How Cancer Cells Spread in the Body

Metastatic cancer cells need to undergo several changes in order to make the arduous journey from the primary tumor where they originated to new target sites

NOT ALL CANCER CELLS ARE EQUAL. Relatively few have the dangerous ability to metastasize throughout the body. If researchers can learn what gives the metastatic cells this ability, then it may be possible to disarm them and develop new cancer therapies. "Metastasis is what kills patients. The difference between benign and metastatic cancer is the crux of the disease." says Lance Liotta of the National Cancer Institute.

The idea that cells from the same tumor could vary in the ability to metastasize dates back to results obtained about 10 years ago by Isaiah Fidler and Margaret Kripke of the University of Texas M. D. Anderson Cancer Center in Houston. Their findings stimulated many investigators, looking at the biology of cancer cells from a variety of angles, to ask just what makes metastatic cells different from nonmetastatic cells. The answers are now beginning to come in.

Last month some 550 of the researchers who are exploring cancer metastasis gathered in Houston to hear the latest results at a symposium* entitled "Critical Determinants in Cancer Progression and Metastasis." Those results show that cancer cells must undergo a myriad of changes to become metastatic.

Bert Vogelstein and his colleagues at Johns Hopkins University School of Medicine have, for example, identified several genetic changes that take place during the development of colon cancers. Some of these work positively to make cells malignant. The Vogelstein group has found that the *ras* gene, one of approximately 50 "oncogenes" that have been implicated in cancer development, becomes activated, usually before full malignancy occurs..

Other changes are negative. Parts of three different chromosomes are consistently deleted, presumably resulting in the loss of genes that would otherwise suppress the development of malignancy. Still other changes involve alterations in the methylation patterns of DNA, which may cause genes to be turned on or off.

One early consequence of the gene changes in an incipient tumor cell is likely to be the acquisition of an increased ability to grow. This may be caused by growth factor production by the tumor cells themselves. But the environment in which the tumor cells live also influences their growth and their ability to metastasize.

This need for a mutual interaction between cancer cells and their environment was another major theme of the Houston meeting, which marked the 100th anniversary of the "seed and soil" hypothesis originally formulated by British surgeon Stephen Paget. Noting that certain types of cancers tend to metastasize preferentially to certain organs, Paget proposed that cancer cells, like plant seeds, may travel in all directions, but can only grow if they fall on congenial soil.

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When human cancer cells are implanted into nude mice, they do not form metastases in the same organs that they would affect in humans, unless, the Fidler group has recently shown, the cells are placed in the correct sites. Kidney cancer cells need to be implanted into the mouse kidney, and colon cancer cells need to be implanted into the colon or spleen if they are to have metastatic patterns similar to those of the cancers in humans.

Fidler suggests that the damage done to the mouse organs during implantation of the cancer cells triggers the release of organ-specific growth factors that are needed to repair the tissue. They also stimulate the growth of the tumor cells. In a similar fashion, a growing tumor may cause damage to the organ in which it originates and induce growth factor release there, too.

Although a high growth rate is often considered a hallmark of cancer cells, it is not sufficient by itself to make them metastatic. It may, however, enable tumor cells to undergo the numerous additional changes required for the development of full metastatic malignancy by providing increased opportunities for gene alterations to occur.

One of the earliest of those additional changes after the increased growth rate is acquisition of a blood supply for the tumor. Judah Folkman of Harvard Medical School and Douglas Hanahan of Cold Spring Harbor Laboratory have results indicating that the onset of the tumor angiogenesis, as the formation of new blood vessels is called, is an independent event occurring a few weeks after cells have begun growing abnormally as the result of acquiring a viral oncogene.

"We found that the oncogene only causes the pancreatic islet cells to grow and become hyperplastic," Folkman says. "After time goes on some of the cells suddenly release a factor to induce the growth of new blood vessels." The ability of the cells to form tumors in mice correlates with the angiogenesis, not with the abnormal growth.

