Induced Pluripotent Stem cells in human medicine

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Content

1. Preface:

One of the most interesting parts of biology is the sheer vastness of different topics. So in order to limit the possible subjects for our term paper, we decided, that we wanted to look into something fresh and interesting. In recent years, there have been many important discoveries in biomedical research, however the topic that seemed to spur fantasies the most, were induced pluripotent stem cells. Or also iPS cells. The researchers that discovered them even won the Nobel prize in medicine in 2012. We were fascinated by the sheer possibilities that they seemingly opened up and wanted to look for ourselves what exactly they could do. So, we ended up with the following lead questions:

- What are iPS cells?
- How are iPS cells created?
- What are iPS cells used for?
- What potential future applications do iPS cells have?

2. Introduction:

For a long time, biologists have been enthralled by the idea of artificially producing cells that have the characteristics of embryonic stem cells. This was partially driven by curiosity whether it could be done and mostly by the everlasting need of pluripotent stem cells. In 2006 it was shown that somatic cells could be returned to a pluripotent stem cell (Takahashi et al. 2006). The experiment was originally more of a proof of concept than an actual method. However, with recent advancements in gene-editing, it became more and more of a viable tool for medical research. In the last few years, there have been a few scientific breakthroughs, which could be accomplished with the use of iPS cells (internet source 1, Sugimura et al. 2017). These breakthroughs include the first artificially synthesized hematopoietic stem cells (internet source 2). iPS cells are especially interesting, as the only alternative to them are embryonic stem cells, which are, in the case of human embryonic stem cells, very hard to come by.

3. The creation and applications of induced pluripotent stem cells:

How induced pluripotent stem cells are created:

To understand the process of how induced pluripotent stem cells are created, it is important to first understand what they are. Induced pluripotent stem cells, short iPS cells, are somatic cells, usually fibroblasts, that have been made pluripotent again. Pluripotent is the scientific term for a cell which has the potential to form every other kind of cell in the body. Re-inducing this pluripotent state is possible because only very few of the transcription factors, that are encoded in the genome of a cell are responsible for this pluripotent property (Takahashi et al. 2006). In a normal somatic cell, these gene are deactivated. Sadly, it is not possible to reactivate these genes yet, so scientists have to introduce new copies of these genes into the cell instead.

The process of creating iPS cells looks the following (fig.1):

1. Somatic cells, usually fibroblasts, are isolated and cultured
2. The transcription factors responsible for pluripotency (Oct4, c-Myc, Sox2 and Klf4) are introduced into the cells. For example, via a viral vector -> iPS cells emerge.
3. The cells are harvested using feeder cells.
4. The emerged iPS cells are cultivated for further processing.

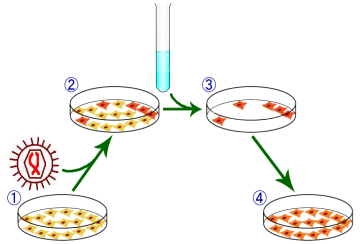

Fig. 1: An infographic about the creation of iPS cells.

Applications of iPS cells:

The applications of iPS cells are very wide, but they can usually be divided into two groups (fig.2):

Disease modeling and drug development:

As iPS cells retain certain properties of the original cell, they can be used to model diseases and then test drugs on these models. For example, one could extract cells from a patient with Alzheimer-disease, and once they are turned into iPS cells, force them to differentiate into neurons, to observe the disease development and test the effects of drugs.

Regenerative medicine:

Since iPS cells are derived directly from the patient, they are not rejected by the patient's immune system. One could use this to cultivate patient tissue by forcing the differentiation of the iPS cell into a certain cell type and then transplant it. This would solve many issues of organ transplantation; however, the possibilities are still being researched.

