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Researchers Spur Growth of Adult Heart Muscle Cells

Howard Hughes Medical Institute researchers have induced adult heart muscle cells to proliferate in adult animals. The researchers say the studies provide a framework for exploring the molecular mechanisms that might one day make possible clinical regeneration of damaged heart muscle.

It has long been believed that after initial development, the heart muscle cells can no longer proliferate. The new findings demonstrate that by eliminating a brake that halts the division of the muscle cells, researchers can then trigger the proliferation of the cells by adding specific cardiac growth factors.

The researchers will publish their findings in the May 15, 2005, issue of the journal *Genes & Development*. The paper was published online on May 3.

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The research team was led by Howard Hughes Medical Institute investigator Mark T. Keating at Children's Hospital Boston and Harvard Medical School. Keating and his colleagues collaborated with researchers from the University of California, Los Angeles and Boehringer Ingelheim Pharmaceuticals in Ridgefield, Conn.

"There has been a longstanding controversy going back more than a hundred years about whether cardiomyocytes (cardiac muscle cells) in adult mammals have the capacity to proliferate," said Keating. "While there have been occasional studies indicating this possibility, the dogma has been that they can't." According to Keating, researchers thought that once an animal's heart has fully developed, cardiomyocytes lose the molecular plasticity that allows them to divide.

In their studies, Keating and his colleagues explored whether an enzyme called p38 acted as a brake on proliferation of adult cardiomyocytes. Although p38 is known to be involved in regulating cell division, very little is understood about its possible role in cardiomyocytes, said Keating.

In their experiments, the researchers explored whether knocking out p38 activity in cultures of rat cardiomyocytes could induce proliferation. The researchers found that knocking out p38 in the cell cultures of both infant and adult rats—in the presence of a cardiomyocyte growth factor protein called FGF1—induced DNA synthesis, an important component in cell proliferation.

They also found other indications that the p38-knockout cells were undergoing mitotic proliferation. For example, they found that the proliferating cells dedifferentiated, meaning they temporarily lost the characteristics of mature heart muscle cells and reverted to a more fetal type of proliferating cell. Additional genetic studies of p38 inhibition showed that it regulates genes thought to be critical for cardiomyocyte proliferation.

Importantly, they found that the cardiomyocytes lacking p38 activity could continue to proliferate through many rounds of cell division in the presence of FGF1. “The fact that we could show that were multiple rounds of division is important, because clinical regeneration of cardiac muscle would require the cells to divide again and again,” said Keating.

“As a result of these experiments, we felt quite confident that we could induce cardiomyocytes to proliferate, at least in vitro,” said Keating. “However, an in vitro system is quite artificial, and there could be many reasons why it would not be relevant in vivo.” So, in further experiments in collaboration with co-author Yibin Wang and his colleagues at UCLA, the researchers tested whether a genetic knockout mouse lacking p38 would show evidence of cardiomyocyte proliferation. Those experiments did yield significant evidence for such proliferation, said Keating.

“These findings represent the first step toward showing that drugs that eliminate p38 activity could reduce scar tissue formation and enhance cardiac regeneration after cardiac injury,” said Keating. The formation of scar tissue in damaged hearts is the major reason myocardial infarctions lead to subsequent abnormalities and compromised heart function, he said.

In future studies, Keating and his colleagues plan to explore whether they can induce significant regeneration of heart muscle by eliminating p38 activity and providing cardiac growth factors in rats with induced myocardial infarctions. “We believe that eliminating p38 is like releasing a brake on the system,” said Keating. “We will still need to induce proliferation with other factors, such as the growth factor that we used.” Should tests in rats show promise, the researchers could proceed to larger animals, and ultimately to human safety and efficacy trials of p38-targeting drugs.

However, emphasized Keating, clinical application of the approach is still far off, and important scientific questions remain. “We still do not know the molecular mechanism of the effect that we have observed,” he said. “And we don't understand other possible mechanisms that regulate cardiomyocyte proliferation. In this study we don't mean to imply that we think p38 is the only pathway that is important in regulating proliferation. We think there are likely to be others as well,” he said.