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Researchers decode biology of blood and iron disorders mapping out novel future therapies

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Two studies led by investigators at Weill Cornell Medical College shed light on the molecular biology of three blood disorders, leading to novel strategies to treat these diseases.

The two new studies—one published online March 17 by *Nature Medicine* and the other March 25 in the online edition of the *Journal of Clinical Investigation*—propose two new treatments for beta-thalassemia, a blood disorder which affects thousands of people globally every year. In addition, they suggest a new strategy to treat thousands of Caucasians of Northern European ancestry diagnosed with HFE-related hemochromatosis and a novel approach to the treatment of the rare blood disorder polycythemia vera.

These research insights were only possible because two teams that included 24 investigators at six American and European institutions decoded the body's exquisite regulation of iron, as well as its factory-like production of red blood cells.

"When you tease apart the mechanisms leading to these serious disorders, you find elegant ways to manipulate the system," says Dr. Stefano Rivella, associate professor of genetic medicine in pediatrics at Weill Cornell Medical College.

For example, Dr. Rivella says, two different gene mutations lead to different outcomes. In beta-thalassemia, patients suffer from anemia—the lack of healthy red blood cells—and, as a consequence,

iron overload. In HFE-related hemochromatosis, patients suffer of iron overload. However, he adds, one treatment strategy that regulates the body's use of iron may work for both disorders.

Additionally, investigators found another strategy, based on manipulating red blood cell production, could also potentially treat beta-thalassemia as well as a very different disorder, polycythemia vera.

Revealing the Third Crucial Player

In the *Nature Medicine* study, Dr. Rivella and his colleagues tackled erythropoiesis—the process by which red blood cells (erythrocytes) are produced—as a way to decipher and decode the two blood disorders beta-thalassemia and polycythemia vera.

Beta-thalassemia, a group of inherited blood disorders, is caused by a defect in the beta globin gene. This results in production of red blood cells that have too much iron, which can be toxic, resulting in the death of many of the blood cells. What are left are too few blood cells, which leads to anemia. At the same time, the excess iron from destroyed blood cells builds up in the body, leading to organ damage.

In polycythemia vera, a patient's bone marrow makes too many red blood cells due to a genetic mutation that doesn't shut down erythropoiesis—the production of the cells.

The researchers studied both normal erythropoiesis, in which a person makes enough red blood cells to replace those that are old, and a mechanism called stress erythropoiesis, which flips on when a person requires extra blood cells—such as loss of blood from an accident. The hormone erythropoietin (EPO) controls red blood cell production, and can also induce stress erythropoiesis. Iron is also essential, says Dr. Rivella. "The two well-known elements needed to switch between

normal and stress erythropoiesis are EPO and iron," he says.

But Dr. Rivella and his team found that a third player is essential: macrophages, the immune cells that engulf cellular garbage and pathogens. Macrophages had been known to digest the iron left when old blood cells are targeted for destruction, but Dr. Rivella discovered that they also are necessary for stress erythropoiesis. He found macrophages need to physically touch erythroblasts, the factories that make red blood cells, in order for more factories to be created so that they can churn out red blood cells.

"No one knew macrophages were a part of emergency red blood cell production. We now know they provide fuel to push red blood cell factories to work faster," says the study's lead author Dr. Pedro Ramos, a former postdoctoral researcher at Weill Cornell.

The research team then looked at diseases in which there are too many red blood cell factories. Polycythemia vera was one of the conditions examined. The researchers disabled macrophage functioning in mice with polycythemia vera and found that red blood cell production returned to normal.

In beta-thalassemia, the body increases the number of red blood cell factories to make up for the lack of viable blood cells—a strategy that doesn't work. As a result, patients develop enlarged spleens and livers due to the overload of erythroblasts in those organs.

The researchers found in mouse models that if they suppress the function of macrophages, the number of blood cell factories revert back to normal levels. However, there was also an additional benefit discovered. One of the functions of macrophages is to put excess recycled iron into erythroblasts. Researchers report if you suppress that function, less iron goes into the red blood cells. "So you then make red

blood cells that have less iron, and they are now closer in structure to what they should be," says Dr. Rivella.

In animal studies, the researchers saw that decoupling macrophages from the erythroblasts not only reduced the number of blood cell factories, but also improved anemia.

