A century ago, newborns with salty skin often did not last long. They would die of malnutrition from cystic fibrosis. Medical advances eventually addressed the issues of nutrient absorption that the disease creates by damaging the pancreas, and today people mostly think of CF as a lung disease. Yet the disease's roots offer the potential for new discoveries. Pitt researchers recently identified several genetic mutations on the CF gene (CFTR) that do not cause pulmonary problems but can develop into debilitating pancreatitis.

MYSTERY CASES SOLVED

CYSTIC FIBROSIS'S CULPRIT GENE UNDERSTOOD ANEW IN PANCREATITIS

BY NANCY AVERETT

A hundred years ago, the picture of cystic fibrosis (CF) looked quite different. "Back then, when a nursemaid kissed a baby and tasted salt, she knew the baby would die," says the University of Pittsburgh's David Whitcomb, who is an MD/PhD professor of medicine, of cell biology, and of human genetics, and chief of gastroenterology, hepatology, and nutrition. Whitcomb is also the Giant Eagle Professor of Cancer Genetics.

CF is caused by abnormalities of the cystic fibrosis transmembrane receptor (CFTR) gene. The CFTR protein forms a channel for chloride and other ions so that they can move in and out of the cells that line certain glands—including sweat glands—and the sinuses, lungs, intestines, and the pancreas.

In fact, the first organ vulnerable to severe damage from CF is the pancreas. CF can cause pancreatic cysts and fibrosis (hence the name for the disease), as well as the inability to secrete digestive enzymes. Today, CF is thought of as a pulmonary disease, but a century ago patients rarely lived long enough to develop lung problems.

As infants, children with CF died of malnutrition because the food they ate could not be digested and passed through the body. "Even though the babies would eat well, they would whither away," Whitcomb says. This pancreatic disease is also called chronic pancreatitis. Though doctors began treatments for the malnutrition issues using replacement enzymes in the early 1900s, it wasn't until the 1980s that they were able to coordinate specialized treatments reliably. Then researchers largely turned their attention to the next major organ that CF overtakes, the lung.

However, Whitcomb and collaborators decided to take a second look at the origins of the disease in patients without lung disease. For Whitcomb it was a quest: "My career goal is to understand why some people, for no obvious reason, get chronic pancreatitis, with ongoing pancreatic inflammation and irreversible scarring." Perhaps a better understanding of CFTR would hold clues.

Here's how the CFTR channel is supposed to work: Movement of ions results in the movement of water (by osmosis), resulting in hydration of mucus and secretion of fluids.

When the channel isn't working, fluid secretion is drastically reduced, mucus builds, and organs are damaged. The lungs become scarred because bacteria cannot be removed; the pancreas is damaged because the digestive enzymes become trapped in the pancreas and destroy it; and sodium chloride cannot be removed from the sweat gland, causing salt to be left on the skin after the water evaporates from sweat.

Although CFTR has been known primarily as a chloride channel, Whitcomb and colleagues have been testing the theory that in the pancreas, CFTR functions mainly as a bicarbonate channel. In the pancreatic duct, bicarbonate stabilizes the key digestive enzyme trypsinogen, which is then secreted into the small intestine to trigger activation of the other pancreatic digestive enzymes to dissolve food into a liquid so that it can be absorbed by the small intestine. When bicarbonate flow is compromised, the enzymes are not secreted; they become active in the wrong place and begin to digest the pancreas itself.

The investigators set out to identify atypical CFTR mutations: those that didn't impede chloride but did affect bicarbonate flow.

Whitcomb, with Pitt mathematics professor Bard Ermentrout, a PhD, first built a mathematical model of the pancreatic duct cells to predict which molecules were critical to bicarbonate movement into the duct. They predicted that the key molecule was CFTR.

The researchers then genotyped patients who had pancreatic disease but not lung disease to see if they had CFTR mutations. "We took 10 years to collect more than 1,000 patients from 30 centers from around the country," Whitcomb says. "We looked at every genetic change that had ever been reported in the CFTR gene in patients with pancreatic disease and then tested to see whether or not those abnormalities were popping up in our cases of otherwise unexplained pancreatitis." They were. The researchers found several CFTR abnormalities occurring in patients who did not have CF but did have pancreatitis.

Then, Min Goo Lee, professor of pharmacology at Yonsei University in Seoul, cloned nine CFTR variations, put them into living cells, and measured whether chloride and bicarbonate went through in the normal way. Chloride did; bicarbonate did not—just as predicted by the mathematical model.

Next, Ivet Bahar, a PhD Distinguished Professor who holds the John K. Vries Chair of Computational and Systems Biology at Pitt, built a computer model of the CFTR molecule, marked where the mutations changed the amino acid sequence, and simulated the effect of the altered amino acid on CFTR channel function. In some cases, the abnormalities were near the opening of the channel through which the compounds flow; the channel was wide enough for chloride but not bicarbonate. In others, the mutations interfered with a hinge that shifts a key molecule so that it secreted bicarbonate rather than chloride.

The researchers found patients who had one or more of the nine mutations were also more likely to have chronic sinusitis. Men with the mutation were also more likely to be infertile. Both the sinuses and vas deferens duct of the male reproductive system also use CFTR to secrete bicarbonate.

