Gene Manipulation as Treatment against Pulmonary Arterial Hypertension

Biology Term Paper

09.04.2018

Armelle Odermatt, Minja Bojic, Rebecca Rietschi

Table of Contents

1. Preface

We wanted our theme to be connected with up to date research on gene manipulation, to help with develop suitable medications for a cardiovascular disease. Therefore, we have chosen a subtopic that is more specific, “application of genetic engineering and biotechnology”.

We decided to write about pulmonary arterial hypertension, short PAH, because we wanted to expand our knowledge about a potential medication, developed with the help of gene technology and a medication that could possibly help further generations to manage and even cure the disease.
What we found particularly interesting about PAH, is that it’s a current subject. Several physicians and biochemists are currently engaged in on-going research all over the world. However, no treatment that can cure entirely PAH has been found yet. About 50 percent of people who are diagnosed with PAH, will die from it within five years. Furthermore, an interesting point is, that the disease appears is less common in general, but occurs unusually more often in young people and children, especially in females and girls.

As we find this subject complex and challenging, we came up with some interesting questions for which we would like to find the answers.

The questions are:

- What exactly does PAH mean and what are the symptoms?
- Why do some people get the disease and other don’t? What are the risk factors of developing PAH?
- Is it a genetic disorder or do we get it by doing or eating certain things? Are there behavioural risk factors?
- How could gene manipulation help to develop a medication for PAH?
- and finally, what do we expect from this medication in the future and could this disease possibly be managed and/or cured?

These are the questions which we are eager to answer. Before writing this paper, we performed research on the internet. We wanted to learn more about PAH, finding answers to our mentioned questions.

The purpose of this paper is firstly to provide an insight look into this fascinating disease PAH and secondly the possibilities of using gene therapy to develop a tool to manage and/or treat the disease by answering questions specified above.

2. Introduction

2.1. What is PAH?

Pulmonary arterial hypertension (PAH) is a cardiovascular disease. It is a form of pulmonary hypertension (PH). Our heart constantly pumps blood through our arteries and veins, sending oxygen to all parts of the body. Oxygen-rich blood coming from the lungs goes out from the left side of our heart through our body and returns to the right side after having delivered oxygen. From there the oxygen-poor (and CO2-rich) blood it pumped to the pulmonary artery and back to our lungs. PAH occurs when the blood vessels in the lung are not working properly.[1] The tiny lung arteries become narrow, so it becomes difficult for blood to flow through them and keep normal blood flow in the cardiovascular system. During PAH, the blood pressure in the arteries that goes from the heart to the lungs (pulmonary artery) is increased compared to normal pressure. The heart has to work significantly faster and stronger in order to pump enough blood through these arteries to the lung (Figure 1). Because of so much constant extra work, the heart adapts to the extra work and increases the right ventricle. Over time it gets tired and weaker. This can lead to a heart failure and often to death. Because of that, considering that the blood of PAH patients has difficulties, getting through the lungs and to pick up oxygen, the blood oxygen level may be lower than normal. In other words, the organs of PAH patients have less oxygen. This has severe consequences on the brain.[2]

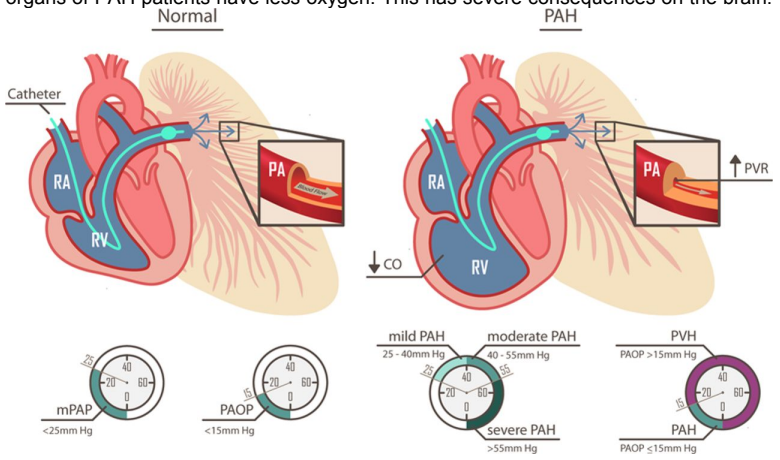

Figure 1: Difference between sick individual’s heart and lung dealing with PAH and a healthy one.

