

Hair Brained

By Martha Ledger

George Cotsarelis might cure baldness, but his discoveries are even more far-reaching.

In March 2004, Diane Sawyer's people woke George Cotsarelis, M.D. '87, G.M.E. '92, at 4:30 a.m. Would he agree to be on *Good Morning America* that morning? Could he get to ABC's studio in Philadelphia by 7?

Cotsarelis, a PENN Medicine dermatologist specializing in scalp and hair disorders and a basic scientist who studies the mini-organ hair follicle, was about to report findings in *Nature Biotechnology*, and a pre-publication press release was attracting attention outside the scientific community. His team had generated new hair follicles by isolating adult stem cells from murine hair follicles and implanting them in immunodeficient mice – and the new follicles had produced hair.

To people with less hair than they would like, this was big news. It suggested a future therapy in which stem cells could be harvested from a person's remaining hair follicles, multiplied *in vitro*, and injected back into that person's bald spots.

"The week the paper came out was insane," recalls Cotsarelis, a totally unpretentious, upbeat, and articulate man (picture Jimmy Stewart with the scrubbed face and talking ability of Brian Williams). "I'd never experienced anything like it." Throughout the month, he appeared on TV and radio and was interviewed for dozens of newspaper articles in this country and around the world.

The off-site interview with Sawyer was what one would expect. Cotsarelis endured bright lights in his eyes and wore an earphone and Sawyer asked him questions, which he answered. But it was a pre-interview detail that Cotsarelis still chuckles about. Before going on-air, he heard a producer on Sawyer's end ask via speakerphone, "Hey Joe, does the doctor have hair? Is he bald?" Would baldness have explained the motivation for Cotsarelis's

research? Would it have convinced viewers that a therapy was really not yet available? For the record, Cotsarelis, 47, still has a good head of hair with maybe a slightly receding hairline.

Cotsarelis had his 15 minutes of fame, but, not long afterward, he had 15 minutes more. In May 2007, his lab was back in the spotlight because people really care about hair. Publishing in *Nature* this time, his team reported that after mice had had a full-thickness patch of skin removed from their backs, new hair follicles had formed in the healing skin. The press went wild. They reported that Cotsarelis had co-founded a start-up biomedical company dedicated to hair regeneration and implied that it wouldn't be too long before a treatment was available – one that would work for everyone. People began e-mailing Cotsarelis, offering themselves for clinical trials.

Both scientific papers do offer hope for people with hair loss, and there are many such people. Five of every 10 men and three of every 10 women suffer a significant degree of age-related baldness by age 50. In addition, there are disorders that cause people to lose scalp hair and sometimes all body hair. And as director of PENN Medicine's Scalp and Hair Clinic – which has a very long waiting list of patients – Cotsarelis is acutely aware of their angst.

Nonetheless, Cotsarelis, the Albert M. Kligman Associate Professor of Dermatology, is not mounting a frontal attack on baldness. His discoveries may lead to a cure for it, but they break ground in other significant areas as well. The *Nature* paper documents the first successful regeneration of a mammalian organ. The experiments it describes shed new light on the road that stem cells travel toward differentiation, which is crucial in the regenerative process.



George Cotsarelis, M.D., examines the scalp of Steve Wlodarczyk.

I recently asked Gerald Lazarus, M.D., former chairman of PENN Medicine's Department of Dermatology, about the scientific significance of this work. "On a scale of one to 10," he said, "I think it's somewhere between eight and nine. Ten," he quickly added, "is relativity."

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A variety of things led Cotsarelis into dermatology. The first was growing up with a brother who had a really bad case of acne. "He'd come back from the doctor," Cotsarelis recalls, "with these terrible marks on his face where pimples had been cut open and expressed. And I was like, boy, I hope I never have to go through that."

Fortunately, he didn't, but his brother's experience made an impression on him. So much so that as a high-school student in central Pennsylvania, Cotsarelis did a report on acne, researching it in the library of the Milton S. Hershey Medical Center. It was there, in the stacks, that Cotsarelis first encountered Albert M. Kligman, M.D., Ph.D., Penn professor of dermatology – his name, anyway, and his writing style. "The best articles, the ones

written so well that even a high-school student could understand them," Cotsarelis says, "were written by Albert Kligman. It was a great thrill when I actually met him, when I became a Penn student."

