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Research reveals how blood flow force protects blood vessels

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It is second nature for most of us that exercise protects against heart attack and stroke, but researchers have spent 30 years unraveling the biochemistry behind the idea. One answer first offered by researchers at the University of Rochester Medical Center is that athletic hearts push blood through arteries with greater force, which alone triggers reactions that protect against dangerous clogs in blood vessels.

In the latest study out of Rochester, published recently in the journal *Blood,* researchers demonstrated that they are very close to understanding every step in one flow-sensitive chain reaction that protects arteries. Each step provides an opportunity to mimic with drugs the proven ability of fast, steady blood flow to open up blood vessels and avert the inflammation and blood clots that come with atherosclerosis.

Past research at the Medical Center and elsewhere had determined that two genes, Krüppel-like factor 2 (KLF2) and endothelial nitric oxide synthase (eNOS), are turned on by blood flow force to reverse atherosclerosis, but not how. The current study found for the first time that flow causes a structural change in the enzyme histone deacetylase 5 (HDAC5), which in turn influences whether the two key genes are turned on.

"Obviously we should all be exercising to get our hearts pumping fast, which increases blood flow force through our vessels with all of these molecular benefits," said Zheng-Gen Jin, Ph.D., associate professor of Medicine within the Aab Cardiovascular Research Institute (CVRI) at

the University of Rochester Medical Center, and corresponding author for the study. "Beyond that, the designers of future therapies may manipulate HDAC5 to fine-tune the action of protective genes."

Forcing It

The current study revolves around a signaling process called phosphorylation, in which enzymes called kinases attach a set of molecules called a phosphate group to a target to switch life processes on or off. In cells lining blood vessels (endothelial cells), the attachment of a phosphate group to an HDAC5 kicks it out of the cell's nucleus, perhaps by hiding a label that says it belongs there.

To study whether blood flow force represents one the signals that cause HDAC5 nuclear export, the team designed a virus to invade the cells and swap out the key building blocks that make possible its phosphorylation via blood flow force. Weiye Wang, also a member of the CVRI and first author of the paper, designed the virus. He also attached a fluorescent tag to HDAC5 in the mutated cells so the team could track it as it moved.

What the team found for the first time is that blood flow force (also called sheer stress) does indeed cause the phoshorylation, and export from the nucleus, of HDAC5 in endothelial cells. Importantly, the team also found that flow, by removing HDAC5 from the scene, forces it to break away from the molecule it usually attaches to in the nucleus: myocyte enhancer factor-2 (MEF2).

When free, MEF2 is known to drive the expression of Krüppel-like factor 2, which calls for increases in the supply of endothelial nitric oxide synthase (eNOS). eNOS then builds more of the nitric oxide that tells muscles surrounding arteries to relax, which increases blood flow and lowers blood pressure. When cells were engineered with HDAC5

incapable of being phosphorylated by flow, HDAC5 never left the nucleus, remained stuck to MEF2 and completely blocked the expression of KLF2 and eNOS.

Furthermore, taking away the ability of fast, steady flow to phosphorylate HDAC5 greatly weakened a second lifesaving benefit of flow: it prevents white blood cells from sticking to the cells lining blood vessels, an early, necessary step in the development of atherosclerosis. Fatty diets cause cholesterol deposits to build up within arterial walls, deposits that white blood cells "see" as infections and home in on to drive inflammatory disease. By increasing KLF2 expression, blood flow force is believed to prevent adhesion molecules on cells lining arteries from snagging white blood cells as they float by.

The team also showed through a series of experiments that flow-induced HDAC5 phosphorylation depends on the well known calcium/calmodulin pathway. The team theorizes that the force of flow changes the shape of calcium channels on the surface of endothelial cells, which enables calcium to rush into the cells and turn on calmodulin, which attaches to an as yet unidentified kinase that phosphorylates HDAC5.

Identifying such an enzyme would complete the first diagram of a flow-sensitive, protective signaling pathway. Jin's lab has zeroed in on calmodulin-dependent kinases as likely suspects, and is designing experiments that will shut down the genes coding for them to see if that stops the phosphorylation of HDAC5 by flow. Should that be the case, the team will seek to screen for drug candidates that encourage the action of these enzymes.

Along with Jin and Wang, the effort was led at the Aab CVRI by Chang Hoon Ha, Bong Sook Jhun and Chelsea Wong. Mukesh Jain led a partnering effort at the Case Western Reserve University School of

Medicine. Much of the early work in area was done in the labs of Bradford Berk, M.D., Ph.D., CEO of the University of Rochester Medical Center, and Jun-ichi Abe, M.D., Ph.D., associate professor within the Aab CVRI. Funding for the work of Jin's team came from the American Heart Association, the American Diabetes Association and the National Heart, Lung and Blood Institute (NHLBI), part of the National Institutes of Health. The article was published online on Dec. 30, 2009.

"If we could free MEF2 from HDAC5 with a drug, we could mimic flow force to enhance KLF2 and eNOS expression and reverse inflammation in vessel walls," Jin said. "That promises to be extremely useful, and potentially to stave off disease underway in the blood vessels of humans."

Provided by University of Rochester Medical Center