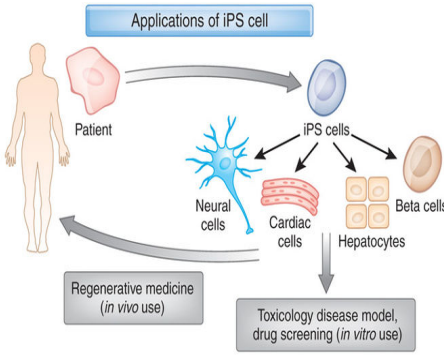

Fig. 2: An image showing the applications of iPS cells

4. Interview with Dr. Philipp Hoppe from Novartis Institute for biomedical research

Question 1: What is your occupation?
Answer:
I am a lab head at the Novartis Institutes for BioMedical Research (NIBR). At NIBR, more than 6000 people worldwide apply innovative technologies in a huge team effort in order to find new ways of treatment for any kind of human disease. My personal job focus is on genetic screening. That means, our team's task is to identify novel drug targets using large-scale genetic methods like CRISPR-Cas9. With that ground-breaking technology, we are able to knock-out all human genes in a one-gene-per-cell way in one single large experiment. By that, it is possible to identify new genes that play a role in a certain disease model. The cell type which we use for such an experiment varies, and among other cell types it is certainly possible to use iPS cells (actually, iPS cell-derived cells). Once we have identified such new genes, NIBR colleagues will try to find ways to manipulate them in order to ultimately cure diseases (e.g. via pill or injections) or use the gained knowledge to develop innovative cell and gene therapies.


Fig. 3: Dr. Philipp Hoppe

Question 2: Have you ever worked with iPS cells before?
Answer:
Yes. iPS cells are nowadays a central research tool because they are pluripotent (as the name tells), which means they have the theoretical capability to differentiate into any of the more than 200 cell types of the human body. In that potential they behave like embryonic stem (ES) cells, as they appear in early embryonic development during pregnancy. The great thing about iPS cells is that they can be generated by hitting the reset button in any kind of mature human cells. The first time this was achieved was with fibroblasts, later with e.g. skin cells, and there are even protocols of generating iPS cells from urine, which contains some shedded cells of the urethra.

Question 3: What potential do you see for iPS cells in human medicine?
Answer:
The most relevant fact about iPS cells is that there are many protocols available which allow the generation of many different mature cell types *in vitro* (e.g. neurons, heart muscle cells, pancreatic cells). For those cells, there are two main applications: One is the theoretical possibility of generating cells or even whole tissues in a dish and transplant them to diseased patients. The huge advantage of using iPS cells is that those cells come from the patient itself. This significantly decreases the risk of rejecting transplanted cells by the patient's own immune system. If necessary, the iPS cells can additionally be genetically modified, e.g. by correcting a disease-causing mutation with CRISPR-Cas9 technology.

The second big application for iPS cell derived cells is that they serve as a useful research tool. A big advantage here is that those cells are human-derived. For practical, but more importantly ethical reasons, researches often don't have access to primary human tissue samples. With the iPS cell technology, the desired cell type can be generated in the lab and used for research purposes. That often allows a more direct way of studying human disease models as compared to using primary cells from other species, e.g. mice. An additional facet of using iPS cells is that one can use cells from a disease patient and compare them to cells of a non-diseased person. By that, genes that are the cause of the disease itself can be identified.

Question 4: What are the main difficulties you see for iPS cells? How could these be resolved?
Answer:
When one compares iPS cells to ES cells (the cells that iPS should theoretically resemble), one can see that iPS cells are not completely the same. Like ES cells, iPS can give to all kinds of mature tissues, but if one looks close enough, there are mostly epigenetic differences (which is a kind of cellular memory). Depending on the research question, this has to be taken into consideration. Another issue is that each iPS cells are always derived from one single person. There is no way of knowing if that iPS cell line is fully normal and one single iPS cell line can anyways never represents the genetic variety of the whole human population. To resolve these issues, it will be necessary to develop better protocols to generate iPS cells in the first place and to be able to constantly monitor they status.

For therapeutic applications, the devil is in the detail: The protocols of generating mature cell types usually try to mimic embryonic development in a very fast way. That leads to the fact that the mature cell types often have an embryonic rather than an adult phenotype, meaning they might be different compared to the cells which one would actually need for transplantations. In other words, the *in vitro* derived cells are not as good as their primary counterparts (e.g. taken directly from adult humans). The best way of developing better protocols usually is trying to understand how the cells develop *in vivo*, but for that one would need to study human development which comes with a lot of ethical issues.

