

Speaker 1:

Welcome to the Eye On the Cure Podcast, the podcast about winning the fight against retinal disease from the Foundation Fighting Blindness.

Ben Shaberman:

Welcome, everyone, to another episode of the Eye on the Cure Podcast. I am Ben Shaberman, Senior Director of Scientific Outreach at the Foundation Fighting Blindness. For this episode, I'm very excited to have with me Dr. Tom Reh. He is Professor of Biological Structure at the University of Washington and also a scientific co-founder of the new biotech called Nayan Therapeutics, that's developing cell-based or cell-associated therapies for inherited retinal diseases. Nayan is also part of the Foundation Fighting Blindness Retinal Degeneration Fund. What I'm excited about is, Tom has been involved in what we call regenerative medicine or often stem cell development for now decades. But his work is really on the cutting edge of regeneration, to try to restore vision for people with inherited retinal diseases, especially those with the most advanced disease. He's really involved in some cool projects, which we'll talk about. So, welcome to the podcast, Tom.

Dr. Tom Reh:

Thank you. Good to be here.

Ben Shaberman:

And again, Tom is coming to us from Seattle, University of Washington. And before we get started talking about some of these emerging therapies, can you tell us, Tom, how you got involved in regenerative medicine? What sparked your interest in this field?

Dr. Tom Reh:

Yeah, it's really very long standing. When I was first learning about science and really back when I was an undergraduate at the university, I first learned about the kind of amazing properties that some animals have for replacing tissues and parts of their body after injury. For example, salamanders and newts, these amphibians that are a little like frogs, that more of your listeners might know about. These animals can repair their body in ways that humans can't do. They can replace their limbs, for example, if they're damaged or lost, the tail can regenerate. But amazingly parts of their nervous system can regenerate, so spinal injury, if they have a spinal cord injury, they can repair that by axon regeneration. And, indeed, if they have damage to the eye, for example, even if the entire retina, the light sensing part of your eye, that entire retina is removed from these animals, it can be replaced.

So, I learned about this and I thought this would be a really fascinating thing to study. And I looked around at that time, and this was back in the '70s amazingly, and there really wasn't anybody studying this anymore. Some of this work had been done classically, but there were no active labs studying eye regeneration at that time. But I thought, "Well, there was a new kind of resurgence of developmental biology," at that time, and particularly neuro-development, development of the nervous system. So, I went to work for a neuro-development lab and during development, of course, all these same tissues like the eye and the brain are generated in the first place. They're made from stem cells during our development, that while we're a fetus and they all have to get generated in the first place. Regeneration that happens in older, in mature animals like frogs, is really a recapitulation, a do-over of regeneration, of normal development.

So, I thought, "Well, I'll study normal development, because maybe that will hold some of the clues for how some of these animals are able to restart this developmental program as adults, and thereby regenerate their tissues." I went to work for a developmental biology lab, and this developmental biology lab eventually got also interested in regeneration, partly through the work I was doing. And so, we began to study regeneration and development, and really try to learn how these things are similar and whether there are some aspects that are different.

So, even since I was a grad student, I've been studying at the interface between developmental biology and regeneration. My work has been going back and forth, looking at how organisms develop their brains and spinal cords and retinas, and then how we can learn from developmental biology to try and use those technologies and ideas and molecular understandings. How we can apply those to understand regeneration, and stimulate regeneration in mammals like ourselves.

Ben Shaberman:

That's really interesting that you're leveraging our knowledge of just basic developmental biology to try to help people regenerate retinal tissue. And, what I think is so compelling about your work is that with most regenerative therapies that are in development, researchers are taking cells from another source and trying to put them in the retina, so these cells can develop into photo-receptors or whatever cells we need, and then to have those cells integrate. Those are two really challenging steps, but your way of doing things, at least one of your projects you're getting a grant from the Foundation for, is to have the retina grow its own new photo-receptors, which is pretty compelling. Can you tell us a little more about that?

Dr. Tom Reh:

Sure. Yeah. A number of years ago, it was in the late '90s, Andy Fischer joined by lab, was a postdoc. And we were trying to understand more about regeneration and how it naturally occurs in animals like fish and frogs. And, one of the things that was known at that time was that, the fish retina in particular, will regenerate from some kind of source within the retina itself.

