Studying Cancer in 3 Dimensions

3-D Models Foster New Insights Into Tumorigenesis

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LIKE ACTORS ASSEMBLED TO PERform a play, hundreds of genes and proteins have been cast in cancer-related roles in the last few decades. But without a script, a narrative of how these characters interact to disrupt normal biological activities and cause a malignancy remains an unfinished story.

For the drama to unfold properly, a stage—a model system—is needed that closely mimics the living environment in which cancer pathogenesis occurs. To this end, some researchers are turning to three-dimensional (3-D) cell culture systems. The reason: limitations of the traditional mainstays of cancer research, cell cultures and animal models.

In conventional cell cultures, researchers can readily manipulate genes to study the genetic changes that drive the cancer process and to investigate biochemical pathways, but they can observe these effects only in a flat, two-dimensional (2-D) sheet of cultured cells. Animal models give a more accurate morphological representation of tumor development, but they are considerably less amenable to large-scale genetic studies.

A 3-D cell culture system, on the other hand, combines virtues of animal models and 2-D cell cultures, explains Joan Brugge, PhD, of Harvard Medical School, in Boston. As an in vitro system that allows cells to develop into structures similar to those in living organisms, 3-D models enable researchers both to perform genetic manipulations and to observe some of the biological changes the genes bring about.

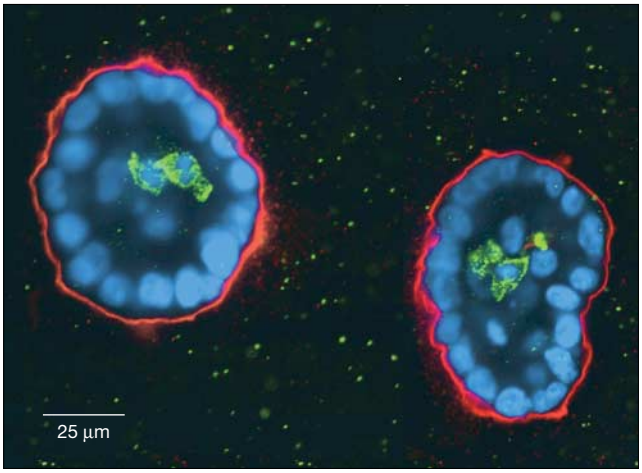
FROM FLAT TO FAT

To probe the function of genes that are altered in breast cancer, Brugge and her coworkers have been using a 3-D model based on a system developed in the laboratory of Mina Bissell, PhD, of the Lawrence Berkeley National Laboratory, in Berkeley, Calif. Over the last three decades, Bissell and her colleagues have used 3-D mouse or human cell culture systems to shed light on how the extracellular matrix (ECM) and tissue architecture shape the way normal and malignant cells receive and respond to signals from their surrounding environment.

An early hallmark of a breast tumor is disruption in the architecture of mammary acini, glandular units at the end of ducts. Healthy acini, which have hollow lumens (from which milk is secreted), are composed of an inner layer of luminal epithelial cells and an outer layer of myoepithelial cells, surrounded by a specialized type of ECM called the basement membrane. But during tumorigenesis, this well-ordered architecture is disrupted as cancer cells escape the controls on proliferation and fill the luminal space.

