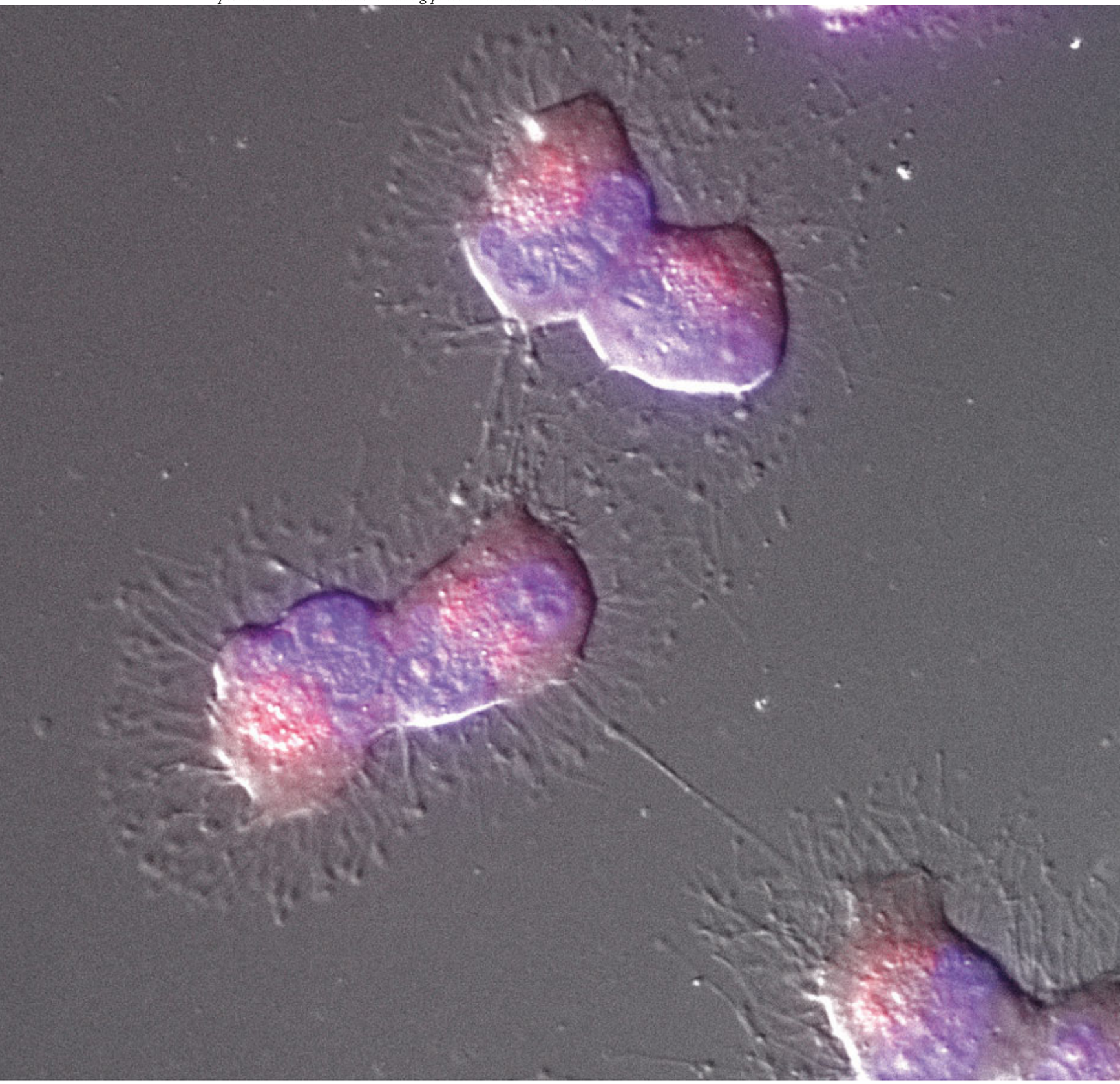

Temozolomide is used to treat glioblastoma multiforme (GBM), the deadliest form of brain cancer, but most patients become resistant to this chemotherapy drug. Here, GBM cells (their nuclei shown in blue) survive after treatment with temozolomide. Pitt scientists have identified genes that may contribute to this resistance.

GENES OF INTEREST

NEW SUSPECTS IN CHEMORESISTANT BRAIN CANCER | BY DANA YATES

Glioblastoma multiforme (GBM) is a disease of extremes. Both the most common and the most lethal type of brain cancer in adults, it is tremendously resistant to chemotherapy. A team of University of Pittsburgh researchers, however, has identified potential weak spots within GBM—and homing in on those targets may make these tumors more sensitive to existing treatments.