At that time, it was clear that if you injured the fish retina, there would be some process that allowed that retina to heal in a way like if we made a ... Let's say you get a cut, a paper cut or something and you slip with a knife or whatever, but your finger will completely heal up again and maybe it'll leave a little scar if it's a particularly bad cut. But in general, it'll heal up, and you won't see any evidence of that injury. How does it do that? Well, it didn't need a transplant from some cells who grew up in a dish. It actually has a source that we would call an endogenous source of repair. And the endogenous source of repair in the skin, it can be thought of as something ... So the fish as something akin to that in its retina. It has something like what helps our skin repair, which is essentially a source of stem-like cells that will allow all the different types of tissues to get regenerated again in the skin.

Particularly, in the hair follicle, this stem cells sits at the base of the hair follicle and near the edge of the hair follicle, and allows new hairs to grow. And then that stem-like cell can actually repair and contribute to the repair of injury after a wound. The retina of the fish has a cell like this called the Müller glia cell. And after an injury, that Müller glia cell will go ahead and replace all the other neurons within that retina of the fish.

Now, it wasn't known what cell was doing this when we started work on this, but Andy Fisher found in the bird retina that when he damaged the retina, a glial cell ... These are support cells in your nervous system that are called glia, and a certain type of this glial cell called a Müller glia was able to make new neurons in the bird retina. Once we knew that, Dan Goldman and others who study fish retina, were

able to find that the same glial cell was making new neurons in the fish retina. And so although before that, we had known the fish retina made new neurons after injury and repaired itself like our skin does, nobody knew what cell that came from.

But after the work in the bird, we now knew that that came from the Müller glia cell. We were able to look for those same cells and this same process in mammals and mice that are more like us. And what we found was that although those same Muller glia cells exist in the mice, they weren't able to reinitiate this process of neurogenesis, so they weren't able to repair. Instead, they just form a scar. So it would be as if after your skin injury, it didn't make new skin with new hair follicles and all the different cells that are normally in your skin. It just made a fibrotic scar. We know that can sometimes happen after a really bad injury to your skin, but that's what always happens in the mouse. It basically forms a scar, rather than forms a healing type replacement of the lost cells.

You have these two processes that exist. One is replacing the cells in a regenerative way, and the other is making a scar that really can't contribute to restoring the function. It's kind of like a balance that happens after wounds. The scar is generally driven by a more inflammatory process even in the skin, and the regenerative process where you make new cells, essentially recapitulating your developmental biology, that process is really associated with a lower level of inflammation. Part of what we've learned is that the fish undergo a less inflammatory process after injury, and so their glial cells will make new neurons like new photo-receptors and that will restore this function. Whereas in mammals, the inflammatory process dominates, and instead of making new neurons that will help, they just make new glia and really what we call reactive glia, and those reactive glia don't restore any function.

Essentially what our task is, is to shift this inflammatory reactive glial process that happens in mammals, to the more regenerative, restorative process that happens in fish and frogs. And that's what we've set out to do, now that we understand the nature of the problem. We find that if we can trigger the restorative, regenerative response in the mammalian retina, by turning on some of these developmental transcription factors that are normally present when your eye develops, but are never re-initiated after injury, but in fish those are the key factors that allow this restorative process. We've learned to kind of trick the mouse retina by turning on these developmental transcription factors. Then essentially, these glia behave like they were back in the fetal retina, when the restorative, regenerative processes dominated, and the inflammatory processes were not established yet in our eyes. We've kind of moved back in time to a time when the cells were being generated and restorative processes were the ones that dominated the injury.

Ben Shaberman:

That's so cool. It sounds almost like magic that you can empower a retina to regenerate itself. So, two questions. How do you turn on those transcription factors to make a mouse retina or a mammalian retina regenerate? How much more work do you have to do until you think you can move this into a clinical trial?

Dr. Tom Reh:

One of the things about science is that it makes an enormous amount of really hard work and typically, a great deal of luck, look like magic. But it's a lot of hard work, it's a lot of failures, and it's also a lot of luck. I have to say not everything we do works, and a lot of times when something works, we don't really know. We can't predict which thing is going to work and which thing isn't going to work. But I think by hard work and luck combined, we often are able to make things that look like magic and that's what we hope to do.

I would say that the way we do this right now is we use technology that was developed for gene therapy. We started our prospects of trying to regenerate this retina by turning these developmental transcription vectors on. We started this project using gene therapy vectors called Lentiviruses or AAVs. Many of the Foundation members were aware of AAVs, because it's how some of these gene therapy technologies are able to be delivered to patients.

