Fundamental beliefs about atherosclerosis overturned

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Gary K. Owens, Ph.D., of the University of Virginia School of Medicine's Robert M. Berne Cardiovascular Research Center. Owens' work shows that efforts to battle atherosclerosis have been built on faulty assumptions about the nature and formation of the plaques. Credit: UVA Health System

Doctors' efforts to battle the dangerous atherosclerotic plaques that build up in our arteries and cause heart attacks and strokes are built on several false beliefs about the fundamental composition and formation of the plaques, new research from the University of Virginia School of Medicine shows. These new discoveries will force researchers to reassess their approaches to developing treatments and discard some of their basic assumptions about atherosclerosis, commonly known as hardening of the arteries.

"The leading cause of death worldwide is complications of atherosclerosis, and the most common end-stage disease is when an atherosclerotic plaque ruptures. If this occurs in one of your large coronary arteries, it's a catastrophic event," said Gary K. Owens, PhD, of UVA's Robert M. Berne Cardiovascular Research Center. "Once a plaque ruptures, it can induce formation of a large clot that can block blood flow to the downstream regions. This is what causes most heart attacks. The clot can also dislodge and cause a stroke if it lodges in a blood vessel in the brain. As such, understanding what controls the stability of plaques is extremely important. "

Until now, doctors have believed that smooth muscle cells - the cells that help blood vessels contract and dilate - were the good guys in the body's battle against atherosclerotic plaque. They were thought to migrate from their normal location in the blood vessel wall into the developing atherosclerotic plaque, where they would attempt to wall off the accumulating fats, dying cells and other nasty components of the plaque. The dogma has been that the more smooth muscle cells in that wall—particularly in the innermost layer referred to as the "fibrous cap"—the more stable the plaque is and the less danger it poses.

UVA's research reveals those notions are woefully incomplete at best. Scientists have grossly misjudged the number of smooth muscle cells inside the plaques, the work shows, suggesting the cells are not just

involved in forming a barrier so much as contributing to the plaque itself. "We suspected there was a small number of smooth muscle cells we were failing to identify using the typical immunostaining detection methods. It wasn't a small number. It was 82 percent," Owens said. "Eighty-two percent of the smooth muscle cells within advanced atherosclerotic lesions cannot be identified using the typical methodology since the lesion cells down-regulate smooth muscle cell markers. As such, we have grossly underestimated how many smooth muscle cells are in the lesion."

Suddenly, the role of smooth muscle cells is much more complex, much less black-and-white. Are they good or bad? Should treatments try to encourage more? It's no longer that simple, and the problem is made all the more complicated by the fact that some smooth muscle cells were being misidentified as immune cells called macrophages, while some macrophage-derived cells were masquerading as smooth muscle cells. It's very confusing, even for scientists, and it has led to what Owens called "complete ambiguity as to which cell is which within the lesion." (The research also shows other subsets of smooth muscle cells were transitioning to cells resembling stem cells and myofibroblasts.)

Researcher Laura S. Shankman, a PhD student in the Owens lab, was able to overcome the limitations of the traditional methodology for detecting smooth muscle cells in the plaque. Her approach was to genetically tag smooth muscle cells early in their development, so she could follow them and their descendants even if they changed their stripes. "This allowed us to mark smooth muscle cells when we were confident that they were actually smooth muscle cells," she said. "Then we let the atherosclerosis develop and progress [in mice] in order to see where those cells were later in disease."

Further, Shankman identified a key gene, Klf4, that appears to regulate these transitions of smooth muscle cells. Remarkably, when she

genetically knocked out Klf4 selectively in smooth muscle cells, the atherosclerotic plaques shrank dramatically and exhibited features indicating they were more stable—the ideal therapeutic goal for treating the disease in people. Of major interest, loss of Klf4 in smooth muscle cells did not reduce the number of these cells in lesions but resulted in them undergoing transitions in their functional properties that appear to be beneficial in disease pathogenesis. That is, it switched them from being "bad" guys to "good" guys.

Taken together, Shankman's findings raise many critical questions about previous studies built on techniques that failed to assess the composition of the lesions accurately. Moreover, her studies are the first to indicate that therapies targeted at controlling the properties of smooth muscle cells within lesions may be highly effective in treating a disease that is the leading cause of death worldwide.

The discoveries have been outlined in a paper published online by the journal *Nature Medicine*: http://www.nature.com/nm/journal/vaop/ncurrent/full/nm.3866.html

More information: KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis, *Nature Medicine* 21, 628–637 (2015) DOI: 10.1038/nm.3866

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