Tumor Angiogenesis
Putting a Value on Plastic GEMMs

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It has long been appreciated that angiogenesis can contribute to the induction or progression of several diseases, including retinopathies, arthritis, psoriasis, and cancer. Because endothelial cells typically exist in a quiescent state in normal vessels, the molecular changes required for these cells to become proliferative and invasive have long been considered potential targets for the control of both neovascularization and the accompanying pathology. To this end, over the past 3 decades, several novel effectors of angiogenesis have been characterized, ranging from growth factors, such as vascular endothelial cell growth factor (VEGF), to signaling effectors, such as RasGAPs, to cell adhesion molecules, including the α integrins featured in the article by Steri et al. Nonetheless, as an aggregate, the efficacy of antiangiogenic agents has only produced a modest impact on disease.

We and others have demonstrated that antagonists of α integrin target and suppress pathological angiogenesis, resulting in decreased tumor growth, inflammatory disease, and retinal disease. In glioblastoma patients treated with these agents, some clinical efficacy was noted, although overall survival was not significantly impacted in recently announced phase III trials. Studies by Hynes et al showed that mice deficient in either α or β3 showed robust blood vessel development. In fact, these animals displayed enhanced endothelial cell VEGF receptor (VEGFR) expression associated with increased tumor angiogenesis. This led the authors to conclude that αβ3 plays a negative role in angiogenesis. How, then, can one reconcile the fact that αβ3 antagonists suppress angiogenesis in mice and humans? In an elegant series of studies, Steri et al examined the effects of acute depletion of endothelial cell integrin αβ3 on neovascularization via Cre-mediated inactivation of a floxed β3 gene. Complementary approaches were used in this multicenter tour de force of mouse genetics, including Tie2-driven expression of Cre and hydroxytamoxifen-inducible expression of Cre driven by platelet-derived growth factor b promoters.

The finding by Steri et al that αβ3 is required for angiogenesis is consistent with previous studies by Byzoya and et al, who showed that mice expressing a signaling mutant of the β3 integrin subunit in which both cytosolic tyrosine residues were mutated to phenylalanine were deficient in angiogenesis. This was associated with decreased interactions between VEGFR2 and integrin αβ3, which were in turn associated with decreased VEGFR2 activation. The deficiency may have resulted from an absence of an active integrin signaling complex, because this mutant eliminated the binding sites for kindlins, which stabilize integrin–ligand interaction.

Steri et al found that although long-term knockdown of integrin αβ3 resulted in no impact on the growth of tumors in either knockdown system, the acute downregulation resulted in immediate decreased angiogenesis and growth of the tumor. The results corresponded to a decreased invasiveness of the temporally induced endothelial cells in aortic ring assays, which suggested that overall competence of endothelial cells to invade the tissue was compromised by acute deletion of integrin β3. However, over the longer-term, the plasticity of endothelial cells, or the tumor cells eliciting the response, permitted a cellular rewiring that promotes alternative means by which endothelial cells execute the key angiogenic functions of invasiveness, survival, and proliferation (Figure). Notably, the authors were able to identify 1 potentially important mechanism: the decreased expression of a crucial integrin downstream signaling protein, focal adhesion kinase (FAK), appeared to permit an increased invasiveness among the integrin-deficient endothelial cells. This corresponded with recent studies implicating haplosufficient endothelial FAK expression in increased tumor growth. Together, the results paint a compelling picture of the adaptations endothelial cells can make to execute a critical physiological program.

Still, all is not as clear as it might be, and certain aspects of these genetic studies defy easy explanation. The depletion of β3 integrin was shown to have an inhibitory effect on the expression of integrin αβ5 in these genetic models, and this feature has not been observed before in β3 integrin–targeted models. It is possible that with the loss of β3, some stabilizing influence on β5 is lost. Given that β3 and β5 seem to have unique and somewhat discrete roles in angiogenesis, the concomitant loss of both integrin heterodimers is surprising and may be significant. A dominant role for a cytokine-activated integrin β5 may cause a de facto requirement for its own downregulation; alternatively, the enhanced expression of VEGF in the β3-depleted cells could result in increased internalization and turnover of β5. Conversely, it is not known if integrin expression changes in response to therapies that target growth factors.

How the findings by Steri et al could, or should, be applied to our current knowledge of tumor therapy remains an open question. For example, if depletion of β3 integrin results in increased VEGF dependence, then is this an indication that...
these tumors will exhibit increased sensitivity to bevacizumab or to other VEGF pathway–targeting molecules?

In a similar vein, if decreased FAK activity is part of a long-term adaptation of integrin loss, then will the use of FAK inhibitors, over time, promote tumor angiogenesis and progression? Interestingly, FAK expression is required for angiogenesis in vivo, and FAK inhibitors have already been linked to decreased tumor vasculature, tumor growth, and metastasis. VEGF-producing tumor cells promote vascular permeability (a critical initial common event in both angiogenesis and tumor extravasation), an effect that is abrogated in the presence of FAK inhibition or genetic expression of kinase dead FAK.

The question regarding whether genetic deletion of an integrin is truly a good representation of what would occur after treatment with an integrin antagonist remains. Given that
integrin antagonism can actively promote cell death, although deletion of the integrin could prevent this, it would seem that this is another example of genetics and pharmacology conflicting. The present study by Steri et al goes a long way to resolve this. Precisely how these 2 systems compare, however, may not be truly known until directed tests are performed using integrin antagonists and genetic models in parallel. Preferably, these would be performed in an orthotopic tumor setting with inducible loss of integrin, alone and in combination. Such a study would permit a clearer elucidation of specific nonendothelial effects of integrin antagonists.

Nonetheless, the acute deletion model described by Steri et al clearly identifies at least 1 aspect of β3 function that may also be disrupted pharmacologically—as a regulator of an endothelial subroutine governing early/initial tumor vascularization. Although the molecular aspects of this subroutine are not identified, it may involve feedback with FAK. By contrast, endothelial cells lacking β3 for an extended period of time do not seem to use, or require, the same subroutine. So, what might this mean in the clinic? Both integrin antagonists and FAK inhibitors are well-tolerated, with minimal toxicity. At a minimum, the antagonism of integrin αvβ3 would be expected to slow the maturation of micrometastases, whereas FAK inhibition could provide a second barrier to tumor spread. However, the final impact of these on patient progression would depend intimately on the disease status itself and the genetics of the particular cancer. Once an agent has been proven in mice, the only way to determine efficacy in human patients is in the clinic. The studies of Steri et al provide useful clues for optimizing approaches for antiangiogenic therapy and, ultimately, may help guide current clinical approaches.

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References


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