Biology Paper

CRISPR/cas9

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Class: 5B

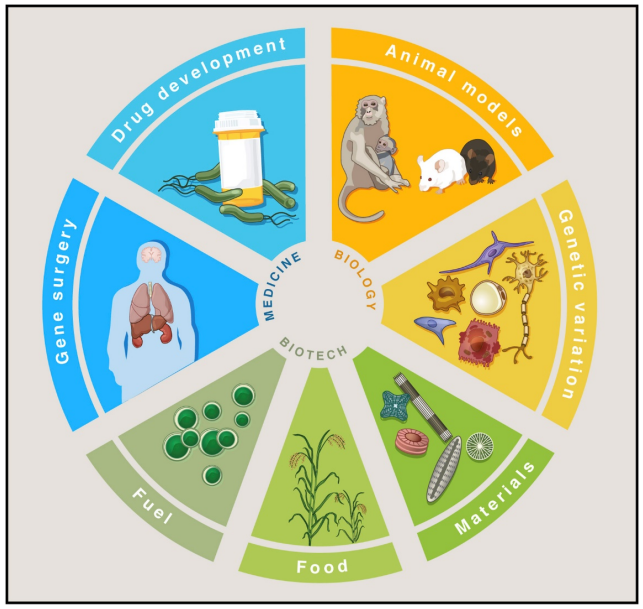

Table of Contents

1. Preface

We found this topic through an article about a dog which gained double the muscle mass because his genes were edited with CRISPR/cas9. We were in disbelief and quite astonished and asked ourselves how this was possible. We then found out how big of a deal CRISPR/cas9 is in biology right now and how many different applications it has. We were also shocked when we found out that two girls were born in china whose genes were edited with CRIPSR/cas9. This technology sounded to us like it came directly out a science-fiction movie or some kind of alien technology. We were both very intrigued and fascinated by this topic so we decided to choose it for our paper. Our main motivation was our curiosity and fascination about CRISPR/cas9.

What we find very interesting is the many different applications for it. It can be used on humans, animals and even plants. Of course, it is also very interesting to find out how CRISPR/cas9 even works. Another interesting aspect is the ethical one. Potentially people could already design their babies and decide exactly how they want them to look. Agriculture is another big topic which can be changed completely by CRISPR/cas9. Some people are talking about ending world starvation by editing plant genes with CRISPR/cas9.

We asked ourselves many questions. How is CRISPR/cas9 even possible? How was this developed? How exactly does it work? Is it safe to use on humans and should it even be used on humans? Is this ethically justifiable?

2. Introduction

Towards the end of 2018 Chinese researcher Jiankui He used CRISPR/cas9 to conduct the first genome-editing operation ever in a human embryo. The goal of the operation was to disable the genetic pathway that allows a cell to be infected with HIV. According to him the operation was successful and the twin girls are as healthy as any other babies. For Jiankui He and his team this was a big step towards the future, but other scientists are skeptical or unhappy with this event. The operation violates ethics guidelines but not any law and it is certainly one more step towards what we used to think was science fiction. Jiankui He defends himself on a YouTube video he uploads shortly after the outrage of the scientific community. He explains that this technology is meant to help people like the father of the two girls who is HIV positive and never thought he could have children. He thinks that this technology should be accessible to all in need but should not be used to create so called designer babies.

Before this event embryos had only been gene edited to investigate the benefit of using CRISPR/cas9 to eliminate disease-causing mutations in labs for research purposes only. But some researchers quickly discovered the risk of off-target effects from CRISPR/cas9. Scientists that disapprove of Jiankui He's work stated "There is, at present, no unmet medical need that embryo editing addresses".[1]

