# Antiangiogenics meet nanotechnology

**A mutant Raf-1 gene loaded onto nanoparticles, delivered to tumor vasculature with an integrin binding compound, provides effective antiangiogenic gene therapy in mice.**

Tumor blood vessels express specific markers that are not present in the blood vessels of normal tissues (Ruoslahti, 2002). Such marker molecules can be present in the endothelium, the pericytes, or the extracellular matrix of tumor blood vessels. Lymphatic vessels in tumors can also be distinct from those in normal tissues (Laakkonen et al., 2002). Many of the marker molecules that are selectively expressed in tumor blood vessels are proteins associated with tumor-induced angiogenesis, the sprouting of new blood vessels (Ruoslahti, 2002). These proteins are often functionally important in the angiogenesis process, and agents that perturb their function can be used to suppress angiogenesis. In a recent paper, Hood et al. (2002) put an angiogenesis marker protein, the $\alpha v\beta 3$ integrin, to a different use as a target for concentrating a gene therapy vector at tumor vessels (Figure 1).

Previous work, mostly from the same laboratory, has shown that the $\alpha v\beta 3$ integrin is selectively expressed in angiogenic vessels, and that antibodies and peptides capable of blocking its activity are antiangiogenic. Nanoparticles containing a contrast agent, an anti-$\alpha v\beta 3$ antibody (Sipkins et al., 1998), and bacteriophage displaying an $\alpha v\beta 3$ binding RGD peptide (Arap et al., 1998) become concentrated in tumor vessels after a systemic injection. Cheresh and colleagues have combined these elements with a dominant-negative Raf-1 gene to assemble a novel vector for gene therapy of tumors in mice. The design is innovative and the first treatment results are impressive.

Delivering the nanoparticles to the $\alpha v\beta 3$ integrin made it possible to achieve 10- to 15-fold tumor targeting selectivity relative to untargeted particles. Moreover, by growing the tumors using a cell line that does not express the integrin, they could restrict the delivery to the blood vessels (and possibly other stromal elements) in the tumor. The $\alpha v\beta 3$ integrin is selectively expressed in angiogenic vessels, and little expression is seen in resting blood vessels. These and previous results by Arap et al. (1998) show that delivering therapeutic agents to $\alpha v\beta 3$ in mouse tumors, including metastases in the lungs and liver, can produce impressive results. However, it remains to be seen how universal these results are, and in particular, how extensively $\alpha v\beta 3$ is expressed in the vasculature of human tumors. It would also be interesting to know whether the endothelial precursor cells that migrate from the bone marrow to sites of angiogenesis (Grant et al., 2002) express $\alpha v\beta 3$. If so, an $\alpha v\beta 3$-based therapy might intercept these cells even before they reach the angiogenic site.

The temporal and spatial expression profiles of other potential target molecules in tumor vasculature may differ from those of $\alpha v\beta 3$, potentially broadening the possibilities for drug delivery. Another way of ensuring a broad effect on tumor vessels might be to deliver the therapeutic agent not only to an endothelial target, but to make use of specific target molecules on the pericytes and extracellular matrix of tumor blood vessels.

The nanoparticles used as a targeting vehicle in this study are bifunctionalized, crosslinked lipid micelles that carry on their surface an $\alpha v\beta 3$ binding compound and a cationic lipid that binds DNA. Any particle injected into the bloodstream tends to be taken up by the reticuloendothelial system (RES). Various treatments, such as coating with polyethylene glycol, are often needed to reduce the RES uptake of particles. The nanoparticles used by David Cheresh's group delivered remarkably little gene activity to the liver, which is the main RES site. The authors only measured gene activity in the liver, so it is possible that the particles were lodged in the liver but that the expression vector DNA was not transduced or was for some other

