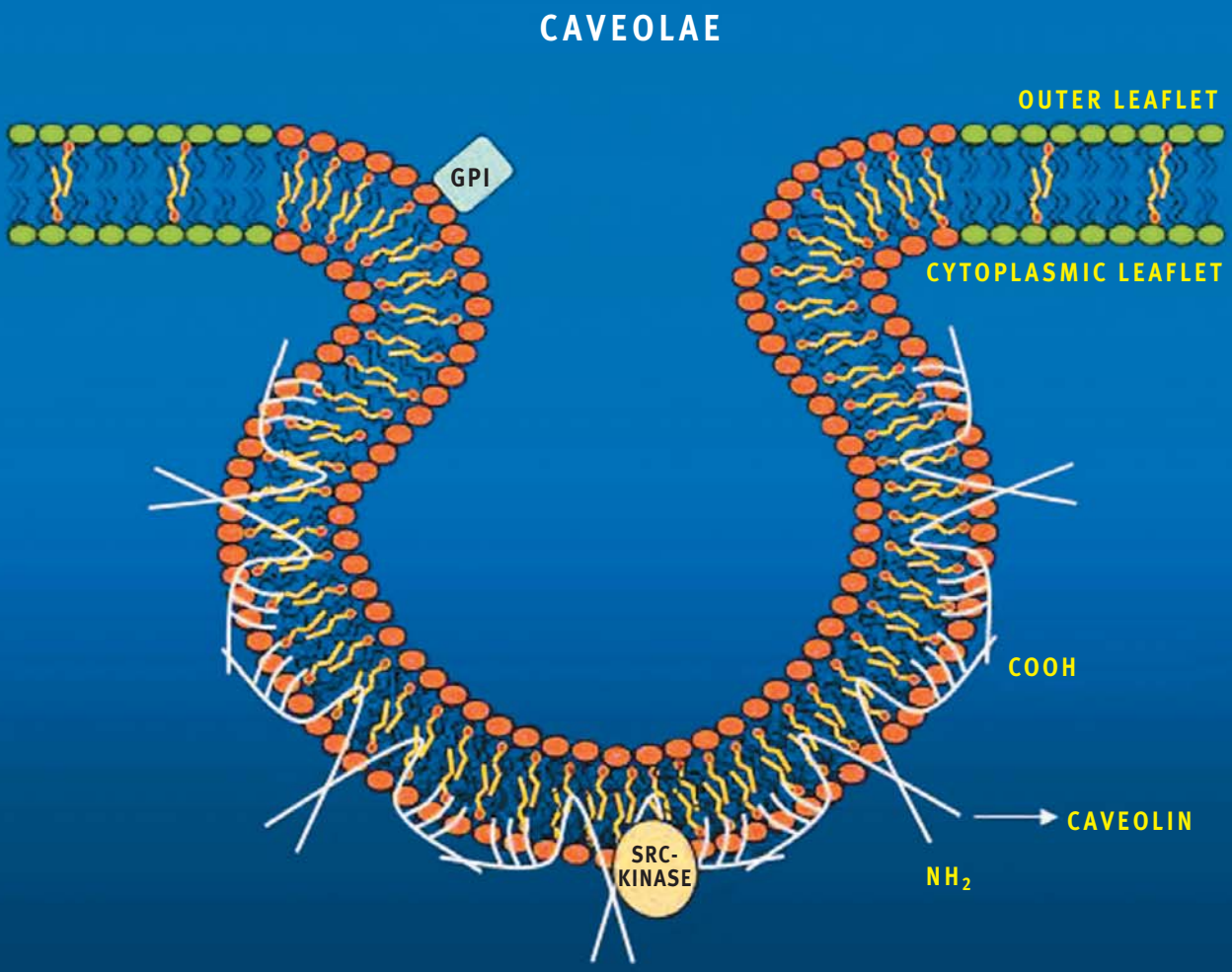


When the protein caveolin-1 is expressed the cell membrane creates caveolae, or "little caves." Through these caveolae, cells execute signaling and transduction. An increase in caveolin-1, Pitt researchers have found, contributes to the development of certain diseases, like emphysema, while reducing the risk of others, including some types of cancer.

