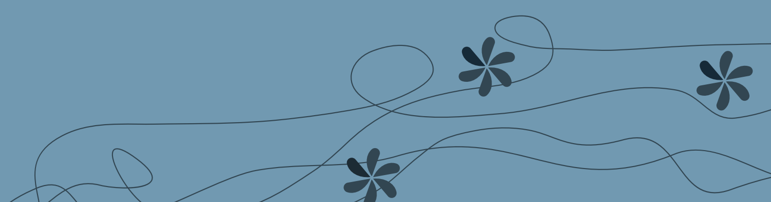
Text: Louise Hagbard, Ph.D. Photo: VUB

MIEKE GEENS

"I do think we're getting closer to getting successful cell therapies. My main concern is safety."



MIEKE GEENS, Ph.D.

RESEARCH PROFESSOR AND GROUP LEADER
AT VRIJE UNIVERSIEIT BRUSSEL, BELGIUM

ABOUT: Research professor Geens' research is mainly focused on exploring the differentiation potential of individual hPSC lines and defining markers that can reliably predict the differentiation propensity of a specific stem cell line. Her research group also investigates whether there are specific chromosomal abnormalities that consistently change the differentiation capacity of hPSCs or give them higher tumorigenic properties.

Photo: ESHRE/ davidadamsonphotography.com

Photo: Alexander Keller

VRIJE UNIVERSITEIT BRUSSEL

Vrije Universiteit Brussel (VUB) is a Dutch-speaking but internationally oriented university located in Brussels, Belgium. It has four campuses: Brussels Humanities, Science and Engineering Campus, Brussels Health Campus, Brussels Technology Campus and Brussels Photonics Campus.

How did you get into stem cell research?

"I actually started working on spermatogonial stem cells during my master's, and my promoter offered me a Ph.D. project, but not on spermatogonial stem cells but on human embryonic stem cells and trying to make sperm cells from them. Because we were looking for strategies to restore fertility, I did my Ph.D. on that. My Ph.D. was the most frustrating years of my life I think, because I never got any sperm cells. As some comfort, it is still difficult to make sperm cells from human embryonic stem cells. Although there is a lot of progression, at that time it was very hard. But I got really interested in human embryonic stem cells, and as a side project, I also derived human embryonic stem cells from single blastomeres. After I finished my Ph.D., I decided to continue working with pluripotent stem cells and I started my Postdoc mainly looking at differentiation propensity and the differences between all the different individual lines. So that's how I started and that's what I'm still doing."

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Photo: Alexander Keller

"We are looking at the differentiation propensity in stem cells and genetic variation."

You then made a career to become a research professor. How did you get to where you are today?
"I get very good support from my boss, professor Karen Sermon. Karen's research focus is mainly on disease models and the origin of chromosomal abnormalities in human embryos. She was actually looking for new team members and she liked my idea of looking into differentiation propensity and the differences between lines. So she's actually the one who sponsored me most of the time. I do get funding from projects for students and stuff, but the main financing for myself is from her. In 2013, I got a 10% professorship at my university so I'm leading my own subgroup of 4 Ph.D. students and a few master students where we are looking at the differentiation propensity in stem cells and genetic variation. I have a colleague, Claudia Spits, who's also working on human embryonic stem cells and is very much into genetic instability of these lines. So, I think we have different trajectories but they intervene quite a lot, so we have a lot of integration between us, between our students."

What is your aspiration for the field? What is your research focus?
"We're doing mainly fundamental research. We're looking into intrinsic variation between lines, why certain lines go well to, for example definitive endoderm, and why certain lines do not. But we are also looking in residual undifferentiated stem cells. After differentiation, often a small fraction of those cells is still present. And if you would transplant those into people there is a high risk of tumor formation in the patients. So that's in a nutshell what we are working on. Now on the differentiation propensity, one of the aims is optimization of differentiation protocols. Where you can say, 'If you use stem cell line, X, and if you add a specific factor, Y, you will get better results deriving this specific cell type, Z'. Or you could just say okay you have 10 stem cell lines here but only these 3 lines give you very efficient differentiation,