For metastasis to occur, a tumor must not only be able to grow, but some of its cells must be able to leave the tumor mass, burrow their way into a blood vessel or a vessel of the lymphatic system, and then find their way back into another tissue. This is an arduous journey, which may help to explain why so few cancer cells make it.

During its journey a metastatic cancer cell will have to cross a basement membrane barrier at several points. Basement membranes, which consist of a complex of proteins, including collagen IV, laminin, and fibronectin, underlie the epithelial cells from which the common cancers are derived. They also surround the smooth muscle of blood vessels. Several researchers at the meeting described adaptations that may help metastatic cells escape from tumors and traverse basement membranes.

The adaptations include the increased secretion of enzymes that may facilitate cell movements through basement membranes by dissolving the membrane proteins. Liotta and his colleagues have found that metastatic cancer cells make higher than normal quantities of type IV collagenase, an enzyme that breaks down type IV collagen, the major collagen of basement membranes. The NCI group has also identified a new member of the "TIMP" (for tissue inhibitor of metalloproteinases) family in tumor cells.

TIMPs ordinarily inhibit collagenases, but this one is different. "The fascinating thing about this member of the family is that it binds to type IV collagenase, but doesn't inhibit it," Liotta says. He suggests that it may help to prevent other TIMPs from inhibiting the enzyme, thereby keeping the enzyme in the "on" position.

In addition, Stuart Yuspa's group, also at

*The symposium, which was held on 6 to 10 March, was sponsored by the University of Texas M. D. Anderson Cancer Center and Smith Kline & French Laboratories.

NCI, has evidence indicating that the *fos* oncogene acts to make cells malignant by turning on the gene encoding transin, another enzyme that dissolves the proteins found in basement membranes and the extracellular matrix. The increased production of these enzymes helps to explain, Yuspa says, how the product of a single gene such as *fos* can produce the broad spectrum of changes that occur when benign cells becomes malignant. By disrupting the extracellular matrix that normally surrounds cells, proteases can disturb the interactions between cells, thereby causing them to become disorganized.

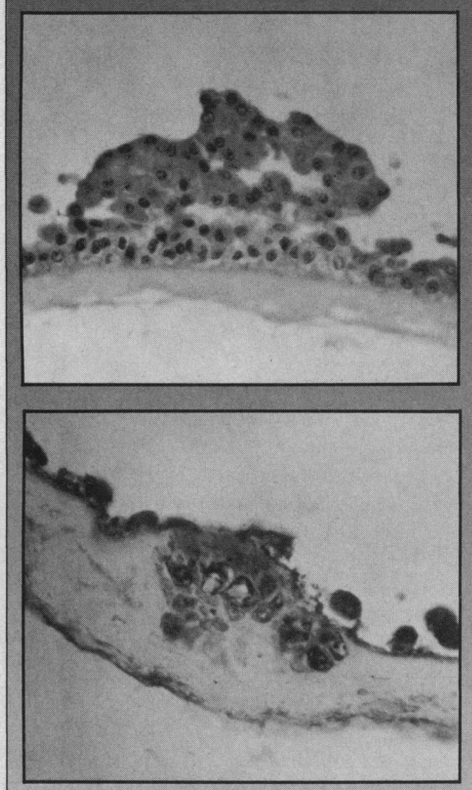
The matrix changes may influence cell behavior by altering gene expression. For example, Mina Bissell of the Lawrence Berkeley Laboratory in Berkeley, California, has found that mammary epithelial cells must be in contact with extracellular matrix proteins to achieve normal synthesis and secretion of milk proteins. As she pointed out at the meeting, "The nucleus is in constant contact with the extracellular matrix. The nucleus modifies the matrix, and the matrix modifies the nucleus."

Metastatic cells also show disturbances in their mutual interactions with basement membrane components that may be related to their ability to invade the membranes. The cells' ability to bind laminin goes up, for example. This may not only help to attract tumor cells to basement membranes, but it may also arm the cells with enzymes for degrading membrane components once they get there. Tumor cell binding to laminin increases collagenase IV synthesis, according to Liotta.

