**The Science breaker**

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**Title: CRISPR-STOP: Stopping a gene is safer than damaging it**

CRISPR is becoming a magical tool in molecular biology. Once known as the bacterial immune system, it now continues to revolutionize fields ranging from agriculture and medicine. As someone who uses this tool and aspires to further develop it, it is great to witness that the word “CRISPR” is being frequently exchanged in daily life conversations and mass media. In its basic form, this technology allows us to “cut” DNA at a specifically targeted site in living cells. Here, the “living cells” part of this sentence needs a special emphasize because scientists were able to cut DNA, copy it and paste it in test tubes for decades. But doing this in a living cell was extremely challenging and in most organisms, it was not possible.

This challenge is now over thanks to CRISPR. The CRISPR tool is composed of a DNA-cutter protein Cas9 and a short guiding RNA called sgRNA. Critically, this short RNA determines where the DNA-cutter is going to bind and cut the DNA. More importantly, the short guiding sequence of this sgRNA can be custom designed; therefore, the DNA-cutter Cas9 can be specifically shuttled to anywhere in the genome to cut the DNA at the desired site. To understand what it means to cut DNA and what happens next, one needs to know the basic structure of DNA. DNA is a long fiber composed of two strands. It is the essential molecule for any living cells. Therefore, during each cell division, this long DNA fiber is replicated and equally distributed to each of the daughter cells. If there is a cut in DNA, i.e., the two strands are broken; there is great a risk of losing a big part of the DNA fiber. Therefore, throughout evolution, cells have developed multiple DNA repair mechanisms to overcome such scenarios. The most efficient one quickly fuses the broken ends together and seals the breaks. This repair system is not perfect. After the repair, random short DNA sequences are either inserted or deleted at the break sites. These insertions/deletions (indel) may have vital consequences depending on where they are. If they happen in the protein-coding part of DNA, even an indel of a single DNA base can shift the frame of information and results in complete loss of protein product. In scientific terms, this is called gene knock out (KO) and it is a highly used approach to understand potential function of a gene.

CRISPR is a very efficient gene KO tool because it is a potent DNA damaging agent. Importantly, DNA damage can be very costly to a cell. If the overall damage is extensive, the damaged cell undergoes a programmed cell death called apoptosis. CRISPR indeed can also induce cell death if the target site is present at multiple sites in the genome. We, therefore, set out to develop an approach to silence genes without breaking the DNA strands. We have hypothesized that such approach will be a safer way of deleting genes compared to traditional CRISPR approach. To achieve this, we use a modified CRISPR tool that enables changing one base into another base without causing DNA double-strand breaks. One of these so-called “base editors” can convert “C” bases into “T” in DNA. Since DNA is composed of four genetic letters (A, G, C, T bases), and the genetic information is translated in triplet codons, there are 64 possible combinations that three letters can form. Of these combinations, 61 code for an amino acid and the remaining three codons, called STOP codons, do not code for anything but signal the end of a gene. We have hypothesized that by changing C into T, we can change some of the normal codons into a STOP codon. Critically, if we do this at the beginning of a gene, this will result in a loss of gene function because the genetic information will end prematurely. More critically, we have hypothesized that this approach will not result in any DNA damage because unlike traditional CRISPR approach, we are not causing DNA double-strand breaks.

To demonstrate the utility of this approach, we used human endothelial cells where we could transfer both WT Cas9 enzyme (traditional) as well as CRISPR base editor. We have showed while WT Cas9 creates random DNA insertions/deletions, our approach specifically edits the genetic code and creates a STOP codon at the target site. Most importantly, we demonstrate that the CRISPR-STOP approach is much safer than the traditional CRISPR approach. We also studied whether CRISPR-STOP was actually safer approach. We therefore compared the traditional and CRISPR approach in terms of the overall DNA damage and the rate of cell death that each approach caused. These comparative analyses showed that CRISPR-STOP results in significantly less overall DNA damage and cell death compared to the traditional WT Cas9 approach. We have demonstrated that CRISPR-STOP is particularly effective when the target site is present in multiple places in the genome. In such situations, WT Cas9 results in cell death due to excessive DNA damage. On the other hand, CRISPR-STOP results in efficient gene silencing without causing significant DNA damage. Notably, we calculated that of the ~20,000 genes in the human genome, we could create an early artificial STOP codon in about 17,000 of genes. Finally, we demonstrate that CRISPR-STOP approach can be used to target multiple genes at the same time in a population of cells. Such approaches enable scientists to interrogate the function of many genes at the same time in a high throughput fashion. Our results demonstrate that CRISPR-STOP approach is compatible with such approaches.

Original Article:

*CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations.*

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