Motherships, Mice, and MicroRNA: Using Nanomedicine to "Turn Off" Atherosclerosis

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"You can think of the carrier as the 'mothership,'" says Haifa Shen, M.D., Ph.D., gesturing toward a blocky pronged cross-section drawn on his office whiteboard. It looks a bit like a castle turret, but the spheres floating between the arms suggest something more unusual. We're not talking about space travel—or even science fiction, for that matter. Instead, the topic is much closer to home and the scale much smaller; Shen is part of a multidisciplinary team pushing the frontiers of cardiovascular research, developing nanotechnology solutions to prevent heart disease. In a review article published in the *Methodist DeBakey Cardiovascular Journal*, Wing Tak Wong, Ph.D.; Shuangtao Ma, M.D.; Xiao Yu Tian, Ph.D.; Andrea B. Gonzalez; Eno E. Ebong, Ph.D.; and Shen discuss the possibilities of using nanoparticles filled with shear stress-induced microRNAs to prevent atherosclerosis.

ATHEROSCLEROSIS 101

In a normal, healthy artery, blood flows smoothly in one direction and massages the vessel's walls with pressure that's called laminar shear stress. In this case, "stress" is a good thing. It causes the vessel's endothelial cells to line up lengthwise, triggering the release of homeostatic factors such as nitric oxide that help keep the vessel's lining smooth and plaque-free.

However, in bends or branches of the artery, blood flow is disturbed, causing eddies and reversals that lower shear stress. Without constant laminar stress, endothelial cells begin to express atherogenic genes. Instead of secreting nitric oxide, the cells produce adhesion molecules and a host of factors that encourage smooth muscle cells and extracellular matrix to proliferate. The endothelium becomes inflamed and "sticky" rather than smooth. Such areas are primed for atherosclerosis; all they need is a trigger.

In individuals with metabolic disorders such as hyperlipidemia, the trigger is cholesterol. Lipid LDL cholesterol molecules attach to the inflamed epithelium then integrate into the vascular walls. As lipids accumulate they oxidize, stimulating an immune response. The endothelial cells secrete more adhesion factors, such as E-selectin, which attract monocytes to attach and penetrate the vessel to ingest the damaged cholesterol.

That's where things start to go wrong. Rather than ingesting the cholesterol and taking off for the liver or spleen for disposal, the monocytes become bloated with lipids and get stuck inside the vessel's walls. These lipid-swollen monocytes transform into "foam cells," the building blocks of atherosclerosis. As more foam cells accumulate, they form a fatty streak, the first sign of an atherosclerotic lesion. The inflammation gets worse, and the cycle perpetuates: E-selectin continues recruiting more monocytes, vascular smooth muscle cells, mesenchymal cells, and extracellular matrix proteins. Ultimately, these coalesce to form the signature form of atherosclerosis: a fatty plaque that gradually expands outward, blocking blood flow. Fast-forward, and atherosclerosis can turn into full-fledged cardiovascular disease, leading to heart attacks, strokes, and over 535,000 deaths each year in the United States.[1]

Until now, physicians had two options to deal with atherosclerosis: reducing its triggers or treating its aftermath. First there's prevention in the form of cholesterol-reducing drugs such as statins or changing lifestyle factors such as diet and physical activity (which is, frankly, still your best bet for avoiding cardiovascular disease). If that fails, surgeons can treat atherosclerosis with arterial bypass surgeries or angioplasties and stents to open the blocked artery. However, these come with their own set of problems.

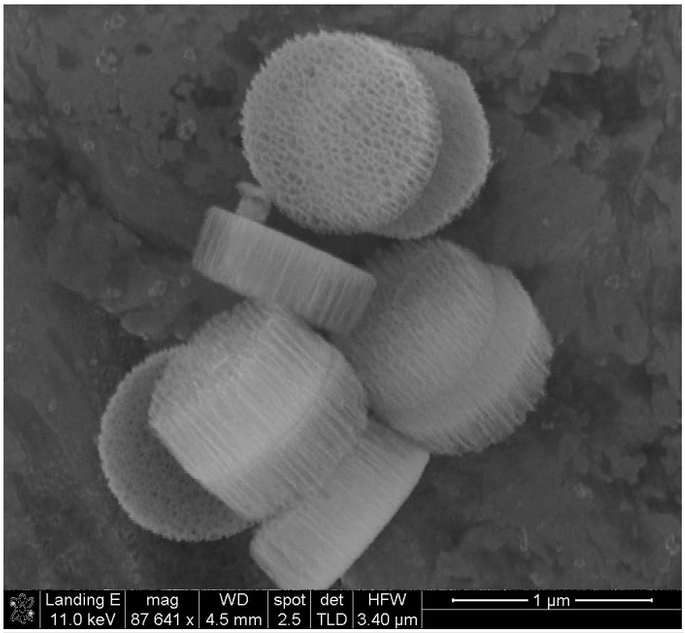
THE PROMISE OF MICRORNA

But now, some scientists are exploring a new approach. What if they could specifically target vulnerable inflamed sections of vessels and "turn off" the atherogenic genes? Could they manipulate intercellular processes to stop plaque from forming in the first place?

Currently, research is still in the "maybe" stage, although in vivo testing is promising (more on that later). But the key to bringing this concept to fruition lies in the recent discovery of microRNAs that can downregulate atherogenic genes.

