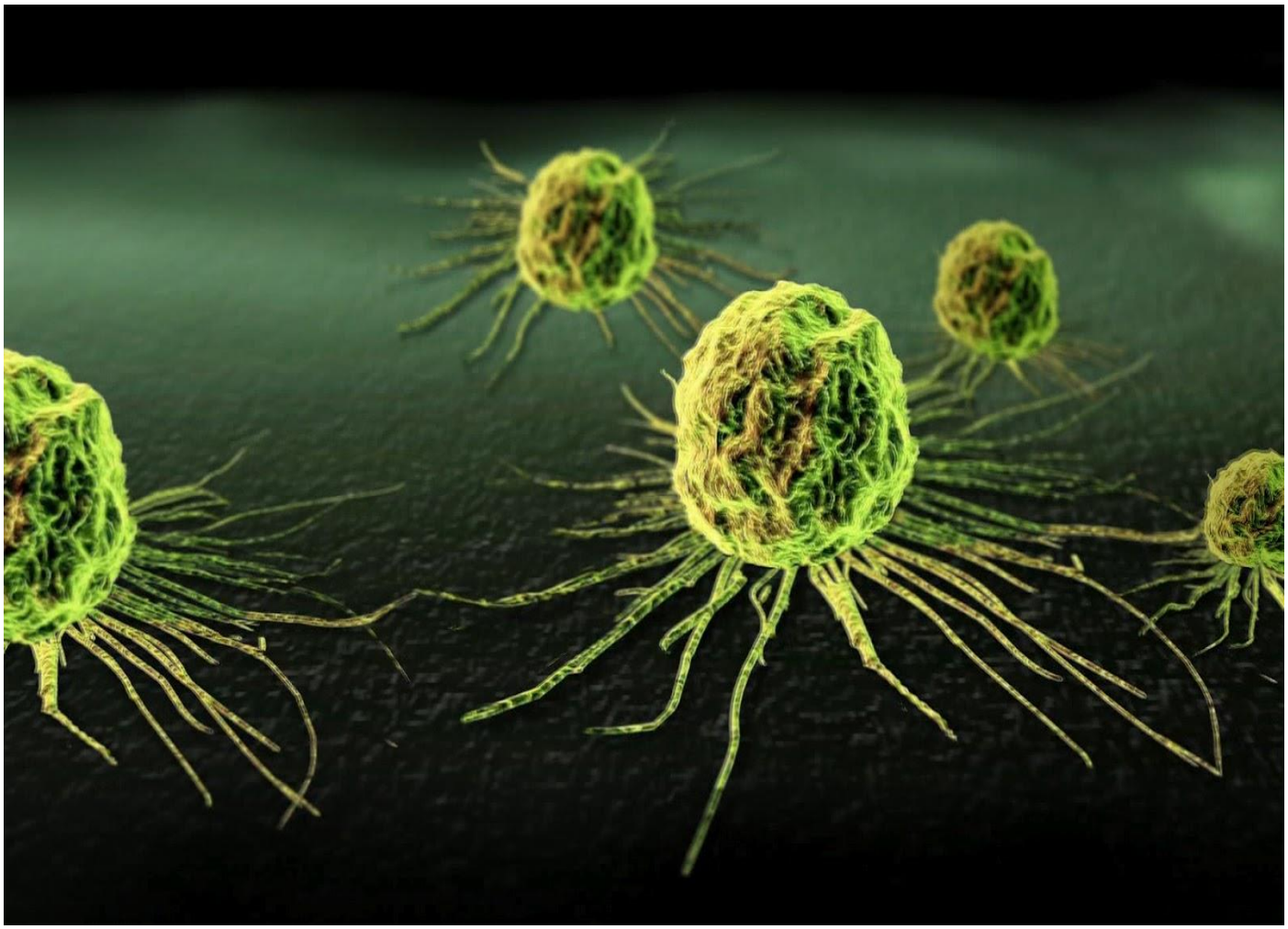
Epigenetics and cancer

biology term paper

by Florian Skelton, David Graf and Moritz Gubler



November 2013 – April 2014, Class 4A, P. Ruggle, Gymnasium Kirschgarten

Index

1. Preface 2
1.1 What was our motivation to work on the topic? 2
1.2 Our questions with respect to the topic 2
2. Introduction 2
2.1 The context of our topic 2
2.2 What is the recent scientific history? 3
2.3 Where and why is epigenetics used? 3
2.4 Alternative treatments 3
2.4.1 Chemotherapy 3
2.4.2 Surgery 3
2.4.3 Radiotherapy 3
3. Description of the engineering technique 5
3.1 Epigenetic functions of histones 5
3.2 Cancer 6
3.3 Causes of cancer 6
3.3.1 Random errors in the DNA 6
3.3.2 Viruses 6
3.3.3 Epigenetic changes 6
4. Discussion 7
4.1 What progress has been made with the application of genetic engineering in the cancer research? 7
4.2 What are the future research steps? 7
4.3 Discussion of ethical aspects 7
4.4 Advantages 7
4.5 Disadvantages 8
4.6 Our opinion 8
5. Visiting the Universitätsklinikum Freiburg 9
5.1 Interview with Stefanie Hölz 11
5.1.1 Summary of the Interview in English 11
5.1.2 Conclusion of the Interview 11
6. Summary 12
7. References 13
7.1 Websites 13
7.2 Pictures 13

1. Preface

1.1 What was our motivation to work on the topic?

Cancer is a major problem in the world. In 2012, 8.2 million people died from cancer and more than 14 million new cases of cancer occurred worldwide[1]. Although the treatments for cancer have improved, the disease remains uncontrollable.

We chose GM in cancer research as a topic because genetic engineering may be able to minimise the risks or even prevent cancer. Some scientists of the cancer research community are able to draw promising prospects about cancer research with genetic engineering. The fact that these scientists rely on this relatively new branch of technology makes it even more interesting. We are able to witness an evolving branch of research.

Cancer is a disease which can strike everybody. It is a threat to us and the people around us. We know a few people in our families who have had to fight cancer. Many people are afraid of cancer and so it has become a problem for society. The importance of this particular branch of research plus the emotional and personal component makes it a very interesting topic.