SMOKED CELLS

EMPHYSEMA LINKED TO PREMATURELY AGING CELLS | BY REID R. FRAZIER

Imagine for a moment that you are a lung cell. You work for a smoker. (Okay, so life's not fair sometimes.) Specifically, you're an epithelial cell in the alveoli, the grapelike sacs at the lung's outer reaches, where blood trades gases with inhaled air—carbon dioxide for oxygen. (Hey, at least you have an important job.)

Then your host lights up. (You've tried to get him to quit, but that's another story.) Smoke, filled with all kinds of ne'er-do-wells called free radicals, surrounds you. You don't feel so good now—scientists haven't quite narrowed down which of the many miscreants in the smoke are so bothersome, maybe all of them—and you instinctively call for help. The body's inflammatory system arrives to help, but it does more harm than good, and somewhere along the line, you stop working so well. This isn't so good for you. In fact, you and your host could die because of this response.

This is roughly what happens in emphysema, a key component, along with chronic bronchitis, of chronic obstructive pulmonary disease. Ninety percent of COPD patients smoke or have smoked. It is a smoker's ailment.

And it is the country's fourth leading cause of death, affecting perhaps 24 million Americans. By 2020, COPD is projected to be the third leading cause of death on the planet.

Yet relatively little has changed in COPD therapies in the past generation.

"We haven't had anything that's improved patient outcomes since oxygen [inhalation] was introduced in 1980," says Steven Shapiro, Jack D. Myers Professor, chair of medicine, and a pulmonary critical care specialist at the University of Pittsburgh. Oxygen therapy helps patients with low levels of blood oxygen, but, Shapiro says, "It's not rocket science."

Pitt researchers have discovered a cellular pathway that could lead to new COPD therapies in the future. They've shown that high levels of a protein called caveolin-1 play a role in triggering smoking-related emphysema, mainly by prematurely "aging" lung cells through a mechanism called cellular senescence. The frontman for this research is Pitt's Ferruccio Galbiati, of the Department of Pharmacology and Chemical Biology. For the past 15 years or so, Galbiati, a Milan-born PhD, has been studying caveolins, the structural protein—or building block—of caveolae (from the Latin for "little caves"). Caveolae are flask-shaped invaginations on cell membranes. They were discovered in the 1950s, but it wasn't until the 1990s that researchers zeroed in on their role in cell signaling and signal transduction. At the time of these discoveries, Galbiati was a postdoc in the Albert Einstein College of Medicine lab of Michael Lisanti, who is at the forefront of caveolin research and one of the country's most frequently cited biologists.

When Galbiati came to Pitt in 2001, he began researching the role caveolin-1 played in cellular senescence, a naturally occurring state in which cells can no longer reproduce or divide. If enough of an organ's cells become senescent, the organ gradually loses function. Premature senescence, a kind of early aging of the cell, can be induced through oxidative stress, an insult brought on by exposure to free radicals, such as those found in ionizing radiation and other environmental agents. Galbiati's research showed that the presence of caveolin increased senescence during oxidative stress. So he decided to look at the role of caveolin in a classic oxidative-stress environment—cigarette smoking and COPD.

Along with Shapiro and other Pitt researchers, Galbiati found that gene-altered mice that produced no caveolin had less severe emphysema when exposed to cigarette smoke than mice with normal caveolin levels. The group found that caveolin in the mouse's lung cells activated the p53 pathway, an important series of cellular interactions that lead to cellular senescence. Caveolin-1 binds a p53 inhibitor, "hiding" it in the caveolae. Without the inhibitor to brake its development, the p53 pathway cascaded through the cell, leading to senescence and later on, to emphysema.

So, lower caveolin-1 levels, and thereby premature senescence, and you lower the rate of emphysema—simple, right? Not quite. Senescence, it turns out, isn't all bad. It slows down certain types of cancers. (Mutations in the caveolin-1 gene have been linked to breast cancer.) So by taking caveolin-1 out of the lung, you could limit emphysema but encourage lung cancer. Researchers need to ensure that any caveolin-related therapy for COPD does more good than harm, Galbiati says.

