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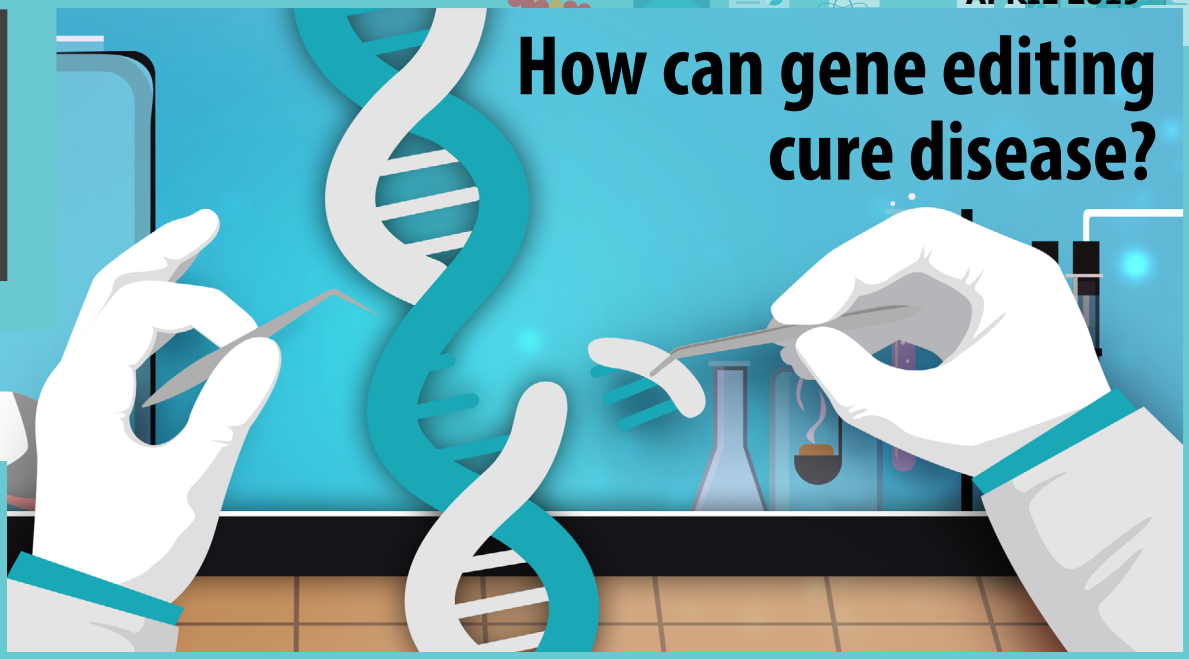
How can gene editing cure disease?



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Abstract

Did you know that the cell copies 50 *nucleotides* (letters of DNA code) per second when it is dividing? And it only makes one mistake per 100 million nucleotides! That's like copying the full 32 volumes of Encyclopedia Britannica twelve times and only making one typo!

Most times even these mistakes are caught and fixed. But sometimes a *mutation* (mistake in the code) gets passed on. In eggs and sperm that means an unborn baby will get one bad copy of that *gene*.

In most cases, even this is okay. The baby is a *carrier* of a bad copy of the gene, but often the good copy from the other parent will work well enough. In rare cases, though, a baby may receive a bad copy from both parents. This means they will have a *genetic disease*.

There are several diseases that are caused by a single *nucleotide mutation*. Scientists have always wanted to use *genetic editing* to correct the bad part of the gene. We found a way to do it in real, live mice!

Introduction

Imagine you misspelled one letter of one word on an assignment, and the teacher gave you a 0% for the whole thing. Not fair!

But this is what happens in diseases like *β-thalassemia*. Just one of the nucleotides in the *DNA* code for a gene is copied wrong, and yet the molecule that gene is supposed to make can't do what it needs to.

β-thalassemia is a blood disorder. People with this disease cannot produce *hemoglobin*, the molecule in red blood cells that carries oxygen through the blood.

