All in the Stroma: Cancer's Cosa Nostra

After focusing for decades on what happens within tumor cells to make them go wrong, biologists are turning to the tumor environment and finding a network of coconspirators

As several spectacular cases have shown, corporate criminals can operate for years, bending office systems to their needs and co-opting others into their nefarious deeds. Eventually, the malfeasance can threaten the entire company. So it is with cancer cells. Cancer biologists have recently been coming to grips with the fact that tumor cells get a lot of help from the cells around them. Such collusion is not the source of disease: More than 30 years of research have shown that mutations in a cell's own DNA initiate the changes that put it on its destructive path. But "people are realizing that the tumor environment is a coconspirator," says Zena Werb of the University of California, San Francisco (UCSF). "There's been a clear shift in interest."

A variety of cells in and around tumors help cancer cells survive, grow, and then spread to new locations where they seed metastases. Investigators are beginning to trace out the biochemical lines of communication that enable this aberrant behavior—information that could help drug developers devise new strategies for combating cancer. "People are excited about potential new [drug] targets in the tumor microenvironment," says Lynn Matrisian of Vanderbilt University School of Medicine in Nashville, Tennessee.

Although this work is still in its early stages, researchers have identified some key molecules in communication pathways that could serve as targets. These include some relatively unfamiliar characters as well as some old friends, such as the protein VEGF, which stimulates angiogenesis, the formation of the new blood vessels that tumors must acquire as they grow. Drugs that inhibit VEGF's action are already in use in the clinic. Their effects are relatively modest, but they do indicate that targeting the tumor environment has promise.

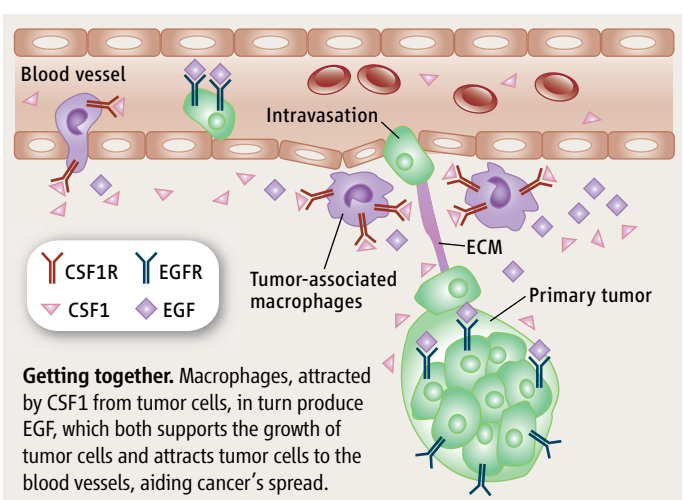
Trouble in the stroma

Researchers have known for many years that a tumor is more than a homogeneous mass of cancer cells. It incorporates several other cells, including fibroblasts, inflammatory immune cells such as macrophages, and the smooth muscle and endothelial cells of the blood vessels—all imbedded in an extracellular matrix that fibroblasts produce. Cancer researchers paid little attention to this tumor microenvironment, or stroma, until the mid- to late 1990s.

