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Commentary

Antiangiogenic gene therapy

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In a previous issue of the *Proceedings*, there are two reports of anti-tumor therapy in mice based on inhibition of angiogenesis by gene therapy. Lin *et al.* (1) used an adenoviral vector to deliver a recombinant Tie2 receptor that blocked activation of the Tie2 receptor on endothelial cells. A single i.v. injection of this construct resulted in a high circulating level of the soluble receptor protein (>1 mg/ml) for 8 days. Growth of two different primary tumors was inhibited significantly. Neovascularization and growth of lung metastases were inhibited almost completely, regardless of whether the metastases arose from tumor cells injected i.v. or from a primary tumor that was later removed. Most important, delivery of the soluble Tie2 receptor–adenoviral construct at the time of surgical excision of primary tumors also inhibited subsequent metastatic growth. This is the first demonstration that gene therapy directed against the Tie2 receptor on endothelial cells will inhibit tumor angiogenesis. Taken together with the prior discovery of ligands for Tie2, angiopoietin-1 and angiopoietin-2 (32, 33), this report provides compelling evidence for another level of regulation of tumor angiogenesis, namely recruitment or repulsion of mural cells (pericytes and smooth muscle cells) to newly formed microvessels by angiopoietin-1 or angiopoietin-2, respectively. The local concentration of angiogenic stimulus, e.g., by vascular endothelial growth factor (VEGF), also may enter into the equation. For example, if, by blocking activation of the Tie2 receptor, the gene therapy described here leaves tumor microvessels relatively devoid of mural cells, new sprouts may continue to form in the presence of high VEGF, but low VEGF may lead to regression of tumor vessels.

The authors (1) laid the foundation for this experiment by previously showing that a single injection of soluble Tie2 receptor protein into a rat cutaneous window reduced tumor vascular length by 40% and inhibited tumor growth inside the chamber by 75%. The soluble receptor had no direct effect on tumors but controlled tumor growth by inhibiting angiogenesis (2). This was the first evidence that disruption of the Tie2 receptor, expressed almost exclusively on vascular endothelium, played a role in pathologic angiogenesis in adult tissues. Previously, the Tie2/Tie2 ligand pathway had been thought to operate only in embryonic vasculature.

Also in that issue of the *Proceedings*, Goldman *et al.* (3) report the use of an *ex vivo* gene transfer method to transfect stably human tumor cells with the cDNA encoding a truncated form of native soluble FLT-1, a receptor for the angiogenic factor VEGF. Soluble FLT-1 inhibited VEGF function directly by sequestering the ligand and in a dominant-negative fashion by forming inactive heterodimers with the receptor for VEGF. The s.c. growth of transiently transfected fibrosarcoma primary tumors was inhibited significantly as was the growth of lung metastases from i.v. injection of the transfected tumor cells. Of interest, human fibrosarcoma cells engineered to persistently express 20-fold more soluble FLT-1 mRNA did not form s.c. tumors in half of the mice implanted. We have observed that this phenomenon of "no take" (T. Udagawa, personal communication) (even in immunodeficient mice) can represent complete blockade of angiogenesis so that only a microscopic *in situ* tumor forms in which the rates of tumor cell proliferation and apoptosis are balanced (34). Mice bearing intracranial human brain tumors that were transfected with the VEGF receptor survived twice as long as control mice. The soluble FLT-1 protein did not affect tumor cell proliferation *in vitro*. The authors previously reported that soluble FLT-1 is a potent and selective endogenous inhibitor of VEGF-mediated angiogenesis. The authors proposed several mechanisms to explain why some tumors escaped inhibition by soluble FLT-1, including the possible emergence of tumor cells expressing angiogenic factors other than VEGF. They emphasized that "this mechanism would not explain the correlation between the increased level of soluble FLT-1 expression and the observed longer duration of tumor growth inhibition." It could be, however, that longer and more effective suppression of tumor angiogenesis decreases the likelihood of the emergence of mutant clones that produce a different angiogenic protein.

When the studies by Lin *et al.* (1) and by Goldman *et al.* (3) are taken together with recent previous reports by other investigators of different types of experimental antiangiogenic gene therapy (4–14), they reveal the emergence of a new branch of gene therapy directed at tumor angiogenesis. Of ≈200 gene therapy clinical trials, at least 50% are for the treatment of cancer, and virtually all of these strategies target the cancer cell. These trials include, among others, the introduction of genes that: (*i*) permit tumor cells to express toxic molecules; (*ii*) prevent or correct genetic defects; (*iii*) increase the immunogenicity of tumor cells; or that (*iv*) increase the sensitivity of tumor cells to drugs (15–18). Although gene therapy of cancer may be inherently less toxic than conventional chemotherapy, it must still overcome other fundamental obstacles that hinder conventional chemotherapy, i.e., limited access to tumor cells, heterogeneity of tumor cells, dependence on cycling cells (i.e., a relatively high growth fraction), and emergence of resistant tumor cells. In contrast, antiangiogenic therapy is directed specifically against microvascular endothelial cells that have been recruited into the tumor bed. Specific antiangiogenic therapy has little or no toxicity, does not require that the therapeutic agent enter any tumor cells nor cross the blood brain barrier, controls tumor growth independently of growth fraction or tumor cell heterogeneity or even tumor cell type, and does not induce acquired drug resistance (20).

