Feature Articles

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Corruption of the Tumor Microenvironment

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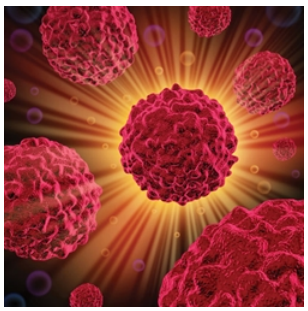

- Researchers are developing novel approaches to disrupt communication between tumor cells and surrounding cells in an attempt to halt tumor progression. [Freshidea/Fotolia]

Accumulating research suggests that the best possible treatments for cancer will not simply target and kill cancerous cells but will also work by making the neighboring cells—the so-called tumor microenvironment—behave more normally.

“Most people recognize that cancer is not just a ball of malignant cells. It’s a complex rogue organ,” says Frances Balkwill, Ph.D., professor of cancer biology at Barts Cancer Institute, Queen Mary University of London. “At least half the tumor isn’t made up of malignant cells at all. It’s all the other cells of the host, particularly immune system cells, fibroblasts, and blood vessel cells, that are recruited and often corrupted by the malignant cells to help the tumor itself grow and spread.”

Dr. Balkwill and her group study the role of tumor microenvironment signaling proteins—cytokines and chemokines—in human high-grade serous ovarian cancer. One of their targets is the cytokine receptor interleukin 6 (IL-6), a known tumor promoter.

“We’ve done one small clinical trial, and now we’re back in the lab trying to learn from that clinical trial about how we might use anti-IL-6 in combination with other cancer treatments,” explains Dr. Balkwill. “[We’re exploring the idea of] blocking cancer-related inflammation, which would help other treatments to work.”

Another target is the chemokine receptor CCR4. “When we use a small molecule or an antibody against CCR4 in mouse models, we find that it seems to switch macrophages from being tumor promoting to being tumor inhibiting, and it has anticancer effects,” notes Dr. Balkwill. This research, which Dr. Balkwill’s lab is carrying out in collaboration with Cancer Research UK and AstraZeneca, is studying the therapeutic potential of a small molecule CCR4 inhibitor. Dr. Balkwill anticipates that the compound will be evaluated next year in a Phase I trial in patients with advanced renal cancer.

Besides this work, Dr. Balkwill's group is also trying to build a completely human tumor model. "We are deconstructing the tumor microenvironment of peritoneal metastasis of high-grade serous ovarian cancer," explains Dr. Balkwill. "We are trying to rebuild as much as we can of that microenvironment in a way that uses stem cell biology, tissue engineering, and biomechanics in a multidisciplinary project."

- **Dormant Disseminated Tumor Cells**

Disseminated tumor cells are cells that travel away from the primary tumor and settle in secondary organs. Whether disseminated tumor cells metastasize or remain dormant may depend on the microenvironment of secondary organs.

The influence of the microenvironment on disseminated tumor cells is being studied by a research team led by Julio Aguirre-Ghiso, Ph.D., professor of otolaryngology and medicine, hematology, and medical oncology at Icahn School of Medicine at Mount Sinai. In one study, Dr. Aguirre-Ghiso and his team compared disseminated tumor cells from lung, a common site for metastasis, and from bone marrow, a tissue where disseminated cancer cells often remain dormant before growing or never metastasize.

"When cells arrive at the bone marrow, they see more TGF-beta2 [transforming growth factor-beta2] and respond to the signal and start making their own TGF-beta2 and sustain this quiescent or dormant phenotype," says Dr. Aguirre-Ghiso. "In the lung, because there is less TGF-beta2 (and more TGF-beta1), they switch to a proliferative mode."

"We discovered that the type 3 receptor for TGF-beta was important to funnel the signal," continues Dr. Aguirre-Ghiso. "If you don't have that receptor, the cells will not become dormant." Silencing of this receptor in breast and prostate cancer correlates with more metastasis.

Dr. Aguirre-Ghiso and his team also identified an orphan receptor in the retinoic acid signaling cascade—nuclear receptor subfamily 2, group F, member 1 (NR2F1)—as a potential dormancy signal. "Perhaps TGF-beta2 plus retinoic acid signaling in the bone marrow is inducing this whole dormancy program," speculates Dr. Aguirre-Ghiso.

In another study, Dr. Aguirre-Ghiso joined Colm Morrissey, Ph.D. a research assistant professor at the University of Washington, to scrutinize expression profiles of bone marrow single disseminated tumor cells from prostate cancer patients. They compared profiles from patients evidencing metastasis to profiles from patients who have been free of the disease for many years, sometimes more than a decade.

"We found that the cancer cells that came from patients that had no evidence of disease for these very long periods carry more of our dormancy gene signatures than the ones that have active disease," asserts Dr. Aguirre-Ghiso. "It is the first time that we found that our markers from experimental models are really correlating with human patients."

- **Inflammatory Chemokines**

For metastasis to occur, the tumor has to activate its microenvironment—particularly nearby blood

vessels. This process appears to involve chemokines, including CCL2 and CCL5, according to researchers led by Lubor Borsig, Ph.D., senior associate scientist in the Institute of Physiology at the University of Zürich.

