Michael Overholtzer: Answering existential questions on entosis

Overholtzer is working to understand how and why tumor cells invade one another.

When a pair of tumor cells break free of their attachments to the extracellular matrix, something very strange can happen. One cell can dive into its neighbor, becoming completely enveloped and usually dying as a result. These cell-in-cell structures are frequently observed in advanced, aggressive cancers, but, until Michael Overholtzer came along, they had not been studied in any detail.

Overholtzer began studying cancer biology as a graduate student with Arnold Levine at Princeton University (1). But he first encountered this process, which he would later name "entosis," while he was a postdoctoral researcher in Joan Brugge's lab at Harvard (2, 3). It immediately enthralled him: Why would a cell choose to invade another cell like this? What biological purpose does it serve? And how does it happen? These questions are now the focus of his own lab (4, 5), which he started in 2008 at the Memorial Sloan-Kettering Cancer Center in New York. We called him to ask what answers he currently has to these questions and what he's looking into next.

OPEN FOR BUSINESS

Did you have any role models when you were growing up?

I think it's interesting that both my sister and I ended up getting PhDs. I think that probably has something to do with the way we were raised by my parents. They did a good job of bringing out their children's curiosity.

I was also close to both sets of my grandparents, but in particular my father's father, who ran a small business called Overholtzer's Radio and TV Service. I think about him a lot these days because, now that I've had my own lab for three years, I've started noticing how running a lab is a lot like running a small business. You have to hire and manage employees, you have a product that you make, and you have to market it in order to keep going. You even have to keep inventory. You have to be on top of everything.

Have you always known you wanted to be a scientist?

Actually, I was interested in biology when I was in high school, but I chose to go to Ithaca College largely on the basis of its strong music program. I loved classical music, mainly violin and voice, and knew I wanted to study it. I ended up minoring in voice. But I didn't really have any sort of career plan at that time.

Ironically, I think part of the reason I became a scientist is that Ithaca College doesn't have a graduate program for the sciences, so the only research there is done by undergrads. That gave me the opportunity to link up with a lab and start doing research. I was also lucky to find a great mentor who gave me the freedom, even at that young age, to feel like I could discover something. I had a real sense that what I was doing was important, and I realized that I love being an experimentalist.

> "That's the $64,000 question: Why would a cell do this?"

Do you still sing?

With a voice minor, it was kind of inevitable that I ended up getting into rock bands. I still have friends who play in this or that band, mostly as a hobby, but I haven't been doing much of that lately. I have three small kids, and, together with starting my own lab, that hasn't left me much time for hobbies.

OPEN-MINDED

Did you have any plans for what to focus on in your graduate work at Princeton?

When I arrived at Princeton I had a very open mind, and I could probably have ended up working on anything. But I happened to really enjoy my lab rotation with Arnold Levine because everyone in the lab had a different project and because they were all trying to ask exciting, big questions. And Arnold was a great mentor for me.

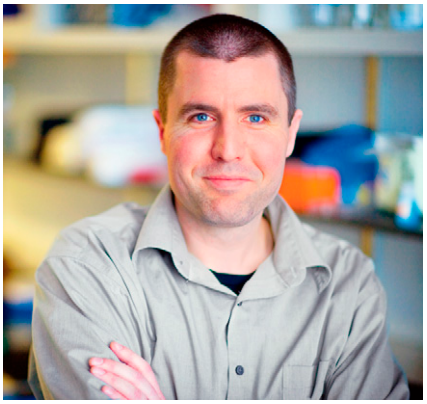

PHOTO BY MATTHEW SEPTIMUS, COURTESY OF MEMORIAL SLOAN-KETTERING CANCER CENTER

Michael Overholtzer

When it came to my own graduate research project, though, I would say I developed it by trial and error. [Laughs] Most of the time our interesting ideas didn't work out. I failed at a lot of projects, but that experience was great for my professional development. Failure is a huge part of discovery.

Why did you join Joan Brugge's lab for your postdoc?

By the time I had finished my PhD, I had met my future wife. I wasn't even sure whether I wanted to do a postdoc, but I talked it over with her and we decided I would give it two years and see how things worked out. When I started looking for postdoctoral opportunities, I was already familiar with Joan's work—really cool and imaginative work modeling breast cancers using 3D culture. So I interviewed with her, and her lab was a perfect fit for me.

But once again, I had multiple projects in Joan's lab, and most of them failed. I kept at it for more than two years, though, because by that point I couldn't imagine wanting to do anything besides research. At the time we made the observations that formed the basis for what my own lab is now working on, I had two or three projects that were in various states of working, or not. But then I noticed something that just blew my mind: I would sometimes see one cancer cell become completely engulfed by another. Neither Joan nor I had any idea what to make of it.

Text and Interview by Caitlin Sedwick
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Our first thought was that we had stumbled upon the mammalian equivalent of something that happens in *C. elegans*, where sometimes one cell eats a neighboring cell that's about to undergo apoptosis, and the engulfing cell plays a role in killing off the dying cell. So I set about trying to trigger this process—which we later called entosis after the Greek word for "inside"—using every approach I could think of, but nothing worked until I realized that I had been trying all my treatments on adherent cells. In fact, entosis only happens amongst cells that are detached from their substrate.

