Adhesion Protein Studies Provide New Clue to Metastasis

Cancer patients usually don't die of their initial tumor. The real killers are the metastasizing cells—the select few that manage to escape the tumor, enter the blood vessels or lymphatics, circulate through the body, and grow into secondary tumors in distant organs, all the while avoiding the immune cells that search out and destroy such stray cells. How the escapees are able to make this voyage, and what genes are crucial to the process, is a mystery that has proved very difficult to solve—much harder than finding the cancer-causing "oncogenes" that caused the tumor to grow in the first place.

But cancer researchers are finally beginning to get a molecular handle on metastasis: In the past few years, they've identified several genes that may be involved. One of the clearest examples is a gene isolated from rat pancreatic tumor cells by Peter Herrlich and Helmut Ponta and their colleagues at the Nuclear Energy Research Center in Karlsruhe, Germany, in collaboration with Margot Zöller and her colleagues at the German Cancer Research Center in Heidelberg. Evidence is steadily mounting that this gene, which codes for a cellular adhesion protein, is important for metastasis. And now the Karlsruhe and Heidelberg groups, based partly on new findings about the protein's normal function (reported on page 682 of this issue) are proposing a tantalizing explanation for the metastasis-adhesion protein link.

"I never thought the gene would make cells metastasize. I thought its effects would be more subtle."

–Peter Herrlich

They suggest that the protein serves as a molecular disguise that helps tumor cells look and act like normal lymphocytes as they differentiate within the lymph nodes. From the lymphatic system, these malignant "wolves in lambs' clothing" then have access to the general circulation and can therefore spread throughout the body.

"Learning about the function of this gene is fascinating," says Hartmut Land of the Imperial Cancer Research Fund in London, an expert on the genetic changes underlying cancer development, "because it's the most promising candidate for a cancer gene which doesn't have to do with growth control, but with later steps in tumor progression." And the discovery could have implications for the diagnosis or treatment of human cancers. Karl-Heinz Heider, who's also at Karlsruhe, and Steven Pals of the Academic Medical Center in Amsterdam have preliminary evidence that the adhesion protein may play a role in human colorectal carcinogenesis, making it a possible target for drug therapy.

Finding the link between metastasis and the adhesion protein was a long, slow process. Herrlich's search for genes that affect metastasis got under way in 1985, and the early efforts were frustrating. "We started with all sorts of ingenious experiments, which didn't work because we didn't know what we were looking for," he says. They didn't know whether "metastasis genes" would induce or suppress metastasis, and they worried that it would take changes in many genes to produce a noticeable effect on metastasis. "After years of hearing how complex [metastasis] is, we thought that we wouldn't be able to see the effect of a single gene," he says.

But surprisingly, success came from a simple approach. In 1986, Achim Wenzel, a student working with Zöller in Siegfried Matzku's laboratory at the German Cancer Research Center, started looking for specific differences between metastatic and nonmetastatic tumor cells by trying to make monoclonal antibodies that would bind to surface proteins on one type of tumor cell but not on the other. Wenzel soon found several antibodies that fit the bill—they bound to metastatic rat pancreatic tumor cells, but not to closely related but nonmetastatic tumor cells. The immunologists then teamed up with Ursula Günthert, a molecular biologist in Herrlich's lab, who cloned the genes for several of these metastasis-specific cell surface proteins.

Sequencing the candidate metastasis genes revealed a big surprise. One of them turned out to encode a new variant of CD44, a well-known cellular adhesion protein found in many different cell types, especially white blood cells. CD44 comes in several forms—nine at last count—that are variants of the so-called standard CD44. The different CD44 proteins have the same sequences at their ends but differ in the middle, which is located just outside the cell membrane. The functions of the standard and variant CD44 molecules haven't yet been pinned down, but they seem to participate in a variety of biological activities, such as chemotaxis and lymphocyte migration and activation.

Then came the critical question. Could the variant CD44 gene induce metastasis? The answer provided yet another surprise—it was an unequivocal yes. When Martin Hofmann, a student in the group, transferred the gene into nonmetastatic tumor cells and injected them into rat foot pads, the cells metastasized efficiently to the lymph nodes. "I never thought the gene would make the cells metastasize," says Herrlich. "I thought its effects would be more subtle, that we would have difficulties seeing what it's doing."

Looking for a function. Since they had no idea how the CD44 variant induced metastasis, they began searching for its function by looking at normal cells. They already knew that only a very few cells types, including certain white blood cells, make it. And when Karin Wirth and Robert Arch in Zöller's group took a closer look, they found that the variant is transiently expressed in certain macrophages and T- and B-cells in response to antigen stimulation. These findings hinted that the variant might be involved in the early steps leading to an immune response. Further experiments confirmed that the CD44 variant is an essential player in the activation of immature lymphocytes into mature immune cells in the lymph nodes. The precise role of the CD44 variant remains to be defined, however.

Once the role of the CD44 variant in lymphocyte activation was recognized, its relevance to metastasis became obvious. As Zöller explains, the researchers saw that tumor cells expressing the variant were in effect mimicking developing lymphocytes, and that "the CD44 variant is probably needed by metastasizing tumors in the lymph nodes for exactly the same reasons it's needed by lymphocytes, whatever that turns out to be."

While they continue trying to pin down the function of the variant, the two groups have gone on to ask if it has any relevance to human cancer. Early results are encouraging. In a small study of colorectal cancer, they found the CD44 variant protein in all the invasive carcinomas and many of the benign polyps that precede them, but not in normal colon cells. This suggests, says Herrlich, that the gene is activated early in colon carcinogenesis. If the link is confirmed in larger studies, the CD44 variant might turn out to be a clinically useful marker of progression in this type of tumor.

Herrlich's group is also investigating the potential of the variant CD44 as a target for drugs designed to lock onto specific tumor cell proteins and kill the cells bearing them. If they succeed, it would bring an ironic twist to the story. The lambs' clothing could turn out to be not a clever disguise but the very garment that spells death for the wolf.

–Patricia Kahn

Patricia Kahn is a free-lance writer based in Heidelberg, Germany.