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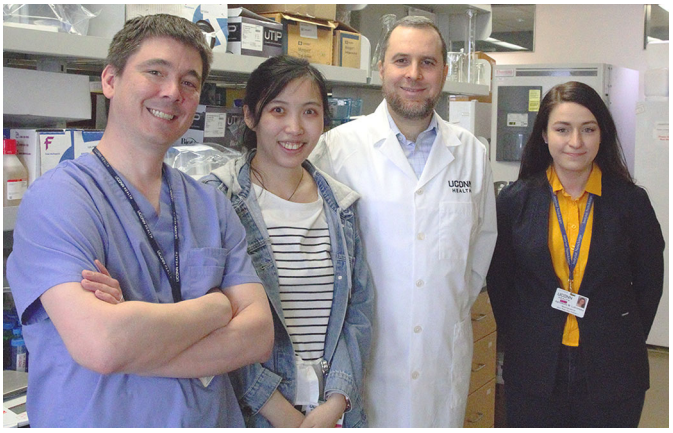
INTERVIEW

The people behind the papers – Jian Xing, Agnieszka Lukomska, Bruce Rheaume and Ephraim Trakhtenberg

The neurons of our central nervous system (CNS) are unable to regenerate axons following injury, which can result in permanent damage. A new paper in Development demonstrates that newly formed oligodendrocytes contribute to axon regeneration inhibition. To hear more about the story, we caught up with first authors, Jian Xing, Agnieszka Lukomska and Bruce Rheaume, and the corresponding author Ephraim Trakhtenberg, Assistant Professor at the University of Connecticut (UConn) School of Medicine.



Left to right: Bruce Rheaume, Jian Xing, Dr Ephraim Trakhtenberg and Agnieszka Lukomska.

Ephraim, can you give us your scientific biography and the questions your lab is trying to answer?

ET: The 'big questions' fascinate me, and they are what motivated me to pursue graduate studies, which eventually led me to focus on neuroregeneration research. I think that this research direction is analogous to reverse engineering: if you succeed in regenerating injured neural circuits and restoring function, this would indicate that you are on the right track toward understating how at least some parts of the brain work – albeit still far from answering the related 'big question'!

I developed an interest in molecular neuroscience during my studies of psychology. After graduation, I enrolled in a master's program in biological sciences at Stanford, where I was mentored by neuroscientists Robert Sapolsky and Luis de Lecea. From there, I continued to a PhD program in neuroscience at the University of Miami School of Medicine in Jeffrey Goldberg's lab (currently at Stanford). Then, I did a postdoctoral fellowship in neuroscience at Harvard Medical School in Larry Benowitz's lab. I started my own lab at UConn Health in 2016, where I built a research program focused on neuroregeneration, which has led to a number of exciting discoveries already.

Jian, Agnieszka and Bruce, how did you come to work in Ephraim's lab and what drives your research today?

JX: I had the opportunity to work with Ephraim during my master's thesis. During that time, I gained valuable experience and knowledge in neuroscience and axon regeneration. Ephraim played a crucial role in helping me to pursue my research interests and further my academic career. He supported me in my application to join the PhD program at the UConn School of Medicine, where I have continued to work on research related to axon regeneration.

My research today is driven by a desire to understand the mechanisms underlying axon regeneration and to develop effective therapies for neuronal injuries and disorders. By furthering our understanding of these mechanisms, we can move closer to the development of novel treatments to improve the quality of life for individuals with neurological injuries and disorders.

AL: I took a postdoctoral position in Ephraim's lab in December 2019. My main motivation for joining his team was the numerous, fascinating research projects on novel approaches to central nervous system regeneration, which has always been at the center of my scientific interests. Today, my research is driven primarily by scientific curiosity, the desire to explore the molecular mechanisms underlying the development of the nervous system, and an attempt to apply this knowledge to the advancement of regenerative medicine.

