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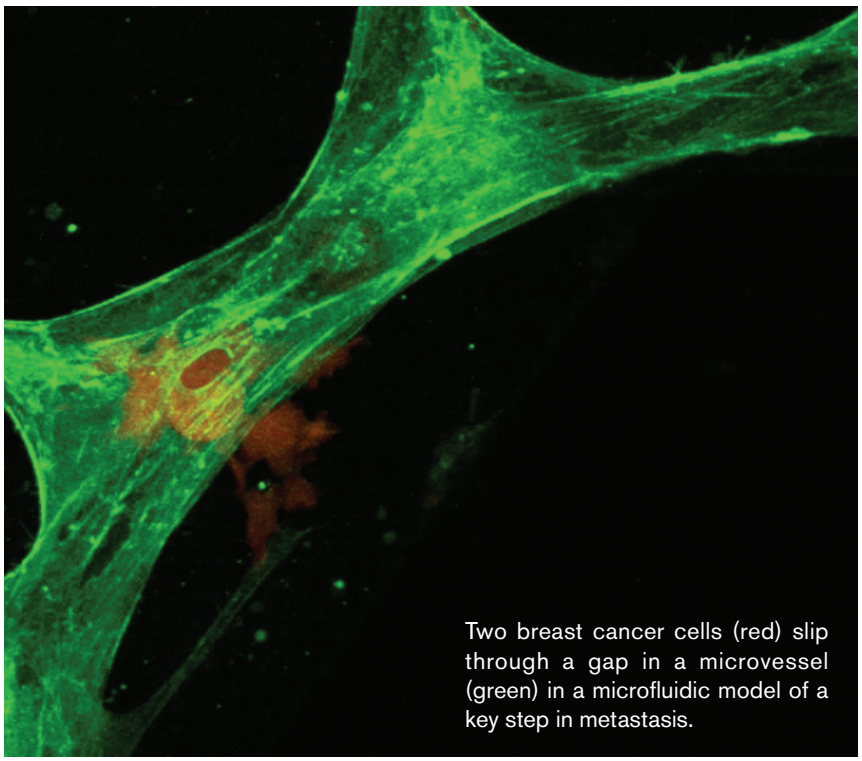

Two breast cancer cells (red) slip through a gap in a microvessel (green) in a microfluidic model of a key step in metastasis.

Tiny Channels, Giant Dreams

Microfluidic devices flow to the fore as a promising way to view and study cancer cells in 3 dimensions

The signs of cancer may appear throughout a cell and range from an enlarged nucleus and extensive ripples in the nuclear envelope to a more deformable outer membrane and increased motility. Existing imaging systems have struggled to pick up such features at a high resolution, however, let alone in 3 dimensions.

A flood of new research in the field of microfluidics, which seeks to control and manipulate the flow of liquids within tiny channels, is aimed at bypassing current limitations in visualizing and characterizing cancer cells. The hope is that precisely designed microtubules and channels will be able to more closely simulate the body's various environments while giving researchers a clearer view of how cancer cells move and behave. The new information, researchers say, could aid the science of early detection, cancer cell analysis, therapy monitoring, and drug discovery.

A New Tool

Alan Nelson, PhD, founder, chairman, and chief executive officer of Phoenix-based biotech company VisionGate, says that the 3-dimensional world of cells is often largely hidden from pathologists. VisionGate is among the companies and research laboratories betting that a more realistic and high-resolution view of cancer cells and their mechanical properties might be achievable through a combination of microfluidics and computer algorithms. "We really have a completely new kind of tool," Dr. Nelson says. "We think we're going to change pathology forever."

For its initial target, VisionGate has picked lung cancer. The company's strategy for early detection is a noninvasive test that picks out rare cancer cells within a patient's sputum. The detection device is a fiber optic tube measuring 67 µm in diameter (only slightly bigger than a cell). "That's what we squirt the cells through," Dr. Nelson says. "We rotate the tube, and the viscosity of the fluid causes the cells inside the tube to also rotate." Each cell, in other words, spirals through the microfluidic tube like a corkscrew on its way past an imaging system that captures 500 images of it over a full 360-degree rotation. The resulting images go into a computer algorithm that reassembles the 3-dimensional cell at a high resolution.

The scanner, dubbed Cell-CT, also enables 590 structural biomarker measurements of each cell. As an example of its power, Dr. Nelson says that the technique can reveal important cancer features such as chromosomal breaks or crossovers and an invagination on the inner surface of the nuclear membrane that "actually looks like cauliflower on the inside of the nucleus." In 2015, VisionGate pegged its system's lung cancer detection specificity at 99.9% and sensitivity at 95.4%, and Dr. Nelson says that the advanced 3-dimensional optics and measurement capabilities could lead to automated lung cancer diagnoses.[1]

AIM Biotech, a Singapore-based spinoff of the Massachusetts Institute of Technology, has also jumped into the microfluidics space. The company is developing a hydrogel matrix-filled device that permits multiple cell types to grow and interact with one another within a more realistic 3-dimensional environment and to be readily accessible for imaging.

Because 90% of all cancer deaths are related to metastasis, AIM Biotech is applying its model to exploring each step in the complex process that begins with cells breaking free from a primary tumor and ends with a new metastatic tumor forming elsewhere in the body. The microfluidic device enabling these investigations consists of a relatively straightforward series of channels of various depths.

In one of the simplest applications, endothelial cells seeded into a channel grow into a confluent monolayer

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and take the form of a blood vessel. In contrast to most animal models, imaging these cells is easily done, says AIM Biotech cofounder Roger Kamm, PhD, professor of mechanical engineering and biological engineering at the Massachusetts Institute of Technology. "Typically, we use confocal fluorescence microscopy so that we can do time-lapse imaging and we can actually see what's going on over time," he says.