CRISPR/cas9 is a relatively new technology but the groundwork for its discovery dates back to 1987. Back then scientists discovered unusual DNA sequences in bacteria which they called clustered regularly interspaced short palindromic repeats, in short CRISPR. In 2007 researchers understood that the Cas9 enzyme was needed to cut the DNA sequence and that it was a process from the immune system of bacteria to protect itself against viruses. And finally, in 2011 Jennifer Doudna and Emanuelle Charpentier developed CRISPR/cas9 as we know it today and made the technology applicable on all living cells. Since then it has been one of the most important discoveries of the century when it comes to gene editing for medical, agricultural and research purposes.[2]

CRISPR/cas9 allows us to edit segments of the DNA in a very precise and easy manner. The cas9 enzyme cuts the DNA strand and an RNA molecule directs the Cas9 enzyme so it cuts in a specific spot. It is much more consistent and therefore safer than older methods. This also means discussions about ethical problems surrounding gene editing become a much more urgent issue than most people thought.

[1] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(18)33080-0/fulltext

https://www.dhushara.com/Biocrisis/18/11c/HIV-CRISPR.Reduce%20to%20300%20dpi%20average%20quality%20-%20STANDARD%20COMPRESSION.pdf

https://www.youtube.com/watch?v=th0vnOmFItc

[2] https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline

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https://www.nzz.ch/wissenschaft/biologie/genschere-crisp-cas9-die-vergessenen-pioniere-ld.117763

The technique is already used a lot by researchers and it's slowly starting to get used for medical purposes. It represents a big step since it has the potential to cure genetical diseases by correcting genetic mutations. In the past this was impossible.

It is also starting to get used to genetically engineer plants. And since it is a natural process it is impossible to tell the difference between a plant that has been bred and one that has been edited with CRISPR/cas9. According to an article about genome engineering you can sum all these applications up into basic biology, biotechnology and medicine.[3]

[3] https://www.sciencedirect.com/science/article/pii/S0092867414006047#fig3

3. Description of engineering technique

The CRISPR/cas9 systems is based on two key molecules. The first one is an enzyme called Cas9. It is responsible for cutting the DNA sequence at the target location in the genome. Once cut you can replace or remove parts of the DNA. The second molecule is a so-called guide RNA (gRNA). This is a pre-designed RNA sequence which is about 20 bases long and is located within a longer RNA scaffold. This RNA scaffold then binds to the DNA that will be edited. The pre-designed sequence within the RNA scaffold guides the Cas9 enzyme to the right part of the genome. The Cas9 then makes a cut across both strands of the DNA. The cell in which the DNA was cut recognizes the damage and tries to repair it. Scientists can use this DNA repair machinery to then insert changes to one or more genes in the genome of a specific cell.[4]

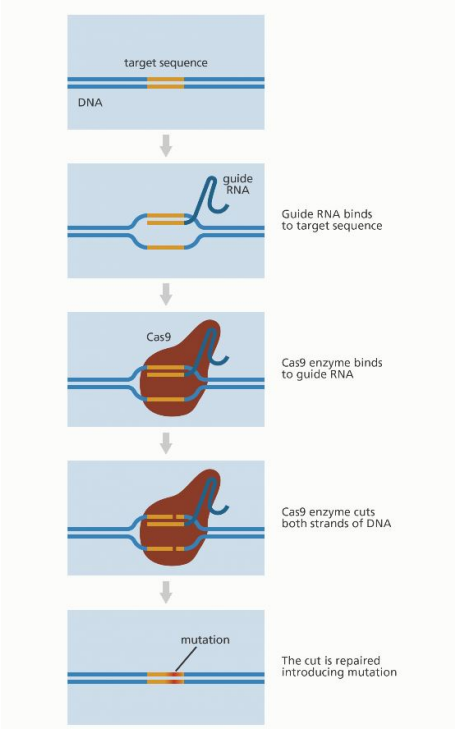

Image 1

[4] https://www.yourgenome.org/facts/what-is-crispr-cas9
https://www.synthego.com/guide/how-to-use-crispr/sgrna
https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting

4. Interviews and pictures of procedure

All our interviews were done via E-Mail conversations. The first interview is with Mr. Prof. Dr. Ernst Hafen who was recommended to us by Tobias`s older brother who studies at the ETH in Zurich and visited lectures from Professor Hafen. The Interview was done in German and then translated to English.

