Tumor-Associated Fibroblasts as "Trojan Horse" Mediators of Resistance to Anti-VEGF Therapy

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While targeting VEGF has shown success against a number of human cancers, drug resistance has resulted in compromised clinical benefits. In this issue of *Cancer Cell*, Crawford et al. (2009) report that tumors resistant to anti-VEGF therapy stimulate tumor-associated fibroblasts to express proangiogenic PDGF-C, implicating it as a potential therapeutic target.

One of the early hopes for the use of antiangiogenic drugs for cancer treatment was that they would be much less likely to lose their therapeutic activity as a result of tumor-acquired resistance over time (Kerbel, 1991). The theory posited that a drug targeting genetically stable (normal) host cells—namely, vascular endothelial cells, rather than highly mutable, genetically unstable tumor cells (a major driving force responsible for acquired resistance to other anticancer drugs)—would be less likely to elicit resistance or tolerance. Aside from the fact that the assumption of genetic stability for endothelial cells in tumor blood vessels may not always be correct (Hida and Klagsbrun, 2005), clinical experience has unequivocally shown that acquired resistance to antiangiogenic drugs is inevitable (Bergers and Hanahan, 2008; Kerbel, 2008). Virtually all patients whose tumors initially respond to drugs such as bevacizumab (monoclonal antibody to VEGF), sorafenib, or sunitinib (small-molecule receptor tyrosine kinase inhibitors targeting VEGF receptors and PDGF receptors, among others) eventually become nonresponsive, often within months of therapy initiation (Kerbel, 2008). In addition, there are significant proportions of patients whose tumors are intrinsically resistant to such drugs, even when the intended drug targets, i.e., VEGF and VEGF receptors (especially VEGFR-2), are present in abundance. As a result, a rapidly growing area in tumor angiogenesis research is the elucidation of the mechanisms responsible for both intrinsic and acquired resistance to antiangiogenic agents (Bergers and Hanahan, 2008).

Although the current literature is limited, the number and diversity of mechanisms that have already been implicated is both biologically fascinating and therapeutically discouraging. With respect to acquired resistance, upregulation of compensatory proangiogenic pathways is one well-known proposed mechanism. Thus, targeting the VEGF pathway may lead to the emergence and overgrowth of tumor cell subpopulations, driven in part by drug-induced elevated levels of tumor hypoxia, which can induce neovascularization simply by producing a different proangiogenic mediator such as basic fibroblast growth factor (bFGF) (Casanovas et al., 2005). Additional mechanisms include selection of mutant tumor cells that have an enhanced ability to survive and grow under elevated hypoxia conditions, rapid remodeling/maturation of the tumor vasculature during treatment, and even co-option of normal vasculature in certain vascular-rich organs (see Bergers and Hanahan, 2008 for review). With respect to intrinsic resistance, aside from the absence of the drug target, alternative cellular mediators of angiogenesis—e.g., the recruitment and infiltration of tumors by proangiogenic but VEGF-independent circulating myeloid $Gr1^{+}CD11b^{+}$ cells (Shojaei et al., 2007)—were reported to be another possible mechanism.

In this issue of *Cancer Cell*, Crawford et al. (2009) report a new mechanism for intrinsic resistance to anti-VEGF antibodies, namely, that tumor cells refractory to anti-VEGF therapy in some manner stimulate adjacent tumor-associated fibroblasts (TAFs) to secrete platelet-derived growth factor C (PDGF-C), which in turn stimulates tumor angiogenesis. The authors studied two transplantable mouse lymphomas, one responsive (TIB6) and one intrinsically refractory (EL4) to anti-VEGF antibody therapy. Using microarray analysis, the authors found that TAFs isolated from refractory EL4 tumors upregulated PDGF-C mRNA. Using a neutralizing antibody, the authors implicated PDGF-C in promoting both angiogenesis and the growth of EL4 tumors progressing under anti-VEGF therapy. Thus, these results show that targeting VEGF can produce yet another compensatory proangiogenic mediator, but surprisingly, in this case, the source of the redundancy was not the tumor cell population per se, but rather one of its stromal cell components.

