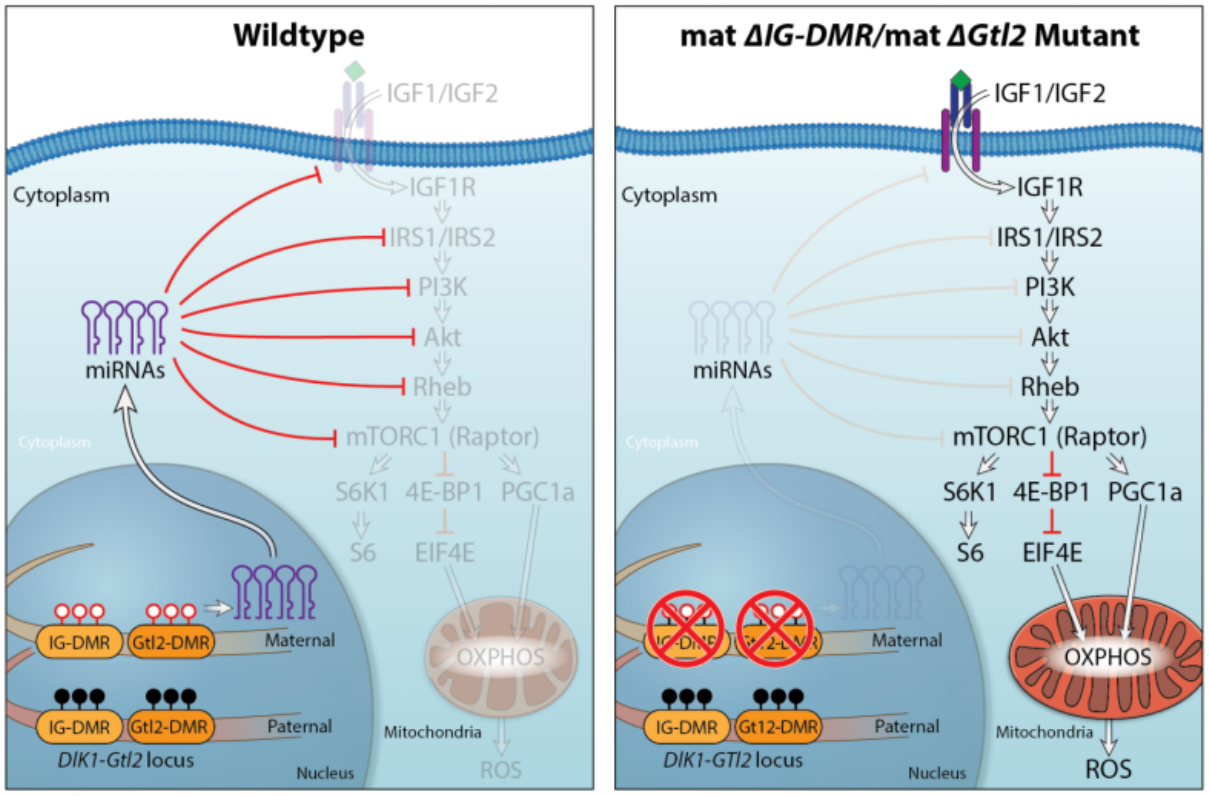
How a genetic locus protects adult blood-forming stem cells

November 25 2015



MicroRNA clusters expressed from imprinted Gtl2 locus maintain adult hematopoietic stem cells through suppression of mitochondrial biogenesis and metabolic activity. Credit: Linheng Li Lab

A particular location in DNA, called the *Dlk1-Gtl2* locus, plays a critical role in protecting hematopoietic, or blood-forming, stem cells—a

discovery revealing a critical role of metabolic control in adult stem cells, and providing insight for potentially diagnosing and treating cancer, according to researchers from the Stowers Institute for Medical Research.

In their study, published online Nov. 25 in *Cell Stem Cell*, Stowers Investigator Linheng Li, Ph.D., and first author Pengxu Qian, Ph.D., along with other collaborators, reveal how the mammalian imprinted *Gtl2*, located on mouse chromosome 12qF1, protects adult hematopoietic stem cells by restricting metabolic activity in the cells' mitochondria.

The research focused on imprinted genes—genes "stamped" according to whether they are inherited from the mother or father. With imprinted genes, one working copy, or allele, is inherited instead of two. Either the copy from the mother or father is inactivated or "silenced." Typically, the paternally inherited allele's expression promotes growth, while the maternally inherited allele's expression suppresses it.

The researchers found that when the *Gtl2* locus is expressed from the maternally inherited allele, it produces non-coding RNAs to curb metabolic activity. Mechanistically, *Gtl2's* "megacluster" of microRNA, the largest cluster of microRNA in the mammalian genome, suppresses the mTOR signaling pathway and downstream mitochondrial biogenesis and metabolism, thus blocking mitochondrial-associated byproducts called reactive oxygen species (ROS) that can damage adult stem cells.

"Reactive oxygen species are like the potentially harmful byproducts that come from industrial manufacturing," says Li. "ROS are unavoidable derivatives of the mitochondrial metabolic process and need to be managed by the cell," he explains.

Hematopoietic stems cells renew themselves and differentiate into other

cells, including white blood cells, red blood cells, and platelets, constantly renewing the body's blood supply in a process called hematopoiesis. Because of their extraordinary transformative qualities, the transplantation or transfusion of isolated human hematopoietic stem cells has been used in the treatment of anemia, immune deficiencies, and other diseases, including cancer.

While hematopoietic stem cells have gained attention in research, it remains largely unknown how cell metabolic states are controlled. The new findings shed light on the delicate metabolic control required to balance hematopoietic stem cell maintenance and action and the associated healthy hematopoiesis.

An upset in that balance can cause cells to grow abnormally and lead to disease. Abnormalities in the *Gtl2* locus on human chromosome 14q32.2 are associated with uniparental disomy in which an individual receives two copies of a chromosome from one parent and no copy from the other parent. Uniparental disomy may cause delayed development, mental retardation, or other medical problems. Differences in gene expression at the *Gtl2* locus have also been linked to fetal alcohol exposure disorder.

But when working properly, the *Gtl2* locus acts as a great protector of cells.

"Most of the non-coding RNAs at the *Gtl2* locus have been documented to function as tumor suppressors to maintain normal cell function," Qian says.

Li's team zeroed in on *Gtl2* by studying hematopoietic stem cells in mice with support from Stowers core centers including cytometry, bioinformatics, histology and electron microscopy, molecular biology, and tissue culture. Other collaborators included researchers from the

University of Kansas; the University of Kansas Medical Center; Tianjin Medical University, China; Christian Medical College, Vellore, India; Tokyo University of Agriculture, Japan; and University of Cambridge, United Kingdom.

Over the three-year study, investigators used transcriptome profiling to analyze 17 hematopoietic cell types and found that non-coding RNAs expressed from the *Gtl2* locus are predominantly located in a subset of the cell types, including adult "long-term" hematopoietic stem cells which have long-term self-renewal capacity. In subsequent experiments, deleting the locus from the maternally inherited allele in hematopoietic stem cells increased mitochondrial biogenesis and subsequent metabolic activity as well as increased ROS levels, with the latter inducing cell death.

The finding opens the possibility for *Gtl2* to be used as a biomarker because it could help label dormant (or reserve) stem cells in normal or potentially cancerous stem cell populations, Li says. The addition of a fluorescent tag to the *Gtl2* locus could allow researchers to mark other adult stem cells in the gut, hair follicle, muscle, and neural systems.

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Provided by Stowers Institute for Medical Research