

JUNE 01, 2007

Muscle Repair Depends on Multiple Cell Types

Researchers have identified a new population of stem cells that act to repair muscle after damage.

Until now, researchers had assumed that all of these cells, which are called satellite cells, had similar properties. They all seemed to follow the same developmental path to becoming mature muscle. The new discoveries show that the developmental fate of a given satellite cell depends on its physical orientation immediately after cell division.

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— **Michael A. Rudnicki**

Adult mouse and human muscle contain a population of cells called satellite cells that were believed to be fully dedicated to the repair of muscle tissue. Using mice as a model, we made the startling discovery that about 10 percent of these satellite cells were in fact a novel population of stem cells, and we developed approaches for their purification and characterization, said senior author Michael Rudnicki, a Howard Hughes Medical Institute international research scholar at the Ottawa Health Research Institute. Rudnicki is director of the institute's molecular medicine program and its Sprott Centre for Stem Cell Research.

The new research comes from a group led by Rudnicki, who is also the scientific director of Canada's Stem Cell Network. The results of the study are published in the June 1, 2007, issue of the journal *Cell*.

A skeletal muscle fiber is sheathed in a tube of collagen. Each fiber has several hundred satellite cells, which sit between the muscle fiber and the collagen. When a muscle is injured or stressed, these cells are activated and help in repair. As satellite cells divide and develop into healthy new muscle, they also give rise to a fresh population of satellite cells that stand by, ready to respond to future damage.

Researchers had for the most part assumed that satellite cells were a uniform population of precursor muscle cells. However, they had long speculated about whether satellite cells were true stem cells, more committed cells, or de-differentiated myoblasts (more mature muscle cell precursors).

A developmental biologist, Rudnicki said he knew of no examples of mammalian de-differentiation, in which a cell backpedals from being a committed cell to becoming a more plastic, multipotent cell. So he and his group—led by postdoctoral fellow Shihuan Kuang—focused on characterizing satellite cells in mice. They used a genetic system to mark cells that expressed a gene called *Myf5*, which is turned on in mature muscle cells.

They found that 10 percent of satellite cells did not express *Myf5*. The group also found that when cells without *Myf5* divided, they sometimes produced *Myf5*-positive cells.

This indicated some sort of developmental hierarchy, said Rudnicki. So we set out to examine that.

The group devised a way to view satellite cell divisions as they occurred in mice. We felt it was important to look at cell divisions *in vivo*, Rudnicki said. We hypothesized that it might matter based on other work, including that of Elaine Fuchs, an HHMI investigator at The Rockefeller University who studies skin stem cells.

What Rudnicki's group saw was startling, even shocking, he said. When satellite cells divided in a planar orientation, meaning both daughter cells were sandwiched between the muscle and the collagen tube, they almost always resulted in two identical daughter cells (either *Myf5*-negative or *Myf5*-positive).

When satellite cells that did not express *Myf5* divided, one daughter cell touched the muscle fiber and the other was in contact with the collagen. When this occurred, the resulting daughter cells were different. Usually, the cell touching the muscle fiber expressed *Myf5*, while the cell in contact with collagen did not.

Finally, Rudnicki and colleagues injected satellite cells into mice that lacked functional satellite cells. Most of the *Myf5*-positive cells underwent terminal differentiation or died. The *Myf5*-negative cells not only survived, but also infiltrated the muscle and reestablished both the *Myf5*-negative and *Myf5*-positive populations of satellite cells.

Taken together, the group's findings indicate that when satellite stem cells divide, those that remain attached to the muscle fiber's collagen sheath become new satellite stem cells. Daughter cells that lose contact with the sheath, however, turn on the *Myf5* gene and become committed to developing into mature muscle cells.

Our understanding of stem cells provides anatomical evidence that the *Myf5*-negative cells are, in fact, stem cells, Rudnicki said.

Ultimately, these discoveries could lead to therapies for muscle diseases. Rudnicki's group is working to develop a better understanding of the genes that are differentially expressed between these populations of satellite cells, so they can identify them in humans. This work could eventually lead to the development of drugs that target satellite stem cells to stimulate muscle regeneration. Another avenue of research would be to isolate and expand the cells for use in transplantation studies.