Optimization of Single-Cell Dissociation Protocol for Efficient Isolation of Intact Spinal Cord Neurons

Popular science abstract – Hannah Weman

Single-cell sequencing is a technique that allows researchers to collect all information regarding genetic expression from individual cells. This technique can be used to very accurately study e.g. embryonal development, cell diversity and disease. As single-cell sequencing can be used within many research fields, the usage has increased substantially in the last couple of years. Single-cell sequencing can also be used to study diversity of different neuronal populations within the central nervous system and I am especially interested in the neurons that are important for pain and itch. To study the diversity of these neurons, it is important that they remain viable and intact. Therefore, for this master thesis project, I tried to optimize a method for isolating single-cell interneurons and projection neurons involved in pain and itch that would result in a high number of viable and intact neurons that can be used for single-cell sequencing.

When we are exposed to a painful or itchy stimulus, neurons in the affected area are activated. The signal is thereafter transferred to the spinal cord, leading to activation of interneurons and projection neurons for modulation, and further transmission to the brain for interpretation and perception. These interneurons and projection neurons are the main topic of our study since we want to gain more knowledge about the neuronal circuitry and diversity. In the long run this could lead to increased understanding of neuropathic conditions, such as chronic pain and itch. In my project, I evaluated the progress of a single-cell dissociation protocol for interneuron isolation using a genetically engineered mouse model. In this mouse model, interneurons important for itch are visible because they are fluorescent. For the projection neurons, I tried to make them visible by injecting fluorescent dye into different brain areas known to receive signal from projection neurons. By comparing how many interneurons and projection neurons I could isolate with my developed protocol to a commercial method, I could see that my protocol was just as well performing in generating viable and intact single-cells. The protocol will now be used for future projects.

- *Interneurons*: Neurons that have their whole structure within the spinal cord. This means that this kind of neurons do not transmit information to the brain. Rather, they are important for modulating which signals that should be sent to the brain.
- *Projection neurons*: This is a kind of neuron that sends ("projects") information from the spinal cord to the brain for interpretation and perception.

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