Up Front

Adult Stem Cell Plasticity Now in Doubt

Challenging current beliefs about how stem cells work, researchers urge caution about proceeding too quickly to clinical trials.

Although adult stem cells contribute to tissues other than the ones from which they originate by fusing with preexisting cells in these tissues, it now appears doubtful that adult cells derived from bone marrow have the plasticity to transform, or "transdifferentiate," themselves into new types of cells, researchers say.

The fusion phenomenon gives the *appearance* that stem cells from bone marrow alter themselves to become mature cells in other tissues, when in fact they do not, concludes HHMI investigator Sean J. Morrison at the University of Michigan, one of the study's senior authors. They published their findings October 30, 2003, in *Nature*. The study suggests that scientists should exercise caution in using adult bone marrow cells in clinical trials designed to generate new cells in other tissues. Such trials—in which, for example, bone marrow cells have been injected into heart muscle in an effort to stimulate the formation of new heart muscle cells after heart attack—are already under way.

The new findings indicate that bone marrow cells contribute to other tissues by fusing with preexisting cells rather than by forming new cells. It remains uncertain whether the fusion of blood cells with cells in other tissues can contribute to the survival or regeneration of cells in those tissues.

Blue Signal

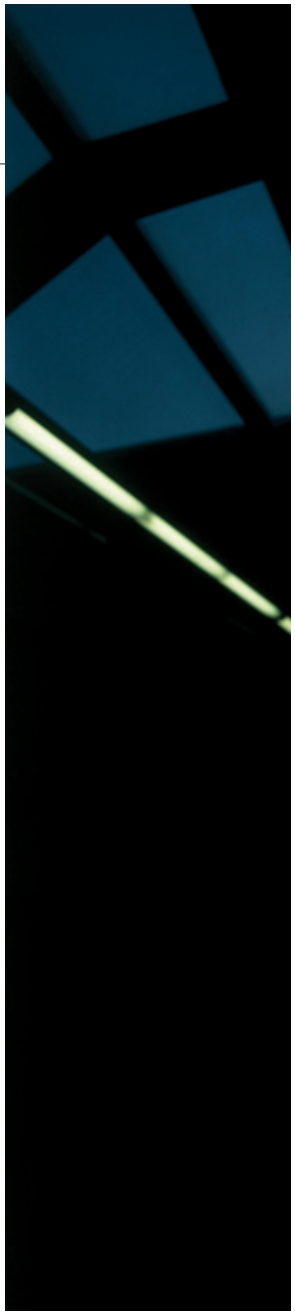
Stem cells have the theoretical potential to differentiate into adult cells of many types. Scientists believe that it might be possible to introduce stem cells to regenerate damaged brain, spinal cord, heart, liver, and other tissues. Initially, adult stem cells were thought to be able to generate cells only from the tissue of origin—for example, blood stem cells had been thought to make only blood cells. However, studies over the past four years have shown that bone marrow cells contribute to unrelated tissues, such as those in the heart, causing some scientists to believe that adult stem cells can mature into specialized cells of unrelated tissue types, in a process known as "transdifferentiation." Thus, for example, hematopoietic, or blood stem cells, could give rise to mature neurons, or vice versa, if they were placed in the appropriate environment.

"The concept of transdifferentiation has been important because papers in major journals over the last few years have suggested that there is widespread potential for transdifferentiation among stem cells from a number of different tissues," says Morrison. "And these findings were the basis for political arguments against the use of embryonic stem cells. Some critics of [embryonic stem cell] research argued that if adult stem cells really had developmental plasticity, there was no need to work on embryonic stem cells."

