Fighting Cancer in 3-D

And what sponges, scissors, fat and ovulation have to do with it

From the Île de la Cité, it’s just a short stroll across the Pont Saint-Michel to the Left Bank and up the sycamore-lined Boul’Mich to the academic heart of Paris with its famous universities.

There, on a mild spring day in 2012, University of Michigan researcher Stephen J. Weiss, M.D., made his way down Rue de l’École de Médecine to give a lecture at a satellite session of the 19th European Congress on Obesity.

The theme of the meeting was fat cells, including the latest scientific developments in the extracellular matrix — the connective tissue that surrounds fat cells and other types of cells throughout the body, lending both structure and support.

And this is what made Weiss’ attendance a bit peculiar. He studies cancer.

“Because I don’t work in fat, nobody there really knew who I was,” recalls Weiss, a faculty member at U-M’s Life Sciences Institute, where his lab is located, and associate director for basic science research at the U-M Comprehensive Cancer Center. “Someone came up to me afterward and said, ‘You don’t know how transformative that paper you published has been for the field.’” (The paper in question, which was published in Cell in 2006, has been cited more than 200 times in journals as diverse as Diabetes, Developmental Cell and Facial Plastic Surgery.)

But to understand how a cancer researcher from U-M wound up at a fat conference in Paris, we have to first talk about scissors — and to do that we need to talk about a desiccated, yellow kitchen sponge that Weiss keeps in a drawer in his office.

“They’re synthetic now, but the sponge that you buy at the store used to be the extracellular matrix of an organism called sponge,” says Weiss, holding up the prop he keeps for explaining his work to...
nonscientists. “They’re tough — the reason you could scrub with them is because everything is cross-linked and held together really well.”

Weiss’ primary focus is on metastasis — not the genetic flaws that give rise to cancer nor tumor growth, but its deadly ability to migrate to far-flung and vital parts of the body. Healthy cells migrate only under special circumstances, such as in the early development of an embryo or when new skin cells and blood vessels move in to repair a wound.

“So we have a few of these controlled invasion programs in normal growth and development,” says Weiss, who is also the E. Gifford and Love Barnett Upjohn Professor of Internal Medicine and Oncology at the U-M Medical School. “And the cancer cell, basically, mobilizes the same kind of invasion program; it’s just not carefully regulated. In my lab, we actually spend about half of our time trying to understand how normal cells invade tissues in order to gain insights into how cancer cells invade.”

For a cancer cell to gain access to the body’s major highways — the blood vessels and lymphatic system — it has to invade through something. And what it invades through is that sponge-like extracellular matrix, which is predominantly made up of collagen.

Traditionally, scientists who want to study cells will culture them in flat, shallow dishes, an approach that dates back to the late 1800s. But this standard, two-dimensional setup is inadequate for studying metastasis.

“Cells don’t invade through plastic,” Weiss says. “So we knew we were going to have to design a three-dimensional construct that recapitulates, as close as we can, what that environment looks like in vivo.”

Weiss’ lab cooked up its own collagen mix for the purpose — one that ended up more closely mimicking important aspects of natural tissue than commercially available alternatives that other scientists were using. And it was using this 3-D culture method, done in clear plastic cylinders that resemble oversized thimbles, that Weiss’ group made its big finding.

“Basically, for the last 10 years or so, we’ve been trying to overturn the discovery we made using these 3-D systems,” he says. “And so far, we haven’t been able to.”

In order to cross through those tough, interconnected fibers of the extracellular matrix, cancer cells employ a pair of molecular “scissors” that are situated on the surface of the cell, Weiss says.

These scissors are really enzymes — known as a matrix metalloproteinase — that can cut the collagen fibers, allowing the cell to move through. Our bodies make hundreds of similar enzymes, 23 of which are specific to degrading the extracellular matrix.

Through rigorous, methodical experimentation, Weiss and his colleagues found that there’s a single pair of these scissors — membrane type-1 matrix metalloproteinase, or MT1-MMP — without which cancer cells cannot snip their way into surrounding tissue.