[1] Arefa Cassoobhoy. November 2017. Visual Guide to High Blood Pressure: https://www.webmd.com/hypertension-high-blood-pressure/ss/slideshow-hypertension-overview (29.3.18)
[2] Suzanne R. Steinbaum. Februar 2017. Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pulmonary-arterial-hypertension#1 (29.3.18)

2.2. Symptoms of PAH

Patients with PAH may suffer from shortness of breath during normal physical activity, fatigue, chest pain and a very fast heartbeat. In some advanced cases, this condition could mirror in fainting, swollen legs, hands or stomach, dry coughing with blood and blue fingers or lips, since they are not getting enough oxygen.[3]

2.3. Who is suffering from PAH?

PAH is a rare disease. People of all ages and sexes can be affected, but most commonly women between the age of thirty to sixty. This heart condition can be inborn, which means that it is carried and passed over by genes and the possibility percentage of getting PAH depends on a family history of this condition. If someone is dealing with other heart or lung diseases it could also lead to PAH.

Other than that, people have more risk of getting PAH by dealing with obesity, using street drugs or even living at high altitudes.[4]

2.4. The genetic basis of PAH

As previously mentioned PAH can be inherited. This is due to a germ-line mutation in genes that are coding for the proteins superfamily Endoglin. These proteins play a crucial role for the growth of blood vessels. In case of PAH, the protein BMPRII, a subfamily of Endoglin, is of importance. There are 144 different BMPRII mutations found in PAH-patients. That means that various genes that are coding for BMPRII can mutate which may affect the patient. Depending on the mutation different effects can occur. But about 70% of these mutations are nonsense or frame-shift mutations, which result in nonsense mRNA without any BMPRII protein production. On the other hand, about 30% of mutations are missense mutations. These mutations affect the receptor function of the cell membrane of blood vessels cell (endothelial cells) and this may lead to dysfunction of the cell and to a proliferation of it, which in turn may lead to a thickening and narrowing of the arteries in the lungs and as a consequence to PAH.[5]

2.5. How is PAH treated?

There is currently no cure for PAH but there are many types of medicines and procedures that can ease the symptoms of the patients who are suffering from PAH.
These medicines can work in different ways. Some of them let the blood flow easier through the arteries in the lungs. Others help the heart and lung to function more efficiently. The different medicines act therefore in many variant ways in order to ease one of the symptoms. One way for example is to block the calcium channels on the surface of the endothelial cells.

As previously mentioned the mutation of a gene in the BMPRII protein affect the receptor function on the surface of endothelial cells. While the receptors are malfunctioning calcium enters and create an over-concentration of calcium in the cell. When this happens the muscles that control the arteries contract and create a narrower gangway for the blood to circulate. By taking this medicine, that blocks the calcium channels the muscle cannot or less contract and therefore no narrow gangway. In a nutshell, this medicine lowers the blood pressure of the PAH-patient by relaxing the muscles that control the arteries.[6]

[3] Brunilda Nazario. January 2018. What is Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pah-overview#1 (29.3.18)

[4] Brunilda Nazario. January 2018. What is Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pah-overview#1 (29.3.18)

[5] Yen-Chun Lai & Karin C. Potoka & Hunter C. Champion & Ana L. Mora & and Mark T. Gladwin. June 2017. PAH, The Clinical Syndrome: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4096686/ (5.4.18)

[6] Brunilda Nazario. January 2018. Treatments for Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pah-treatment-options#1 (5.4.18)

3. Description of engineering technique

3.1. What is gene therapy?

"In the medicine field, gene therapy is the therapeutic delivery of nucleic acid into a patient's cells."[7] The goal of it is to modify human DNA in such a way that we can treat or prevent disease. There are various ways to manipulate human DNA. Depending on the disease that has to be cured, a gene can be inserted in the patient's cells that replaces a mutated gene, or an additional gene can be placed. As a result, a different protein will be formed that will help to fight, reduce and/or stop the disease.[8]

