CANCER RESEARCH

Peptide-Guided Cancer Drugs Show Promise in Mice

As any cancer patient who has endured chemotherapy knows, most regimens walk a fine line between killing the tumor and killing the patient. That's because chemotherapeutic drugs spread throughout the body, reaching not only the tumor but also healthy organs such as the gut and bone marrow, where they kill off normal dividing cells. To make matters even worse, tumor cells are also quick to mutate and become resistant to the drugs. Now, on page 377, a team led by Erkki Ruoslahti at The Burnham Institute in La Jolla, California, reveals a strategy that may get around both problems.

Ruoslahti and his colleagues have devised a way to target cancer drugs to the new blood vessels that nourish the tumor. They found small peptides that zero in on the cells lining these newly formed blood vessels, then linked the peptides to the chemotherapeutic drug doxorubicin. By addressing the toxic drug specifically to the tumor, the strategy spares other tissues. And because the tumor vessel cells are not cancerous themselves, they are much less likely to develop resistance to the drugs than are the highly mutable cancer cells. Indeed, when the researchers gave the peptide-drug combination to mice with large tumors, it killed off the blood vessels, stopped tumor growth, and allowed the mice to survive the cancer.

Other researchers, such as Judah Folkman at Harvard Medical School, have shown that inhibiting angiogenesis, as new blood-vessel growth is called, can block tumor growth in animals. Indeed, such work has made angiogenesis a hot research area in recent years (*Science*, 24 January 1997, p. 482). But progress on that approach has been slowed because the best angiogenesis inhibitors are proteins that are expensive and laborious to produce in bacteria. Ruoslahti's method lacks that disadvantage. "In theory, this is immediately translatable into the clinic," says tumor biologist Bob Kerbel, of the Sunnybrook Health Science Center at the University of Toronto, "because you are dealing with a drug that is already available and clinically approved. And it would not be difficult to produce these peptides."

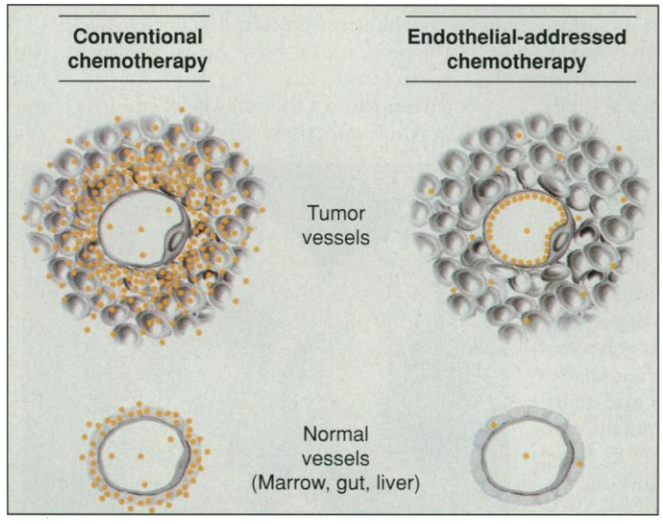
What's more, the technique used by the Ruoslahti team to identify peptides that home in on tumor vessels can identify peptides that bind specifically to the blood vessels of other organs. This means that peptides could be developed to carry drugs to many different tissues to treat conditions other than cancer. "Ruoslahti can address drugs wherever he wants to in a nontoxic way," enthuses Folkman. "A few years from now, this will be the basis of a new pharmacology."

The idea of ferrying chemotherapeutic drugs to tumors on the backs of other molecules isn't new. But large, ungainly proteins such as antibodies have generally been used as the vehicles, with mixed success. To find small peptides that could deliver drugs specifically to tumors, Ruoslahti and then-postdoc Renata Pasqualini turned to a technique developed in the late 1980s by George Smith at the University of Missouri in Columbia. Called phage display, it involves engineering bacterial viruses called phages so that each displays a different random peptide on its surface. The technique had been used to find peptides that stick to particular proteins, by exposing a mix of peptide-displaying phages to a surface coated with the protein. Ruoslahti reasoned that if the phages were injected into an animal, the technique might be able to identify peptides that stick to specific tissues.

