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CRISPR is an Incredible Tool for Treating Cancer

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a relatively new gene editing technology. It can be used in many ways, including cancer research and treatment. CRISPR and CRISPR associated enzymes (Cas) were discovered as an adaptive immune system in bacteria in 2013, and have already been used in clinical trials. In treatment, it binds to or removes mutated genes, canceling their effects. In research, this is used to test the necessity of the gene for cell survival or drug sensitivity. There are still several difficulties in using it, but it is already far better than previous tools, and will become better as research continues.

Keywords: CRISPR-Cas9, Cancer, Biotechnology, Gene editing

1. Introduction

The relatively recent discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has given researchers an incredible new way to treat and study cancer. Cancer is a disease characterized by genome instability and mutations that contribute to rapid cell growth. Researchers have attempted to treat cancer using genome editing for many years, but CRISPR appears to be the most successful. Many researchers have contributed to discovering and adapting CRISPR as a cancer treatment, especially Dr. Doudna and Dr. Charpentier, who were awarded the Nobel Prize in Chemistry for their work on CRISPR. CRISPR-Cas has been adapted for two main uses in cancer: as a key tool in gene therapy and as an invaluable research tool. In gene therapy, Cas, most commonly a version called Cas9, can function in numerous ways to edit the genome. For instance, it can remove the mutated gene, bind to the gene so it is inaccessible, and more. CRISPR screens are a research tool for testing how important a gene is to a cell's survival or to its sensitivity to a cancer drug. Researchers have worked hard to make CRISPR as reliable as possible, but more work is needed on the delivery of CRISPR, to make it a useful widespread treatment tool.

2. History of CRISPR as a gene editing technology

The history of gene editing begins with the discovery of the DNA double helix in 1953, and has exponentially progressed since then. CRISPR was first discovered in 1987 in *E. coli* bacteria by Ishino et al., but it was not investigated until the early 2000s (Doudna et al., 2014). Zinc-finger nucleases (ZFNs) were the first targetable gene editors, first used by Kim et al. in 1996. While they were useful, and certainly set the ball rolling for future gene editing, they were difficult to work with. Because of how they are programmed, the use of ZFNs is time-consuming and expensive. (Chandrasegaran, 2017) Their successors, transcription activator-like effector nucleases (TALENS) were much easier to use. TALs were first described in 2009 (Boch et al., 2009), and in 2015, were used as the first genomic treatment of cancer in humans (Qasim et al., 2017). CRISPR-Cas9 and other variants quickly became the most effective gene editing technique, beginning in 2011, due to the discovery of a second RNA strand essential to

Vol. 2024 (4) 166 – 169
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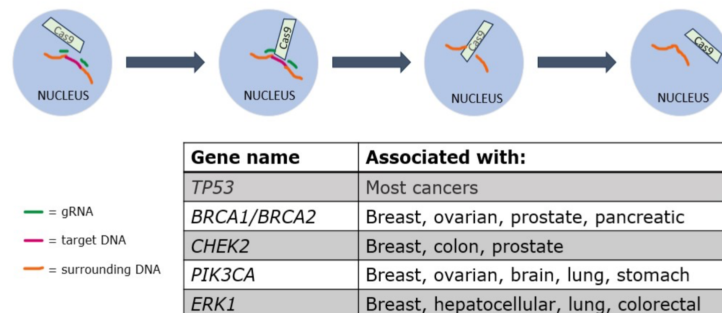
creating an *in vitro* CRISPR-Cas9 system by Charpentier et al (Charpentier et al., 2011). In January 2013, five articles were published saying that CRISPR-Cas9 had successfully been used to edit the genome of a human cell.

