Stem Cells Derived from Adult Testes Produce Wide Range of Tissue Types for Therapeutic Organ Regeneration

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After a decade of research, Howard Hughes Medical Institute scientists have succeeded in reprogramming adult stem cells from the testes of male mice into functional blood vessels and contractile cardiac tissue. The research offers a promising new source of stem cells for use in organ regeneration studies.

Some scientists think that organ-specific adult stem cells may offer the same therapeutic potential as embryonic stem cells, without the ethical concerns or the risk of immune rejection that are associated with embryonic stem cell therapies. However, adult stem cells may lack the plasticity and pluripotency of embryonic stem cells' capacity to generate any cell type. The study of adult stem cells has also been limited by their relative scarcity in various organs and the attendant difficulties in identifying and harvesting them, as well as differentiating them in large quantities into functional vascularized tissues.

HHMI investigator Shahin Rafii and his colleagues at Weill Cornell Medical College appear to have solved some of these problems in male mice. Using spermatogonial progenitor cells obtained from the mouse's testes, the researchers reprogrammed the cells to form multipotent adult spermatogonial-derived stem cells. If the same can be done with human cells, they say, adult stem cells may be a promising source of new therapies for men, for diseases such as vascular diseases, heart disease, Alzheimer's, Parkinson's, stroke, diabetes, and even cancer.

Scientists have had good success in deriving pluripotent stem cell lines—those with the ability to develop into multiple cell types—from adult testes cells. But only a small subset of cells from the testes has the potential to become pluripotent, and until now, investigators have lacked a means to identify and isolate them.

In a paper published in the September 20, 2007, issue of the journal *Nature*, Rafii and colleagues at Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center report that they have identified a novel cell surface marker that is expressed on a unique set of cells within adult testes known as the spermatogonial stem and progenitor cells (SPCs). The marker, GPR125, enabled the scientists to identify and harvest a large number of SPCs from adult mouse testes, then propagate and reprogram them in the lab to become stem cells that could differentiate into many cell types.

The researchers demonstrated that these multipotent adult spermatogonial-derived stem cells (MASCs) could develop in vivo into working blood vessel (endothelial) cells and tissue, as well as contractile cardiac tissue, brain cells, and a host of other cell types. They also injected MASCs from culture into mouse blastocysts—embryonic cells—that they implanted in mature female mice. When the blastocysts developed into mice, the researchers could see that the MASCs had differentiated into many kinds of tissue. These data suggested that the MASCs are truly multipotent: reprogrammable to differentiate into functional tissues.

Ten years ago, Rafii observed that human testicular cancer cells share many characteristics with adult stem cells. As an oncologist, he also noticed that a large number of patients with testicular cancer develop tumors called teratomas, which contain different types of tissue. Based on these observations, he reasoned that spermatogonia, whose sole function is to generate the precursors to sperm, have the potential to readily give rise to pluripotent cells. As such, he thought, they might prove more amenable to reprogramming than other adult stem cells.

Using gene screening studies, Rafii and colleagues discovered a potential specific surface marker on SPCs. Comparison of all cells in the adult testis showed that this G-protein coupled receptor, known as GPR125, was expressed on SPCs, but not other mature germ cells. With GPR125 in hand, Rafii could isolate large numbers of SPCs from adult mouse testes. They also established a highly sophisticated culture system in which the progenitor cells rapidly grow and divide, creating a large population of cells that can be converted into MASCs.

"It appears that these specialized GPR125-positive spermatogonial cells could be an easily obtained and manipulated source of stem cells with a similar capability to form new tissues that we see in embryonic stem cells," said Rafii. For male patients, he believes, "It could someday mean a readily available source of stem cells that gets around ethical issues linked to embryonic stem cells. It also avoids issues linked to tissue transplant rejection, since these autologous cells come from the patient's own body."

Rafii's team is currently pursuing a similar study of human testes to determine whether stem cells derived from their spermatogonial progenitor cells share the pluripotency of the mouse MASCs. "We believe this to be an easily obtainable goal in the near future," he said.

If they succeed, several steps remain before such stem cells could be applicable to humans. "We still have to learn the exact biochemical and epigenetic `switch' that tells GPR125-positive SPCs to convert into MASCs," said Marco Seandel, a senior post-doctoral fellow in Rafii's laboratory who is the first author of the *Nature* paper. "Discovering that switch will be crucial to our being able to create MASCs on demand,"

There is a chance that implanted cells derived from MASCs may trigger cancer in the recipient. This is an area that requires further investigation, Rafii said. However, he noted, "So far, we haven't seen any cancer or evidence of pro-cancerous activity in adult mice that are implanted with differentiated MASC cell tissue derivatives."

Rafii and his team have worked out the growing conditions that coax spermatogonial progenitor cells to develop into MASC germ lines—genetically stable stem cells that continue reproducing indefinitely. Stem cell studies have been limited to date by the scarcity of germ cell lines. "None of these GPR125-positive germ cell lines was previously readily available for genetic, biochemical, and cellular analysis by other laboratories," says Rafii. "We intend to share them with other researchers."

Rafii's lab is now investigating whether GPR125 can be used to isolate cells from other adult tissues that can be converted into multipotent stem cells. His group has also begun pursuing a similar effort in ovaries. "It's much more difficult," he said. "However, it is possible that reprogrammable stem cells with similar properties to GPR125-positive SPCs may also exist, although at very low numbers, in adult mouse or human ovaries." His lab is actively investigating this intriguing possibility, Rafii said.