Figure 1 Danio rerio

The Regeneration of Photoreceptors in Zebrafish *(Danio rerio)*

A Scientific Paper

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1.Preface

Over the last few decades life expectancy increased drastically, bringing age related diseases with it. Macular degeneration (AMD in short) is the most common chronic illnesses causing severe visual handicap or even blindness for people over the age of 50 years. In fact, 32% of blind or visually impaired people above the age of 50 suffer from AMD. [1] The blindness is caused through the dying off of sensory cells in the retina. This damage is irreversible for humans. Zebrafish on the other hand, were proven to be able to regenerate their sensory cells. It is known that zebrafish are good at regeneration, they can even regenerate parts of their fins and brain.

It is no wonder, that us humans want to copy this skill. In this term paper we want to discuss, how and if we are able to do so. We want to discuss the following questions:

- How can zebrafish regenerate their retinal cells?
- How can we apply this technique in human retina?

We think this topic is becoming progressively relevant. There are numerous studies going on, especially because this topic is closely linked to the regeneration of other types of cells.

2.Introduction

Zebrafish have the ability to regenerate their cells. We chose the topic of regenerating the retina with the neurotransmitter GABA (Gamma-Aminobutyric Acid). This neurotransmitter activates the gene to regenerate cells. In this case it activates the gene to regenerate the retina cells. During our research we realised that this topic is closely linked to the epigenetic changes, so we included it as well.

The genome of zebrafishes and humans are very similar so zebrafishes are perfectly suitable for the research of healing disease in the retina of humans. For example, Macula degenerations could be healed with this method of regenerating the retina. Sadly, there is no other method to regenerate the retina and the regeneration with the GABA neurotransmitter has never been applied to humans.

Recently, researchers found out that there is a Müller cell dependent retinal regeneration. That works with dedifferentiated Müller cells because they will re-enter into the cell cycle and regenerate some types of neurons. Then 2018 researchers found a two-step reprogramming approach to induce mouse Müller cells into photoreceptors. This results in partial vision restoration in a mouse model of congenital blindness and offers new therapeutic perspectives to treat retinal degenerative diseases. [2]

3.Description of Engineering Technique

3.1 The Anatomy of the Retina

To understand the possible techniques, we first have to look at the anatomy of the retina. For its understanding, it is okay to assume that fish and human retina are the same. The retina is composed of several layers. The first one is the ganglions, they send the impulses to the nerve. In the second layer are Amacrine, Bipolar and Horizontal cells. They help with the communication between the neurons and photoreceptors. The Müller cells are also located in this layer. For our topic they play a crucial role, because they do not only serve for communication and transmission of impulses, but they also have stem cell like abilities. The fourth layer is composed of the photoreceptors and lastly there is the retinal pigment epithelium.

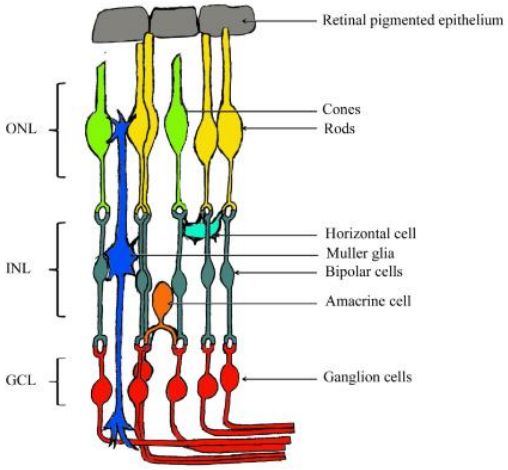

Figure 2 Anatomy of Retina

The Müller cells are the most important part looking at the regeneration of retinal neurons. They normally act as a transmitter of light impulses in our retina, but they also serve as stem cells. Müller cells in zebrafish retina are able to divide into a post-miotic Müller cell and a neuronal progenitor cell. [3] These neuronal progenitor cells then undergo further cell division, before they eventually develop into newly differentiated retinal cells. In the following, we describe two methods that try to imitate this skill.