The results of Crawford et al. add a new twist by which TAFs can influence tumor angiogenesis. For example, previous studies have implicated such cells as a possible major source of endogenous VEGF driving tumor angiogenesis (Fukumura et al., 1998). With respect to drug-induced changes in TAFs, trastuzumab treatment was shown to upregulate the endogenous angiogenesis inhibitor thrombospondin 1 (TSP1) in TAFs, which may

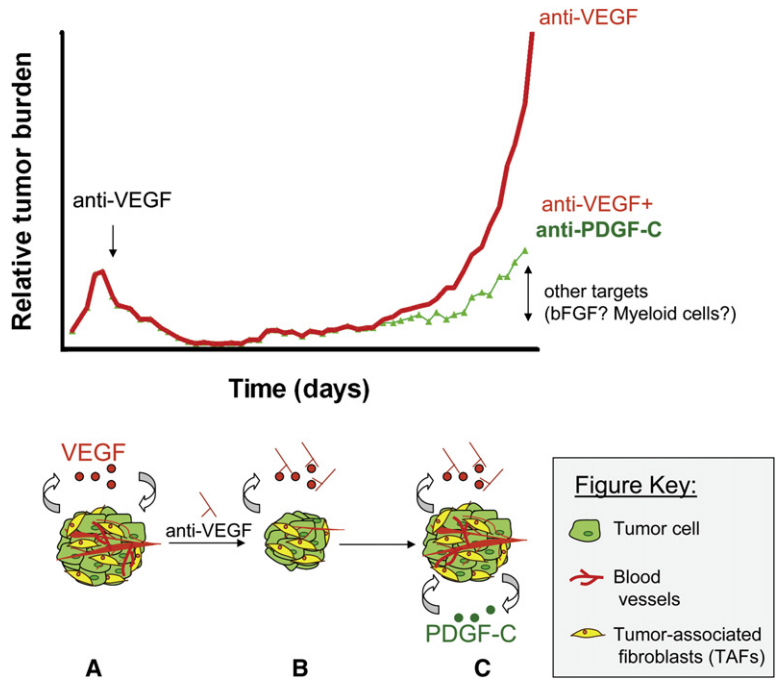

Figure 1. Model of Resistance to Anti-VEGF Therapy Mediated by Tumor-Associated Fibroblasts

Schematic graph shows relative tumor growth over time of a tumor responsive to administration of anti-VEGF therapy (A), leading to initial regression (B). Crawford et al. (2009) report that tumors refractory to anti-VEGF therapy stimulate tumor-associated fibroblasts (TAFs) to secrete the proangiogenic factor PDGF-C, which compensates for the neutralization of VEGF and promotes tumor angiogenesis (C). Although their results were obtained with tumors intrinsically resistant to anti-VEGF therapy (as in C), their results imply that the same mechanism may arise in tumors that develop acquired resistance and suggest that PDGF-C targeting may be an effective second-line therapy.

account for some of the antiangiogenic "side" effects of this drug (Izumi et al., 2002). Similarly, the antiangiogenic effects of low-dose metronomic chemotherapy may be caused by upregulation of TSP1 (Bocci et al., 2003), occurring in part within the TAF population (Hamano et al., 2004). However, in contrast to the results of Crawford et al., such changes do not facilitate resistance to such drugs, but rather the contrary.

The secretion of PDGF-C by TAFs once again highlights the importance of the tumor microenvironment in tumor biology. It also provides further evidence that nonmalignant stromal cells are not necessarily innocent bystanders in the tumor milieu. Rather, they can be active consiglieri, conspiring to stimulate tumor growth, metastasis, and perhaps even response to antiangiogenic therapy (see Figure 1).

Like any new and provocative finding, the Crawford et al. study raises many questions. The authors used two transplantable lymphomas for their studies in part because fibroblasts can be isolated relatively easily from such tumors. Will the results apply to carcinomas and sarcomas? The studies undertaken involved mainly intrinsic resistance and as such lead one to ask whether acquired resistance to VEGF pathway-targeting drugs might also be mediated by upregulation of PDGF-C in TAFs. What might the implications of the findings be for small-molecule drugs such as sunitinib or sorafenib that target not only VEGF receptors but also PDGF receptors, which can bind PDGF-C? Could this be a factor in their robust single-agent activity in renal cell or hepatocellular carcinoma patients, in contrast to bevacizumab, which is currently approved for use only in combination with chemotherapy? Also with respect to the issue of clinical relevance, could the results of Crawford et al. help explain the phase III clinical trial failure of bevacizumab treatment (when combined with weekly gemcitabine) for pancreatic cancer? As noted by the authors, pancreatic cancers are often heavily infiltrated by fibroblasts. Conversely, in situations where bevacizumab does provide a benefit when combined with chemotherapy—something not modeled in the Crawford et al. studies—would PDGF-C upregulation provide escape from such combination treatment regimens, or solely from anti-VEGF monotherapy? And what about mechanisms of acquired resistance to drugs such as sunitinib or sorafenib? Such resistance infers additional pathways of resistance. Indeed, the VEGF-refractory EL4 tumor model was previously reported to recruit and subsequently "prime" bone marrow-derived circulating $Gr1^{+}CD11b^{+}$ myeloid cells to stimulate tumor angiogenesis, even in the presence of VEGF-neutralizing antibodies.

