

## INVESTIGATIONS

*Explorations and revelations taking place in the medical school*



The U.S. Department of Agriculture recommends that about 66 percent of dietary fat should be unsaturated. But don't overdo it. An excess accumulation of these fats seems to be harmful in obese patients with pancreatitis.

# UNSATURATED SOLUTION

## RESEARCH INTO PANCREATITIS DEATHS YIELDS AN UNEXPECTED CULPRIT

BY MELINDA WENNER MOYER

Vijay Singh was perplexed. The assistant professor in the University of Pittsburgh's Division of Gastroenterology, Hepatology, and Nutrition was using rodents to study acute pancreatitis, a rapid-onset illness in which the pancreas becomes swollen and inflamed. But time and time again, what Singh learned from his average-weight animals didn't explain what happened to people when the condition was severe: They often died within days. The consensus based on animal studies was that pancreatitis was caused by malfunctioning proteins that ate up the organ; yet researchers had developed drugs to tame these vicious proteins, and all had failed miserably in clinical trials.

"After being in the field for 10 years, one starts thinking, 'What is going on?'" Singh says. To find out, he teamed up with his wife, Pitt's Sarah Navina, an assistant professor of pathology, who had access to the postmortem bodies of pancreatitis victims. Seventy-four autopsies and a handful of lab experiments later, Singh and Navina published a paper in *Science Translational Medicine* uncovering a shocking new cause for deadly pancreatitis: unsaturated fat.

Obese people with pancreatitis are much more likely than lean people to succumb to multi-organ failure. When Singh and Navina compared the pancreases of lean and obese people who had died, they began to see why. Whenever Singh asked his wife to point out a pancreatic region that was necrotic—that is, where cells had died a particularly violent death—"it would be around a chunk of necrosed fat," Singh recalls. In other words,

fat and dead pancreatic cells seemed to go hand in hand. They also found that those with severe pancreatitis were more likely to be obese and have considerably more pancreatic fat than were healthy subjects.

Singh still didn't know whether fat cells caused pancreatic cell death. Their juxtaposition might be a coincidence. When the pancreas' pyramid-shaped acinar cells become damaged, as they do in pancreatitis, their normal processing fails and the cells begin spilling their powerful enzymes everywhere. Back in his lab, Singh placed acinar and fat cells side by side so that everything the acinar cells secreted came into contact with the fat cells and vice versa. The acinar cells soon died. Inside the shared liquid in which the cells were cultured, Singh found high levels of unsaturated fatty acids—essential fats that we get through our food. But the cells survived when Singh added a molecule called orlistat, which inhibits lipases that break down triglycerides. (Orlistat prevents the body from absorbing the fats and is sometimes prescribed for patients so they don't regain lost weight.)

Singh surmised that the spilled digestive proteins prompted the fat cells to release their stored unsaturated acids. Then, lipases (also part of the cocktail of digestive proteins) broke down the unsaturated fats. But what he still didn't know was how the unsaturated fats' byproducts were harming the pancreatic cells. When he looked inside dying acinar cells, he saw that the byproducts were blocking two key steps in the cells' energy-production pathway. Unsaturated fat was literally starving pancreatic cells of energy and killing them.

This discovery explains how unsaturated fats harm pancreatic cells. But how do they cause multi-organ failure? Because blood travels from the pancreas directly to other organs, including the kidneys and lungs, Singh believes that unsaturated acids reach those organs and damage them, too. As the final piece of his puzzle, Singh gave orlistat to obese mice with pancreatitis. They did not develop multi-organ failure and die. "I was very, very, very surprised," says Singh.

The U.S. Department of Agriculture recommends that 66 percent of dietary fat should be unsaturated. Singh speculates that unsaturated fats are only dangerous when we eat too many of them and they are stored. In smaller quantities, these fats are used up rapidly.

"The secret lies in the excess accumulation," he says.

Singh's work was funded by the National Center for Research Resources, Pitt's Clinical and Translational Science Institute, and others. His next goal is to determine which pancreatic lipases break down unsaturated fats and attempt to inhibit them with drugs to protect obese pancreatitis patients. (When taken as a pill, orlistat doesn't get absorbed enough to inhibit pancreatic lipases, says Singh.) But this work could apply to other acute conditions, too: For example, burn victims have higher-than-normal blood levels of unsaturated fats.

There are far more questions than answers at this point, but one thing seems certain: Unsaturated fats aren't harmless. "What is chanted about unsaturated fats being very good—we have to take that with a pinch of salt," Singh says. ■

# SCHOOL OF FISH

A NEW DIRECTION IN  
PARKINSON'S RESEARCH  
BY JUSTIN HOPPER

**E**dward Burton, an MD, looks forward to the day when the two sides of his work coalesce. As a practicing clinician in UPMC's Department of Neurology, Burton spends a few days each week seeing patients suffering from Parkinson's and other neurodegenerative diseases. As a research scientist in the University's Pittsburgh Institute for Neurodegenerative Diseases (PIND) and assistant professor of neurology and of microbiology and molecular genetics, Burton spends the balance of his time with an unlikely subject—zebra fish.