But it was Brian Jegasothy, M.D., then

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vice chairman of dermatology at Penn and Cotsarelis's first-year medical advisor, who made the field seem especially appealing. "He was a very nice man who seemed quite satisfied with his life," Cotsarelis recalls, "so I considered it." By his third year, he had decided. He dropped into the lab of Robert Lavker, Ph.D., then associate professor of dermatology, and asked if he could do some work there.

"Medical students," Lavker explains, "would – and still do – come in for two or three months hoping for a letter of recommendation for their residency application and something interesting to talk about in their interviews." He's not sure that Cotsarelis's motivation was any different, only

that he turned out to be the real thing.

Lavker and his collaborator Tung-Tien Sun, Ph.D., were looking for the stem cells that maintain the cornea, and they set Cotsarelis to work on a variety of experiments to find them. He used a tech-

nique based on the slow-cycling nature of stem cells. Since these cells rarely divide, they retain a radioactive label much longer than normal cells that divide frequently and halve their marker with each division. After a period of time, the slow-cyclers can be detected through autoradiography. Cotsarelis also devised his own experiments to monitor corneal repair, "very creatively developing his own ointments to stimulate the eye," according to Lavker, who is now professor of dermatology at Northwestern University.

Cotsarelis was able to show that all of the stem-cell-like activity was coming from the peripheral cornea, or limbus, where the white of the eye meets the

pigment. These findings led to a first-author paper in *Cell* and, not long after, a change in the way corneal transplants were done. At that time, ophthalmologists normally grafted only the central part of the cornea, and if the patient had suffered a full-thickness burn, the graft lasted only about six months. When surgeons began incorporating limbal cells in the transplant, these grafts lasted as long as the patient did.

Cotsarelis was hooked on research. Lavker explains: “Once you get findings like these . . . you just can’t get enough of it.” Plus, the laboratory was fun. “We laughed a lot. It wasn’t at all dictatorial, and there was freedom to do what you wanted.”

It was so good that Cotsarelis considered postponing graduation and spending another year in the lab. Lavker, for one, argued strenuously against it. “I was looking at it solely as a parent would,” he says.

In the end, Cotsarelis decided to finish his medical degree on schedule. He interned in medicine at Geisinger Medical Center, and in 1988, before he started his residency at Penn, he returned to Lavker’s lab as a research fellow. It was then that he started studying the hair follicle.

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“The reason the hair follicle is so fascinating is its cycling,” Cotsarelis explains with enthusiasm. “A hair grows, falls out, and another grows in its place. It’s the body’s only organ in which there’s a total loss of [hair-generating] cells and then a total regrowth of those cells. And this cycling goes on throughout your lifetime.

“The cells that make a hair are some of the most rapidly proliferating in the body – and quite suddenly, they shut down and die. The follicle’s stem cells are very quiescent – and quite suddenly, they awaken and set regrowth in motion. How do cells know when to do these things?

What signals are they responding to?” says Cotsarelis. “These are fundamentally important questions relevant to treating cancer and wounds, where you want to turn off or turn on cells.

“An understanding of the follicle may lead to a treatment for hair loss,” he says, but adds that he hopes studies like his “will lead to a lot of other advances as well.”

In the lab, Cotsarelis started looking for the stem cells that control the follicle’s cycling process. The hypothesis was that stem cells would be in the bulb, the rapidly growing bottom part of the follicle. Cotsarelis didn’t find any slow-cycling cells there, but he did find them in another part of the lower follicle, in a shallow pocket that had no name. “You must be doing something wrong,” Lavker and Sun said. “Go back and do it again.” Says Cotsarelis: “I kept doing it and kept getting the same results.”

Cotsarelis continued the work into his residency. While researching the follicle to make a Rounds presentation, he consulted a 1956 book by biologist William Montagna, Ph.D., that described

the work of P. G. Unna, a 19th-century German scientist. In 1876, Unna had noted the presence of the follicle’s wulst [bulge], which he said seemed prominent during fetal development but whose function was unknown. Looking through his microscope, Unna also noted that he rarely saw cell division occurring there. Cotsarelis realized at once that this bulge area was exactly where he was seeing slow-cycling cells. He had indeed found the follicle’s stem cells, as well as the name for the structure that housed them. In 1990, still a resident, Cotsarelis had another first-author paper in *Cell*.