1.2 Our questions with respect to the topic

- How does cancer evolve?
- How could genetic engineering influence cancer?
- Is cancer research an important branch of genetic engineering?
- What are the prospects for the treatment and prevention of cancer?
- What consequences can epigenetic processes have on cancer?
- What will change with this new branch of research?
- What does the work of the scientists in this particular branch of research look like? What are the problems they have to face?

2. Introduction

2.1 The context of our topic

Different genetic factors are responsible for the development and the growth of cancer. At present, cancer can neither be prevented nor cured. New treatments and methods of diagnosis are needed. In this term paper, we will focus on epigenetics. Epigenetics promises new forms of cancer therapy.

But what exactly is epigenetics? Wikipedia defines it as follows[2]:

Epigenetics is the study of heritable changes in gene activity that are not *caused by changes in the DNA sequence; it also can be used to describe the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable.*

Epigenetic modifications include DNA methylation and histone modification. With the knowledge and methods of

1 http://www.cancerresearchuk.org/cancer-info/cancerstats/keyfacts/worldwide/
2 http://en.wikipedia.org/wiki/Epigenetics

epigenetics, scientists try to find out how cancer develops and how it could be prevented.

2.2 What is the recent scientific history?

Both epigenetics and cancer research are very important branches of science. Cancer is a big health problem over the whole world. The need for better treatments and methods of diagnosis pushes cancer research forward.

Epigenetics is a very new branch of research which originally comes from the USA. The term *epigenetics* was only defined in 2008 at a Cold Spring Harbor meeting[3]. There is still a lot to find out about genetics, but maybe the answer to prevent cancer lies in the study of epigenetics.

