Identity-shifting cells protect against rupture in atherosclerosis

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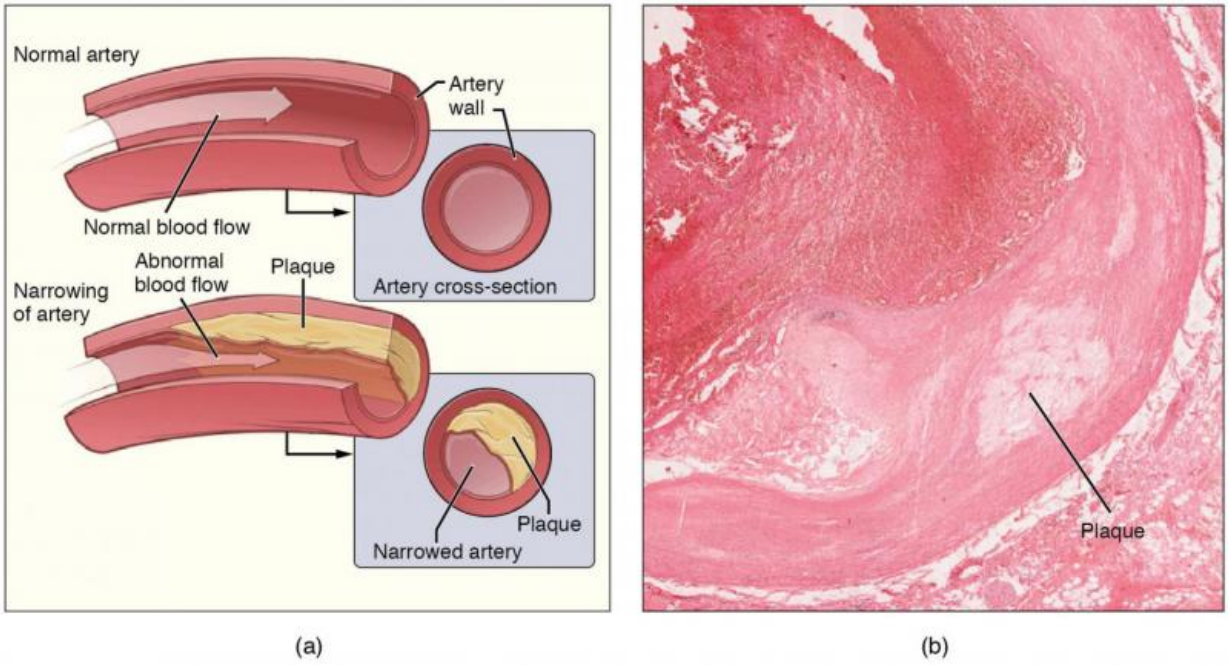

Atherosclerosis is a condition affecting the cardiovascular system. If atherosclerosis occurs in the coronary arteries (which supply the heart) the result may be angina pectoris, or in worse cases a heart attack. Credit: Wikipedia/CC BY 3.0

Changing your identity to protect others might sound like something reserved for comic book vigilantes, but a study led by researchers at the Stanford University School of Medicine has found a select group of cells

in artery walls do just that.

For these cells, the identity shift happens in a disease called atherosclerosis, which occurs when arteries get clogged by plaque, a buildup of fats, cholesterol and molecular particulate.

"We know that things like poor diet and lack of exercise contribute to atherosclerosis," said Thomas Quertermous, MD, professor of cardiovascular medicine at Stanford. "But molecularly speaking, researchers still don't know how the disease progresses or, conversely, is hindered." This new work, he said, takes a big step toward addressing that question.

Plaque grows within the layers of tissue that form the artery, as opposed to inside the tube itself, causing the blood conduit to narrow. Too much plaque tears open the tissue, allowing the built-up gunk to flood the interior of the tube. That leads to a clot, which can cause artery blockage and often a heart attack.

In people with atherosclerosis, cells that make up the artery wall transform and invade the area containing the plaque, or lesion, and form something called a fibrous cap, which acts kind of like a lid to prevent the plaque from bursting into the artery. Now, Quertermous and his colleagues have characterized the identity of these transformed cells, giving key insights into something called plaque stability, which determines the likelihood of a plaque bursting. The more robust the fibrous cap, the more stable the plaque and the less likely it is to rupture.

The team has also pinpointed a gene that seems to be behind the cells' transformation. What's more, when they looked at populationwide genomic data, they saw that individuals who had more activity in this particular gene were at a decreased risk for heart attack.