"The general dogma is that DNA makes mRNA and mRNA makes proteins," explains Shen, associate professor of nanomedicine at the Institute for Academic Medicine and Houston Methodist Research Institute. "But about 15 years ago, it was discovered that certain mRNAs—what we call microRNAs—don't make protein at all. They're the anti-mRNA. Their job is to regulate the DNA–mRNA–protein process by causing certain mRNAs to make fewer proteins."

Endothelial cells generate these anti-atherogenic microRNAs in response to laminar shear stress. Although scientists still don't understand the exact relationship between shear stress, microRNAs, and atherosclerotic plaques, current research suggests that this set of microRNAs bind to atherogenic



This scanning electron microscopy image shows multistage vectors (MSV) produced at the Houston Methodist Research Institute. Each MSV measures 1 µm wide and 400 nm high. Nanoparticles containing microRNAs fit inside the 40-to-80-µm MSV pores. (Image courtesy of Dr. Haifa Shen, Houston Methodist Research Institute)

The discovery of shear-stress-regulated microRNAs gave researchers a potential tool to manipulate intercellular processes to prevent plaque formation. However, a critical question remained: How could they deliver the microRNAs to specific regions of an inflamed, low-shear-stress artery? As Wong and Shen's team reports, microRNAs are unstable outside of cells, so bare microRNAs can't simply be injected into the bloodstream. Viral vectors were also ruled out. Although they have been used successfully in other RNA applications, loading a virus with microRNAs risks integrating the viral DNA into the endothelial cell genome, potentially causing a new set of problems. Researchers needed a new vector that could seek out an inflamed endothelium and deliver functional microRNAs into the target cells. For that, they turned to nanotechnology.

SHARING A NANO-TOOLBOX

Cancer researchers have been paving the way in nanomedicine, so cardiovascular researchers often borrow and modify technologies developed for tumors for use in the heart and vasculature. For instance, when Wong's group needed a vector for their microRNAs, they turned to a silicon multistage vector (MSV) that Shen helped develop on a cancer project; the MSV was originally designed to deliver nanoparticles containing cancer-treating drugs to tumor cells.

Shen's MSV is a prime example of the cross-discipline overlap. "Cancer cells and atherosclerosis lesions secrete the same kind of inflammatory cytokines. We are taking advantage of this similarity and applying the nanotechnology platform originally designed for cancer research to send microRNAs directly to the inflamed atherosclerotic lesion," says Shen.

Thus we arrive back at Shen's "mothership." The mothership is the MSV's outer silicon shell that carries microRNAs to the target cells. The MSV is designed with surface molecules that seek out and bind to E-selectin adhesion factors, thus delivering the package directly to inflamed endothelial cells.

"Inside the mothership are nanoparticles, and inside the nanoparticles are the microRNAs. We use the mothership to send nanoparticles, and thus microRNAs, to the atherosclerotic lesions," explains Shen. "The mothership stays on the surface of the cells, then gradually degrades in the blood. Then the nanoparticles go into the disease lesion cells. Once inside the cell, they break open and the microRNAs are released. So, in a sense, the carrier is the first stage, the nanoparticles are the second stage, and the microRNAs are the third stage."

FROM MICE TO MEN

Wong et al. has their tool, microRNAs, and the carrier, Shen's MSV, to get the microRNAs to the low-shear-stress lesion. But is that enough to stop an atherosclerotic plaque from forming?

The initial results are promising. Wong and Shen's team tested the system in mice fed a high-fat diet, comparing plaque formation when mice were injected with microRNAs encased in free nanoparticles to that of mice who received the MSV-microRNA treatment. The mice treated with microRNAs delivered via MSV developed significantly less plaque compared to mice in the free-nanoparticle group.

Of course, testing MSV-delivered microRNA in mice is a long way from using it in a clinical setting for humans. But the results are certainly exciting. Moreover, Shen is even farther along on a different project using MSVs to deliver multiple cancer-treating drugs. Shen's team is performing "good laboratory practice" studies in animals, the final step before seeking FDA approval to begin human trials. He anticipates starting clinical trials for MSV-packaged cancer drugs in 12 to 18 months. "Once that MSV is approved for clinical use, then this kind of particle can be applied to other drugs, either for cancer or cardiovascular disease," Shen says.

Conflict of Interest Disclosure:
Laura Gerik is assistant managing editor at the *Methodist DeBakey Cardiovascular Journal*.

THE FUTURE OF NANOMEDICINE

Even though Shen is not a cardiovascular expert—his focus has been cancer treatments— his work is a sign of the times in this brave new world of nanomedicine. "To really develop something novel and effective, you need a multidisciplinary research team," says Shen, echoing sentiments from many of nanomedicine's pioneers. "You need to work with people in nanotechnology, medicine, materials engineering, and mathematical modelling. We all have to work together in order to create breakthrough treatments for human disease."

Does nanotechnology represent the future of cardiovascular medicine? As Wong and Shen's studies suggest, it certainly has the potential to revolutionize the way we treat inflammatory diseases. Nanodevices that deliver therapeutics directly to diseased cells could lead to more effective treatments using lower concentrations of drugs, thus reducing negative side effects. In a broader sense, nanomedicine could lessen the need for invasive interventions and allow physicians to target diseases by controlling cellular activities, treating patients quite literally from the inside out. It's an exciting time for nanomedicine's multidisciplinary researchers who have big dreams for the tiniest particles.

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