Just as clinical trials of a widely heralded cancer treatment are about to be expanded, two groups report that they couldn't get it to work, indicating again how fickle and mysterious the compound remains

Setbacks for Endostatin

Harvard University's Judah Folkman electrified cancer researchers 5 years ago when he and his colleagues reported on a new compound that could shrink tumors in mice virtually to nothing. A surgeon at Children's Hospital Boston, Folkman had long pursued a strategy of fighting cancer by cutting off the blood supply to tumors, rather than by poisoning patients with toxic drugs. Using a substance called endostatin, the Harvard group obtained dramatic results; clinical trials soon followed. But some other researchers who attempted to follow this lead were unable to find endostatin's seemingly miraculous properties. Now two new studies, published in the April issue of *Molecular Therapy*, take aim at endostatin again, both reporting that it had no effect on tumors in mice.

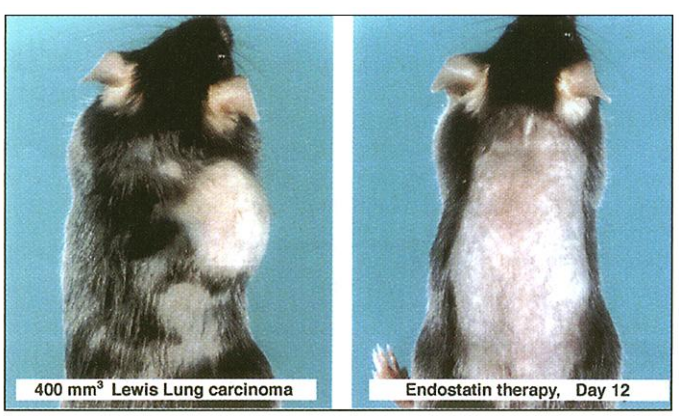
Although these papers are not the first to raise questions about endostatin, they are among the most pointed. One title speaks of "the unfulfilled promise of endostatin" in a type of gene therapy for mice with leukemia. And the other reports that, "despite continuous, high-level secretion of endostatin" in the bloodstream of mice, "we detected neither inhibition of [blood vessel growth] nor antitumor activity." In a companion essay, *Molecular Therapy*'s editor, Fintan Steele, writes that "results from these two groups certainly contradict much of what has appeared in prior publications." The confusion about which data are reliable prompts Steele to ask whether there is "sufficient basic science to understand what endostatin is and what it does"—and whether it makes sense to expand clinical trials built upon the early reports.

Folkman and Michael O'Reilly, the researcher in his lab who discovered endostatin, see no reason to pause. Although Folkman acknowledges that some gene transfer experiments such as those reported in *Molecular Therapy* have not worked out, he says others have been more promising. He and O'Reilly, who is now at the M. D. Anderson Cancer Center in Houston, Texas, also argue that the simpler approach of injecting endostatin directly has yielded positive results in animals that justify expanding clinical trials. So far, fewer than 200 patients have taken part in tests designed to measure safety. No cures were expected, and none have been reported.

Conscious of endostatin's mixed record, some leaders in this field agree that the picture is not as simple as it seemed 5 years ago. As Robert Kerbel of the University of Toronto says, the pharmacokinetics of compounds designed to prevent blood vessel growth (antiangiogenics) may be "very complex," and the method of administration can have a "huge impact" on efficacy. Folkman himself views the complexity as intriguing, adding that even negative reports are useful because they may help unravel the mysteries.

Lapsed believer

When O'Reilly and Folkman first described endostatin in the 24 January 1997 issue of *Cell*, it seemed like an ideal anticancer weapon. This naturally produced, nontoxic compound selectively shrank blood vessels and repeatedly caused tumors in mice to shrink to microscopic size. A later paper in *Nature*, with still more promising results, triggered bold predictions, including a report in *The New York Times* quoting Nobel laureate James Watson to the effect that Folkman would "cure cancer in 2 years." This led to front-page stories and turned Folkman into a reluctant hero. He also became the subject of a popular book, *Dr. Folkman's War*, published last year.



