THE DEPTHS OF CELL MIGRATION

A growing number of researchers are trying to tease out the cellular mechanisms of tumor metastasis. Sarah Webb examines the tools they are using to probe the mercurial movements of cancer cells.

Image Credit: Jeff C. Hsu and Kenneth M. Yamada.

For decades, researchers have crafted a variety of exquisite techniques aimed at teasing apart the complicated biology of cell migration. Their quest to study the forces that spur cells to break free, move within an organism, and invade a new tissue has been motivated by the knowledge that cell migration supports many biological processes, including development, immune processes, and disease pathology.

Cancer cell migration, however, presents unique challenges. Tumors are heterogeneous masses with just a small percentage of cells capable of acquiring migratory characteristics and moving into new tissues. Metastatic cells display mercurial migration patterns, swapping strategies based on microenvironments even as chemical and physical factors play off of each other.

Here is where researchers face critical choices when studying migration. On one hand, they often need simplified *in vitro* systems to test specific hypotheses and parse out motility mechanisms. At the same time though, they want to understand those mechanisms within the context of tissues and living organisms. These conflicting motivations have driven more and more researchers towards the use of three-dimensional (3-D) model systems and intravital imaging (microscopic imaging of living tissue) techniques in recent years. But with the convergence and contradictions

that can come up within these approaches, researchers also need to figure out how to standardize data and integrate diverse experimental data sets.

3-D modeling

Until very recently much of our current knowledge about biological and biochemical mechanisms in cells came from 2-D experiments—cells grown on slides or plates—according to Kenneth Yamada of the National Institutes of Health's National Institute of Dental and Craniofacial Research.

But the molecular mechanisms observed in 2-D haven't always provided answers that are fully consistent with the migratory behavior observed in tissues. For example, classical 2-D migration experiments show that the proteins Rac, Cdc42, and PIP₃ facilitate actin polymerization at the leading edges of polarized cells. But when researchers examine cell migration in either a 3-D cell-derived matrix system or an explanted organ system from a mouse's ear, migration occurs quickly, but not through this sort of polarized signaling, Yamada says. "It's become quite clear that a significant percentage of the conclusions in 2-D may not apply in specific 3-D systems." Signaling is a striking difference, he notes, but there are also differences in cell shape and in the biophysical mechanisms of cell migration.

Tracking migration in 3-D. Small changes in cell locations due to movement can be tracked using video imaging. Here in the upper left corner, changes in cell location lead to changes in color patterns (green). Credit: K. Yamada.

This doesn't mean that data from 2-D and even one-dimensional (1-D) systems aren't important. 1-D systems can characterize the way cells move along fibers, and migration along collagen fibers is one potential mechanism by which tumor cells infiltrate other tissues. Cells can use a variety of migration strategies, and those that show up in threadlike, flat or 3-D scenarios can all play roles in tumor metastasis (1).

Still, according to Peter Friedl of the Radboud Institute for Molecular Sciences in Nijmegen, The Netherlands, the development of 3-D models has been critical to the study of cell migration over the last decade. These sophisticated models allow researchers to control factors such as scaffold porosity and stiffness that may be critical for cell movement. Researchers can choose the ligands, cells, and conditions that are relevant to the question that they hope to address. In fact, Yamada's group recently used 3-D culture to discover an entirely new migration mechanism in normal and tumor cells where the cell propels itself forward using the nucleus like a piston (2).

Today's 3-D culture systems evolved from the collagen gels of the 1980s. More recently Matrigel—a tumor extract designed for growing epithelial cells and tumor cells—has proved a popular choice for studying epithelial biology. Now a range of support materials—from biomaterials to a variety of synthetic polymers—are used in 3-D models for studying cell migration, Yamada says. Depending on the problem that researchers wish to study, they can add in adhesion promoting peptides, growth factors, or other agents that can make a 3-D in vitro culture system look more like a living organism.

"In vitro environments allow us to test environments that we think are right and get results that show us those possibilities," says Friedl. For example, by looking at a culture in a matrix with pores of a defined size, researchers can see whether the size of the pores relative to the size of a cell nucleus limit whether a cell can migrate, he says. "It's a way to decide 'does this matter?'"

Microfluidic flow

Microfluidic devices and sandwich assays are another important way to test migratory hypotheses. In addition to providing a 3-D space, these devices allow researchers to examine the

effect of fluid flows, compression, and stretching with substrates that are deformable.

