Podcast - Speaking of Mol Bio - Season 1 Episode 5

Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a new podcast series about molecular biology and its trending applications in life science. I'm Steve Lewis.

Dr. Gabriel Alves 00:19
And I am Dr. Gabriel Alves.

Steve Lewis 00:21
In our first season of Speaking of Mol Bio, we're focusing on conversations on four exciting application areas: CRISPR cell engineering, multi-omics, exosomes, and single cell analysis. And today we're continuing our exploration of exosomes with Dr. Johan Skog.

Dr. Gabriel Alves 00:40
Johan is the Chief Scientific Officer of Exosome Diagnostics, which has been a pioneer in the field of exosomes since he founded the company in 2008. ExosomeDx developed the world's first liquid biopsy tests using exosomes and remain on the forefront of exploration in the industry. We started our conversation with Johan by asking him about his research before founding Exosome Diagnostics, and how it led to the work he does today.

Dr. Johan Skog 01:13
I was studying tumor stem cells at the time. And when you do that, you look at them through the microscope, of course, look at the health of them. And what was really fascinating to me at the time was that tumor stem cells, they do a lot of blebbing, which means that there's like large vesicles coming off from these, these cells that you can actually see in a light microscope. I was not aware of any studies that have properly characterized them and I got sort of curious what these large blebs really were. So, I took my cells to the electron microscopy facility at that time and spent some time looking at these larger vesicles that I could see in the light microscope, but at the time, I found something else. And that was that these cells were covered by a smaller vesicle type that I hadn't seen, of course, in the light microscope, because they're too small for that. And that was even more fascinating. So, you have this spectrum of vesicles released from these primary tumor cells. Very small ones that were very abundant and larger ones that were a little bit less abundant, but they were still clearly there. Of course, you do what you do, so you start to read up trying to learn about this, what these could be, and found at the time that these smaller vesicles have been studied for decades. And, and they, they were, of course, they were called exosomes. Decades of publications highlighted that they had some interesting proteins and lipids, but stated that they did not contain nucleic acids like RNA. And my work at the time included some RNA profiling of these tumor stem cells. So, I wanted to really make sure that these vesicles did not have RNA. I'm a virologist, and these smaller vesicles, they really look like viruses. They're the same size as a virus particle. So, I had adopted a protocol to isolate viruses from these—from on these exosomes and was really fortunate that the first time I tried actually there was quite a lot of RNA present in these samples. I knew that these tumors, brain cancer tumors, carry certain types of mutations. And if I could find these mutations on RNA, inside these exosomes, that would open up a whole new field where you can actually utilize these exosomes for diagnostics. And we showed that

these exosomes carry not only RNA, a wide diversity of RNA, but they also carry tumor specific mutations. So, we figured if we could take a sample like a plasma sample, that is, and isolate the exosomes from there and find these tumors specific mutations, then we've proven that really cancer-specific exosomes make it into the circulation, and that they are stable enough for us to use them as biomarkers.

Dr. Gabriel Alves 04:52
That is an awesome, great story. What molecular biology techniques do you use to profile those exosomes?

Dr. Johan Skog 05:00
One reason that exosomal RNA was so elusive for decades and people did not really study it was that there's fairly low amounts of RNA in an exosome sample. And you have to be very careful. When you have a low yield RNA preparation where there could be RNase activity, you have to take extreme care of either removing the RNase activity or washing it off before you actually lyse them. And so you can have very efficient ways of the RNA isolation. After that, of course, you reverse transcribe the RNA and you use PCR-based methods usually to assess targeted content. So, you can look at specific genes. And really, what made exosomes so fascinating here is that everyone thinks that RNA is unstable, so you can't really work with them as biomarkers. But when they are released from the cell, they're tightly packaged up into these RNA-protein complexes inside these exosomes and they're released into the biofluid. They remain very stable. We've shown that if they're stored properly in freezers, we can isolate good quality or RNA to do global RNA profiling on samples, that's been stored for decades.

Steve Lewis 06:25
Do you mind kind of just describing the process from a cellular perspective, going from the DNA to what results in the extracellular vesicles?

Dr. Johan Skog 06:37
So, RNA is typically seen as the messenger of information. So, if a cancer cell or another cell wants to create an action, it produces—it starts production of an RNA that has a specific function. So, when that RNA is increased in that cell, the exosomes are being formed inside the cytoplasm, and they capture the RNA content in that cell. And then when that RNA content is released into the bio-fluid, we can harvest that information. And we can track things longitudinally. We can see—you take a sample at time point zero. And compare that too, for example, after treatment with a specific therapeutic. You can see if that molecule, that specific RNA molecule, increased or decreased over time, which makes it possible for you to use this for stratification, responders versus non-responders, etc. So, there's a lot of basic biology and basic research that can be done with these exosomes.