We can use those same vectors remarkably. We can target them just to the glia cells, using other technologies that allow specific cell targeting. So, instead of targeting these to the rods or the cones, we target these Müller glia with these transcription factors, using an AAV approach similar to what would be used for gene therapy. And then we can sort of trick these Müller glia to express the transcription factors that'll allow us to reprogram them back in time to when they were neuro-progenitors. And those glia in a sense think, "Hey, now we should just make new cones. There aren't any cones around, that we should make new rods, or we should make new ganglion cells," any of the cells that were lost.

And we find we have to, if we use one of these transcription factors, which we used to call Mash, or it's now called ASCL1, but it's a gene, it's a transcription factor that's normally important for retinal development. And we find if we put this into an AAV vector or an antiviral vector when we do our first initial screen, when we put this into viral vectors and we infect the glial cells with it, the glial cells get moved back to the stage when they were a progenitor cell. And so they start to make new neurons. And then we can direct which type of neurons they make by adding a second transcription factor, one that will direct them to either the ganglia cell fate, or the amacrine cell fate, or the bipolar cell fate. And so we have a second layer of control where we first nudge them back into their developmental program, and then we direct them down a particular pathway to where we make just the neurons that we want to make.

That's our ultimate goal. And we're learning now to use these other transcription vectors to control which cell types are made by the glial cells, the glia derived progenitors. So, that's how we're working this right now. We got real lucky where two of these transcription factors are, when we put them together, we're able to make nearly 80% of the glial cells have got infected into retina neurons. So it's remarkably efficient when we combine two transcription factors together and into one AAV. We hope to be able to deliver this to the Müller cells and Müller glia cells, and then direct those cells with efficiency into these new neurons.

So far we've done this in mice. We're working now to move this to monkeys. We think monkeys will be really important to use, but we don't want to do very many monkeys. So we're trying to do this on the minimum number of animals, but what we do feel is that since they have a phobia and people have a phobia, it will be necessary to try this in these animals with a phobia.

So luckily we've been able to obtain Müller glia from animals that others are using in their investigations. And so when those investigators are finished using the retina, we can obtain those retinas and we can use the glia from those retina to test whether the same factors work in mice, will work in the monkey. And we're working on that right now. This year, we'll be studying whether those glia derived from the monkeys will work, in the same approach that we've tested in mice. And then we also have the ability to get new Müller glia from human retinal organoids. And so we grow a little organoids in dishes. This is a remarkable technology that's been developed over the last 15 years, 20 years where embryonic stem cells can be directed into retina, and we can get human Müller glia now for the first time. So we're growing human Müller glia, and we're putting these same transcription factors into human Müller glia, to see if the same things will work in human.

So, most of the stuff is done in a culture dish. So obviously, we can't test these initially in humans, but once we know that it'll work in the culture dish in monkey Müller glia, human Müller glia, and we're doing these tests in right now, this year from foundation grants, to see if the same thing that we've

found in mice can be tested in human and monkeys. And then hopefully, if all goes well by next summer, we hope to do the first tests in vivo, in monkey Müller glia, in live animals to see if the same approach using the AAV and using these vectors will stimulate making new neurons in those animals. And if so, then I think we can begin to attract the kind of investment that will make, allow us to move the technology into people, but that's going to come after we know that it works in the monkey, in vivo. So probably if you were to interview me next year at this time, hopefully, I can tell you whether the whole thing works in monkeys and in humans. And I think, that's our goal for this year.

Ben Shaberman:

Okay. So if we can wait a year on this highly cutting edge approach to regeneration, we should have a much better idea of whether this has the potential to work in humans well. And all I can say is, I know you have a lot of listeners out there, we have a lot of listeners that are rooting for you. So good luck moving forward.

So, I wanted to spend a couple of minutes talking about another project. So everything you've talked about thus far, the regeneration is funded directly by the foundation, but this other project is part of the Nayan Therapeutics, the small biotech's portfolio. And this is something being funded through our RD fund, our venture philanthropy fund. And I wanted to explain to listeners, as I think many of our listeners know, that in a condition like retinitis pigmentosa, the condition begins in rod photo-receptors. And ultimately, the vision loss that happens in RP, happens because of the degeneration of rods. But once the rods go, the cones will follow. There's this symbiotic relationship between the rods and the cones. And with that knowledge, you're developing a molecule to really, if I understand correctly, change the identity of rods so they don't degenerate, so you can save your cones. Do I have that correct? Can you tell us more about that?