The processes of gene therapy include several steps. First, the DNA first has to be presented to the body, then it must reach the specific cell where it should be active. Therefore, it must enter the cell and then either express or disrupt a protein. There are multiple delivery techniques that have been explored and identified. One of the most used delivery technique, is to insert the manipulated gene into a virus, that acts as a vector. The modified virus enters the specific cell and brings its nuclear acids, with the new gene, in the chromosome of the human cell (Figure 2).[9]

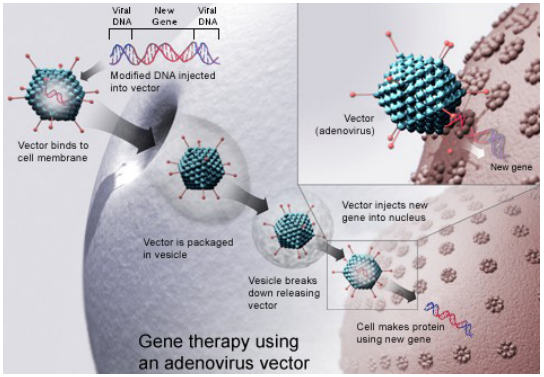

Figure 2: The engineered virus with new DNA sequence enters in a cell and delivers the modified sequence.

3.2. The gene therapy for PAH

Even if the survival rate of PAH-patient has increased thanks to the medicines over the past two decades, PAH-patients have a much-reduced life expectancy. Because of this, researchers are looking for a cure that could save PAH-patients. Gene therapy could be an important improvement. In recent studies, a gene therapy was investigated. It is, however, not yet tested on humans, but on rodents and pigs. The tests showed much success and confirmed, that this gene therapy not only improved the heart and lung function, but also reduces and even reversed cellular changes caused by PAH.

As previously mentioned the thickening and narrowing of pulmonary vessels is triggered by abnormal calcium levels within the endothelial cells. The calcium concentration is regulated by proteins, that act as a calcium pump and then prevent the calcium to proliferate in the vessel wall. However, if there is a mutation that affects the BMPRII protein, the receptor of endothelial cells and among them also the calcium pump will be affected and will subsequently malfunction.

The new gene therapy for PAH aims to deliver through a virus a modified DNA sequence, that will create more of those proteins, that act as a calcium pump. A down-regulation of these proteins leads to the proliferative re-modelling of the vasculature. By inserting this key DNA sequence, more of the calcium pump protein are produced. Their concentration increases as the calcium concentration inside the cell decreases until it reached a normal level. This will hinder the pulmonary vessel to grow and to create PAH.

The modified DNA sequence is as mentioned transported in the cell by using an aerosolized virus. This means the engineered virus contains the foreign DNA and has to be taken via an inhalation spray.[10]

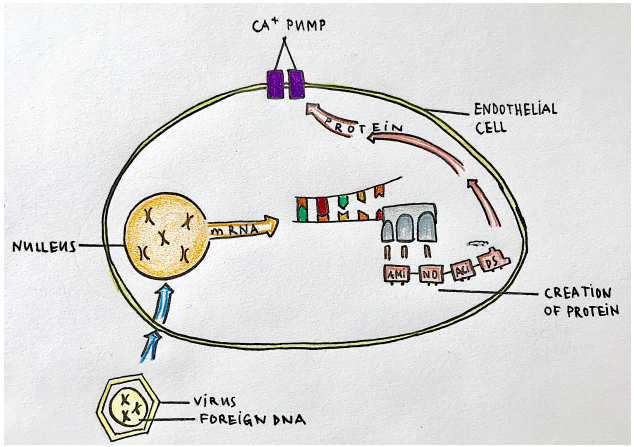

Figure 3:Self-made of the viral vector process and how DNA changes in a protein.

[7] Wikipedia. April 2018. Gene therapy.
[8] USA.gov. April 2018. What is gene therapy: https://ghr.nlm.nih.gov/primer/therapy/genetherapy (4.4.18)
[9] Melvin Y. Rincon & Thierry VandenDriessche & Marinee K. Chuah. August 2015. Gene therapy for cardiovascular disease: advances in vector development, targeting, and delivery for clinical translation.
[10] Professor Roger J. Hajjar, MD. April 2016.The Journal of the American College of Cardiology (JACC).

