Laidlaw Scholars Program
Research Proposal

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Research Focus: Regulation of Runx3 expression in early proprioceptor development

Proposed Start Date: June 15th, 2021

Introduction:

Somatosensation underlies how we perceive and interact with our surroundings. Diversification of sensory neuron subtypes enables the distinction among various stimuli. Having interned at Dr. Joriene De Nooij's laboratory as a high school student, I am interested in delving deeper into proprioceptor development. Proprioceptors are sensory neurons that sense limb position within space and are important for motor control (Lallemend & Ernfors, 2012). Runt-related transcription factor 3 (Runx3) is critical for proprioceptor development, but the regulation of Runx3 expression is unclear (Lallemend & Ernfors, 2012). Thus, I propose a two-part study consisting of a literature study and data analysis component to study the mechanisms of Runx3 induction and maintenance within the proprioceptor lineage. Through the Laidlaw Scholars Program, I aim to apply knowledge and research skills gained from the classroom and the lab to an original independent project, ultimately developing a set of testable hypotheses and conclusions. On a broader scale, I hope to contribute to the elucidation of the mechanisms of sensory neuron development. In turn, this project will inform neurogenesis studies and have potential clinical applications for approaching ataxias and peripheral neuropathy disease modelling and treatment.

Research Question:

Cell bodies of sensory neurons are located in dorsal root ganglia (DRG), which flank the spinal cord (Ahimsadasan, 2018). DRG sensory neurons (SNs) serve as central collaterals, extending their axons to targets in the spinal cord and in the periphery, where they receive sensory information and communicate it to the brain and spinal cord (Inoue et al., 2002). SNs are divided into nociceptors (pain), mechanoreceptors (touch), and proprioceptors (limb positioning) (Lallemend & Ernfors, 2012). Each primary subtype is characterized by the expression of a different neurotrophin receptor—tropomyosin receptor kinase (Trk) A, TrkB, and TrkC, respectively—as well as transcription factors Runx1 and Runx3 (Lallemend & Ernfors, 2012). My research proposal will focus on the regulation of Runx3 expression in the context of cell fate specification and commitment to the proprioceptive lineage.

The proprioceptor lineage derives from the interplay between Runx3 and TrkC, the receptor for ligand neurotrophin-3 (Levanon et al., 2002; Kramer et al., 2006). Runx3 plays a critical dual role in proprioceptor development: (1) maintaining TrkC expression and (2) encouraging the proprioceptive fate rather than the mechanoreceptive fate through repression of Shox2, a transcription factor that maintains TrkB expression (Kramer et al., 2006; Abdo et al., 2011). Although Runx3 is essential for driving the proprioceptive phenotype, the mechanisms by which Runx3 expression is induced in and restricted to proprioceptor progenitors are unclear (Kramer et al., 2006; Lallemend & Ernfors, 2012; Faure et al., 2020).

Previous studies have demonstrated that the onset of Runx3 expression occurs after TrkC expression, and that it does not depend on TrkC signalling, implying a TrkC-independent

signalling system for Runx3 induction (Kramer et al., 2006). Moreover, it has been shown that pan-neuronal transcription factor Brn3a is important for Runx3 expression (Dykes et al., 2010). However, as Brn3 transcription factors are expressed in all sensory neurons, the mechanisms by which they help regulate Runx3 expression specifically in proprioceptor progenitors rather than other neuronal classes remain unclear (Dykes et al., 2010).

Thus, my research question is: During the development of dorsal root ganglia sensory neurons, what are the mechanisms that induce, restrict, and maintain Runx3 expression in the $TrkC^+$ proprioceptive neuronal population?

Methodology:

The goal of this proposal is to develop a set of hypotheses that elucidates the mechanisms of Runx3 regulation. This study will be a two-part study consisting of a literature study and preliminary data analysis component.

For the literature study, I will perform a PubMed research study and consult online databases to:

1. Examine what is known about Runx3 expression patterns in both proprioceptor development and other developmental systems in which Runx factors are expressed (e.g. thymocyte development).
2. Identify the signalling pathways in early proprioceptor development that are expected to be active during changes in Runx3 expression.
3. Develop a list of candidate regulatory molecules associated with Runx3 expression such as transcriptional regulators and epigenetic modifiers.

For the data analysis component, I will analyze single-cell RNA sequencing (scRNA-seq) data from developing mouse proprioceptors to provide insight into Runx3's transcriptional induction and maintenance.