2.3 Where and why is epigenetics used?

Epigenetic research is mainly conducted in the USA and in Germany. Scientists at the *Urologische Klinik* of the *Klinikum der Universität Freiburg* are trying to find out what consequences the genetic engineering of cancer cells has on the development of cancer. If this will be a new way of treating cancer is still unclear.

2.4 Alternative treatments

2.4.1 Chemotherapy

Definition derived from Wikipedia[4]: *Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract, and hair follicles.*

Chemotherapy is often used in conjunction with other cancer treatments, such as radiation therapy, surgery, and/or hyperthermia therapy. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.

2.4.2 Surgery

Definition derived from the Cancer Research UK Website[5]: *Surgery is one of the main treatments for cancer. The tumour and some normal tissue from around the cancer (known as a clear margin) as well as the lymph nodes nearest to the cancer (in case they contain cancer cells) are removed. With some types of cancer, surgery can help people to live for a long time and may sometimes lead to a cure. Surgery is likely to cure small, early stage cancers that have not spread to other parts of the body.*

2.4.3 Radiotherapy

Definition derived from the Cancer Research UK Website[6]: *With the help of X-rays, radiotherapy destroys the cancer cells in the treated area by damaging the DNA within these cells. The damaged DNA can't divide any more and dies.*

3 http://genesdev.cshlp.org/content/23/7/781.full
4 http://en.wikipedia.org/wiki/Chemotherapy
5 http://www.cancerresearchuk.org/cancer-help/about-cancer/treatment/surgery/surgery-to-treat-cancer
6 http://www.cancerresearchuk.org/cancer-help/about-cancer/treatment/radiotherapy/

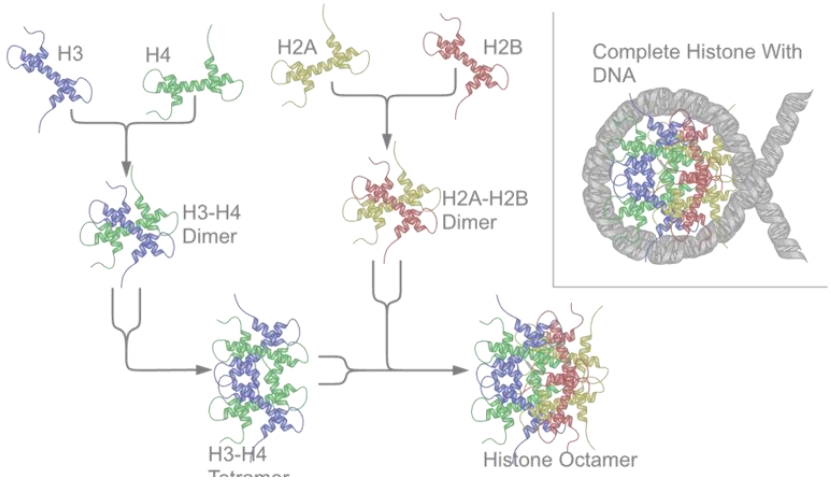
Radiotherapy can be given in various ways:

- *From outside the body as **external radiotherapy**, using X-rays from linear accelerator machines, electrons, and more rarely other particles such as protons.*
- *From within the body as **internal radiotherapy**, by drinking a liquid that is taken up by cancer cells or by putting radioactive material in, or close to, the tumour.*

The response of a cancer to radiation is described by its radiosensitivity. Highly radiosensitive cancer cells (e.g. leukaemia) are rapidly killed by modest doses of radiation. The majority of epithelial cancers are only moderately radiosensitive, and require a significantly higher dose of radiation (60-70 Gy) to achieve a radical cure. Some types of cancer are notably radioresistant, that is, much higher doses are required to produce a radical cure than may be safe in clinical practice.