Unlikely, that is, for a practicing MD. Besides being home-aquarium favorites, zebra fish have long been found in labs studying developmental biology. In the early days of their lives, zebra fish are transparent—a state made permanent in the human-engineered species called Casper zebra fish. This gives researchers the opportunity to view physiological changes in a living, growing vertebrate. But in 2004, Burton looked at Pitt's world-class zebra fish facilities—aquarium tanks stacked 10 feet high filling a football-field-sized room—and began to think, “What could these animals tell us about Parkinson's disease?”

“You can do things with zebra fish you can't do with other animals,” says Burton. “If we had a zebra fish model for Parkinson's, because they're transparent we could look at the biochemistry [of the disease and potential

treatments] in a living animal.”

Before zebra fish can be effectively used to study Parkinson's, someone needs to create a model—to understand how the disease affects a zebra fish's behavior and biochemistry in ways that can be correlated with the same effects in a human. (Since 2004, several other labs around the world have begun working on creating this model, often using Burton's publications as groundwork.)

Behavior is relatively easy: Zebra fish are active critters with fast-paced lives, and Burton can tell a Parkinsonian fish within two hours (that makes it possible to do quick, large-sample tests for drug effectiveness). In fact, Burton and others at PIND showed that scientists could make a zebra fish observably Parkinsonian by knocking out its dopamine system.

The biochemistry is tougher to sort out but has come a long way recently, as evidenced by a paper Burton and colleagues published late in 2011 in *The Journal of Biological Chemistry* titled “Hypokinesia and Reduced Dopamine Levels in Zebrafish Lacking  $\beta$ - and  $\gamma$ 1-Synucleins.” This research has made an important advance towards establishing



COURTESY E. BURTON

**A 7-day-old zebra fish larva used to study neurodegenerative disease swims in the well of a plate in Burton's laboratory.**

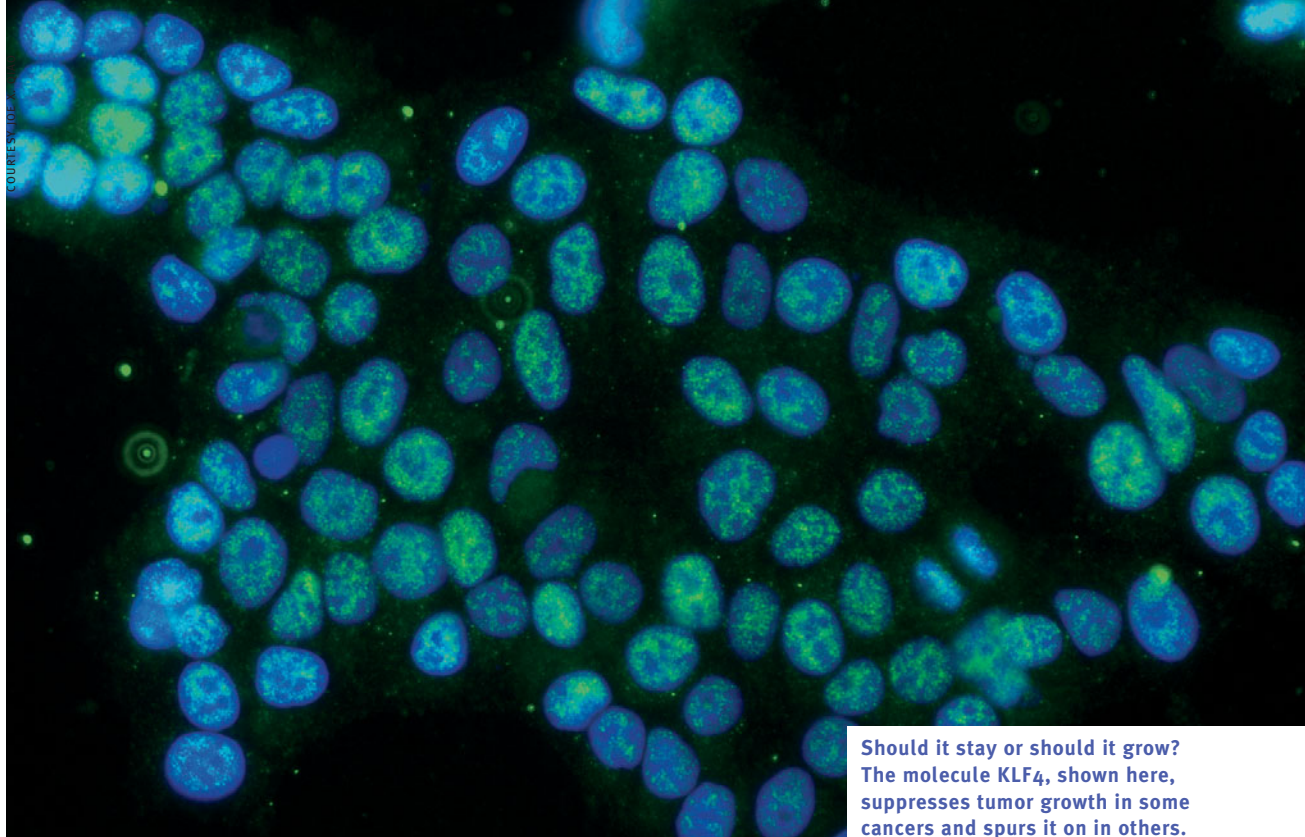
Burton's zebra fish model for Parkinson's by mapping zebra fish synucleins—proteins present in neural tissue that are heavily implicated in Parkinson's research.