4. Documentation and pictures of research institutions visited

4.1. Idorsia

On the 19th of March 2018, Idorsia invited us to an Interview with Mr. John Gatfield. Idorsia is a new biopharmaceutical company, with a strong scientific department. It is located in Allschwil, Switzerland. Indorsia is specialized in the discovery and development of small molecules, to provide innovative therapeutic options.

4.2. The Interview with Mr. Mr. John Gatfield

Mr. John Gatfield is an Associate Director of Indorsia and Principal Scientist

1. What are you' re main tasks in the lab?

We work with cells, especially focusing on cellular biology. To be exact, we are interested in cardiovascular biology as well as the study of fibrosis. We deal with diseases of the cardiovascular system or organ fibrosis and induration of the tissue. All of this can lead to organ failure and can affect lungs, kidneys and liver. Finally, we want to develop medicines for various types of fibrosis or cardiovascular diseases, including hypertension and special forms of hypertension, such as PAH.

The mechanisms of fibroses are always similar, regardless which organ is affected. This means, that we think, that the drug developed for one organ could also dissolve or prevent fibrosis in other organs.

We work with cells, our favorite cells we work with are vascular muscle cells.

These are the cells that are arranged around the blood vessels (arteries, veins). The muscle cells that regulate blood pressure, cause the muscles to contract or relax. We are also working on the development of fibroblasts. These are the cells that multiply excessively in the fibrosis. This is not cancer but in a way comparable. The fibrosis cells replace the functional cells.

The fibroblasts are mostly obtained from humans. We can use them for experiments. Our intention is, to use these cells to assess whether a compound or a possible drug can change the behaviour of a particular cell, in other words giving it a push in the right direction.

Here in this laboratory, one of our tasks is to obtain a certain object in such a way, that the protein we want to be influenced by, for example blocking it with a drug or a substance, forces the protein to become inactive. This means we do work a lot with cells and sometimes also with isolated proteins. We get chemical compounds from chemists, we test them on the cells and see whether the protein is inhibited. In other words, this is what we do.

2. Clearly you produce medicaments, am I right?

Yes, you're right. Each week we receive roughly hundred chemical structures, which could be potentially fit for a successful medication.

3. Consequently, you do perform gene manipulation?

Not necessarily on these so called primary cells, which originate directly from human beings for direct usage and therefore don't need any gene manipulation. We also work with transgenic cells. These are cells in which we insert a gene from outside (DNA), thus it simply holds unfamiliar DNA in its cell nucleus.

In result, the trans-genetic cell produces one more protein – the protein for our own benefit. The advantage is, that the transgenic cells are very easy to cultivate. These cells contain inserted DNA, which very often is the object that interests us.

For example, we may say: "I would like to create a mutated version of the "target" in which an amino acid is missing". There are several gene sequences that can be introduced or removed. Our aim is to introduce a clearly defined protein into a simple cell each time an attempt is made and then examine this protein in the cell.

4. Could you use this technique to cure hypertension?

Yes of course! We know from hypertension, that there are several receptors on the cell surface, that bind the contractile hormones, subsequently cause the blood vessels to contract and as a result causing high blood pressure. These "poor-quality" hormones are called angiotensin. When angiotensin binds to the receptors on the smooth muscle cells, a signal is generated within the cell, which in this case leads to an increase in calcium (figure 4).

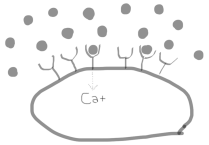

Figure 4: The event when angiotensin, the dark dots, binds to the receptors of a smooth muscle cell

As the calcium concentration increases, the muscle contracts in response. Inside the cell the muscle fibers contract, resulting in a narrower vessel diameter. If the calcium concentration cannot decrease again, the muscle remains tense and the vessel remains narrow. This causes high blood pressure in blood vessels.

However, the key to this would be, to find a substance that can bind to the receptors on the muscle cells and prevent angiotensin from binding instead.

By this means, no calcium increase can take place as well as contraction of the muscle. So basically, you're trying to develop a competitor. This is called competitive commitment.