3. Description of the engineering technique[7]

In epigenetics scientists try to understand the process of inheritance of traits, which are not fixed in the DNA-sequence. In the introduction we mentioned that epigenetics deals with the mechanisms of the spatial structure of DNA and the biochemical processes of the cell. The packaging materials of DNA, the histones[8], give us important information about the development of cells and the possibilities of mutations. Histones can activate as well as deactivate genes with the help of their chemical groups (methylated or acetylate). The histones give their properties to their daughter cells. It is believed that this process of methylation, acetylation and phosphorylation of histones can be influenced by environmental factors and food.



Schematic representation of the assembly of the core histones into the nucleosome

The histones have a leading role in the spatial structure of the DNA. The histones pack together and order the DNA and reduce its size 50,000-fold. We distinguish between five different histones types: H1, H2A, H2B, H3 and H4[9]. All of them fulfil a different task in addition to the packaging of the DNA. These different types exist because histones can have a methyl, acetyl or phosphate group at different places. Depending on the chemical composition between the histones and DNA, these are loosely or very tightly bound together. If the package is loosely bounded, the genetic code can be read. Otherwise this process is at present not possible Thus, the histones function is to control gene activity. They determine which genes are going to be used by loosening or tightening the connection between the DNA and the histones. This is a very important mechanism because each cell in the human body has identical DNA molecules with the same genetic code. Thus each cell has the ability to produce all proteins. However they do not need the whole repertoire of building blocks because not all proteins are needed everywhere in the body. The skin cells, for example, only need those proteins which can make new skin cells.

For this reason the histones determine which parts of the DNA should stay deactivated and which parts should be read. They ensure that everything runs correctly. Each protein is produced based on this information, which in turn leads to the formation of the corresponding cell.

3.1 Epigenetic functions of histones

The methylation of histones is accomplished by the enzyme methyltransferase and by proteins that bind to the methyl groups of the DNA[10]. In this process the methyltransferase attaches to the C5-position of cytosine and binds a methyl group to that location. The numbers of these enzymes are regulated by methylation and by particular proteins.

7 http://www.bristol.ac.uk/biochemistry/
8 http://www.nature.com/scitable/definition/histone-histones-57
9 http://en.wikipedia.org/wiki/Histone#Classes
10 http://www.nature.com/scitable/topicpage/the-role-of-methylation-in-gene-expression-1070

Depending on the composition of the methylation pattern, the properties of the histones change so they are not able to break apart from the DNA strand. This would mean that the specific gene is going to be deactivated. But if the methylation pattern leads to a loosening of the packaging material, the DNA transcription can begin.
Even more epigenetic processes such as phosphorylation (adding a phosphate group) can lead to a separation of the histones from the DNA. However, if an error occurs during these epigenetic control procedures, it can lead to excessive cell division, as is the case with cancer.

3.2 Cancer

If one has cancer there is a large increase in the gene activity, which leads to uncontrolled growth of the cell. These mutated cells push the healthy tissue aside and can severely damage it. The movement of cancer cells from their point of origin to other parts of the body is called metastasis[11]. External factors such as UV light, poor nutrition, toxic chemicals and smoking increase the risk that a cancer cell develops from a normal tissue cell.

3.3 Causes of cancer

3.3.1 Random errors in the DNA

An error in the DNA, which does not disappear, is called a mutation. Not every mutation results in cancer, since not every change in the DNA sequence results in a change of the genetic information. The cell has also certain preventive measures which can erase an error in a DNA strand. But certain mutations are very dangerous for our body because the cell can begin to grow unhindered or old and sick cells do not die off.

3.3.2 Viruses

There are some viruses[12] which can cause cancer by incorporating their own genetic material into the DNA of their host cells. If the virus is present in the place that is responsible for the regulated cell division it could lead to unregulated cell division. There are even some viruses that are able to get into the host cell and then manipulate the monitoring mechanism so they can build proteins. In this case, the cell no longer able to have a regulated cell division.

