Interview Transcript – prof. dr. Maurice van Steensel

We're in the lab of Dr. Maurice van Steensel, at the Academic Hospital in Maastricht. My name is Jill Woodward and I'm speaking with Dr. van Steensel, who's just hosted the Third BHD Symposium. What research or findings have excited you the most?

Well, apart from my own results of course, what really struck me during this conference is the link that is now emerging between Folliculin and AMP kinase signalling, so basically the work that was presented from Arnim Pause's lab who are using this *C. elegans* model. He quite convincingly shows that there is a quite a deep connection between Folliculin and longevity and energy sensing. So we all quite had the idea that it must be connected but this is really the first time it is quite obvious also what Folliculin's place in this entire cascade is.

And next to that work, there's Ravi's results obviously showing for the first time and pretty convincingly, as far as I'm concerned, that at least the C-terminus of Folliculin, which is kind of an evolutionary innovation, is a GTPase. So that immediately sets you thinking and presents a whole host of possible new directions for research to take. And the idea of Folliculin being a load-carrying protein, that is hydrolysing GTP to actually do its work, in conjunction with this FNIP1 and FNIP2 complex, that's hugely attractive in terms of understanding how it functions, and certainly when you compare that to the yeast orthologue, which is a known transport protein, so all of a sudden things start to make sense and give you ideas of where to go next.

And moving on to what you are doing, tell us about research that you're working on right now.

Well we're interested in cellular signalling, that's where we come from. So I've worked in gap-junctional communication for several years for the simple reason that people have skin diseases caused by defective intracellular communication. Our work in BHD also is about communication mostly so what we're interested in is in how Folliculin is picking up signals from the outside world and transducing into the inside because we think that this is what it's involved in. And we are thinking this because we are finding Folliculin in the primary cilium. The cilium; that's a highly conserved structure - it's an organelle on the cell that looks like an antenna actually. So resting cells when they stop dividing and grow onto confluence, they will put out this antenna-like structure that can be longer than the cell itself actually. And they

use this antenna to pick up signals from the outside. So in kidney, for example, they're sticking into the ducts and collecting information about flow and pressure and chemicals that drop by and growth signals and the like. What we're thinking is that this function in kidney, and in lungs, and possibly in skin also, is disrupted. And this way cells become confused and no longer really know where they are and start to form cysts. Rather than these nice tubules you have in the kidney, you get these cysts. And we really want to know why, because these cysts, we think, are the very very early stages of kidney cancer. So if you understand cyst formation, by extension, you might eventually understand kidney cancer and understand how to prevent this from eventually happening. Because the cysts are probably a really early event but if you can arrest them in their tracks, you might be able to prevent kidney cancer from ever happening. And what we're finding is that Folliculin is in the cilia and it's also in the nucleus and it's at mitochondria, that's what Andrew Tee has found. So it looks as if it has some sort of communication function there and we are very interested in finding out what that is and that's what this research here is all about.

Have you met any challenges or difficulties in getting the work completed?

Oh tonnes; it's first of all not very easy to image Folliculin at the cilia and cilia are vulnerable structures. So for example if you transfect your cells, cilia tend to just go away because they really don't like it. They also are intimately connected to the cell cycle, so if your cells are not resting, if you don't have your culture conditions right, they won't ciliate. We've found that technical issues such as permeablisation, for example how much Triton X, which is a soap basically you use to make sure that your antibodies can see all the structures inside the cell, is really very important. We've had to solve issues with cell types not being type-advertised, we've had contaminations of various cell types. It's been pretty challenging over the years. And certainly it's getting all the conditions right, to actually have reliable data and reproducible data, and being able to show that basically Folliculin is in every single type of ciliated cell type that we've so far looked at: that's been pretty challenging because of the imaging.

Now you're a clinician.
Yup.

How does that affect your role as a researcher?

Well, it kind of defines my role as a researcher because basically every research question that we have has started as a clinical observation. So we went, we started looking into cilia because when doing the screening for the people with BHD, we found that they have cysts in their kidneys and kidney cysts and liver cysts, which they also have, and pancreatic cysts which we've also seen, are really a hallmark of disorders where ciliary function is disrupted, so ciliopathies. So we got started in BHD also because of a family that I met whom we diagnosed eventually with BHD after finding out that they had fibrofolliculomas. So however, interesting the basic questions are, you know in terms of what's Folliculin's function deep deep down—that's a very basic question—but we're always keeping in mind the eventual applications of that. That's also in our collaboration with the Tee lab. Drug screening is very close to the top of our priorities.

Because there's two approaches that you can take to solve the issue of BHD. You can go into drugs that you know affect certain aspects of Folliculin's function, that we have elucidated, such as for example the interaction with HIF-1 transcriptional activity, that's something you can target using drugs. The autophagy angle is druggable again. The processes related to autophagy are all druggable.