1. Which application of CRISPR/cas9 are you most interested in?

CRISPR/Cas9 as medicine. Instead of inhibiting an enzyme or another protein with an antibody or a chemical substance, you can edit the DNA with CRISPR. A 3D (protein structure) is reduced to a 1D (1 dimension) DNA sequence problem.

https://www.ethz.ch/de/news-und-veranstaltungen/eth-news/news/2018/10/erbkrankheit-mittels-genkorrektur-geheilt.html

2. Which new research possibilities does CRISPR/cas9 offer?

Cc (CRISPR/cas9) in medicine development (see above).

Cc in gene therapy.

Cc in agriculture.

3. How close are we to using CRISPR/cas9 as therapy to fight heritable illnesses?

5-10 years.

4. Do you think interventions in the DNA before birth should be allowed, what consequences could such interventions have?

If these therapies are safer and more efficient and cheaper. In most cases, you can predict and prevent homozygous heritable illnesses by prenatal diagnostic.

5. How can we explore the consequences on later generations that could possibly show up if we use CRISPR/cas9 before birth like in china?

This requires a lot of research. Although Cc is a technology developed by nature 3 billion years ago. It was not created by humans it was only applied by them.

6. Do you think CRISPR/cas9 will be used in agriculture in Europe in the near future?

It is ethically not justifiable to not use our knowledge and our research funds to follow this path. Even if we do not (yet) need aridity- and salt resistant plants such developments could contribute to securing world food supply.

7. Is it possible to compare the plants, which are formed by this technique, with traditionally grown plants or is there a big difference?

In normal breeding, we do not know what we are doing at the genome level. We only select for a larger fruit. At the same time, in normal sexual exploitation, all gene variants of father and mother are reshuffled. With Cc we specifically alter a gene. The problem

is that many features do not come from single genes, but from the interaction of 100-1000 genes. So, Cc cannot be used in humans to make smarter humans.

8. Do you think CRISPR/cas9 can be applied ecologically to agriculture?

Sure.

9. In which direction do you think CRISPR / cas9 will develop in the next 10 years, how important is it for the future?

See above and the interview by J Doudna in NZZ Folio.

The second interview was done with Priya Satalkar. She is a Biomedics Ethics Expert and works at the university of Basel.

1. Which applications of CRISPR/cas9 are the most problematic from an ethical standpoint?

Any applications that try to edit human genome with CRISPR/cas9 are deeply problematic. So are the applications in somatic cell editing where, first it is not scientifically or medically needed to edit genome, second the editing does not provide benefits that outweigh the risks and where the long term safety and efficacy is unknown, and third where effective treatment or prevention strategy currently exists for a disease.

2. Are there applications without any ethical problems at all at the moment?

It is hard to say that there are applications without any ethical problems. Ethics does not take into account just individual welfare but also that of society and future generation. Ethics also tries to ensure that the benefits from an application are not accessible, available and affordable only to a select group of people or nations but rather those benefits can be shared globally. Having said that, if the technique over time grows to be very selective, with least off target effects and fewer chances of mosaicism, diseases with single gene inheritance could be the applications where some nations and funding agencies will allow research with CRISPR/Cas 9. Examples of such diseases would be sickle cell anemia or muscular dystrophy. However this will still be basic research and not translated into clinical setting unless and until we are very sure of the effectiveness of intervention in preventing disease, long term effects of such editing, and there has been extensive multi-stakeholder discussion where it is agreed that we might have reached a stage when clinical application is permissible with rigorous monitoring in place.

3. Do you see any differences between carefully bred agricultural plants and plants modified with CRISPR/cas9?

I am not an expert in field of botany and hence can’t really make a judgement on this.

