KEYWORDS

iPSCs, Co-culture model, microglia, motor neurons, motor neurone disease, PhD.

Intro

Hello, it's Ritika Katie and Neddy and you're listening to the context cast.

Katy

In this episode I interviewed Dr. Björn Vahsen, who currently works as a postdoc in Professor Kevin Talbots lab. He's using induced pluripotent stem cells to explore the molecular mechanisms behind motor neuron disease, and developed the first co-culture the motor neurons and microglia during his PhD research.

Thank you so much for joining us today. Can you summarize your journey in academia so far?

Björn

Yeah. So first of all, thanks for having me. So I'm originally from Germany. And I did my undergraduate degree in medicine back in Germany, and then decided that I was mostly interested in neuroscience and came to Oxford to do the MSc in neuroscience, and then stayed on to also do a PhD in neuroscience and clinical neuroscience and now I'm a postdoc in neuroscience. Yeah, I did my PhD and my postdoc now in the same lab, so I'm a postdoc in Kevin Talbot's lab, who was one of the PI's of the Oxford Motor Neurone Disease Center.

Katy

Perfect. And so you've moved from medicine, studying a medical degree into research. And I know that that's something that quite a lot of people do end up doing. How did you make that decision?

Björn

I think I might have to explain the sort of German medical system a bit because it's slightly different from from the UK system. So in Germany, we actually have two different academic degrees. So we have one that allows you to qualify as a physician, so to essentially practice medicine. But then we also have an academic degree, which is called an MD. So it's a medical doctorate, but it's completely disconnected from working with people as an academic doctorate, essentially. And many people ended up doing this medical doctorate in parallel with normal medical studies. So I ended up doing my main research project for this medical doctorate degree in a lab in a neuroscience lab. And that was what I realized, then really what I wanted to do, because I really liked going into the lab, and I had a really good time. Obviously, it's a very lucky thing, ending up in a good lab. But I think that made me realize that I was more interested in research neuroscience research, specifically, rather than actually practicing medicine

Katy

What was that first project in?

Björn

Yeah, so I worked in a lab that was focusing on translational neurodegeneration. So I was working closely with the postdoc who had set up the whole project and then sort of joined at some point. So everything was there. And we worked on axon degeneration. So essentially, the deterioration of nerve cell projections in a very basic model system. So we use cells from rats, which are called

primary neurons. And I studied essentially how the process of degeneration of these nerve cell projections, works. And I'm particularly focused on autophagy, which is essentially self-digestion of nostra projections of nerve cells. And that was looking at how a particular protein in this autophagy process is involved in the degeneration of excellence.

Katy

So these are models of human disease, but they're obviously not within the humans themselves. So you were working on rats at that point? Was that in cell systems still in sort of petri dishes?

Björn

Yes. Yeah. So I've done all of our projects with cell culture, because I really like cell culture, it kind of stuck with me.

Katy

So you've moved to human IPSC at some point, so induced pluripotent stem cells. When was that move was that when you moved to Oxford

Björn

That was actually during my PhD because my master's project. So my first master's project was Kevin Talbot was using, essentially neuroblastoma cell lines. So that was human cells, but not induced pluripotent stem cells. My second project was actually with mice. So I didn't really start working with IPSC until I started a PhD, which in a way might be good, because maybe I didn't quite fully know what I was getting myself into. But yeah, so that was very much the PhD.

Katy

So what were you getting yourself into? Was it harder than you expected?

Björn

I mean, yeah, you know, I think if you've worked with cells before, then, you know, there's, it's quite time consuming. There usually is some weekend work involved, but I don't think you fully appreciate how time consuming IPCs are until you really start working with them. I think it's good for everyone starting at PhD with IPSC to have some prior experience. I ended up liking it very much. But you know, could have also gone the other way, I guess. Yeah.

Katy

Yeah, I get the impression that it's like having a child you have to sort of go and visit and feed them every day. (Pretty much-yeah) So you generate these cells from patients, can you explain a little bit about how that process works?