BR: I met Ephraim at UConn in the summer of 2016, while I was on a rotation in the Neuroscience department before I began my medical training for the MD/PhD program. His excitement and ambition for his projects, focused around CNS axonal regeneration, were contagious. I saw that his lab was using the latest technologies and innovative approaches to answer questions that have long been unanswered in science and medicine, while also aiming to translate the outcomes into therapeutics, and I was excited to be a part of it. I worked in the lab when I could find time during my first and second years of medical school and then did a rotation in the lab when I started the graduate phase of the program. There, I was able to hone the skills I had while also learning several new, powerful skills and techniques. I joined the lab during my rotation, and I defended my thesis in 2022. The drivers of my research are curiosity and hope. I have always been curious about nature, and it is this curiosity that has steered me toward a career in science. The hope I have is for individuals who have debilitating diseases for which there are currently no curative therapies. Channeling my curiosity in order to develop life-changing therapies that help sick people, while also directly caring for them through clinical medicine, is exactly what I had imagined I would be doing as a physician-scientist.

Before your work, what was known about stimulating axon regeneration following injury?

JX, AL, BR & ET: In the mammalian CNS, long-distance projection axons do not regenerate spontaneously after injury, even if the neurons themselves have survived the injury. This is despite the fact that, to various extents, compensatory neuroplasticity can

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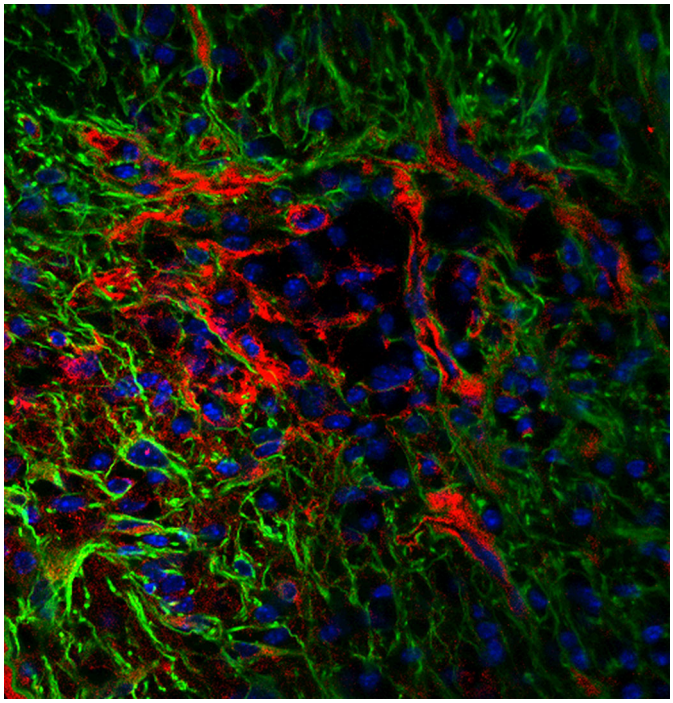

Post-injury-born immature oligodendrocytes (labelled for CC1, red) repopulate the injury site and are surrounded by reactive astrocytes (labelled for GFAP, green).

help recover some of the lost functions. Among the most prominent examples of this clinical problem are paralysis after spinal cord injury and blindness after optic nerve damage. Decades of research discovered a number of mechanisms underlying the failure of axon regeneration in the CNS. Some of these mechanisms involved a number of genes and signaling molecules within the neurons themselves that regulate axonal growth during normal development; targeting these directly in the surviving neurons was found to stimulate some axon regeneration. Other, non-neuronal factors, such as the glial scar and inflammation, involved multiple molecules and different cell types that mostly inhibited axon regeneration, although some growth factors stimulated modest regeneration. Later, researchers started combining the different approaches in the hope that co-targeting several factors would lead to a more robust axon regeneration. Although axon regeneration was improved, the majority of the axons stalled growth before reaching their targets in the circuitry. This motivated us to search for other mechanisms that may contribute to stalling the growth of axons that were experimentally stimulated to regenerate.

Can you give us the key results of the paper in a paragraph?

