homeostasis and integrity, oncogene activation, or inflammatory insults result in the induction of chemokines and cytokines that shape the local microenvironment. Recruited immune cells and CAF, as well as altered epithelial and neoplastic cells, continue to produce cytokines (such as IL-6) that activate oncogenic transcription factors (i.e., STAT3), further sustaining tumor-associated inflammation. This paradigm applies not only to pancreatic or esophageal cancers, but also to the majority of solid malignancies. This implies that drugs that disrupt tumor-associated inflammation, either by targeting STAT3 activating kinases (e.g., JAK2) or key proinflammatory cytokines, such as IL-6, should have significant therapeutic and preventive effects in a variety of cancers, regardless of their origin.

ACKNOWLEDGMENTS

S.I.G. was supported by Crohn's and Colitis Foundation of America (CCFA #2693), whereas M.K. is an American Cancer Society Research Professor, and research in his laboratory is supported by the National Institutes of Health.

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Multiple Effects of Angiopoietin-2 Blockade on Tumors

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DOI 10.1016/j.ccr.2011.03.016

In this issue of *Cancer Cell*, Mazzieri, Pucci, and colleagues describe the marked effects of inhibiting the proangiogenic cytokine, Angiopoietin-2, on tumor angiogenesis and progression in spontaneous tumor models, as well as the proangiogenic functions of TIE2-expressing macrophages.

A number of antiangiogenic agents have now been developed to inhibit vascular endothelial growth factor (VEGF) signaling pathway in tumors. Like the majority of existing anticancer therapies, their clinical efficacy is limited by the transient nature of their inhibitory effects on advanced tumors. In several mouse tumor models, VEGF inhibition results in marked suppression of tumor angiogenesis, which often leads to reduced tumor growth and even tumor shrinkage. However, tumors usually revascularize and grow back upon prolonged treatment. This escape from VEGF blockade is thought to be due to various mechanisms, including the upregulation of alternative proangiogenic growth factors, the enhanced invasive/metastatic activity of tumor cells (to locate an alternative blood supply), and the increased recruitment of proangiogenic bone marrow-derived cells to the tumor site, where they promote tumor revascularization and growth (Bergers and Hanahan, 2008).

A major finding in the latter area has been that certain inflammatory cell types convey tumor resistance to antiangiogenic therapies. For example, Shojaei et al. (2007) showed, in murine tumor models, that tumor-infiltrating CD11b+ Gr1+ myeloid cells can induce tumor resistance to VEGF therapy via their release of Bv8 (prokineticin-1), a proangiogenic cytokine stimulated by their exposure to tumor cell-derived granulocyte colony-stimulating factor (G-CSF). In this way, certain mouse tumors treated with anti-VEGF drugs are able to circumvent their dependency on VEGF and render themselves resistant to anti-VEGF therapy.

Proangiogenic myeloid cells are also thought to be recruited in response to the elevated release of such chemoattractants as CXCL12 (stromal cell-derived factor-1, SDF1) by hypoxic areas of therapy-damaged tumors (Kioi et al., 2010). Among the most essential of these are a highly proangiogenic subset of monocytes/macrophages that express the angiopoietin receptor, TIE2, and so are termed