Robert Sobol, associate professor of pharmacology and chemical biology and of human genetics, is senior author of a study that identified new targets for drugs that are aimed at destroying glioblastoma multiforme. The investigation, which was led by David Svilar, a PhD student in the School of Medicine's Medical Scientist Training Program, was the December 2012 cover story of *Molecular Cancer Research*.

According to the National Cancer Institute, GBM accounts for about 15 percent of all brain tumors. It's an extremely aggressive cancer with no effective long-term treatments; as a result, patients with these tumors typically survive less than 15 months after diagnosis.

The standard treatment for glioblastoma multiforme involves surgery to remove as much of the tumor as possible, followed by radiation and the chemotherapy drug temozolomide. The chemical agent damages the genome of GBM, which, in turn, prompts the tumor cells to die.

But some genes in the tumor repair the damage; other cells soldier on no matter what is thrown at them, says Sobol.

So, with funding from the National Brain Tumor Society, the National Institutes of Health, and a NYSTAR James D. Watson Investigator Program Award, the research team set out to stop the genome-repair process. Along the way, the researchers discovered that a supplementary treatment—one that could act as a powerful ally of temozolomide—may do the trick.

The team treated tumor cells with temozolomide, but only enough to cause a small percentage of the cells to die. Using siRNA—small RNA molecules that interfere with gene function—the researchers then tested more than 5,200 genes derived from GBM. The idea was to determine which ones helped temozolomide work better and whether there were additional genetic components that could be targeted to improve treatment response.

Eventually, from the original collection of 5,200 genes, the researchers were able to identify 125 "genes of interest"—that is, those that produce proteins that bind tightly with small molecules in order to spur various important activities in the cancer cells, including metabolism, protein synthesis, and cell division. These proteins, therefore, could potentially serve as druggable targets. For example, an accompanying drug therapy could be developed to affect these specific proteins, inhibiting them from doing their jobs and ultimately improving a patient's response to temozolomide.

Now Sobol's team is investigating the genes of interest and their attendant proteins in greater detail. The goal: sleuth out exactly what is happening in the genes and pinpoint the precise functions that are governed by the proteins. It may take years to develop a new drug to support temozolomide, Sobol says, but he is hopeful that his detective work at the molecular level will one day make a major difference in the clinic for those with the deadliest form of brain cancer.

The genes of interest oversee 12 broad categories of cell activities. And while the actions vary greatly, each one appears to be critical to helping the tumor survive the onslaught of treatment.

The genes of interest oversee 12 broad categories of cell activities. And while the actions vary greatly, each one appears to be critical to helping the tumor survive the onslaught of treatment.

"This demonstrates the complexity of the cellular response when exposed to temozolomide. Cells respond in a multitude of ways," says Sobol. ■

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BIG DATA BIG HELP

TYPOGRAPHY: ELENA CERRI

MAKING SENSE OF A POSTGENOMIC WORLD

BY LILY DAYTON

Ansuman Chattopadhyay was a biochemistry postdoc at Vanderbilt University in the 1990s—a time when cloning was hot and Dolly the sheep dominated the headlines.

"Everyone wanted to compare gene sequences, but they needed computer software," he says. "I had no formal training, but I saw the need. I started playing with [data-analysis] tools."

He was a molecular biologist by day, and by night, he became his peers' go-to guy for questions about how to harness the ever-growing amount of genomic data to ask biological-research questions.

Soon after he completed his postdoc, Chattopadhyay went to work for a start-up software company, where he began his training in bioinformatics—the branch of science that connects biology with information science and computer science. The start-up did not survive the dot-com collapse, but something else awaited Chattopadhyay. In 2002, the University of Pittsburgh's Health Sciences Library System (HSLS) was looking for someone with his knowledge and skill set to lead one of the first bioinformatics programs in the country, the Molecular Biology Information Service (MBIS). Chattopadhyay came aboard and built the service from scratch, drawing from his own experience as a young researcher in a postgenomic world. He put himself in the shoes of Pitt scientists and asked, *What do they need?*

When Chattopadhyay held his first library workshop for scientists, the demand was so high he had to triple the number of programs offered. Demand continues to increase as more genomic, proteomic (the full set of proteins encoded in genomes), and other genetic information becomes available. In this information tsunami, it can be difficult for researchers to find the information they need, let alone the tools to process it.