Galbiati's investigations into the link between COPD, oxidative stress, and senescence could provide a new way to understand the disease, says Shapiro, who has been on the front lines of COPD research for several years:

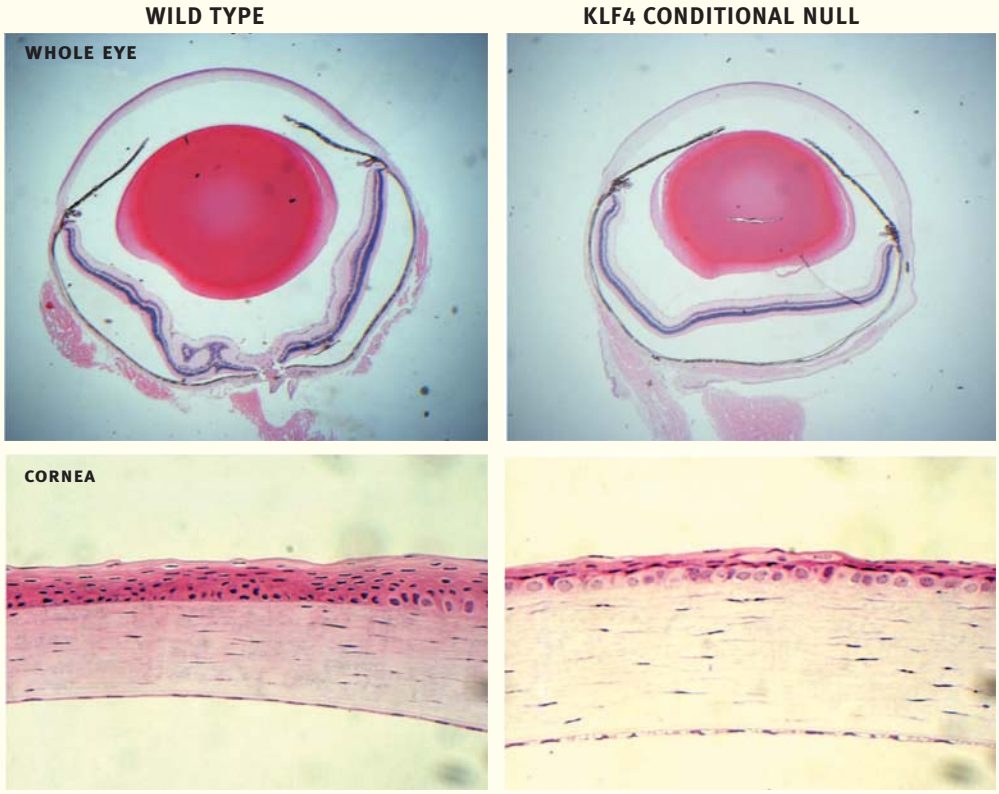
"He's really teased into some of the cellular mechanisms and signal transduction pathways" that initiate emphysema during smoking. "People are just starting to think about these things, so he's ahead of the game." ■

IF YOU CAN READ THIS,

THANK YOUR TRANSCRIPTION FACTORS

A NEW APPROACH TO WARDING OFF DRY EYE

BY ELAINE VITONE



COURTESY S. SWAMYNATHAN

Transcription factor Klf4 plays a key role in eye development and maintenance. Here, an animal study compares a normal eye (top left) and normal cornea (bottom left) with those of a mouse that lacks Klf4 (right column).

Every time you rub your eyes, a few cells in your cornea die. Without all the protective layers and health-promoting moistening mechanisms in your peepers, you'd be in the dark. But until recently, nothing was known about how these layers of protection form and stay healthy.

During his postdoc fellowship at the National Eye Institute, PhD Shivalingappa Swamynathan became interested in studying the role of gene regulation in eye development and maintenance.

