Cancer's New Context

Old Concept or New, the Tumor Microenvironment Is Today's Hottest Idea in Cancer Research

Pathologists at New York-Presbyterian Hospital see lots of cancer slides—they screen 40,000 cases a year—but they'd never seen anything like the images that John Condeelis showed them. Condeelis, who is a cell biologist at Albert Einstein College of Medicine in the Bronx, had laser-illuminated videos of invasive breast cancer cells moving rapidly toward macrophages. These are white blood cells that normally engulf pathogens and cellular debris. The macrophages were perched on endothelial cells along a blood vessel and, as Condeelis explained, calling to the invasive tumor cells, sending out a chemical homing signal for each crawling cancer cell to follow and relay.

It was a chilling view of a deadly process—metastasis. It is the spread of cancer cells from a primary tumor into the blood or lymph system and then throughout the body, invading distant sites where they grow out as murderous secondary tumors. In cancer, it is metastasis that kills. Understanding exactly how cells migrate is one of the great quests of basic cell research, and bringing that knowledge to bear on cancer treatment one of the great hopes of clinical medicine.

The Condeelis cell videos of metastasis were illuminating, but slides are what pathologists know. Condeelis teamed up with pathologist Joan Jones at New York-Presbyterian Hospital to develop a tissue staining method for human samples to find sites on blood vessels where macrophages attract tumor cells. The result was triple-stained breast cancer samples on which were highlighted the three different cell types—tumor, macrophage, and endothelium. Where the three stained types overlapped, Condeelis believed that they formed a "tumor microenvironment for metastasis" (TMEM). It was this intersection of cell types—an anatomical compartment, Condeelis called it—that was driving metastasis. He predicted that the more TMEMs in a given sample, the greater the likelihood of metastasis.

Until fairly recently, the idea of metastatic cancer as the product of a "tumor microenvironment" would have been considered slightly flaky. Today, it is the hottest idea in cancer research. For decades, the dominant paradigm for cancer research focused on "oncogenes," genes key to controlling cell growth. When mutated or overexpressed, oncogenes helped otherwise normal cells go wild, multiplying without end and spreading without control. Oncogenes have been the focus for thousands of studies and the targets for hundreds of drugs. Some oncogene-directed therapies like the cell cycle–disrupting drug Gleevec have been effective, but their overall impact on the cancer death rate, especially for metastatic cancer, has been limited. Now tumor microenvironment theories are offering radical new approaches, especially for metastatic cancer.

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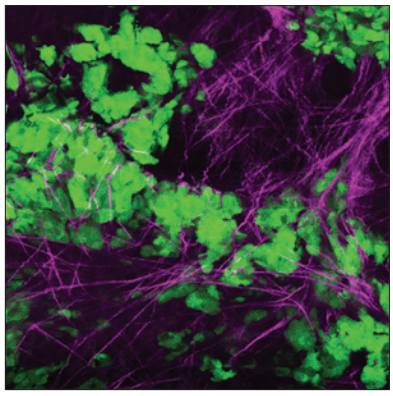
No Marker Close

Condeelis's experiment was one of the first limited trials of a tumor microenvironment–based test in a clinical setting. Once Jones and her team of pathologists learned to recognize the TMEM compartments, they prepared sets of triple-stained slides drawn from a breast cancer tissue bank. The pathologists were "blinded" as to which were from patients whose breast cancer had metastasized and which were from patients whose primary tumors had remained localized. The results of this limited experiment, published in April 2009, were startling. The number of TMEMs in a given sample area correlated with only one cancer dimension—metastasis. No other traditional marker came close to TMEM

density in predicting the metastatic potential of a tumor sample.

The TMEM test for metastatic breast cancer is now moving toward wider clinical trials. Elsewhere scientists are applying tumor microenvironment theory to other cancers such as melanoma and to reevaluating existing cancer treatments like tumor irradiation.

"It's almost trendy," says Richard Hynes of the Massachusetts Institute of Technology (MIT), who was one of the pioneers in the study of cell adhesion and the molecular "glues" that hold cells together and in place on the extracellular matrix (ECM). "But it's not a new idea, although it is now becoming more understandable in molecular terms," Hynes explains.



Deep inside a living breast tumor, green-labeled tumor cells move on red-emitting collagen fibers toward a blood vessel (black hole in upper right). Micrograph courtesy of Masen Sidani, Jeff Wyckoff, and John Condeelis, Albert Einstein College of Medicine.

The central concept, Bissell explains, is that "Phenotype can be dominant over genotype if conditions are right," that is, cancer cells with powerful oncogenes do not act like cancer cells unless they are surrounded by the "right" tumor environment of other cells, circulating growth factors, and cues from their immediate surroundings.

The term, "tumor microenvironment," may be five or so years old but the idea goes back to 1889 and the British surgeon Stephen Paget, who first proposed the "soil and seed" theory of cancer metastasis. Paget observed that primary tumor cells were like seeds let loose in the body that took root only in certain tissues. For example, primary breast cancer tumors typically spread to secondary sites in bone. Paget suggested that there was something in the bone environment—the soil—that attracted and nurtured the cancer seeds.