Whitcomb says the study will allow doctors to identify more patients at risk for pancreatitis earlier and to offer better treatment. Their most recent paper was published in the July issue of *PLOS Genetics,* with Pitt PhDs Jessica LaRusch, Ignacio General, and also South Korean researcher Jinsei Jung as first authors.

"This was a reverse engineering problem," says Whitcomb, "and it required many different kinds of engineers to figure it out." ■

HULTON ARCHIVE/GETTY

A Pitt team has discovered how NSAIDs deter colon cancer. Here we see dying cells in a colon tumor from a patient treated with NSAIDs. The red areas indicate the activation of an enzyme by the cell-suicide trigger known as BID.

COURTESY ZHANG LAB

HOW NSAIDs SEND COLON CANCER ON A SUICIDE MISSION

BY HEATHER BOERNER

LIFE AND CELL DEATH

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have long been associated with reduced rates of colorectal cancer; now scientists are gaining insight into the whys and hows behind these life-saving effects: suicide pathways, death receptors, and warped genes.

"What our study provides is a clue as to why NSAIDs selectively kill precancerous cells and leave healthy cells alone," says Lin Zhang, a PhD professor of pharmacology and chemical biology at the University of Pittsburgh and the University of Pittsburgh Cancer Institute, as well as a partner with UPMC CancerCenter. Zhang is coauthor of a paper on NSAIDs with PhD research associate Brian Leibowitz that was recently published in the *Proceedings of the National Academy of Sciences*.

Leibowitz, Zhang, and colleagues started with a mouse model that has a mutation in codon 850 in one copy of the adenomatous polyposis coli (APC) gene—a gene long known to be associated with the development of cancerous polyps in the colon. Then they unleashed NSAIDs on the mouse's cells.

The NSAIDs attacked cells they'd normally pass over. Bingo.

The team found that the loss of the remaining copy of the APC gene sends out a signal to NSAIDs to destroy, helping them get rid of cells that might turn into colorectal cancer.

"Here, we show that's how NSAIDs work, by targeting cells with a specific mutation," says Zhang. "It's really exciting."

They got the same results in human cells.

But how exactly NSAIDs manage to seek and destroy APC-mutated cells that lead to cancer was still a mystery. So Zhang and colleagues set up another experiment to track the progress of NSAIDs in the cell.

The answer, it turned out, was what's known as BID—the BH3 interacting domain death agonist—which triggers cell suicide, also known as apoptosis. It appears that BID is activated by the so-called "death receptor" pathway, and signals to cells containing the mutated genes to self-destruct.

Once it is activated by the death receptor, BID then activates a protein called Bax, which presses a second self-destruct button—another suicide pathway. Put BID and Bax together, and the cells don't seem to stand a chance. And although scientists knew how the death receptor and the suicide pathways worked before, what Zhang and colleagues showed was that BID is the trigger that sets cell death in motion.

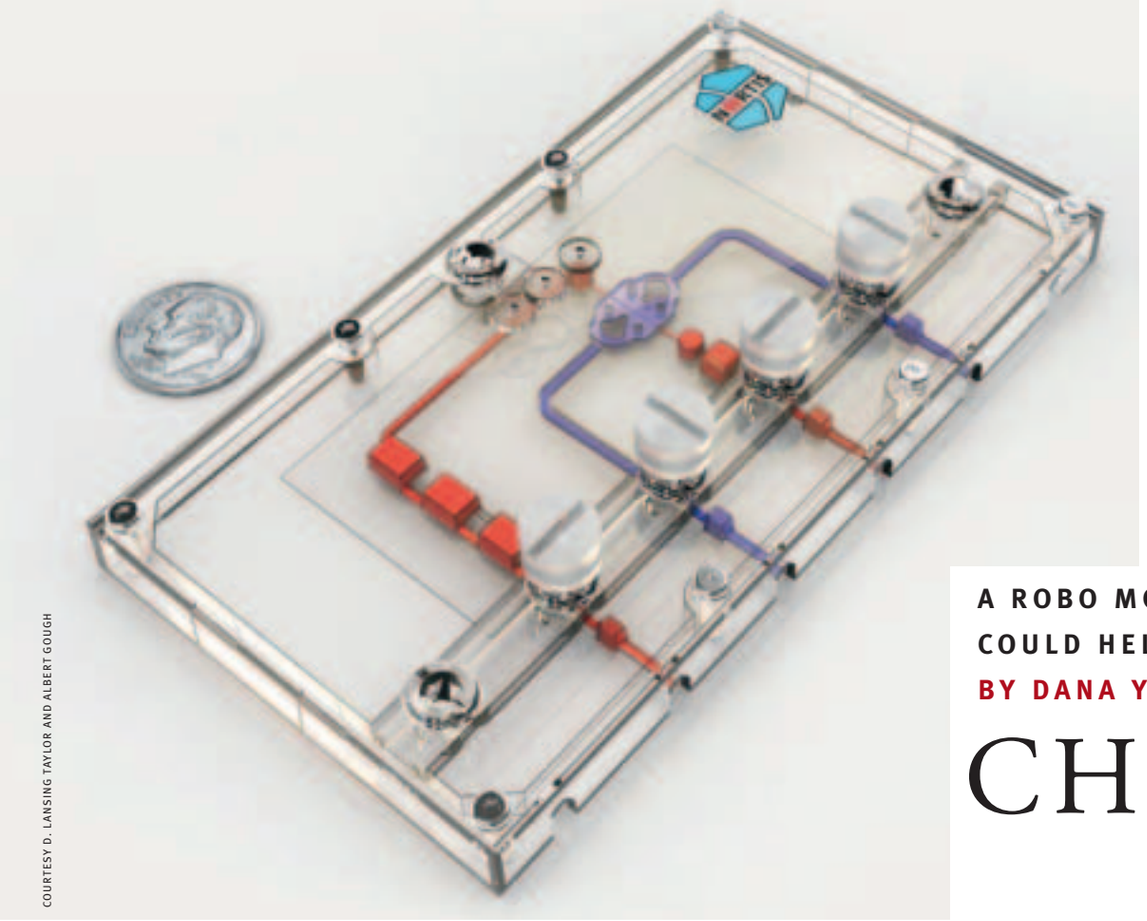
"BID is the cross-talker, the connector in this process," says Zhang. "It's the two pathways together that kill the cells."

