PEDIAPOD APRIL 2022 TRANSCRIPT

Geoff Marsh
Hello and welcome to Pediapod for April 2022. This month an integrated analysis of miRNA-mRNA interaction in pediatric dilated cardiomyopathy.

Dilated cardiomyopathy or 'DCM' is a rare but serious condition of children and often progresses to heart failure. The outcomes for children with DCM are poor, with 50% of pediatric patients dying or needing a heart transplant within five years of diagnosis. Whilst more is known about the etiology of adult DCM, the pediatric disease is more idiopathic and enjoys no effective therapeutics. In this episode, we meet Carmen Sucharov, a professor at the University of Colorado Anschutz campus and the director of the Pediatric Cardiology Research Laboratories. She and her team have been studying the regulation of microRNAs and their putative target genes in the pediatric DCM heart that may contribute to the distinctive phenotype of this disease in children.

Carmen Sucharov
So in dilated cardiomyopathy, pediatric or adult, the heart muscle becomes thin and can't efficiently pump blood into the circulation. So the heart cannot meet the needs of the body.

Geoff Marsh
How common and how serious is it for children?

Carmen Sucharov
It's serious but it's uncommon. In adults, it's about one in 100 have heart failure. In children, dilated cardiomyopathy is about one in 100,000 children. And worldwide there's about 100,000 children with dilated cardiomyopathy. So in adults, the incidence is about one in 100, in children it's about one in 100,000. When we think of dilated cardiomyopathy and heart failure, we think of older adults. Although it can affect younger adults it's common in the older population. In kids that progression to disease can be very fast and about 40% of the kids may either need a transplant or they may die two years after diagnosis. So although it's rare when it's diagnosed, the progression can be very fast.

Geoff Marsh
So what are the current therapies used to treat DCM?

Carmen Sucharov
That's part of the problem. There are no therapies with strong evidence that they work. So this is different to the adult population. There have been multiple clinical trials in adults with dilated cardiomyopathy or heart failure. So in adults, the most common cause is ischemic heart disease or heart attack, but there have been multiple trials in the adult population. And that is because the numbers are higher. And because clinical trials are led by drug companies, that's how it is, governments don't have the money to lead clinical trials. Because of that, kids have been treated like

adults. So there has been an assumption that if this drug works for adults, it's going to work for kids. So there haven't been a lot of trials that have looked at the effects of a drugs in the pediatric population.

Geoff Marsh
And in terms of the biological causes of DCM, is it fair to say that we have a better handle on the adult situation because they've got a lifetime of medical histories, and we can tell where it's maybe coming from, but that's not the case for children. That's much more elusive?

Carmen Sucharov
So that's an interesting question. If we look just at dilated cardiomyopathy in adults, in the last few years people that work with genetic causes of disease have been able to show that several cases of dilated cardiomyopathy in adults are due to mutations in this one protein called titin. In kids, titin mutations don't seem to be a major player in developing the disease. Mutations still play a role- there are familial cases. Pediatric dilated cardiomyopathy can also be due to viral infection so myocarditis can be a cause of the cardiomyopathy. But the majority of the cases in kids are still idiopathic. So we don't know the causes.

Geoff Marsh
You've done some previous work looking specifically at microRNA expression? Can you just tell us briefly about that previous work and what it told you?

Carmen Sucharov
Sure. We have a pretty big tissue bank at the University of Colorado. This was started in the 80s. Every time there's a transplant we have a team of people that go into the operating room, they collect the heart and they bring it back to the lab. So we have over 1200 adult hearts in our tissue bank and over 400 pediatric hearts. Those include also control hearts, hearts of people that have died and their hearts could not be used for transplant for whatever reason. So we can compare what is happening in the diseased heart to what's happening in the control heart. What changes are there in the kids in terms of levels of proteins, levels of RNAs, that are unique to the pediatric population. Specific to microRNAs, we were the first group that published changes in microRNA levels in the adult population, both in the dilated cardiomyopathy population and in the ischemic population. And that I think was about 15 years ago. And then subsequent to that, we did the same study in kids. And we wanted to evaluate if microRNAs were changing in kids and adults. We first did an array-based study and array based studies are much more limited and RNA-Seq. And then this last publication was with RNA-Seq which allowed us to look at many more processes, then an array-based one.

Geoff Marsh
So how exactly do you study the differential expression of microRNAs in these children?