Dr. Gabriel Alves 07:55
One of our previous guests, Jim West, he mentioned that one of the biggest challenges in exosome, or extracellular vesicle research is reproducibility. Do you agree with Jim?

Dr. Johan Skog 08:06

I don't. Because this is typically a problem in academic research when you're using methods that are not reproducible. At Exosome Diagnostics, we've developed platforms for extremely robust exosome isolations for decades, and we have methods where urine exosomal RNA or plasma exosomal RNA can be isolated with high throughput and in an extremely reproducible way, so that you get exactly the same result every time. As you may know, in 2016, we launched the world's first exosome-based diagnostic test, which is the EPI prostate cancer test. Of course, we wouldn't be able to launch a diagnostic unless there was a very reproducible platform for the exosome RNA isolation. And we've shown that over dozens of operators and years of isolations that it's very robust, and has about the same variability as you would expect from a standard qPCR variability. And that's quite remarkable.

Steve Lewis 09:32
Pulling on the thread of diagnostics, when you're thinking about exosomes as biomarker candidates, is there any difference related to selectivity or specificity compared to maybe protein-based biomarkers?

Dr. Johan Skog 09:50
It depends on what you're looking at. As you can imagine with exosomal RNA, it depends on the mark—specific marker. Where there are certain RNAs that could have similar features as the actual protein that it codes for. But when it comes to specificity, for example, mutations are very specific to cancers. And you can find these mutated RNA sequences, and you can harvest those, and those are very specific in itself. But what's really interesting here is that while that gives you a good specificity that there's a cancer, the field of diagnostics in oncology is also moving towards understanding outcome. Where in the case for prostate cancer, we know today that the majority of prostate cancers are fairly indolent and it's actually better off not finding them and diagnosing them in the first place. One of the big drawbacks or criticisms of PSA-based testing has been that when PSA-based testing for prostate cancer was introduced, we started to find a whole lot more prostate cancers, but the reduction in deaths was not as dramatic as you would have hoped. There's a lot of side effects of treating indolent cancers and removing the prostate from men that have a cancer that actually would not kill them in their lifetime. So, we want to understand, not only do you have cancer, but do you have a cancer that is treatment worthy. If you think about mutations today, as far as I know, there are no mutations in the prostate cancer that will tell you if you have that type of cancer that will actually kill you. But when you combine sort of the exosomal RNA here, and that RNA signature can actually differentiate and find and rule out actually patients that have high-grade prostate cancer. We're going to see much more of that in the future, where mutations need to be combined with RNA transcriptome to better type these type of cancers and understand not only if they're there, but how should it be treated, and should it be treated. And I think there's just this wealth of information that these exosomal RNA components can contribute.

Dr. Gabriel Alves 12:42
One of the things that I've been reading, and it's part of my class that I teach at Western is about the microbiome. And bacteria also release exosomes, and one of the diseases that is very—affects a lot of people is the Crohn's disease, and it has to do with—a lot of people, when they get diagnosed with Crohn's, they change their diet, changing their microbiome to a, I would say, a healthier microbiome. Do you think that exosomes play a role in that inflammatory, chronic inflammatory disease?

Dr. Johan Skog 13:20

That is a great question. I haven't seen any larger studies on that topic. However, precisely as you outline also, bacteria release a type of vesicle to the surrounding and we've shown that bacterial vesicles can be isolated also from stool samples. That opens up this wonderful opportunity, I think, for gastrointestinal diseases and inflammatory diseases. Because your gut health depends on the epithelial cells in your gut, your immune cells in your gut, as well as the bacteria or the microbiome in your gut. All those three components release vesicles into your stool and they can be harvested. So, it's going to be interesting in the future to see if we can actually combine that trifecta; looking at vesicle profiles that are being released from the microbiome as well as signals coming from the epithelial cells and the immune cells in the mucosa to study those diseases, and understand even if there are certain therapeutic targets that can be identified from that type of research.

Steve Lewis 14:46
We hope you're enjoying this episode of Speaking of Mol Bio. We wanted to take a moment to tell you about the Invitrogen School of Molecular Biology. It's a great educational hub for molecular biology with rich and reliable technical content designed for new and experienced molecular biologists alike. Check it out today at thermofisher.com/ismb. And now back to our conversation.

For aggressive tumors like glioblastoma, you mentioned you had worked on, how much does time series analysis related to exosomes, as a tumor develops, how much is that studied, either by yourself or in the field?