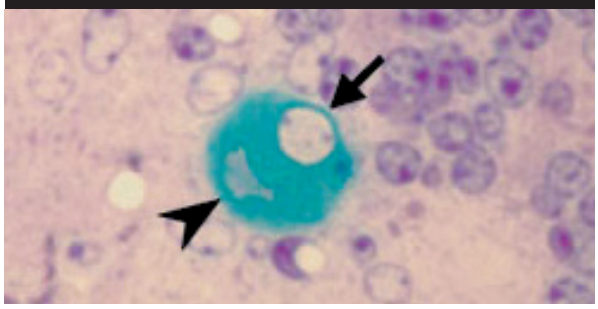
Other studies meanwhile raised the possibility that the seeming plasticity observed in adult stem cells might result from cell fusion. Morrison, Arturo Alvarez-Buylla (of the University of California, San Francisco), and their colleagues set out to develop a technique that could directly and unequivocally determine whether cell fusion actually took place in vivo.

They installed in bone marrow cells a kind of genetic switch, called Cre, that has the capability of turning on a "reporter" gene whose activity could be detected by a characteristic blue staining of tissues. In genetically engineered mice, this reporter gene is normally turned off in cells of different tissues; only when cells with the blue reporter gene fuse with cells with the Cre protein can the Cre protein turn on its telltale blue expression.

In initial experiments in vitro, Alvarez-Buylla and his colleagues showed clear evidence that the system worked by signaling in blue when cell fusion took place. Next, researchers in both laboratories endeavored to determine whether cell fusion occurred in live mice. In those studies, they transplanted bone marrow cells containing the Cre protein into mice whose endogenous marrow cells had been eliminated by irradiation. Cells throughout the bodies of those mice con-



Tale of Two Nuclei *Fusing bone-marrow-derived cells with Purkinje neurons, researchers found cells with two nuclei—one irregularly shaped like those in typical Purkinje cells (arrowhead) and the other more spherical (arrow)—suggesting that the nuclei had different origins. The investigators found no evidence of transdifferentiation without fusion.*



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DONNA TEREK

tained the reporter gene that could be switched on by Cre.

"In these mice, we consistently found small numbers of blue neurons in the brain, blue hepatocytes in the liver, and blue cardiac muscle cells in the heart," said Morrison. Many of these blue cells had two or more nuclei, which further confirmed that they had been formed by fusion.

Alvarez-Buylla and his colleagues went a step further and performed indicator experiments that were designed to detect transdifferentiation in the cells of mice. They used a second reporter gene in the bone marrow cells whose expression did not depend on fusion and found no evidence that the bone marrow cells transdifferentiated into brain, heart, or liver cells.

"Responsible stem cell researchers have argued all along that it is important for research to continue with both embryonic stem cells and adult stem cells."

—SEAN J. MORRISON

Red Flag

According to Morrison and Alvarez-Buylla, the results of their studies should caution researchers who are planning, or are already doing, clinical trials based on the plasticity of adult stem cells. "Our findings raise a red flag about going too fast to clinical trials based on the assumption that transdifferentiation is the mechanism by which stem cells give rise to other cell types," says Alvarez-Buylla. "Our paper suggests that previous claims of transdifferentiation may be explained by cell fusion." The scientists say they cannot rule out that transdifferentiation might be occurring, but they saw no evidence of it in their experimental system.

In any case, according to Morrison, the findings emphasize the importance of using a wide range of studies to determine the properties of stem cells. "Responsible stem cell researchers have argued all along that it is important for research to continue with both embryonic stem cells and adult stem cells," he says. "And I think these findings further support that idea by providing evidence that the plasticity of the adult stem cells was overestimated."

He adds: "In this paper, we described a relatively simple method for looking directly for evidence of fusion. And I hope that future studies of transdifferentiation will use methods like this to determine whether the contribution of bone marrow under other conditions could also be accounted for by fusion."

According to Alvarez-Buylla, the findings of the two laboratories are also significant because they might reveal a new biological mechanism. "Although this remains quite speculative, cell fusion might be a physiologically relevant phenomenon," he said. "While investigators have long used cell fusion as an experimental tool to explore the relative influence of one cell's cytoplasm over another's nucleus, they never suspected that fusion was occurring naturally."

He and Morrison will now use their fusion-detection method to search for fusion in other tissues and to determine whether it does indeed play a rescue role in damaged cells.

—DENNIS MEREDITH