We would like to fix the section of their DNA. Then they could produce the oxygen-carrying molecule on their own! This is called genetic editing. If we correct the bad letter of the DNA code we can cure a person for life!

That's very difficult, though: bodies are very good at destroying the molecules we use to do genetic editing. Most of the time this is a very good thing. Outside of the lab, most unfamiliar molecules are from a virus or infection. But it makes our job very hard.

We thought we could solve this problem using *nanoparticles*.

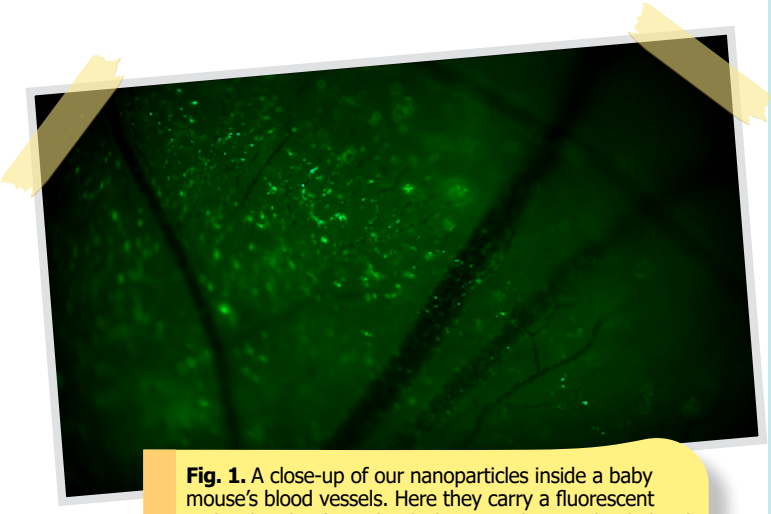


Fig. 1. A close-up of our nanoparticles inside a baby mouse's blood vessels. Here they carry a fluorescent molecule. They're so bright because they are loaded with lots of molecules, but they're still so small that they can travel through the tiniest blood vessels! (See the full video on our website!)

What is a nanoparticle?
Nanoparticles are much bigger than the molecules we use for genetic editing. These particles can carry hundreds of our genetic editing molecules! Our molecules stay inside the nanoparticle until it reaches its destination. This means the body will not break them down. But nanoparticles are still *very* small. They are about a thousand times smaller than a grain of sand! This means they can still travel to where we want them to go (Figure 1).

Methods

What do we need to genetically edit DNA?

Essentially we need just three components:

1. A *donor DNA* with the correct sequence - it serves as a template. We want the cell to use this correct sequence to replace the mutated section of the gene.
2. An *editor molecule*. This molecule physically breaks the DNA backbone of the mutated gene and inserts the donor DNA in its place.
3. A *specificity molecule*. This molecule tells the editor molecule exactly where the DNA strand needs to be cut for our donor DNA to get added.

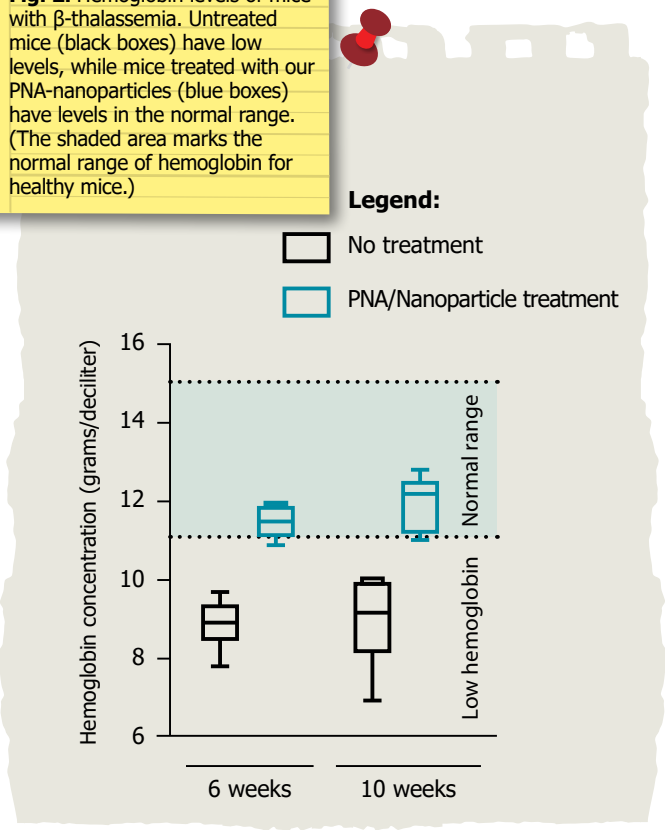
The editor molecule makes permanent changes to DNA. We need a very accurate one, or we might create new mutations! So we decided to use the safest editor molecule out there: the cell's own *DNA repair* molecules. The native DNA repair molecules can detect unusual structures, and swap in the donor DNA to fix them.