“Synucleins are important in Parkinson's. For example, rats lacking  $\alpha$ -synuclein become resistant to toxins commonly used to model Parkinson's,” says Burton. “We wanted to know, ‘Do zebra fish have  $\alpha$ -synuclein?’”

The answer, it turned out, was no. Which makes it somewhat more complicated, but by no means impossible, to create the model. His lab will attempt to genetically engineer a fish with  $\alpha$ -synuclein.

That fish is next on Burton's to-do list. He believes there will be a big payoff in terms of the ability of scientists to witness Parkinsonian biochemistry and degeneration in a transparent model. “It's a long road, because we've got to invent everything ourselves [in terms of techniques and reagents],” says Burton. “Creating an effective treatment for human patients is a multidecade process.” ■

**ONE MOLECULE,  
TWO EFFECTS  
ON CANCER**  
BY DANA YATES



Should it stay or should it grow?  
The molecule KLF4, shown here,  
suppresses tumor growth in some  
cancers and spurs it on in others.

## QUITE A RANGE

**T**he human body can work in contradictory ways. Take, for instance, Kruppel-like factor 4 (KLF4)—a molecule that suppresses tumor growth in colon cancer but spurs it on in breast cancer. So what drives these different responses? According to a University of Pittsburgh researcher, it could all come down to one protein.

Yong Wan, an associate professor in the Department of Cell Biology and Physiology and a member of the University of Pittsburgh Cancer Institute, has studied KLF4 for several years. The molecule is of great importance to current cell research: In 2006, researcher Shinya Yamanaka at Kyoto University discovered that KLF4, in combination with three other molecules, could be used to turn mature cells into a type of cell very similar to embryonic stem cells. Because stem cells are a possible starting point for cancer, KLF4—and its flip-flopping effects on cancerous cells—is of particular interest to researchers such as Wan.

“It was an exciting development, and I wanted to know what triggered the switch and how the process worked,” says Wan. His research on the subject, which was funded by the National Institutes of Health and the

American Cancer Society, was published earlier this year in *Molecular Cell*.

To solve the KLF4 conundrum, Wan and his research team grew cancer cells in the laboratory. From there, the researchers separated proteins produced by the cancer cells from the larger groups in which they normally work. Using sophisticated techniques to examine the interactions of individual proteins, Wan’s team began to focus on a protein made by the von Hippel-Lindau gene. Called pVHL, this protein binds to KLF4, activating a process that ultimately causes KLF4 to lose function.

KLF4 affects the fate of cells by stimulating or inhibiting a network of genes that are involved in a variety of cellular functions. Those tasks include everything from cell-cycle regulation and metabolism to stem-cell renewal and cell death. Essentially, KLF4 is akin to an actor with an impressive range. In some cases, KLF4 acts as a villain, increasing cell numbers and encouraging them to transform into stem cells—two scenarios that lead to cancer. But, as the researchers found, KLF4 can also play the role of a superhero. In fact, in some cells it prompts the production of proteins that stop new cells from forming.

Enter pVHL stage right. When pVHL is

high, the lifespan of KLF4 is cut short; if it is low, KLF4 lasts longer and cell numbers multiply. But there’s more to the story. Cancer cells are abnormal, and as such, they produce atypical proteins. Wan believes these unusual proteins could also influence the interaction between pVHL and KLF4. And for that reason, Wan’s work to better understand these processes is ongoing. Down the road, Wan’s research could contribute to a number of important outcomes. Among them, a new way to predict a future cancer diagnosis and a novel target for drug therapy.

Additionally, Wan’s work may one day change how doctors treat estrogen-receptor-positive breast cancer. Currently, some women with this type of cancer take tamoxifen to interfere with the activity of estrogen, the naturally occurring and tightly controlled female hormone that can be hijacked to promote tumor growth. Tamoxifen, however, can have serious side effects, such as blood clots, strokes, uterine cancer, and cataracts. Moreover, many women develop resistance to the drug. Wan’s research could lead to a new endocrine therapy for hormone-sensitive breast cancer.

“It all starts with knowing how KLF4 is regulated,” he says. ■