Researchers identify possible drug target for deadly heart condition

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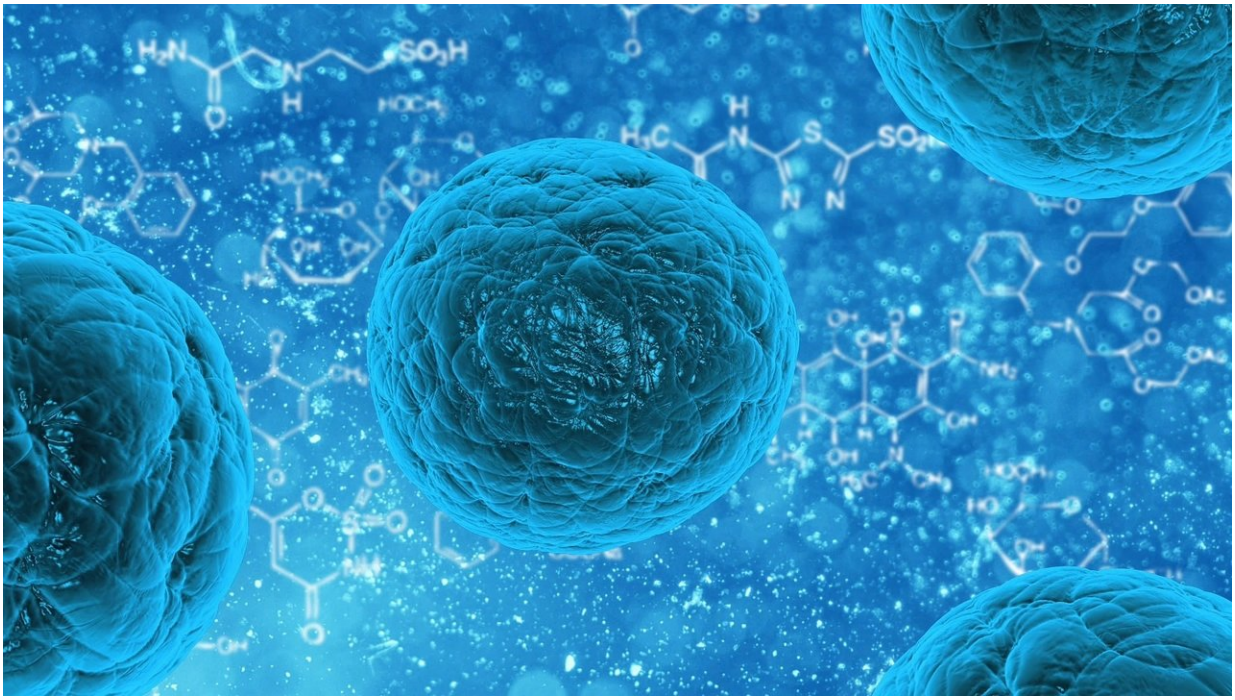

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A genetic mutation linked to dilated cardiomyopathy, a dangerous enlargement of the heart's main pumping chamber, activates a biological pathway normally turned off in healthy adult hearts, according to a study by researchers at the Stanford University School of Medicine.

Chemically inhibiting the pathway corrected the mutation's effects in

patient-derived heart cells in a lab dish, the study found. The researchers accomplished this with drugs already approved by the Food and Drug Administration.

The findings, which will be published online July 17 in *Nature*, suggest that existing drugs could one day be repurposed to treat dilated cardiomyopathy. More broadly, the study demonstrates how patient-derived heart cells can help scientists better study the heart and screen new candidate drugs.

"With 10 milliliters of blood, we can make clinically usable amounts of your beating heart cells in a dish," said the study's senior author, Joseph Wu, MD, Ph.D., director of the Stanford Cardiovascular Institute and a pioneer of the technique. "And if you tell me you're taking some kind of medication for your heart—like beta-blockers or statins—we can add that to see how it affects your heart. That's the beauty of this approach."

The researchers studied heart muscle cells grown from patients with a genetic mutation associated with dilated cardiomyopathy. Heart cells with a mutation in lamin, which forms part of the nuclear envelope, failed to beat properly—just like in patients with the disease. The scientists found that the defect was the result of a surge in the platelet-derived growth factor pathway. This pathway is important in the formation of blood vessels and normally only activates when the heart first forms or is under stress. Treating heart cells with existing drug inhibitors of the pathway restored regular, rhythmic beating.