4. Where lies your biggest concern with CRISPR/cas9 applied on humans?

The biggest concern is permanently editing human genome which will be passed on to future generations, where the change is irreversible and could make individuals or group of peoples susceptible to other conditions as a result of gene editing. I personally also do not agree with use of this tool for enhancing treatment as in improving person's muscle or eye sight so that one could see in infra-red light.

5. Do you think it should be used as therapy to cure genetic disorders, if long term consequences are identifiable?

After sufficient data has been collected about long term consequences through robust follow-up, if scientific experts opine that the benefits of editing outweigh the risks, if there are no off target effects, even then we need to consider many other questions including societal views, views of minority groups, patient groups before one could use it as a treatment option. If there are already existing, safe and effective treatment or prevention options against a disease, then just long term consequences being identified does not make it acceptable to go ahead and use it as therapy to cure genetic disorders. Before any new experimental treatment modality is accepted as a therapy, it has to undergo rigorous pre-clinical and clinical trials.

6. On which aspect of CRISPR/cas9 should researchers focus in the near future?

As explained earlier, disease with single gene inheritance without effective prevention and treatment options will be the first focus of research using CRISPR/cas9 near future. Furthermore, we also need to extensively study the specificity of editing, off target effects, reducing mosaicism in cells where a person shows a mixture of edited and non-edited cells. Another key focus of researchers should be rigorous peer review of research by their peers and engagement in public debate so that the general public can also be informed about the technology, its potential and limitations.

7. What ethical issues are there with Gene Surgery in the prenatal state? (like they did in china https://www.youtube.com/watch?v=th0vnOmFItc)

There are number of issues with the case of human genome editing that took place in China.

First of all, it was not in line with local Chinese regulation. Global community of scientists had also called for moratorium on clinical application of genome editing till enough safety, efficacy data was obtained. He did not follow that. The informed consent procedure with the couple had many inadequacies and flaws. The baby's born, one had mosaicism, which means some of her cells had CCR5 gene edited and some did not, which defies his purpose of making them resistant to acquiring HIV infection. His action to edit this gene makes these girls susceptible to other infections such as west Nile virus. Some of the changes or effects of gene editing do not become evident until much later in life, we do not know what those effects will be in these girls unless they are carefully followed up over their life span.

8. Do you think it could be considered unethical to not use this technology to help people?

At this stage with our current knowledge about the technology, its effects, blind spots in scientific knowledge and lack of input from larger societal conversation and debate,

considering using this technology will be unethical. At this stage, I am not sure we will help people by using this technology, we might be exposing people to significant harm that will be passed down to future generation. So, we are far from the point where our knowledge and scientific evidence is so compelling that it will be considered unethical not to use the technology to edit germ line. Even if scientific evidence is robust and appealing, unless society at large is also engaged in actual debate, going ahead with use of this technology is likely to harm the discipline and reduce public trust in science. We are not there yet as to edit human germline and we should not do it.

Pictures of procedure

Image 2

The first thing you need is cells. They are placed in petri dishes.

Image 3

The CRISPR/cas9 is then injected into the cell with a needle.

Image 4

After the cells are edited, they are stored in frozen vials and can be transported or stored and used for whatever they are needed.

5. Discussion

By editing the genome of agricultural plants, we will soon be able to make them more resistant to environmental deprivation or pathogenic infection. Also, will it be possible to make more nutritious food and even allergy-free food. In the USA researchers are currently researching which genes and combinations of genes are responsible for which properties. In Europe the progress in this domain is slower because of stricter guidelines and regulations.

The issue here is that some consider plants with an edited genome by CRISPR/cas9 a genetically modified plant while others say that it can be considered a plant like any other since the same mutation could occur with normal breeding processes. Since CRISPR/cas9 leaves no trace of the genome-editing even organic farmers that do not use any pesticides would plant CRISPR/cas9 edited plants.[5]

In the USA it has already been decided that this technology is not considered a genetical modification thus the first products may hit the market soon.

More cost-effective biofuels could also be developed by creating efficient metabolic pathways for ethanol production in algae or corn. This would create a really attractive new renewable energy source.

