



Darwin Prockop and his colleagues study how adult human stem cells might exert a therapeutic influence on neighboring cells, helping to repair dysfunctional cells in damaged tissues.

Unlocking the Mysteries of Stem Cells

Scientists explore the therapeutic mechanisms of adult stem cells.

BY SANDRA J. ACKERMAN

Several years ago, Darwin Prockop of Tulane University and a group of medical students set out to solve a mystery that might have been dubbed the Case of the Disappearing Stem Cells. Their findings, to date, have led to two ground-breaking papers and a handful of new insights into one of nature's essential tools for repairing damaged tissues. In fact, clinical studies based on Prockop's animal research may ultimately help to improve the treatment of cardiac, bone, and neurodegenerative disorders, and possibly some forms of cancer.

Scientists first observed the mysterious disappearance of stem cells while testing experimental therapies for animal models of inherited or acquired diseases. In some cases, infusion of healthy adult stem cells significantly improved an animal's condition. But even when the treatment was clearly effective, the stem cells themselves soon became virtually undetectable.

"The tissues got better," says Prockop, director of Tulane's Center for Gene Therapy, "but we couldn't find many of the cells afterward." For example, in a mouse model of myocardial infarction, or heart attack, Prockop's research team injected half a million mesenchymal stem cells, or MSCs. Derived from bone marrow, MSCs can differentiate, or mature, into almost any tissue in the body except blood cells. MSC infusion spurred the repair of muscle tissue in the heart, helping the animals to regain a stronger and faster heartbeat. Yet, seven weeks after the infusion, the scientists found fewer than five MSCs in the entire heart muscle.

As the principal investigator of the NCRR-supported Adult

Mesenchymal Stem Cell Resource, Prockop is thoroughly knowledgeable about these cells. He oversees their preparation, testing, and distribution to scientists all over the world (see box).

Prockop found his way to stem cells through collagen, the fibrous protein that is the principal source of strength for bone and other tough tissues of the body. With research that continued over decades, first at the National Institutes of Health and later at the University of Pennsylvania and the Robert Wood Johnson Medical School in New Jersey, Prockop and his colleagues defined the unusual pathway by which cells synthesize collagen.

The investigators then isolated the human genes for collagen, along with a series of mutations that interfere with either the normal production or the normal function of collagen. These mutations cause a rare and sometimes disabling disorder known as brittle bone disease, or osteogenesis imperfecta. Children with severe forms of the disease die before or shortly after birth. Children with milder forms easily fracture their bones and often stop growing altogether.

Prockop's group provided critical preclinical data to colleagues Edwin Horwitz and Malcolm Brenner at St. Jude Children's Research Hospital. Studies published by Horwitz and Brenner in 1999 and 2001 describe clinical research in which normal MSCs were given to a small number of children with severe osteogenesis imperfecta. Each young patient received first a bone marrow transplant from a healthy sibling, then an infusion of MSCs from the same individual. Because the patient's immune system had already been "replaced" with cells from a



healthy sister or brother, scientists expected that the new stem cells would not be rejected by the patient. Within a few months, children who received the stem cell infusions began to grow rapidly, and 80 percent were able to sit up unassisted for the first time in their lives. These encouraging results raise the prospect of new

and more effective treatments not only for osteogenesis imperfecta but also for other bone disorders, including osteoporosis.

Prockop arrived at Tulane in the summer of 2000, accompanied by more than a dozen scientists who had already spent several years preparing and studying adult MSCs. The NCRR resource opened shortly thereafter, and the search for the disappearing stem cells was under way. The group published one possible solution to the mystery in the *Proceedings of the National Academy of Sciences (PNAS)* in December 2005. When MSCs are implanted in the brain of a mouse, the scientists found, the cells did not proliferate but rather stimulated the proliferation of neighboring neural stem cells, which are found in small numbers in the adult brain. The researchers suggest that the MSCs stimulated neural stem cell production by secreting chemical signals known as chemokines and cytokines. The new neural stem cells then dispersed throughout the brain and began to differentiate into several types of mature neural cells in normal fashion, although few of the original MSCs survived.

The Tulane research team presented another possible solution in January 2006, again in *PNAS*. This time the scientists made an unexpected discovery while studying the cellular mitochondria, known as “the powerhouse of the cell” because they supply the energy needed for normal cellular functions. In culture, the scientists demonstrated, the mitochondria of MSCs can actually move or transfer themselves, or their DNA, into cells with malfunctioning mitochondria. By giving their own mitochondria to other cells, the MSCs can help to repair the effects of serious maladies such as heart disease, spinal cord injury, or stroke.

Alternative explanations for disappearing stem cells may still be forthcoming, as Prockop and his collaborators continue to pursue their investigations. Meanwhile, the Adult Mesenchymal Stem Cell Resource provides researchers worldwide with the means to pursue their own lines of inquiry.

The stem cell resource receives some funding from HCA Healthcare Corporation and the Louisiana Gene Therapy Research Consortium, yet the essential agency at the outset was the NCRR, Prockop says, because its support offered his group the time and the equipment they needed. The stem cell center continues looking for more ways to serve the research community. “This is an important service being provided to the scientific community by Tulane and by NCRR,” Prockop says. “These cells offer significant potential for biomedical research that will define the basic biology of adult stem and progenitor cells and the possible use of these cells for treating a large number of human diseases.”

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About the Adult Stem Cell Resource

NCRR established the Adult Mesenchymal Stem Cell Resource in 2003 to open up a bottleneck that had hindered progress in the promising field of stem cell research—that is, scientists lacked a reliable, standardized, and fully characterized source of adult stem cells. Each shipment from the Tulane-based resource lists the characteristics of the enclosed stem cells. Because the resource staff consistently maintains cell characteristics from one batch to another, researchers can compare results over

time or among various studies.

The resource’s stem cell distribution system fills a unique niche in the international scientific community, says Brian Butcher, associate director of the resource and a research professor of medicine at Tulane. Indeed, the original grant application to create the stem cell resource received 164 letters of support from researchers worldwide, Butcher says. The resource offers adult rat, mouse, and human mesenchymal stem cells for in vitro or animal studies. Administer-

ing these cells to patients or using them for commercial purposes is prohibited.

The cells are supplied frozen in liquid nitrogen in small vials, with approximately 1 million cells per vial. Each shipment includes a second vial as a backup. The current fee of \$150 can sometimes be waived in cases of hardship.

For more information, or to place an order, scientists should contact Roxanne Reger at msc@tulane.edu or visit the Web site at www.som.tulane.edu/gene_therapy/distribute.shtml.