Neuropathic pain research

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[00:00:05] **Dr. Benjamin Greenberg:** We're going to be transitioning now for the afternoon to talk about updates and research. And there's a lot of different areas to discuss and every year when we're going through this, we have to make difficult decisions about what topics to include. And so, we have four research updates today that we want to do. The first one is going to be an update in neuropathic pain research. And there was a question very early on in the day I apologize, I don't remember exactly who asked it, but it was about banding pain and what causes the banding pain. And that's what this talk is going to partially address.

[00:00:41] The basis of neuropathic pain. And we are delighted to have Doctor Ferreira with us from the University of Texas, Dallas. So, Diana received her master's and training while in Portugal and then went to the UK to the University of Sheffield to get a PhD in Pain Neuroscience. You didn't think there could be a PhD in Pain Neuroscience but there is. Then came here to Dallas. Why on earth would somebody leave Sheffield come to Dallas? Well, it turns out there is an international epicenter of neuropathic pain research with our colleagues in Doctor Ted Price's lab at the University of Texas Dallas, asking the very fundamental question biologically, why do some people develop neuropathic pain after different injuries versus others? And so, we'll get an update on that research today and I'm happy to turn things over to Diana.

[00:01:40] **Dr. Diana Tavares:** Well, thank you so much for having me here. So, as it was mentioned, I am not a medical doctor. So, it is a little intimidating after all the questions about pain management to come here to talk about pain. But I hope that if you can take only one thing from this talk is that we scientists are doing our best to try to find novel targets that have the least side effects. And I will also mention that we don't always have the opportunity to talk to patients or even to talk to clinicians sometimes. So, this has been an incredible opportunity. I've been learning so much.

[00:02:25] And I will also say that if you have any questions at any time, please feel free to ask. So, the sensations of touch, proprioception, temperature, and pain help us sense the world. And they are, I think I touched something. Yes, and these sensations, they help us sense the world and they are transmitted by the system of nerves fibers. And so, this system transmits the external stimuli from the periphery to first to the spinal cord and then to the brain where these sensations are perceived.

[00:03:16] So, what we are particularly interested in is I'm going to try to use this. So, what we are particularly trying to understand is this sensory neurons that are located next to the spinal column. So, sensory neurons are a very special type of cell with axons extending for a large distance. So, they can reach the target structure that they are innervating the skin. But the cell body or where the nucleus or where all the things that keep the cell alive are located in this thing here that we call Dorsal Root Ganglia or DRG, which is right outside of the spinal cord.

[00:04:04] And up until recently, we were characterizing the sensory neurons in three main categories. So, we had the myelin large diameter neurons that are called a-beta fibers. So, those are the neurons that innervate the skin and are responsible for transmitting light touch. So, when somebody touches our hand and we can feel that. So, these are the neurons that get activated. Then we also have proprioceptors which innervate the muscles and other structures to give us a sense of where our limbs are located in space.

[00:04:41] Then we have lightly myelinated medium-diameter neurons which can detect both harmful stimuli but also light touch. And finally, we have the unmyelinated small-diameter C fibers. And those are the special type of neurons that are specialized to detect potentially damaging or damaging stimuli. So, they can transmit painful stimuli. And obviously, in our research, we are particularly interested in this nociceptors. They are also called nociceptors. And but we know that in the tissue, there are not only neurons, there are other cells that can interact with the neurons and these other cells can release mediators that can then activate the neurons.

[00:05:33] So, I'm also going to talk a little bit about these interactions between immune cells, glial cells, and the neurons. And so, pain in a normal circumstance can alert us for danger. But when it becomes chronic or when it is the result of a nerve damage, it can have a huge impact in patients' lives as we have been hearing today. And in fact, chronic pain is estimated to affect over 50 million people in the US. And is one of the most common reasons that adults seek medical care and has been linked to restrictions, mobility and just an overall reduced quality of life.

[00:06:18] It's also associated as we heard today with other conditions and the current available treatments, as we also heard are also either ineffective or have unacceptable side effects. And this you may know the most of the studies that are conducted before we reach a clinical trial are done in animal models, which are not always representative of humans. And in fact, we actually have not had a completely molecular characterization of human sensory neurons. So, we don't know exactly what molecules are present in these neurons.

[00:07:05] So, the work in our lab focuses on characterizing these sensory neurons that I mentioned before. And I will just here this slide to talk a little bit about the technologies that we are using. So, we are particularly interested in the RNA. So, as you probably know in our cells, we have DNA, which is like our genome or it has all the information that will make our cells make us. And then this DNA is transcribed into RNA and then later on can be translated into protein. And we are focusing on RNA. And I will also use the terms RNA or genes or a transcriptome, which are kind of interchangeable. And the transcriptome is the study of the genes of the RNAs that are present in a tissue.