“How can it be that there’s only one really critical enzyme that both normal cells and cancer cells have to make in order to move through any kind of three-dimensional extracellular matrix that’s dominated by type 1 collagen?” Weiss muses. “It makes no sense, but that’s what the evidence points to, and we’ve done our best to prove ourselves wrong.”

The expectation would be that with so many different types of molecular scissors out there, each tuned to different components in our tissues, the invasion process would be regulated by a whole network of contributors working together.

“The rule in biology is that for anything that is really important for the cell to do, there’s built-in redundancy, like the five computers that operate the space shuttle,” Weiss continues. “If you’re missing...
one thing, the others can rescue the problem. And even if there’s not redundancy, there’s some kind of compensatory mechanism — several elements that can work together to circumvent a loss."

In this case, not so much. All of the findings to date point to MT1-MMP as the lynchpin. You can delete it from a cancer cell and leave all of the other myriad scissors intact, and the cancer won’t be able to go anywhere.

Even more unexpected was what Weiss and his colleagues discovered in research mice lacking the gene to make MT1-MMP.

e-Hwa Chun, M.D., Ph.D., a postdoc in Weiss’ lab, noticed something amiss while looking over the shoulder of a colleague working with skin from an MT1-MMP knockout mouse.

“I noticed in pictures of the mouse tissue that there were no fat cells beneath the skin,” recalls Chun, now an endocrinologist leading his own lab in the U-M Medical School’s Department of Internal Medicine and Biointerfaces Institute.

The missing fat cells were an important clue in understanding how MT1-MMP remolds collagen and how this process might be co-opted by invading cancer cells, Weiss says.

According to the National Cancer Institute, 80 to 90 percent of cancers are carcinomas, meaning they originate in epithelial tissue — which includes skin, as well as breast tissue and the linings of the lungs, prostate and colon, to list just a few examples.

Epithelial cells grow on top of a thin, specialized layer of protein called the basement membrane. So a cancer cell wanting to cross into the collagen-rich extracellular membrane, and from there invade other parts of the body, first needs to be able to penetrate the basement membrane.

“When someone is diagnosed with cancer, if the cancer cells are still above the basement membrane, the prognosis is actually quite good,” Weiss says. “But if the cancer has crossed the basement membrane and has moved into the underlying matrix, the outlook is much more dire.”

As it turns out, mature fat cells are completely surrounded by a basement membrane, he says.

“Now you might be thinking, ‘Why do I care if fat cells are surrounded by a basement membrane?’ ” Weiss continues. “Well, fat cells make MT1-MMP, our favorite protease, but they don’t invade. It’s not like fat cells escape their basement membrane and move from your belly up to your lungs.”

The fat cells do, however, need
to reshape their basement membrane to give themselves room to grow and expand. And they use MT1-MMP to accomplish this.

“So, studying how a normal cell interacts with the basement membrane can tell us lots about how a cancer cell must be interacting with it in order to remodel it during invasion,” says Weiss.

The line of inquiry also highlighted the advantages of using a 3-D cell culture platform.

Fat cell progenitors lacking MT1-MMP grew just fine in a 2-D petri dish. It wasn’t until they were cultured in a more true-to-life setting, like the 3-D collagen matrix, that they failed to differentiate and function normally.

“What’s unique about our study is that it showed that although MT1-MMP is not required for adipocyte differentiation on a plastic dish, it’s indispensable for differentiation in a collagen-rich environment, like in our body,” notes Chun, who was the lead author of the 2006 Cell article — which led to Weiss’ unusual invitation to the conference in Paris.

Now, one might ask, are there any examples where normal, healthy cells actually cross through the basement membrane? The answer is yes — and, to Weiss, it suggests that cancer’s malicious spread may not be random, but is instead an inappropriate hijacking of a familiar genetic program.

A n unfertilized egg cell can’t travel from the ovary, into the fallopian tube and thence to the uterus until it escapes from a protective package known as the follicle — whose envelope includes a basement membrane.

