People & Ideas

Valentina Greco: Got hair?

Greco studies tissue regeneration and regression in the hair follicle and in cancer.

PHOTO COURTESY OF RENEE GAUDETTE, YALE CANCER CENTER

Valentina Greco

In mammalian hair follicles, follicular tissues and new hairs are generated during a cyclical growth cycle. The growth phase is followed by a period of degeneration and then by entry into a resting state that terminates with a new round of growth. The transitions between different stages of the cycle are triggered by interactions of hair follicle stem cells with underlying mesenchymal cells, but the detailed cellular and molecular mechanisms involved in this process are still being worked out.

Valentina Greco became interested in stem cells' role in hair follicle regeneration as a postdoc in Elaine Fuchs's lab (1). Greco's own group at Yale has shown how the positioning of stem cells within the hair follicle's stem cell niche affects their activity and contribution to successive rounds of tissue regeneration and regression (2, 3). Recently she has also begun investigating parallels between regenerative processes in the hair follicle (4) and in a form of regressive skin cancer (5). We called her to talk about some of the hairy details of her work.

LEAPING A BARRIER

You're from Italy…

Yes. I grew up and did all my schooling through my undergraduate degree in Palermo, Italy. It is a big city, so I felt no need to go abroad. I felt I was born to live in Palermo for the rest of my life.

Why didn't you stay in Italy?

I spent the last two years as an undergraduate working full time in Aldo di Leonardo's lab, which studied the activity of tumor suppressor genes in mitotic cell division. When I finished my undergraduate degree, I wanted to stay in Palermo. I applied to the graduate program there, but I was not accepted. That turned out to be a good thing because it pushed me to consider other places for my PhD.

My friend Eugenia Piddini, who had shared lab space with me when we were undergraduates, was accepted to the EMBL graduate program. She kept telling me how I had to come too, so I applied to EMBL and was selected for an interview. I went two weeks ahead of time because my English was extremely poor. To help me prepare for the interview, Eugenia and all her friends spoke to me only in English for those two weeks. Then I had to go through 21 interviews with different professors, all in English. Somehow I made it.

Why did you choose Suzanne Eaton's lab for your PhD?

During my interviews Suzanne and I had a great interaction. She wasn't afraid of my not knowing English. She patiently explained everything to me, and she was extremely passionate. We talked about planar polarity and the problem of tissue patterning. I made it clear that I was very interested in these subjects, and Suzanne decided to bet on me.

> "We knew we were ready to start … live imaging. The game was on."

The initial eight months of my project were completely different from what I worked on at the end. That always happens. [*Laughs*] But that project wasn't going in any clear direction, so under Suzanne's mentorship I switched to a different project studying how morphogens can travel long distances in tissues. It seemed like a long-shot project at first, but it ended up being a very important question in Suzanne's lab.

THE WAY FORWARD

What were you looking for in your postdoc?

It felt like I didn't really have a plan. I was in love with the topic I had been working on in Suzanne's lab, but I wanted to take on a new challenge in a different subject. I chose to join Elaine Fuchs's lab because I was interested in stem cell biology and there were so many exciting people working there. The lab had three major research foci at the time: transcription factors, stem cells, and the cytoskeleton. I felt I could learn a lot there.

When did you start thinking about setting up your own lab?

Very late. My postdoc wasn't very smooth. On the basis of a straight knockout we knew that ephrins regulated hair morphogenesis, so I worked for two and a half years to establish a double conditional ephrin knockout and to overexpress a dominant-negative ephrin receptor within the skin epithelium. But I got no phenotype. That was very painful. I was also pregnant, and my husband, Antonio Giraldez—a very talented scientist—had gotten a job two hours away. I spent a lot of time reflecting on whether or not I was cut out to run my own lab.

But then I started working on a project I was very passionate about, which involved communication between the mesenchyme and epithelium in the hair follicle. At that time the dogma was that stem cells located in a structure called the bulge received some kind of signal from the nearby mesenchyme to fuel the growth of the hair follicle. But the data I was seeing didn't match with that model. I realized that the center of action was in a different compartment: in the stem cell progeny located in the hair germ, which is situated between the bulge stem cells and the mesenchyme. This work reconciled a long-standing paradox on how stem cells can be quiescent while still being capable of

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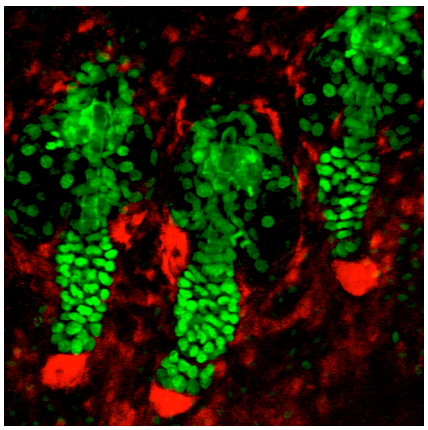

IMAGE COURTESY OF PANTELEIMON ROMPOLAS

An image showing epithelial nuclei (green) and mesenchymal cells, including dermal papilla (red).

active growth. It demonstrated that stem cells exist in two different pools in mammalian skin: an activated one and a quiescent one.