Powerful result. Endostatin therapy dramatically shrank mouse tumors in a 1997 experiment that raised high hopes for antiangiogenesis treatment.

Since then, Folkman's group has expanded its work to other compounds that inhibit blood vessel growth and explored dozens of ideas for new therapies. Many other groups around the world also have plunged in. A private company—EntreMed Inc. of Rockville, Maryland—obtained rights to manufacture endostatin and since the late 1990s has sponsored small clinical trials. The National Cancer Institute (NCI) provided support too, funding a couple of clinical trials and animal studies on endostatin and other antiangiogenics carried out in well-established laboratories. But the two papers in *Molecular Therapy* have raised new red flags, including a report from one lab that it couldn't repeat the original 1997 experiment.

Philippe Leboulch, a contributor to both papers in *Molecular Therapy* and the senior author of one of them, has turned from endostatin enthusiast to skeptic. A molecular geneticist, Leboulch investigates gene therapy techniques with a joint position at the Massachusetts Institute of Technology (MIT) and Harvard Medical School. He also has a small company, Genetix Pharmaceuticals Inc. in Cambridge, Massachusetts. Inspired by early data from Folkman's lab, he embraced endostatin in 1995.

When Leboulch first connected with Folkman's team, he says: "We were very excited about collaborating." One barrier to research in the early days, Leboulch explains, was that endostatin was hard to get. The endostatin for the successful 1997 Folkman lab mouse experiment had been produced in the bacteria *Escherichia coli*. But output was low, and the product was an insoluble aggregate. The MIT group—including Robert Pawliuk, Thomas Bachelot, and Omar Zurkiya—took a different route: This team spliced the endostatin gene into mouse hematopoietic stem cells, the progenitors of blood cells that live in bone marrow. This looked like a great strategy for getting endostatin expressed continuously and at high levels in animals.

Gene transfer worked "as we had planned," recalls Leboulch. "We got very high levels of secretion" in the bloodstream of mice: about 746 nanograms per milliliter (ng/ml), he says. Leboulch estimates that this systemic concentration, on average, was 750% higher than that naturally found in the animals. Eighteen mice received endostatin-expressing stem cells, and 10 received cells that didn't express the protein.

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To look for effects on blood vessel formation, Leboulch collaborated with Yihai Cao of the Karolinska Institute in Stockholm, who is an expert on angiogenesis. Cao compared five endostatin-treated and four control mice and saw no antiangiogenic effect. "In theory, [endostatin gene transfer] should have worked," says Cao. "I don't see why it didn't." He speculates that the protein produced by the transplanted gene may have been misfolded—a possibility Leboulch concedes. But no one knows how the active form of endostatin is folded, or whether a change in folding would make a difference.

Not only did the MIT experiment have no effect on blood vessel growth, but it also failed to control tumors. The MIT researchers injected fibrosarcomas—one of the tumors used in the O'Reilly-Folkman mouse experiment—into mice in different ways to simulate local and metastasized tumors. Again, they found no difference between endostatin-treated mice and controls.

The second experiment reported in *Molecular Therapy*—run by a group at the British Columbia Cancer Agency in Vancouver, Canada, including Connie Eaves and Wolfgang Eisterer—took a similar approach. This group targeted a cancer of the blood, acute lymphocytic leukemia (ALL), for endostatin therapy. The Vancouver group withdrew ALL cells from four patients and implanted them into immune-deficient mice. With gene transfer, the researchers also got mice to express relatively high levels of endostatin—180 ng/ml—in the blood. But when they compared the endostatin-producing mice with controls, they found no difference in cancer burden.

Leboulch says his group took steps to see that the endostatin it produced was as close as possible to the original form. The researchers tested the protein produced by the transplanted gene to ensure that it inhibited endothelial cell proliferation, examined its amino acid sequence, and ran confirmatory antibody checks.

