Muscle stem cell identity confirmed by Stanford researchers

September 17 2008

A single cell can repopulate damaged skeletal muscle in mice, say scientists at the Stanford University School of Medicine, who devised a way to track the cell's fate in living animals. The research is the first to confirm that so-called satellite cells encircling muscle fibers harbor an elusive muscle stem cell.

Identifying and isolating such a cell in humans would have profound therapeutic implications for disorders such as muscular dystrophy, injury and muscle wasting due to aging, disuse or disease.

"We were able to show at the single-cell level that these cells are true, multipotent stem cells," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor of Pharmacology. "They fit the classic definition: they can both self-renew and give rise to specialized progeny." Blau is the senior author of the research, which will be published Sept. 17 in the online issue of *Nature*.

"We are thrilled with the results," said Alessandra Sacco, PhD, senior research scientist in Blau's laboratory and first author of the research. "It's been known that these satellite cells are crucial for the regeneration of muscle tissue, but this is the first demonstration of self-renewal of a single cell."

One-tenth of the body's mass is skeletal muscle. Satellite cells hang out between a muscle fiber and its thin, membrane-like sheath, waiting to spring into action when the fiber is damaged by exercise or trauma.

When necessary, they begin to divide to make more specialized muscle cells. This property alone, however, doesn't qualify them as stem cells. That designation requires them to be able to also make copies of themselves for future use.

Although many researchers suspected that the satellite cell population included muscle stem cells, it was difficult to prove because not all satellite cells are identical. It was possible that one subpopulation was responsible for making lots of specialized muscle cells, while another replenished the supply of satellite cells.

This divide-and-conquer approach might be efficient, but doesn't have the same exciting clinical applications as identifying a true stem cell. However, analyzing the specific properties of a single cell is technically difficult, and usually requires hundreds of hours of painstaking microscopic analysis of tissue slices from many laboratory animals.

Sacco used a trick to overcome these hurdles. She isolated satellite cells from a mouse genetically engineered to express a glowing protein, luciferase, first identified in fireflies. She then used a novel imaging technique developed at Stanford to follow their fate after transplantation into living animals that did not express the protein. Because this non-invasive method allows repeated imaging of the same animal, fewer mice are needed for the research.

"To be able to detect the presence of the cells by bioluminescence was really a breakthrough," said Blau, the director of the Baxter Laboratory of Genetic Pharmacology. "It taught us so much more. We could see how the cells were responding, and really monitor their dynamics."

Sacco transplanted a single satellite cell expressing the glowing protein into the hind leg muscles of each of 144 mice; in six of the mice, these cells went on to proliferate and self-renew in the recipient's existing

muscle. The relatively low success rate is most likely due in part to the fact that not all of the satellite cells are stem cells and also to the difficulty of keeping a lone cell alive and happy during isolation and transplantation.

The leg muscles of these six mice were repopulated with between 20,000 to 80,000 glowing progeny of the original satellite cell. Many cells made new muscle fibers or contributed to the recipient's muscle fibers. Most exciting, several of the glowing cells expressed cell markers specific only to satellite cells, indicating the original cell was also making more copies of itself and confirming that it was a stem cell.

In another set of experiments, Sacco and her colleagues transplanted between 10 and 500 satellite cells expressing the glowing protein into each mouse leg muscle. These cells also engrafted and proliferated extensively, increasing approximately a hundredfold in number after transplantation and a hundredfold more in response to muscle damage. They contributed extensively to the recipient's muscle, both by forming new fibers and by fusing with injured fibers. Furthermore, once the need for reinforcements had been met, the satellite stem cells stopped proliferating; that is, unlike tumor cells, the transplanted cells were responsive to local cues.

Finally, the researchers were able to induce a second and third wave of proliferation of the glowing satellite cells with repeated incidences of damage, showing that the stem cell function persisted over time.

"Now we can monitor the same mouse over time, and see how various treatments affect muscle regeneration," said Sacco. She and her collaborators are now turning their attention to isolating similar muscle stem cells from humans.

In addition to visually following the fate of the glowing cells, researchers

can also use the intensity of the signal to assess the speed and strength of the stem cells' rescue response under a variety of conditions - an important feature that will allow researchers to directly compare the function of putative stem cells in a variety of injury and disease models.

"This technique provides the first quantitative way to compare stem cells in solid tissues," said Blau. "By providing a means of assessing the efficacy of a range of stem cell therapies in a variety of tissues, I think it will greatly impact not only the study of muscle stem cells in regenerative medicine, but also the stem cell field in general."

Source: Stanford University