Björn

Yeah, so always starts with a patient that very generously decides to provide their cells. And then they come to the clinic. And we take a skin sample, which is very small. So you perform a small skin biopsy, then you take these cells into the laboratory, grow the skin cells, and then we essentially revert them to a stem cell stage. So when you have these skin cells, they're not the stem cell -like they're just skin cells. But what we can do as we can just add a few different factors, which are known to essentially revert them to a stem cell stage, which means that they can then be sort of made into pretty much any cell type that you're interested in. And then once we have reverted them

to the stem cell stage, we refer to them as induced pluripotent stem cells IPSC. And then once we have the stem cells, we can then generate all sorts of cell types, neurons, immune cells, astrocytes, whatever you're interested in. Yeah, and that's how it works.

Katy

So they're quite powerful things, because it's one of those direct ways we can study humans, or at least their cells, when many of us in neuroscience are working on models that have got lots of disadvantages. How similar are mouse's and fly is to a human? Who knows? What would you think about? It's the two of the applicability of IPSC's for human disease in a full organism? What do you think about them as a model system? Obviously, you spent a lot of your career on them. So you clearly have some faith?

Björn

Well, I think because we're studying human disease, obviously, you should be studying human cells. So that's very important. And then you kind of want to study the cell type that is reflecting the cell type that is dying, or degenerating, and the disease that you're interested in, I think IPSC are very powerful, because you can essentially create the cells that you're interested in, in the human context. So that's very powerful. And I think it's the best way of studying human disease the moment obviously, it does have some disadvantages. We know that IPSC, developmentally very immature. So you're trying to model maybe a late degenerative process and very immature cells. But nonetheless, you have the human background. And I think that's very important. And the best model system that we have at the moment.

Katy

Yeah, one of the amazing things you did in your PhD was this co-culturing, wasn't it of human motor neurons and microglia. And so I think one of the disadvantages is often we get very simple models, but you've created something a bit more complex, there? can you tell us about what it was like to develop that model?

Björn

Yeah, I think having one cell type can already be perhaps a bit challenging, depending on the protocol. But if you combine two different cell types, then just thinking about the media that they grow, and you kind of need to optimize the media compositions, and then you keep both cell types happy, because usually you have specific factors in the media that will keep your nerve cells alive, or your immune cells alive, or whatever side you're culturing. And if you co-culture them, say you have them in addition, at the same time, then you kind of need to find a media composition that actually makes both cell types happy, then quite a bit of time in the beginning, actually optimizing the media composition to find the factors that are crucial for nerve cell survival, but also the ones that could be removed from the media to then also allow the co-culture with microglia. But actually ended up being quite lucky, because it was fairly straightforward, optimizing the media composition. But then you have to make sure that the cell system says that you want to learn to grow, right? So you need to make sure that the neurons as they're firing, you need to make sure that the microglia are doing what they're meant to do. So one of the things that they do is move around. So we did some live imaging, for example, to actually confirm that they're still moving around, and stuff like that. So that, of course, it comes with some challenges, but it's actually very exciting. So I can clearly remember the first time looking at the cells and kind of remember that thinking, okay, that probably happy

together. So this is probably going to work out. And I think that yeah, that makes it worth it that in the end, yeah.

Katy

That's amazing. And from my perspective, I didn't quite realize that two different types of cell would actually require very different media. Given that they both live in the brain. Normally, it's sort of quite surprising. Are they found in those niches, chemical niches within the brain naturally, or is it just a product of how we grow them in the lab?

Björn

I think, well, they just have different factors that they need to survive. So for example, for neurons, you need BDNF GDNF, because that's external stuff that's secreted in the brain and keeps them alive. microglia, for example, need interleukin 34 to stay alive, which then humans don't really care about. Yeah. So essentially, you try to add in the factors that are present in the brain that have potentially presented by the cell types that you don't add right? because it's challenging and very nice to have two cell types, but in the end, there's way more cells in the brain than just those two cell types.

Katy

Yeah, gosh, these two working out which bits of the complex picture to add without (exactly) one of the key things about stem cells and actually getting them to produce the right kind of cells? Can you add a little bit more to the idea of this imaging of the cells and working out whether they're firing in the right way and how you're validating the model?

