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Brian Martin: From UPMC Children's Hospital of Pittsburgh, welcome to That's Pediatrics. I'm Brian Martin. I'm the vice president of medical affairs here at Children's.

Carolyn Coyne: And I'm Carolyn Coyne. I'm a scientist in the Division of Pediatric Infectious Diseases. Joining us today is Dr. Bernhard Kuhn. Dr. Kuhn is a cardiologist who studies cardiomyocytes, the cells of the heart muscle, and discovering ways to make them replicate and proliferate so as to enable the heart to heal itself in the cases of congenital heart disease or heart failure. Dr. Kuhn is also the associate director of the Richard King Mellon Foundation Institute for Pediatric Research here at Children's Hospital of Pittsburgh. And thank you for joining us.

Bernhard Kuhn: Thank you, Carolyn and Brian.

Carolyn Coyne: So I always like to start these with a little bit of background. Why don't you tell us a little bit about yourself, maybe your influences, your training, and what brought you to research?

Bernhard Kuhn: Well, really I went to medical school mostly on the account of my pushing of my parents and very quickly got involved with research and spent three years in a lab in which MD researchers and PhD research were side by side, and the same standards were applied for graduation. And I tremendously enjoyed that. That was the leading pharmacology lab in Germany at that time. And my direction was really set for becoming a pharmacologist and never use a stethoscope.

Bernhard Kuhn: And in my final year, I won a stipend to spend in the United States at different places. So at Yale, I really fell in love with pediatrics, and I chose pediatrics for elective. I thought, "It's good to know this kind of stuff when you have a family, to know when to call a doctor, sort of some basics might be useful." And I saw a physician-scientist in gastroenterology really being a competent clinician in a very narrow area and a really competent researcher, and that was enabled by this particular structure that enables physician-scientist to be good doctors and good researchers. And so I thought, this is really what you want to do.

Bernhard Kuhn: And then I came for residency to Yale in New Haven and cardiology fellowship in Boston, and that then really gave a lot of satisfaction, the clinical aspect of it. But I always felt that really, you could amplify what you're doing by developing new understanding or new therapies that enable to help many more patients so to increase the impact. So that really fascinated me about and kept me going with research after clinical training.

Bernhard Kuhn: And then I met my postdoctoral mentor totally by chance in my interview. I didn't even know, "Why did they put this person on the program?" And it turns out, Mark Keating had cloned the first three lethal human arrhythmia genes. It was really, really famous in a field that I was not very familiar with. And we hit it right off, and I decided to join his lab. He took his fame in human molecular genetics to switch to regeneration genetics using zebrafish as a model. So then I was in a regeneration lab and applied what I learned in pharmacology, and then it became sort of a self-propelling self-stimulatory circuit.

Brian Martin: What drew you to cardiology in the first place?

Bernhard Kuhn: Well, so at that point, I was 30 years old, and like many professionally less mature people, we make our decisions not so much on rational considerations but on mentors.

Brian Martin: My wife would concur.

Bernhard Kuhn: So my program director, Alan Friedman at Yale, is a happy, very happy pediatric cardiologist with a very happy family. And I thought that, "Wow, this is happy clinician."

Brian Martin: That looks pretty good.

Bernhard Kuhn: So that is how that came. And then plus a lot of pharmacology, early classical pharmacology comes out of cardiology, right, like beta receptor before we knew what beta receptors are. We knew heart rate regulation to certain hormones and such. So that was sort of the first baby steps in molecular cardiology, maybe some of that last goes back a hundred years. So I felt like there was a reasonably good connection between what I was doing and research as a student and what I could do as a clinician.

Carolyn Coyne: So what is your research focus now? So are you still looking at this idea of regeneration in the context of a particular disease, or what is your lab studying currently?

Bernhard Kuhn: Yeah, so as a postdoc, I was, like I said, in Mark's lab, and Mark was an adult cardiologist that didn't practice, and so he wanted to regenerate the adult heart. And it became over postdoc on early junior faculty years clear that regenerating the adult human heart is a very tough target. So we have then done work in human infants on heart regeneration that indicated on a very descriptive level that heart regeneration may be easier achievable in infants.