Dr. Johan Skog 15:36
So, the time series is a very important component, I think, where it can give us more information about where the tumor is going. And especially important in studies on minimal residual disease, etc. When you do a baseline sample, you can find certain RNA targets, mutations, or transcriptome profiles, and they can be used to stratify patients at that initial decision point. However, if you're able to do longitudinal samples from each patient, you can actually open up a new wealth of information because you're actually looking at the directional change of the tumor over time. Tumors change very quickly. You do need to have real time information about what's going on inside these tumors and the liquid biopsy approach opens up those opportunities. Since you have this wealth of information on the RNA transcriptome, you have the entire RNA transcriptome there, you can start to find biomarkers that you may not find at the baseline. Think about it this way, when you're trying to find a biomarker, that is finding the level of protein or an RNA or whatnot, compared to a reference gene. The biomarkers need to be increased or decreased compared to that reference gene. And it needs to be within a fixed interval. However, cancer is different, and we are different, we are all genetically different. And finding a biomarker that behaves exactly the same at that baseline across every tumor, every tumor type, and every ethnicity, it's very challenging. However, when you have repeat testing, you can actually let that patient's baseline sample be the reference, even if you start with a biomarker at a different level, you can look at the directional change over time, is it increasing or decreasing? And that opens up opportunities for biomarkers that are not useful at baseline but they become very useful for that particular patient over time.

Dr. Gabriel Alves 18:08

If a test is sensitive enough, or precise enough, doesn't that take away the need of purification steps on exosome identification?

Dr. Johan Skog 18:21
Yeah, so, I assume that you're thinking if something in blood is so specific for the tumor, you can just take the blood itself and precipitate everything and you'll find it. And that may be the case for some things. However, for mutations, for example, we've shown that if you do that, you may actually overwhelm the system. If you take up a plasma sample. A lot of people think that plasma, while itself free of course, but all RNA there is probably not in a vesicle exosome. However, the majority of RNA in plasma is from platelets. And we showed in a publication, for example, that if you take the platelet fraction, there can be millions of copies of the BRAF molecule, but that BRAF mRNA, is all wild type and doesn't share that actual mutation. And if you're including that in your sample, yes, you get more BRAF molecules out, but the copy numbers of that mutated BRAF that you're actually trying to identify is just drowning in that sea of wild type and the methods that we have available will not be able to find them. Because even, for example, the reverse transcription process has errors, right? And that baseline error rate would be higher than that allelic frequency that you see in that sample. So, the same is true for RNA sequencing. We published a study earlier this year actually, together with NASA, where we were profiling patients with higher intracranial pressure. So, to better understand the molecular mechanism and what's going on, in these in these individuals, they, of course, cannot take a biopsy of the brain of living astronauts. So, the second best would be to do a molecular profiling of the exosomal sample. But they also asked that same question, can we just take a blood sample? Where we withdraw the sample into a PAXgene tube that preserves all of the RNA that is there? And then you just sequence it. Will you be able to see these molecular changes in the patients with higher intracranial pressure versus the ones that don’t have it? And is there a difference if you do that process over that exosomal isolation? And the answer was a resounding yes, it does matter. You get more RNA in your sample if you take a whole blood sample, of course, because it has all in the cells and all of that, but the RNA is coming from irrelevant processes, as the majority of the reads you're getting are from globins and things that you're not really interested in. Whereas the exosomal sample, or the EV sample, had much more distinct, signature, relevant targets in these patient populations. So, that was a really intriguing study that we actually did, where we show that more is not always better.

Dr. Gabriel Alves 22:00
Right. And if you know, for example, your target, you're looking for specific RNA with, we talk a lot about molecular biology products in this podcast, oligos, for example. Wouldn’t if you have a specific oligo if you're looking for that specific RNA or that several oligos, look for a larger population of RNAs? Do you see the field going that way? Having those different types of genomics involved?

Dr. Johan Skog 22:36
Absolutely. So, for some diagnostic applications you want to go broad, and you want to look at transcriptome with next-generation sequencing, etc., but in specific diagnostic applications, you may just want to go and look for a specific target. And it can be protein, it can be an RNA, it can be a mutation, and you have to adopt your analytical method that best suits the purpose. PCR can sometimes be more beneficial than sequencing because it's quicker, it's in some cases more sensitive, it's cheaper, and has a lot of those diagnostic advantages. So, you have to tailor your downstream