3.3.3 Epigenetic changes

The order of the methylation pattern is disturbed in cancer cells[13]. Often there are some accidental changes during methylation which can lead to the deactivation of certain genes. Especially tumour suppressor genes are affected. These particular genes carry the code for the formation of proteins, which in turn are responsible for the suppression of uncontrolled cell division. If no excessive methylation in tumour suppressor genes is present, they prevent the development of cancer. This discovery allows an early detection of the illness because it can be detected before the cells mutate. The reversal of these epimutations promises to become a completely new direction in cancer therapy.
An inhibitor must be found to stop the methylation at the tumour suppressor genes. This inhibitor slows the enzyme DNA - methyltransferase down. This is part of the current research.

[11] http://en.wikipedia.org/wiki/Metastasis
[12] http://www.dkfz.de/de/forschung/schwerpunkte/fsp-f.php
[13] http://jco.ascopubs.org/content/22/22/4632.full

4. Discussion

4.1 What progress has been made with the application of genetic engineering in the cancer research?

Genetic engineering has made huge progress in recent years. Epigenetics may bring even bigger advances in cancer diagnosis and therapy in the future. Cancer can be detected at an earlier stage leading to a higher chance of healing. Factors that increase the risk of cancer can be defined more precisely. Therefore better protective measures can be developed. Epimutations which enable cancer growth in the body can be reversed so that the tumour suppressor genes may perform their function again.

Most of these possibilities still lie in the future, but there are already some methods in practice based on epigenetics. In Germany, for example, the substance azacitidine was allowed in 2008 for the treatment of certain blood cancers[14]. Because this drug has not been fully developed, all cells are affected by it and thus preventing healthy blood cells from being able to divide. This makes only small dosages possible.

Using genetic engineering more information about cancer and its origins can be found out, thus contributing to the production new medicines.

4.2 What are the future research steps?

Epigenetics has become an important sector of genetic engineering. This new field of research is definitely capable of development. With the development of new more efficient machines, the possibilities of genetics research will be increased.

Examples of specific goals:

- Find epigenetic markers[15], which advance cancer therapy
- Supporting the immune system to detect and fight cancer
- Prevention of cancer by defining risk factors that may cause epigenetic changes

4.3 Discussion of ethical aspects

In many laboratories the scientists work with mice and mice embryos which die after the experiments. From an ethical point of view this is certainly reprehensible. But we think animal experiments in the field of scientific research belong in a different category. If someone discovers a method to prevent or cure cancer, it is acceptable to use animals as research objects.

4.4 Advantages

Chemotherapy damages not only the tumour cells; it also harms the entire body. Genetic engineering might be able to find new healing methods for cancer which are painless and reduce the risk of relapse. Epigenetics may be able to prevent cancer by finding out how epimutations work exactly. Epigenetics could also succeed in other areas than cancer therapy. It promises to revolutionize other medical, agricultural and industrial fields.

[14] http://de.wikipedia.org/wiki/Azacitidin
[15] http://sciencev1.orf.at/news/147120.html

4.5 Disadvantages

Unfortunately genetic engineering is a very expensive field of research[16]. It is still necessary to invest a lot of money for substantial progress. Despite all new equipment and techniques, scientists cannot guarantee promising inventions. The field of epigenetics has not been sufficiently explored yet. It might take a few years to see if all the studies have paid off.

Another disadvantage is that the experiments are conducted with laboratory mice which is unethical in the eyes of some people.

In extreme cases, the knowledge of genetic modifications can be used to harm others. During the Second World War, for example, the Nazis tried to find out how they could sterilize all the "lower races" so that the Aryans could take over. They performed horrible experiments on humans in the concentration camps[17].