Next up for Zhang and his colleagues: Identify the exact molecules to which NSAIDs bind for BID activation. Knowing that should allow researchers to develop small-molecule drugs that bind to those receptors and have the same effects on established cancer cells that NSAIDs have on nascent ones.

Zhang and his team are also searching for other, relatively nontoxic natural supplements—such as dietary components and herbal medicine treatments—that might target the same APC mutations and use the same pathways.

Zhang says he would like to give patients options to prevent cancer before it starts and bring evidence-based medicine to the widely unregulated world of prophylactic supplements.

"Right now, people aren't sure which natural products have the right mechanism," he says. "We can provide clues for other products that are well tolerated and can be used for a long period of time to prevent cancer." ■



COURTESY D. LANSING TAYLOR AND ALBERT GOUGH

Small chip, big change. A team of Pitt researchers is further developing a 3-D, microfluidic model that mimics the structure and function of the human liver's acinus.

A ROBO MODEL OF THE ORGAN COULD HELP DRUG DEVELOPMENT

BY DANA YATES

CHIPPED LIVER

The liver is a master multitasker. The largest internal human organ, it performs hundreds of functions. Among those: fighting infections, producing proteins and hormones, and controlling blood sugar. And, of particular interest to drug researchers, the liver also plays a key role in metabolizing medications. As part of an effort to enhance predictions of drug safety and effectiveness, researchers at the University of Pittsburgh received $5.8 million from the National Institutes of Health (NIH) to develop a miniature model of a key component of this mighty organ. They are creating what's known as a "tissue chip."

Pitt's NIH funding supports the next phase of the agency's Tissue Chip for Drug Screening program, which is refining existing chips and then combining them into an integrated system to simulate the human body's complex functions.

"We've seen huge advances in this microphysiology systems research during the last two years," says Pitt principal investigator D. Lansing Taylor, a PhD, the Allegheny Foundation Professor of Computational and Systems Biology, and director of Pitt's Drug Discovery Institute. "It will take time," says Taylor, "but there's a good possibility that we will one day replace animal testing. This is a powerful tool for basic physiology research."

MODEL UNIT

The new liver model will focus on the acinus, the smallest functional unit of the liver. The Pitt team is further developing a 3-D model that mimics the acinus's structure and activities. The chip will simulate nonalcoholic fatty liver disease, primary liver cancer, liver cancer that has spread from the breast, and other kinds of liver damage.

DRUG DE-LIVERABLES

Pitt researchers will integrate their liver chip with other human tissue chips of the kidney and gut—vehicles that are also vital to drug absorption and metabolism. (Those models are being developed at Johns Hopkins University and the University of Washington.)

"Animal [models] aren't very predictive of human disease. In fact, rodents are only 50 percent predictive of a human [liver's] reaction to a treatment. That's basically a coin toss," says Taylor.

According to the National Academy of Sciences, more than 30 percent of medications that show promise in preclinical studies involving animal models ultimately fail in human trials because they are determined to be toxic. The cells in tissue chips mimic human organs; so scientists are hoping the new technology will let them better predict how well drugs will perform in clinical studies. Those data could reduce the time and money that it takes to develop effective drugs.

THE RECIPE

The process begins by bringing together the fields of nanotechnology, biology, and tissue engineering to make an organ. To develop their model, the Pitt researchers are using cells from resections, surgeries that remove all or a portion of the liver. (Eventually, they hope to use liver cells obtained from stem cells, as well as three other cell types.) The microphysiology systems are built on transparent microchips that feature human cells as well as intricate fluid delivery systems.

HEPATO-TRACKING

The liver platform will have fluorescence-based biosensors that provide real-time physiological readouts and spectrometry measurements that determine toxicity.

The team also will build a database to manage, track, analyze, and model the data collected from the miniature platform. ■