Outside Influences

A cancer cell's physical environment controls its growth

By JOHN TRAVIS

Open a box containing fragile objects—dishes, wineglasses, vases—and you will frequently find Styrofoam peanuts surrounding and protecting them. Most people ignore this cushiony material, furiously shoveling the pellets out of the way to get at the treasures within the package.

Similarly, cell biologists have neglected the complex natural environment surrounding their objects of study. For most of their field's history, they examined individual cells, or groups of cells, away from the dense, fibrous network of proteins and sugars that normally envelops them. The scientists viewed this extracellular matrix as mere packing material, little more than the body's own Styrofoam peanuts.

Over the last 2 decades, the extracellular matrix has gradually gained the attention and respect of cell biologists. They have found that components of the matrix, proteins with names like laminin and fibronectin, bind to specific molecules, called integrins, on the cell surface. Through these 20 or more integrins, the matrix sends cells various signals that regulate what genes are active, ultimately influencing whether cells proliferate, specialize, migrate, or even kill themselves.

"Why doesn't your nose turn into your elbow?" jokingly asks Mina J. Bissell of the Lawrence Berkeley (Calif.) National Laboratory. The serious answer, she and many other scientists now believe, lies largely in the matrix's ability to command cells to use particular, tissue-specific genes.

Indeed, investigators have found that cells from very different organs and tissues become almost indistinguishable when removed from their normal extracellular matrix and placed on glass or plastic surfaces. Only when the correct matrix molecules are added to these surfaces do the cells begin to behave like blood vessels, mammary glands, or other specialized tissues.

"It's now a universal concept that the microenvironment outside cells, of which an important component is the extracellular matrix, confers tissue specificity," says Bissell, president of the American Society for Cell Biology.

While most studies of the extracellular matrix focus on its influence over normal cells, researchers have recently begun to explore its importance in the development of cancer.

The results so far have surprised many scientists. Bissell's group, for example, has recently shown that antibodies blocking the function of certain integrins seem to transform malignant breast cells into normal mammary cells, a finding that suggests new anticancer strategies.

Most human cancers arise from epithelia, the layers of cells that form the outer skin and line the internal and external surfaces of most organs. These cells sit on a laminin-rich matrix called the basement membrane, which separates them from the rest of the organ to which they belong.

Investigators have known that cancerous epithelial cells often find a way to chew through the basement membrane, an action that allows them to metastasize, or spread to other regions of the body. The protein-degrading enzymes that enable tumor cells to rip through the matrix are called proteases. While proteases have been implicated in metastasis, new results suggest that they are also vital to the early development of a tumor.

In collaboration with a research group led by Zena Werb of the University of California, San Francisco, Bissell's team gave mice a copy of the gene for stromolysin-1, a matrix-degrading protease. Cells in the breast normally secrete this protein during involution, the remodeling of the extracellular matrix that occurs after pregnancy, when the breast becomes smaller and stops making milk.

The researchers had designed the added gene to turn on much earlier, during the middle or the end of a pregnancy. Unexpectedly, large numbers of the mice developed breast tumors.

"It always shocks people," says Bissell. "We're simply destroying something outside the cell, and stromolysin-1 isn't considered an oncogene [a cancer-causing gene], yet these animals get tumors."

The exact mechanism by which stromolysin-1 promotes cancer remains unclear, but its activity does suggest that a normal extracellular matrix has some ability to suppress tumor formation, says Bissell.

Another piece of evidence supporting that view has recently come from a test developed several years ago by Bissell's group and a team led by Ole W. Petersen of the Panum Institute in Copenhagen (SN: 4/2/94, p. 222). At that time, the researchers were tackling a practical problem facing cell biologists: How does one tell a breast cancer cell from a normal mammary cell?

Pathologists perform this task by surgically removing tissue from the breast and looking for telltale differences in cell shape. Yet if researchers use enzymes to disintegrate the extracellular matrix in those tissues and then place living cancer and normal cells on a petri dish, they'll find it almost impossible to distinguish between the cells.