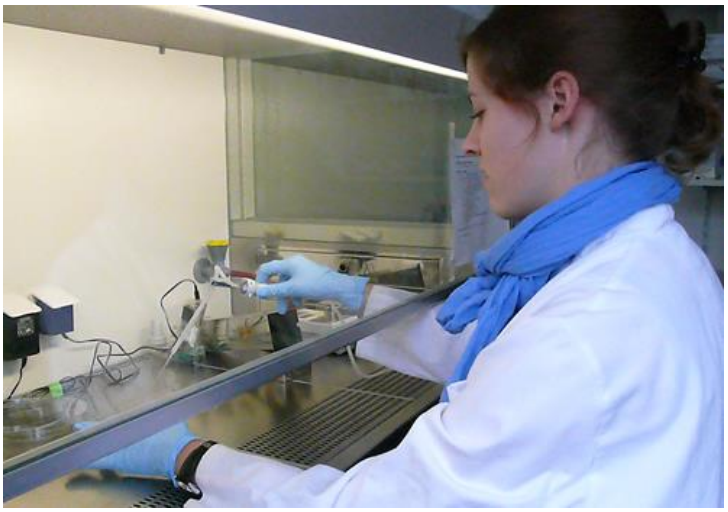
4.6 Our opinion

We consider the application of genetic engineering on cancer to be a good thing. We also think that epigenetics will help to find a successful treatment for cancer. Hopefully these scientists will be successful and will be able to alleviate pain and save lives.

Before we wrote our term paper we only had a vague idea what cancer really was. We had not thought yet about how cancer could be prevented in the first place. Now we know how important this part of research is and how many lives it may be able to change for the better.

[16] http://www.researchandmarkets.com/research/krh74h/global
[17] http://www.arte.tv/guide/de/047372-000/die-auschwitz-arzte-des-todes

5. Visiting the Universitätsklinikum Freiburg



We contacted Stefanie Hölz, the girlfriend of Florian's cousin which is about to doctorate in molecular biology. She has been working for a year at the University of Freiburg in the department of Urology. On Monday, the 3rd of March we visited Stefanie Hölz at her laboratory in Freiburg in Breisgau. She showed us various tools and facilities. First we saw the withe laboratory mice.

The co-worker of Stefanie uses the mice as research objects.

With mice embryos, they can test the effect of the protein LSD1. She noticed that lung tumours formed with the loss of LSD1. The embryos died before they were born.

She thinks that the problem must lie in the stem cells. Stefanie's task is to find the relevance of the protein LSD1 in stem cells.

Then Stefanie let us try out special laboratory pipettes which required a lot of skill. After that we went to the experimental laboratory. She showed us the chapel (picture on the left). Everything is strictly sterile in there. The vents suck the polluted air in. One has to work with a lab coat and disinfected gloves. At the end, Stefanie showed us the campus of the university.

At the end Stefanie took us through the university hospital. She showed us the freezing chamber where it is always four degrees cold.

After our little tour we went to the cafeteria and made the interview.

It was a very interesting and informative day, as we were able to get an insight an insight in a very different world. We would like to think Stefanie Hölz for her kindness to show us around and let us be part of the laboratory for one afternoon.

5.1 Interview with Stefanie Hölz

Stefanie Hölz intended to make the interview in German. We didn't translate the interview because we were afraid that we would change the meaning of it. We will conclude the interview in English at the end of the chapter.

1. Stefanie, danke für das Interview. Wie sieht ein ganz gewöhnlicher Arbeitstag einer Doktorandin aus?

Die Hauptarbeitszeit in unserem Labor ist von 9 bis 18 Uhr. Als Doktorand wird von dir natürlich mehr Einsatz erwartet, das heisst längere Arbeitszeiten - meistens bis um 19:00 Uhr, je nachdem, wie viel man zu tun hat. Es gehören ja immer noch Gespräche über deine Ergebnisse mit dem Chef dazu und man kann nie genau planen, wie lange die manchmal dauern.

Naja und dann gehört eben auch dazu, dass man meistens am Wochenende kommen muss, um sich um die Zellen zu kümmern! Die Stammzellen mit denen ich arbeite, da muss man fast täglich frisches Medium dazu geben. Bis jetzt hatte ich noch keinen ungewöhnlichen Arbeitstag, da ich noch so gut wie nie vor 18:00 Uhr aus dem Labor gegangen bin.