Bernhard Kuhn: So we have used the general principles that we have developed using adult models and implemented them and emulated them in pediatric hearts, right? So mechanisms of heart regeneration are active in adult hearts as well. They are just very low grade, and you might say inefficient, not sufficiently fast and strong enough to replace the quarter of the heart that's lost after large heart

attack. So in pediatrics in our patient population, we're challenged with loss of cardiomyocytes as well. It's just not a large body of literature about this. So there's a lot of very basic work that needs to be done, and that's fairly hard.

Bernhard Kuhn: So yeah. Carolyn, your question about what is your focus at present, I like to think of this as several narrow areas of focus on a spectrum between very basic research to clinical research. So at the very basic end, we are doing gene discovery using single cell transcription profiling. We are doing basic cell biology using a lot of live cell imaging. We're using confocal microscopy. We are now using also super-resolution microscopy. And then on the more translational end, we have neonatal mouse heart injury models that we use to test mechanisms that we discover in the dish in an animal. And then we're now doing human descriptive observational clinical research so where we are taking either human heart muscle cells and analyze them transcriptionally. And we're now also measuring heart muscle regeneration in human infants with congenital heart disease.

Carolyn Coyne: So tell us a little bit, and this is probably a very basic question, and I'm not really familiar with probably the complexities of it, but how does the heart regenerate? And you mentioned before that there was this difference between adults and infants, and so how does the heart regenerate, and what is the mechanism for that difference? What explains that differential sort of ability?

Bernhard Kuhn: So at the molecular level, I don't think we have a clear understanding yet what are the molecular signals that change between development and adults. Maybe we have a glimpse in that the amount and the degree of the presence of proliferation factors that stimulates proliferation of heart muscle cells and of progenitor cells is a lot lower in the adult heart, right? But on the other hand, there must also be some autonomous effects that is adult heart cells are locked in a non-proliferous state, and that thought comes from the concentration that cancer of the heart is nearly unknown, right? There is nearly zero cancer in the heart at any point of life.

Bernhard Kuhn: Now in terms of what mechanisms do we pursue, the work in infants and in neonatal mouse models has taught us that there is probably a high degree of similarity between developmental mechanisms in regeneration that you find in neonatal mice and in young humans. So we've started this in looking at adults, but now, we're finding ourselves on the other end of the spectrum. We're doing a lot of basic developmental and cell biology, cell biology that is situated within developmental biology. And that in of itself sometimes is a deficiency in the field.

Brian Martin: Can you speak a little bit about your collaboration with cardiac surgery and the sort of translational kind of crosswalks that you have? [crosstalk]

Bernhard Kuhn: That is fantastic here. What we're doing with fresh warm human heart muscle out of the operating room requires very close communication between the research team and their clinical team. And so we are getting a heads up page or

call. We're getting the call to go to the OR and get the piece of heart muscle, so we can get warm fresh heart muscle with a very short line of custody. And talking to my colleagues at other institutions, that is fairly remarkable. That simple collaborative chain of communication thing, as simple as it sounds, once it works, is a barrier at many other institutions.

Carolyn Coyne: So again, we talked about this difference between neonates, infants, and adults, and presumably, this is sort of a fluid process that gradually changes over time. Is there a specific age where you see in particular that that change occurs in terms of the ability to regenerate?

Bernhard Kuhn: The literature would suggest yes, there are some deflection points at which this is more dramatic, the decline of regenerative ability. I would agree with that to a certain degree, but there is also evidence that suggests that it is not quite as falling off a cliff. So the literature would suggest, well, soon after birth. And some of that thinking is I believe is derived from the fact that mouse still is our most favorite lab animal, and in mice, it happens nearly like on a cliff. When you look in humans, it is not quite so dramatic. When you look in sheep, some of these mechanisms happen before birth. So this may potentially be more complex than we think.