JX, AL, BR & ET: Myelin debris in and around the glial scar is a major component of the inhibitory environment that blocks axon regrowth. We found that, while all of the oligodendrocytes in the optic nerve injury site die by 1 day post-injury, the resulting myelin debris is cleared by immune cells in the injury site 2 weeks later (although beyond the injury site, new myelin debris will appear later as severed axons undergo Wallerian degeneration). We then found that, as the myelin debris is cleared, new oligodendrocytes are born from the progenitor cells (that were known to be present there) and repopulate the injury site. Then, using scRNA-seq, we found that these post-injury-born immature oligodendrocytes expressed axon growth-inhibitory molecules of a similar composition to those presented by the myelin debris. Thus, as the myelin debris is cleared, new oligodendrocytes appear and present on their surface similar axon growth-inhibitory molecules as the myelin debris had done previously. That is why we believe that axon regeneration stimulated by a treatment may stall as newly born oligodendrocytes start interacting with the axons in an attempt to prematurely myelinate them while they are still growing, before these axons reach their targets. We also think that the surviving mature oligodendrocytes located far from the injury site along the path of axon regeneration may further contribute to inhibition of regeneration.

> **"Axon regeneration stimulated by a treatment may stall as newly born oligodendrocytes start interacting with the axons in an attempt to prematurely myelinate them"**

Were you surprised that oligodendrocytes could inhibit axon regeneration?

JX, AL, BR & ET: We knew that, during normal developmental axon growth, oligodendrocytes do not appear until after the axons have reached their postsynaptic targets, and that, in addition to improving conductivity through myelination, they also stabilize axons, including by preventing promiscuous sprouting. That is why we thought that if oligodendrocytes could interact with an axon while it is growing, as occurs in mature CNS during experimental axon regeneration, it may also stall the growth. The surprise was that we were correct.

What are the implications of your research for stem cell therapies?

JX, AL, BR & ET: Stem cells could secrete growth factors that could promote survival of injured neurons as well as axon regeneration. If these stem cells are pre-differentiated to generate neurons, then studies show that such neurons may form 'bridges' between the disconnected circuits within the spinal cord. However, this approach does not work in the optic nerve. Instead, researchers have developed stem cells that can give rise to new retinal ganglion neurons, which after transplantation will, hopefully, integrate into the retinal circuitry, survive long term and regenerate axons that reach their targets. However, if the transplanted stem cells are pre-differentiated to generate oligodendrocytes and used for treating lesions that involve transection of CNS axons that now need to regenerate, then it would be better to transplant such stem cells after the axons have already reached their post-synaptic targets. Otherwise, such stem cell therapy may interfere with the concurrent axon regeneration therapy.

You recently had another paper accepted in Development that focuses on the axon regeneration response to Pten knockdown. How do the two papers intersect?

JX, AL, BR & ET: Aside from the non-neuronal factors involved in blocking the growth of regenerating axons, CNS neurons themselves lose their ability to grow axons after they fully mature. Therefore, a number of approaches targeted molecules within the injured neurons in an attempt to reactivate their ability to grow axons. One such approach involved the inactivation of a tumor suppressor molecule called Pten. Most axons stimulated to regenerate via this approach stalled after only short-distance growth, and for an unknown reason only a few types of neurons responded by regenerating long-distance axons. We discovered

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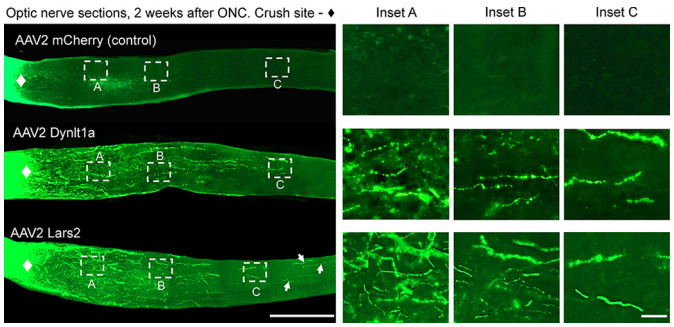

Dynlt1a and Lars2: previously unreported axon-regenerating factors. Regenerating axons (labelled for CTB, green) are present beyond the injury site in the optic nerves treated with Dynlt1a or Lars2, but not in mCherry control.

