INVESTIGATIONS

Explorations and revelations taking place in the medical school

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Fighting off prostate cancer is one thing; keeping it away is quite another. In fact, 20 to 30 percent of men who have been diagnosed with the disease experience a relapse, according to the Prostate Cancer Foundation. At the University of Pittsburgh, though, researcher Jian-Hua Luo is developing a genetic test that could identify tumors that are most likely to return.

Luo, an MD/PhD and professor in the School of Medicine’s Department of Pathology, is director of Pitt’s High Throughput Genome Center. He is also the senior investigator of a study that showed that genetic changes in the blood and tissue of prostate cancer patients could predict whether their malignancies would reappear and just how aggressive they would be. The findings were published online in the *American Journal of Pathology* in May.

The American Cancer Society reports that one in six men will be diagnosed with prostate cancer during his lifetime. Occurring primarily in men who are older than 65, the disease can be serious, but most men will not die of it (5 percent of those with the cancer will). That’s because prostate tumors grow slowly in the majority of cases. Fast-growing cancers, however, are a significant concern.

“To ensure that men get the timeliest and most appropriate treatment, we need a screening tool that can tell us if a tumor is aggressive,” Luo says. “This information would improve therapies and put doctors on high alert for the likelihood of metastasis.”

Currently, physicians check prostate-specific antigen (PSA) in the blood to monitor tumors. An elevated PSA result is typically considered the first sign of a possible growth. But the tool isn’t perfect; a PSA test can provide normal results when a man actually has cancer and abnormal results when he does not. (For an in-depth discussion of this issue, see “To Screen or Not To Screen,” p. 18.)

Furthermore, even when a PSA test detects cancer, there is no way to conclude whether the situation truly is dire. To know definitively whether or not a tumor is dangerous, a patient must undergo a needle biopsy or surgery to remove his prostate gland. The former carries a slight risk of infection, bleeding, and pain. The latter can dramatically affect a man’s quality of life, leading to problems with sexual function and incontinence.

To find a better way of detecting dangerous tumors, Luo used a mathematical algorithm to study gene abnormalities. Specifically, his process looked at copy number variation (CNV)—the deletion or amplification of areas of DNA within chromosomes.

In a study funded by the National Cancer Institute, Luo and his fellow researchers at Pitt analyzed the genomes of samples from men who had prostate gland removal, prostate tumor samples, blood samples from prostate cancer patients, and samples of benign prostate tissue surrounding the tumors. The samples came from three patient groups: those whose cancer had come back and whose PSA level had doubled in less than four months (usually a sign of particularly aggressive prostate cancer), those whose cancer had recurred with a slowly increasing PSA level that doubled in more than 15 months, and those who had not experienced a relapse more than five years after undergoing surgery.

The researchers discovered that elimination or increased redundancy of DNA fragments occurred in the chromosomes of prostate cancer tumors and even in blood samples and noncancerous tissues adjacent to tumors.

What’s more, when the CNV results were compared to the different patient groups, the CNV analysis consistently predicted relapses in 70 to 80 percent of the cases.

Now, Luo is conducting a larger study to validate his earlier findings. He hopes that CNV measurement will become a routine screening tool for prostate cancer.
Scleroderma’s progress is unpredictable. Some individuals with the autoimmune disease never experience much more than a few spots of thickened skin and a tingling and sensitivity to cold in the fingers and toes. Others, however, are plagued by fibrosis—an overgrowth of connective tissue—not only in their skin, but also in blood vessels, lungs, and other organs. One of the most damaging effects of the disease occurs when collagen, the main component of connective tissue, begins to hijack the lungs, causing scarring that interferes with breathing. So far, not a single treatment can counter fibrosis, says Carol Feghali-Bostwick, a PhD and associate professor of medicine and pathology at the University of Pittsburgh. “There’s nothing that’s effective.”

Feghali-Bostwick may have uncovered a new path to treatment, however. She has been studying the biology underlying fibrosis—particularly in scleroderma and a lung ailment called idiopathic pulmonary fibrosis—for more than 20 years. Her interest is more than academic. Shortly after starting graduate school at New Orleans’ Tulane University in 1988, she was diagnosed with scleroderma herself. “There’s nothing that’s effective.”

Feghali-Bostwick came to Pitt in 1993 as a postdoc with former chief of rheumatology Timothy Wright. She started her own lab in 2002. A few years later, Feghali-Bostwick’s team found two sister proteins—transporters of growth factors within the cell—whose levels were elevated in diseased tissue of people with scleroderma. To see how these proteins contributed to the disease, the researchers trawled for other molecules whose levels were either revved up or dampened by these proteins. One intriguing finding from their fishing expedition had to do with endostatin, a fragment of collagen naturally produced when collagen is cleaved. When other researchers looked back at fibrotic tissue, they found it had significantly more endostatins than their control samples had. Because increased collagen production drives fibrosis and endostatin is cut from collagen, Feghali-Bostwick assumed that endostatin would also promote the condition. But, to her surprise, the opposite occurred—cells bathed at fibrotic tissue, they found it had significantly more endostatins than their control samples had. Because increased collagen production drives fibrosis and endostatin is cut from collagen, Feghali-Bostwick assumed that endostatin would also promote the condition. But, to her surprise, the opposite occurred—cells bathed in endostatin made less collagen and other connective tissue components, and injecting the molecule into explants of fibrotic human skin decreased its thickness. “It was not the result we were expecting,” she says.

