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Advanced Methods In Parkinson's Disease Research

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Abstract

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Discussion

Parkinson's disease (PD) is a common neurodegenerative disease resulting in progressive motor and cognitive decline, along with other symptoms. Over 200 years have passed since its discovery, and yet disease-modifying-therapeutic approaches remain elusive, thereby causing millions of patients and carers around the world a significant burden. Standard molecular biology techniques are no longer enough to study this disease, and, therefore, it is important that we as a research community produce clear and reproducible methods that can accelerate research in this field. We have, therefore, created this method collection comprising articles spanning advanced methodologies from *in vitro* to *in vivo* techniques, that can help tackle questions associated with the pathophysiology of PD. This collection presents different *in vitro* and *in vivo* techniques, that will enable researchers to study the pathophysiology of PD and explore therapeutic relief.

Mitochondrial dysfunction has been extensively linked to Parkinson's disease in the literature[1,2,3,4]. This has prompted intensive research on mitochondrial biology, morphology, and function in different PD models. Seahorse technology has been widely used in order to investigate mitochondrial function[1,5]: this technology measures oxygen consumption through several sensors after the sequential addition of different inhibitors or activators of mitochondrial

respiration. *In vivo* use of this technology has been challenging, as this technique was originally designed for cell culture applications. In this method collection, Zhi et al. presented a 24-plex method for the evaluation of mitochondrial respiration in striatal brain slices[6]. In this article, they apply their method to young and aged *Pink1*-/- and wild-type mice and show that basal oxygen consumption is reduced in *Pink1*-/- aged mice. Their method will allow PD researchers to evaluate oxygen consumption in their models, and provide a platform for the *in vivo* evaluation of mitochondrial dysfunction in PD[6]. Likewise, Ciceri et al. show that the morphology of mitochondria can be evaluated in post-mortem mouse tissue sections[7]. Several studies suggest that abnormal mitochondrial morphology correlates with functional dysregulation in the context of PD[1,4,5], and so the evaluation of mitochondrial morphology in such tissue sections will enable the widespread evaluation of mitochondrial health without the use of specific transgenic models.

Dopaminergic neuronal loss in the substantia nigra pars compacta (SNc) remains one of the golden standards for the development of PD rodent models[8]. Likewise, its reduction remains one of the current golden standards for PD therapeutic treatments[8]. The quantification of tyrosine hydroxylase-positive (TH+) neurons is, therefore, an essential technique for translational and pre-clinical research laboratories. Unbiased stereology is the preferred technique for the quantification of TH+ neurons, and it is not only laborious and time-consuming, but also often impossible to access for lower-budget laboratories as it requires the use of expensive proprietary software. In their article, O'Hara et al. describe a step-by-step protocol for the assessment of TH+ neurons that can detect reductions in this neuronal population that are comparable to those produced by unbiased stereology[9]. Finally, they also show that they can detect a reduction in this neuronal population in a *SNCA*-transgenic model[9]. Together this research will enable multiple laboratories to investigate PD models in order to assess the potential of treatments at reducing or increasing this neuronal population.

Induced pluripotent stem cell (iPSC) technology provides unique possibilities for human disease modeling[10]. Differentiation protocols for a plethora of cell types are widely available and used by the research community in order to model disease. This has been especially impactful for the neurodegenerative research community as it cannot readily access biopsy material due to the nature of the tissue involved. While we have learned from iPSC-derived cell cultures, these cultures suffer from significant variability: cross-site reproducibility in iPSC-based molecular experiments is poor, and therefore, quality control and rigorous protocols for the differentiation of iPSC are critical, and finally, collaborative studies are needed to reveal systematic biases to improve reproducibility[11]. This highlights the need for robust protocols with exquisite attention to detail for the development of these cultures and the measurement of their associated phenotypes. In this method collection, Crompton et al. present a protocol in order to reproducibly produce ventral midbrain astrocytes, essential for TH+ neuronal survival[12]. These cells are also intimately associated with the pathophysiology of PD[12,13,14], and, therefore, represent good candidates for the *in vitro* study of disease modifiers. Likewise, Roberts et al. have produced an efficient, robust, and scalable protocol for the phagocytosis of cellular material by iPSC-derived macrophages[15]. Together these two articles will aid the research community by providing robust modeling resources for glial cells in PD, which are too often still discarded as support cells. These

protocols will also provide much-needed attention to the neuroinflammatory aspect of PD.

In conclusion, the biggest unmet need in the field of PD remains the lack of disease-modifying therapy for PD patients, and so, this collection of articles will help the field move forward by providing a solid methodological basis for several highly relevant techniques for the research community.

Disclosures

The author has nothing to disclose.

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