

INVESTIGATIONS

Explorations and revelations taking place in the medical school



UPCI Summer Academy scholars take a break under the Roberto Clemente Bridge during a bicycling trip. FROM LEFT: Natalie Nash, Matt Miklasevich, Sam Rest (getting horizontal — “planking” in Internet fad-speak), Ishan Chatterjee (crouching behind), Andrew Shin, and program mentor William Buchser.

SMART FUN

IN THE SUMMERTIME

HIGH SCHOOLERS SHINE IN CANCER ACADEMY

BY MARC MELADA AND ELAINE VITONE

On a mid-July morning in 2011, Dilafruz Khakimova wakes up at 5 for her hour-and-a-half commute on the 67 bus from Monroeville to Shadyside. It's a typical workday in a laboratory at the Hillman Cancer Center—you know, interrogating DNA-replication fork progression and the stability of DNA-repair complexes in human cells.

Not too shabby for a 17-year-old, right?

Khakimova is one of 25 high school students participating in the third-annual University of Pittsburgh Cancer Institute (UPCI) Summer Academy. The program was started in 2009 to promote careers in cancer care and research to rising juniors and seniors from both the local area and beyond (students also come from Maryland, Michigan, Indiana, Texas, New Jersey, and California). The students, referred to as scholars, pair up with a mentor researcher at the UPMC Hillman Cancer Center, Pitt's Department of Biomedical Informatics, Pitt's Department of Computational and Systems Biology, or the Magee Womens Research Institute. Each scholar completes a research project during the eight-week program.

Khakimova arrives at her project site, the lab of Chris Bakkenist, Pitt assistant professor of radiation oncology, and opens an incubator filled with petri dishes containing lung cancer cells. With the articulation and enthusiasm of a grad student, she explains their hypothesis: The DNA-repair pathways known as ATM and ATR have a functional relationship and work in tandem. If the hypothesis is right, the popular cancer drug gemcitabine, which targets ATR to stall cancer-cell replication, could

be made even more effective, she says.

There's a lot to do: tending to four different cell lines; collecting the cells with a centrifuge and dividing them when they get too crowded; treating the cells with various pathway inhibitors and examining cellular proteins to make sure those inhibitors are working; and, finally, determining whether the inhibitors kill the cancer cells.

"The thing I've learned about lab work is that it's different every day," Khakimova says. Today, she's conducting a western blot, probing for protein expression in lung cancer cells by running them through the shaker (the pink-and-white contraption known around the lab as "the belly dancer").

Lazy days of summer? "Seems like they never existed," says Khakimova. But she wouldn't have it any other way. This is science. This is what she loves. Besides, it takes some doing to outsmart oncogenesis. "My PI always says, 'Remember, Dela, we want to kill the cancer cells without damaging the other cells.'"

In addition to her experimental research, throughout the Summer Academy program, Khakimova's days are filled with traditional classes as well as field trips (to the National Cancer Institute in Bethesda, Md., for example) and events like crab night at program director/instructor Michael Lotze's home in Shadyside. One of Khakimova's favorite experiences was donning scrubs and observing bypass surgery. "It was different than I imagined. They played happy music and were laughing in the operating room."

The scholars are issued security passes. They're given the same intellectual freedom and expected to show the same level of matu-

rity as any med student or MD. Their mentors provide ideas to start with, but, "[The scholars] make the projects work," says Lotze. He adds that these responsibilities inspire the scholars to work hard, be imaginative, and take on meaningful tasks.

Lotze's own Summer Academy mentee (from 2010 and 2011), Ishan Chatterjee, hopes to study at MIT and Pitt med. At the Intel International Science and Engineering Fair in Los Angeles last spring, he presented part of the project he started with Lotze, and he placed second in cellular and molecular biology. Natalie Nash, a fellow Summer Academy scholar, also attended the event.

Before the summer, Khakimova had been thinking about going to med school to become a gastroenterologist. She knows now that she wants to work directly with patients. "My mom says I make connections with people," she says. But lately Khakimova has been thinking it would be fun to be a researcher, too. "They're the ones coming up with all these treatments," she says.

In its first three years, the academy class has already grown from five students to almost 30. Many of the scholars are from economically disadvantaged backgrounds. The program is one of the med school's several mentoring programs for high school and undergraduate students. (It begins accepting applications in January.)

