Interview Transcript - Dr Laura Schmidt

We're at the NIH in Bethesda, Maryland, my name's Jill Woodward and I'm speaking with Dr Laura Schmidt, who's a staff scientist at the NIH. Dr. Schmidt, you've been a pioneer in BHD research, you were part of the group that identified the Folliculin gene and there was a landmark 2002 paper published on that. Talk about the current state of the research and what you'd like to see in the near future.

Well, since 2002 so much has happened. I mean it's very exciting for me to have been part of that initial discovery phase. We discovered, as almost everybody knows, this novel gene whose function was totally unknown and its sequence didn't give us any clues as to what the gene might do. And now, over the last 8,9,10 years, the information that we've gained from all of the research that's been going on has been phenomenal. We now have identified a number of interacting protein partners, that's been one of our focuses as a lab, to try to find other proteins that interact to give us clues as to what Folliculin does and we have identified two of those, we've called them FNIP1 and FNIP2. And although they were also novel proteins, they in turn interact with a very well-known protein, called AMPK which is known, a very well-known, energy sensor in cells and it's very important in the regulation of a pathway that controls cell growth called the mTOR pathway. So we have this clue now as to what Folliculin may do in the cells and the mTOR pathway is known to be disregulated in cancer. So it makes sense that Folliculin could be involved in that pathway. We've also since that time— we now have these very exciting mouse models, animal models. Now again the urologic oncology group has contributed to that; we have a number of animals models that we've used to try and get at the function of Folliculin by inactivating Folliculin in those mouse models, in this case, and then looking at the phenotype: what happens to the animal when you knock out Folliculin and it's given us some really interesting insights into Folliculin function. And the most exciting thing for me right now, we've just learned about this at the BHD meeting in the Netherlands, is that now we have the crystal structure; this is coming out of the Cambridge lab, Ravi Nookala and Tom Blundell's lab. That's very exciting; that really gives us the three-dimensional structure of the protein. So this information we're gaining from the crystal structure I think is really going to be an important clue that will direct our research towards perhaps understanding whether Folliculin may play a role in some cell process that requires energy because a GTPase would hydrolyze GTP and release energy.

So what would you like to see happen in the near future in BHD research?

What I'd really like to see is labs pursue several different avenues of research. We've been focusing on protein-protein interaction looking for new interacting partners to Folliculin as a way to get at mechanism, we've been focusing on animal models—we know that there's a *C. elegans* model and a zebrafish model so we have other models coming along. But there are a couple of areas I think that are lacking in research direction. One of them is those people who study the lung. BHD lung is a really important aspect of the manifestations that the patients experience. Currently, there really aren't very many pulmonologists or pulmonary research labs that are focused on BHD. So I think that's actually an area where we could use more research efforts. And I think we need more structural biologists to support the work being done by the Cambridge group and to help think of new approaches and ways that we can expand upon on their discoveries. And at the same time we need some good biochemical bench people to test the potential of Folliculin as a GTPase at the biochemical level, right at the bench.

Can you talk about how you discovered the FLCN gene?

We had families that had kidney cancer; it was clearly in the family. But they were tested for the *VHL* gene mutation, they were tested for the *MET* gene mutation—which were the two genes known at that time that gave rise to inherited kidney cancer syndromes. These patients did not have mutations in those two genes. And then when some of these families were re-evaluated by a dermatologist, they noticed the skin bumps. So doing a literature search they discovered there was this rare dermatologic disorder called Birt-Hogg-Dubé syndrome in which patients had skin bumps. Then they began to see that there was a correlation between the skin bumps and the kidney cancer in some of the family members here at the NCI. So that got us to thinking: well, maybe patients with kidney cancer and fibrofolliculomas have Birt-Hogg-Dubé syndrome. And we should look for the gene for that because now we have families here. So we began to do what's called linkage analysis where you take a DNA sample from the affected individuals in the family through the generations, and you look for the inheritance of a segment of chromosomal DNA through the generations along with the kidney cancer in our case, and that helps you narrow the focus of where the gene might lie that causes that kidney cancer. And then once you have sort of a narrow region, you can go in and mine all the genes in the region and—it's kind of brute force—you go in and you test each one for mutations, in

those individuals that you've identified as having this inherited kidney cancer syndrome. And eventually we found mutations in this unknown gene—novel gene—that had been sequenced but for which there was no known function. And once we found mutations in this novel gene in one family, we began to test all our BHD families and we started seeing different mutations but in that same gene. So we knew we were on the money there. So we had discovered the gene.

You've also been involved in breakthroughs in VHL and hereditary papillary renal carcinoma research. What perspective has this given you on your BHD work?