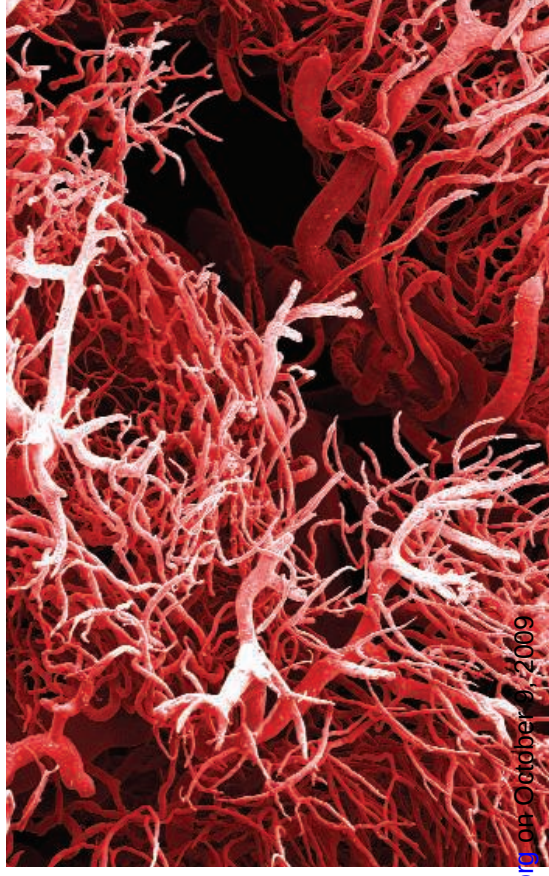
At the time, one of the few investigators systematically pursuing the question of how the tumor microenvironment influences cancer development was Mina Bissell of Lawrence Berkeley National Laboratory in Berkeley, California. Bissell's team got interested in cell surface proteins called integrins that help assemble organized tissues by forming contacts between cells and with the basement membrane. In 1997, Bissell and her colleagues reported that treating human breast cancer cells with an antibody directed at an integrin caused them to behave more like normal cells. In mice, for example, they formed fewer tumors than untreated cancer cells.

Conversely, antibodies directed against a different integrin could make normal cells behave like cancer cells. These results showed that simply disturbing cellular interactions, and thus tissue architecture, can dramatically alter cell behavior. Bissell says this is evidence for what she has long argued: "Structural integrity needs to be maintained for signaling to be maintained," she says. "When that doesn't happen, you get tumors."

Other research in the late 1990s implicated so-called tumor-associated fibroblasts (TAFs) as important coconspirators in the development of the common solid tumors, such as those of the breast, prostate, lung, and colon. These cancers originate in epithelial cells, which form the inner linings of the intestines and lungs and of the ductwork of the mammary and prostate glands. In 1999, Gerald Cunha and colleagues at UCSF showed that nonmalignant prostate epithelial cells grown in culture with prostate TAFs acquired the ability to form tumors when transplanted into mice. The researchers concluded that TAFs had undergone changes that resulted in their production