Mingming Wu is a bioengineer at Cornell University who has long been interested in microfluidics. Eight years ago, her lab developed an agarose gel microfluidic device that, during a sabbatical with Melody Swartz (now at the University of Chicago) jump-started her into studying cancer cell migration. "If you want to observe how fish swim, it's better to put them in a fish tank than to look at them in the ocean," she says when describing the importance of microfluidics.

According to Wu, microfluidics overcomes some of the disadvantages of the classical Boyden chambers so often used to assess migration. Boyden chambers are simple to use, allowing researchers to observe how groups of cells respond to a chemical signal. But that is limiting when it comes to cancer cells. "You don't see single cell behavior [with a Boyden chamber], which is very important for cancer." Microfluidic devices on the other hand provide spaces that are closer to the size of cells with the opportunity to control the environment, including the flow of liquids and chemicals through tiny channels.

Initially, Wu and her colleagues wanted to test chemokine gradients to understand how those external cues affected breast cancer cells in lymph node tissue. But along the way, they discovered that liquid flowing around the cells could actually change their migratory behavior. In the presence of a fluid flow, cells crawled in an amoeboid pattern, but without flow they showed a polarized, leading edge migration pattern. As a result, Wu says, her research group is now focusing on physical factors, such as flow and matrix mechanics, at work in cell migration. (Learn more about microfluidic devices for studying cancer metastasis in the March Technology Feature "Designing a Microscale Lab.")

Move to mammals

Over the last decade, studies of cell migration have moved from basic mechanisms toward disease pathology. "People have become more and more interested in mammalian model systems, specifically the mouse, and in trying to model disease states more directly," notes Andrew Ewald of Johns Hopkins University.

Cell migration experiments with transparent organisms such as the amoeba *Dictyostelium discoideum*, the nematode *Caenorhabditis elegans*, and the zebrafish *Danio rerio* have contributed important fundamental insights about in vivo cell migration, notes Friedl. But to look at mammalian tissues, researchers had to get a little more creative. “The basic challenge is the optical and experimental inaccessibility of mammalian models,” Ewald says. Because mouse tissue is not transparent, visible light penetrates only up to 100 micrometers below the surface. And although there are great mouse models of disease, it can be expensive and time consuming to produce a sufficient number of mice for in vivo studies.

One way to get around this problem is 3-D culture using tissue explants, a strategy Ewald’s laboratory relies on. In their studies of cell migration in mammary tissue, they isolate epithelial tissue from a mammary gland and explant whole pieces, each containing a few hundred cells, into culture. “We’re explanting what we call a tissue organoid,” Ewald says. “We try to manipulate it as minimally as possible to get it in to culture.”

Despite the challenges that come with in vivo work, an increasing number of groups are pursuing studies of cell migration in mice using a suite of techniques broadly known as intravital imaging. The basis of the technique relies on transgenic mice and a toolkit of fluorescent reporters to highlight tumor cells which can then be tracked using microscopy.

To peer inside the mice, researchers use imaging windows, transparent portals surgically implanted in mouse tissue. Such windows have long existed for studying the brain, where the skull provides the necessary structural support, says Jacco van Rheenen of the Hubrecht Institute in Utrecht, The Netherlands. But now there are window chambers that researchers can implant in the dorsal skin fold, near the mammary glands, and in the abdomen (3). With multiphoton microscopy, which allows researchers to combine multiple doses of longer wavelength, deeper penetrating infrared and ultra-infrared light to activate reporters, researchers can approach millimeter imaging depths within mouse tissues.

Fifteen years ago, van Rheenen started out working on cancer cell migration using in vitro models. But following a postdoc with John Condeelis at Albert Einstein College of Medicine, he is now focused on in vivo work in mice. Individual cells can accumulate a variety of DNA mutations, which produces

genetic heterogeneity in tumors. Cells on one side of the tumor could be vastly different from those on the other side, he says. The microenvironment of an individual cell—such as whether it’s sitting next to a macrophage or not—can change its behavior.

In a 2012 paper (4), van Rheenen and his colleagues developed the abdominal imaging window and used it to follow the growth of micrometastases from cells that traveled to the liver. Through the window, they were able to follow cells as they escaped from the primary tumor and look at their behavior as they invaded healthy tissues. As these cells arrived in the liver, they proliferated and formed a clone that retained its migratory behavior and remained isolated from other tumor cells. In clones where migration was inhibited, those cell groups did not mature to micrometastases, suggesting a new way to target this process.

