

## News

# Micro-RNA's Function to Maintain Stem Cell Phenotype

Johns Hopkins Kimmel Cancer Center scientists have found a set of “master switches” that keep adult blood-forming stem cells in their primitive state. Unlocking the switches’ code may one day enable scientists to grow new blood cells for transplant into patients with cancer and other bone marrow disorders.

The scientists located the control switches not at the gene level, but farther down the protein production line in more recently discovered forms of ribonucleic acid, or RNA. MicroRNA molecules, once thought to be cellular junk, are now known to switch off activity of the larger RNA strands which allow assembly of the proteins that let cells grow and function.

“Stem cells are poised to make proteins essential for maturing into blood cells, but microRNAs keep them locked in their place,” says cancer researcher Curt Civin, M.D., Ph.D., who led the study. The journal account appeared online the week of February 5 in the early edition of the Proceedings of the National Academy of Sciences.

To halt protein assembly, microRNAs pair up with matching full-length RNA, then fold and twist it, rendering the larger RNA useless. But the RNA pairings are not perfect, and one microRNA can latch on to several hundred RNA strands. “They act like a single circuit breaker to efficiently control hundreds of RNAs,” says Civin, the Herman and Walter Samuelson Professor of Cancer Research.

“We’re looking for ways to flip these microRNA switches, to control when stem cells grow into new blood cells,” says Robert Georgantas, Ph.D., research associate at the Johns Hopkins Kimmel Cancer Center and first and corresponding author of the study.

To identify the key microRNAs, Georgantas sifted through thousands of RNA pieces with a custom-built, computer software program. Its algorithms let the software, fed data from samples of blood and bone marrow from healthy donors, match RNA pairs. The outcome was a core set of 33 microRNAs that match with more than 1,200 of the larger variety RNA already known to be important for stem-cell maturation.

Georgantas and Civin currently are testing whether these pair predictions are valid by using a non-reproducing virus to insert genetic instructions for each of the 33 microRNAs into adult stem cells. They’ll then be cultured in Petri dishes. MicroRNA-155—the first microRNA tested—was predicted to stop stem cells from developing into red and white blood cells. As expected, stem cells without microRNA-155 matured: they formed approximately 75 red and 150 white blood cell colonies per dish. Stem cells with microRNA-155 matured into far fewer red and white cell colonies—about seven and 30 per dish, respectively.

“Using microRNAs to stall an adult blood stem cell in its early stage could help us grow new ones in test tubes, and perhaps give us more insight into stem-cell maturation for other tissue types,” says Civin.

Civin and his team have filed for patents on the microRNA technology. The research was funded by the National Institutes of Health, National Cancer Institute, National Foundation for Cancer Research, and Kimmel Foundation for Cancer Research.

Additional authors include Richard Hildreth, Sebastien Morisot, and Jonathan Alder from Johns Hopkins; Chang-gong Liu, George

A. Calin, and Carlo Croce from Ohio State University; and Shelly Heimfeld from the Fred Hutchinson Cancer Research Center.

The Johns Hopkins University holds patents on the CD34 monoclonal antibodies and inventions related to stem cells. Civin is entitled to a share of the sales royalty received by the University under licensing agreements between the University, Becton Dickinson Corporation and Baxter HealthCare Corporation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict-of-interest policies.

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# Clinical Benefit of Antisense Therapy in a Phase III Trial in Patients with CLL

The first “antisense” drug to be tested in chronic lymphocytic leukemia (CLL) shows benefit in a phase III clinical trial for a specific subset of patients - those who are still sensitive to a chemotherapy drug often used to treat this cancer.

Researchers at the University of Texas MD Anderson Cancer Center, reported in the early on-line edition of the March 20, 2007 Journal of Clinical Oncology, found that the agent, oblimersen (trade name: Genasense) produced a four-fold increase in “CP/nPR,” a clinical response defined by no definitive evidence of disease, in patients who were sensitive to the chemotherapy drug fludarabine, compared to patients who no longer responded to fludarabine.

“The results make sense because oblimersen is designed to work alongside chemotherapy,” says the study’s lead author, Susan O’Brien, M.D., professor in the Department of Leukemia. “We found in this study that oblimersen enhances sensitivity to chemotherapy, and so we think it deserves further study in a population of CLL patients who are sensitive to chemotherapy agents,” she says.

CLL, a cancer of the blood and bone marrow, is the second most common type of leukemia in adults. More than 15,000 new cases of the disease will be diagnosed this year, according to the American Cancer Society, and about 4,500 people will die from the cancer.

Fludarabine is a newer chemotherapy drug that is now being used as first treatment for many patients, according to O’Brien. Oblimersen is an experimental agent that inhibits the production of a protein known as Bcl-2 in cancer cells. This protein can stop a cell from destroying itself, and is often over-expressed in cancer. As an antisense drug, oblimersen provides a complementary genetic strand to the messenger RNA that produces Bcl-2, inactivating it and preventing the protein from being produced. “It gets rid of Bcl-2, and cells that have less Bcl-2 are more sensitive to chemotherapy,” O’Brien says.

This study focused on patients who had relapsed after a prior fludarabine—containing regimen or were refractory to fludarabine; refractory patients had failed to respond to a prior fludarabine-based regimen or had progressed within six months of treatment. It enrolled 241 patients at cancer centers worldwide who were randomized to receive fludarabine combined with cyclophosphamide chemotherapy or the same regimen combined with oblimersen.