But we also had to pick the right specificity molecule. *Peptide nucleic acids (PNAs)* bind to double-stranded DNA to make a strange-looking triple-stranded PNA-DNA segment, which is recognized by the native DNA repair molecules.

Lastly, we needed to pick the right kind of vehicle to deliver our editing molecules. We decided to use a *biodegradable polymer* nanoparticle that is known to break down to safe natural molecules after its job is done.

DNA replication is the fastest in *fetal* (unborn) animals, so we decided to genetically edit mice *in utero* (before their birth). We then injected the donor DNA and PNA-loaded nanoparticles into fetal mice with the β-thalassemia mutation and waited for the results!

Fig. 2. Hemoglobin levels of mice with β-thalassemia. Untreated mice (black boxes) have low levels, while mice treated with our PNA-nanoparticles (blue boxes) have levels in the normal range. (The shaded area marks the normal range of hemoglobin for healthy mice.)



Did you know that this type of plot is called "box and whisker"? It is a graphical representation of a range of data points. The box represents the middle half of the values (if you were to line all the values up in order, from ¼ to ¾ of the way through), and the whiskers show the full range of the data values.

Results

We saw most of the nanoparticles injected *in utero* traveled to the developing liver. This is great news! The fetal liver makes the cells that produce hemoglobin after the mouse is born.

Next, we checked the mice six and ten weeks after they were born to see if we had correctly edited the hemoglobin gene. Our genetically edited mice made as much hemoglobin as a healthy mouse (Figure 2). They were effectively cured!

The genomes of these mice showed that 6% of all hemoglobin-producing cells were successfully edited. Just as importantly, no new mutations happened from our molecules editing at the wrong site.

Lastly, these mice lived a full, normal life span!

Discussion

These results are fantastic news! Our nanoparticles delivered our molecules to the right cells and corrected the hemoglobin gene in 6% of the cells we wanted to edit.

Does 6% sound small? In diseases like β-thalassemia, 6% is fine. The corrected cells made enough hemoglobin to keep the mice healthy. Still, this might not be enough for correcting other diseases.

However, human babies take nine months to develop. Mice develop in just 20 days! This means we could give a human baby multiple doses of our nanoparticles rather than just one. Each dose could increase the percent of edited genes.

Of course, we aren't testing this treatment on human babies just yet. We need to be completely sure these PNA-loaded nanoparticles are safe and effective before we try it on humans. But we *are* one step closer!

Conclusion

Until recently we could only dream about editing away genetic disease. With our PNA-loaded nanoparticles, we might just make that dream a reality!

β-thalassemia is just one genetic disease. With the right PNA and donor DNA, our nanoparticle method could theoretically cure any disease caused by a DNA-copying mutation. Some examples are sickle cell anemia, cystic fibrosis, and Huntington's disease.

Doctors already have the tools to detect these genetic diseases *in utero*. Soon we may also be able to correct the error before the baby is born!

