Lloyd Trotman: Of mice and men, cancer, and PTEN

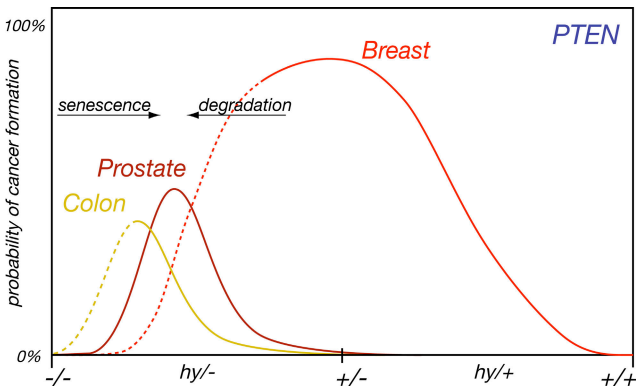
Lloyd Trotman is investigating the role of the tumor suppressor PTEN in cancer using molecular biology and animal models.

p53 and PTEN are two major tumor suppressors that keep cell growth in check (1). Loss of tumor suppressor activity through deletion or mutation contributes to many human tumors.

Lloyd Trotman was lured from Zürich, Switzerland, to do his postdoctoral work with Pier Paolo Pandolfi at Sloan-Kettering in New York City. There, he and his colleagues found that loss of one allele of PTEN triggers cancer, whereas biallelic loss exposes cells to growth suppression by a senescence pathway mediated by p53, findings that contradict the classic two-hit model of tumor suppression (2, 3). He also showed that PTEN shuttles between the cytoplasm and nucleus (4)—odd behavior for a protein thought to act mainly at the plasma membrane.

"The one thing that I really never wanted to do after high school was biology."

Now directing his own research group at Cold Spring Harbor, Trotman is working to understand the relevance of PTEN nuclear localization to PTEN function and cancer and to grasp the importance of the p53 senescence pathway in human cancer. We called him to discuss how he tackles big questions and dogmatic concepts in biology.



***PTEN* haploinsufficiency results in tumors, but complete loss triggers senescence.**

FONDNESS FOR FUNDAMENTALS

When did you decide you were interested in science?

It was always clear that I liked certain sciences quite a bit—I was really drawn to physics and chemistry because I wanted to be able to understand things on the most basic, fundamental level. On the other hand, I have to say that I also was always flirting with the social sciences. Even after I had entered Zürich University to study biochemistry, I took a break once for a year to study art history and theory of science in a different country. I am married to an art historian. The funny thing is that the one thing that I really never wanted to do after high school was biology. I was drawn back to it much later.

How did you select Urs Greber's lab for your graduate work?

At the end of my university studies, I didn't really know what I wanted to do most. I'd been working with very basic biochemical questions in biology, like functional peptide and protein design, but I wasn't really sure if that field was making substantial progress. So I took some time and worked in a *Drosophila* lab, where I was exposed to the power of genetics.

Later on, there was an opening in Urs's lab there in Zürich, where they were using viruses to tackle cell biological questions. Viruses have developed together with their host cells for tens of thousands of years, so you can study the interaction of a virus with the cell and ask it to reveal the cell's secrets that it is exploiting to its own ends. I liked this approach of studying something fundamental in a peculiar way.

Lloyd Trotman

CHALLENGING DOGMA

You made a big switch to studying cancer biology in your postdoctoral work.

Our work with viruses was touching on many aspects of cell biology, and one of them was nuclear architecture. I was trying to tackle questions of how adenovirus gets its DNA through the nuclear pore complex, and one of the steps had to do with a nuclear structure called the PML body, which Pier Paolo Pandolfi had discovered in a completely different context: leukemia. I was fascinated by the fact that the PML protein is essential for forming a nuclear structure; if you don't have the protein, this whole structure falls apart, and this structure is clearly involved in cancer. After talking to him, it was clear I wanted to work with Pier Paolo.

When I got to Pandolfi's lab, I originally wanted to work on PML, but nobody was working with PTEN at the time, and he convinced me very quickly that I should really look at PTEN. Our first problem was that there were so many mouse models that we could have been using at the time. We finally decided to look at PTEN gene dosage in cancer. It paid off big time.