3.2 GABA as Regeneration Factor

Researchers found out, that the lack of GABA (Gamma-aminobutyric acid) causes these Müller glia to produce those neuronal progenitor cells. GABA is a neurotransmitter that normally enables local communication between neurons. [4] When GABA levels are high, stem cells stay quiet, when they are low, they start reproducing. This was proven in an experiment, where they injected GABA into zebrafish retina, which then could not recover from damage. In contrary, when they injected a GABA inhibitor into an undamaged zebrafish retina, they detected regenerative response. Other studies showed that the GABA neurotransmitter could be generally linked to cell regeneration. When GABA is injected into the brain and pancreas of mice, the stem cells stay quiet.

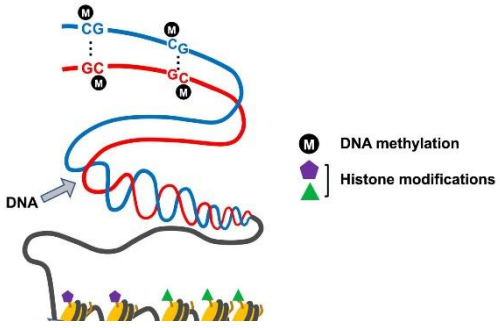

Figure 3 DNA Methylation and Histone Acetylation

3.3 Epigenetics

All of our cells have our complete genome in it. The "only" thing that differentiates Müller cells from Photoreceptors are the genes that are expressed. Parts of our DNA, that are not used in differentiated cells are wrapped around histones, thus are not transcribed and translated. [5] The methylation of DNA and the acetylation of histones are both epigenic modifications. Methylation silences genes through binding methyl groups directly to the DNA

with the help of DNA methyltransferase. [6] In certain cases, this can also lead to the expression of other genes, when a gene coding for a repressor for other genes is silenced. Acetylation is associated with transcriptional activation. The adding of acetyl groups to histone proteins leads to the detaching of the DNA, that is wrapped around the histones. This process is catalysed by enzymes called histone acetyltransferase.

4.Interview

For our Term Paper we interviewed Emily Bayer. She is in a research team at the Biocentre Basel and has already done research on zebrafish.

1. **Question:** Could you tell us something about your research topic and what you did?

   Answer: Right now, I'm interested in the connection between sensation inside your body and your brain. Your brain always has to be keeping track of what is going on in your body. I am interested in the connection between your brain and all of your organs because the brain is directly connected to all of them. One of the reasons why I think this research is great to do in zebrafish is that the early development in contrast to for example a mouse is in an egg so everything is external. Also, the egg of the zebrafish is also completely transparent so you can see the whole animal during the development. That allows us to do a lot of good research in terms of actually being able to see the neurons that connect the brain and the body.

2. **Question:** Is there a special gene for the regeneration of body cells? (2.47min)

   Answer: This is a topic which is very interesting to people. You have to pay attention to how big your fish is in contrast to how big your container is. That is because fish never really stop growing so theoretically a fish can just get bigger and bigger. That is one of the reasons why people think the brain keeps growing too, because the body never stops growing. But also, there are other kinds of regeneration that zebrafish are really good at. There are some extreme cases where for example you cut of a fin of a fish and the fish will regrow that fin. That is because the fish can reuse its genetic program (which made the cells grow in first place) to regrow cells. This reusing technique of the genetic program is really interesting to us humans because why can't human regrow an entire arm? Everything we would need to do that is reuse the genetic program that we had for the first place growth. But sadly, we don't know how to turn this program back on.

3. **Question:** Would it hypothetically be possible to take the gene and put it in a human embryo so the human has the ability to regenerate just like the zebrafish does?