On the other hand, you have this really fascinating protein that seems to be everywhere: and it's hugely important and it's hugely conserved in evolution, and yet when mutated causes a phenotype that as far as we know is eminently survivable and relatively mild when compared to disorders, such as for example von Hippel-Lindau syndrome or Tuberous Sclerosis, which are related disorders but far more severe in their early manifestations. So now it looks as if Folliculin in terms of its cellular physiology is all about fine-tuning of processes. It's twirling various knobs, you know volume, bass, treble etc. etc. All those systems have to be just right in order for them to function. And so what you're seeing in BHD is things getting slightly out of balance and ever more and ever more and ever more and ever more and then eventually you end up developing cancer. That's a very, very basic question: how is it doing that? And we also want to understand that, being scientists and all. But from a clinical point of view we're just like: we go straight towards the goal and we don't care if there's a black box in between. Because you put in A and out comes B and we can prevent B, I don't care what's in the black box. So there's basically two approaches that you can take in parallel and that's what we're doing.

The Third Symposium has just finished; can you talk about the values of these meetings and collaborating?

I think apart from being fun and being able to share your ideas, there's always a very motivating aspect of collaboration. Because I really don't like keeping things for myself, if you have something exciting and new, basically my reflex is to share the stuff. Another aspect, and that's something that we've really seen, as far as collaborating very, very closely with the Tee lab and now also with Ferenc Mueller and Eamonn Maher in Birmingham, is that you have this synergy. So stuff that you'd do on your own—that takes a long time. You have to solve these technical problems etc. etc., you have to repeat your experiments. But if there's two labs or three labs all going in the same direction, you can actually share experiments and move much faster. And because you have synergy, because you can discuss your results, you can actually move more than twice as fast as the first two labs—you can maybe move three times as fast. Now there's a Dutch funding scheme that addresses orphan disease such as Birt-Hogg-Dubé. So what we're trying to do now is take the European Consortium and the Canadian group, and trying to build an even larger collaboration where we're going to address several aspects of BHD and Folliculin functionality and drug screening and animal models and animal model development, and put all of that in a large collaborative project for five years. And basically tell the funding agency: give us a couple of million Euros, and we're going to, at the end of this, have actually useful drugs.

What advice would you give a young scientist considering moving into the field of BHD research?

Well certainly do so, because it's hugely fascinating and you can basically focus on whatever process interests you. It doesn't necessarily need to be about cancer. It turns out that there are links with longevity as well, so if you're interested aging, you can go in Birt-Hogg-Dubé syndrome. If you're interested in emphysema, you can go into BHD. If you're interested in kidney cystic disease, you can go into BHD. There's tonnes of clinical work yet to be done. So if you're interested in entering the field, do go and talk to one of the BHD people who are in a lab near you. The list is on the internet and it's pretty well known who the people are who are serious about BHD research. And you've probably seen at the Symposium that all of them are really, really happy to talk to you about doing research and are really keen also on finding

good people who actually want to carry on. Because any lab is always in flux: people come, people go. And whenever you write a grant and are funded, then your next problem becomes finding people who can actually do the work and are enthusiastic and are willing to give their best.

And what specialisations do you think are needed?

I think it would be very, very good to be conversant with, say, zebrafish and *C. elegans* models. And there also happens to be a very interesting fruitfly model around that's been largely neglected and that really ought to be resurrected. And there are several aspects of BHD that in my opinion are best studied in yeast, so yeast people I think are most welcome in the field. And people who know their zebrafish or *C. elegans* and are good at imaging, that's really what we need. Because it turns out that Folliculin is a very dynamic protein and I can imagine that there's some really, really interesting imaging experiments that you could also do to understand the protein's basic behaviour. We're certainly moving in that direction. So in terms of specialties that are needed, that would be my take on the way it's going. There's mice already, there's plenty of people who know their mice. Some more structural biology might also be a good idea because there's loads of interactions that might be druggable targets. And for that obviously we need crystal structure in order to be able to design drugs that target these interactions that you might be interested in. So also structural biologists.

The Third Symposium is now finished. What can you tell us about the next meeting?

This Symposium now, it's a two-day Symposium with over 40 abstracts submitted and more than 80 attendants. And considering the level of discussion and the high level of the presented work, it's become a very, very attractive symposium to go to. You get two days of really good science. So we're going to have every year a symposium; I guess for now alternating between Europe and North America. So USA, Canada, Europe. I definitely think that this Symposium also has a function of outreach, which is why we also like patient sessions during the conference. So people can also have interactions with researchers and can actually see for themselves the work that's being done. For example, at this Symposium we had the patients share the clinical sessions with researchers on the morning of the first day. I think that's very good, so you can actually seen how clinicians and basic scientists are trying

to get their heads around how to best screen people and how to best help them live with this disorder.

Dr. van Steensel, thank you very much for talking with me today.

You're welcome, it's been my pleasure.