Overall Timeline:

As this proposed project is a remote literature and data study, there is no necessary training prior to the start date. During the 6 to 8 week project duration, for the literature study, I aim to complete Methodology Goal 1 within Weeks 1 to 2, Goal 2 within Weeks 3 to 4, and Goal 3 within Weeks 5 to 6; for the data analysis component, I will set weekly goals according to the progress made learning the program R Seurat and knowledge gleaned from the literature component. Altogether, I aim to complete Summer 1 within 6 weeks with a 2-week buffer period for any necessary additional inquiry. Throughout the duration of the research project, Dr. De Nooij, Mr. Gautam Kumar, and I will regularly meet via Zoom. During the first half of the meeting, Dr. De Nooij and I will discuss my progress and establish a plan for the week. During the second half of the meeting, Dr. De Nooij, Mr. Kumar, and I will discuss data analysis using R Seurat. Each Friday, I will send Dr. De Nooij a report of my weekly progress. In the interim, Dr. De Nooij, Mr. Kumar, and I will communicate via Zoom or email as necessary.

This proposal is a remote research study that will be conducted entirely online. Should COVID-19 restrictions be lifted, I hope to test my hypotheses in a wet laboratory setting during the second summer of the Laidlaw Scholars Program.

Interdisciplinary and/or International Focus:

With intersections in the fields of neuroscience, molecular genetics, developmental biology, and bioinformatics, my proposed two-part study takes an interdisciplinary approach to elucidating the regulation of Runx3 expression.

Research Advisor & Collaborators:

I am grateful to be under the mentorship of Dr. Joriene De Nooij of Columbia University's Department of Neurology as well as to receive data analysis tutoring from Mr. Gautam Kumar of The University of Maryland Graduate School.

Dr. De Nooij will (1) provide weekly feedback on my progress, (2) suggest additional research articles and areas for further study, (3) provide scRNA-seq data from early mouse proprioceptors for the data analysis component, and (4) oversee weekly meetings with Mr. Kumar. Data analysis will be performed under the tutelage of Mr. Kumar, a collaborator of the De Nooij Laboratory.

Outcomes:

The final deliverables of this study are a set of hypotheses on the candidate regulatory mechanisms of Runx3 expression developed on the basis of both the literature study and data analysis component. These can be experimentally tested during the second summer of the Laidlaw Scholars Program.

Ethics Review:

This two-part research project is remote and does not require an ethics review.

Works Cited:

Abdo, H., Li, L., Lallemend, F., Bachy, I., Xu, X. J., Rice, F. L., & Ernfors, P. (2011). Dependence on the transcription factor Shox2 for specification of sensory neurons conveying discriminative touch. *European Journal of Neuroscience*, *34*(10), 1529-1541. https://doi.org/10.1111/j.1460-9568.2011.07883.x

Ahimsadasan, N., & Kumar, A. (2018). Neuroanatomy, dorsal root ganglion.

Dykes, I. M., Lanier, J., Eng, S. R., & Turner, E. E. (2010). Brn3a regulates neuronal subtype specification in the trigeminal ganglion by promoting Runx expression during sensory differentiation. *Neural development*, *5*(1), 1-18. https://doi.org/10.1186/1749-8104-5-3

Faure, L., Wang, Y., Kastriti, M. E., Fontanet, P., Cheung, K. K., Petitpré, C., ... & Hadjab, S. (2020). Single cell RNA sequencing identifies early diversity of sensory neurons forming via bi-potential intermediates. *Nature communications*, *11*(1), 1-15. https://doi.org/10.1038/s41467-020-17929-4

Inoue, K. I., Ozaki, S., Shiga, T., Ito, K., Masuda, T., Okado, N., ... & Ito, Y. (2002). Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. *Nature neuroscience*, *5*(10), 946-954. https://doi.org/10.1038/nn925

Kramer, I., Sigrist, M., de Nooij, J. C., Taniuchi, I., Jessell, T. M., & Arber, S. (2006). A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron*, *49*(3), 379-393. https://doi.org/10.1016/j.neuron.2006.01.008

Lallemend, F., & Ernfors, P. (2012). Molecular interactions underlying the specification of sensory neurons. *Trends in neurosciences*, *35*(6), 373-381. https://doi.org/10.1016/j.tins.2012.03.006

Levanon, D., Bettoun, D., Harris‐Cerruti, C., Woolf, E., Negreanu, V., Eilam, R., ... & Groner, Y. (2002). The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. *The EMBO journal*, *21*(13), 3454-3463. https://doi.org/10.1093/emboj/cdf370