To Lazarus, who is currently director of the Center for Wound Healing at Johns Hopkins Bayview Medical Center, Cotsarelis has the right attitude to do great science. “A lot of scientists have an end in mind – a hypothesis that they’ve got to prove so they can write papers about it. When George started working, he didn’t have an enormous vested interest in what the result was going to be. He expected Y to happen. Y didn’t happen. But Z did. George was really good at dealing with



Mayumi Ito, Ph.D., left, who worked in the Cotsarelis lab as a postdoc, consults with Zaixin Yang, M.D., a research specialist.

Z, asking ‘what do these results mean?’ This kind of maturity is very unusual, and George had it from the very beginning.”

Lavker notes that Cotsarelis also had “the trick of writing, of presenting your stuff in an appealing and understandable way.” What he didn’t have upon completion of his residency, however, was training in molecular biology or experience with anything beyond the most basic, labor-intensive investigative tools. Supported by a Howard Hughes Medical Institute Postdoctoral Research Fellowship, he acquired the background he needed.

Still, Cotsarelis initially made less progress than he had hoped. He was trying to find genes specifically activated in the bulge area, but after four years he had identified only a few, and they were also activated elsewhere. One day, he talked with Stephen Lyle, M.D., Ph.D., a pathology resident at Penn, who was studying melanoma. Lyle told Cotsarelis that a part of the hair follicle was routinely being stained by a protein-seeking antibody he was using. The marked area turned out to be the bulge, and Lyle soon identified the tagged protein as keratin 15 (K15).

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This was the turning point for Cotsarelis. He identified the section of DNA that controls K15 expression and began using it to target the bulge cells with genes that produced a fluorescent protein. Soon after, he was able to isolate the bulge stem cells and show that they are multipotent – that is, they generate all the different types of epithelial cells in the lower hair follicle and shaft.

Cotsarelis had expected follicle stem cells to contribute to maintaining the epidermis as well. But this turned out not to be the case. To prove the independence of the epidermis, his research team created a transgenic mouse with a “suicide gene” in its follicle stem cells. They

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grafted a piece of its skin onto an immunosuppressed mouse and then activated the gene, which destroyed the follicle. The hair on the graft fell out, but the epidermis survived. This result explains why cosmetic removal of unwanted hair is at least theoretically safe.

If the *skin* is wounded, however, follicle stem cells do participate in repairing the epidermis. Using transgenic mice whose follicle stem-cell progeny could be tracked, Cotsarelis’s researchers demonstrated that cells migrated from the bulge to the area of skin rebuilding, eventually making up 25 percent of the healing wound. There, they began acting like epidermal cells, expressing epidermal rather than follicle stem-cell genes. This display of plasticity was both remarkable and temporary. As the wound healed more thoroughly, these cells disappeared. The findings have therapeutic implications: Once scientists understand the signals that summon bulge cells to a wound, they will be able to augment their presence and more successfully treat wounds and aging disorders like epidermal atrophy.

In the course of his research, Cotsarelis took advantage of newly emerging technologies for molecular studies. He ticks off the arsenal: transgenic mice; rapid gene-sequencing techniques “that let you do in a day or two what took years before”; micro-array chips, “where you can look at 30,000 genes at once instead of just five or 10”; and fluorescent-activating cell sorting. “The last is an incredible technology. You separate skin cells using an enzyme, add an antibody with a fluorescent tag to a cell’s surface protein, and then shoot the cells through a machine where a laser hits each one. If the cell fluoresces, it gets separated out from the rest of the batch. That’s how we ended



up sorting out the stem cells – because we could make them fluoresce.”

During the wounding studies, Mayumi Ito, Ph.D., a postdoc from Japan, who had come to the United States in 2003 to work with Cotsarelis, noticed structures very similar to embryonic hair germs forming in the healed wound.

This fact was unexpected. “The dogma,” says Cotsarelis, “was that you were born with the total number of hair follicles that you were ever going to have. Their loss was considered permanent.” Moreover, he explains, mammals don’t normally regenerate their tissues. Instead, they’ve evolved to repair themselves. They form scar tissue, which has thick collagen bundles that lack hair follicles and sebaceous glands.

So the question was, where were these new follicles coming from?

Ito searched the literature and found three articles from the 1950s that discussed the growth of new hair after wounding. Two of the papers dealt with wounded rabbits. The third, by Albert

Kligman, noted the phenomenon in biopsies done on dermabraded patients. The possibility of regeneration was debated for about 10 years after these papers, but, in 1967, researcher William Straile published a review article arguing that established follicles had simply migrated in from the wound's edge.