"The cells, when you take them out of the body and place them on plastic, look the same and grow at the same rate. No one had an assay to distinguish normal and malignant cells within a culture dish," says Bissell.

To solve the problem, the investigators drew upon their knowledge of the extracellular matrix's role in determining how cells specialize. For example, capillary epithelial cells roll up to form normal blood vessels only if grown on the proper matrix molecules.

Bissell and her colleagues therefore tried to construct a "virtual breast" by creating a solution that contains laminin and other basement membrane molecules. They then poured mammary cells into the mix and let it all gel. "It's just like making Jell-O," says Bissell.

Within this gelatinous mass, normal and cancerous mammary cells behave quite differently. The normal cells start secreting the components of basement membrane, says Bissell, and "make these beautiful, well-organized structures that look identical to the normal cells in a breast."

Indeed, the laboratory-grown cells even begin secreting milk proteins. The cells also ceased dividing, "which is very exciting, because normal human epithelial cells [placed] on plastic don't stop growing," says Bissell.

In contrast, malignant breast cells placed within this three-dimensional gel continue to proliferate, piling into huge masses with little or no structure.

"The tumor cells are laying down a lot of extracellular matrix, but it is totally disorganized," says Bissell.

These experiments prompted the scientists to wonder why the cancer cells didn't establish a normal tissue structure. In the April 7 JOURNAL OF CELL BIOLOGY, Bissell and her colleagues offered some answers.

To create their cancer cells, the investigators grew normal mammary cells without the aid of the chemical growth factors ordinarily present in the cell culture medium. "The cells, little by little, developed mutations," says Bissell. The genetic abnormalities ranged from duplications of entire chromosomes to specific gene mutations known to promote cancer.

The researchers determined when the cells had become malignant by periodically testing their ability to form tumors when injected into mice.

The investigators observed that the distribution of several different integrins changed dramatically when the cells became malignant. In normal cells, different integrins appear in separate, well-defined regions, whereas integrins occur largely at random on cancer cells. Moreover, integrins called beta-1 were more abundant on cancer cells, and beta-4 integrins were less abundant.

That wasn't too surprising, notes Bissell, since many other researchers have shown that normal and cancerous breast cells display different kinds and amounts of integrins. However, nobody knew what those integrin differences meant, she adds.

Using the three-dimensional cell culture, Bissell and her colleagues found that when they blocked beta-1 integrins with antibodies, malignant breast cells reorganized within days into balls that were structurally similar to normal cells. Most important, the cells stopped growing.

Bissell finds that result "mind-boggling"—because the cells still harbor the genetic mutations that drove their malignant growth. The antibodies apparently reduced the beta-1 integrin signals within the cell, and that drop triggered the dramatic halt in cancerous behavior.

"We're showing that the ratio of [integrins] at the cell surface governs tissue organization, growth, etc. It regulates the entire gamesh," says Bissell.

The scientists even showed that they could switch the cells back and forth between normal and malignant-looking by alternately treating them with antibodies and cleansing them of antibodies.

Finally, the investigators treated breast cancer cells with beta-1 integrin–blocking antibodies and injected the cells into a small number of mice. Only 7 of 16 such mice developed tumors, whereas 15 of 16 mice that received untreated cancer cells did.

A group in Japan, notes Bissell, recently used antibodies to the same integrins to tackle cancer in an even more dramatic fashion. The Japanese scientists, she says, injected the antibodies into mice that had well-established tumors; the therapy shrank the cancers significantly.

Other integrins appear vital to cancer as well, the researchers stress. While antibodies that block the beta-4 integrins do not correct malignant cells, normal cells treated with such compounds lose their organized structure. This suggests that eliminating the ability to sense the extracellular matrix through beta-4 integrins may be an important step in cancer development, the scientists say.

