Genome editing improves blood clotting in mice with hemophilia B

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(Medical Xpress) -- Scientists have used a gene therapy tool that acts like intelligent molecular scissors to correct the key gene defect in mice with hemophilia B, a disease that can lead to uncontrolled bleeding. The intervention improved the animals' blood clotting enough that their severe disease was reduced to a mild form.

Hemophilia B affects about 3,000 men and boys in the United States. People with hemophilia can experience uncontrolled bleeding, including spontaneous and life-threatening bleeding into the joints or the central nervous system. In severe cases, patients must undergo a lifetime of clotting factor infusions to control bleeding.

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Katherine A. High.

The disease is caused by defects in the blood clotting factor called Factor IX. Repairing that gene defect could eliminate the disease. One possible gene therapy strategy is to use molecular tools called zinc-finger nucleases, which allow scientists to snip out and correct specific DNA defects. So far, this approach has only been used successfully to edit genes in isolated cells, but now, in what may be a significant advance in gene therapy research, Howard Hughes Medical Institute investigator Katherine A. High has broken that barrier. Her team's work is published in the June 26, 2011, issue of the journal *Nature*.

Zinc fingers are a natural part of the genetic machinery in organisms from yeast to humans, and each one's finger-like structure binds to a specific sequence of DNA. By linking these DNA-binding molecules to DNA-snipping enzymes called nucleases, scientists have created molecular scissors that can be targeted to specific parts of the genome.

The zinc-finger approach is more precise than conventional gene therapy, in which whole genes are introduced to cells to counteract the action of mutated genes. When those genes insert into the cell's genome, it's anybody's guess where they'll land. "There is some possibility of integration of the donated DNA in untoward sites that may, for example, promote tumor formation," says High explaining one risk of conventional gene therapy. "If you could do genome editing, you could get a site-specific correction."

In laboratory cell cultures, zinc-finger nucleases can be targeted to home to a specific site on the genome and snip double-stranded DNA in two. Sensing the disruption, the cell rushes to repair the break, often by copying nearby homologous DNA. Scientists take advantage of that tendency by administering a strand of flawless DNA designed to correct the mutated gene along with the molecular scissors. If all goes well, the cell's own DNA repair proteins copy the flawless strand into the cellular DNA, and, voilà, a perfect gene where a broken one once lived.

"People had already shown you could do this kind of genome editing, but only in cells you could take out, manipulate in the laboratory and put back in the patient," said High, whose lab is at the Children's Hospital of Philadelphia. In the new work, High's team repaired the defective gene responsible for hemophilia in living mice, and the correction was maintained in new cells when the treated cells divided.

Key to making this work, High said, was using adeno-associated virus to carry both the DNA strand and the zinc-finger nuclease into the mouse

liver. This virus, engineered from naturally occurring parvovirus, heads straight to the liver when injected intravenously. The liver is the main source of the blood clotting factors missing in hemophilia.

To be used for gene therapy, zinc-finger nucleases must be designed to specifically target the defective segment of DNA. Working with collaborators at Sangamo BioSciences, High tested a number of zinc-finger nucleases that the California company had engineered to target various portions of the gene that encodes Factor IX, the clotting factor that fails to work in patients with in hemophilia B.

Serendipitously, the zinc-finger nuclease that most efficiently snipped out the Factor IX DNA was the one that allowed her to simultaneously target all of the gene's commonly mutated segments. "We didn't know whether among all of the [zinc-finger nucleases] that were designed, this one would succeed," she said. "This turned out to be the most efficient, which was fortuitous."

When the mice with severe hemophilia B were treated with the Factor IX zinc-finger nucleases, the clotting factors in their blood increased to a level that is considered mild disease. Most individuals with hemophilia B have less than 1 percent of normal clotting factors. An increase of clotting factor to 5 percent of normal transforms severe hemophilia into mild hemophilia. "That doesn't mean you can play football, but it does mean you can play tennis," High said. "In these mice we got levels ranging from 3 percent to 7 percent."

"I think that there were a couple of keys to our success," she said. "We used a highly efficient method of delivery to the liver, and, secondly, the zinc-finger nucleases we used were very efficient."

High says further work will be needed to translate her team's work in the laboratory into safe, effective treatments for patients, but the new

findings are promising for the future of gene therapy.

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