Cotsarelis's team would have to disprove Straile's conclusions. To eliminate the possibility of migrating follicles, Ito created a wound that was large enough (1/2-inch by 1/2-inch) to still leave an area open for examination after the wound contracted. She also removed the full thickness of skin, so there could be no existing follicles.

In a few weeks, new skin formed in the wound, and a few weeks after that, hair began to grow from its center. "I was so excited to see the result, I cut a section by myself," she says.

"We knew what we had," Cotsarelis explains, "because we studied the skin at several time intervals after wounding and saw under the microscope that hair follicles were developing. They looked identical to embryonic follicles. We just had to prove they were the same molecularly."

Which the lab did. As they reported in the 2007 *Nature* paper, hair-follicle differentiation markers were notably absent from the epidermis for several days after wound closure, but these markers reappeared when hair germs started to develop. The regenerated follicles also expressed the same proteins and enzymes (KRT17, Lef1, alkaline phosphatase, Wnt10b, and Shh) that are present during embryonic follicle development. The researchers showed, too, that Wnt proteins played a major role in the regenerative process: When Wnts were blocked, no follicles formed; whereas excess Wnts doubled the production of follicles.

Through genetic-lineage analysis, the researchers also demonstrated that there was a negligible number of bulge-cell

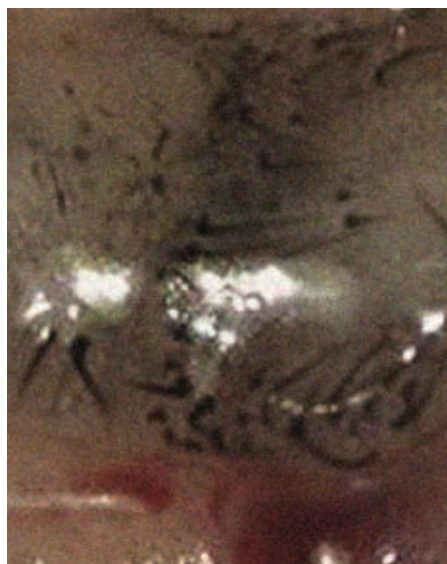
offspring in the new follicles, which took care of William Straile's explanation once and for all.

Most importantly, the researchers demonstrated that the new follicles arose from epidermal cells. That discovery suggests that wounding makes significant remodeling possible: epidermal cells turning into follicle cells and, as the earlier wounding experiment showed, follicle cells turning into epidermal ones.

"When cells move in to close a wound," Cotsarelis explains, "they are trying to make a decision: Should I make epidermis or should I make a hair? If there is a lot of Wnt around, they choose to become hair follicles.

"Maybe – and we're not sure yet – what's going on is de-differentiation," says Cotsarelis, "where cells that are usually committed to one lineage actually revert back to a state where they can become something else."

If this proves to be the case, a window of opportunity exists in which to manipulate cells. In terms of treating hair loss, this regeneration model suggests a different kind of therapy from stem-cell implants, one that might combine dermabrasion with a topical application of something containing Wnts or other factors.



Hair follicle stem cells isolated from an adult mouse generate new hair follicles when injected into another mouse. Each black line is a newly generated hair.

But the patch of new hair on a mouse's back might be a more far-reaching breakthrough, one that opens a door to organ replacement, a kind of science-fiction idea that at least some people think is the future of medicine. Some scientists are definitely moving in this direction.

In 2007, two research teams elsewhere were able to turn an adult cell into a pluripotent cell (one with unlimited differentiation possibilities) by inserting four genes. But, as Ito notes, "even though the cells have the capacity to differentiate into many types of cells, it's still very difficult for them to make a whole organ. In order to make an organ, cells must know how to collaborate with each other. In our model, the entire process of organ generation can be induced and studied at the molecular level." And at present, this is the only model scientists have for the complete regeneration of a mammalian organ.

"These are important and seminal observations," Lazarus says. They were possible, he adds, because Cotsarelis has been totally focused throughout his career. "One thing built upon another, and the applicability of his initial observations keeps broadening."

Ito, who recently established her own lab at New York University, has an additional idea about why the Cotsarelis lab has been so productive. "George gives freedom. If he wanted us to look only at hair-follicle bulge cells, this regeneration project would not have happened. Some of us work on his hypotheses. Some of us work on our own interests. He identifies questions. We identify questions, too. He gives us a chance and room to think from zero. And then he is very patient. He can wait. He can leave us alone to get the results of our ideas.

"So the lab never narrows," Ito says. "And that's a good thing for science." ♥