When did you recognize the potential significance of your observation?

Not until I'd been working on it for some time and happened to show my data to a pathologist, who said, "You know, you can see this in cancer. It's not hard to find." Pathologists had noticed it a long time ago, and they call it a "cell-in-cell structure." We'd had no idea! You can't do a PubMed search on something if you don't know what search term to use.

Point of grammar: Is entosis done by the engulfing cell or the one being engulfed?

I think the word entosis really just refers to the engulfment mechanism that occurs between pairs of cells. As far as we know, the cell that is being engulfed is the one that is driving this process. It relies on Rho signaling to myosin and the actin cytoskeleton to undergo something resembling an invasion of the enclosing cell. But the enclosing cell probably also participates in the process, in ways that we just haven't uncovered yet.

The simplest way to think about it is that the cells are initially connected by a normal epithelial junction. But if the cells aren't adherent to some surface, they don't have any way to counter the contractile forces they are exerting against each other. So if one cell is contracting more than the other, it just keeps pulling the junction around itself until it becomes engulfed.

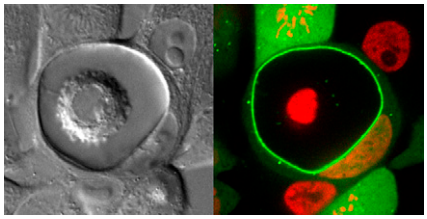

IMAGE COURTESY OF OLIVER FLOREY

An entotic cell is contained within a large vacuole inside its host cell.

OPEN QUESTION

What purpose does entosis serve?

That's the $64,000 question: Why would a cell do this? We don't know the answer yet. From what we know today, the cell that goes in seems to be an active participant, but it's unclear whether it could get any benefit from doing this. It may be a suicide method, because most of these cells will die. However, engulfment is not sufficient to trigger death. Instead, the internalized cells have to be executed by their hosts, or else they can escape. In fact, we recently showed that host cells use a process involving the cellular autophagy machinery to kill entotic cells.

> "As we tackle the biology of entosis, we're always keeping an eye out for ways to turn it on or off."

It's also unclear what the larger biological outcome of this process is. When it happens in tumors, then one tumor cell is swallowing and often destroying another. Joan and I had shown that, after a cell division, one daughter cell often invades into the other and is killed, which could block tumor outgrowth. So if it occurs at high rates in a tumor, then it could be tumor-suppressive.

But at the end, you are still left with a tumor cell, and, in one of the first papers from my own lab, we showed that that cell is often endowed with more aggressive characteristics. That's because the presence of an entosed cell can block cytokinesis, causing polyploidy or aneuploidy in the enclosing cell. Aneuploidy is often lethal to individual cells in the short term, but in the long term in cell populations aneuploidy promotes tumorigenesis. My instinct is that entosis' overall impact on tumor growth is situational; it'll depend on the context of the tumor or the timing of the event.

Where do you go now?

Overall, we're looking at entosis on two levels: One, what can entosis teach us about basic biology? What basic processes are at work here, and what triggers them? Our recent paper on the role of autophagy on entotic cell death is a good example of that approach, and half my lab is following up that finding by looking at what triggers this death pathway. The other half is working on questions related to cell–cell adhesion: How does a cell junction undergo the morphological changes required to engulf an entire cell? But we're also interested in the bigger picture. What does this phenomenon mean for human cancers? As we tackle the biology of entosis, we're always keeping an eye out for ways to turn it on or off.

I think a lot of students these days feel that there's so much that's already known, they haven't really had a chance to make a big discovery. I don't think they realize that there could be whole processes out there that are still waiting to be discovered. There are still big things to find.

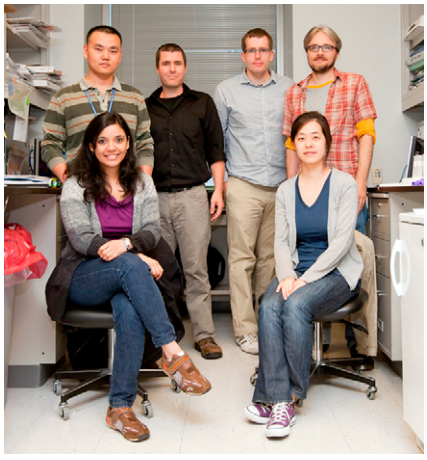

PHOTO COURTESY OF MATEJ KRAJCOVIC

Overholtzer and members of his lab are working to get to the bottom of entosis.

1. Overholtzer, M., et al. 2003. *Proc. Natl. Acad. Sci. USA*. 100:11547–11552.
2. Overholtzer, M., et al. 2007. *Cell*. 131:966–979.
3. Overholtzer, M., and J.S. Brugge. 2008. *Nat. Rev. Mol. Cell Biol.* 9:796–809.
4. Krajcovic, M., et al. 2011. *Nat. Cell Biol.* 13:324–330.
5. Florey, O., et al. 2011. *Nat. Cell Biol.* 13:1335–1343.