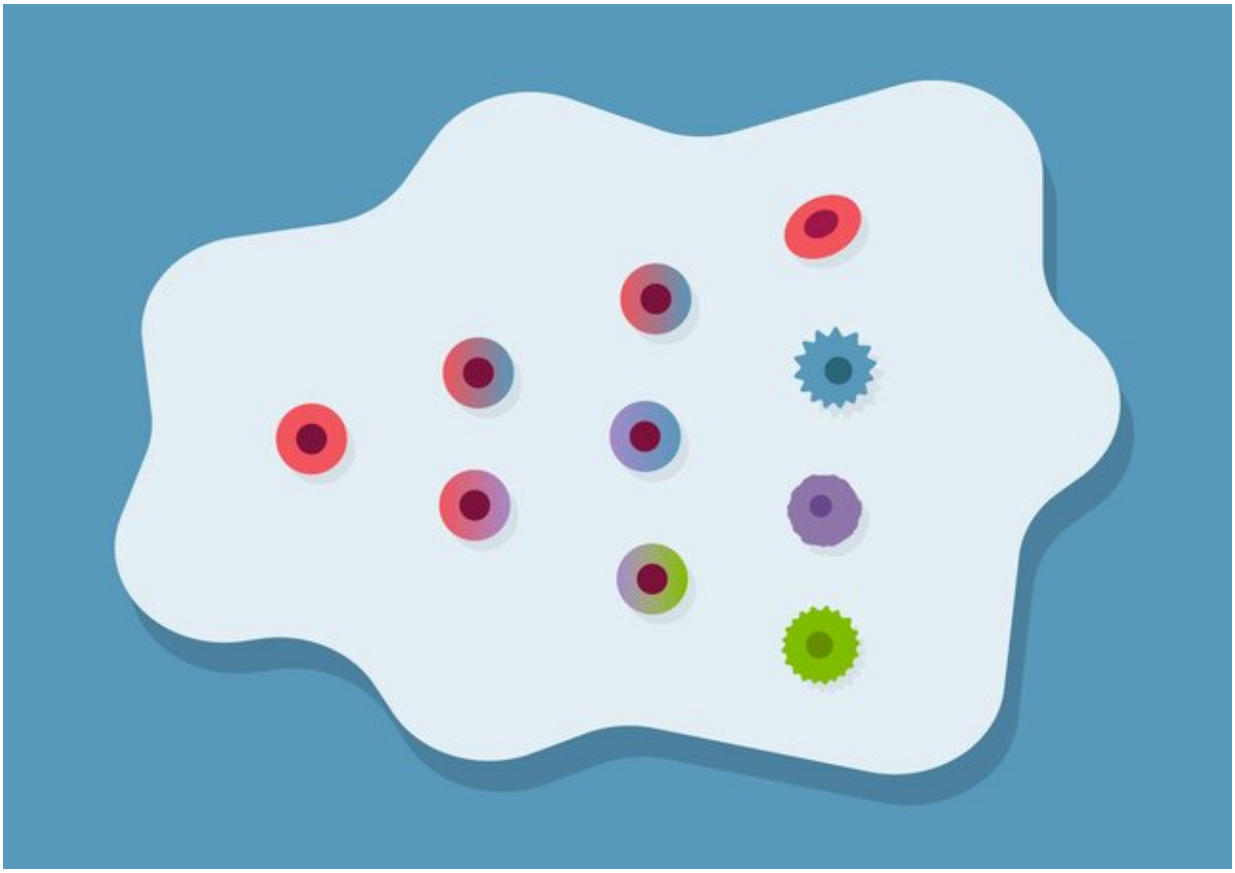
Researchers use base editing to probe blood cell biology

May 3 2023, by Sarah C.P. Williams



Credit: Sonja Vasiljeva, Broad Communications

Researchers have used a highly precise genome-editing technology called base editing to make hundreds of direct edits to blood stem cells from

patients' bone marrow.

Their work, published today in *Cell*, is the first time that such high-throughput base editing, which can make many single-base substitutions in DNA in many cells at once, has been applied to blood stem cells. The research team showed how such single-nucleotide changes in genes can affect the biology of blood cells and contribute to the treatment of diseases including leukemia and sickle cell disease. The findings suggest how the technology can help scientists learn more about the role of gene variants in disease in multiple cell types.

"Our approach allows us to understand not just whether a particular gene might be implicated in human disease, but exactly how individual changes to that gene are playing out at a molecular level," said Vijay Sankaran, a senior author of the new study and an associate member at the Broad Institute of MIT and Harvard. "This finer resolution gives us a new roadmap for how diseases occur and how to treat them."