3. Mechanism of CRISPR

CRISPR has several enzymes associated with it that are necessary to its function. There is the Cas protein, an endonuclease, which actually binds and cuts the DNA, and there is the guide RNAs (gRNAs). A gRNA is a short RNA sequence which defines the genomic target as the DNA that will be complementary to their sequence. Cas9 - the enzyme variant most commonly used in cancer treatment - uses that to bind to where it needs to cut. Cas9 is used in relation to cancer more commonly than other variations because it is a type II system. Type II systems require one protein to function, whereas other types may require multiple proteins, making them much larger. (Brouns et al., 2008).



Gene name	Associated with:
TP53	Most cancers
BRCA1/BRCA2	Breast, ovarian, prostate, pancreatic
CHEK2	Breast, colon, prostate
PIK3CA	Breast, ovarian, brain, lung, stomach
ERK1	Breast, hepatocellular, lung, colorectal

Figure 1. CRISPR-Cas9 system can be used to edit genes associated with cancer. Colored lines represent DNA and gRNA. The green line represents the gRNA, which is complementary to the red target DNA. A simplified model is shown for clarity. The table represents a few common genes associated with cancer that the red DNA could represent.

4. CRISPR is an incredible cancer research and gene therapy tool

In cancer treatment, CRISPR can be used to directly remove genes, replace genes, and enhance or repress the transcription of genes. The gRNAs can be used to direct the Cas enzyme to the target gene, where it will cut through the DNA. As it is stitched back together, there will be a few nucleotides either removed or added, so that the undesired gene can no longer be transcripted (Chiruvella et al., 2013). This is the most common way of forming breaks, called non-homologous end joining (NHEJ). It is also possible to fix breaks by adding a new gene, called homology-directed repair (HDR). Although it is not as common, as it is mostly limited to certain phases in dividing cells, it is more precise and less error-prone than NHEJ (Ran et al., 2013)

Deactivated Cas (dCas9) paired with transcription repressor can bind to and inhibit a gene's transcription. This inhibition is called CRISPR interference (CRISPRi). CRISPRi is valuable because preventing the transcription of a gene can reduce the effects of that gene's expression. This can be better than simply removing a gene for various reasons. It can potentially remove some off target effects of completely cutting the genome. It can also block more than one gene at a time (Yao et al., 2015). One benefit of CRISPRi as opposed to knockout treatment is that knockout completely removes the gene, restructuring the genome. With CRISPRi, the genome is still intact, but transcription of the target gene is inhibited. The effects of CRISPRi are also reversible, so it doesn't have to be a permanent change.

CRISPR activation (CRISPRa) uses dCas9 paired with an activation effector to enhance the transcription of target genes (Wang et al., 2022). It is essentially the opposite of CRISPRi. Where CRISPRi is used if the gene is being overexpressed or having negative effects on the cell, CRISPRa is used if a gene is being underexpressed or another gene is inhibiting its expression.

CRISPR screens are a powerful research tool. gRNAs are transduced into Cas9 or dCas9 expressing cells, at a low rate to ensure that there is only one gRNA per cell. With only one gRNA per cell, this ensures only one gene is knocked out per cell (He, 2021). Some cells will then be treated with a drug, while others are left alone as a baseline. After a set amount of time, the remaining cells will be sequenced, which can lead to a better understanding of what genes are responsible for certain things (Aguirre et al., 2016). For example, if a cell survives that has a gene knocked out in the untreated condition, that means the gene that was knocked out is irrelevant to survival or actually inhibits survival. If a cell does not survive that has a gene knocked out, then that gene was required for survival.

Vol. 2024 (4) 166 – 169
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Some screens also compare the baseline to what happens if the cell is treated with a drug (Bock et al., 2022). If, when treated with a drug, the cell survives, the gene that was knocked out was probably responsible for sensitivity. This can be checked against what happened without the drug. The opposite is also true. If, when treated with a drug, the cell dies, the gene that was knocked out was responsible for resistance. This is important because it allows for patient-centric, personalized therapy and maximizes efficiency in all therapies.