Björn

Yeah, I guess, protocols have been very much informed by what we know about the normal, the normal embryological development. So if you look at how an embryo develops, you can identify the different pathways, the different chemicals sent out involved in essentially making a neuron A neuron, for example. And we very much mimicking that, in addition, by just adding the factors that have been identified, to then push the cells towards the self aid that we interested in. So for example, for motor neurons, in order to push them towards the spinal cord, because we're interested in modern humans in the spinal cord, which we're mimicking in ALS, you need to add in things like retinoic acid, for example, and agonist which will push the cells towards the expected identity in the spinal cord, suggest adding these chemicals kind of ensures that you have the correct cell types, but then you also need to validate down right and you can, for example, do a transcriptomic analysis. So you look at the gene expression in the cells and compound this with cells that have been isolated from post mortem, brain spinal cords, and then you can essentially confirm that the correct genes are being expressed. Or you can do patch clamping. So you look at the activity of the neurons and make sure that they're actually firing. For microglia, it's the same, you can also look at the transcriptomic profile, you can do imaging, so essentially, express a certain fluorescent protein in the cells, which you can then observe under the microscope and make sure that they're moving around. And microglia are also very active, active cells in terms of secretion, so you can also look at what they're secreting, and make sure that they're, for example, responding to different stimuli. Because ultimately microglia should protect the brain, for example, from vaccine. And you can sort of mimic this by adding different particles, bacteria particles, these particles, and then see how they behave. So that's, that's a few of the things that we do.

Katy

So it's mostly degeneration, you've studied in the context of different diseases, can you tell us sort of about these conditions and a little bit more detail of sort of what processes you're looking at in the cells that are then applying to these conditions?

Björn

Well, so I'm currently focused on ALS, which is a mild trophic lateral sclerosis, which is also called called Motor Neurone Disease, what I'm focusing on now is essentially trying to understand if it's just modern humans that are degenerating and the disease, so ALS is essentially, it's rather late onset degenerative disease that is characterized by progressive death of the motor neurons, so the nerve cells that control muscle movement, so ALS, patients will then develops progressive problems walking, swallowing, speaking. So that means the primary cell type that is affected our motor neurons. But what we are starting to understand now is that it's probably not just motor neurons that are being affected. There's lots of other cell types in the brain. And one of the cells that have been essentially identified based on animal studies are microglia, which are essentially the resident immune cells in the brain. And what I'm trying to understand is in a human context, so using these human IPSC, if the microglia also contribute to the disease process in motor neuron disease, by looking at whether, for example, the microglia lead to less or more death of the neurons. So that's sort of the key phenotype that I'm interested in.

Katy

So, because you've got a cell culture, you can really look at the molecular mechanisms, how have you studied different molecular pathways? And what questions can you answer with this system?

Björn

Yeah, I think there are different questions that you can answer. So focusing on ULK1, for example, which is what I worked on before, we essentially use the virus to downregulate the protein and then you can look at the effects on the different pathways that it's involved. So that's one of the things that we tried to answer in my my first project regarding C9orf72, the reason why we are looking at this is because mutations in the C9orf72 gene are the commonest mutations that have been associated with motor neurone disease. And what we're trying to understand is if cells with this mutation, so this gene error and C9orf72 behave abnormally Compared with cells from a healthy individual, and then you can look at the different genes, so the different cytokines or whatever being dysregulated and understand a bit more about what's different between the disease cell versus the control cell, we actually generated microglia from patients with this mutation in C9orf72. And we essentially identified that in the mutant microglia versus controls. The mutant microglia were more pro inflammatory than the control microglia, which suggests that the expression of the mutation already skews microglia to more pro inflammatory profile. And we then focused on one specific mediator called matrix metalloproteinase nine MMP nine, which was more highly expressed in the mutant microglia then draw microglia. And we then also perform some co-culture experiments using this co-culture model that I sent out before. And what we found is that if you add the mutant microglia to neurons, they caused more toxicity than control microglia. And that this toxicity is partly mediated by MMP nine because when you add inhibitor against MMP nine, this toxicity is not completely but partly ameliorated. And that's interesting because that firstly suggests that ALS patients with this mutations in an C9orf72 microglia probably contribute to the disease plan. And sort of is the foundation for future work to identify more, what sort of different mediators are involved in this process and potentially, also to see if we can target this therapeutically?

Katy

Do you think this kind of work is going to start influencing stuff in the clinic?

Björn

We would hope so. I mean, what I'm trying to do in my work is kind of also have a bit of a link between the Clinic and the cell models, because obviously, we're taking cells from the patient, we're then identifying what's essentially going on in the cells in the dish. And then you would hope that this could translate back into the clinic either by helping some sort of drug screening approach to then identify new drugs, which could be tested in the clinic, or what we also did in this context is we essentially identified that one particular protein called DPP4 was upregulated. In the cockroaches, and we are now trying to see DPP4 perform, maybe it's also upregulated, in patient samples. So I think it's not just identifying different targets for drug screening, it's also maybe identifying biomarkers, which are linked with this toxicity or pro inflammatory microglia profile, which you can then look at the patient and see if the same thing happens in the patient essentially.