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Check your understanding

1. Why did we use nanoparticles?
2. What could go wrong if our editor molecule wasn't specific enough and edited other spots in the genome?
3. Look at Figure 2. Why do you think we checked twice, even though we got the results we wanted at 6 weeks?
4. Our method could theoretically be used for any disorder caused by one bad gene. Can you think of a disease where our editing method would not be helpful?
5. Why did we use biodegradable nanoparticles?
6. Some people consider genetic editing unethical. All powerful tools can be used for good or for bad. If we can genetically edit away a disease, do you think we should?

Glossary of Key Terms

β-thalassemia - a genetic disease where the red blood cells cannot properly carry oxygen. This disease results from receiving two sets of bad genes from their parents, as one good set can produce enough of the oxygen-carrying molecule (hemoglobin).

Biodegradable polymer - a large molecule that is made of many small biologically natural molecule "parts," linked together into a long chain. When a biodegradable polymer breaks down, these parts are either useful to the body or are easily removed through the body's waste elimination processes.

Carrier - a person who "carries" one bad copy and one good copy of a gene. In most cases, the bad copy just can't do what it is supposed to do, and the one good copy is enough to keep them healthy. The only concern is that they may pass on the bad copy to their own children.

Complementary strand - DNA is double-stranded, meaning two strands form the DNA double helix *(duplex)*. The letters that make up the genetic code "pair" with their complementary strand. A binds to T on the complementary strand, while C binds to G.

DNA - the molecule found in each cell of a living organism. It contains all the instructions the cell needs to grow from a single cell into an entire person and every cell type they have in their body! It is what a cell uses to store the genetic code.

DNA repair molecule - the molecule in cells that locates and corrects DNA copy errors. It is the cell's natural editor molecule. While the DNA duplication molecules only make one mistake every 100 million nucleotides, the DNA repair molecules reduce the number of errors by *another* 100 fold! This molecule detects when something unusual is going on with a section of DNA, like when our PNAs bind to it.

Editor molecule - a molecule that breaks DNA chemical bonds and inserts a new DNA section in its place. Editor molecules are needed to genetically edit DNA, but they can cause more harm than good if they edit sections of DNA that we did not want them to!

Fetus - a developing animal or person before they are born. Each cell in a fetus will divide many times as it grows. Genetically editing a mutated gene in a fetus's cells is much less work than editing the gene in all the cells of a full-grown adult body.

Genetic editing - modifying the code of a gene. This also alters its function.

Genetic mutation - a change to a gene's DNA code that makes that gene not function how it is supposed to. Sometimes a mutation can be a single nucleotide, like in β-thalassemia. Other times it can be a duplication, deletion, or rearrangement of an entire section!

Hemoglobin - the molecule inside a red blood cell that allows it to carry oxygen through the blood. Hemoglobin is what gives red blood cells their color!

In utero - inside the womb. Babies grow and develop inside of their mother's uterus, so all fetal treatments must be done *in utero.*

Nanoparticle - molecules that are much smaller than what we can see with the naked eye, so small that they can move throughout the tissues of a body. Drug delivery is just one thing nanoparticles can do.

Nucleotide - the individual letters that make up the DNA code. While DNA stores all of the genetic information, it has just four letters to do so! The four nucleotide letters in DNA are G, C, A, and T.

Peptide nucleic acid (PNA) - a molecule that can tightly bind to a specific section of DNA. It looks similar to a strand of DNA, but it is synthetically made in the laboratory. It still has the G, C, A, and T letters, but its backbone looks more like an amino acid than a nucleotide! They are designed to bind to the major groove of DNA (forming a triplex structure), directly to the DNA bases, or even to do both!

Specificity molecule - this molecule directs the editor molecule where it needs to edit. In our case, the specificity molecule had to bind to the correct spot on the mutated hemoglobin gene to flag down the DNA repair molecule. It did so by creating an unnatural triple-strand, which changed the DNA's shape.