Pasqualini made the idea work. Two years ago, she and Ruoslahti reported in *Nature* that they had identified peptides that home in on the blood vessels of the kidneys or brains of mice. "We practiced first with normal organs," says Ruoslahti, "although, all along, our aim was to look at tumors." And in this issue, the team reports that when they tried the scheme on mice with tumors, they found several peptides that stick to molecules found only in tumor-associated blood vessels.

One of the peptides the team identified binds to $\alpha_v\beta_3$ integrin, a cell-adhesion protein that David Cheresh's group at The Scripps Research Institute in La Jolla had already shown to be concentrated in angiogenic blood vessels. But the group also identified "a whole panel of other peptides," Ruoslahti says, that bind to as-yet-unidentified molecules specific to angiogenic vessels. Not only do those peptides represent alternate means of conveying drugs, but they also will be useful probes for studying the proteins they target.

Next, the team chose two of the peptides and hooked them individually to the anticancer drug doxorubicin, to see if they would guide the drug to tumors and kill them. They did. When given to mice that had large tumors derived from human breast cancer cells, even tiny amounts of the peptide-linked drug were better at stunting tumor growth than was free doxorubicin, which was hampered by its toxicity.



Zeroing in. In standard chemotherapy (left), drugs (orange) flood both tumors and normal tissues. Drugs targeted to tumor blood vessels (right) spare normal tissues.

Indeed, some of the mice treated with the doxorubicin-peptide conjugate lived for 6 months after the treatment, while those treated with doxorubicin alone died either of tumors or of drug poisoning at the high doses. "We were never able to find a concentration of free doxorubicin that was anywhere near as effective as our conjugate," Ruoslahti says. The tumors don't disappear completely, he notes, but what remains seems to be inactive scar tissue. "The mice live very long, so it doesn't seem to bother them."

Ruoslahti thinks that the drug conjugates act primarily by killing blood vessels that feed the tumor, although the drug may also diffuse into the tumors and kill cells directly. "The technique probably targets both the vessels and the tumor cells. That is its big advantage," says tumor-cell biologist Bruce Zetter of Harvard Medical School. "It gives you a kind of double-pronged therapeutic effect that should be quite powerful." And, of course, a similar double-punch could be achieved with other drugs. "There may be things we haven't used because of their high toxicity that could be used in a more directed way," says Zetter.

Ruoslahti's isn't the only team to kill tumors by directing drugs to their blood vessels. Early last year, Philip Thorpe's group at the University of Texas Southwestern Medical Center in Dallas reported that a blood-clotting factor targeted to tumor blood ves-

sels with an antibody caused massive clotting and killed the tumors. And in November, a University of Minnesota team led by S. Ramakrishnan reported in the *International Journal of Cancer* that it had slowed tumor growth in mice by targeting diphtheria toxin to tumor blood vessels using vascular endothelial growth factor, a protein that binds to a receptor that is plentiful in new vessels.

Thorpe notes that his work relied on experimentally engineered tumor cells, so it "didn't directly extrapolate to humans" as Ruoslahti's does. And many in the field favor the latter approach over others that use proteins, because the small peptides are easy to make and use and the technique can be tailored to many different tissues.

The path to clinical trials of the peptide-doxorubicin conjugate looks fairly clear, but oddsmakers know only too well that the favorite out of the gate will not necessarily be the first across the finish line. "We are in very early stages of anti-angiogenesis therapy," says Zetter. "We have to test them all, and go after the best." Where peptide conjugates finish will be apparent only after the race is run.

–Marcia Barinaga

INFECTIOUS DISEASE

Sequence Offers Clues to Deadly Flu

The Hong Kong "bird flu" that has killed four people, sickened more than a dozen, and prompted the mass slaughter of more than 1.5 million chickens in the last month is still perplexing to scientists. Researchers are trying to discover why this virus is so deadly and why, unlike most known avian viruses, it can infect human cells. Now, in a report on page 393, a team from the United States and Hong Kong provides the most careful look yet at the virus—a complete sequence of the genes that code for its surface proteins and partial sequences of the remaining genome. Although the sequence so far can't reveal all of the virus's biological tricks, it offers clues as to how the virus infects cells, and it lays the groundwork for understanding what makes the bird flu a killer.