The approval of the first antiangiogenic agents for cancer therapy set off a wave of excitement. Now, the dawning realization is that tumors possess an embarrassment of riches when it comes to intrinsic, inducible, and/or acquired mechanisms to evade antiangiogenic therapies. Uncovering such mechanisms, of which PDGF-C upregulation is the latest addition, should hopefully lead to strategies that cause growth delays in tumors that evade and then relapse to first-line antiangiogenic therapies (Figure 1). In this scenario, the ultimate target will not likely be a single molecule, but rather the gradual yet significant extension of survival brought about by additional lines of therapy aimed at multiple different targets.

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Unholy Matrimony: Aurora A and N-Myc as Malignant Partners in Neuroblastoma

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Aurora A is a mitotic kinase that is essential for regulation of the G2/M checkpoint. In this issue of *Cancer Cell*, Otto et al. report that Aurora A interacts with *MYCN*, a potent oncogene in human neuroblastoma, and sequesters it from proteolytic degradation. This surprising finding further enhances Aurora A's potential as a therapeutic target.

The Aurora kinases have attracted intense scrutiny in recent years due to accumulating evidence that they often act as oncogenic drivers in many human cancers (Gautschi et al., 2008). The Aurora family consists of three known gene paralogs (*AURKA*, *AURKB*, and *AURKC*) that are key regulators of mitosis. The genes each encode serine/threonine kinases with a significant degree of homology in the C-terminal catalytic domain, suggesting that the divergent N-terminal domains distinguish their diverse effects on the cell cycle and mitosis. While little is known about Aurora C, and Aurora B appears to play a regulatory role throughout mitosis, recent evidence strongly suggests that Aurora A has a more restricted role in the cell cycle and is absolutely required for the G2/M transition via phosphorylation of polo-like kinase 1 in concert with the cofactor Bora (Macurek et al., 2008; Sasai et al., 2008). In addition, Aurora A is critical for mitotic spindle assembly and stability, as well as regulation of centrosomal and kinetochore formation (Marumoto et al., 2005). It is therefore not surprising that Aurora A expression is tightly regulated throughout normal development and the cell cycle and that engineered *Aurka* deficiency in mice is early embryonic lethal (Sasai et al., 2008). Finally, *AURKA* amplification/overexpression is commonly seen in a variety of human neoplasms, and there has been interest in leveraging this fact therapeutically (Gautschi et al., 2008).

Likewise, the Myc family of transcription factors is commonly deregulated in cancer, via chromosomal translocation events, gene amplification, and interference with normal protein degradative pathways. In the childhood cancer neuroblastoma, *MYCN* is highly amplified in about 20% of cases, and these are uniformly very aggressive neoplasms with patients showing a poor survival probability. Importantly, there are another 20%–30% of cases that behave in an equally aggressive fashion but in which the tumors do not harbor amplification of the *MYCN* locus or other mechanisms for *MYCN* overexpression. Strikingly, these tumors typically overexpress *MYC* via mechanisms yet to be determined (Liu et al., 2008). While both the Aurora and Myc gene families seem to be obvious candidates for anticancer drug development, the Auroras theoretically provide a much more tractable therapeutic target since kinases are currently more easily druggable, especially compared to promiscuous and weak transcription factors like Myc and N-Myc.

In this issue of *Cancer Cell*, Otto et al. (2009) identify Aurora A and N-Myc as oncogenic partners in neuroblastoma, with Aurora A functioning to sequester N-Myc away from ubiquitin-mediated proteolytic degradation. Using a synthetic-lethal screening strategy in neuroblastoma cell line models, the investigators knocked down 194 separate genes selected to be candidates for allowing neural progenitor cells to survive deregulated *MYCN* (forced overexpression of *MYCN* in neural progenitor cell models or *MYCN*-nonamplified neuroblastomas results in immediate induction of programmed cell death). These were genes overexpressed in *MYCN*-amplified tumors and/or genes with direct evidence for being a Myc target. *AURKA* was one of 17 genes that showed selective antiproliferative effects in the *MYCN*-amplified cells when the protein was knocked down. In a series of elegant and well-controlled experiments, Otto and colleagues demonstrated that Aurora A stabilizes the N-Myc protein through a direct physical interaction and interferes