“An increase in chemokines occurs during the original metastatic seeding,” says Dr. Borsig. “Chemokines not only recruit leukocytes or inflammatory monocytes, they also act as signaling molecules for activation of the endothelium.”

According to Dr. Borsig, the interaction of chemokines with their receptors on endothelium cells makes the vasculature more permeable, paving the way for possible metastasis. “The first initiation step for this externalization, leaving the blood vessels and getting to the tissues, can be significantly promoted by chemokine-regulated recruitment of monocytes and activation of the local epithelium and distant organs.”

At present, Dr. Borsig’s lab is using chemokine knock-out mouse models to examine the crosstalk between different types of chemokines. “There are inflammatory chemokines that are always upregulated when there is some kind of inflammation or even tumor growth or metastasis,” explains Dr. Borsig. “We have evidence that they have complementary functions, which can modulate not only recruitment but also activation of cells in the environment. There seems to be some type of specificity for their pro-tumorigenic activity.”

As Dr. Borsig notes, chemokine inhibitors have promise as cancer therapeutics, but understanding how chemokines work in animal models is key to furthering clinical research: “It is a matter of providing convincing evidence from a number of different animal models that targeting these molecules would really benefit patients.”

- **Regulating Metastasis**

“Half the secret of life is outside the cell. That’s true for cancer. It’s true for the issue of metastasis,” says Zena Werb, Ph.D., professor of anatomy at the University of California, San Francisco School of Medicine. Dr. Werb and her lab study microenvironment changes that set the stage for metastasis.

One protein of interest for the group is the transcription factor GATA3. “It is the transcription factor that is the master regulator of luminal differentiation in the breast,” explains Dr. Werb. “It is the third most mutated gene in breast cancer.” Additionally, patients with the so-called triple-negative form of breast cancer who have a poor disease prognosis have very low expression of GATA3.

“If you take human or mouse breast cancers that have little or no GATA3 and you put it back in, it’s very clear that you inhibit metastasis,” asserts Dr. Werb. According to Dr. Werb, reintroducing GATA3 causes tumor cells to differentiate and become more like normal epithelial cells. “But the other thing that is happening is that you are turning off the production of a whole group of genes—like vascular endothelial growth factor, angiopoietin-like 4, a bunch of collagens, lysyl oxidase—molecules that are independently pro-metastastic.”

The lab found that expression of the microRNA miR129 is initiated by GATA3. “If you put just this microRNA back, you suppress a whole family of more than 30 genes,” notes Dr. Werb. “You have

one molecule affecting essentially a node of function. It turns out it is affecting the microenvironment."

These findings appear to be relevant to cancer treatment. "If you can make the microenvironment more normal, then the tumor cells will follow suit. [This approach] is advantageous because you don't have to change every cell," asserts Dr. Werb. "Everyone thinks about getting rid of their cancer, but having cancer stop behaving like cancer is okay as well."

- **Highly Multiplexed Tumor Imaging**

"I wanted to have an imaging method that actually visualizes the cell within its microenvironment," recalls Charlotte Giesen, Ph.D., scientist and Branco Weiss Fellow at the Institute of Molecular Life Sciences at the University of Zürich, where she works in the laboratory of Bernd Bodenmiller, Ph.D. "The current treatment in cancer focuses on the cancer cells, but the tumor microenvironment drives many hallmarks in cancer and is therefore as important if not even more important."

According to a release issued by Dr. Bodenmiller's laboratory, determining a tumor's cell profile, its neighborhood relationships, and the circuit structure within and in between cells is a highly complex endeavor because the biomarkers—that is, the specific molecules of the various cell types and their circuits—have to be measured in their spatial relationships.

While working in Dr. Bodenmiller's laboratory and collaborating with ETH Zürich analytical chemists Hao A.O. Wang, Ph.D., and Detlef Günther, Ph.D., Dr. Giesen developed a new imaging method that can simultaneously visualize multiple biomarkers in a single sample at a subcellular resolution. "We are currently able to record up to 32 biomarkers simultaneously," asserts Dr. Giesen. "In the near future, we will have 100 biomarkers available."

The technique is based on immunohistochemistry. "We use metal isotopes, which are used as a tag on the antibodies," explains Dr. Giesen. "The metal tags are detected by a mass cytometer." The CyTOF mass cytometer is combined with a laser-based imaging setup that has a resolution of one micrometer.

Dr. Giesen and Dr. Wang used this technique to examine biomarker expression in human breast cancer tissue. They found that some tumors suffer from internal oxygen deficiencies whereas others misuse the body's immune cells to drive their growth. "Such findings had been described in the literature," continues Dr. Giesen. "In our work, however, we were able to see all that simultaneously without having to go protein by protein. We learned a lot about the individual tumors and the individual patients that may not have been obvious during the regular diagnosis."

Dr. Giesen is now using the technique to study cellular communication and metastasis formation in hopes of identifying predictive biomarker patterns. Down the line, the technique could be used for diagnostics and for determining the weak points in cancer signaling networks that could be drug targets.