analytic to really, what's the purpose, what are we actually doing? And I think this applies not only to the analytical methods to assess the biomarker, but this applies also to the upstream exosome isolation. I know a lot of efforts have been to try to standardize and find this exosome-isolation method that can be used for everything. We took a very different approach early on at Exosome Diagnostics because we quickly learned that there's no single optimal exosome-isolation method for any application because the method needs to be tailored to your analytical targets so that the method for exosome isolation we use for a protein-profiling project may not be the same as the method we use for an exosomal-RNA analysis out of urine, for example. We have several different approaches that we take depending on what the analytical readout is in the exosome. Whereas if it's exosomal protein, or exosomal RNA, or DNA, or a combination of cell-free DNA and exosomal RNA. That actually leads us into a very important part of the liquid biopsy concept where I think in the past a lot of people have been laser focused on a certain application. So, they work on exosomal RNA only, or cell-free DNA only, which is another type of liquid biopsy, or circulating tumor cells. And I think diseases like cancer, unfortunately, requires all our tools in the toolbox to be combined to really find the optimal process. So, we've found for some applications, we actually combine the biomarker on the cell-free DNA plus the exosomal RNA. And we're finding more value in that combination over just looking at one analytical parameter at the time. And I think that multi-analyte approach is going to be critical in the future as we move into more sophisticated diagnostic methods.

Dr. Gabriel Alves 25:57
Where do you see the exosome, or extracellular vesicle research going in the next five years?

Dr. Johan Skog 26:03
The research field is booming, right? The number of publications are skyrocketing across the field in a variety of different applications. And here at Exosome Diagnostics, we have this catalyst where we actually have the world's first exosome-based test that's been now utilized to help patients and we're just trying to translate that now into the other applications. And that is a mix of medical oncology applications as well as normal oncology areas. And I think we'll see a huge boom on actual targeted, clinically useful diagnostic tests coming from the exosome field.

Steve Lewis 26:50
For somebody who might be interested in getting into the field, what are some of the other areas of research you would recommend, or maybe some lessons learned along the way for you in your career?

Dr. Johan Skog 27:03
The field has matured a lot in the last few years. We have a very active international society for exosome research, for example, that can be helpful for new researchers getting into the field. But also look at some of the success stories, and we're trying to replicate some other success stories in other fields of exosomal research. The future is very bright for the EV (extracellular vesicle) RNA field in general.

Steve Lewis 27:35

Great. And as we come to close for the interview, is there anything that we may not have touched on that you might want to share about whether it's related to your work or any information you might want to share with our listeners out there.

Dr. Johan Skog 27:52
One thing that we haven't touched upon this single vesicle analysis that is also something that is growing in the exosome field. It's important to realize when we talk about the exosome material, I have highlighted that you can do global RNA profiling and transcriptome profiling and look at all these interesting proteins, etc. But that is not true for single vesicles. Right? So, these exosomes are very small, they're the size of a virus particle that can normally package somewhere around 10 kb nucleic acids, right? So, the entire transcriptome is not present in every vesicle, you have only maybe a few RNA targets per vesicle, but when we're doing our exosome isolation, we get millions, if not billions, of these exosomes out, and that combined exosome batch will give you that global profiling ability. We just have to set our expectations right when we're doing it, when we're looking at individual vesicles that we may not see the same diversity as we can do from more of a batch exosome perspective.

Dr. Gabriel Alves 29:20
What are some of the advantages of looking at one single vesicle and not the batch?

Dr. Johan Skog 29:26
I think a lot of work has gone into trying to figure out exactly what molecules are associated with single vesicles. When we look at, for example, exosomes released from a cell culture that is overexpressing an EGFR on the surface. If that EGFR receptor molecule is there, you can find them on the surface of the exosome as well so you can use that to fish out that particular exosome. However, because of the size of this virus particle, or size similar to a virus particle, we know that the entire proteome of the cell cannot be represented in a single vesicle. So, maybe only 20–30% of the exosomes released from that cell type will carry that molecule on the surface. Right? So, to do single vesicle and single analyte readout has that limitation that most vesicles from that cell type will not have that particular target. So, you'll have to adopt the way where you can actually look at single vesicles but at a very high throughput similar to what we do with cell profiling in a flow cytometer. Right? You're looking at single cells and the content on single cells with a flow cytometer. However, you're rarely making conclusions based on single cells. It makes conclusions based on thousands, if not hundreds of thousands, of cells in mapping the surface content on many, many cells to make a conclusion.

Steve Lewis 31:25
That was Dr. Johan Skog, founder and chief scientific officer of Exosome Diagnostics. If you're interested in hearing even more of today's conversation, you can view the extended video version of this interview by visiting the URL in the episode notes, and if you've enjoyed our interview with Johan, please consider leaving us a review wherever you're listening to this podcast. It helps other people find our show. This episode was produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Thanks for listening.