Dr. Tom Reh:

Yeah, so again, this goes back to developmental biology and over the years we've learned that, the rods in the cones come from a common precursor. And there are molecules, similar kinds of molecules called transcription factors that allow, that act like little switches and molecular switches. And they allow switching, just like the Müller glial are able to be switched to a progenitor by turning on the transcription factor ASCL1, the rods develop in the first place by turning on a transcription factor called NRL. And NRL then turns on a second transcription factor, NR2E3, and it's this NRL/NR2E3 combination that switches these cells into rods, allows them to become rods. If those genes aren't present, if they don't get expressed in a cell, then that cell becomes a cone. This rods-cone precursor goes off in the cone direction.

Now you can then, this bi-potential precursor exists to make both rods and cones during your development. But it turns out that you need the NRL and the NR2E3 throughout your whole life to keep cells, to keep them being rods. A lot of times we think, "Oh, after development, cells are stable in their identity," but it turns out that's not always true. And in the case of rods and cones, if you get rid of the NRL, even in adult mice, those cells start to become more like cones. Now what's interesting is since a lot of retinitis pigmentosa is due to mutations in genes that are only expressed in rods, we thought, "Well, if we made those rods into cones, then they wouldn't express the genes that caused them to die." So, that was really the basis of this approach. And we looked for genes and molecules that would affect the expression of the NRL and the NR2E3, and we found some molecules that would affect the expression of the genes in this NRL pathway, these NRL transcription factors.

And sure enough, in mice, when we cause these to be down-regulated or inhibited in their function, we found that the rods shifted to a more cone-like state. Not becoming completely cones, but becoming a lot more like cones. And they became enough like cones that they no longer died from mutations that affected rods. So we were able to decrease the expression of rod genes enough so that the rod cells were no longer susceptible to those mutations and to leading to their death. And by keeping the rod-like cells, you can call them rones or cods, or there's some immediate like cell, but by keeping them or by keeping them around, then their normal cones also survived.

So that's the therapy that we're developing right now in Nayan, and we have moved this. The goal is to move this from mice, to try and test it in animals with larger eyes, to know if this could work in an animal with a bigger eye, say a pig or a dog. And then also to try and test this in monkey cells and human cells, to see if the same thing works. So those are the things we're working with in Nayan right now. And yeah, hopefully, we'll have some news soon to tell the Foundation about.

Ben Shaberman:

That's great. And what I want to confirm and emphasize if I am correct here. For both this approach and the earlier approach that you talked about, the regenerative approach and the change of identity, these are designed to work independent of whatever mutated gene is causing the patient's disease. So this should hopefully work for a pretty large swath of people affected by RP, and potentially other conditions where the-

Dr. Tom Reh:

That's right. These are not dependent on the particular mutation. It's never easy to say, "It'll work for everybody," right? I mean, we all know that there are different stages of the disease and there are certainly complications that arise from the disease, that also limit how widely applicable they can be. But I would say the following thing. For both of these approaches, we'll start out looking at a very specific population. Of course, because it's really, I think your odds of getting a real improvement and a real repair are going to depend on a lot of these other factors. But I would say if we can see a positive result in anybody, then these will be much more widely applicable than some of the existing gene specific approaches. So, because they have the potential to be reapplied more widely, that's not to say we won't start with a subset of the available treatable population. But once we see a positive signal in those people, yes, there would be no reason why it couldn't be applied more broadly.

Ben Shaberman:

Very cool. Very cool. Tom, I really appreciate the time you've taken to talk about these two different approaches. And as I said before, these are really cutting edge methods for potentially saving and restoring vision, because you're not really introducing new cells into the retina, which while that's still a new thing, relatively speaking, it's kind of the old school way of using regenerative medicine. And I think what's also cool is you've learned so much about developmental biology along the way in evaluating these approaches, that even if they're not successful, you've done wonders for imparting knowledge to just the general field about how the retina can be regenerated or might be regenerated for other scientists working in this field. So, thank you.

I want to remind our listeners, if you have a question, you can send it to [podcast@fightingblindness.org](mailto:podcast@fightingblindness.org). Again, that's [podcast@fightingblindness.org](mailto:podcast@fightingblindness.org). Tom, thanks again for your time. You got a lot of people out there, including me, rooting for your success, and keep up the great work. And again, thank you for taking time out of your busy day to do this podcast and let us all know about your great research.

Dr. Tom Reh:

Well, thanks so much for giving me this opportunity. And again, thanks so much to the foundation for the support of the current work, and all the support you've given my lab over the years. Thanks.

Ben Shaberman:

Great. Thank you. And thank you as always, listeners, for tuning in and stay tuned for the next episode of Eye on the Cure. See you later.

Speaker 1:

This has been Eye on the Cure. To help us win the fight, please donate at [foundationfightingblindness.org](http://foundationfightingblindness.org).