"Logically, it makes sense—the more cells that help form the fibrous cap, the stronger the protection against plaque rupture and therefore the less risk of a heart attack," said Quertermous, who is the William G. Irwin Professor in Cardiovascular Medicine.

A paper describing the details of the study will be published July 29 in *Nature Medicine*. Quertermous and Juyong Kim, MD, instructor of medicine, are the senior authors. The lead author is Robert Wirka, MD, instructor of cardiovascular medicine.

Smooth muscle cells to the rescue

Under healthy conditions, the smooth muscle cells that make up the wall of arteries control the vessel's dilation, expanding and contracting to regulate blood flow and blood pressure. But when plaque in the artery starts to build, smooth muscle cells begin to shift.

The cells actually move toward the plaque lesion, Wirka said. The genes that make the smooth muscle cells begin to shut off and, in their place, new genes turn on. Then, like Clark Kent to Superman, the smooth muscle cells ditch their everyday identity for a heroic version of themselves—the fibromyocyte, similar to a fibroblast, a cell type known for its role in connective tissue and collagen production. The fibromyocytes then form a protective cap over the cholesterol, fat and molecular debris that compose arterial plaque.

"It's kind of like a scab over a wound," Quertermous said. "Only in this case, the scab also keeps the plaque stable."

Researchers have known that smooth muscle cells reinvent themselves during atherosclerosis, but it wasn't clear exactly what their new identity was. Scientists thought these cells could have a beneficial role, but also suspected they could transform into dysfunctional immune cells that

promote inflammation and worsen the condition.

To figure out the smooth muscle cells' intentions, Wirka, Quertermous and their colleagues used an experimental technique in mice called lineage tracing, which allowed the scientists to track the whereabouts of specific cells and cells derived from those cells. The group labeled arterial smooth muscle cells in the mice with a special chemical that turns the cells red under a microscope. Then, after inducing a mouse version of atherosclerosis, they checked the arteries for signs of smooth muscle cell movement. They observed that some of the red-labeled smooth muscle cells had moved into the plaque from their original homes in the artery.

New place, new name

Wirka and Quertermous then profiled all the cells in the artery, analyzed the collection of cells—immune, smooth muscle, fibromyocyte and more—and ran gene expression analyses to see which genes were "on" in each individual cell. According to the gene expression analysis, the red-labeled smooth muscle cells that migrated to the plaque were sporting a new look.

"These cells exhibited a sort of swap: Patterns of gene activity that track with smooth muscle cells decreased, and activity of genes that give rise to fibromyocytes increased," Quertermous said. "The data allowed us to, beyond a shadow of a doubt, characterize these particular cells in the plaque as smooth muscle cells that have turned into fibromyocytes." Remarkably, Wirka said, the researchers found no evidence that smooth muscle cells transformed into plaque-destabilizing immune cells, resolving a long-standing question in the field.

Next, Quertermous and Wirka used a form of computer modeling to bridge mouse biology to humans. They took tissue samples from human

patients with atherosclerosis who'd received heart transplants. The scientists analyzed cells from the human arteries with the same single-cell gene expression method used in the mouse tissue.

With data from both human and mouse atherosclerotic tissue, the computer model accurately identified cell types, regardless of species. Importantly, the researchers found the same phenomenon occurring in the human arteries: Smooth muscle cells were also transforming into fibromyocytes during human disease.

The gene behind the transition

Quertermous and Wirka went even one step further, identifying the gene that seems to drive the transition from smooth muscle cell to fibromyocyte during atherosclerosis. In Quertermous' earlier work, he identified one particular gene, TCF21, that was associated with a person's risk for coronary artery disease.

"It's been my theory all along that TCF21 gets reactivated in the vessel wall and is a key contributor to this cell type transition," Quertermous said.

So he tested that theory in a mouse model of atherosclerosis, disabling the TCF21 gene to see if it exacerbated the disease. He and Wirka saw that mice without TCF21 formed fewer fibromyocytes overall, fewer fibromyocyte cells in the plaque and a less-sturdy fibrous cap.

Quertermous and Wirka said that TCF21 could likely help guide them toward a new therapy for coronary artery disease. But before taking steps in that direction, there's still more to understand about TCF21 and how it mediates this transformation at the molecular level, they said. "Now we have good evidence that the ability for smooth muscle cells to undergo this transformation to fibromyocytes is important to protect

against clinically significant coronary disease, but the timing and extent of this transformation is likely also important," Wirka said.

More information: Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis, *Nature Medicine* (2019). DOI: 10.1038/s41591-019-0512-5

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