Confronting ALS: understanding multicellular contribution to neurodegeneration : computational analysis and hiPSCs in vitro modelling as a multidisciplinary approach

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Summary in English

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Computational analysis and hiPSCs in vitro modelling as a multidisciplinary approach

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterised by a progressive loss of motor function. While it is known for the eponymous sclerosis of the spinal cord observed upon autopsy, ALS is the result of extratelencephalic Cortico-Spinal Motor Neuron (CSMN) degeneration, connected to gradual loss of cortico-spinal tracts and degeneration of spinal cord motor neurons which results in loss of control over muscles and respiratory failure. It remains unclear why classes of motor neurons are selectively affected by the disease. Although genetics studies of familial ALS have tremendously increased our understanding of this condition, the vast majority of ALS cases are sporadic (90%), occurring without a family history and most often without a known genetic cause. Indeed, whether familial mutations and sporadic insurgence might converge on similar molecular pathways is still unknown and how different cellular subtypes might contribute to these changes remain a subject of investigation. The fundamental inaccessibility of brain tissue and the uncertainty surrounding the disturbances triggered by the disease in specific cell types poses two main hurdles: first, the need for a better understanding of what molecular processes are disrupted in patients at a cell type resolution; second, the need to build different systems to model these mechanisms *in vitro*.

This body of work seeks to answer aspects of these questions by starting with an effort to understand the unique molecular properties that sensitise motor neurons to ALS. To do so, we performed RNA sequencing of 79,169 single nuclei from cortices of patients and age-matched, controls. In unaffected individuals, we found that expression of ALS risk genes was significantly enriched in THY1$^+$ layer-5, extratelencephalic (L5-ET) neurons and not in other cell types. In patients, these genetic risk factors, as well as genes involved in protein homeostasis and stress responses, were significantly induced in THY1$^+$ L5-ET and a wider collection of extratelencephalic neurons. Examination of oligodendroglial and microglial nuclei revealed patient-specific gene expression changes that were at least in part a response to disease-associated alterations in neurons. Our findings suggest that the selective vulnerability of extratelencephalic neurons is partly connected to their intrinsic molecular properties sensitising them to genetic alterations produced by ALS pathology.

In an effort to create new paradigms to study these mechanisms we will then discuss the generation of a new protocol to differentiate motor neurons from human Pluripotent Stem

Cells (hPSCs). In this study, we coupled the overexpression of the neuralising transcription factor Neurogenin2 (*Ngn2*) with small molecule patterning to differentiate hPSCs into lower induced Motor Neurons (liMoNes/liMNs). Using an *Hb9*::GFP-reporter line, we showed that this approach induced activation of the spinal motor neuron (MN) specific transcription factor *Hb9/MNX1* with up to 95% of cells becoming *Hb9*::GFP^{+}. These cells acquired and maintained expression of canonical early and mature MN markers. liMNs resembled *bona fide* hPSC-derived MN differentiated by conventional small molecule patterning, exhibited spontaneous electrical activity, expressed synaptic markers and formed contacts with muscle cells *in vitro*. Pooled, multiplex single-cell RNA sequencing on 50 cell lines revealed reproducible populations of multiple anatomically distinct MN subtypes of cervical and brachial, limb-innervating MNs that in part resemble their *in vivo* counterparts in the human embryonic spinal cord. We conclude that combining small molecule patterning with Ngn2 overexpression can facilitate the high-yield, robust and reproducible production of multiple disease-relevant MN subtypes, which is fundamental in propelling our knowledge of motor neuron biology and its disruption in disease.

Finally, we put together a compendium of protocols to differentiate hPSCs into different brain cell types that could be used to further dissect multicellular contributions to neurodegeneration. In this instance, we mostly focus on studies that have tested the efficacy of transplantation of these cellular products in an effort to repopulate cells lost to degeneration but we also provide a detailed and valuable resource for researchers to draw from to model biology of the central nervous system *in vitro* and dissect mechanisms disrupted by ALS in different cell types.

This work aims to shed a light on what molecular mechanisms are disrupted in the disease at single-cell resolution, offer options on tools to study these disruptions in human models and further discuss how future experiments using multi-disciplinary approaches could expand our knowledge on the disease in an effort to find new therapies and a possible cure.