Pandolfi also has this willingness to think of the fundamental things that we know about cancer in totally different

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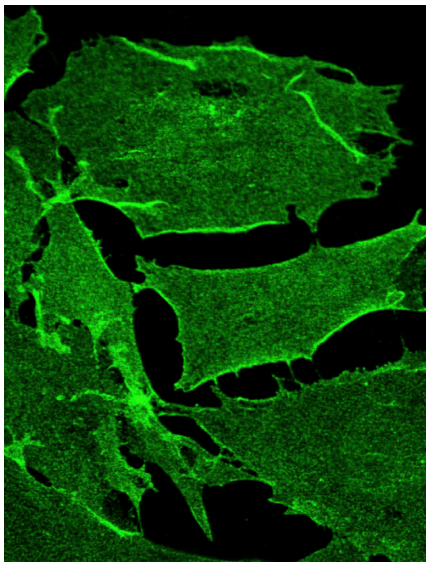
ways and to toss them overboard if new ideas make more sense. I could take my background in transport biology, nuclear import, and nuclear biology into what we were studying to do something that is totally nonmainstream. For example, I wanted know why there is PTEN in the nucleus when it's supposed to act at the membrane. Is that at all important? How does it get in and what is it doing there? After my second week, I had these ideas, but it was only four tough years later that we actually got clear answers to those original questions.

What has been the biggest challenge in your work so far?

The biggest conceptual challenge is connected to one of the biggest discoveries that came out of our lab at the time: the senescence principle, that the complete loss of PTEN causes senescence. At the time, that concept was just impossible; loss of both alleles of a tumor suppressor was supposed to cause cancer.

Amazingly, I'd had the result for some time, that complete loss of PTEN doesn't allow a cell to proliferate. I had the job of creating PTEN-null mouse embryonic fibroblasts in the Cre/Lox system using adenovirus to deliver Cre, but I just could not get cells to grow after adding the virus.



Cells that completely lack PTEN do not form tumors unless they can avoid senescence mediated by Akt (green) and p53.

We transferred the task to a technician, who got the same result with a different virus, but since he was also crossing PTEN-nulls with p53-nulls, he clearly saw that embryonic fibroblasts that have neither protein would proliferate very well.

Pier Paolo had this idea—could it be that p53 triggers senescence when you lose PTEN? To me, that was going too far. I just felt it was too much. But I went back to my adenovirus experiment and included all the right controls, testing if a PTEN-null cell actually had a growth disadvantage. Everything all of a sudden started to make sense. It was clear that if they only lose one copy of PTEN, cells proliferate a lot more, but if they lose both, they become senescent. It was a lesson in how hard it is to overcome a preconceived notion that is totally entrenched, that less tumor suppressor will always give you more of a proliferative advantage.

On the other hand, our biggest technical challenge had to do with understanding a mutation in PTEN that was not in a place that was expected to affect PTEN function. We had clearly seen that PTEN is modified at the mutation site by something that's added onto it. I spent four years trying to identify what is added (it turned out to be ubiquitin), but during this whole time it wasn't really clear if what we were looking at was something that really plays a role in cells. The only thing that kept us going was that somebody had identified a patient family that had a mutation on our major target site, and that having this mutation there would give you a disease. We finally got some histology slides from these patients, and it was clear that the mutant PTEN really had a problem in going into the nucleus.

OF MICE AND POSTDOCS

It sounds like you have had to put in a lot of time in the mouse room for this work.

Yes. That's something that Pandolfi insisted upon. We did everything with the mice ourselves, and it was key to be in intimate contact with your colony so you knew exactly what was going on. That was essential for realizing some important details that could have been easily missed. That's what I tell my students and postdocs now, "Anything that you want to cross, you want to follow up. There's going to be minimal help from a technician, because I want you to be there with these mice, and I want you to realize if there's a problem with something like the Mendelian ratio at which they're born or if they look different at birth—whatever it is, I want you to be on top of it."

"We shouldn't forget that you cannot solve the big problem by *trying* to solve the big problem."

What questions are you focusing on now in your own lab at Cold Spring Harbor?

One thing we are looking at in my lab is what PTEN does in the nucleus. It could be that it's a protective mechanism to have PTEN there, because in the nucleus PTEN is not degraded as easily as in the cytoplasm. We also want to understand whether the known (or novel) targets of PTEN are in the nucleus in a relevant fraction, or if they shuttle between the nucleus and other compartments. We will need mouse models to ask if any aberration in this regulation will actually cause cancer, and we will work with patient samples to look at its importance for human cancer. There's also another whole layer involved in the senescence pathway in human tumors. We think the PTEN haploinsufficiency and senescence paradigms could form the basis of understanding this cancer from the bottom up.

I also think we shouldn't forget that you cannot solve the big problem by *trying* to solve the big problem. What you have to do is try to solve a small, tractable problem that you believe to be extremely relevant. JCB

1. Trotman, L.C., et al. 2003. *Cancer Cell.* 3:97–99.
2. Trotman, L.C., et al. 2003. *PLoS Biol.* 1:E59.
3. Chen, Z., et al. 2005. *Nature.* 436:725–730.
4. Trotman, L.C., et al. 2007. *Cell.* 128:141–156.