Well, it's interesting, each of the rare inherited renal cancer syndromes that we have discovered and found the genes for, and that includes hereditary leiomyoma renal cell carcinoma and succinate dehydrogenase-associated renal cancer. Those are two other rare renal cancer syndromes that have been worked on by the UOB group and the focus here at the NCI. In each of those cases, we found that the syndromes are associated with mutations in specific genes, which are different one from the other and they seem to predispose to different subtypes of kidney cancers, which we call the histologies, so they look different under the microscope. And BHD is no exception. So the *Folliculin* gene when mutated gives rise to kidney cancer in BHD patients but the way it looks under the microscope, the histology is quite different, say, from the VHL-associated kidney cancer, for the most part. So I think one of the things that I've taken from my research in these areas is: don't get tunnel vision, don't think that all kidney cancer is going to be the same because it isn't. There are a variety of epithelial tumour types each of which can be described or associated with one of these different rare syndromes and we've learned so much by our study of each of those independent genes, the *VHL* gene, the *MET* gene, the *Fumarate Hydratase* gene and *Succinate Dehydrogenase* gene as well as the *Folliculin* gene. But at the same time, as we're understanding those pathways better, it's starting to look like maybe they will converge on sort of a common endpoint. So that brings us back full circle and helps us learn from each of those studies looking at the different syndromes, how we can apply that scientific knowledge to our current syndrome, which is BHD.

The Urologic Oncology Branch is involved in both clinical and the basic side of research. How has that been important to your work?

That's really, really been helpful. Because we are probably the largest medical institution in the United States, and in North America perhaps, that sees the largest number of BHD patients, perhaps in the world. I probably shouldn't say that, but certainly in North America. So we have this rich resource available to us. We have patients that come here: they are very carefully screened and evaluated for all of their manifestations. We have at our disposal a blood sample from each of the affected individuals, we can determine their mutation and we can compare it with the manifestations that they have developed for Birt-Hogg-Dubé, and see if we can see any kind of correlation between the type of mutation or the location of the mutation within the gene and whether they develop kidney cancer, fibrofolliculomas, or lung cysts. That's called genotype-phenotype correlation. That's a really important part of understanding the gene function and how that then translates into the manifestations in the patient. And we've learned a great deal from the VHL story, exactly along these lines. And there is a clear genotype-phenotype correlation in VHL. It's directly connected to the VHL function in the cell, as a substrate recognition site for HIF. And so hopefully we will learn the same kinds of information about the Folliculin gene from the studies that we do using clinical samples available to us. Of course, the availability of the tumours is just fantastic because we can do the kinds of studies that I was just mentioning. If you want to do next generation sequencing of those tumours, we bank all tumours that are surgically removed here and we have those at our disposal to use in the future. We can look at the histology of the tumour, we can look at the subsequent genetic changes in the tumour and correlate it with the mutation in the gene. So it's just a wonderful rich resource and it's right here at the UOB, ready for us to use.

What effect do you think new technologies will have on BHD research?

Well, I think as a rule we've been using the old-fashioned methods right up until this point, but I think this next generation sequencing which is coming along where we can do whole genome sequencing is really going to help us a lot in our ability to identify additional genetic changes that may predispose to tumour progression or ultimately to metastasis. So for example, one could take multiple tumours removed surgically from a BHD patient. Each of them is going to have a different environment, they may be different sizes, they may be at different levels of progression in terms of their development in that patient, and what we can do is we can extract DNA from each of those tumours, sequence the DNA and look for genetic modifications in addition to that germline *Folliculin* change. And eventually if we look at

enough tumours, we should be able to correlate these additional genetic changes with the state at which that tumour is: whether it's small or large, whether it's very early onset tumour or late in its progression. Of course if it's metastatic it would be quite different from those early-onsets. And hopefully these genetic profiles will give us a lot of scientific information that we can use in our research to help us understand those pathways of progression and metastasis and obviously consequently to develop good therapies to treat those patients. So I think that's where next gen sequencing is really going to be fantastic for this research. And that actually is one of the things we're thinking about doing in the lab, so that's why I shared it.

What advice would you give to an early stage researcher and what is the most important thing that you've learned about the research process?

I think it's very easy when you're a young researcher starting out to—two things. You can jump on the bandwagon and do everything that comes across your plate. So you see lots of interesting leads and you want to follow them all. In that case, you spread yourself a little too thin. And one of the lessons I learned from my mentor Bert Zbar was to reign myself back in and try to stay focused and do the best work you can as you focus on a particular research path. And obviously you can do one or two or three, but you can't do ten things at the same time because you dilute yourself too much. On the other hand, you can also get very discouraged because research is hard—it's hard business and we probably fail more than we succeed in our lifetime in research. So you have to always have a positive attitude and say: you know I'm going to do this, I'm going to put my nose to the grindstone and give it my best effort; but try not to be discouraged when things fail, because they will.

Dr Laura Schmidt, thank you very much for your time today.

Well thank you very much. It was a pleasure.