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For Immediate Release

LJI researchers shed light on devastating blood diseases

Scientists show how a mutated gene harms red and white blood cells

LA JOLLA, CA—Scientists at La Jolla Institute for Immunology (LJI) have discovered how a mutated gene kicks off a dangerous chain of events during blood cell production.

The study, published recently in the *Proceedings of the National Academy of Sciences*, reveals how a mutated gene called *ASXL1* is involved in a disease called clonal hematopoiesis, a precursor to malignant diseases such as myeloid malignancies and chronic monomyelocytic leukemia.

"We know that many diseases—and all cancers—are driven by mutations in the genome," says LJI Instructor Zhen Dong, Ph.D., who served as first author of the new study. "We showed how mutated *ASXL1* may give rise to dysfunctional cells in your bone marrow and blood.

Dong co-led the study with LJI Professor Anjana Rao, Ph.D. Rao is a well-known expert on the genetic drivers behind immune cell dysfunction and cancer development.

Mutated blood cells take over

For the new study, the scientists investigated a potential link between mutated *ASXL1* and an age-related disease called clonal hematopoiesis. Patients with clonal hematopoiesis have trouble making healthy red and white blood cells. Instead, these patients start to produce blood cells with scrambled genomes, which eventually develop into cancers.

All blood cells are derived from special cells called hematopoietic stem cells (HSCs), whose differentiated progeny normally repopulate the blood. Clonal hematopoiesis develops when a single HSC acquires growth-promoting mutations and begins to churn out many, many clones of itself. Eventually, these mutated blood cells become over-represented in the blood—which can lead to major health issues, such as cancer and inflammation, especially inflammatory heart disease.

"Your genome becomes very unstable," says Dong. "These 'bad elements' cause new mutations and harm your normal, healthy cells."

For some patients with clonal hematopoiesis, the repetitive DNA sequences make cells think they are infected by a virus. This confusion triggers immune defenses within blood cells themselves, leading to harmful inflammation that damages healthy tissues as well. This is an especially big problem for the heart, which then has to deal with a flood of inflammatory molecules as it pumps blood through the body.

"The mutation-containing HSCs give rise to proinflammatory immune cells—exacerbating heart inflammation and cardiovascular disease—and may induce or accelerate heart failure," says Dong.

For other patients, the scrambled genome causes additional problems in expanded cells already predisposed to grow more quickly than normal. These patients end up with a rapidly expanding number of abnormal HSCs and myeloid cells—which can lead to myeloid malignancies, lymphoid malignancies, chronic monomyelocytic leukemia, and other forms of leukemia.

"The abnormal repetitive and transposable element sequences can de-repress certain silent genes that could promote cell growth by allowing cells to adapt more efficiently to the changing environment," says Dong. "And that's good for highly proliferating cells, which become 'addicted' to the bad proteins that hurt normal cells."

So what triggers clonal hematopoiesis?

ASXL1 mutations are very common in patients with clonal hematopoiesis and some other blood diseases. In fact, Rao led a previous *Nature Communications* investigation into the role of mutated *ASXL1* in myeloid leukemias. Still, scientists haven't known exactly how this mutated gene affects blood cell development, growth or function.

The LJI team used mouse models with mutated *ASXL1* to solve this mystery. Because clonal hematopoiesis develops as a person ages, Dong worked with mice between 10 months and two years old, the equivalent to about 70 years old in humans.

The researchers used a technique called mass spectrometry to uncover exactly how mutated *ASXL1* altered the normal hematopoietic stem cells—the cells that serve as precursors to both red and white blood cells. This work was done in collaboration with LJI's Global Autoimmune Institute Assistant Professor Sam Myers, Ph.D., who was a researcher at the Broad Institute at the time.

Rao, Dong, and their colleagues discovered that mutating the *ASXL1* gene wrecked havoc on the normal workings of a tightly packed form of DNA called heterochromatin.

Heterochromatin is important for blood cell development because it "silences" potentially harmful genes—and genes that are normally shut off as a cell differentiates. Thanks to heterochromatin, hematopoietic stem cells can develop and mature correctly into all types of blood cells, such as red blood cells or white blood cells (which have specialized jobs as T cells, B cells, and more).

In the new study, mutating the *ASXL1* gene spelled disaster for blood cells. Without heterochromatin working properly, hematopoietic stem cells never matured into normal blood cell lineages, including the lymphocytes, immune cells, and normal red or white blood cells.

The researchers could see that the mutated ASXL1 proteins interacted with a protein complex in cells—called the EHMT1-EHMT2 histone methyltransferase complex—to kick off a dangerous domino effect. The altered protein complex reduced H3K9me2 and H3K9me3, two repressive histone marks needed for silencing heterochromatin, making the tightly packed heterochromatin uncoil. This change led to the reactivation of previously silenced genes and transposable elements.

This alternation was also a huge problem for cells. Heterochromatin normally suppresses a very large number of repetitive and transposable elements, also called "jumping genes," which are repressed in healthy cells. Transposable elements can randomly be excised and re-inserted into the genome, leading to genome instability.

Even if they cannot jump, transposable elements can become highly expressed and in turn inappropriately activate the expression of nearby genes, especially those in heterochromatin. This process adds to the genome confusion and aberrant gene expression caused by mutant ASXL1 proteins.

The researchers then compared the mutated mouse models to normal mice. They found that the *ASXL1*-mutated mice had the same scrambled genomes and harmful DNA repeats found in chronic monomyelocytic leukemia patients with mutated *ASXL1.*

Next steps for preventing disease

Dong says the LJI team is investigating how mutated ASXL1 proteins interact with other important players in our immune cells. So far, their work suggests that ASXL1 also interacts with a protein called TET2, which is also frequently mutated in clonal haematopoiesis and in many blood cancers. Functional TET2 proteins are critical for normal DNA demethylation, so there's an urgent need to understand how these genes affect each other.

"Both the *TET2* and *ASXL1* genes can be mutated, individually or together, in clonal hematopoiesis, leukemia, and a premalignant form of cancer called myelodysplastic syndrome," says Dong. "We need to ask how these two genes might cooperate in these diseases."

Dong adds that further research might also shine a light on the origins of some neurodevelopmental disorders. Other genes from the *ASXL* family can be mutated in neural stem cells, which may lead to speech difficulties, motor delay, intellectual delays, and even death for some pediatric patients.

"I'm very inspired and motivated to continue this project," says Dong, whose son has a neurodevelopmental delay. "We need to figure out more about all the members of the *ASXL* gene family."

Additional authors of the study, "A mutant ASXL1-EHMT complex contributes to heterochromatin dysfunction in clonal hematopoiesis and chronic monomyelocytic leukemia," include co-first author Hugo Sepulveda, Leo Josue Arteaga Vazquez, Chad Blouin, Jenna Fernandez, Moritz Binder, Wen-Chien Chou, Hwei-Fang Tien, Mrinal Patnaik, Geoffrey J. Faulkner, and Samuel A. Myers.

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