   Answer: I think that the human embryo already has it and is using it but the problem is that the gene just turns itself off and we cannot turn it back on so you have it but it is just turned off. I think people are more interested in gene therapies. For instance, gene therapy right now is being use to reverse some kinds of blindness that people have where something goes wrong in your eyes. Whereas the cells sense light they will die and then you inject the corrected version of it and then the cells will survive and you won't go blind. You have to address the problem where it is happening so you have to do gene therapy in an older individual rather than be able to fix it in an embryo ahead of time.

4. **Question:** Would you inject DNA/ RNA or how does that work?

   Answer: They are often injecting DNA and there are some viruses called retroviruses that insert themselves into your DNA and so for instance if you have a mutation in your gene they'll inject the correct version of this gene in a retrovirus so it inserts itself into your genome but just locally and then you have a correct copy in your eye.

5. **Question:** In what stage is the gene extracted?

   Answer: If you want to get the gene you collect a lot of embryos and then you can purify the all the DNA from this in a tube. And then you'll amplify the gene that you want and you can insert any modification that you want in it. This modification is going to be in another tube and that will be injected into the egg and then the next generation will have the modification.

6. **Question:** What special traits could human want to copy from zebrafish? (19.45min)

   Answer: I think regeneration in zebrafish is definitely the top one. But also, the fact that zebrafish are able to continue making new neurons in the brain is interesting for a lot of diseases. For example, if you have a brain injury.

7. **Question:** Do you think it would be ethically correct to inject the zebrafish gene into humans?

   Answer: It depends on wether you would know what would happen or not. If there is only a single mutation in one single gene then it is not an ethical concern at all because we would know what would happen and we know what the gene acts like when it's being fixed. But unless you are not 100% sure what happens after the injection you should not do it because then it is an experiment.

5. Discussion

5.1 Progress in research

There were several studies on the neurotransmitter GABA during the past few years. In 2017 they proved that GABA represses the regeneration of Müller glia in zebrafish retina. The injection of GABA in mammalian retina does not have the same effect, as another study shows. [7] Current research tries to find out more about the effects of epigenetics and the differentiation of retinal progenitor cells. [8]

5.2 Future research steps

A future step in epigenetic research is finding the genes that are responsible for dedifferentiating into their progenitor cells. Parallel to that, research on the neurotransmitter GABA could investigate more about the process in mammalian retina and other factors that play a role in photoreceptor regeneration. [9]

5.3 Ethical aspects

All changes in the genome must be ethically justifiable. Epigenics have the benefit, that the amino acid sequence stays the same, only the gene expression is modified. Thus, no genetic information is lost or added. Epigenetics hold big potential, the dedifferentiation of cells would change a lot, especially in medicine. The threat is, that epigenetics are a rather new topic in research. For example, we still do not quite know, how epigenetic changes are inherited and if the changes fade over time.

Strengths: The amino acid sequence is not changed, thus no genetic information is lost or added.

Weaknesses: In consequence to the fact that no genes are added, we cannot adopt certain skills/traits from other species or individuals with epigenetics.

Opportunities: Our complete genome is present in each of our cell. Theoretically we could modify cells with epigenic changes to dedifferentiate back into stem cells and then differentiate into any other cell.

Threats: Epigenetics are fairly new in research. For example, we still don't quite know, how epigenetic changes are inherited or if the changes will disappear over time.

6. Summary

Zebrafish are very fitting animals to work with in research. Not only because their genes are very similar to human genes but also because their embryos are completely transparent and grow fast. But the most interesting thing about them is their ability to regenerate their retina. This ability could help in the research for healing retinal diseases, for example age related Macular Degeneration. This disease is caused by the dying off of retinal neurons. The Müller, which are crucial to the regeneration of the retina, normally serve for communication and transmission of impulses. If there is a lack of GABA (Gamma-aminobutyric acid) in the system, then these Müller glia will produce neuronal progenitor cells and in contrast if there is a high level of GABA, then the Müller glia will stay quiet. Through this finding it is possible to regenerate the retina in laboratory animals.

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Figure 3: Epigenetics | National Cancer Center Reseach Institute (ncc.go.jp)

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