Current Methods In ALS Research

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Editorial

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects roughly 1 in 400 people in their lifetime. The disease initially presents as upper and lower motor neuron impairment and eventually progresses to paralysis and death as a result of respiratory failure within 2–5 years after symptom onset[1]. ALS can be hereditary, with over 30 different genetic mutations but only 4 gene variants (*C9orf72*, *FUS*, *SOD1*, *TARDBP*) accounting for about 55% of familial ALS. The majority of ALS cases, approximately 90%, represent sporadic ALS, for which the leading causes are still not fully understood[2]. There is an urgent need to unravel the mechanisms of ALS by using the appropriate tools and model organisms. In this methods collection, we provide an overview of the recent research progress in terms of mimicking this disease and, hopefully, ultimately finding treatment options. For example, the application of induced pluripotent stem cells (iPSCs) that can be differentiated into motor neurons or astrocytes offers a humanized model system[3,4,5]. Additionally, in this methods collection, animal models are presented, such as *Drosophila* to study glucose uptake and the neuromuscular junction (NMJ) *in vivo*[6,7], mice to study cortical neurons[8], and *C. elegans* or zebrafish to investigate motor impairments[9,10] and post-mortem patient tissue[11].

Zebrafish larvae are transparent, and their motor neurons are directly visible, making them a perfect tool for non-invasive *in vivo* studies. Asakawa *et al.* show the phase transition of optogenetically expressed TDP-43 in single spinal motor neurons[9]. After irradiation, the cytoplasmic relocation of TDP-43 can be observed and analyzed. The aggregation of cytoplasmic TDP-43 is a hallmark of degenerating motor neurons in ALS. This method allows for the functional study and analysis of ALS-associated proteins in a subcellular, temporal manner.

Employing super-resolution structured illumination microscopy (SIM), Coyne and Rothstein detail a protocol that isolates the nuclei and describe how to investigate nucleoporin complexes[11]. Nucleoporin complexes consist of multiple copies of about 30 different nucleoporin proteins (Nups). Nucleocytoplasmic transport (NCT) impairment and Nup alterations have been shown to be early hallmarks of many neurodegenerative diseases, including ALS. By extracting the nuclei, it is possible to investigate the individual

Nup proteins within the NPC and nucleoplasm in 3D. Interestingly, this can be applied to not only iPSC-derived cells but also to post-mortem tissue.

Currey and Liachko describe two assays to discriminate between mild, moderate, and severe motor impairment in *C. elegans* models of ALS[10]. In the radial locomotion assay, crawling on a surface is measured, making this an easy and cost-effective assay. In their second method, the swimming assay, thrashing movements can be measured using a computer-based tracking method. The authors use this to study TDP-43 and tau.

Hayes *et al.* also describe a method to study NCT[8]. They apply a permeabilization method to neuronal cultures. Using primary mouse cortical neurons, they describe a method that maintains the nuclear membrane integrity by using hypotonic lysis combined with a bovine serum albumin cushion. By doing so, nuclear import still functions in an energy-dependent manner, thus providing a high-content microscopy and analysis platform. This platform will have broad applicability in the future for studying passive and active nuclear transport in primary neurons.

The quick assessment of how manipulation, disease-related proteins, or RNA impact synaptic processes and whether therapeutic drugs can restore these functions is essential for ALS research. Using iPSC-derived motor neurons as well as primary neurons from mice, Krishnamurthy *et al.* present a protocol that enables the real-time monitoring of presynaptic calcium influx dynamics and synaptic vesicle membrane fusion[3]. The authors demonstrate that C9orf72-$(GA)_{50}$ transfection impairs synaptic transmission, highlighting the suitability of these methods for detecting mutation-based differences in synaptic function.

Altered glucose uptake is one of the pathobiological characteristics of ALS. In this *Drosophila* model, Loganathan *et al.* describe a FRET-based method to measure intracellular changes in glucose uptake in specific cells[6]. Using a genetically encoded glucose FRET sensor, they validate their method with TDP-43 expression neurons, which display higher glucose uptake. In the TDP-43^{G298S} mutant line, increased glucose uptake is only detectable upon glucose stimulation. This method provides an important tool for studying glycolysis not only in ALS but also generally in relation to motor neuron regeneration.

Dissection techniques preserving the NMJ architecture are of the utmost importance for studying changes in the motor neurons along the *Drosophila* leg over time. Stilwell and Agudelo utilize a technique that allows the characterization of the NMJ for identifying motor neuron arbors using immunocytochemistry[7]. Interestingly, the adult neurons are present throughout the lifetime of a fly, which is approximately 90 days. Comparing a SOD1^{H71Y} mutation to the wild-type, the authors demonstrate different markers for age-dependent bouton swelling, protein aggregates, and enlarged mitochondria.

The innovation of mimicking an NMJ using a co-culture system meets the urgent need to study the dissociation between motor neurons and myotubes. In terms of this method, Stoklund Dittlau *et al.* describe how to cultivate human iPSC-derived motor neurons and human primary mesoangioblast-derived myotubes to form functionally active NMJs[4]. The authors show their functionality by the activation

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of motor neurons with potassium chloride and calcium influx in Fluo-4-labeled myotubes thereafter, which was abolished by the administration of NMJ blockers.

Recently, co-culture systems have gained increasing attention. Studying not only one but multiple cell types in a dish has the benefit of mimicking physiological conditions better than methods using monocultured cells. ALS-related pathobiology, such as astrocyte-mediated toxicity and neuronal hyper-excitability, can be studied using this approach. In the video by Taga *et al.*, the generation of cortical neurons and astrocytes in a co-culture combined with a multi-electrode array (MEA) setup is shown for monitoring electrophysiology[5]. The functional activity can be monitored over time, allowing flexibility in cellular composition as well as different culture conditions. This additionally provides a platform to test the therapeutic potential of drugs and their influence on functional activity.

Currently, there are only three FDA-approved treatments for ALS, all with limited application potential. To find more promising treatments, future research must understand the pathobiology better by employing multiple model systems and approaches. Without a doubt, human iPSC-derived models will provide an interesting platform to investigate the underlying molecular mechanisms. This, combined with model systems such as zebrafish, *C. elegans*, *Drosophila*, or rodents, will lead to progress in the field. Furthermore, future epidemiologic research will hopefully provide more insights into how environmental factors play a role in the development of ALS[12]. With the expanding datasets and bioinformatics developing at high speed, it will become easier to unravel the common denominators of neurodegenerative diseases in the future. This will lead to new avenues for therapy or even prevention.

Disclosures

The authors have nothing to disclose.

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