Carmen Sucharov
So the way that we do this is we get a small piece of that heart tissue and then you extract the total RNA from that tissue. And then once you have the total RNA there are different methodologies that allow us to study the expression level of the microRNAs. One of them is array-based so there are several little wells, and there are probes in there. So you only detect the microRNAs that are on the

chip. RNA-Seq is different- what you're doing is that you're actually sequencing the RNA that is in that tissue. So by doing that, you were detecting all the microRNAs that are present, you're not limited just to the ones that are present on the chip. And then you'll confirm the findings through RT-PCR. So RT-PCR is a very targeted approach, you choose one microRNA that you found in your study to be interesting. And because RNA-Seq technologies are expensive, in our study we did 10 controls for the RNA-Seq and 20 for the dilated cardiomyopathy patients. For the PCR because it's a very targeted approach and we can choose which microRNAs we are interested in from the RNA-Seq data, we can then expand the number of patients that we are looking at to confirm the RNA-Seq data.

Geoff Marsh
How do you choose the most interesting differentially expressed microRNAs?

Carmen Sucharov
So the first thing we did was we did a cut off and we looked at the top 100 most expressed microRNAs in terms of microRNA numbers. And that is an advantage of the RNA-Seq data, you cannot get that from the arrays, but you can get that from RNA-Seq. So we knew how many copies there were of each of the microRNAs. And we just chose the top 100 of the ones that were most expressed. From that top 100, we looked at the ones that were significantly dysregulated between disease and control.

Geoff Marsh
How do you go from these interesting microRNAs to perhaps what they're doing, what their target genes are and then what pathways might be disrupted?

Carmen Sucharov
So we had also done mRNA sequencing work. So in the same hearts, we had done mRNA-Seq work in the past. So what we were able to do is we used the same tissue, the same hearts that we had microRNA sequencing and the ones that we had mRNA sequencing, and there are software programs that can predict the pairs, MicroRNA- mRNA pairs based on the sequence of the mRNAs. So we use that software to narrow down which mRNAs would be targeted by those top microRNAs. And then we looked at our mRNA sequencing data set to see if that gene A was dysregulated in the pediatric heart. So we were able to really narrow down which mRNA-microRNA pairs were there in the pediatric heart. Of course, this is all predicted. To *prove* that that's the case takes years of study.

Geoff Marsh
So how do you go from that predictive miRNA-mRNA pairing to actually figuring out if they are having an effect in the pediatric DCM heart.

Carmen Sucharov
So what we try to do in our approach is to go from the characteristics of the heart itself, so the phenotypic characteristics of those hearts, to try to understand what these pathways are telling us. For example, different to adults, pediatric patients don't have an increase in cell size, myocyte hypertrophy. The myocytes when they are stressed, they don't divide anymore, they're terminally differentiated so they get bigger. That's very common in adults. We have shown in the past that myocyte hypertrophy is not a characteristic of the pediatric heart so their hearts don't get bigger. Fibrosis is also very minimal in

the pediatric heart which is different to the adult population. Others have shown that inflammation seems to be suppressed. So we can look at those as pathways of interest because we are seeing those differences. So we do see hypertrophy signaling, we see pathways related to inflammatory responses. So that is what makes the whole story interesting is that we are seeing characteristics of the heart that are then being seen at the level of these microRNAs and mRNAs.

Geoff Marsh
How could you use that information then to create drug therapies that are specifically targeted to that pediatric population?

Carmen Sucharov
So if hypertrophy is not a major player in the disease, or if inflammation is not a major player, we shouldn't be targeting those processes. So one of the things we are doing right now is that we are pursuing one of these microRNAs to understand their biological consequences. And we have an interest in the lab on mitochondrial function. So another thing we can do is when we get those fresh hearts is that we can study the mitochondrial function from those fresh hearts. One of these microRNAs seems to impair mitochondrial function. So we know that the pediatric hearts have mitochondrial dysfunction and now we know that one of these microRNAs negatively affects mitochondrial function. So we are pursuing that. So that's the next step, out of all those microRNAs that we're seeing dysregulated in kids, what are the biological processes that they are affecting? So we are doing these studies now in an animal model and in a cell culture model. So we use primary cardiomyocytes from rats, and in a human IPS cardiomyocyte model. So we're using all three to try to understand the effect of this microRNA.

Geoff Marsh 12:07
It sounds like you've got several years of functional studies ahead of you?

Carmen Sucharov
Yeah, that's the problem. We have several years of functional study, always. We're very interested in circulating microRNAs and their effect on the cells. So we are finishing some analysis now on how kids can recover from heart failure. And we are finding that circulating microRNAs can tell us, even though these kids all have heart disease in the acute setting, so they all come into the hospital, it's an acute heart failure, at that time point, the circulating microRNAs - the ones in the serum - can predict which ones are going to recover from heart failure and which ones are going to need to be transplanted.

Geoff Marsh
Great. Well we look forward to more Pediatric Research submissions, I suppose.