathogenesis of chronic and acute myelogenous leukemias.

Human cancer stem cells, identified in acute myelogenous leukemia (Bonnet and Dick, 1997), myeloid blast crisis of chronic myelogenous leukemia (Jamieson et al, 2004), breast cancer (Al-Hajj et al., 2003), and brain tumors (Singh et al., 2003), share functional properties with normal stem cells, such as high proliferative potential, some differentiation capacity, and the ability to be serially transplanted (reviewed in Passegué et al., 2003). Signaling pathways involved in the regulation of normal stem cell self-renewal are frequently mutated or epigenetically activated in cancer, indicating that self-renewal, i.e. a cell division that produces progeny identical to the parental cell, is a vital property of cancer stem cells (reviewed in Reya et al., 2001). Targeted disruption of cancer stem cell self-renewal would represent a novel therapeutic strategy that could significantly reduce the capacity of a tumor to propagate itself, and could be employed in the eradication of a broad spectrum of cancers, including leukemias.

Chronic myelogenous leukemia (CML) and most types of acute myelogenous leukemia (AML) are induced by leukemia-associated fusion proteins that generally function as aberrantly activated signaling mechanisms or positive or negative transcriptional regulators, and directly interfere with the hematopoietic differentiation program. While their mechanism of action is relatively well understood, little is known about their developmental requirement for transformation and the role of self-renewal in this process. In this issue of *Cancer Cell*, Huntly et al. (2004) have studied in the mouse the target cell requirement of two human leukemia-associated fusion proteins, MOZ-TIF2 and BCR-ABL. MOZ-TIF2, an AML-associated fusion gene resulting from the inv (8)(p11q13)-induced juxtaposition of the MOZ chromatin remodeling gene and the TIF2 nuclear receptor transcriptional coactivator, is thought to modulate the transcriptional activity of target genes through aberrant histone acetylation. In contrast, BCR-ABL, the hallmark of CML, gives rise to a constitutively active protein tyrosine kinase, which endows primitive stem and progenitor cells with a proliferative and survival advantage (reviewed in Daley, 2004). Using retroviral gene transfer combined with in vitro serial replating assays and leukemic transplantation into lethally irradiated recipient mice, they compared the capacity of MOZ-TIF2 and BCR-ABL to enhance the self-renewal potential of normal murine bone marrow mononuclear cells, highly purified hematopoietic stem cells (HSC), and more committed progenitors including common myeloid progenitors (CMP) and granulocyte-monocyte progenitors (GMP). They also included critical controls, such as MOZ-TIF2 point mutants that lacked transforming activity, to exclude a contribution by retroviral insertional mutagenesis to the observed leukemogenic effects. The results presented in this paper demonstrate that MOZ-TIF2, but not BCR-ABL, endows myeloid progenitors with self-renewal