The large number of different integrins indicates that the extracellular matrix's control over cells is probably vast and complex. In fact, investigators are slowly discovering that integrins may be as important to tumor formation as the much more thoroughly studied cell surface proteins that react to the body's chemical growth factors, says Alan F. Horwitz of the University of Illinois at Urbana-Champaign.

Horwitz notes that compounds inhibiting the integrins used during a tumor's formation of blood vessels are already being tested on people. Without a steady blood supply, tumors cannot grow to dangerous sizes, he explains.

Bissell believes that her group's recent experiments with the integrin-blocking antibodies offer a compelling argument that cancer researchers must focus as much on a cancer cell's altered connections to its surroundings as on the internal changes that spur its excessive proliferation.

"The structure of the tissue is dominant over [the genes in the cell]," she says. "That is 180° different from current dogma, which says that once a tumor cell, always a tumor cell."

"The notion that cancer is reversible was heresy 5 years ago," agrees oncologist Harvey Schipper of the University of Manitoba in Winnipeg.

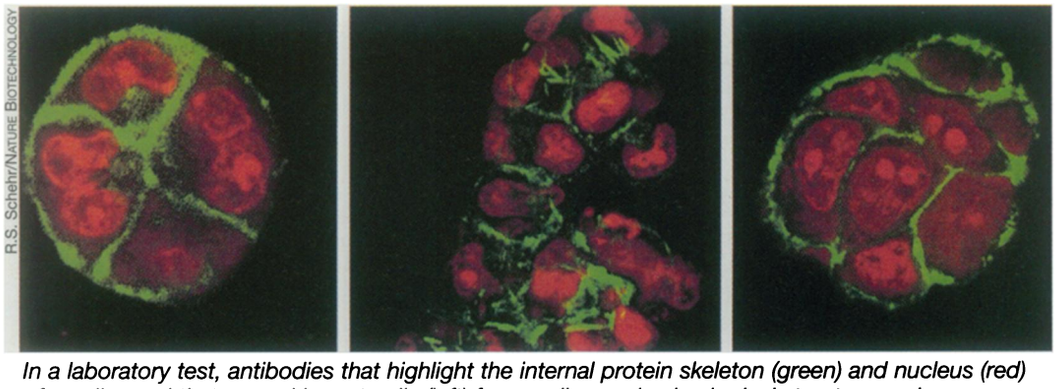
In recent articles, Schipper and other investigators have questioned whether physicians must kill every single cancer cell in the body or whether it might prove easier to find therapies that reestablish normal growth controls over malignant cells. The new work by Bissell and her colleagues provides additional evidence that the latter course is achievable, he says.

"It's certainly surprising that such simple manipulations can change [cancer cells] so drastically, and it gives you hope that if you figure out how to do the same in people, you would have something useful for therapy. On the other hand, cancers seem to uniformly outmaneuver the host in the end," comments cancer geneticist Bert Vogelstein of Johns Hopkins Medical Institutions in Baltimore.

Eventually, some cells develop genetic mutations that enable them to evade even the powerful growth restraints imposed by the extracellular matrix. Otherwise, there wouldn't be any cancers to combat, he explains.

The most important aspect of the extracellular matrix research by Bissell's group, contends Vogelstein, is its attempt to study cancer in an environment similar to that of tumor cells in the body. Investigators have documented scores of genes mutated in cancers, he notes, but they have rarely explained completely how those alterations affect a cell's behavior outside its artificial life in petri dishes.

"We've just begun to understand what happens in the microenvironment of a tumor, which is so much more complex than an isolated cancer cell growing in a test tube. Hopefully, Bissell's at the forefront of what will blossom, in many years, into a real understanding of what goes on in vivo," says Vogelstein. □



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In a laboratory test, antibodies that highlight the internal protein skeleton (green) and nucleus (red) of a cell reveal that normal breast cells (left) form well-organized spherical structures, whereas malignant cells (center) do not. Treating cancerous cells with antibodies that block signals from the extracellular matrix allows the cells to develop a more normal organization (right).