Crispr-CAS9

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# What is CRISPR-CAS9?

CRISPR stands for clustered regularly interspaced short palindromic repeats. This technology allows the scientists to edit genes. It is a revolutionary discovery that completely changes the whole biomedical field of research. It enables us to modify, make adjustments and correct possible errors inside the genome. Furthermore, it makes it possible for us to enable and disable the genes from cells or organisms at low costs, high speeds and, most importantly, without difficulty. Previous applications have proven that CRISPR is capable of repairing damaged DNA from rats, healing them from the genetic diseases that they had. This discovery led to theories that infer the fact that human embryos can be altered in a similar fashion.

CRISPR can also be used in the clinical field for applications such as treating HIV and other infectious disorders, gene therapy and projecting genetic material cultivated on the patient in order to treat cancer and other disorders.

# How does CRISPR-CAS9 work?

For this method to function, two vital components need to be present: CAS9 which stands for CRISPR-associated protein 9, and a guide RNA that needs to match the target gene. CAS9 is an endonuclease that generates a double-stranded rupture to the DNA, making it possible to modify the genome.

# How can this technology be used in the clinical field?

Although briefly mentioned above, I do consider important to clarify the role of this new discovery for the clinical field. The most exciting and important applications of this technology lays in its potential to cure genetic diseases provoked by single-gene mutation. Some of the disorders that fit in that category are:

* haemoglobinopathies
* CF (cystic fibrosis)
* DMD (Duchenne's muscular dystrophy)

It is important to note that the method has been only tested in preclinical models, with the intention of implementing it into the clinical practices soon.

The CF disorder has been treated using CRISPR-CAS9 by Gerald Schwank et al. They collected mature intestinal cells from two patients suffering from cystic fibrosis and they managed to rectify the most generic mutations that were causing cystic fibrosis in the intestinal organoids. Moreover, they proved that by repairing once the mutation, the functionality of the CFTR (cystic fibrosis transmembrane conductor receptor) has been restored.

In the foreseeable future, haemoglobinopathies could also be treated using the CRISPR-CAS9 technology. The mechanism has been used by the researchers in order to perturb the DNA at a specific part called the BCL11A enhancer. This disruption can trigger a gene that generates fetal haemoglobin both in mice and in human primary erythroblast cells. Hopefully, this method will enable fetal haemoglobin to be inserted into patients that have irregular adult haemoglobin. This new, but roundabout strategy, would enable adults to produce fetal haemoglobin because it is extremely hard to simply produce adult haemoglobin. However, this approach should relieve some of the associated symptoms.

The DMD disorder has been researched using CRISPR-CAS9 by Mohammadsharif Tabebordbar et al. They used AAV (adeno-associated virus) for the delivery of CAS9 endonuclease inside a mouse suffering from Duchenne's muscular dystrophy. The purpose was to repair all of the expressions of dystrophin from that model mouse by removing the exon that had the original mutation. The result of this intervention was a truncated, yet viable protein. After the experiment, the lab rats showed signs of recovery and they managed to regain muscle functionality. Furthermore, it has been proven that the dystrophin can be edited into muscle stem cells, and these stem cells can be used to repair muscle tissue that is mature. This crucial piece of information assured the scientists that the CRISPR-CAS9 treatment will not fade or disappear over the time.

# Will there be a cure for HIV?

Fortunately, there exists antiretroviral therapy, which delivers an efficient treatment against HIV, but there is no cure for HIV due to the viruses’ constant integration inside the hosting genome. Wanqing Hu et la demonstrated that the CRISPR-CAS9 technology can identify the genome activity of HIV-1. This made the HIV gene integration mechanism inactive and it stopped the virus from cloning itself inside the cells that were predisposed to infestation, all without causing any toxic repercussions. Moreover, the targeted cells would also be immune to future HIV-1 infections. This discovery can lead up to a treatment that would cure organisms that already suffer from HIV.

# Limitations

Despite all of the benefits, there are still a lot of problems to be solved before this technology can reach all of the patients in need. One issue is arisen by the means of transportation of the gene editing to the cells in need when the treatment is made in vivo. For a safe delivery, there needs to exist a suitable vector, one that has no toxicity associated, one option used before is AAV (adeno-associated virus). The problem with AAV is that it is too small for efficient transport of the CAS9. Another option is to develop a smaller CAS9 gene that can be transported, but this approach affects efficiency.

Another problem is represented by the possibility of editing the genome in an undesired part, unwillingly, by misfire. This can have unwanted long-term effects.

# Bibliography:

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