For most 17-year-olds, achieving a leftward shift on an optical-density graph is not the highlight of the summer. But as Khakimova makes her way around the lab, it's clear that there is no other place she would rather be. ■

STRONGER HEARTS

MOLECULE GOES THE DISTANCE
TO REGROW BLOOD VESSELS
BY DANA YATES

A heart attack is a trauma that the body never forgets. In fact, even after an arterial blockage has been cleared, patients are still at risk of heart failure down the road. But Yadong Wang has developed a method that may one day help people heal from heart attacks once and for all.

Wang is a PhD associate professor of surgery in the University of Pittsburgh School of Medicine and of bioengineering in Pitt's Swanson School of Engineering. He is also on the faculty of Pitt and UPMC's McGowan Institute for Regenerative Medicine.

"The body is able to heal itself in many ways. Just look at broken bones," Wang says. "But when it comes to the heart, the body's natural reaction isn't enough."

Specifically, Wang is interested in heart disease, the leading cause of death worldwide. Caused by a buildup of plaque in the arteries, coronary artery disease can slow down or stop blood flow to the heart. Once a heart attack occurs, there's no looking back. Dead muscle is replaced by stiff scar tissue, and the body's healing process actually causes adverse changes in the heart's shape, size, and function.

This process, called pathological remodeling, involves thinning of the damaged ventricular wall, weakening and over-dilation of the ventricles, and enlargement of the heart. Taken together, these changes can lead to congestive heart failure.

That said, Wang is focused on reducing scarring and disrupting the downward spiral of remodeling by spurring the development of new blood vessels. And his weapon of choice in the fight against heart disease? A substance

called growth factor.

The body makes use of several growth factors, each targeting various areas and handling different functions, including cell differentiation and cell migration. In light of this potency, growth factor is strictly controlled by the body, which releases the substance only when absolutely necessary.

"It's an efficient system," says Wang. It's also a difficult one to manipulate. Previous research has shown that injections of growth factor are unproductive; the body destroys the substance too quickly for it to take proper effect.

Wang wanted to buy growth factor more time to do its job. So, with his research team, he explored how to control the release of growth factor, bundling and delivering it in a way that enabled the body to harness and use the substance efficiently. The solution: Bond a molecule called heparin to the growth factor. As one of the molecules that binds growth factor to its receptor on a cell's surface, heparin may stabilize growth factor and increase its activity.

After converting the resulting water-soluble compound into a coacervate—a collection of oil droplets—the researchers injected fibroblast growth factor-2 under the skin of laboratory mice. In so doing, the Pitt team of bioengineers and stem cell researchers—which includes Hunghao Chu, Jin Gao, William Chen, and Johnny Huard—became the first

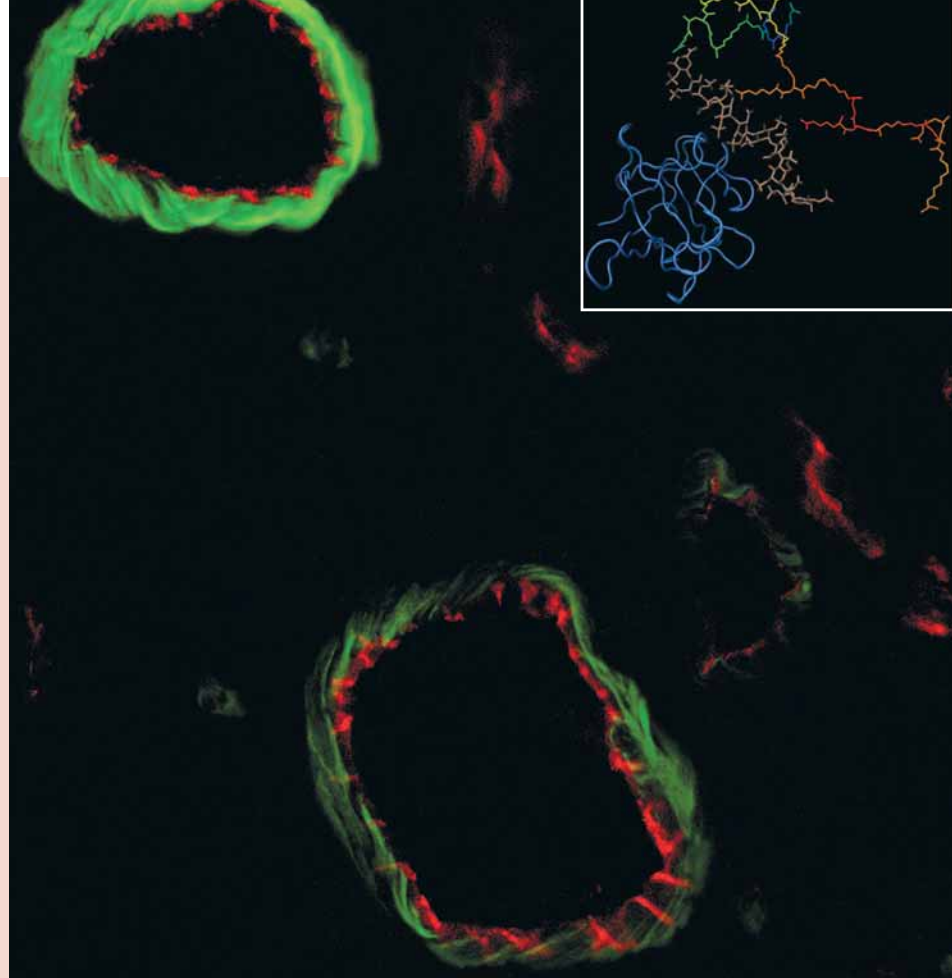
Attempts to fortify damaged blood vessels with growth factor have turned out vasculature that quickly breaks down—until a Pitt team concocted a unique compound (inset), which stimulated blood vessel growth in mice. BACKGROUND: The vessels held strong after a month.