2. Wie bleibst du trotz wochenlangen erfolglosem Experimentieren motiviert?

Das ist ziemlich schwer zu beantworten, da du eigentlich die meiste Zeit keine beziehungsweise schlechte Ergebnisse hast und immer irgendwas nicht funktioniert. So musst du dir immer überlegen, woran das jetzt liegt und das ist manchmal so richtig nervig. Eigentlich kann ich dazu nur sagen, dass du richtige gute Nerven brauchst als Forscher und vor allem viel Ehrgeiz! Naja, und ich muss sagen im Labor motiviert man sich immer gegenseitig. Dann ist ein Tag mal schlecht, aber der andere ist dann mal wieder gut - ein richtiges auf und ab! Aber abschalten kann man oft nicht so schnell, zumindest ich. Unter der Woche fast gar nicht und am Wochenende eben nur bedingt, da ich, wie gesagt, auch dann oft hin muss. Motivierend ist nur, dass ich weiss, dass ich mit einem Doktortitel meine Arbeitsstelle besser aussuchen kann und somit hoffentlich auch in der Industrie arbeiten kann.

3. An was forschst du gerade?

Das ganze Labor forscht am Protein LSD1. Eine Kollegin von mir hat die Frage beantwortet, warum Mäuse, die kein LSD1 mehr haben sterben, bevor sie geboren werden. Die Stammzellen haben dann Probleme zu differenzieren. Meine Aufgabe ist es jetzt herauszufinden, was LSD1 genau in den Stammzellen macht, sodass diese differenzieren können, wenn sie sollen. Das ist jetzt ziemlich allgemein gesagt und alles andere wäre viel zu kompliziert, da sehr komplexe Mechanismen dahinterstecken, sodass sogar einige im Labor manchmal etwas durcheinander bringen.

4. Bei euch wird auch mit Prostatakrebs geforscht. Was wird aktuell gemacht?

Es gibt zwei Versionen von Prostatakrebs

a) die anfängliche Version, der Tumor ist dabei androgenabhänig. d.h. für sein Wachstum ist er von Testosteron abhängig und man kann diesen gut behandeln, indem man Hormonblocker gibt, sodass der Mann kein Testosteron mehr hat und der Tumor dann aufhört zu wachsen.

b) Dann gibt es die weiter fortgeschrittene Version des Tumors, der androgenunabhängig wächst.

Man weiss, dass LSD1 in den Prostatatumoren stark über exprimiert wird. Die Frage ist jetzt, was macht LSD1 in den

Tumoren? In androgenunabhängigen wissen wir schon, dass es zusammen mit Androgen und dem Rezeptor bestimmte Stellen an den Histonen demethyliert. Wenn diese Stellen methyliert vorliegen, können die Gene nicht mehr abgelesen werden. Die Demethylierung von LSD1 führt dann aber dazu, dass die Gene wieder abgelesen werden können. Das sind sogenannte reprimierende Marks. Diese Gene sind zum Beispiel für das Wachstum einer Zelle verantwortlich. Die Frage ist jetzt, was macht LSD1 in den androgenunabhängigen Tumoren?

5. Was sind die häufigsten Probleme in der Forschung auf?

Die Konkurrenz schläft nicht… man muss immer schneller sein als die anderen. Da Epigenetik erst seit ein paar Jahren ein Forschungsgebiet ist es top aktuell und viele Gruppen forschen daran. Es machen also viele das Gleiche, und die anderen können natürlich schneller sein als du!

Ein Standardproblem beispielsweise ist, dass bestimmte Methoden nicht funktionieren und man muss herausfinden, wieso das so ist.

5.1.1 Summary of the Interview in English

Working as a doctoral candidate is a really tough job. They have long working hours and have to go to the laboratory even on weekends. They basically live for their work.