HIGHLIGHTED PUBLICATION

Higher-Density Culture in Human Embryonic Stem Cells Results in DNA Damage and Genome Instability
Jacobs K. et al.
Stem Cell Reports, 2016, doi: 10.1016/j.stemcr.2016.01.015

In this article the authors demonstrate a direct correlation between medium acidification linked to culture density, and the occurrence of DNA damage and genomic alterations in hESCs grown on feeder layers. The DNA effects are rapid and occur in the short time span of a single passage. Increasing the frequency of the medium refreshments minimizes the levels of DNA damage and genetic instability. Culturing hESCs on Biolaminin 521 (hr laminin 521) was also shown to decrease the propensity of acquiring DNA damage.

“First of all, we need to do something about this genetic instability. The problem is that it’s very difficult to make a risk assessment.”

so if you can choose, choose those 3. For residual stem cells I hope that we can find ways of preventing them or at least become better in identifying them so that we can prevent the insertion or the transplantation of those cells into patients. So we are not really focusing on specific cell types or on the differentiation process toward specific cell types. We're focusing more on genetic heterogeneity. But we use different differentiation protocols because we need models, like hepatic differentiation and definitive endoderm, retinal cells, cardiomyocytes and muscle cells."

If you look back on when you started culturing stem cells to where we are today, is there a big difference in how to culture stem cells?

"The difference is huge. One of the main advantages is that it is so quick and easy to passage cells. Now you passage the cells in five minutes and for my Ph.D. students that's very, very nice. Another great advantage is that the cells are not the limiting factor anymore, whereas before, scale up was a big issue. If we needed to have a lot of cell culture dishes for an experiment it could take us weeks to get there. And in the meantime, there could be issues of cells not attaching, risk of getting an infection, reagents not arriving on time, cell passage getting too high or other things that forced us to go back to one dish and start over. It was horrible. Now, we can passage our cells 1 in 20 or even 1 in 50 so that we can start experiments every single day. So that's an enormous improvement! And, the fact that we don't have any variation anymore, that made the life for my students much easier.

You're working most with human embryonic stem cells. Do you also use iPS cells? Do you see any differences regarding safety between these two cell lines?

We started the work in the lab with human embryonic stem cells, but since the last five years or so we've also been working on human induced pluripotent stem cells. Mainly in the project of disease modeling, and then mainly because we have iPSCs from patients. We also have a small project running on trying to improve iPSC reprogramming using oocyte factors to try to improve the epigenetic status of those cells."

"I'm not sure if iPS cells will be much more unsafe. With the reprogramming techniques that are in place now, in my opinion they're about in the same safety level. I have more concerns about the mutations that they acquire during culture."

"If I look back on when I started culturing stem cells to the way we are doing it today, the difference is huge. Now, it is so quick and easy to passage cells and scale up is not a big issue anymore. And, the fact that we don't have any variation anymore, that made the life for my students much easier." Photo: Alexander Keller

"Belgium has actually a very open-minded legislature regarding the use of human embryos and pluripotent stem cells for research."

Do you have any idea of what a normal stem cell is?
"That's a difficult question. As I mentioned, we were trying to improve iPSC reprogramming. We're analyzing the cells at the epigenetic level whether we have improved the process or not and our standard to compare to is human embryonic stem cells. But as we know, these are culture artifacts too, so yeah, I don't know."

From our perspective and what we try to communicate is that it is important to have a defined culture so that we better can control the culture conditions. How is your thinking around these things?
"I agree. Our lab has shown in a publication that the pH of the culture medium is one of the main factors inducing double stranded DNA breaks and therefore the genetic instability of the cells. So just by changing the medium twice daily we could get the DNA damage to control levels. We also showed that the use of laminin cell culture substrates had a protective impact. So, I think that a big part is trying to optimize the protocols so that the cells are less stressed and more stable. It will probably never be 100%, but I think we will get much more homogenous cultures if we drastically improve the culture conditions."

