Spinal cord organoids add an extra dimension to traditional motor neuron cultures

Since Lancaster et al. (2013) first described the formation of self-organizing cerebral organoids for modeling neurodevelopmental disorders, it became evident that three-dimensional (3D) neural organoid cultures are more superior systems for modeling neurodevelopment and neurodegeneration in human. The use of a spinning bioreactor to grow organoids allows better nutrient absorption and enhances formation of neuroepithelial-like zones, making it a great tool to study neurodevelopment and neurodegeneration. Neural organoids are 3D cell culture systems formed by proliferating, differentiating, migrating and self-organizing pools of neural progenitors. They mimic brain structures in their cell type composition, cytoarchitecture, and to some extent maturity and functionality (Lancaster et al., 2013). Because of these unique properties, neural organoids have been used extensively to study diseases associated with neurodevelopment and neurodegeneration. As neural organoids recapitulate early stages of neurogenesis, neural organoids have been applied to model microcephaly, a neurodevelopment disorder; demonstrating that cerebral organoids from microcephalic patients have fewer proliferator progenitor cells and smaller cell bodies. This shedding light into the underlying mechanism of microcephaly (Lancaster et al., 2013). Forebrain organoids were also used to understand the association between Zika virus infection and the destruction of neural progenitor pools (Garcez et al., 2016). The self-organizing properties of brain organoids allow one to model neuropsychiatric disorders where circuit formation and refinement is impaired, allowing identification of underlying molecular and cellular mechanisms (Mariani et al., 2015).

Organoids have also shown potential in mimicking maturation and late-onset phenotypes that are undetectable in a traditional two-dimensional culture system. Neurenmelain, the adult substantia nigra-specific pigment, was observed in dopaminergic neurons within a midbrain-like organoids and not in two-dimensional cultures, demonstrating that the 3D environment in the organoid aids in the maturation of the neurons (Jo et al., 2016). Studies have also demonstrated that cerebral and midbrain organoids can be used to study Alzheimer’s disease and Parkinson’s disease, respectively. For instance, 3D cell cultures were able to recapitulate extracellular amyloid aggregation, and demonstrate an accumulation of hyperphosphorylated Tau proteins, allowing for the differentiation of different spinal cell types, mimicking the ventral spinal cord (Figure 1). The presence of both branchial and thoracic cell types within the same organoid suggests that cell-cell interaction in a three-dimensional environment plays an important role in allowing the repression of lower brain genes, creating a neural network that more closely resembles that of the in vivo condition. For instance, Quadrato et al. (2017) found through transcriptional profiling of organoid-derived cell types are similar to that of their in vivo counterparts. As an example, Quadrato et al. (2017) found through single cell RNA-seq that human brain photoreceptive organoids comprise an extensive diversity of neural cell types, each containing homogeneous for lumbar markers, which again do not represent the rostro-caudal patterning in the spinal cord.

Other than motor neurons, the spinal cord also consists of other neuronal populations such as the interneurons that provide inhibitory and excitatory signals to the motor neurons, sensory neurons and neurogial. It has been found that these cells play a role in disease pathogenesis and progression in motor neuron diseases. For instance, SMA interneurons have been found to have a smaller soma size and reduced VGLUT1 synapses was observed in the motor neurons of a SMA mouse model, hypothesizing that interneurons may play a role in SMA pathology (Thirumalai et al., 2013). However, in the traditional two-dimensional cultures, spinal interneurons are largely absent and hence not able to mimic the complex cellular interactions that exist between various spinal cell types that contribute to a functional spinal cord unit.

Recently, our group developed an approach to generate a 3D spinal cord organoid from human induced pluripotent stem cells (iPSCs) (Hor et al., 2018). The adoption of the culture system used by Lancaster et al. (2013), we encapsulated retinoic acid-treated and caudalized embryoid bodies within Matrigel droplets that were allowed to expand and grow in spinner flasks. Remarkably, the resultant organoids resemble the ventral spinal cord in multiple ways: First, we are able to derive different spinal cell types including limb-innervating motor neurons, excitatory V2a interneurons, inhibitory Renshaw interneurons and spinal astrocytes in our spinal cord organoids. Second, these organoids were patterned along the rostrocaudal axis, where we observed HOX B4+ branchial and HOXC8+ thoracic spinal cell type, in the absence of exogenous GDF11. This suggests that the 3D microenvironment in these organoids creates the morphogen gradient, allowing for differentiation of different spinal cell types, mimicking the ventral spinal cord (Figure 1). The presence of both branchial and thoracic cell types within the same organoid suggests that cell-cell interaction in a three-dimensional environment plays an important role in allowing the repression of lower brain genes, creating a neural network that more closely resembles that of the in vivo condition. For instance, Quadrato et al. (2017) found through transcriptional profiling of organoid-derived cell types are similar to that of their in vivo counterparts. As an example, Quadrato et al. (2017) found through single cell RNA-seq that human brain photoreceptive organoids comprise an extensive diversity of neural cell types, each containing homogene
Spinal cord organoids can be generated from human-derived iPSCs via small molecules to direct differentiation, forming self-organizing pools of neural progenitors in a three-dimensional spinal environment. Spinal cord organoids can generate a diversity of spinal cell types that are specific to the spinal cord. In addition, the spinal cord organoids pattern closely to the spinal cord rostrocaudal axis and are functionally capable to form neuromuscular junctions, demonstrating its potential in modeling motor neuron diseases. With cell-type heterogeneity arising from the three-dimensional environment, single cell analysis can be useful to provide resolution to cell-type specific changes and uncover underlying disease mechanism on selective motor neuron vulnerability in spinal muscular atrophy and amyotrophic lateral sclerosis. Hence, spinal cord organoids serve as an excellent model to uncover the mysteries underlying selective motor neuron vulnerability in SMA and ALS (Figure 1).

Although spinal cord organoids recapitulate major features of spinal cord development and disease phenotypes, a number of improvements have to be made in order for them to mimic the in vivo spinal cord more closely. Currently, we are unable to generate both ventral and dorsal structures within a single spinal cord organoid. Moving forward, microfluidic devices that can maintain distinct gradients of morphogens over time can overcome this problem (Lim et al., 2019). Ogura et al. (2018) reported the formation of separate dorsal and ventral spinal cord organoids by modulating concentrations of bone morphogenetic protein 4 and Sonic Hedgehog, respectively. These dorsal and ventral spinal cord organoids may be manually fused together to form a functional sensory-motor circuit. Previous evidence indicated that in a fused organoid model, cortical interneurons migrate from medial ganglionic eminence organoids to functionally integrate into cerebral organoids (Xiang et al., 2017). We anticipate that similar strategies to fuse ventral and dorsal spinal cord organoids can assemble the sensory-motor circuitry that is critical for investigation on motor-sensory disorders.

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