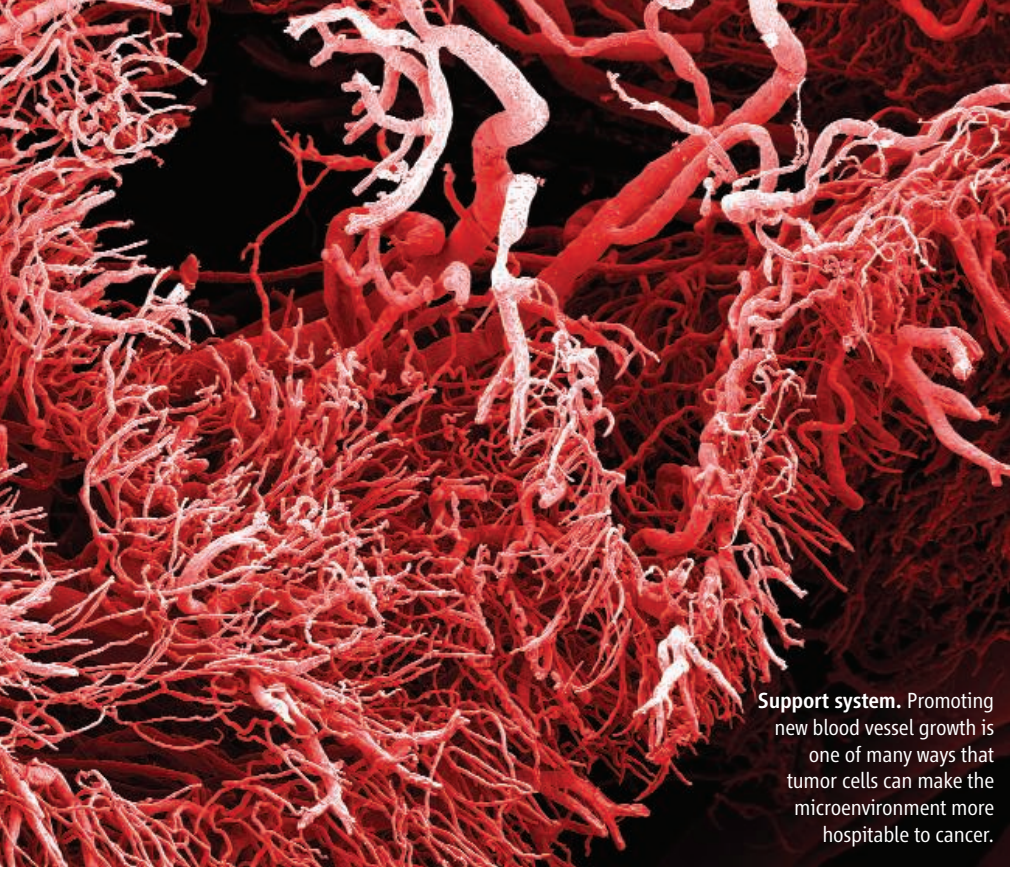


Getting together. Macrophages, attracted by CSF1 from tumor cells, in turn produce EGF, which both supports the growth of tumor cells and attracts tumor cells to the blood vessels, aiding cancer's spread.



CREDITS: CLOUDS HILL IMAGING LTD./PHOTO RESEARCHERS INC.; A. MORALES/*SCIENCE*; SOURCE: CONDEELIS *ET AL.*, *NATURE REVIEWS CANCER* 3 (2003)

Published by AAAS



Support system. Promoting new blood vessel growth is one of many ways that tumor cells can make the microenvironment more hospitable to cancer.

CREDIT: D. L. GORDEN *ET AL.*, *INTERNATIONAL JOURNAL OF CANCER* **121**, 495 (2007)

of growth factors or other substances that can make cells cancerous.

Since then, cancer biologists have been finding that essentially all components of the tumor stroma contribute to cancer's growth and spread. This includes the cells involved in forming the tumor blood vessels, the focus of pioneering work begun more than 2 decades ago by the late Judah Folkman. More recently, the role of macrophages and other inflammatory cells in promoting cancer has come in for a lot of attention (*Science*, 5 November 2004, p. 966).

Cancer stimuli

With the role of the microenvironment now well established, researchers are investigating how the various stromal components interact with cancer cells to promote growth and metastasis. "The question now is how do these things talk to each other," Werb says. Matrisian cautions, however, that answering that question won't be easy. "There's incredible complexity," she says. "For 35 years, we've been working on the tumor cells. Now we're adding five to six cell types."

One of the important communication molecules to emerge from this jumble is transforming growth factor-β (TGF-β), a protein best known as a suppressor of tumor growth. About 4 years ago, Harold Moses and colleagues at Vanderbilt University School of Medicine provided evidence that TGF-β doesn't have to act directly on cancer cells to inhibit their growth. As described in the 6 February 2004 issue of *Science* (p. 848), when the Vanderbilt team inactivated the receptor through which TGF-β exerts its effects in mouse fibroblasts, the animals developed early signs of prostate cancer and also more advanced invasive carcinomas of the stomach.

Turning to a different form of cancer, Moses and his colleagues transplanted mammary carcinoma cells, together with fibroblasts lacking the TGF-β receptor, into mice. Those animals, Moses says, "got more aggressive cancers and many more metastases" than when normal fibroblasts were used. The altered fibroblasts appear to stimulate cancer growth by producing transforming growth factor-α and hepatocyte growth factor. Loss of the ability to respond to TGF-β might therefore be one of the changes that cause fibroblasts to stimulate cancer growth.

The conspiracy hatched in the stroma does more than help cancer cells grow; it can also help them move—and metastasize. More than 20 years ago, a group of enzymes called the matrix metalloproteinases (MMPs) came in for a lot of attention as researchers found that some of them could help cancer cells spread by breaking down the extracellular matrix (ECM) and other barriers that would otherwise hold the cells in place. This early work culminated in clinical trials conducted primarily in the 1990s to test whether MMP inhibitors could extend life in human patients. But the trials "were spectacular failures," says Matrisian, an early MMP pioneer.