Do you feel that researchers are aware about these issues, how much the cell culture conditions affect the genetic stability of the cells?
"I'm probably biased because I meet a lot of researchers who work on genetic abnormalities and heterogeneity. But when I see that that people are still doing G-banding to test the genetic content of their cells, knowing that many of the abnormalities that might occur you just cannot see with these techniques, it's a bit disappointing. Especially if they want to go to clinical trials showing that the cells are stable after 10 passages because they did G-banding. Then my heart breaks a bit. But yeah, I hope that more and more people are getting more aware of this."

From your point of view, what do you think about the progress of regenerative medicine? Will it be as big as we hope?
"I think we're getting there. There are quite some clinical trials running. My main concern is safety. Probably because my lab is focused on this genetic instability, we see that actually we have not a single human pluripotent stem cell line in culture that is normal. There is such a high heterogeneity and I think one of the main focuses the next years will need to be to investigate this further. We need to do something about it before we transplant cells. Nowadays more and more groups start using single cell genetic analysis, which will certainly drastically improve our insight in this topic."

If you look at the pluripotent stem cell research environment in Belgium today compared to when you started, what is your thoughts?
"When I started within this research field, it was fairly new. But Belgium has actually a very open-minded legislature regarding the use of human embryos and pluripotent stem cells for research. In our clinic, for example, we have a program for donation of embryos that are left over from IVF treatments, not only to make embryonic stem cells but for all research areas. It's not permitted to make human embryos for research unless there are no other ways of doing the research that you would like to do. For example, while we were trying to derive the stem cells from single blastomeres we needed good quality four cell stage embryos and those are not available in the clinic because those are going to the patients. So, for this research project we were allowed to make human embryos to derive human pluripotent stem cells."

Photo: Alexander Keller

"My big breakthrough is still to come because there are so many nice papers in the pipeline."

What is your feeling regarding the common public opinion? Do they have a positive attitude regarding stem cell research?
"I think so. We have a project, the science and ethics of stem cell derived gametes, and the idea is to make sperm cells and oocytes from human pluripotent stem cells, and a big part is the ethics around this. For this we actually had workshops with laypeople to see what their view on it is. In the beginning they were quite against it, until we started to explain what the possibilities were and what the risks are. In the end, it completely changed their mind. So, I think the more you inform people, the more permissive their minds become to any kind of research actually. It's often the lack of information that's making the people not want to do certain things."

Is there something specific in your career that you are a bit extra proud of?
"One of the things I am proud of is that we were able to make human embryonic stem cell lines from single blastomeres, because that meant that we would not have to destroy a human embryo to make human embryonic stem cell lines. And the fact that we were the first to xenograft human spermatogonial stem cells and make them survive quite a long time, that was good. Well, I hope that my big breakthrough is still coming. There are so many nice papers in the pipeline so I'm hoping in a year or two that there will be something on differentiation propensity of different lines towards definitive endoderm and hepatic cells."

Is there a specific person that has been a big inspiration to you?
"I think one of the researchers that I really look up to, mainly because he's also working on the things that I'm interested in is Nissim Benvenisty from Israel."

So, what kind of advances in the field would you hope to see in the next coming five years?
"First of all, we need to do something about this genetic instability. I think there are still big advances necessary. The problem is that it's very difficult to make a risk assessment. Because there is such a big heterogeneity, we have so many different factors that may play a role that it's impossible to study all of them. I know that in Japan they have a list of genes that are involved in cancer and if your mutation is not in one of those genes then they assume that your line is safe. I can follow the thought but I'm not sure if it's true. Also, if you transplant one abnormal cell probably it will not survive. If you transplant 5 or 10 maybe it's not a problem either, but 100, we don't know. No one knows. So, I think that's the main issue that we have no idea what the risk is. On the other hand, I think we need to focus on the maturation of the cells. We're able to differentiate into many cell types, but often we're kind of stuck in a more fetal phenotype. So, I think good maturation of cells will be important, also safety wise." •

MIEKE GEENS ON FUNDING

Let's say that you had almost unlimited amount of funding, like $100 million dollars, what would you spend it on?

"Can I say a house in the south of France? No really, I would try to do even more high throughput screening of pluripotent stem cells, to focus on the heterogeneity to be able to make some kind of risk assessment. To get a better understanding of how safe or unsafe it is to use the cells that we are making. I think I would mainly spend the money on that."

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