In conclusion, we prepare cells, “throw” different substances on them that we hope will prevent calcium from being released into the cell.

1.3. Pictures of the Laboratory

Figure 5: The laboratory in the cell biology department of Idorsia.

Figure 6: A container holding liquid Nitrogen which helps preserves cell preparations to keep them alive.

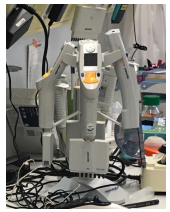

Figure 7: 12-channel adjustable volume pipettes. Such pipettes are used to generate multiple same (pro)portions of a solution. This is a faster way preparing substances.

Figure 8: A working booth in the chemistry department which is sterile because of the exhauster which would catch any liquid droplets advancing into the booth.

Figure 9: This fully automatic enormous machine equipped with a robotic arm is used to conduct experiments with analysing and collecting data at the same time and afterwards comparing them virtually on a screen.

5. Discussion

The developments of gene therapy for PAH is a new medical research. By now it has only been tested in animals, more precisely in rodents and pigs. In these studies PAH cured. Viral vectors were used for this gene manipulation. Viral vectors are actually more efficient than non-viral vectors because they show a long-term gene expression potential. We think this is a very big step in medicine, because if we found a way to treat PAH in certain animals, it gives us hope, that in a few years this method may be successfully tested on humans and may potentially save many lives in future. Doctors, chemists and pharmacists also lay a lot of hope in this new medication.

Gene technology and gene manipulation have a number of important advantages. They are, for example, that existing defective cells which cause harm, may be replaced with some new, healthy ones. With this technology, several diseases can be cured and many lives can be saved. This part of medicine is very important, because it shows great potential and is very promising for future treatments and develop new medications. It opens new possibilities manipulating genes in ways, that have not been discovered yet.

Another advantage of gene manipulation is that some diseases could be completely destroyed from the root, without leaving a trace or coming back. The method in cases of severe illness is also very promising for the future of medicine. By using somatic gene therapy, that will not interfere gene manipulation with germ cells and cells for sexual reproduction and not be passed sexually to the next generations.

Gene manipulation is more successful than drug therapy, as gene therapy can provide a cure for diseases, while drugs can only ease patient symptoms.

Some of the disadvantages of this technology are that gene manipulation could damage the gene pool of humans, which could be very serious and very dangerous for us as individuals and maybe even for the whole population. This means, that some of our genes could be changed or even that human abilities could be modified, which could lead to some serious problems and damages. As much as gene manipulation can fight and even destroy a disease, it could also encourage the potential rising in other diseases.

Gene therapy is theoretically a new breakthrough in the world of science. It is not yet sufficiently studied. It relies more on theory rather than proved facts.

When looking at this technology in detail, there are a lot of ethical, religious and moral concerns that could influence future development of gene manipulation.

There are also some concerns about the implications of viral vectors in this therapy, because it is not entirely clear, whether the vector will end up at the wanted destination or not. If not, it could cause damage to genetic structure, as well as to the patient.

The other disadvantage is that the results achieved with gene therapy were only short-lasting till now. To obtain long-term results, more research, studies and experiments have to be done.[11]

[11] March 2016. 6 Advantages and Disadvantages of Gene Therapy: https://futureofworking.com/6-advantages-and-disadvantages-of-gene-therapy/ (5.4.18)

6. Summary

PAH is a cardio vascular disease that can currently not be cured. PAH occurs when the tiny arteries in the lungs become narrow, so it becomes difficult for blood to flow through them and keep normal blood flow in the cardiovascular system. This means that the blood pressure in the pulmonary artery is higher and that the heart has to work significantly faster. PAH can lead to a heart failure and therefore to death. There are already many different treatments for individual handling of PAH, but none of the current treatments can cure the disease, only ease the symptoms. About 50 percent of the patients, who are diagnosed with PAH, will die from it within five years. That's why scientists and researchers are still searching intensively for a treatment that can cure PAH and save the ones that are suffering of it. In 2016 a study had shown, that there is a potential cure for PAH. This cure deals with gene therapy but is not yet clinical tested on humans.