Katy

So, to move slightly away from pure academia, I know that you've mentored a few students while you've been in the lab, what do you look for in a potential lab mate in someone who's going to start cell culture work?

Björn

I think you want someone who is first of all keen to learn and motivated. I mean, ideally, obviously, someone who has a bit of prior experience in the lab even better, and the tissue culture. But I think most importantly, you need someone or you want someone who is keen to learn. I mean, with IPS work, you also need to put in the hours, I think that's that's also important to appreciate, because it is just fundamentally very time consuming work. It's important to point that out when you meet students, because they should be aware of what they're getting themselves into. And if that's clear from the start, then I think the expectations are clear. And then you just need to be there for them as well. Right? There's I think there's no point in just getting someone to execute the work that you want them to do, I think you also want to teach someone something. And that requires being there in the lab. And very often also means responding to what Sam said, 11pm In the evening, but I think you know, I think that's fine. And that's that's, that's fun.

Katy

Yeah, amazingly committed to your work. It's really cool. And the other thing I noticed, in my research was how many scholarships you seem to get and now on a fellowship, how do you write such brilliant applications?

Björn

I think, well, there's always an element of luck, right? And there's lots of applications that are not successful, which are that are not listed on the website, for example, right. So I think it's maybe slightly skewed in that sense, because I also applied for lots of things that I didn't get, but in the end, I think, just need to keep trying. I think that's very important. So I think it's good to just apply for whatever you can.

Katy

I always like the quote is one my dad really likes it like luck is when preparation meets opportunity.

Björn

Well, I guess Yes. You have to also kind of put in the work (Yeah). And you have to be, you have to expect failure in a way as well. And particularly with the big round applications, the success rate is just so low, I think that you need to brace yourself for being frustrated.

Katy

I think that's true in all science as well. Like, I bet when you were developing these models, you had months where everything didn't work. What was it easier than that?

Björn

Yeah, I think yes. Because, you know, if the differentiation fans for some reason, which you can't always control, then you easily lose two or three months of work, which you can't control. And that's frustrating. So I think you need to have some sort of resilience ready to deal with that.

Katy

Why do you still do it? And it's so hard? What what do you love about science? What is it?

Björn

I think the fun bit is asking a question and then being being able to answer that question yourself, right? So you have an idea to generate a hypothesis, you're interested in a particular thing. And then you just execute the project? And you'll find the answer. That's not always the answer that you hoped for. But at least you answer the question that you posed in the beginning. And I think that's fundamentally, just really cool. Because you also have the freedom to explore within the sort of grand scheme of things that is imposed by the funding buddy and your PI, maybe, but you've got the freedom to explore whatever you want. That's just really cool. Yeah.

Katy

mean, you get to ask new questions. No one's asked before your case, make cultures that no one has made before.

Björn

Yeah. And that's fun. Because then in that moment, you want to save someone else's doing it at the same time. But in that moment, you think you're potentially the first one to see this. And that's, that's quite cool.

Katy

It's very, very cool. First person in the world to do motor neurons with microglia, when it might be super important for a really awful disease.

Björn

Yeah. No, I think there's lots of lots of things going on. But we can't really saying that we've identified an attack and the cell culture, and this has sort of changed someone's life, which obviously is what we would want. I don't think we can say that at the moment. But there's lots of things going on, which are being identified and being explored. And I think that's very important.

Neddy

In this interview, led by Katy Doctor Björn Vahsen discusses the importance of using induced pluripotent stem cells as a model understand neurodegenerative diseases. He gave insight into the work developing cell culture models, being the first to co-culture human motor neurons and microglia to better understand Motor Neurone disease, with potential impact on drug screening and biomarker discovery. In addition, Dr. Vahsen shares valuable insights into the dedication required instance of research, providing tips on navigating successful grant applications, and the resilience needed for successful scientific career.

Katy

Thanks for listening in on our conversation today. We hope you enjoyed it as much as we did. Please keep an eye on our social media to find our next one.