Now, however, MMPs have been identified as mediators of the communication between tumors and their microenvironment. Matrisian and others have found that MMPs are largely produced by various stromal cells rather than by the tumor cells themselves. The enzymes can appear early in tumor development and may contribute to tumor growth and spread in several ways.

About 4 years ago, for example, work by Douglas Hanahan's team at UCSF implicated MMP-9 produced by macrophages in the so-called angiogenic switch: the activation of the machinery that produces the blood vessel tumors need to grow and metastasize. Working with a mouse model of cervical cancer, the researchers found that macrophages in the tumors began producing the enzyme just at the time new blood vessels began to form. In addition, the drug zoledronic acid, a nonspecific MMP-9 suppressor, inhibited angiogenesis and slowed tumor growth. Later research suggests MMP-3 inhibition results in suppression of the pro-angiogenic protein VEGF.

Trojan horses. When carried in by MSCs, IFN-β inhibits the growth of metastatic tumors in lungs (top row), whereas the interferon alone has little or no effect (second row) as shown by comparison to untreated controls (third row). Normal lungs are in the bottom row.

The finding that MMPs can work early to promote tumor progression may help explain why inhibitors of the enzymes worked so poorly in clinical trials: Therapy may have come too late for these patients who had advanced disease.

The MMP situation is complicated, however; not all of the enzymes foster cancer development. Matrisian and her colleagues have found that stroma-derived MMP-12 actually protects against the development of non–small cell lung cancer. And even MMP-9 can be protective very early in the development of melanoma tumors in mice, says Raghu Kalluri of Harvard's Beth Israel Deaconess

Published by AAAS

Medical Center in Boston. "We're not just talking about positive influences on tumor growth," Kalluri says. "Some cancers can be held in check by the stroma."

More conspirators

Macrophages are apparently essential for the angiogenic switch. As Jeffrey Pollard and his colleagues at Albert Einstein College of Medicine in New York City reported in the 1 December 2006 issue of *Cancer Research*, the onset of the switch was greatly delayed in mouse mammary tumors that can't accumulate the cells. Indeed, in more than 40% of the animals with such tumors, the angiogenic switch had not been turned on by the time they were 16 weeks old; in all of the normal mice of that age, the tumors had progressed to advanced metastatic disease.

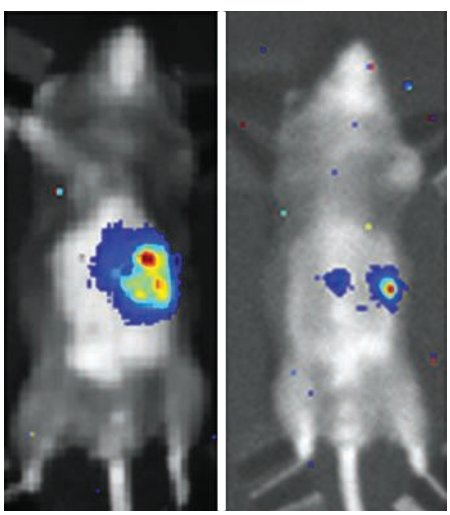
But macrophages and other inflammatory factors do more than just foment angiogenesis. They actively aid the cell movements that produce metastases. John Condeelis and his colleagues at Albert Einstein College of Medicine have devised methods that allow them to visualize cell movements in mammary tumors growing in live mice. Using these methods, the Condeelis team, working with Pollard's team, observed a few years ago that mammary tumor cells migrate very quickly along the fibers of ECM to blood vessels.

The Condeelis-Pollard team has found that tumor cells are called to the vessels by macrophages. The specific lure is epidermal growth factor, a protein produced by macrophages that can stimulate both the growth and the movement of cancer cells. More recently, the Condeelis-Pollard team showed that tumor cells escape into the blood vessels in direct association with macrophages. "They follow the macrophages like little trained dogs," is how Condeelis describes it. (The results appeared in the 15 March 2007 issue of *Cancer Research*.)