[00:08:04] So, there are three technologies that are going to be talking about today and I'll just briefly mention that. So, all RNA sequencing is basically you take the whole tissue that you are interested in studying. For example, the sensory neurons, we would take the Dorsal Root Ganglia. And we can characterize the RNAs and the genes that are present in that tissue, but we don't know from what cells they are coming from. So, we can also use single-cell RNA sequencing which in this case is very similar approach. But this time we can profile individual cells. So, we know from which cell the RNAs are coming from.

[00:08:45] And then we also use spatial transcriptomics, which basically means that we can take in consideration the spatial position of the different cells. So, we can see if certain immune cells are closer to neurons and we can see exactly what genes or what RNAs are present in those cells. And so, our lab focused mostly on using human tissues. And we obtain our tissues from the collaboration with Southwest Transplant Alliance, which is organ donor organization here in Dallas. We're very grateful for organ donors that can also donate their other tissues for research.

[00:09:31] And we can recover a variety of tissues including the DRGs, the spinal cord and we can perform those technologies that I mentioned to characterize the molecularly characterize those tissues. And we can also do like cell culture and then try to test certain compounds and see how they perform. And here, just out of curiosity, this is an image of a human DRG here and then how it is connected to the spinal cord. And I'm just going to skip here. So, if you remember a few slides ago I mentioned that we could classify the neurons in three main categories.

[00:10:19] What you can see here is that once you actually profile the molecules the RNAs are present in these sensory neurons, there are actually many more sub-types. They are not just three. And for example, in the A-beta fibers. So, the fibers that can transmit light touch, you can see here on this graph that there are three sub-types of them that we identify based on these different markers that we were able to identify. And then for the C fibers which are the neurons that transmit pain, the nociceptors, we actually identify six sub-types. And I'll just highlight a couple which are here on the left side of this graph.

[00:11:03] We identify based on genes that these are the neurons that transmit cold sensation. So, the cold or cooling sensation is transmitted by neurons that have this receptor called topiramate. And then another interesting one is this one here called Peritogen receptor which are the itch nociceptor. So, they transmit the sensation of itch. And so, we were able to characterize all these various sub-types. And just to go in a little detail into, what do I mean by molecularly characterizing these sensory neurons?

[00:11:47] What you can see here is that we have a list of all the genes. And this is a very like selected top genes that are expressed in each nociceptor that I was mentioning. And what this graph shows is that the larger the circle, the more cells express that gene and the darker the color, the higher is the expression. So, what we see here is that this gene called SCN11A which the protein is called NAV1.9. So, patients with the mutation in this gene have a congenital insensitivity to pain or have a partial loss of pain sensation. But reports in the literature also show that these patients that do not have pain, have an intense itch phenotype.

[00:12:38] And so, we think that because this gene is regulated in this particular sub-type, we think that mechanisms associated with this enrichment might explain this phenotype. But obviously, we are trying to validate this. As I mentioned, also a lot of the studies for identifying novel targets are conducted in animal models, particularly mice. And even though there are some similarities, mice are not smaller humans. And so, there are also some differences that we are able to identify by conducting this study. And I'll just highlight particularly this gene called TRPV1. So, this is the gene and then also the neuronal sub-type that gets activated when we eat something spicy gives that hot sensation.

[00:13:35] Actually the investigator that discovered this channel, this receptor was awarded the Nobel Prize I believe two years ago. What you can see here. These graphs are similar to the ones I've showed before. That again, the larger the circle, the more cells express them. On the left you have the human expression. So, you can see it is expressed in more than one sub-type. When you look here in the mouse, it is particularly high in this one. And actually, there was a drug that was being in clinical trials targeting this receptor for the treatment of pain that has failed because the side effects that was causing, which was a higher body temperature.

[00:14:25] And at that time, we didn't have this molecular characterization of human tissue. So, we couldn't know how the drug was going to act. One thing that I want to mention in the interest of time is that there are other cells present in this tissue. So, it's not just neurons. So, how are we taking into account the interactions between other cells that might release inflammatory mediators and activating the neurons? So, what you can see here and I'll just mention briefly is a technique that labels the cells present in the tissues. So, here in pink, on the top, we have neurons.

[00:15:11] So, neurons are these large cells and then in white you have the T cells. So, this is a marker for T cells. So, you can see that in humans immune cells are very closely located to human sensory neurons. And then here on the right, you have similar image but from mouse DRG. So, what you can see, first of all is that the neurons in the mice are much smaller. But also that at basal level without inducing pain in this animal, they don't have these immune cells present. So, again, it's just like another difference that we identify that may suggest why sometimes animal models might not be as representative of humans.

[00:16:04] And one of our goals in our work is to create a liken receptor. So, interact a map and investigate how these interactions change in not just in the in peripheral neuropathies but in other conditions. Because here in blue, you can see these dots represents neurons. And then here is a representative image we have kind of an orange color CD4, which is a marker for macrophages, which is another type of immune cell. So, you can see that there are a lot of immune cells present in human and very close to the neurons.