Bernhard Kuhn: There is a line of thinking that the change into the normally oxygenated state at birth has a role in this. There's some other data out there that would conflict with that. So we're looking at this fairly broadly and taking into consideration findings like in sheep that if it happens before birth, there must be that is proliferative arrests and terminal differentiation in some cardiomyocytes happens before birth. Then that suggests that there are tractable molecular mechanisms that one could try to understand and then work with these mechanisms and see if they're altered in infants with congenital heart disease and if so, if we can change in a positive way.

Brian Martin: Can you tell us a little bit about what are called "expectation modulation" in terms of parents and families that you may have interacted with in the past because there's a lot of, sort of segueing over into popular literature, right? People hear stem cells. They hear regeneration. They hear the pathway to this. Do you find the fork is... It's complex. It's detailed clearly. Where in the continuum of discovery would you say are we? Are we in the infancy? Are we in early childhood in terms of the ability to grow new heart muscle and to modulate this type of regenerative recovery?

Bernhard Kuhn: Well, so to grow new heart muscle, that has to hold up to certain scientific standards. I think, to this day, many leaders in the heart regeneration field would say we have not proven that we have regenerated heart muscle in humans. And even in mammalian model systems, that proof is pretty hard. So I think the stem cell field now is going into a direction that injection of stem cells into the heart, no matter who the host is, does not lead to substantial generation of new heart muscle cells.

Bernhard Kuhn: Now, there is functional benefits from injecting stem cells reproducibly in some studies. And the thinking is that that is due to factors that are released from stem cells such as growth factors or exosomes. And so there is a whole line of research that goes into understanding how that works at the molecular level, and then you could use those molecules to administer them, right?

Bernhard Kuhn: We're looking at the natural way how the heart muscle grows in development, and that is by division of heart muscle cells. And that is accepted, proven mechanism of heart muscle growth before birth, and we have demonstrated it's a mechanism of heart muscle growth in humans after birth, and others have proven that this is part of heart muscle growth after birth and myself. And it is the mechanism of heart regeneration in zebrafish, the other model system that is used to study regeneration.

Bernhard Kuhn: So yes, I think in our line of work, we are probably looking at having to do a lot more basic science because we want to understand how this happens naturally and then emulate this or the critical elements of that, emulate them for a therapeutic approach. Whereas many of the stem cell research is in a very applicatory direction, and that is, is there a functional benefit? If so, is it reproducible? And if yes, and there's otherwise no harm, then we can do it in humans.

Carolyn Coyne: You mention that the ultimate goal of your research of course is to treat a lot of congenital heart disease. Tell us a little bit about that. What are the different disorders that exist?

Bernhard Kuhn: Yeah, so the congenital heart disease is a heterogeneous mix, right? It's the most common birth defect with 1% of live born babies having congenital heart disease and approximately half of them having severe congenital heart disease. That means intensive care and potentially surgery. So it is very heterogeneous. And that distinguishes it from adult heart disease where really coronary artery disease is the number one, two, and three offending disease.

Bernhard Kuhn: And so in congenital heart disease because so little has been done in that direction for good reasons because it is technically and infrastructurally challenging, we have worked a lot with tetralogy of Fallot, which is the most common type of cyanotic congenital heart disease that is when patients who have blue cyanotic skin color. Tetralogy of Fallot is reasonably common. We do many cases every year. It is anatomically relatively homogeneous. And nearly always when our surgeons operated, they take a piece of heart muscle out when they recreate normal blood flow that enables normal oxygenation. And that piece of heart usually has been thrown away. And so we are taking this now for our research. So while tetralogy of Fallot may not be the greatest menace within the box of congenital heart disease. It is, for practicality reasons, our first disease that we are working with.

Bernhard Kuhn: Now, the next step might be something like dilated cardiomyopathy, and again, that's the number one reason for heart transplantations. And there is a real dire medical need for therapies other than transplantation.

Carolyn Coyne: Well, thank you very much for joining us, Bernhard.

Bernhard Kuhn: Thank you.

Carolyn Coyne: You can find other episodes of That's Pediatrics on iTunes, Google Play Music, and YouTube. Be sure to subscribe to keep up with new content. Leave a review and tell us what other topics you'd like our experts to cover. Thank you for listening.