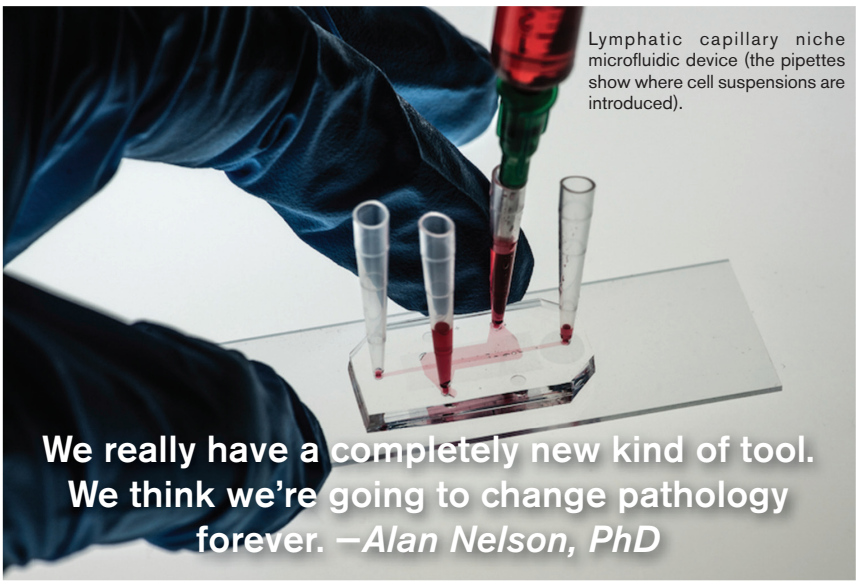
In its tumor modeling work, AIM Biotech has introduced tumor cell aggregates that simulate a primary tumor into its microfluidic device and then observed how the tumor cells break away from the primary tumor as the first step in metastasis. The company has since published the results of experiments on various drugs that might prevent that step, which is known as the epithelial–mesenchymal transition.

Slithering, Stretching, and Whirling Cells

Sofia D. Merajver, MD, PhD, scientific director of the Breast Oncology Program at the University of Michigan's Comprehensive Cancer Center in Ann Arbor, is likewise focused on metastasis. One microfluidic device created by Dr. Merajver and colleagues, called a lymphatic capillary niche, contains strictures that limit the movement of cells. Her laboratory's previous experiments in mice suggest that curbing the activity of a signaling protein called p38γ interferes with cancer cell motility. When the researchers forced cancer cells through the microfluidic device's strictures, they found that highly metastatic ones were able to "slither through," whereas those that got stuck had reduced p38γ expression that limited the cell's ability to deform its cytoskeleton. "It's very specific knowledge that you wouldn't be able to ever have without a device," she says.

A related device contains multiple channels, with cancer cells loaded at one end and a variety of environmental stimulants and factors at the other. With the long channels, the researchers can measure how fast and how far the cancer cells move in response to the various stimuli and even recover them for further study.

An even more complex microfluidic device called a brain niche simulates the blood-brain barrier and is providing Dr. Merajver with new clues about how some metastatic cancer cells breach the barrier and infiltrate the brain. "There is nothing more terrible that you will tell a patient of any type of cancer than, 'You have brain metastasis.' That's the worst day," she says. The device has already suggested how cancer cells "throw a leg" between 2 endothelial cells in the first layer of the blood-brain barrier to pry open an entryway and disrupt the barrier's electric gradient; this is akin to creating a weak spot in an electric fence and allowing other cells to slip through. Eventually, she hopes to use her device to study patients' primary breast tumors upon diagnosis. "If any of the cells go through our device, we'd like to recover them and find out how to kill them," she says.



Lymphatic capillary niche microfluidic device (the pipettes show where cell suspensions are introduced).

Greater deformability has been linked to cancer cells; it potentially allows them to more easily slide through tissue and invade other sites, but the correlation has lacked solid evidence. Dino Di Carlo, PhD, director of the Cancer Nanotechnology Program at the University of California Los Angeles, is measuring the deformability of thousands of cells per second by stretching them with fluid as they pass through microchannels. The deformation of each can then be imaged and measured via a high-speed camera system that captures approximately 600,000 frames per second.

"That allows us to increase the throughput orders of magnitude beyond what was done previously," he says. "So I think that's allowing us to actually think about mechanics as a practical measure now in cytopathology type of investigations." On the basis of deformability measures, Dr. Di Carlo has confirmed that mesothelioma cells are more deformable than normally stiffer mesothelial cells. The same generality holds for other cancer cells: "They would all be slightly different sizes and of slightly different deformability depending on the type of cancer, but in general they appear to be more deformable."

A separate microfluidic technology sorts out desired cells by creating tiny whirlpools in a microchannel that trap those larger than a certain size. "So for example, we accumulate circulating tumor cells from blood while red blood cells and white blood cells pass into the vortices and then pass out," Dr. Di Carlo says. Once the sorting period is over, scientists can lower the flow rate to dissipate the vortices and aggregate the tumor cells for DNA sequencing or downstream cytopathology tests. "The important point is that the downstream purity becomes enriched by a million-fold or so because there are so many white blood cells that interfere with assays," Dr. Di Carlo says.

It is yet another example of how a field focused on tiny channels and tubes may soon play an outsized role in helping clinicians better understand and target cancer. ■

Reference

1. Wilbur DC, Meyer MG, Presley C, et al. Automated 3-dimensional morphologic analysis of sputum specimens for lung cancer detection: performance characteristics support use in lung cancer screening. *Cancer Cytopathol.* 2015;123:548–556.

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