The CRISPR/cas9 technology could also be used as a tool for gene therapy to correct harmful genetic mutations causing inherited disorders. Patients with cystic fibrosis, sickle-cell anemia, or Duchenne muscular dystrophy could be permanently cured.

In the case of muscular dystrophy experiments on animals have already proven that CRISPR/cas9 can be used as therapy. Researchers used it on dogs and one treatment was enough to permanently stop the disease. People or animals who suffer from muscular dystrophy don't produce a protein which is essential to all muscles including the heart. Therefore, many who have the disease do not live a very long life. Thanks to CRISPR/cas9 it was possible to delete the malfunctioning dystrophin gene and replace it with a fully functioning one. In humans there have been some minor successes, but there is still much research to be done to be certain of the safety of the method.[6]

The main issues concerning safety are that like in genetic mutations there is a really small possibility that the Cas9 enzyme cuts at the wrong place. This is completely harmless when it comes to plants since the mutations would also occur with traditional breeding, but it is really dangerous when it comes to treatments, since an off-target editing could make things much worse than they were to begin with. Another issue is that at the moment we use harmless viruses to introduce the Cas9 enzyme and the

[5] https://labiotech.eu/tops/crispr-applications-gene-editing/
https://www.deutschlandfunk.de/molekulare-pflanzenzuechtung-debatte-um-crispr-cas-pflanzen.676.de.html?dram:article_id=417139

[6] https://www.sueddeutsche.de/wissen/gentherapie-genschere-heilt-muskelschwund-bei-hunden-1.4111951

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guide RNA molecule, but some patients have a too weak immune systems and risk dying because of the operation.

When you add genome-editing of embryos into the equation lots of new problems arise because of the changes the genome will pass on to the next generations. There is still very little known about the consequences of CRISPR/cas9 and this will surely be the main focus of future research. These high risks also explain why the Chinese CRISPR-twins are so controversial especially since it was a disease that could have been treated without the use of CRISPR/cas9.

This high-risk high reward dilemma is the core of the ethical problems surrounding CRISPR/cas9.

Of course, many people would benefit from this technology and currently have very few effective alternatives but in the wrong hands this technology could also be abused since it is so powerful.

In an interview Jennifer Doudna, one of the creators of the method, states that she once had a nightmare where Hitler told her he was interested in the technology, which must have been terrifying but at the same time she sees a bright future for the medical applications and hopes that one day we will be able to cure genetic diseases and better the lives of many people.[7]

This is the most represented ethical stance: the technology should be used to help patients who cannot get any other medical treatment and it should be used to improve our agriculture so we can adapt it to climate change and feed more people.

On the other hand, it should be forbidden to use it to create designer babies or anything similar.

In the next few years research should focus on the potential damage that mistakes with CRISPR/cas9 could provoke and on how to use it in the safest manner possible.[8]

[7] https://folio.nzz.ch/2019/mai/heiliger-strohsack-das-wird-krass

[8] https://www.aerztezeitung.de/politik_gesellschaft/medizinethik/article/964538/medizinethik-crisprcas-methode-zwischen-zauberwerk-hexenkunst.html

6. Summary

-CRISPR/cas9 is one of the most significant discovery of the century.

-CRISPR/cas9 has a wide range of applications, the most noteworthy being the medical field and agriculture.

-It was discovered in 2011 and seven years later the first CRISPR-babies were born.

-CRISPR/cas9 can edit the genome by cutting DNA sequences more precisely and easily than ever before.

-CRISPR/cas9 has two main components: the Cas9 enzyme and the guide RNA.

-In agriculture CRISPR/cas9 could allow us to create stronger more nutritious crops.

-In medicine CRISPR/cas9 could correct harmful gene mutations which cause disease.

-The ethical issue is that there is still too much uncertainty surrounding CRISPR/cas9 and that the bad things it can do could potentially still outweigh the good ones.

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Cover Image

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Image 1

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Image 2

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Image 3

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Image 4

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