LOOKING MORE THAN SKIN DEEP

What approaches have you used to study this topic in your own lab?

In my own lab, the questions we wanted to ask essentially were: Are the hair germ and bulge stem cells functionally different? Are their functions influenced by the niche, and, if so, how? The first approach we took was to build new conditional expression mouse lines that would allow us to restrict the expression of a reporter or eliminate alleles in either of the two populations. We spent a lot of money and got nothing out of it.

On the side, I decided to try a high-risk/high-reward approach in collaboration with Ann Haberman, a principal investigator at Yale who also runs an intravital imaging facility. We played around with a two-photon microscope and with different markers until I found a way to observe live cell division events in the hair follicle for the first time. It took a year from the day I first walked into my own lab to reach the point where we knew we were ready to start setting up the system for live imaging. The game was on.

What were the keys to getting this to work?

In the skin, intravital microscopy can view about 150 micrometers deep. Hair follicles on the heads of mice grow at an angle, so they're close enough to the surface that we can capture their whole length. We also figured out that the best marker to track cells in the follicle is a simple epithelial nuclear marker. Looking back, it seems trivial now, but there were a lot of roadblocks to overcome.

We wanted to investigate the functional roles of the different hair compartments—bulge and hair germ—during hair regeneration and whether they are actually important. Panteleimon Rompolas, a postdoc in the lab, demonstrated that, depending on their position within these compartments, cells cover different functions. Thus, the functional contribution of hair germ cells is mostly to give rise to differentiated cells, while the stem cells located in the bottom part of the bulge will become new hair germ cells in the next cycle. We also learned that both hair germ and bulge stem cells are dispensable. If these cells are removed through laser ablation, follicles can do without them because nearby epithelial cells can come in and do their job. Conversely, when the mesenchymal niche was removed, regeneration stalled.

> "It placed more emphasis on the stem cell niche than on the stem cells themselves."

This was very surprising because it placed more emphasis on the stem cell niche than on the stem cells themselves. Now we would like to understand how these niche signals are integrated to drive tissue regeneration and also how the undifferentiated and differentiated cells are eliminated during the regression phase of the cycle.

You've also recently started studying tumor biology…

Yes. A colleague here at Yale, Christine Ko, introduced us to a type of tumor that spontaneously regresses, called keratoancanthoma. "Because some regress, keratoancanthoma can be benign, but they can also give rise to malignant, squamous tumors. We were interested in these tumors because we felt that our knowledge of hair follicle growth and regression might help us understand how tumor regression can occur. For example, Elizabeth Deschene and Peggy Myung in my lab showed that expression of stabilized β-catenin in hair follicle stem cells recruits neighboring wild-type cells to participate in ectopic follicle growth through the secretion of Wnt. Giovanni Zito, a postdoc in the lab, observed that Wnt is also important for fueling the growth of keratoancanthoma, and we identified a signaling cascade through which retinoic acid switches off Wnt. We also found we could induce regression in the squamous form of the tumor by applying retinoic acid. Now we are investigating what features distinguish the benign from the malignant tumor forms.

How do you balance your work with your life outside the lab?

It's not a balance; it's a dynamic equilibrium! [*Laughs*] Between my children Lola and Gael, my husband, my faraway family, my friends, and the lab members, my life is quite full. But it's the best life I could ever imagine to have. It's an intense life because juggling family and a lab is not easy, but if it were easy it would be boring for me.

1. Greco, V., et al. 2009. *Cell Stem Cell*. 4:155–169.
2. Rompolas, P., et al. 2012. *Nature*. 487:496–499.
3. Rompolas, P., K.R. Mesa, and V. Greco. 2013. *Nature*. 502:513–518.
4. Deschene, E.R., et al. 2014. *Science*. 343:1353–1356.
5. Zito, G., et al. 2014. *Nat. Commun.* 5:3543.

PHOTO COURTESY OF DIONNA KASPER

The Greco lab has a question for you.