They have to keep their motivation up. They often get stuck with their work and have to double-check every decision they make. They need a lot of patience and above all ambition.

Stefanie Hölz's laboratory researches the mode of action of the protein LSD1. She is trying to find out what LSD1 does to the stem cells exactly. But her work is really complicated to explain.

There are two types of prostate cancer. Type one is a tumour which only grows with the help of testosterone. With the help of hormone blockers it is fairly easy to treat. Type two is a more advanced type. This one is more complicated. Stefanie Hölz and her colleagues are trying to find out what the LSD1 is doing to the tumours. They are always under pressure to produce results because the other scientists are also researching.

5.1.2 Conclusion of the Interview

We were really impressed how patient these scientists were and how much work they put into their research every day. The visit allowed us an insight in a very different world. We realised how much work had to be done to find out what we are learning at school now.

We were really fascinated by the beauty of the prostate cancer cells, which formed a beautiful symmetrical pattern.

6. Summary

Cancer still counts as a dangerous illness which is hard to treat. However, the chances of recovery have increased rapidly in the last years. With the help of genetic engineering, new methods of treatment and prevention of cancer have been discovered. We have limited our paper to the field of epigenetics, which deals with biochemical processes and the packaging of the DNA. That is why it has an impact on many genetic processes. We were very interested in the methylation pattern of the histones which decides about the activity of a gene. New results show a change in the methylation in cancer cells. The reason for this phenomenon is still unclear.

Genetic engineering is going to be very important for our medicine, agriculture and industry in the future. However, epigenetics has also some disadvantages. A lot of laboratories need animal experiments for their research. Nevertheless, epigenetics promises huge success such as new ways of treatment and a better understanding of cancer.

7. References

7.1 Websites

1. http://www.cancerresearchuk.org/cancer-info/cancerstats/keyfacts/worldwide/ (last visited: 16.02.2014)
2. http://en.wikipedia.org/wiki/Epigenetics (last visited: 22.04.2014)
3. http://genesdev.cshlp.org/content/23/7/781.full (last visited: 22.04.2014)
4. http://en.wikipedia.org/wiki/Chemotherapy (last visited: 22.04.2014)
5. http://www.cancerresearchuk.org/cancer-help/about-cancer/treatment/surgery/surgery-to-treat-cancer (last visited: 16.02.2014)
6. http://www.cancerresearchuk.org/cancer-help/about-cancer/treatment/radiotherapy/ (last visited: 22.04.2014)
7. http://www.bristol.ac.uk/biochemistry/ (last visited: 26.03.2014)
8. http://www.nature.com/scitable/definition/histone-histones-57 (last visited: 26.03.2014)
9. http://en.wikipedia.org/wiki/Histone#Classes (last visited: 25.04.2014)
10. http://www.nature.com/scitable/topicpage/the-role-of-methylation-in-gene-expression-1070 (last visited: 26.03.2014)
11. http://en.wikipedia.org/wiki/Metastasis (last visited: 25.03.2014)
12. http://www.dkfz.de/de/forschung/schwerpunkte/fsp-f.php (last visited: 22.04.2014)
13. http://jco.ascopubs.org/content/22/22/4632.full (last visited: 22.04.2014)
14. http://de.wikipedia.org/wiki/Azacitidin (last visited: 22.04.2014)
15. http://sciencev1.orf.at/news/147120.html (last visited: 22.04.2014)
16. http://www.researchandmarkets.com/research/krh74h/global (last visited: 22.04.2014)
17. http://www.arte.tv/guide/de/047372-000/die-auschwitz-arzte-des-todes (last visited: 25.04.2014)

7.2 Pictures

- Cover picture Cancer cell: http://guardianlv.com/wp-content/uploads/2013/11/Cancer-Cell1.jpg (last visited: 28.04.2014)
- Schematic representation of the assembly of the core histones into the nucleosome http://en.wikipedia.org/wiki/File:Nucleosome_structure.png (last visited: 28.04.2014)