Paget's soil theory was pushed aside in the 20th century as knowledge of genetics and cell biology exploded. Researchers fastened on the seed, the tumor cell itself, looking for cancer's genetic causes in oncogenes. There were dissenters, among them Mina Bissell at the Lawrence Berkeley National Laboratory in Berkeley. "Everyone was working on the concept of single oncogenes in the '70s and '80s," Bissell recalls. "But the idea that single gene mutation alone could completely explain cancer, especially epithelial cancers, didn't make much sense to me."

Oncogene Dogma

In the 1980s, Bissell published a series of experiments that seemed to contradict the dogma that once an oncogene, always an oncogene. Bissell and her collaborators injected chicken embryos with Rous sarcoma virus (RSV), the first "oncovirus," discovered in 1911. It carries RNA coding for an aggressive cancer in chickens. Bissell's injected chicken embryos developed essentially cancer-free, despite expression of the active oncogene.

But when cells were isolated from embryonic tissues and grown in cell culture, they assumed a malignant behavior overnight. Something about the embryonic state prevented the oncogene from causing cancer. Bissell explains, "The architectural context, where the oncogene was placed, and the embryonic state were determining whether or not a tumor would form."

Bissell's RSV papers were met by an embarrassed but understandable silence, she recalls. Over time, Bissell expanded the notion of context, moving her work into mammalian systems to examine its relevance in breast cancer. She explored the three-dimensional context of both normal and cancer cells, pointing out the effects of cell geometry, signaling networks, the ECM attachment, adhesion, and inflammation.

Bissell says that in the mid-1990s the scientific tide began to turn. David Lyden and Shahin Rafii of Weill Cornell Medical College, for example, began talking about the microenvironment of metastatic cells and the premetastatic niche. Call it context or "tumor microenvironment," it is now a respectable and fundable idea in cancer research, says Bissell. "I'm delighted to tell you that the field has arrived," Bissell reports. "People are recognizing its importance now."

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Condeelis cheerfully admits to being a latecomer to tumor microenvironment. He came into the field from an unlikely direction—following the slime mold *Dictyostelium.* "Dicty" is a famous amoeba, valued in laboratories for its hardy unicellular existence and for its ability to act socially in times of peril.

Condeelis was studying how individual Dicty amoebae, in the face of starvation, follow a signal from a "founder" cell, migrating epic distances (for slime molds) to form a multicellular protective mass called a slug. Dicty does this by "relay chemotaxis," says Condeelis. Individuals home in on the founder's chemical signal, while reproducing it for relay to neighbors. Mass movement guided by a relayed signal reminded Condeelis of contexts beyond the slime mold world—the self-organization of the human embryo into specialized tissues and the movement of metastatic cancers.

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To see if the analogy was real, Condeelis developed an artificial blood vessel to capture metastatic tumor cells from mammary cancers. With these live metastatic cells in hand, Condeelis and colleagues Sumanta Goswami at Einstein, Paola Nistico at Rome's Regina Elena Cancer Institute, and Frank Gertler at MIT identified a subset of breast cancer cells that express a high level of a protein, Mena, that allows them to crawl vigorously. In metastasis, the crawling cancer cells headed for macrophages perched on top of endothelial cells along blood vessels. "The tumor cells were homing in on the macrophages, but it was the macrophages around the blood vessels who kicked off the whole process," Condeelis reports.

Cancer as Embryonic Recap

This suggests to Condeelis that cancer metastasis is a recapitulation of embryonic development: a highly motile cell interacting with surrounding cells in an elaborate epic of tissue remodeling. "The metastatic breast tumor is trying to make another breast. All of this is caused by the microenvironment," he says.

All of which also suggests to Condeelis that predicting the outcome of untargeted bench research is impossible. Something as seemingly obscure as Dicty signaling led him to something as clinically important as predicting cancer metastasis. "This brings the cell biology of signaling, motility, and classic embryology together at the bedside in a way that you could never have predicted," says Condeelis.

For Hynes, tumor microenvironment theory is a welcome new slant on the cancer equation. "It's been apparent for some time that the oncogenes didn't explain everything," Hynes explains. So far, tumor microenvironment theory hasn't made the explanation any simpler. Instead, it has brought in a host of new participants—normal cells like macrophages and platelets plus noncellular tissues like the ECM, growth factors, adhesion molecules, and microfactors. Suddenly all are players in the tumor microenvironment. Says Hynes, "It's a very complex cast of characters, but there's no point in pretending that it isn't."

Cancer is so complex that Hynes doesn't see any single theory leading to a single cure. Previous research breakthroughs on oncogenes or in blocking tumor blood supply led to drugs that worked up to a point, according to Hynes. Those drugs are being improved, he says. "But the [cancer] cells evolve. They get around the inhibition. What we need is a bigger armentarium so we can hit tumors with several things at once." A good clinical parallel is combination therapy that uses two or more drugs against resistant bacteria, parasite-borne diseases, or HIV. Hynes thinks that tumor microenvironment research will eventually yield new "druggable" targets that could be attacked at the same time as other cancer elements, say, oncogene proteins or inflammation factors.

"One has to be careful in science not to overpromise. People overpromised on the idea that under all the oncogenes would be the answer. It wasn't the whole story," warns Hynes. "I'm incredibly optimistic that this [tumor microenvironment] approach will be successful, but will it work right here and right now? No. Still, it's an incredibly exciting time to be a cell biologist." ■

—John Fleischman

"It's an incredibly exciting time to be a cell biologist," Hynes declares.