Don't stop the beat

In dilated cardiomyopathy, the heart's main pumping chamber, the left ventricle, expands so much that the heart can no longer beat regularly. Patients experience shortness of breath, chest pain and, in severe cases, sudden and deadly cardiac arrest. Approximately 1 in every 250

Americans suffer from a form of dilated cardiomyopathy of which the exact cause is not known, though 20% to 35% of these cases run in families.

Previous studies correlated mutations in lamin to familial dilated cardiomyopathy, but it seemed like an odd connection. Lamin forms part of the nuclear envelope, a structure that separates DNA from the rest of the cell and regulates the movement of molecules in and out of the nucleus—not exactly an obvious candidate for regulating heart function.

"We were puzzled," said Wu, the Simon H. Stertzer, MD, Professor and professor of medicine and of radiology. "Why would a mutation in a nuclear envelope protein not involved in squeezing of the heart, such as sarcomere protein, or in electrophysiology of the heart, such as an ion channel, lead to dilated cardiomyopathy?"

To solve the mystery, the researchers needed to study the lamin mutation in heart muscle cells. Excising a tissue sample from a patient's heart, an invasive medical procedure, was not a good option. Mouse tissue was another possibility, but mouse findings don't always hold up in humans.

Instead, the scientists generated heart cells by turning back the clock on patient-derived skin cells to make induced pluripotent stem cells, which can become any of the specialized cells found throughout the body. While the researchers used skin cells in the study, Wu said that the same technique can also be done with 10 milliliters of blood—roughly two teaspoons.

Heart muscle cells grown in a dish pulse rhythmically, just as they do in the body. But cells from members of a family with lamin mutations and a history of dilated cardiomyopathy beat noticeably off-rhythm and had irregular electrical activity. The defect could be fixed by swapping in a normal copy of the gene with a gene-editing technology. Introducing the

mutation into cells from healthy patients caused those cells to beat off-rhythm too. Cells with the lamin mutation had abnormal levels of calcium, a key ion that regulates muscle contractions.

Getting back on rhythm

As part of the nuclear envelope, lamin interacts with a tightly packed form of DNA known as heterochromatin. Interestingly, the researchers found by various DNA sequencing techniques that cells with the lamin mutation had fewer regions of heterochromatin. Since DNA packing affects what genes get activated or shut off, the researchers looked at gene-activation patterns to see which pathways went awry in cells with the mutation—and what they could do about it.

"Although we did all this sequencing and other experiments, without a specific target, we cannot provide the right therapy," said the study's lead author, Jaecheol Lee, Ph.D., a former postdoctoral scholar who is now an assistant professor at the School of Pharmacy at Sungkyunkwan University in South Korea.

They found nearly 250 genes that were more highly activated in mutated cells than in normal cells. Many of the genes were part of the platelet-derived growth factor, or PDGF, pathway. When the researchers tested heart tissue from dilated cardiomyopathy patients with a lamin mutation, they saw signs that the same pathway was activated.

But did activation of the PDGF pathway cause abnormal rhythms or the other way around? To test this, the researchers treated heart cells with two drugs, crenolanib and sunitinib, that inhibit a key PDGF receptor. After treatment, heart cells with the lamin mutation began beating more regularly, and their gene-activation patterns more closely matched those of cells from healthy donors.

These two drugs are FDA-approved for treating various cancers. But previous work from Wu's team shows that the drugs may damage the heart at high doses, which will make finding the right dose or a safer alternative critical.

The current study is part of a broader effort by the researchers to use these patient-derived cells in a dish to screen for and discover new drugs. It's why the Wu lab has generated heart muscle cells from over 1,000 patients, including Wu, his son and daughter.

"Our postdocs have taken my blood and differentiated my pluripotent stem cells into my brain cells, heart cells and liver cells," Wu said. "I'm asking them to test some of the medications that I might need to take in the future."

More information: Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy, *Nature* (2019). DOI: 10.1038/s41586-019-1406-x

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