This particular virus was isolated from a 3-year-old boy in Hong Kong, who died in May after coming down with a flulike disease that did not match any of the known human influenza strains (*Science*, 12 September 1997, p. 1600). It did, however, match a bird strain, called H5N1 because of the varieties of the proteins hemagglutinin and neuraminidase on its surface. H5N1 had infected and killed thousands of chickens in Hong Kong a few months earlier, but no one expected it to jump to humans.

To infect cells, viruses must attach to specific binding sites on the cell membrane, and human and bird sites are different enough that researchers assumed a single flu virus could not infect both species. Avian strains generally have to mix with human flu viruses in an intermediate host, such as pigs, to produce a new variety dangerous to humans. When H5N1 broke this rule, it triggered a public health alarm. Because people had never before been infected by the bird strain and therefore have no immunity to it, epidemiologists worried that the strain could trigger a pandemic.

By analyzing the DNA sequence of the virus, researchers led by Kanta Subbarao of the U.S. Centers for Disease Control and Prevention in Atlanta have now confirmed experts' first hunch: The virus is indeed derived from an avian influenza strain, evidently without an intermediate host. This is probably not the first time such a leap has happened, but it's the first time scientists have been able to observe it directly, says Subbarao.

The team members also have uncovered a possible clue to what makes the strain so deadly to both birds and people. When they sequenced the gene for hemagglutinin, they found an insertion that is common among especially virulent bird viruses but had never been isolated from a human. The insert codes for several additional amino acids right next to a crucial spot where cellular enzymes cleave the hemagglutinin protein. That cleavage helps the protein coat break apart, allowing the virus to infect cells.

Scientists suspect that the cleavage site is key to a virus's infectivity. The enzymes that cleave the most common protein are abundant in the digestive and respiratory systems of birds, and most flu strains can infect only those cells, says team member Michael Perdue of the U.S. Department of Agriculture's (USDA's) Southeast Poultry Research Laboratory in Athens, Georgia. But the extra amino acids may provide an easier—and less specific—target for enzymes, allowing the virus to infect other tissues, including heart, brain, and blood vessels.

APPLE DAILY/AP PHOTO

Chicken fever. The death of Hong Kong's chickens will help protect humans.

Virologist Robert Webster of St. Jude Children's Research Hospital in Memphis, Tennessee, suspects that the insert "allows the virus to become systemic. Instead of just replicating in the respiratory tract, it now can spread through the bloodstream." Poultry victims of the virus suffered general hemorrhaging and death within a few days. It is still not clear whether the virus works the same way in humans, however. None of the human victims hemorrhaged, although several had suspicious kidney failure, says Webster.

The virus isolated from the boy is deadly to chickens, however. The Subbarao team experimentally infected 24 chickens with it, and all but one died. That raises concerns for the USDA, says Perdue. If a human were to carry the flu back from Hong Kong, it could be devastating to U.S. poultry.

What the scientists still don't know is exactly how this flu strain manages to infect humans. To solve that question, Subbarao says, researchers are closely examining a range of avian flu viruses, hoping to pinpoint how this H5N1 strain is different. Webster says he and his colleagues have uncovered one potential clue. In work in press at *The Lancet*, he and his colleagues report that the hemagglutinin of viruses isolated from Hong Kong chickens in March contains a carbohydrate near the site where it binds to cell surfaces, but that molecule is missing from the H5N1 strain isolated from the boy. Webster says the change "may have great influence" on the virus's ability to bind to human cells.

However the virus has altered to allow bird-to-human infection, it still doesn't pass easily between humans. So far, there has been only one suspected case of transfer from one person to another: The toddler's doctor has antibodies to the virus but never got sick. And for now, there has been a drop in new infections—the latest individual became ill on 28 December. But epidemiologists are keeping a wary eye on Hong Kong, especially as the yearly flu season begins. Although no new cases have been reported, officials fear that a currently circulating version could mix with a strain more adept at infecting humans, sparking a pandemic.

The chances of that pandemic are greatly reduced, say most researchers, now that Hong Kong's millions of chickens in open-air markets have been killed. "The slaughter was absolutely essential," says Webster. "The big question is whether the stable door was shut in time."

–Gretchen Vogel