Moreover, blocking the binding to laminin inhibits metastasis. Laminin is a large, complex protein with several sites for binding to cells. George Martin and his colleagues at the National Institute of Dental Research have shown that a synthetic peptide corresponding to one of these binding sites reduces the lung metastases produced in mice by injected melanoma cells. Because of the speed with which the peptide breaks down in the body it is unlikely to be of use in preventing metastasis in human cancer patients, however.

Whereas laminin binding facilitates metastasis, binding of cells to another basement membrane protein, fibronectin, apparently has the opposite effect, causing the cells to settle down on the matrix and behave more normally. Metastatic cells have a reduced capacity to make and bind fibronectin.

According to Richard Hynes of the Howard Hughes Medical Institute at the Massachusetts Institute of Technology, the reduction in fibronectin binding may be the result of changes in the receptors, known as integrins, by which cells bind to fibronectin. "These cells are changing their matrix receptors in response to transformation and that may well change the way they interact with the matrix," Hynes says. It may also influence gene expression, as the integrins are instrumental in relaying information about matrix binding to the cell interior.



Lance Liotta, National Cancer Institute

Noninvasive cancer cells *multiply, and simply pile up on a basement membrane (top). Invasive cancer cells (bottom) readily penetrate the membrane, however.*

James Dennis of the Mount Sinai Hospital Research Laboratories described still another change in cell surface molecules that may contribute to metastatic ability. Dennis and his colleagues have found that metastatic cells bear a type of complex branched carbohydrate that is not usually seen on mature cells. Growing cancer cells in the presence of a chemical that prevents the synthesis of the branched carbohydrate markedly inhibits their ability to produce tumors in mice.

According to Dennis, the carbohydrate may help tumor cells enter blood vessels by increasing their adhesion to the vessel walls. It may also facilitate the cells' subsequent movements out of the vessels and into the surrounding tissues by decreasing their adhesion to the extracellular matrix.

Once a tumor cell has completed the first phase of its journey and entered the bloodstream or lymphatic system, it runs a major risk of being wiped out by immune cells that are on the lookout for cancer cells. Tumor cells may escape this danger, however, by losing the cell surface molecules, called major histocompatilibity proteins, that are needed for recognition by some immune cells.

Both Robert Goodenow of the University of California, Berkeley, and Michael Feldman of the Weizmann Institute of Science in Rehovot, Israel, described experiments in which they made cancer cells much less tumorigenic by transferring genes encoding appropriate histocompatibility molecules into the cells. Such gene transfers can, Feldman says, "reduce a highly metastatic phenotype to a hardly metastatic phenotype."

Tumor cells may also protect themselves against immune attack by sticking together. According to Avraham Raz of the Michigan Cancer Foundation in Detroit, melanoma cells that do not aggregate into small clumps do not form lung metastases. The ability to clump is apparently related to the presence of large amounts of a sugar-binding protein on the surface of the tumor cells.

Not all of the tumor cells that actually arrive at a new organ site are capable of growing there, however. The seeds have to be able to respond to the soil. The response may be dependent upon growth factors made by specific tissues. For example, Philip Cavanaugh and Garth Nicolson of M. D. Anderson have identified a growth factor, made by lung tissue, that promotes the growth of melanoma cells that readily form lung metastases. According to Feldman, the metastatic cells may become responsive to the growth factor by switching on the gene encoding the receptor for it.

Any or all of the adaptations that tumor cells must undergo to become metastatic might provide points of attack for therapies aimed at preventing or treating disseminated cancer. That does not mean that the job of producing such therapies will be easy. Martin Raber of M. D. Anderson pointed to a major obstacle to prevention. Undetectably small metastases are often present by the time a patient's cancer is first diagnosed. "Most patients already had metastases when they walked into your office with local or regional disease," Raber told the symposium participants.

Moreover, cancer cells have a well-known ability to develop resistance to the drugs used to combat them. Nevertheless, researchers are hopeful that the better understanding of metastasis they are gleaning will ultimately prove useful in the clinic. "The notion that metastasis is selective is a very optimistic one, because we know that we can study it. Once you understand something you may outsmart it," Fidler says.

■ JEAN L. MARX