He also says an earlier version of their paper was turned down for publication by *Science* because it lacked a "positive control"—a substance illustrating effective tumor control to compare with the endostatin failures. To remedy this, Leboulch tried to repeat the original 1997 mouse experiments. Leboulch's postdoc, Bachelot, asked Folkman's group for samples of the original *E. coli* precipitate but never received any. So Bachelot made injectable endostatin using the original *E. coli* recipe. This also produced no effect.

Failed experiments such as these often don't get published, but Leboulch says he decided to submit the results partly because his ex-postdocs wanted this work to get out, and partly because "some of my colleagues at Harvard encouraged me to make the data available." One former Harvard researcher, asking not to be named, grumbles that he and "thousands of postdocs" have had the same disappointing experience. Although Leboulch admires Folkman and endorses his antiangiogenic program, he says: "We think we will get out of this endostatin business."

The beat goes on

The failure of these two experiments points up what Folkman calls "a paradox": Endostatin delivered to the body by gene therapy appears to be less effective than when the protein is simply injected. Last year, in a paper co-authored by Folkman in the *Proceedings of the National Academy of Sciences*, Richard Mulligan's group at Harvard compared the potency of five antiangiogenic compounds delivered by modified adenoviruses to mice. Ranked by efficacy, endostatin was at the bottom.

Quiet celebrity. Judah Folkman with Senator Edward Kennedy (D–MA) and on the cover of a recent book.

"The mechanism of this paradox is unknown," Folkman writes in a comment faxed to *Science*. The high concentrations of the protein produced by gene therapy, he speculates, might lead to "protein aggregation" that renders endostatin inactive. Mouse receptors might become overloaded at high serum concentrations, although the identity of the receptor is not known. And the gene-produced molecule might be more vulnerable to degradation or metabolic processes.

Yet even this paradoxical behavior is not consistent. Folkman notes that a gene therapy experiment by Andrew Feldman and Steven Libutti at NCI did produce some promising results. Feldman and Libutti transplanted an endostatin gene into mouse liver tumor cells and implanted the cells into mice. As they reported in the *Journal of the National Cancer Insitute* last year, the implants expressing the highest amounts of endostatin were most strongly inhibited from growing. Although Folkman speculates that high levels of endostatin may overload receptors, Libutti thinks that endostatin concentrations of 1 μg/ml or more—higher than described in either *Molecular Therapy* report—are needed locally to have an effect.

To O'Reilly, the fact that some groups have seen at least modest tumor inhibition in gene therapy experiments suggests a simple explanation for the failure of the two studies reported in *Molecular Therapy*: The proteins produced in both experiments were defective.

In contrast to gene therapy experiments, Folkman says protein-injection studies have yielded many positive reports. A recent one, co-authored by Folkman, Oliver Kisker, and other Harvard scientists in *Cancer Research* last October, reports "tumor regression" in immune-deficient mice treated with endostatin delivered continuously by a small implanted osmotic pump.

The researchers used a soluble, yeast-produced form of human recombinant endostatin, the same material that EntreMed gives patients in its clinical trials. They calculated that the minipumps delivered systemic doses of 200 to 300 ng/ml. Although this is lower than in the Leboulch gene therapy experiment, Folkman notes that this method of delivery was up to 10-fold "more effective" at controlling new blood vessels than periodic injections in most studies were—with the exception of the remarkable effects seen in the 1997 study.

O'Reilly agrees that it makes sense to investigate all of the discrepancies and puzzles in the results with endostatin so far. But he argues that these investigations should not hold up clinical trials, because "patients with advanced cancer are desperate" and "don't have the luxury of waiting." EntreMed has received clearance from the U.S. Food and Drug Administration to expand its clinical trials to investigate responses to different doses. Even Leboulch says that clinical trials are now likely to provide the best new information on whether endostatin really works.

–ELIOT MARSHALL

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