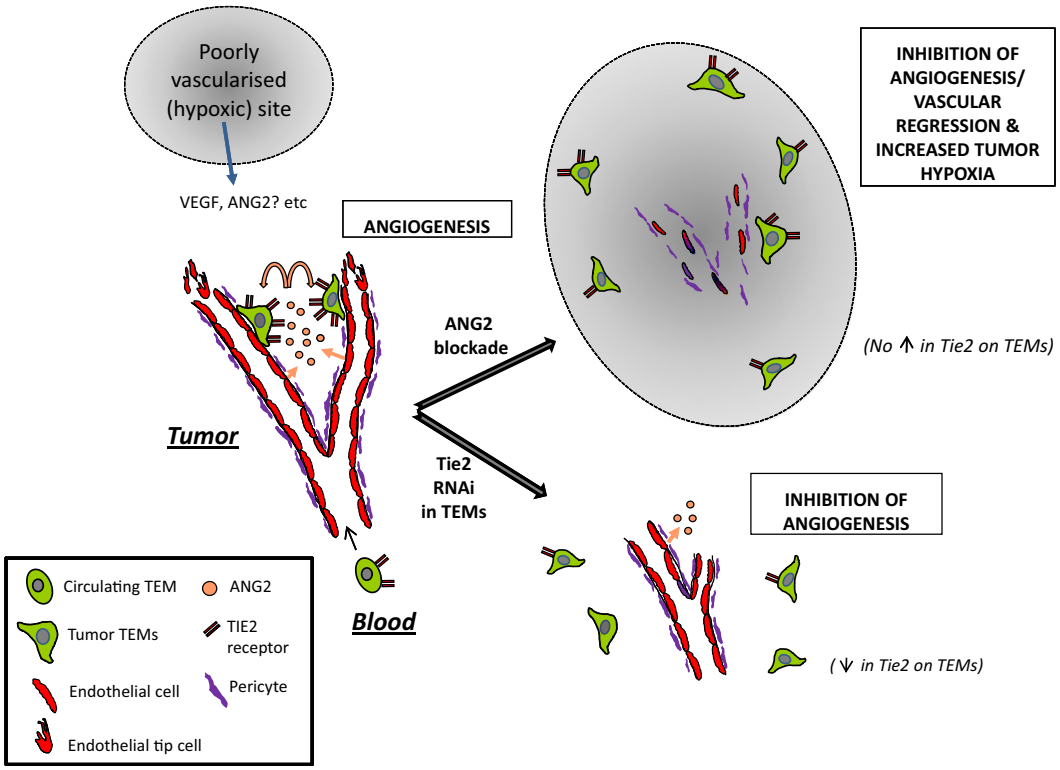

Figure 1. Targeting ANG2 or TIE2 on TEMs Has Pronounced Effects on the Tumor Vasculature
(Left panel) TEMs upregulate TIE2 upon entry to tumors and locate around angiogenic blood vessels. At these sites, they are exposed to ANG2 (released by the vessels as well as possibly cells in hypoxic areas).
(Top right panel) Treatment with ANG2 antibody causes vessel regression, increased hypoxia, blocks the increase in TIE2 on tumor TEMs, and causes TEMs to locate to sites distant from remnant blood vessels. This phenotype is associated with decreased metastasis to distant sites.
(Bottom right panel) Conditional knockdown of TIE2 in TEMs results in inhibition of tumor angiogenesis and again blocks TEM association with tumor vessels.

TIE2-expressing monocytes/macrophages (TEMs). These cells preferentially infiltrate tumors where they upregulate their expression of TIE2, express an aggressive, tumor-promoting phenotype and are essential for tumor angiogenesis and progression (De Palma and Naldini, 2009). Although TEMs have yet to be implicated in tumor resistance to anti-VEGF therapy, they appear to be involved in tumor recurrence after local irradiation (Kioi et al., 2010).

An interesting picture has emerged recently of one way in which the tumor microenvironment stimulates the tumor-promoting functions of TEMs. Angiopoietin-2 (ANG2), a cytokine upregulated in tumors mainly by endothelial cells and some malignant cells, stimulates tumor angiogenesis in concert with other proangiogenic factors, namely VEGF (Augustin et al., 2009). However, tumor-derived ANG2 also stimulates the proangiogenic functions of TEMs (Coffelt et al., 2010), suggesting that new therapeutic agents targeting the ANG2–TIE2 axis may not only inhibit tumor angiogenesis directly (by targeting autocrine signaling on activated endothelial cells) but also indirectly by inhibiting TEMs. Furthermore, ANG2 is upregulated by tumor hypoxia—a known consequence of anticancer therapies—so it may further enhance the tumor-promoting functions of TEMs in treated tumors.