Macrophages are not alone in their ability to stimulate metastasis. Researchers have recently discovered that a group of immunosuppressive cells called MDSCs can promote cancer development (*Science*, 11 January, p. 154). Earlier this year, Moses and his colleagues found that these cells contribute to cancer spread. Inactivation of the gene for one of the receptors through which TGF-β exerts its effects in mouse mammary tumor cells resulted, they found, in an influx of MDSCs that ended up primarily at the invasive edges of the tumors.

Moses and his colleagues identified what they consider to be a trigger for the influx: increased production of two chemokines (SDF-1 and CXCL5) by the receptor-deficient mammary cancer cells. Drawn by the chemokines, MDSCs promote tumor metastases by producing at least three MMPs that stimulate the migration of cancer cells, presumably by digesting the extracellular matrix.

Several research groups have identified still another type of cell—the mesenchymal stem cell (MSCs)—as a prominent component of the tumor microenvironment. Last fall, a team led by Robert Weinberg of the Massachusetts Institute of Technology (MIT) in Cambridge reported evidence that these cells can also promote metastasis. The researchers injected mice with human breast cancer cells labeled with green fluorescent protein either with or without



Aiding cancer spread. Normal mice show much greater growth of liver metastases (*left*) than mice lacking the enzyme MMP-9.

MSCs. Mice given both cell types developed many more lung metastases—up to seven times more—than animals injected with only the cancer cells.

MSCs rev up the metastatic potential of the breast cancer cells by secreting the cytokine CCL5, which triggers a signaling pathway that sparks the cancer cells' migratory abilities. This change is not permanent, however. When the MIT team isolated cancer cells from lung metastases and injected them into new mice, the cells formed no more lung metastases than did the original cells injected without MSCs. "They're educated to be metastatic," Weinberg says. "But when they're moved, they forget that education." The discovery suggests that it might be possible to develop a therapy that blocks the metastatic changes.

There may be another way to enlist MSCs in the fight against cancer. Because the cells concentrate in tumors, researchers are trying to turn them into Trojan horses. "Tumors recruit these cells from the circulation," says Frank Marini of the University of Texas M. D. Anderson Cancer Center in Houston. "That means we do have access to the tumor" through MSCs. It may be possible to use them to deliver drugs or cancer-fighting cytokines.

For example, Marini, working with M. D. Anderson colleague Michael Andreeff, has genetically engineered MSCs to produce interferon-β. In mice carrying either melanoma or breast cancer tumors, the engineered cells proved much more effective at suppressing lung metastases and extending life than did simple injections of the interferon-β protein. Mice given the protein by itself lived no longer than controls, whereas those that received the cells lived roughly twice as long as the controls. Marini hopes to begin clinical trials of the engineered cells in a year.

It may even be possible to control cancer growth by targeting the stroma rather than the cancer cells themselves. Hans Schreiber's team at the University of Chicago in Illinois has been trying to develop immunotherapies but, like other investigators in that field, has often been thwarted by cancer cells' propensity for losing their antigens. When that happens, they can escape detection by immune cells that have been trained to recognize them.

About a year ago, Schreiber and his colleagues showed that by targeting stroma cells, they could eradicate well-established tumors in mice even though the tumor cells expressed little antigen. The researchers first treated the tumors with local radiation or chemotherapy. Although this won't eliminate the tumors, it apparently killed enough cells so that their antigens were picked up by the stroma. Subsequent injection of killer T cells finished off both the stroma and the tumor cells, which apparently succumbed to a "bystander effect."

In a paper out last month in *Cancer Research*, the Chicago team reported that immune cells directed against the stroma alone halt tumor growth, although in this case, the tumor cells weren't killed. "When you just target the stroma, tumors stay in long-term equilibrium—close to a year—without relapse," Schreiber says.

At this point, it's too early to tell whether strategies directed at the stroma will pay off in better cancer therapies. But evidence is building that it will be necessary to corral the entire cancer gang to truly get the cancer problem under control. **–JEAN MARX**

This article is Jean Marx's 610th in a 35-year career. Sadly, she has decided it will be her last as a staff writer.

CREDIT: GORDEN *ET AL.*, *INT J CANCER* 121, 3 (2007)