The discovery could be translated into an experimental therapy by finding the molecule that physically binds a macrophage to an erythroblast, and then targeting and inhibiting it. "We need macrophages for good health, but it may be possible to decouple the macrophages that contribute to blood disorders," Dr. Rivella says. "I estimate that up 30 to 40 percent of the beta-thalassemia population could benefit from this treatment strategy."

Dr. Rivella also made another connection. He says polycythemia vera "is sort of a tumor of the red cells, because you make too many of them." And he notes that previous research on macrophages found that they are very important in cancer metastasis. "I see a parallel between the activity of macrophages in supporting the proliferation of cells that are under stress conditions—growing tumors and red blood cells that need to grow," he says. "It seems to us that macrophages are important in supporting a switch between normal growth and increased growth."

Too Much Iron As Well As Anemia

In the *Journal of Clinical Investigation* study, researchers from Weill Cornell and from Isis Pharmaceuticals of Carlsbad, Calif., examined the body's exquisite regulation of iron. Too little iron causes anemia. Too much iron in the body results in organ toxicity such as heart attacks and liver failure. Beta-thalassemia and hemochromatosis are two disorders in which affected individuals accumulate too much iron in their bodies.

Now, Dr. Rivella, with his partners at Isis Pharmaceuticals Dr. Brett P. Monia and Dr. Shuling Guo, have revealed the ballet of molecules that controls iron absorption, as well as what goes wrong and how to potentially correct the deficit.

Iron control is regulated, first and foremost, by hepcidin or Hamp, a hormone secreted into the bloodstream by the liver. Hamp controls the so-called "iron gate" in the intestines, a protein known as ferroportin. Ferroportin allows the body to absorb iron from food to help make red blood cells. (Iron latches on to the oxygen that the blood cells carry.) If iron levels are too high from iron-rich foods that are consumed, Hamp levels increase, which shuts the door on ferroportin's iron gate, blocking iron absorption, says Weill Cornell's Dr. Carla Casu, a postdoctoral researcher in Dr. Rivella's laboratory and one of the two lead authors of this study with Dr. Guo at Isis Pharmaceuticals.

Patients with beta-thalassemia and hemochromatosis have levels of Hamp that are too low, so the body absorbs more iron than is healthy. Hemochromatosis occurs because of a deficit in the HFE gene that controls the Hamp hormone. "Hamp is sleeping. It doesn't wake up when iron comes along, so too much iron is absorbed," says Dr. Rivella. The defect in beta-thalassemia is due to a defect in the globin gene that helps make hemoglobin. So Hamp is shut down because the body senses the anemia, and believes that more iron is required to make red cells. As a result, there is iron overload."

The researchers found an answer to the iron overload in both diseases by studying a third disease, a childhood disorder in which a mutation in a gene called Tmprss6 causes Hamp levels to rise too high, so not enough iron is being extracted from the diet. Tmprss6 keeps Hamp levels high during childhood and adolescence, so a body cannot use iron successfully to grow.

They reasoned that if they could create the conditions of Tmprss6 mutation—high levels of Hamp hormone and repression of the body's use of iron—in patients with thalassemia and hemochromatosis, they could treat those conditions. "If we block Tmprss6, we increase the expression of Hamp to normal levels, with the consequence that iron does not now accumulate," Dr. Monia says.

The research team leaders, Dr. Monia and Dr. Guo at Isis Pharmaceuticals, developed an antisense drug that blocked Tmprss6 "in order to wake up Hamp expression." An antisense drug works by administering a chemically modified, stable DNA-like molecule that targets specifically an RNA sequence that is produced by the gene. This sequence binds to the natural gene RNA product, forming a double-stranded RNA/DNA hybrid duplex. This duplex is recognized by enzymes in the cell that cause degradation of the natural RNA. "When you destroy that RNA, you destroy the ability of the Tmprss6 to make any protein," Dr. Monia says.

Both potential therapies offer new solutions to old blood disorder diseases. They need more studies before they can be brought to the clinic, although the antisense technology can be rapidly modified for its applications in humans, Dr. Rivella says.

"These studies are like putting together pieces of a complicated puzzle, which then offers you the big picture, as well as ways to creatively improve the view," he says.

Provided by Weill Cornell Medical College