Another practical issue with iPS cell-derived cells is how they can be transplanted into patients. In e.g. diabetic type I patients, the beta-cells of the pancreas get destroyed, but they are deeply embedded into the tissue and there are no easy ways of transplanting the *in vitro* derived healthy cells to their correct location. To circumvent this, one would either need to transplant whole tissues rather than just individual cells, or one needs to find ways of generating new cells directly *in vivo*, but then this will probably be independent of the use of iPS cells.

And last but not least, unfortunately, the potential of any kind of stem cell is always connected to cancer. The definition of a stem cells is that it can self-renew and give rise to mature cell types. The self-renewal aspect is always at risk of generating out of control. Therefore, whenever stem cells or stem-cell derived cells are being transplanted, one needs to be sure that the cells are as normal as possible. This holds especially true, when cells are genetically modified.

Question 5: Does the methylation of the original cell have an impact on the iPS cells?

Answer:

Absolutely. Reprogramming to iPS cells always seems to be an incomplete process. Deeper investigations of different iPS cell lines showed that the methylation status shows similarities to the methylation status of the original cell. Lineage-specific genes of the original cells tend to be hypomethylated whereas lineage-specific genes of other cell types tend to be hypermethylated. That will lead to the consequence that these iPS-cell lines will rather not make problems in re-differentiating into the original cell type, whereas the differentiation into other cell types might be more difficult or inefficient.

Question 6: Are there alternatives to using Oct4, c-Myc, Sox2 and Klf4 to achieve an iPS like state?

Answer:

These four "Yamanaka factors" are transcription factors, so proteins that directly regulate gene expression by binding to specific DNA sequences. In the original protocol, those four factors were delivered as retroviral elements, which leads to the integration of foreign genetic material into the host cells. This is something which absolutely has to be avoided, because besides constant expression of a transgene which per se is already problematic, also the integration site of the virus might alter expression of nearby genes and, in the worst case, lead to cancer. Therefore, iPS cells with the purpose of therapeutic application have to be reprogrammed without the integration of genetic material in the host genome. This can be achieved by delivering of nucleic acids transiently. Alternatively, chemically defined small molecules can be used as well. These small molecules are able to mimic to a certain extent the changes that transcription factors would induce by interacting with signaling pathways.

Question 7: Are there ethical issues concerning stem cells?

Answer:

The term "stem cell" in the media often refers to ES cells, which indeed come along with a lot of ethical issues: In order to harvest true human ES cells, embryos would need to be sacrificed, which for good reasons is forbidden, of course. It is also possible to collect ES cells by using *in vitro* fertilized eggs and their subsequent daughter cells. While it is forbidden to generate ES cells in that way just for research purposes, there is an ongoing debate whether such cells should be used when they are generated for women which cannot get pregnant naturally. During this procedure, usually spare cells which won't be implanted into the woman's uterus would get discarded. Some people argue that is it better to use those cells for research instead of trashing them. Official regulations around that topic vary from country to country.

"Stem cell" can also mean adult tissue stem cells, e.g. hematopoietic stem cells (HSCs). This is one single stem cell type at the top of the hierarchy of the blood system. In mice, it can be shown that one single adult HSC is able to generate to whole blood system of an individual animal which is pretty remarkable. That is why bone marrow transplants work so

well and are being used for decades already in the clinic to treat people with e.g. leukemia. Unfortunately, for other tissues it is often much less clear if such adult stem cells exist at all and how they could be used directly *in vivo* for regenerative medicine. Of course, it would be great if we could make a pill that makes a certain type of damaged tissue regrow, but that seems to be wishful thinking. There are not many issues around adult tissue stem cells, the biggest challenge is how to identify and harvest the stem cell type of interest in humans.

One possibility would be to generate adult tissue stem cell via iPS cells, but there is still a lot of research to be done before that. As mentioned before, any cell type could be used to generate iPS cells, even cells from urine, so in a non-invasive way. Therefore, considering the huge potential of iPS cells, they don't face huge ethical issues in pratical terms. One very important aspect, though, is that every iPS cell line is derived from a human being. Therefore, the iPS cell line must only be generated and used with the consent of that person. Every iPS cell line contains the whole genetic blueprint of one individual. This information is very private, and every individual must be able to be in control of that information, of course.

Question 8: What ethical issues could potentially be resolved via the usage of iPS cells?

