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## Sending Cancer a Suicide Note

Researchers have dreamed of thwarting cancer by using proteins that halt cell growth and kill the tumor. In theory, the approach should work. In practice, however, scientists have not had an easy time of getting tumor suppressor proteins into cells where they can block growth effectively.

Now, those barriers are crumbling thanks to an ingenious technique engineered by Howard Hughes Medical Institute researchers at the University of California, San Diego (UCSD). Roughly half of all human cancers share in common the loss of a critical tumor suppressor gene, *p53*. The researchers reasoned that if they could somehow introduce a message into cancer cells that reactivates *p53*, they might be able to stop cancer in its tracks.

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— Steven F. Dowdy

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They tried the strategy in mice with aggressive human cancers and found that the introduced suicide message is ignored by normal cells, but prevents the growth of cancer cells. Administering the *p53* protein fragment, or peptide, greatly increased the survival of mice, in some cases producing apparently disease-free animals.

According to the researchers, the results constitute a proof-of-concept that the technique could form the basis of new cancer treatments based on protein therapy. These treatments would use tumor suppressor proteins to stop cancer growth. Currently, biological treatments have been limited to those that act outside the cell. Significant technical barriers, such as engineering a vector that could cross the cell membrane efficiently, have hampered development of strategies that would allow researchers to deliver proteins to intracellular locations.

The research team, which was led by Howard Hughes Medical Institute investigator Steven F. Dowdy at UCSD, published its findings in the February 2004 issue of the journal *Public Library of Science Biology*. The lead author was Eric Snyder, a former graduate student of Dowdy's who is now at Washington University School of Medicine in St. Louis. The researchers also published a related article on February 8, 2004, in the

advance online edition of *Nature Medicine* in which they provide additional details about the mechanism by which the protein TAT enters cells.

In the published articles, the researchers described how they sought to reactivate the protein p53 in cancer cells. This protein is a key activator of genes in the signaling pathway by which cells commit suicide when they are genetically damaged or no longer needed during development of an organism.

“The tumor suppressor protein p53 is inactivated in half of all human malignancies, preventing the DNA damage associated with cancer cells from triggering cell death,” said Dowdy. “Thus, the cells are freed to undergo continuous proliferation. So, if you could reactivate or reconstitute p53 function, you could selectively kill the tumor cells but not the surrounding normal cells, because the normal cells don't have DNA damage.”

Dowdy and his colleagues built on previous work by David Lane at the University of Dundee in Scotland and other researchers, who discovered that a short piece of the p53 protein, called a peptide, could induce reactivation of aberrant p53 proteins. However, major problems remained in getting the peptide through the barrier of the cell membrane, and in protecting it from degradation once inside the cell.

To introduce the p53 peptide into the cells, Dowdy and his colleagues used a technique they had developed previously. Using this method, they attached large molecules to be introduced into a cell to a harmless protein called TAT, derived from HIV. Due to a biochemical quirk, TAT can breach the cell membrane, carrying with it any attached peptide. In the *Nature Medicine* article, the researchers described new information about how the TAT-containing protein enters cells. This knowledge will enable them to add yet another peptide from influenza virus that enhances entrance into cells and release of the peptide.

To eliminate vulnerability to degradation once inside the cell, the researchers synthesized the peptide using tricks done with chemical “mirrors.” The researchers began with amino acid components that were mirror images of those amino acids found in nature. From these “D-isomers,” they created a p53 peptide with the reverse sequence from the natural peptide. Because the resulting “retro-inverso D-isomer” peptide retained essentially the same surface shape as the natural peptide, it was still capable of switching on p53 in cancer cells, but was in effect invisible to enzymes that degrade foreign proteins.

Initial studies in cancer cells grown in the laboratory showed that the manufactured peptide successfully stopped proliferation of cancer cells and activated the p53 pathway.

For their *in vivo* tests, the researchers chose the human cancer, peritoneal carcinomatosis, because it is an aggressive, chemotherapy-resistant cancer that is highly lethal. “It's one of the most deadly malignancies in terms of the overall percentage of patients who succumb to it,” said Dowdy. “It's also a

real mimic of a broad range of other human cancers. And we wanted to demonstrate this technique on a malignancy that was a hard one to tackle, because it would give us a much better justification for moving this treatment forward toward clinical use.”

The researchers found a dramatic reduction in tumor number after they introduced their peptide into mice with peritoneal carcinomatosis. Those animals lived more than six times as long as mice that were not treated with the peptide.

They also tested the treatment on mice with another aggressive human cancer, peritoneal lymphoma, which had a different type of p53 malfunction. Fifty percent of the mice treated in the experiment were still alive after more than 200 days, whereas the average survival time for untreated animals was 33-35 days.

While Dowdy sees great promise for treating cancers with the new approach, he cautions that much more work is necessary. “Clearly, we have shown that this is a proof-of-principle that we can treat terminal malignancies in mice that mimic a real-life human disease,” he said. “However, we don’t know how many tumors in the real world would respond to this treatment. Also, my guess is that this is not the exact molecule that one would go forward with in the clinic, but that it needs further refinement. We would want to learn to activate p53 even more effectively and to test molecules that are even more tumor-selective.”

Although activating p53 is a highly important objective in attacking cancer cells, Dowdy said that other cancers present additional vulnerable targets that could be activated using the same basic delivery system for other molecular cargoes.