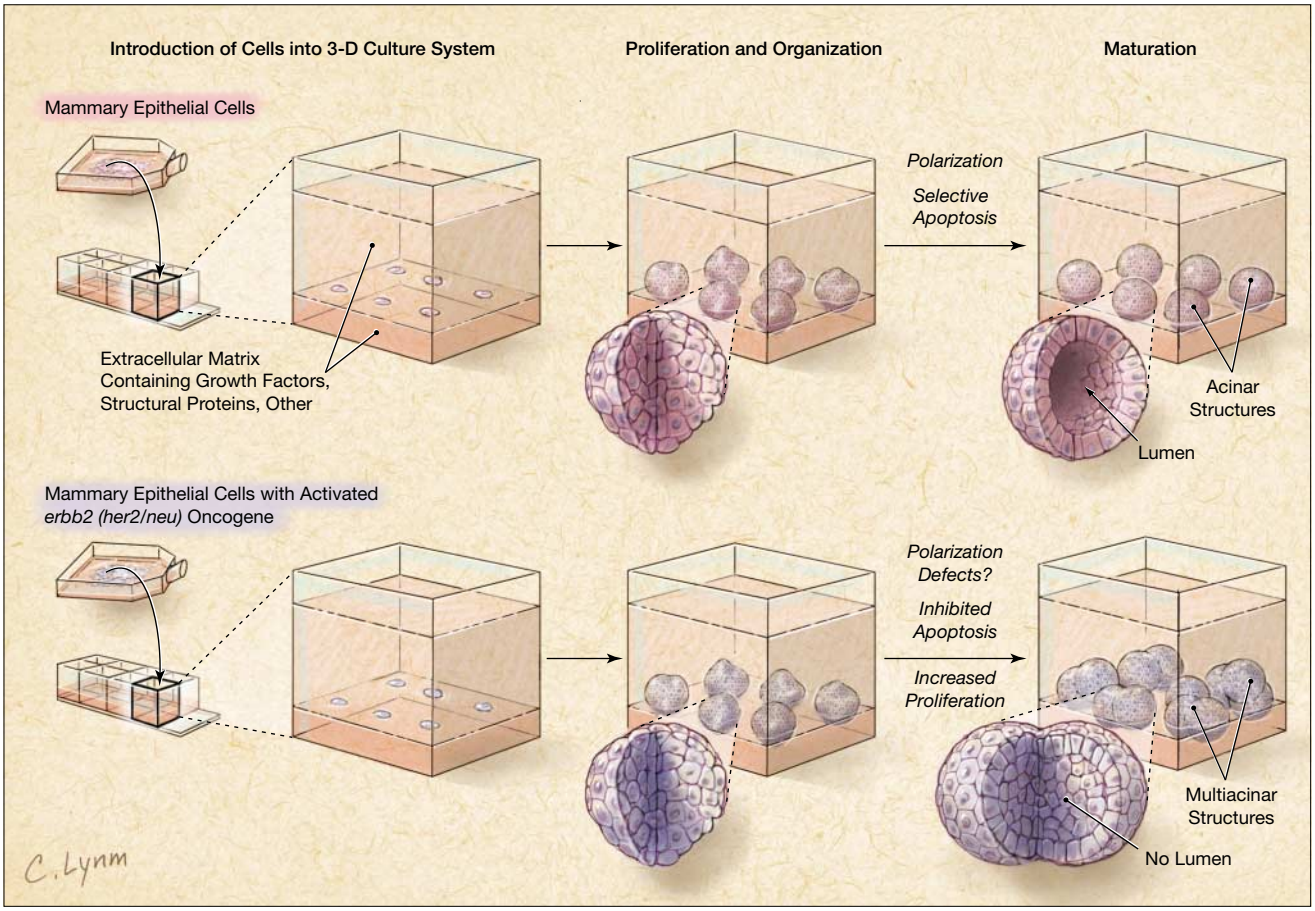
In the Brugge laboratory, mammary acini are grown by placing epithelial cells on top of a layer of reconstituted basement membrane—usually a commercial mix called Matrigel, composed primarily of substances obtained from the ECM of a type of mouse tumor. A dilute suspension of Matrigel is then spread over the cells, simulating their natural microenvironment. Initially, the cells divide to form a solid, structured mass, but as the structure matures, the cells in the center of the sphere undergo programmed cell death, or apoptosis, creating a hollow lumen.

Acini grown in 3-D matrices allow researchers to study the molecular mechanisms necessary to form and maintain normal glandular architecture, and to manipulate them to determine how cancer-related genes disrupt the structure. Brugge and her colleagues are introducing genes associated with cancer



Jay Debnath

3-D culture systems can allow cells to develop into structures similar to those in living organisms and reveal how cancer genes disrupt those structures. This image shows mammary epithelial cell acini after 8 days of 3-D cell culture. The cells in the center (green immunofluorescence) are undergoing normal programmed cell death (apoptosis) to form a hollow space.



In 3-D cell culture systems, normal mammary epithelial cells develop into acinar structures through the processes of proliferation, polarization (directional orientation of cells on the outer edge of the cell mass), and apoptosis of cells to create a lumen (top panel). Cells with the activated oncogene *erbb2* (*her2/neu*) display increased proliferation, appear to contain polarization defects, and do not undergo apoptosis; hence, they do not develop normally (bottom panel).

into breast epithelial cells to tease out the underlying biological processes responsible for the different grades and stages of breast tumors. Using this approach,they hope to discover the mechanisms that help initiate lesions while maintaining the gland's architecture; the processes involved in more advanced stages of breast cancer in which tumor cells are able to fill the lumen; and the events involved in metastasis.

"Ultimately, what we'd like to understand are the most significant changes responsible for invasive carcinoma," said Brugge. "What underlying processes allow those cells to emerge from that cocoon that they're growing in, break down the basement membrane, break down the cell junctions, and move into the surrounding tissues?"

Work by the Brugge laboratory also suggests that the 3-D breast cancer cell model also may be useful for screening the activity of potential cancer therapies. Jayanta Debnath, MD, recently demonstrated that rapamycin (also called sirolimus), a targeted cancer drug now in clinical trials, can reverse the abnormal cell behavior driven by an altered gene, *Akt*, without affecting normal cell behavior—a result that's encouraging for using this compound therapeutically.

UNIQUE PHENOTYPES

So far, Brugge's team has screened about 275 genes out of about 1000 that have been implicated in one way or another in breast cancer. At the annual meeting of the American Association of Cancer Research in July, she described experiments in which they examined a number of different oncogenes that promote uncontrolled cell proliferation.

Not surprisingly, the researchers found that when various oncogenes were introduced into the cells, the cells grew in an uncontrolled fashion. What was unexpected, said Brugge, was that in each case, the unfettered growth did not give rise to the same biological and morphological phenotypes. Instead, each gene gave a unique twist to the acinar structure.