In this treatment, the foreign DNA sequence is brought into the cell by a virus. The DNA sequence produces a protein, that acts as calcium pump and reduces the thickening and narrowing of pulmonary vessels, triggered by high calcium levels within the endothelial cells.

This treatment had been tested on rodents and pigs and came as a success and showed that this gene therapy not only improved the heart and lung function, but also reduces cellular changes caused by PAH.

The aim of gene therapy is a modification of human DNA in such a way, that will lead to treating and hopefully preventing diseases. There are many ways to manipulate human DNA, depending on the disease, that should be cured. According to the type of disease a gene can be inserted in the patient's cells that will replace a mutated gene.

This method is one of many milestones in medicine. It has potential to cure many diseases. However, it has its advantages and disadvantages.

7. References

1. Suzanne R. Steinbaum. Februar 2017. Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pulmonary-arterial-hypertension#1 (29.3.18)
2. Melvin Y. Rincon & Thierry VandenDriessche & Marinee K. Chuah. August 2015. Gene therapy for cardiovascular disease: advances in vector development, targeting, and delivery for clinical translation: https://academic.oup.com/cardiovascres/article/108/1/4/266142 (29.3.18)
3. Professor Roger J. Hajjar, MD. April 2016.The Journal of the American College of Cardiology (JACC).
4. Arefa Cassoobhoy. November 2017. Visual Guide to High Blood Pressure: https://www.webmd.com/hypertension-high-blood-pressure/ss/slideshow-hypertension-overview (29.3.18)
5. Brunilda Nazario. January 2018. What is Pulmonary Arterial Hypertension: https://www.webmd.com/lung/pah-overview#1 (29.3.18)
6. MD PhD Atsushi Yao. November 2012. Recent advances and future perspectives in therapeutic strategies for pulmonary arterial hypertension: https://ac.els-cdn.com/S0914508712001980/1-s2.0-S0914508712001980-main.pdf?_tid=9f835c74-5ec6-459d-932a-740b95ee9f8b&acdnat=1522522771_a2427459092a9eb320e0199de7d4bf06 (29.3.18)
7. USA.gov. April 2018. What is gene therapy: https://ghr.nlm.nih.gov/primer/therapy/genetherapy (4.4.18)
8. Wikipedia. April 2018. Gene therapy: https://en.wikipedia.org/wiki/Gene_therapy (4.4.18)
9. Osmose. November 2017. PH – causes, symptoms, diagnosis, treatment, pathology: https://www.youtube.com/watch?v=Dx4QgdN_hI4 (1.11.17)
10. Wikipedia. June 2017. Adeno-assoziierte Viren: https://de.wikipedia.org/wiki/Adeno-assoziierte_Viren (5.4.18)
11. Wikipedia. January 2018. Adenoviridae: https://de.wikipedia.org/wiki/Adenoviridae (5.4.18)
12. Yen-Chun Lai & Karin C. Potoka & Hunter C. Champion & Ana L. Mora & and Mark T. Gladwin. June 2017. PAH, The Clinical Syndrome: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4096686/ (5.4.18)
13. Wikipedia. March 2018. Endoglin: https://en.wikipedia.org/wiki/Endoglin (5.4.18)
14. March 2016. 6 Advantages and Disadvantages of Gene Therapy: https://futureofworking.com/6-advantages-and-disadvantages-of-gene-therapy/ (5.4.18)
15. Brunilda Nazario. January 2018. Treatments for Pulmonary Arterial Hypertension:

https://www.webmd.com/lung/pah-treatment-options#1 (5.4.18)

Pictures

Picture on the front page: https://majestynews.com/?p=31254#.WsuMJFe5mRs

1. https://www.google.ch/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwiJia_RwqfaAhWGC-wKHfGcCE0QjRx6BAgAEAU&url=http://circresearch.com/gallery/pulmonary-arterial-hypertension-the-clinical-syndrome-4/&psig=AOvVaw1wNx162njTyXCvTmdS5ii0&ust=1523168391137526
2. https://en.wikipedia.org/wiki/Gene_therapy#/media/File:Gene_therapy.jpg

3.& 4. Self-made drawing

5.- 9. Photos taken with an IPhone 6