Answer:

iPS cells could eventually lead to the complete avoidance of ES cell usage. Besides that, if we ever manage to find ways of generating whole organs and are able to successfully transplant those organs, organ donation could become obsolete. Unfortunately, we are not there, yet. There is still a lot of effort necessary and since iPS cells need to be compared to ES cells in order to judge them properly, ES cell research still seems to be inevitable the moment.

5. Discussion

What impact did iPS cells have on human medicine?

With the emergence of iPS cells, researchers got a very potent tool for disease modeling and drug testing. It is now possible to model and observe diseases that were previously very difficult to observe, by recreating the disease state with iPS cells, which are derived from a patient with the disease. This method is especially useful for the modeling of diseases, that affect the central nervous system, like Parkinson-Disease or Alzheimer-Disease. It is also possible to use iPS cells for drug development, by observing the effects of a drug on cells, which differentiated from iPS cells (internet source 5).

Potential research on iPS cells:

There are a number of problems that are currently hindering iPS cells (internet source 6):

- The efficiency of iPS cell production is very low.
- iPS cells have been correlated to cancer.
- When modifying cells genetically, there is a risk of mutagenesis occurring.

For any of the three problems mentioned, there is still a lot of research to be done. If we are able to solve these problems, we could use iPS cells for regenerative medicine.

Advantages vs. Disadvantages:

In disease modeling and drug testing:

The advantages of using iPS cells for screening purposes are numerous: They are easily accessible and can be used to model most diseases and the effects of drugs on any human cell can be tested, without having to extract them or using embryonic stem cells, which are hard to come by. However, the production efficiency of iPS cells is very low, which is the main restriction in using them. It is also currently unknown, whether they have any unwanted effects.

In regenerative medicine:

The main advantages of using iPS cells in regenerative medicine are, that one would not need to rely on organ donations anymore and that there would be no rejection of the implant. However, iPS cells have been linked to cancer and there could be unknown side effects.

Ethical concerns:

There are no special ethical concerns when using iPS cells for screening purposes. iPS cells even managed to resolve an ethical issue, as now, one doesn't have to use embryonic stem cells anymore. However, if one would use it in regenerative medicine, there are several ethical problems which arise:
Firstly, iPS cells have been linked to cancer growth. It is also possible, that there are undiscovered side effects. Lastly, there is the possibility of mutagenesis in the production of iPS cells. These circumstances are the main reason, why iPS cells are currently not used for regenerative treatment. However, there are a lot of medical trials that look to resolve this problem.

6. Summary

The possibilities of iPS cells are seemingly limitless and it is very easy to get lost in fantasies about what they could do. They seem especially potent in regenerative medicine, where they could provide useful resources. However, when taking a closer look, it becomes apparent, that our current understanding of the processes involved in the usage of iPS cells is lacking. In addition to this, the production of iPS cells is very resource inefficient and the success rate is very low. Due to these circumstances, iPS cells are not yet ready to be used in regenerative medicine. They are nonetheless a very potent tool for disease modeling and drug testing, as they retain certain properties of the original cell. They have also lessened the need for embryonic stem cells. In conclusion, we can say that iPS cells are currently only useful for a small range of application, but in future, as medicine advances, they will become a much more potent tool for a large variety of medical purposes.

7. Glossary

Alzheimer-disease:
A chronic neurodegenerative disease. It is responsible for 60% ~70% percent of dementia.

Feeder cells:
A special kind of cell which is responsible for removing unwanted objects in our body. (For example, dead cells)

Fibroblasts:
A connective cell from which tissue forms. They can register injuries and play an important role in wound healing.

Hematopoietic cells:
Hematopoietic cells are cells, that can divide into any type of blood cells. (for example, bone marrow)

Mutagenesis:
The occurrence of mutation in the genome.

Parkinson-Disease:
A degenerative disease in the central nervous system, that mainly affects the motor system.

Pluripotent:
The potential to form every other kind of body cell.

Somatic cell:
A cell that has already differentiated.

Transcription factors:
Chunks of DNA which encode certain proteins.

Viral vector:
Using a special lab-virus to introduce DNA into a cell.

8. References:

Figures used:

Fig. 1: *An infographic about the creation of iPS cells*
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Fig.2: *An image showing the applications of iPS cells*
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Fig 3: *Dr. Philipp Hoppe*
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