[00:16:44] In here on the right, you have a list of many interactions that exist between immune cells and neurons. And our goal is to take into account their location and see and eventually design better models to test drugs for the treatment of pain. And now in the last few minutes, I'm just going to briefly talk about some clinical cases that we've also been working on. And I have not worked directly with neuro immune disorders but the places that I'm going to be talking about have neuropathy so like nerve damage and can be similar to other conditions.

[00:17:38] So, in this case, here is a collaboration with MD Anderson Cancer Center in Houston. Where patients that have tumors on their spine sometimes this tumor can compress the nerve roots and cause pain. And during the removal of the tumor, it is medically recommended to also remove the DRG. So, if you remember that's where the sensory neurons are located. And what we've done is again characterize these tissues. And what we observed is that here on the left is all the ligands that were identified in these tissues.

[00:18:22] And here on this right in gray, you have the receptors present in the neurons. Again suggesting that there is in this painful condition, there is high interaction between other cell types such as immune cells and neurons. And lastly, I will mention about peripheral neuropathy which you may have heard about. But it is one of the most prevalent complications of diabetes and symptoms include pain, abnormal sensations, and also numbness.

[00:18:57] So, it depends a lot on the patient. And one of the clinical characteristics of diabetic neuropathy is that it affects the axon. So, it affects the nerve fibers that innervate the hands and the feet. And this is for collaboration again with the Southwest Transplant Alliance and also with UT Southwestern. And here, this is a very kind of complicated and colorful slide. But basically, the idea is that we have these tissues from these patients that have a history of diabetic neuropathy. And we can identify what cells are present in these tissues.

[00:19:43] As you can see, there are nociceptors, neurons, and also immune cells. And highlighting the immune cells we can see that if you see here, we can see that there are macrophages and T cells mostly within the immune cell populations. And interestingly the control. So, these were recovered from organ donors that did not have a history of neuropathy. You can see that there is an increase in this population here. So, if you

notice the number of each dot corresponds to a cell. So, in the diabetic neuropathy or DPN you can see an increase in these cells. And then in this cluster here, in this blue circle, you can see there is a decrease compared to the controls. So, this suggests that there are changes in what immune cells are recruited closer to the neurons in certain neuropathies.

[00:20:42] We are working on to identify what are those specific populations and what ligands they are releasing so that then we can test and see if we can prevent that. Here is just a few more details about some of those genes. I'll just highlight for the interest of time. This one here, CD163. This gene it is a marker for macrophages. You can see that macrophages are particularly increased in the diabetic neuropathy condition. When we look at the genes that are changed overall, you can see that there are many pathways that are involved in.

[00:21:26] But mostly they are involved in the release of cytokines and inflammatory mediators that then can sensitize the neurons and activate them to transmit the painful sensation. We also study the peripheral nerves. So, again, as I said, here is the nerves that innervate our feet. Sural nerve and tibial nerve particularly. And even in this samples, we can see that there are different levels of damage. So, here is an example of a nerve that has normal axonal disease. So, it has a normal number of nerve fibers present. And this is, you can tell this by this dark blue round shapes here which correspond to the myelin sheet around the nerves.

[00:22:19] And then in neuropathy, you can see that there is severe in some cases, even severe axonal loss. And again, we can use the technologies that I mentioned and characterize the cells that are present in there. We can also look at the molecules that are expressed. So, here, you can see there is an increase, so this brownish and amber yellow colors, you can see there is an increase in immune cell markers in the nerves that had increase in a severe nerve damage.

[00:22:59] And then in conclusion, I'll just say that so transcriptomics are the study of the genes that are present in the tissues. These technologies allow us to molecularly characterize the tissues involved in pain. And we are using these technologies to characterize potential interactions between neurons and other cells present in the tissues. And we identify species differences in the transcriptoming on neurons and immune cells.

[00:23:32] And as I mentioned, we are hoping that these data sets can be a key resource to facilitate a translation of targets that have been discovered in animal models and can also be used to improve existing models. And with that, I'll just thank, so this is our team at UT Dallas and our collaborators. Again, we're very thankful to all the organ donors and the patients that contribute to our research because without them, we cannot do this. And if you have any questions, I'll be happy to thank them.

[00:24:26] **Audience Member 1:** I don't have the proprioception and I lack sensation of touch but do have the light touch. So, you were talking about RNA and those things. Are y'all just concerned with developing medications to help treat neuropathic pain or even potentially restoring like the proprioceptor cells that would help restore a sensation of touch?

[00:24:52] **Dr. Diana Tavares:** Yes, that's a great question. So, we obviously are mostly focused on nociceptors and developing novel targets for the treatment of pain. But I think these technologies that I described can also help in studying how proprioception is lost in people and patients that don't have it. And so I think that could be also useful to apply these technologies to the study of that.

[00:25:29] **Audience Member 1:** That would be really supportive, thank you.

[00:25:30] **Dr. Diana Tavares:** Yeah, I think that it's, I will say that I don't think it is impossible. I do think there's a lot of work to be done though but yes, I think hopefully one day.