"My colleagues found that transcription factors Klf4 and Klf5 were present in the cornea in high amounts," says the assistant professor of ophthalmology, "and yet nobody knew what they were doing." Two years ago, Swamynathan established the Laboratory of Ocular Surface Development and Gene Expression at the University of Pittsburgh to discover their role in the ocular surface.

In many organs throughout the body, Klf4 helps maintain a barrier to mitigate injury, it also keeps bacteria out and life-sustaining water and nutrients in. When you breed mice without Klf4, they die within hours—all the moisture in their bodies evaporates through the skin. This poses a problem if you want to study Klf4's role in the cornea, which doesn't fully form in mice until they're 6 to 8 weeks old.

In 2006, Swamynathan used bacterial CRE recombinase—a method of targeting a specific sequence of DNA and splicing it—to create a new strain of mouse that lacked Klf4 only in the lens, cornea, and conjunctiva portions of the eyes. In the case of the conjunctiva—a clear membrane over the white of the eye—the mice failed to form goblet cells—the cells that produce mucus, which protects and moisturizes the eye.

This finding could one day mean good news for patients with dry eye, a condition that affects as much as 45 percent of the population older than 65. Women are two to three times more likely than men to get it, and hormone-replacement therapy can make it worse. The irritation of dry eye is more than a nuisance—in time, it undermines the health of the cornea, which is vital to our vision. As the body fights to heal itself, the cornea can cloud with immune cells and scars. Eventually, dry eye can even lead to blindness.

A small number of dry-eye patients suffer from the condition out of a lack of goblet cells. And for many others, regardless of the underlying cause, if the condition continues for too long, it can *cause* the loss of goblet cells, fueling a vicious cycle.

Swamynathan used microarray analysis to identify more than 1,000 genes that Klf4 influences. He found that, of each of the five layers of the cornea, all are affected by these genes—from the outermost corneal protective barriers to the innermost corneal endothelium.

And that's just Klf4. Still to come are his Klf5 studies; he's breeding a line of mice for that now.

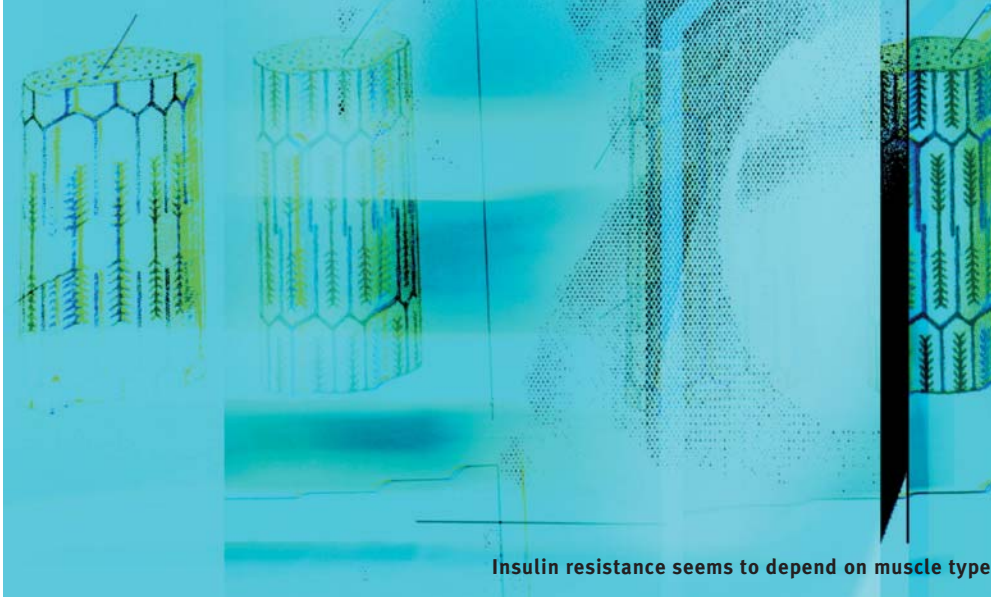
Klf4 and Klf5 are structurally similar and bind to the same sequence of DNA; however, their functions are very different. Klf4 is a suppressor of cell division and Klf5 an activator. Swamynathan looks forward to seeing how they complement one another and what happens when one is absent, or even both are absent.