For example, Senthil Muthuswamy, PhD (then with the Brugge laboratory, now at Cold Spring Harbor Laboratory in New York), found that introducing the *erbb2* (*her2/neu*) oncogene (which is amplified, or found in increased numbers, in about 30% of human breast cancers) caused the cells to proliferate in many directions (*Nat Cell Biol*. 2001;3:785-792). Some grew into the lumen and filled it; others divided to outside the acinus to form struc-

tures resembling those seen in an early breast cancer lesion called comedo ductal carcinoma in situ.

In contrast, when oncogenes such as *CCND1* (which encodes cyclin D1) or *E7* (an oncogene present in human papillomavirus type 16) were introduced into cells, the cells grew into the lumen, but then underwent apoptosis, thereby preserving the lumen's structure. Only when a gene that blocked apoptosis was introduced into the model were the cells in the lumen able to resist the death signal and survive (*Cell*. 2002;111:29-40). Additional genes brought about still other growth patterns, which causes Brugge to speculate whether such patterns "reflect the different spectrum of carcinoma in situ lesions detected in human breast cancer."

Brugge's group also has focused on unraveling the processes involved in converting a noninvasive phenotype into an invasive one. Using a set of genes associated with invasive behavior, the Harvard researchers are dissecting the pathways involved in this process.

For example, amplification of *erbb2* alone does not appear to enable a tumor able to invade surrounding tissues. But the Harvard team found that by combining the *erbb2* gene with genes encoding growth factors—transforming growth factor (TGF) β1, β2, or β3—the cells became aggressively invasive. This corroborates evidence in animal models indicating that TGF-β genes and *erbb2* collaborate to cause invasion, said Brugge.

WHY CONTEXT MATTERS

While a 3-D culture system provides a more lifelike environment for tissue development than conventional cell cultures, other factors are needed to create a milieu that mimics a microenvironment that resembles what happens in nature, namely the chemical substances that exist in a given tissue in vivo, explains Bissell, a 3-D cell culture pioneer. These substances provide the proper signals for the cells to grow in an organized fashion.

"Breast cells in gels of stromal collagen, for example, behave much differently than they do in gels of basement membrane, even though both are 'ECM' material in '3-D,'" she explained.

There's considerable evidence demonstrating that the ECM and cellular context not only play important roles in the normal functioning of cells, but that loss or damage to ECM may lead to tumorigenesis, as well. A few years ago, Bissell's laboratory provided a dramatic demonstration of the importance of matrix proteins and their signaling in breast tumor development: By manipulating ECM-receptor signaling, they coaxed malignant breast cancer cells, which form aberrant acinar structures in 3D, to revert to forming normal ones (*J Cell Biol*. 1997;137:231-245).

In this study, when the researchers treated human breast cancer cells in 3-D culture with an antibody that blocks β-1-integrin, a receptor for ECM molecules, the tumor cells' abnormal acinar structure reorganized into one that looked and functioned normally. Genetic analysis indicated that all the genetic alterations the cells carried were still present, suggesting that tumor structure can trump genetic information, at least in some situations.

In more recent work, Bissell and coworkers used 3-D models to examine how tissue structure might influence whether malignant and nonmalignant cells develop resistance to chemotherapeutic agents that induce apoptosis (*Cancer Cell*. 2002;2:205-216). As Bissell pointed out, the results indicate that tumor architecture, in addition to genes and growth factor signaling, plays an important role in the emergence of drug-resistant tumors, information that may eventually help researchers in designing more effective anticancer agents.

Bissell is moving ahead with evermore ambitious plans. The goal is to develop increasingly complex models, and, ultimately, to incorporate other cell types "to literally make a complete model of the breast and breast tumors," she said. In recent work using epithelial and myoepithelial cells grown in a 3-D model, she and Olé Petersen, MD, PhD, of the Panum Institute in Copenhagen, have identified an important role for myoepithelial cells in normal and tumor cell growth (*J Cell Sci*. 2002;115:39-50).

Bissell stressed that 3-D models are needed for other kinds of tissue. "Ideally each organ and tissue will have its own model," she said.

"Of course, other tissues may be more complicated than the breast," conceded Bissell. "But our experience shows that it can be done, and can help shorten the time to get there." □

Gene Studies, Anti-TNF Therapy Take Lasker Honors

Mike Mitka

AN AMERICAN SCIENTIST WHO helped advance the understanding of gene expression and two British researchers who discovered treatment for rheumatoid arthritis and other autoimmune diseases were presented with this year's Lasker Awards for medical research.

Robert G. Roeder, PhD, of Rockefeller University, New York, won the 2003 Lasker Award for Basic Medical Research for his pioneering studies on eukaryotic RNA polymerases and on the cellular machinery for gene transcription, which led the way to biochemical analysis of gene expression in animal cells. Marc Feldmann, PhD, and Ravinder N. Maini, BCh, of the Kennedy Institute of Rheumatology at Imperial College, London, won the 2003 Lasker Award for Clinical Medical Research for their discovery of anti-tumor necrosis factor (TNF) therapy as an effective treatment for autoimmune diseases.

The Lasker Awards, given by the Albert and Mary Lasker Foundation and first presented in 1946, are sometimes called "America's Nobels." Sixty-six