that the subtypes that were able to regenerate long-distance axons had deviated less from their embryonic state as they matured than the other subtypes had. Retaining features of the embryonic cell state primed these neuronal subtypes to respond to the treatment by partially de-differentiating and reinstating more features of embryonic cell state, so that they were able to regenerate long-distance axons. We then found two molecules in these neuronal types, which were affected by the treatment, and discovered that targeting these molecules also directly stimulated axon regeneration. Even long-distance regenerating axons still commonly stall their growth, but when we co-treated with a toxin that reduced emergence of post-injury born oligodendrocytes, we found that the length of axon regeneration stimulated by inactivation of Pten molecule was increased. Thus, future studies will need to continue co-targeting molecules within injured neurons, in order to stimulate their axon growth mechanisms, and concurrently target non-neuronal factors that block axonal regrowth.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

JX, AL, BR & ET: There were several striking realizations, for example: (1) the fact that some types of neurons in the adult CNS (far from the two neurogenic regions) retained features of the embryonic state; (2) that Lars2, a previously unreported factor that we discovered is downstream of Pten inhibition and promotes axon regeneration on its own, is required for translation of mitochondrial DNA-encoded genes more than any other molecule of its class, and thus could be a limiting factor for axonal regeneration; and (3) that as myelin debris is cleared, new oligodendrocytes appear and present on their surface similar axon growth-inhibitory molecules as the myelin debris did, so that this aspect of the inhibitory environment is continuously preserved by these two mechanisms.

And what about the flipside: any moments of frustration or despair?

JX, AL, BR & ET: Of course, but we focus only on the positive.

What is next for you after this paper?

JX: I'm in the last year of my PhD. After completing it, my next goal is to pursue a computational biology-related position in biotech companies where I can apply my knowledge and skills to tackle complex biological problems. I am passionate about using computational methods to gain insights into biological systems and develop innovative solutions to medical problems. I am interested in leveraging my knowledge and skills in computational biology to conduct cutting-edge research and contribute to the development of new treatments. I am particularly interested in biotech companies that focus on developing treatments for neurological disorders. I hope I can make contributions to better understand the underlying mechanisms of the diseases and develop more-effective treatments with my research experience and my familiarity with a range of techniques and technologies.

AL: The next step is, of course, the finalization of the remaining projects conducted in the Neuroregeneration Lab. I am looking for a broad approach to the problem of regeneration of the nervous system after damage, taking into account extrinsic and intrinsic factors, with the use of solutions successively published by us. Moreover, after completing all ongoing projects, I intend to continue to develop the field of regenerative medicine. I hope that one day I will be able to effectively implement the best of the established scientific solutions into pharmacological and clinical practice.

BR: As a physician-scientist, I plan to continue to pursue my passion for basic discovery and clinical medicine. I currently have a clinical case study manuscript under review, and I am drafting another case study manuscript and clinical review. In the Neuroregeneration Lab we also have several projects that we are wrapping up and a book chapter that Ephraim and I are currently drafting, and I look forward to presenting all of these works to the scientific and medical communities in the coming months. From a long-term perspective, I plan to eventually become involved in clinical trials; as an example, I hope to help bring the therapeutic targets we discovered in the Neuroregeneration Lab to the clinical phase of development. Overall, I will continue to work in any and all realms of discovery that align with my training and offer potential benefits for patients.

Ephraim, where will this story take your lab next?

ET: We will be following up on these and on our other recently published and yet unpublished discoveries, with the ultimate goal of developing an approach that could be translated to clinical relevancy and help blind people (who suffered optic nerve damage) to see again and paralyzed people (who suffered spinal cord damage) to walk again.

Finally, let's move outside the lab – what do you like to do in your spare time?

ET: Raising my daughters, spending time with family and friends, traveling, ping-pong, swimming and thinking about the 'big questions'.

JX: In my spare time, I enjoy hiking with my dog and exploring new trails in the area. It's a great way for me to get some exercise and clear my mind after spending hours in the lab. I also enjoy reading books on a variety of topics, both scientific and non-scientific. I find it to be a great way to relax and learn new things outside my field of research.

AL: I try to lead a balanced active lifestyle, so I spend my spare time actively in the gym or in nature. I also enjoy traveling and getting to know the cultures of other countries.

BR: I most enjoy spending time with my wife and son and our extended families. I also enjoy traveling, snowboarding, golfing and flying airplanes.

Reference

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