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Critical Stem Cell Survival Factors Found

As researchers attempt to take advantage of the potential of adult stem cells in regenerative medicine, understanding the mechanisms that delimit lifespan and longevity of stem cells will be critically important. Researchers have now identified a family of proteins that contributes to the survival and regenerative potential of blood-forming stem cells. According to the researchers, their findings in hematopoietic stem cells might be relevant to stem cells in other tissues, and provide insights into potential strategies to enhance the longevity of stem cells.

The new knowledge, gleaned from animal studies, could help enhance the viability of blood stem cells used for bone marrow transplants for patients with leukemia.

The lead author of the study, which was published in the January 25, 2007, issue of the journal *Cell*, was Zuzana Tothova, an M.D./Ph.D. student in the laboratory of Howard Hughes Medical Institute investigator Gary Gilliland. Tothova and senior author Gilliland are at Brigham and Women's Hospital, the Harvard Stem Cell Institute, and Harvard Medical School.

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- D. Gary Gilliland

FoxO genes produce proteins called transcription factors that regulate the activity of other genes. Previous research had shown that the *FoxO* proteins regulate cell survival and proliferation in many tissues, acting in response to signals from growth factors outside the cell, said Tothova. Studies had shown that aberrant genes that cause some leukemias and lymphomas inhibit *FoxO* genes, enabling blood stem cells to escape their regulation and proliferate

uncontrollably.

“The puzzling finding until now, however, has been that knocking out single members of the *FoxO* family didn't seem to have much effect on the hematopoietic system,” said Tothova. “That led us to the hypothesis that they must be functionally redundant in the hematopoietic system,” she said. Thus, Tothova and her colleagues in the lab of Ronald A. DePinho at the Dana-Farber Cancer Institute decided to generate a transgenic mouse in which they could switch off all three relevant *FoxO* genes—*FoxO1*, *FoxO3* and *FoxO4*—in the hematopoietic system of adult animals.

The mice that they created had far fewer hematopoietic stem cells than normal animals. Furthermore, these stem cells could not produce blood for more than a few months - in essence, they appeared to “burn out” and undergo extinction, Tothova said. When the scientists searched for the cause of these defects, they found that the hematopoietic stem cells in the *FoxO*-deficient mice had abandoned their normal quiescent state and launched into active cell division, or cycling. This cycling compromised the cells' ability to give rise to other stem cells - a process known as self-renewal. The stem cells also underwent excessive programmed cell death, or apoptosis.

Tothova explained that the abnormal cell cycling was restricted to hematopoietic stem cells and not found in more differentiated cells, such as myeloid progenitors, which are among the most proximal progeny of stem cells. Myeloid progenitors mature into certain types of white and red blood cells, whereas the less mature hematopoietic stem cells retain the ability to become a broader range of hematopoietic cell types. “This was a very curious finding—that these effects were restricted to the stem cell compartment and not present just one step further in their differentiation,” said Tothova. “So, we searched for the mechanism that caused this very stem-cell-specific ‘burnout.’”

Tothova found that the stem cells in the mice lacking all three FoxO proteins contained more reactive oxygen species (ROS) than normal cells. These are highly reactive chemicals that contribute to normal signaling within the cell, but increased levels can be toxic or even lethal to cells. In a key experiment, Tothova found she could restore the stem cell number and function to normal by introducing an antioxidant chemical that reduced ROS levels.

“The finding that ROS appeared to be the critical determinant of stem cell life span was quite surprising,” said Tothova. “Showing that we could rescue the cells with an antioxidant suggests that the increased levels of these reactive oxygen chemicals were responsible for the changes in the cells that we observed.” In contrast, Tothova and her colleagues found ROS levels to be unaffected by FoxO loss and normally much higher in myeloid progenitor cells, which use the reactive molecules as weapons to destroy invading microbes.

Gilliland said he, too, found Tothova's results surprising. “For one thing, since the *FoxO* transcription factors are important targets of some of the leukemia-causing genes we study, we thought that knocking them out would remove the brakes, and we would get a fulminant leukemia phenotype. But initially the animals seemed to do fairly well without them.

“And the second surprise was that, while few of the differentiated myeloid cells took a hit, losing the *FoxO* family members took the brakes off hematopoietic stem cell proliferation. This made them exhaust themselves and become extinct. That finding gives us a sense that the stem cells in this and other adult tissue compartments—and maybe even in germ cells—rely on this pathway for their longevity and lifespan,” said Gilliland. He noted that previous studies in the DePinho laboratory have indicated that the *FoxO*-deficiency causes a similar “burnout” in other tissues, such as follicle cells in the ovary.

“These findings give us a better understanding of the molecular mechanisms that limit the lifespan of hematopoietic stem cells,” Tothova said. “This understanding could be beneficial in our ability to extend the lifespan or modulate longevity of stem cells in adults.”

“Although it is really just a pipe dream at this point, it may be that augmenting this protective pathway against reactive oxygen might prove useful in enhancing longevity in tissues, or perhaps in entire organisms,” Gilliland noted.