Chattopadhyay and colleague Carrie Iwema, an information specialist in molecular biology, advise researchers at various stages of their projects—whether they're searching for a particular gene, deciphering the structure of a protein, or trying to find the underlying cause for complex disorders like cancer or schizophrenia.

"We don't analyze data for them," says Chattopadhyay. "We train them and give them tools so they can do it themselves."

Between workshops and graduate and undergraduate courses, the two MBIS specialists trained 844 researchers in 67 hands-on bioinformatics sessions in 2012. They also offered one-on-one consultations to 431 researchers last year.

Researchers are not only trained in how to use leading commercial software programs in their research, but they are also given free access. The license for many of these packages costs around $10,000—a high price for most labs. With 1,353 registered users last year, the HSLS program saved Pitt researchers more than $6.2 million in licensing fees. The HSLS site (www.hsls.pitt.edu/molbio) also hosts videos, tutorials, and search engines to locate software, databases, and other resources.

Pitt's approach has proven so successful that it's been used as a model for other molecular library programs cropping up across the country, including those at the University of Southern California, the National Institutes of Health, the University of Rochester Medical Center, and the University of Florida.

"We train people to navigate the human genome—the ultimate blueprint for finding disease," says Chattopadhyay.

"Everything is there; the question is how to find what you want to know." ■

CORBIS

SCRATCHING THE SURFACE

THE EMERGING NEUROBIOLOGY OF ITCH

BY ELAINE VITONE

Pain and itch have an interesting relationship. If you are bitten by a mosquito, you can ease the itch by scratching your skin. And if you take a dose of a powerful painkiller like morphine, you're likely to itch. The interrelatedness of these two experiences has made deciphering the neurobiology of itch—the least understood of our somatic senses—a real head scratcher for scientists. Many have reasoned that the circuits must be the same for both itch and pain.

But Sarah Ross, a PhD assistant professor of neurobiology at Pitt, points out that we experience pain and itch very differently. Pain can happen anywhere in the body, but itch is only on the skin. And we react completely differently to these two sensations: Your hand flies away from the hot skillet, and right at the mosquito bite.

As a postdoc at Harvard, Ross studied a protein called Bhlhb5—a transcription factor known to play a key role in healthy development of the nervous system. She developed a mouse that lacked Bhlhb5 and found that it rubbed little bald spots in its coat. "It looked like it was suffering from itch," she says. She compared the sensory-stimuli responses of these mice to those of normal mice and found that the mutants had a heightened response to itch—but not to pain.

Next, she began looking at more selective removal of Bhlhb5 from the components of the nervous system. She found that a Bhlhb5-free brain produced a normal mouse. A Bhlhb5-free system of primary sensory neurons (where we first encounter sensation) in the mouse's body didn't have any effect either. But when she removed Bhlhb5 only from the spinal cord, the mice had a heightened itch response. She then examined what had gone wrong in the spinal cords of these mice and found that they lacked a particular population of inhibitory neurons—nerve cells that dampen the sensation of itch. They'd been wiped out by the loss of Bhlhb5.

"In retrospect, the spinal cord seems like a logical place to look," she says. "Because primary sensory neurons convey their information to the spinal cord, and that's the first place that the information is processed and modulated." A failed brake system there would send itch signals firing through the body willy-nilly.

Ross tagged this special population of inhibitory neurons, which she dubbed B5-I, in normal mice, providing the first molecular handle on the circuits that have eluded neurobiologists for so long.

Her studies are the first to offer a model of chronic itch as a loss of neurological inhibition (loss of inhibition also underlies chronic pain, interestingly). Her team published these findings in *Neuron* in 2010. Last fall, her grant application to continue her work received a perfect score from the National Institutes of Health.

Now, Ross's team is beginning to draw the circuit for itch in the spinal cord. "And, eventually, we'll trace that circuit further up into the brain." She adds that one exciting thing about this project is the mystery of it: Nobody knows which neurons in the brain give rise to the experience of itch or how the brain distinguishes itch from pain. Lots of things can cause either itch or pain (certain chemicals, for example), yet people tend to experience one or the other, not both. How the nervous system tunes such sensory input isn't clear.

Ross hopes her work might eventually lead to better, more targeted treatments for people who have chronic itch, an underappreciated problem that can devastate quality of life.

"But we're not stopping with itch," she says. "We're going to use the same approach . . . to look at other types of sensation, as well. And this is going to have broad implications for pain, which is another huge problem for people worldwide." ■