The catch was that endostatin starves blood vessels of oxygen—a property that makes it a promising tumor-fighting agent. (It’s being tested against cancer in clinical trials.) It also means endostatin can destroy tissue.

Feghali-Bostwick and her team broke down the molecule into three fragments and tested those molecular regions in living mice, as well as in cultured human skin. Both in living mice and in segments of human skin, one of the fragments was found to carry the magic combination of features: it blocked fibrosis—and even reversed it—but did not affect blood vessels. The study was published in Science Translational Medicine in May.

Now, the group is trying to figure out exactly how endostatin conveys its antifibrotic properties. One possibility, Feghali-Bostwick thinks, is it dials down levels of an enzyme that stabilizes collagen—thus making its rigid structure more prone to degradation. Additionally, endostatin appears to meddle with a molecule called EGR1, which is a central regulator of fibrosis. That suggests that endostatin could effectively treat fibrosis that presents in other organs because of various causes.

Feghali-Bostwick is looking for industry partners who can help move the molecule into clinical trials. Discoveries like hers emphasize the importance of basic research at a time when cuts loom for science funding, she says. “A lot of people assume that discoveries for potential therapies can come only from drug companies,” Feghali-Bostwick says. “This is where it starts. This is where the observations are made.”
Most people wouldn’t think high blood pressure and a deadly bacterial infection have much in common. Yet recent findings by Michael Butterworth, a PhD assistant professor in the University of Pittsburgh’s Department of Cell Biology, imply a link. His work—which spans two vastly different biological systems—suggests that both conditions may arise in part because of the misdeeds of a common sodium-shuttling protein.

Butterworth started studying the epithelial sodium channel (ENaC) more than a decade ago when he was a graduate student at the University of Cape Town in South Africa. ENaC is a protein that embeds itself inside the cell membranes of kidney, lung, and colon cells, allowing sodium ions to pass in and out. It is well known among kidney experts: Mutations in ENaC’s gene can result in two serious diseases, Liddle’s syndrome and pseudohypoaldosteronism type 1, which cause chronic hypertension and hypotension, respectively. Researchers believe that ENaC plays a role in the development of general hypertension, too, a common condition that increases the risk for heart and kidney disease. When too many ENaC channels become active on a cell’s surface, sodium floods the cell along with water—a cascade that leads to a boost in blood volume and thereby blood pressure.

Butterworth’s most recent work suggests that regulating ENaC might be easier than previously thought. Researchers have long believed that cell-membrane protein channels (there are many types, and they are responsible for moving molecules in and out of cells) are transported to and from cell membranes from the interior of the cell in tandem and in response to hormonal cues. But Butterworth’s study suggests that ENaC actually has its own dedicated transportation vesicle.

“This suggests that you can regulate ENaC separately from other transporters,” Butterworth says. So it should be possible to ramp down ENaC’s activity with drugs without also affecting other membrane-bound proteins and causing dangerous side effects.

ENaC influences more than just blood pressure, however. People with cystic fibrosis—a disease characterized by a mutation in the membrane channel for chloride, causing thick mucus to build up in the lungs—are at a much higher risk of deadly respiratory infection with the bacterium Pseudomonas aeruginosa, and Butterworth’s work suggests that ENaC could be a reason why. He and his colleagues recently reported that ENaC affects moisture levels on the surface of the lungs, influencing the lungs’ vulnerability to infection. In a series of experiments, Butterworth showed that the bacteria secrete a protein called alkaline protease, which cuts the loops of the ENaC protein on the surface of lung cells, making ENaC more active. This process causes more sodium and water to get pulled inside lung cells.

Ultimately, the sequence of events dries the outer layer of the lung and prevents the lung’s cilia, tiny spindles that work like molecular mops, from being able to clear away lurking bacteria and other unwanted debris. “The cilia stop beating in those areas, and the bacteria can take hold,” Butterworth explains.

There is good news for these patients, too. To protect their own proteins from being clipped, the bacteria release small amounts of an alkaline protease inhibitor, which could potentially be used in patients to prevent P. aeruginosa from transforming the lung environment to the bacterium’s favor. Butterworth is testing whether this inhibitor, applied to lung cells, could stave off infection. If so, it might one day be possible to give people with cystic fibrosis an inhalable drug containing the protective molecule.

Then the problematic bacteria would become part of the solution.