“This means that every time a mouse or a woman ovulates, that little oocyte has to invade through that basement membrane,” Weiss says.

Weiss was recently contacted by Ariella Shikanov, Ph.D., an assistant professor of biomedical engineering at U-M, who has been working on the opposite problem, keeping oocytes inside their basement membranes.

The goal of Shikanov’s research, which also employs a 3-D platform, is to help women facing damaging cancer treatments. Shikanov hopes to preserve immature egg cells and allow them to mature outside of the body to a stage where they can be used for in vitro fertilization at a later date.

“She was looking for new ways to preserve them, but I’m going, ‘Wait a minute, you’re telling me that if you leave it in a plastic dish, the oocyte knows how to eat a hole through the basement membrane and escape?’ ” Weiss says. “I’m thinking that oocyte may be turning on the same genetic program that cancer cells are inappropriately activating.”

For Weiss, this is the fun part of the research process.

“I’m not wedded to the answer being one thing or another,” he says.
“Even if I’m wrong — and we’re going to have to prove that I’m wrong — the theory matches the evidence at hand. There’s clearly a set of underlying instructions that is telling this normal cell to cross a basement membrane, move through collagen-rich tissues and to travel to a remote spot where it proceeds to multiply itself like mad.

“Usually when you get an idea like this, you go into the literature and find out that somebody in the 1930s thought of it first,” Weiss continues. “But the key isn’t to say, ‘Gee, isn’t this a cute little explanation.’ It’s to go, ‘Hey, let’s attack this theory experimentally.’ ”

Even as Weiss continues to chip away at fundamental questions about how normal cells and cancer cells behave, and to pursue MT1-MMP as a potential therapeutic target, he is also using the 3-D platform to go after cancer in a different way — by leveraging its power to discover and develop cancer-fighting antibodies.

“While all this other stuff is intellectually stimulating, at the end of the day there’s no guarantee it will lead to anything of therapeutic benefit to anybody,” he says. “So simultaneously, we’re also doing what we call an unbiased screening approach.”

The researchers start by embedding cancer cells in their 3-D matrix, where, unlike in a 2-D environment, the cells grow much as they would in human tissue. Then the scientists inject the cancer-collagen tissue composites into mice, whose immune systems generate thousands of antibodies directed against what they believe are foreign invaders.

In this case, Weiss’ preferred analogy is chewing gum.

“Pretend I’m going to randomly stick wads of chewing gum on a car,” he says. “If I put a wad of gum on the aerial, on the door, on the headlight, on the seat — it won’t make one whit of difference to how the car runs. Similarly, most of these antibodies hit that cell surface and don’t do squat. But the idea is that if you stick enough wads of chewing gum on the car, eventually you’re going to hit on the fuel line or the ignition, and bingo, the car is paralyzed.”

Weiss’ pioneering approach received early support from the LSI’s Innovation Partnership — a program conceived of and designed by the LSI’s Leadership Council to offer expertise and early-stage funding to the institute’s most propitious projects. More recently, Resonant Therapeutics, a Houston, Texas-based company, is sponsoring work in the Weiss lab to further develop several of the most promising antibodies that have been discovered to date.

“This discovery by the Weiss lab was research that required nontraditional funding to transition their work out of the lab and on a path toward patients,” says Roger Newton, founder and scientific advisor at Esperion Therapeutics and member of the LSI Leadership Council. “Through the Innovation Partnership, the institute was able to conduct the experiments necessary to attract external support.”

Meanwhile, like a true experimentalist, Weiss waves off broader questions about the biggest challenges facing cancer research.

“Nobody will want to hear this,” he says, “but we still know very, very little about how cells function normally. We can look more deeply into cell function than ever before, and at speeds we never dreamed of a decade ago. But the complexity is mind-boggling, and every question we answer raises 10 new ones.

“So, how the cellular machinery goes awry is pretty difficult to pin down when you don’t fully understand the normal workings of a cell,” he adds. “Slowly but surely, we continue to grow our understanding of the basic machinery of the cell — and that’s why support for basic research is still vitally important.”

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