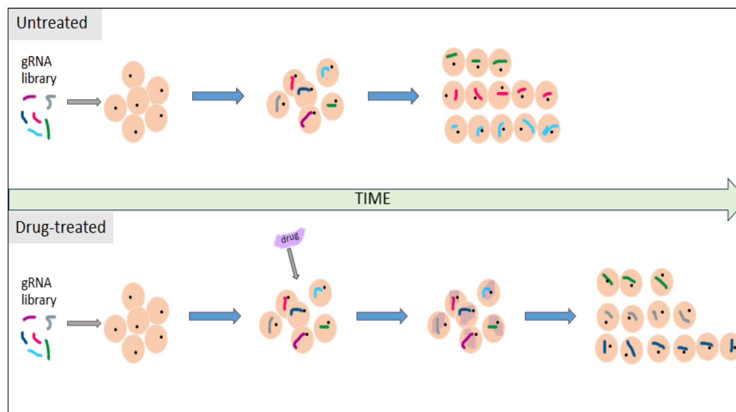

Figure 2. CRISPR screens are a research tool for studying genes that are critical to cancer progression or suppression. The dot in each cell represents Cas9, already integrated into the cell. Different gRNAs are represented by different colored lines. In the untreated panel, colors represented in the end show that the removal of those genes did not affect the survival of the cell. Colors that are not represented in the end represent genes that affect a cell's survival. In the drug-treated panel, colors represented in the end represent genes that contribute to sensitivity to the drug, as losing those genes causes those cells to survive more. Colors not represented in the end represent genes that contribute to resistance, as losing these genes causes those cells to survive less.

5. Difficulties in using CRISPR *in vivo*

There are two major difficulties in using CRISPR. One is the method of delivery. CRISPR is a large protein, so finding a carrier able to transport it is difficult. There are several methods, however, categorized into two major groups - viruses and nonviral. Viral methods can be divided into adenoviruses (AV), adeno-associated viruses (AAV), and lentiviruses (LV). Each method has its advantages and disadvantages. AAV are a fairly reliable method of delivery, especially *in vivo*, but they have a low cloning factor (Yang et al., 2016). AVs can transduce into any cells, and are widely used in clinical trials, but they are more likely to trigger an immune response that will prevent the AV from reaching its destination (Lee et al., 2017). LVs have a larger cloning capacity and can efficiently transduce into dividing and non-dividing cells. However, they have a tendency to randomly integrate with the genome, creating harmful mutations (Yip, 2020). There are several physical delivery methods, but these are impractical for *in vivo* delivery (Fajrial et al., 2020).

The other major difficulty is off target effects. These are things that may happen with the genome that are not on or near the target of the Cas9 protein. This can be a huge problem, as it could cause other difficulties for the cell or organism. The causes of target effects are not well understood, and neither are the actual effects.

There are ethical difficulties to using CRISPR as well. There are side effects of gene therapy that can drastically change a person's life, and researchers and doctors must consider what are acceptable consequences. They must also be careful of ethical laws, such as those against human experimentation (Gostimskaya, 2022). As CRISPR becomes more common, discussions of the ethics surrounding its use will become more important.

6. CRISPR now

Researchers have made enormous progress in utilizing CRISPR as a genetic tool. It will be extremely valuable if it can be widely used on patients, and will hopefully help with many genetic diseases and disorders. It has progressed incredibly quickly towards that point in only a decade. In 2013, its potential as a genetic tool was realized. Now, in 2023, there are many clinical trials for patients with cancer, some of which have been going on for years. Many genes associated with cancer have been identified, which is extremely useful for researchers (Gonzalez-Salinas et al., 2022). CRISPR can be targeted at these genes to remove or edit them, and can be used to identify drugs that can be used against cancer. Off-target effects and delivery methods continue to be a problem, but researchers are finding ways to avoid these difficulties. If researchers continue their studies and work to overcome the current challenges, both scientific and ethical, it may be possible to have a reliable cure to cancer with minimal effects, something that has only been dreamed of before.

Vol. 2024 (4) 166 – 169
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