The study reported by Mazzieri et al. (2011), in the current issue of *Cancer Cell*, employed both a monoclonal antibody to ANG2 (clone 3.19.3) and a novel conditional lentiviral system for specifically knocking down TIE2 in TEMs to demonstrate the essential role of the ANG2/TIE2 axis in driving TEM-mediated tumor angiogenesis in various tumor models. ANG2 blockade induced vascular regression and durable growth inhibition, irrespective of whether it was administered to early or late-stage spontaneous tumors. Importantly, no signs of tumor resistance were evident in their tumor models, including a spontaneous islet tumor model known to develop resistance to anti-VEGF/VEGFR therapy (RIP1-Tag2) (Bergers and Hanahan, 2008). At first glance, these findings appear to be at variance with the fact that tumor xenografts grown in ANG2-deficient mice showed no sign of reduced tumor angiogenesis and progression (Nasarre et al., 2009). However, they accord well with other recent studies demonstrating the antitumor efficacy of specific ANG2 inhibitors in mouse tumor models (Huang et al., 2010). An equally fascinating finding by Mazzieri et al. (2011) is that the ANG2 blockade also suppressed the metastatic spread of spontaneous mammary (MMTV-PyMT) tumors. Further studies are now warranted to investigate the ANG2-driven mechanisms promoting metastasis in this and possibly other tumor models.

Taken together, the above findings highlight the potential therapeutic value of specific ANG2 inhibitors, some of which are currently being tested in clinical trials. However, the degree of inhibition of tumor growth elicited by AMG386, a peptide-Fc

fusion protein that blocks both ANG2 and its sister molecule ANG1 (another TIE2 ligand, which has the opposite effects to ANG2 on endothelial cells), appears to be model-dependent (Coxon et al., 2010). Both ANG1 and ANG2 were targeted by AMG386 because ANG1-specific antagonism was seen to increase the inhibitory effects of ANG2-specific antagonism on tumor growth (suggesting that ANG1 and ANG2 may have cooperative effects on tumor growth in some models). Although specific ANG2 inhibitors show efficacy in a wide array of tumor models (Huang et al., 2010; Mazzieri et al., 2011), it remains to be seen whether they are also effective in a broad spectrum of human tumor types.

The paper by Mazzieri et al. (2011) also generated a number of unique insights into the regulation of TEMs by ANG2 in tumors (Figure 1). First, they showed that the ANG2 blockade resulted in increased TEM recruitment to tumors (possibly via a hypoxia/CXCL12-driven pathway) but prevented their upregulation of TIE2 upon entry to the tumor site. This suggests that ANG2 regulates TIE2 expression in TEMs, although whether this operates via a direct effect on these cells or indirectly via endothelial cells has yet to be ascertained. Intriguingly, disruption of the ANG2-TEM axis using either their ANG2 antibody or the selective knockdown of TIE2 in TEMs impeded TEM association with nascent tumor blood vessels and disabled the proangiogenic activity of TEMs. Although not directly addressed in their paper, the authors propose a causal link between the latter two events. Finally, they show that TIE2 knockdown in TEMs led to reduced tumor angiogenesis and vascular perfusion but not the vessel regression and tumor growth inhibition seen with the anti-ANG2 antibody. They speculate that this could be due to the nonexhaustive knockdown of Tie2 in TEMs. However, an alternative interpretation is that the growth-promoting effects of ANG2 on tumors may not be mediated by TEMs. Future studies will clarify and extend these aspects of the study.

The full efficacy of anti-VEGF and other antiangiogenic therapies is unlikely to be realized until the mechanisms underlying tumor resistance are fully understood and then selectively inhibited. The results described by Mazzieri et al. (2011) are encouraging and suggest that targeting the ANG2-TIE2 axis may represent a promising antiangiogenic treatment that inhibits not only key events in tumor angiogenesis and metastasis, but also the insidious myeloid cells recruited by tumors to kick-start their recovery. However, it should be remembered that, to date, evidence for murine and human tumors employing similar reparative mechanisms after antiangiogenic therapy remains scant. Phase II clinical studies have recently shown that AMG386 improves progression-free survival in ovarian cancer patients (Huang et al., 2010), but we will have to wait to see whether any of the selective ANG2 inhibitors currently in clinical trials have the marked antitumor effects predicted by the current study—either alone or in combination with cytotoxic or other antiangiogenic agents.

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