Sankaran, who is also the Lodish Family Chair at Boston Children's Hospital, the Jan Ellen Paradise, MD associate professor of pediatrics at Harvard Medical School, and a New York Stem Cell-Robertson Investigator, led the new work in collaboration with Ramnik Xavier, a core institute member of the Broad, and other colleagues. Jorge Diego Martin-Rufino, a Ph.D. candidate in Sankaran's lab, was the study's first author.

"Impacting patients with blood disease requires experiments that are informed by processes causing disease. This remarkable multi-institutional collaboration between bench scientists, biotechnologists, and physicians has outlined a path to map disease-associated variants in relevant cell types implicated in blood disorders," said Xavier, who is also director of the Klarman Cell Observatory at the Broad, the Kurt J. Isselbacher Professor of Medicine at Harvard Medical School, director

of the Center for Computational and Integrative Biology and core member in the Department of Molecular Biology at Massachusetts General Hospital.

The convergence of technologies

Billions of nucleotides comprise a person's genome, and a change to just one—called a single nucleotide variant—can trigger disease. In recent decades, scientists have become increasingly adept at pinpointing the associations between these variants and disease. However, understanding exactly why or how each variant causes disease is more difficult.

"Unless we understand the mechanisms by which these variants are causing disease, we often can't do anything about them," said Sankaran. "When we can map out what is happening at a molecular level, it gives us a lot more opportunities to intervene."

While base editing is efficient for editing genomes of cultured cell lines, it is far more challenging to use it for editing genes in cells taken from patients.

In this new research, Martin-Rufino, Sankaran, and colleagues successfully adapted base editing to work at scale in patient-derived hematopoietic stem cells, the bone marrow cells that give rise to a diverse array of blood and immune cells. They also used single-cell RNA sequencing, which lets researchers observe which genes are active in any given cell at any time. Using both these approaches, they could study how the edited cells differed from unedited cells as they progressed through various stages of development.

Unlike previous research methods that simply link a gene with a disease, the new method allowed the team to see exactly how changes to a gene altered the overall RNA fingerprints of many different cell types.

"This is a very powerful platform to identify novel genetic therapies directly in the same cell types that will be the target of curative therapies for diverse human diseases," said Martin-Rufino. "With our approach, we can not only introduce specific changes in the sequence of DNA but also know exactly what effects these changes have in different cell types."

Linking nucleotides to disease and treatment

To test the utility of their new base-editing screen, Sankaran's group used it to edit genes known to have a role in disease.

In one case, the group edited the CD33 gene, which encodes an immune checkpoint receptor that is often present at higher levels in the blood cells of people with acute myeloid leukemia (AML). Existing immunotherapy drugs aim to treat AML by directing immune cells to attack cells expressing CD33, but these therapies destroy both leukemia cells and healthy hematopoietic stem cells. Using base editing, the researchers studied which single-nucleotide changes to CD33 could reduce its expression in healthy cells, potentially preventing these cells from being impacted by CD33-targeting immunotherapies.

In another set of experiments, the researchers made multiple different single-nucleotide edits to areas of the genome potentially related to fetal hemoglobin production in red blood cells. They discovered a set of edits that could activate fetal hemoglobin production, pointing toward ways to potentially treat conditions that impact the adult form of hemoglobin, like sickle cell disease.

Finally, Sankaran and collaborators made hundreds of different edits to the GATA1 gene, which has been found to have variants in patients with a handful of different rare blood diseases. In most instances, though, physicians are not sure whether a patient's GATA1 mutations are

directly causing their disease or not. After profiling nearly 300,000 individual cells at four different stages of blood cell development, Sankaran's group described how many of the GATA1 mutations change the expression of other genes in blood cells, altering the cells' overall biology and potentially causing disease.

"What this really let us do was prosecute many of these variants as being causative in disease, which is incredibly satisfying," said Sankaran. "Knowing the exact causes of these patients' diseases is the first step toward developing ways to treat them."

Sankaran's team is planning future base-editing screens for other genes of interest, including other types of blood and immune cells. They also hope to soon be able to use new types of base editing that expand the types of changes that can be made to nucleotides.

"Each of these advances will be very helpful and enable us to keep filling in the gaps between genetic changes and human diseases," Sankaran said.

More information: Jorge D. Martin-Rufino et al, Massively parallel base editing to map variant effects in human hematopoiesis, *Cell* (2023). DOI: 10.1016/j.cell.2023.03.035

Provided by Broad Institute of MIT and Harvard