"We have really a treasure trove of genes to study," he says, a glimmer of kid-in-the-candy-store in his voice.

"I will be characterizing these genes for the rest of my life." ■

GOODPASTER ON DIGESTIVE DOMINOES

BY SARA GOUDARZI



GETTY IMAGES

Insulin resistance seems to depend on muscle type

DIABETES: WHAT'S MUSCLE GOT TO DO WITH IT?

How well our skeletal muscles do their job seems to be a key factor in the development of type 2 diabetes. The specifics, however, are unclear.

Bret Goodpaster, a PhD associate professor of medicine at the University of Pittsburgh, thinks that insulin resistance depends on muscle type and its ability to accumulate potentially harmful lipids that affect sugar absorption.

We rarely think about how our bodies extract energy from food. After a meal, carbohydrates are absorbed into the bloodstream. The rest is pretty much a digestive domino effect: The pancreas secretes insulin, which attaches to cell receptors and activates other receptors to allow sugar absorption. The absorbed glucose is either oxidized or stored for later use.

At least, that's how it's supposed to work.

For about 23 million people in this country who suffer from diabetes, there's a glitch in the process. The pancreas of those with type 1 diabetes can't produce insulin. For people with type 2 diabetes—that's about 90 percent of all diagnosed diabetes cases—the pancreas secretes insulin, but it's either not enough or their bodies' cells don't respond to the hormone.

"It's not a case of insulin not being present, it's just not able to do what it needs to do to stimulate that glucose metabolism," says Goodpaster. Exactly why some people have this inability to respond to insulin has been a mystery to the scientific community, and one that Goodpaster hopes to solve.

A lifelong cyclist, Goodpaster has always been interested in exercise and human performance. He became more serious about the field when his father, at the age of 39, had quintuple coronary bypass surgery. Goodpaster was in high school at the time.

"I made the study of biology, health, disease, and exercise my passion and career," says Goodpaster.

Goodpaster has a master's degree in exercise physiology from Ohio's Kent State University and a PhD in human physiology from Ball State University, in Muncie, Ind. In addition to focusing on type 2 diabetes, he studies age-related loss of functional capacity and mobility.

Goodpaster takes advantage of novel methods, such as the acquisition of muscle and fat tissue from biopsies. By examining proteins and genes in skeletal muscle samples from human subjects, Goodpaster and his colleagues can translate findings back and forth from humans to animals and cell systems and models.

"We can literally look inside the muscle cells and see what might be some of the underlying mechanisms for this insulin resistance," he explains. "We have found that muscle cells accumulate a variety of lipids, some of which appear to be used as fuel for the muscle, whereas others appear to have a negative impact on the way the muscle effectively uses glucose."

He has recently begun to elucidate how diet and exercise seem to work at the muscle level in improving insulin resistance for type 2 diabetes.

"[We] look at accumulation of lipids in muscle to see how this might be related to this fatty acid metabolism, mitochondria function, and then, in turn, how all this potentially plays a role in the development of type 2 diabetes," he says.

Earlier this year, Goodpaster and his team convincingly showed that when older adults lose weight by dieting alone, they also lose significant amounts of muscle mass—which could affect their mobility and independence. But when they combine calorie restriction with exercise, they can nearly completely prevent that loss of muscle associated with dieting.

His group is also one of the few in the world to use positron emission tomography to look at insulin resistance in glucose metabolism of skeletal muscles. In his study, volunteers are infused with a radioactive tracer that emits positrons when glucose is metabolized; that way, researchers can pinpoint locations in the body where it's metabolizing. Physicians typically use this technology in cancer patients to determine whether a tumor is active based on how much glucose it's breaking down.

"We're essentially doing the same thing, except in fairly healthy people, looking at glucose metabolism in muscle response to insulin to see if we can get more mechanistic information about their insulin resistance," Goodpaster says.

This mechanistic information, Goodpaster hopes, will help the digestive dominoes fall into place for the 8 percent of the population suffering from type 2 diabetes. ■