to use a coacervate for the controlled delivery of growth factor.

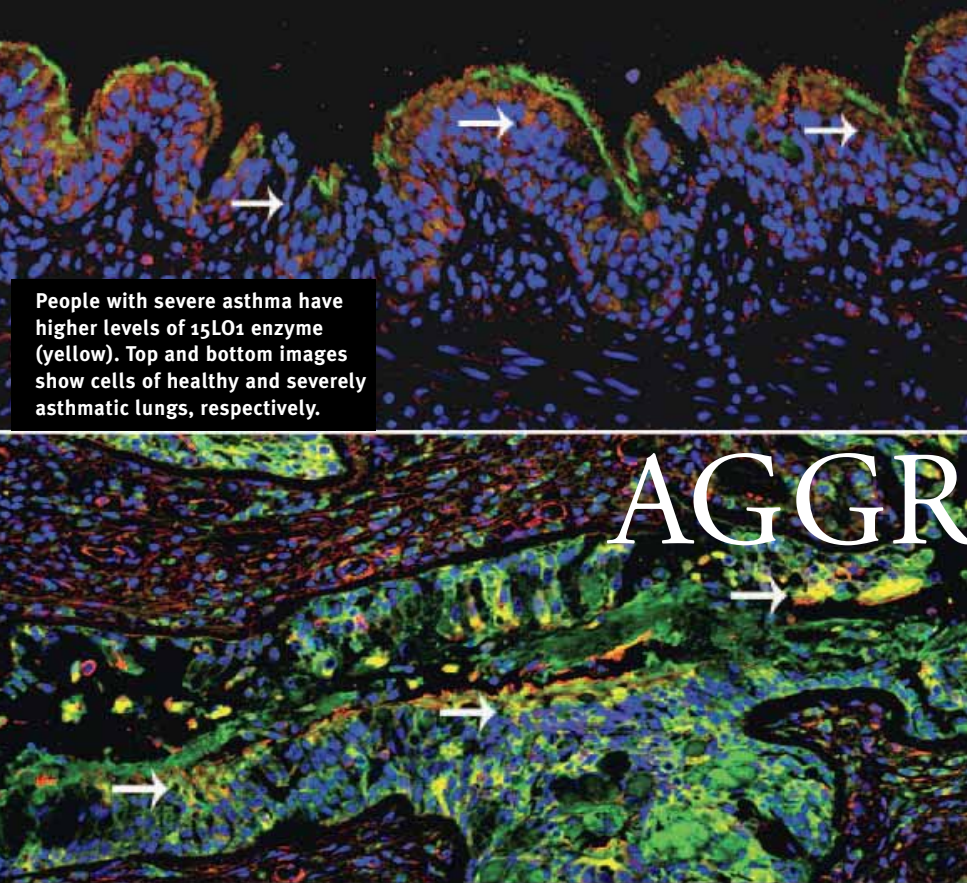
Their findings, which were published in the Aug. 1 issue of *Proceedings of the National Academy of Sciences*, were encouraging. The compound led to the extensive formation of new blood vessels—ones that were robust and resembled arterioles, the small but critical pathways that connect arteries to capillaries. In addition, the new blood vessels were long-lasting. After just one injection of the growth factor compound, the new structures were still intact at least a month later.

