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Researchers use stem cells to pinpoint cause of common type of sudden cardiac death

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When a young athlete dies unexpectedly on the basketball court or the football field, it's both shocking and tragic. Now Stanford University School of Medicine researchers have, for the first time, identified the molecular basis for a condition called hypertrophic cardiomyopathy that is the most common cause for this type of sudden cardiac death.

To do so, the Stanford scientists created induced pluripotent stem cells, or iPS cells, from the skin cells of 10 members of a family with a genetic mutation that causes the condition. The researchers then coaxed the cells to become heart muscle cells so they could closely study the cells' behavior and responsiveness to the chemical and electrical signals that keep a heart beating normally. They also used these bioengineered heart cells to quickly pinpoint the drugs most likely to be effective in human patients and to study their potential as preventive medications.

"For obvious reasons, it's difficult to get primary human heart tissue from living patients for study," said cardiologist and stem cell researcher Joseph Wu, MD, PhD. "Moreover, animal hearts are not ideal substitutes either because they contract differently and have a different composition than human hearts. As a result, it has been difficult to show the specific cause of heart failure, whether it's due to enlargement of the organ or if it's caused by abnormalities at the single-cell level."

The research highlights what many experts consider to be some of the main advantages of iPS cells—the ability to quickly create patient-specific cells of nearly any tissue type for study, as well as to allow rapid

and safe drug screening.

Wu, an associate professor of medicine and the co-director of the Stanford Cardiovascular Institute, is the senior author of the research, which will be published Jan. 3 in *Cell Stem Cell*. Postdoctoral scholars Feng Lan, PhD, and Ping Liang, PhD, and graduate student Andrew Lee are co-first authors of the work.

Hypertrophic cardiomyopathy, which affects about 0.2 to 0.5 percent of the population, is a condition in which the muscle of the heart is abnormally thickened without any obvious physiological cause. It is also a leading cause of sudden cardiac death in young, seemingly healthy athletes. Clinical symptoms, including arrhythmia and chest pain when exercising, typically emerge in late teenage years or young adulthood, but can occur at nearly any age.

Although clinicians have known for some time that the disorder can be caused by any one of several genetic mutations, until now it has not been clear how these mutations cause the thickening and eventual failure of the heart muscle.

The Stanford team compared cells from family members of a newly diagnosed 53-year-old woman with a mutation in the MYH7 gene, which partially encodes for a protein in the heart called beta myosin. Mutations in this gene have previously been associated with hypertrophic cardiomyopathy. Four of the woman's eight children had inherited the mutant copy of the gene from their mother; the other four carried two healthy copies of the gene.

The father of the children did not have the mutation.

The two oldest affected children, aged 21 and 18, displayed slightly enlarged hearts; the youngest affected children, aged 14 and 10,

displayed a slight increase in blood volume (another symptom of the condition).

Wu and his colleagues collected skin samples from all 10 family members and used them to create iPS cells in the laboratory. They then compared iPS-generated heart muscle cells, or cardiomyocytes, from the family members who have the mutation to those without it. They found that although all the cardiomyocytes appeared normal at first (e.g., beating rhythmically in a laboratory dish), the cells with the mutation began to change after about 30 to 40 days in culture.

"When we compared samples from the whole family, we discovered that these cardiomyocytes would start to display abnormal rhythms and elevated calcium levels over time," said Lan, one of the co-first authors. "Although it had previously been speculated that calcium processing may involved in hypertrophic cardiomyopathy, this is the first time the calcium's role has been demonstrated conclusively in human cells. In the past, much of the focus had been on whether the abnormal growth, or fibrosis, seen in affected hearts could itself be the cause of the arrhythmia experienced by patients."

Under normal conditions, waves of calcium entering heart muscle cells cause the muscle to contract to pump blood throughout the body. Efficient contraction depends on a tightly managed system that controls when, how and where calcium is admitted into the cell. Perturbations in this system can cause abnormal rhythms, but until now there was no way to prove the calcium processing is the culprit in patients with hypertrophic cardiomyopathy.

"In our study, we demonstrate that this is actually happening at the cellular level," said co-first author Lee. "Fortunately, this happens much more quickly in a laboratory dish than it does in an intact human organ. In a human subject, we would have to wait a decade or more to see signs

of disease."

The accelerated development may be due to the physiological stresses of growing in a laboratory dish, or perhaps because, unlike the microenvironment in a whole heart, there are no neighboring support cells to compensate for emerging deficiencies in single cells, the researchers speculate.

In the study, the affected cardiomyocytes were then treated with drugs currently approved for patients with hypertrophic cardiomyopathy or other arrhythmias. The Stanford team found that drugs that modulated the activity of channels in the cell membrane through which calcium passes could restore normal rhythms to the affected cells. One drug in particular, known as verapamil, was also able to prevent the hypertrophy, or abnormally large size, of affected cells. This finding suggests that earlier treatment might be better than waiting for symptoms to arise.

"Our results indicate that we may need to rethink our current treatment strategy," said Wu. "Maybe by the time a person begins to exhibit clinical symptoms, the damage could not be easily undone. Earlier intervention may soon be possible in the near future. The hope is to be able to use genetic techniques, such as DNA sequencing, coupled with iPS cell-derived cardiomyocytes to identify potential patients at risk at a much earlier stage. We may also be able to treat patients earlier with the right medications to prevent enlargement and damage of the heart muscle from taking place in the first place."

The Stanford researchers have started to study iPS cells from patients with other mutations associated with hypertrophic cardiomyopathy, as well as to test other known drugs and new drugs under development. "Instead of conducting clinical trials on patients, which is a much more costly and painstaking process, we may one day be able to do the first stages of a trial primarily on cells in a dish. These iPS cells would consist

of cells from patients of different ages, genders, ethnicities and cardiovascular disease backgrounds for us to assess drug effects on a population level," said Wu.

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