Although there are no current clinical trials of antiangiogenic gene therapy, the appearance of the two important reports in the previous issue of the *Proceedings* point the way to the feasibility of such clinical application. Therefore, it may be prudent to consider certain principles of antiangiogenic therapy that could facilitate the design of antiangiogenic gene therapy trials.

Local vs. Systemic Antiangiogenic Gene Therapy. In the most complete review of antiangiogenic gene therapy to date, Kong and Crystal (21) argue that antiangiogenic gene therapy

The companions to this commentary are published on pages 8795–8800 and 8829–8834 in issue 15 of volume 95.

should be used exclusively in a local or regional setting, for example in human glioblastomas or in other primary tumors that may be untreatable by conventional modalities. Their argument rests mainly on the possibility that: (*i*) The "bystander" effect of antiangiogenic gene therapy may be greater than other forms of gene therapy directed only against the cancer cell; and (*ii*) potential side effects of systemically administered antiangiogenic gene therapy may be avoided. The enhanced "bystander effect" is illustrated by an experiment in which "liposome-mediated transfer of the p53 gene to mammary tumors in mice led to less than 5% tumor cell transfection, but was associated with a 60% reduction in the number of blood vessels in the treated tumors" (22). The antiangiogenic effect most likely resulted from increased production of thrombospondin-1, an angiogenesis inhibitor known to be under the control of wild-type p53 (23). Kong and Crystal were correctly concerned about the potential side effects of systemic antiangiogenic therapy based on a report from my laboratory that systemic administration of the angiogenesis inhibitor AGM-1470 (TNP-470) (a synthetic analogue of fumagillin) to nonpregnant mice inhibited endometrial maturation and corpus luteum formation and that, in pregnant mice, it induced regression of embryo growth (24). My colleagues and I also reported that AGM-1470 delays wound healing by ≈17% (25). Therefore, I assumed that probably all angiogenesis inhibitors, especially those that inhibited angiogenesis more potently than AGM-1470, would interfere with female reproduction and wound healing. This assumption may not be valid. Current ongoing studies suggest that neither angiostatin (26) nor endostatin (27) delay wound healing in mice and that pregnancy as well as growth of neonatal mice are not affected by endostatin (angiostatin has not been tested to date) (Richard Rohan and Jennifer Marler, personal communication).

The report by Lin *et al.* (1) in the previous issue of the *Proceedings* and a prior report (28) that angiostatin gene therapy administered i.v. inhibited tumor growth at a remote s.c. site demonstrate some of the advantages of systemically administered antiangiogenic therapy. In the future, systemic antiangiogenic therapy may be used: (*i*) after surgery or after radiotherapy to prevent recurrence of distant metastases; (*ii*) in combination with conventional chemotherapy; (*iii*) in combination with vaccine therapy or immunotherapy; or (*iv*) in combination with other types of gene therapy, for example, delivery of tumor suppressor genes.

Although we had assumed that antiangiogenic therapy of any type might have to be delivered for the rest of a patient's life, or at least for many years (analogous to tamoxifen), our thinking was changed by the demonstration that 80–185 days of cycled therapy of large tumors of three different types in mice was followed by permanent tumor arrest during which tumors remained at a microscopic dormant size with blocked angiogenesis even after therapy was discontinued (20). Current ongoing experiments indicate that cycling may not be necessary to achieve the dormant state (M. S. O'Reilly, personal communication). Furthermore, combination therapy with angiostatin and endostatin eradicated tumors in mice (20). Also, combinations of angiogenesis inhibitors and conventional cytotoxic chemotherapy cured tumors in mice when either therapy alone could not accomplish this (29). Another advantage of systemic antiangiogenic gene therapy is that it may reduce the expense of prolonged protein therapy with angiogenesis inhibitors such as angiostatin and endostatin for either human or veterinary use. Antiangiogenic gene therapy could begin simultaneously with protein therapy, after which the protein therapy could be discontinued in several months if blood levels of the inhibitor were maintained by host production of the genetically engineered protein. Experimental data also suggest that effective antiangiogenic therapy requires the continuous presence of the inhibitor in the blood, perhaps more efficiently achieved by gene therapy than by bolus protein therapy. Primary tumors that inhibit angiogenesis in their metastases by production of either angiostatin (26), or thrombospondin-1 (30), appear to maintain effective blood levels of the inhibitor with expenditure of considerably less total protein than would be required for daily s.c. injection.

Direct vs. Indirect Antiangiogenic Therapy. During the development of antiangiogenic gene therapy, it should be recognized that there may be subtle differences between antiangiogenic therapy targeted specifically to endothelial cells (direct) vs. antiangiogenic therapy that interferes with a tumor-derived angiogenic factor or the receptor for it (indirect). The latter may engender a higher risk of "drug resistance" because the genetic instability of tumor cells eventually may yield clones that produce a different angiogenic factor.

This risk is theoretical at this writing and cannot be predicted. It should not, however, preclude clinical trials of gene therapy directed at tumor-derived angiogenic factors. It may turn out that potent inhibition of tumor angiogenesis dampens the emergence of variant tumor cells, mainly because expansion of tumor mass is restricted.

The reports by Lin *et al.* (1) and Goldman *et al.* (3) provide exciting experimental evidence that translation of antiangiogenic gene therapy from laboratory to clinic may soon become a reality. Of interest, this new therapeutic opportunity has resulted from the joining of two fields, angiogenesis research and gene therapy research. At the same time, gene therapy for therapeutic myocardial angiogenesis appears to be emerging from a similar synthesis (31).

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