The coacervate is not viscous, so it can be injected into the heart using a needle as thin as a strand of hair. The procedure, which could be performed immediately after a heart attack or even a few days later, would be much less invasive than open-heart surgery.

The experimental treatment still must pass muster in clinical trials and be commercialized before it finds its way to patients. In the meantime, Wang is excited about the potential: "We are using nature to help people regenerate and recover," he says. ■



COURTESY HUNGHAO CHU



People with severe asthma have higher levels of 15LO1 enzyme (yellow). Top and bottom images show cells of healthy and severely asthmatic lungs, respectively.

ASTHMA AGGRAVATORS

FINDINGS THAT MAY HELP THE MOST SERIOUS CASES

BY MELINDA WENNER MOYER

COURTESY JINMING ZHAO

When people with asthma suffer an attack, the muscles around their airways tighten and their lungs fill with thick, sticky mucus. More than anything else, it is this mucus that makes it impossible for them to catch their breath. “The generation of mucus is considered to be one of the most important pathological changes in asthma,” says Pitt professor of medicine Sally Wenzel, director of the University of Pittsburgh Asthma Institute at UPMC. This is what led her to piece together the details of a key signaling pathway that activates mucus production—a pathway that she hopes to dial down so that one day people with severe forms of asthma, an inflammatory lung condition that causes 1.6 million emergency room visits annually in the United States, can breathe more easily.

Some asthmatics can effectively manage their symptoms using existing treatments like corticosteroid inhalers. But a small subset cannot, and research suggests that these patients have a unique biological signature characterized by immune cells that overproduce inflammatory proteins. One inflammatory protein in particular, interleukin-13 (IL-13), has interested Wenzel in part because it is known to play a role in mucus production. In 2010, she and colleagues at a handful of institutions assessed the efficacy of an injected inhibitor of this protein on 243 asthma patients, but they were disappointed to discover that it did not significantly help.

Part of the problem with blocking this inflammatory protein is that “the levels that you can find in humans are infinitesimally small,” Wenzel says—it’s difficult to inhibit something that is only present in the body in tiny amounts. Might it be possible, Wenzel wondered, to control mucus production differently, perhaps by targeting mucus-stimulating molecules downstream of this inflammatory protein? Wenzel knew of earlier studies suggesting that the protein activated an enzyme called 15-lipoxygenase 1 (15LO1) and its breakdown product, both of which are present at higher levels in the lung cells of those with severe asthma than in those of healthy people. She also knew that the inflammatory protein turned on an enzyme called extracellular signal-regulated kinase (ERK), which is ramped up more in asthma lung cells, as well. Wenzel wondered whether 15LO1 or its product might somehow help to turn on the kinase and thereby control mucus production in asthma.

To find out, she exposed lung cells taken from 65 asthma patients to the inflammatory protein. Levels of active kinase spiked within minutes. But when she exposed the cells to the inflammatory protein for a week—and then removed the immune chemical and added it briefly again—kinase levels were far higher. This finding suggested to her that perhaps the inflammatory protein stimulates the kinase both directly and indirectly, recruiting other proteins that, over time, take over the activat-

ing job. Suspecting that 15LO1 or its product might be one of these recruited proteins, Wenzel performed the same experiment while inhibiting the production of 15LO1 to see what would happen. This time, the kinase was activated in much smaller amounts, which suggested that 15LO1 or its product plays an important role in stimulating it.

Wenzel believes that the inflammatory protein turns on 15LO1 and then that 15LO1 or its product initiates a feedback loop that causes sustained kinase activation (and ultimately mucus production). She published her findings in August in *Proceedings of the National Academy of Sciences*. Ultimately, it may be that “some of these downstream pathways are going to be more important than IL-13 itself” when it comes to making mucus, she says.

So how exactly does 15LO1 or its product activate the kinase? The kinase gets turned on when its activator molecule breaks away from its natural inhibitor. In additional experiments, Wenzel showed that both 15LO1 and its product bind to this inhibitor and pull it away from its activator, allowing the kinase to get turned on.

Ultimately, Wenzel hopes to find ways to inhibit the activity of 15LO1 or its product (or both) in order to slow mucus production and ease asthma symptoms in people who don’t have other viable treatment options.

“That is definitely, absolutely our goal,” she says. ■