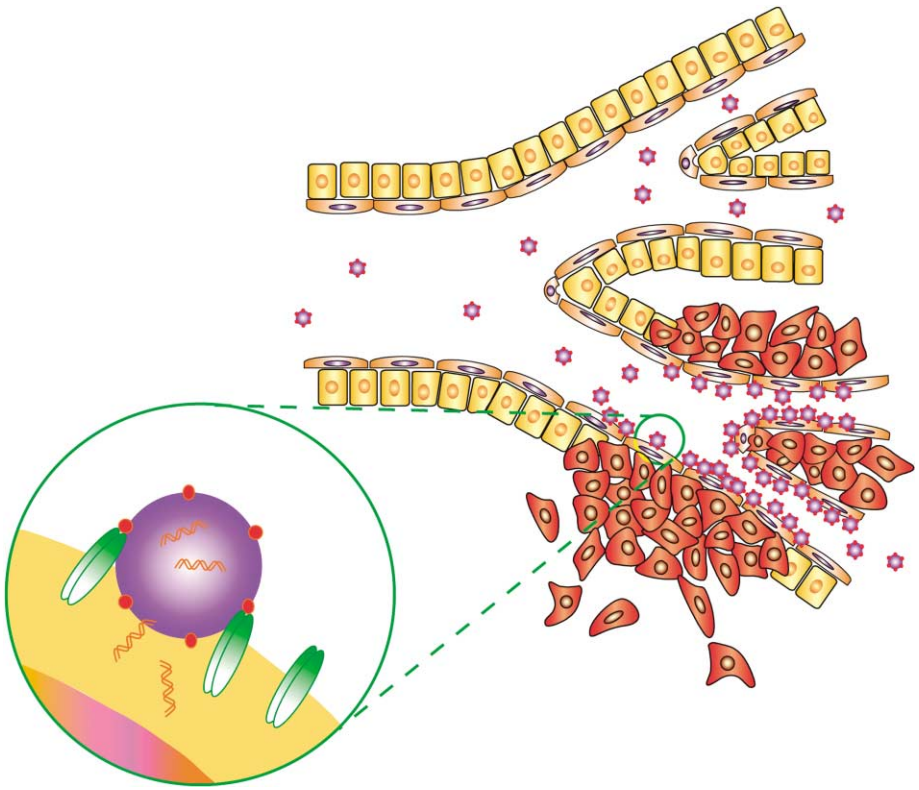

**Figure 1.** Nanoparticles specifically target tumor vasculature

Circulating nanoparticles bind to integrin on the endothelial cells of tumor vessels, but do not bind to the vessels in normal tissue. The bound particles discharge their DNA cargo into the cells. Thanks to Dr. Masanobu Komatsu for preparing the illustration.

reason not expressed. While the effects on normal tissues require further study, the vehicle clearly produced the desired differential gene expression in the tumor.

The specificity of the treatment effects achieved in this study is likely to be a net effect of three factors: accumulation of the vehicle at the tumor vasculature, selective transduction of the payload DNA into the $\alpha v\beta 3$-expressing cells, and possibly, selective susceptibility of the proliferating endothelial cells in angiogenic vessels to inhibition of the Raf-1 pathway. Future gene therapies for cancer are likely to use such combinations of selective effects. Targeting will increase effectiveness and reduce the likelihood of side effects, as will the selectivity of the targeted gene.

The treatment devised by Cheresh and collaborators was very effective as they were able to achieve tumor regression in mice, and some of the mice survived prolonged periods of time with no gross evidence of tumors after a single injection of the targeted nanoparticles. One would expect antiangiogenics to be able to prevent further growth of a tumor, but not to affect the existing parts of the tumor where the established blood vessels should not be affected. The tumor used in this study grew fast and maximal tumor burden was reached in less than 3 weeks. Most of the blood vessels in these fast-growing tumors would be "new" and likely to carry angiogenesis markers such as the $\alpha v\beta 3$ integrin, which could make these tumors particularly responsive to antiangiogenic therapy. As human tumors grow over years, a greater proportion of their vessels may be more mature and less susceptible to antiangiogenics than fast-growing experimental tumors.

Destroying established tumor vessels is likely to require a combination of antiangiogenic and antivascular therapy. As blood vessels mature, they acquire a pericyte coating and associated extracellular matrix. Tumor-specific changes in these vascular elements, such as the NG2 proteoglycan in pericytes (Ozerdem et al., 2001) and oncofetal fibronectin in the matrix (Xu et al., 2001; Halin et al., 2002), may make it possible to develop targeted delivery approaches for antivascular therapy. If sufficient damage to the vessels is achieved, thrombosis of the affected vessels may be initiated, providing a natural amplification mechanism for the treatment (Huang et al., 1997).

Tumor blood vessels may also have specific markers, the expression of which is not regulated by angiogenesis. In various normal tissues, the blood vessels carry tissue-specific markers (Ruoslahti, 2002). In analogy with this, tumors may put a signature on their vasculature. Discovery of such marker molecules in tumor blood vessels would further expand the vascular targeting possibilities, as may targeting through the lymphatic vessels (Laakkonen et al., 2002). The future of antivascular tumor therapies is likely to be similar to current tumor treatments, which rely on combinations of drugs; the most effective approach may be a concerted attack at more than one of the vulnerabilities in tumor vasculature.

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