Data sensibilities

With multiple chemical and physical factors contributing to cell migration and the numerous methods used to study them, cell migration researchers can grapple with an overwhelming amount of data. Cell migration is a plastic process defined by multiple inputs and modules such as adhesion, contractility, stiffness, membrane fluidity, ion channel activity, proteolytic activity, and the environment, explains Friedl. “Ideally they have to be monitored in context to get the whole picture.”

The challenge is combining and interpreting the results from in vitro and in vivo studies. “There’s a tendency to publish in vivo data because it’s more interesting,” Friedl says. But prioritizing in vivo findings can pose a risk that researchers have overlooked other complications. Some studies, particularly in vivo ones, rely on data from relatively few cells. Friedl notes that it can be tempting to focus on the single video confirming a hypothesis and ignore everything else. “In vitro, I don’t see this as a challenge anymore,” he says.

Even with the tension between the two, in vitro and in vivo modeling clearly rely on each other. “I don’t know a single in vivo imaging lab that does not do in vitro work. And I see more and more in vitro labs working on cell migration that feel the urge to try in vivo,” Friedl says. In vivo work is expensive, which leads researchers to rely on collaborations and consortiums to support the expensive infrastructure.

And data standardization is another challenge for a field that Erik Sahai of the Francis Crick Institute in London says needs to become more quantitative.

Peter Friedl says that for cell migration studies, in vivo and in vitro experiments can be used to inform biology. Credit: Radboudumc, Nijmegen.

TAKE ACTION AGAINST CONTAMINATION WITH QMI® SAFE-SEPTUM

Sample, Inoculate Or Add Nutrients
To Your Bioreactor Aseptically !

The QMI Safe-Septum is:

- Aseptic
- Pressure & Temperature Safe
- Pre-Sterilized
- Easy To Retrofit
- Validated

651-501-2337

Email: info@qmisystems.com

www.qmisystems.com

Mingming Wu, a Cornell bioengineer, has a long interest in developing microfluidic solutions.
Credit: M. Wu.

"There are so many different migration assays, and different people get different answers from roughly the same experiment, and it makes your head hurt. I think good quantification, both of the extent of cell migration, but also exactly what the assay setup was will be helpful for unpicking some of the difficulties in discrepancies."

Friedl is quick to emphasize the wealth of existing primary data—movies and other cell migration information—sitting on hard drives around the globe. He and Sahai were co-authors on a recent paper (5) proposing an open-access cell migration database. "People want to share", he says, "[and] we must find smart ways to use secondary data."

References

1. **Friedl, P. and Wolf, K.** 2009. Plasticity of cell migration: a multiscale tuning model, "J. Cell. Biol. 188:11-19.
2. **Petrei, R.J. et al.** 2012. Nonpolarized signaling reveals two distinct modes of 3-D cell migration. J. Cell. Biol. 197:439-55.
3. **Ellenbroek, S. and van Rheenen, J.** 2014. Imaging hallmarks of cancer in living mice. Nature Reviews Cancer. 14:406-418.
4. **Ritsma, L. et al.** 2012. Intravital microscopy through an abdominal imaging window reveals a pre-micrometastasis stage during live metastasis. Sci. Trans Med. 4:1-11.
5. **Masuzzo P. et al.** 2015. An open data ecosystem for cell migration. Trends in Cell Biol. 25:55-58.

Written by Sarah Webb, Ph.D.

BioTechniques 59:8-12 (July 2015)
doi: 10.2144/000114305

Cell migration in silico

Cell migration has long interested computational biologists and mathematical modelers. But now computational models are becoming increasingly important for researchers who are studying these processes experimentally. "Many migration papers today come with a model," Friedl says. The fields of mathematics and migration analysis are merging.

Sahai and his colleagues have used computational work to simulate the 3-D environment of cells and how they affect the organization and movement of cells. In a computer simulation, they

can perfectly control the organization of the matrix, the adhesiveness, or other factors and explore how those play out within the computational scenario. That highly controlled system allows them to generate new hypotheses and build new experiments to test them.

Computational models also provide a way to explore seemingly contradictory results from different assays. "So essentially, it's a great intellectual playground for refining ideas and exploring hypothesis, and asking the, 'what if,' questions," Sahai says. --S.W.