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Putting the Brakes on Blood Stem Cells

Howard Hughes Medical Institute researchers have discovered the first regulatory molecule that puts the brakes on the proliferation of blood stem cells. The molecule also preserves the integrity of those stem cells and enables them to produce functional blood cells over a long period of time.

Blood stem cells are immature cells that sustain blood production throughout life. The researchers are hopeful that their discovery will improve knowledge about how blood-cell production is regulated.

Ironically, the same molecule, called growth factor independent 1 (Gfi-1), acts as an accelerator of growth in immune cells, emphasizing the important role that cellular context plays in the regulation of stem cells.

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This discovery offers valuable lessons for researchers seeking to produce stem cells for use in therapy, said the study's senior author, Stuart Orkin of the Howard Hughes Medical Institute. Scientists are working toward using an array of stem cells to grow mature specialized cells that could regenerate damaged or diseased skin, brain, heart or other organs.

"Investigators who are searching for important genes in stem cells very often think that such genes have to be specific to stem cells alone, which isn't necessarily true," he said. "Because this protein is in a number of different types of cells, and may perform different functions in different cells, it is a wake-up call that the stem cell is very versatile," Orkin said.

The researchers published their findings in the October 21, 2004, issue of the journal *Nature*. The article was published online earlier in an advance online publication of *Nature*. Lead author of the paper was Hanno Hock in Orkin's laboratory at the Dana-Farber Cancer Institute, Children's Hospital, Boston, and Harvard Medical School.

Prior to embarking on their experiments, Orkin's group was aware of the existence of regulatory proteins that switched on the proliferation of hematopoietic, or blood-forming, stem cells. Previous studies had revealed that Gfi-1 functioned as a growth-promoter in immune-system T cells.

“In that setting, Gfi-1 was known to promote cycling of T cells that become malignant,” said Orkin. “But our experiments showed the reverse in hematopoietic stem cells—that it puts the brakes on.”

In their studies, Hock, Orkin and their colleagues produced mice that lacked a functioning gene for Gfi-1 and studied how the loss of that gene affected blood production. Their studies showed that knocking out *Gfi-1* produced a complex set of effects.

“When these mice were young, they had normal or elevated proliferation of blood cells,” he said. “But as they aged, they began to lose them because the whole integrity of the stem-cell system appeared to depend on the expression of this protein. Our evidence suggests that if you remove this brake, and the cells cycle too much, they can exhaust themselves.”

The researchers also transplanted stem cells from the Gfi-1-negative mice into animals in which the blood stem cells had been eliminated by irradiation. This experiment sought to reveal the properties of the Gfi-1-negative cells in a neutral setting with no other blood-forming cells. The researchers also did competition experiments in which they introduced both Gfi-1-negative and normal stem cells into the irradiated recipient mice. Both of these kinds of experiments confirmed Gfi-1's role as a brake, said Orkin.

“We saw that if you transplant the Gfi-1-negative cells alone, you can get blood formation in the recipient, which is similar to what we see in the mutant mice. But if you put almost any number of competitor cells in there, meaning normal cells, they just can't compete. They probably don't have the right brake, and they probably exhaust themselves.” Similarly, when the researchers produced chimeric mice containing cells from both Gfi-1-negative and normal mice, the negative cells tended to disappear from the blood-forming system.

The researchers next studied the activity of the Gfi-1-negative cells, confirming that without the brake, the cells overproliferate, “so the system is just running at top speed, but yet they can't make effective cells,” said Orkin. When the scientists measured the activity of some of the other components of the cell proliferation machinery, they found evidence of accelerated

proliferation.

“So, our conclusion is that this is an important brake on the system that we previously didn't know of,” said Orkin. “And it also highlights the fact that a protein may have quite different roles in terms of control of the cell cycle, depending on the cell context. In T-lymphoid cells, Gfi-1 seems to drive proliferation, but in the setting of stem cell it seems to do the opposite.”

While the findings do not have direct clinical relevance, said Orkin, “anything we can learn about how to regulate stem cells is very important in developing approaches to amplifying stem cells in vitro, which is key to their therapeutic use.”

The latest finding identifies Gfi-1 only in isolation as an important regulatory brake on blood stem cells, but does not reveal the control pathway by which it functions, said Orkin. His group is planning additional studies that they hope will uncover the control pathway.