Review

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Modeling motor neuron disease: the matter of time

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Stem cell technologies have created new opportunities to generate unlimited numbers of human neurons in the lab and study neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Although some disease hallmarks have been reported in patient-derived stem cell models, it is proving more difficult to recapitulate the full phenotypic extent of these disorders. The problem with these stem cell models lies in the disparity between the advanced age of onset of neurodegenerative disorders and the embryonic nature of the *in vitro* derived cell types. In this review we discuss experimental methods of *in vitro* aging of neural cell types as a means to elicit late-onset symptoms in induced pluripotent stem cell (iPSC) models of neurodegenerative disease.

Aging and motor neuron disease

The prevalence of late-onset neurological diseases such as ALS and Alzheimer's disease has been increasing along with the aging of the world population. Many such neurological disorders still have no cure or treatment options owing to a fundamental lack of understanding of the disease mechanism. Human neurological diseases have been among the most difficult to study because of the lack of availability of affected patient tissues. Biopsy of live neurological tissue is highly invasive and presents considerable risk without ascertainable benefit to the patient. Isolation of post-mortem tissue circumvents this issue; however, owing to the poor expansion and survival of these degenerating cell types their potential use in disease modeling and drug screening remains limited.

The differentiation of neural populations from pluripotent stem cells has presented an exciting opportunity to obtain large numbers of human neuronal cell types for research, and already some molecular hallmarks of disease have been observed in several patient stem cell disease models including Parkinson's disease (PD) and motor neuron disorders [1–6]. However, most *in vitro* differentiation protocols generate cell types which are embryonic in nature, whereas disease symptoms present either postnatally or even much later in life, making it difficult to recapitulate fully the disease as it occurs in patients. Consequently, extended *in vitro* maintenance is required for end-stage phenotypes to appear in culture [5–7], which are therefore difficult to distinguish from general degenerative effects that are inherent to *in vitro* cell culture.

Thus, although pluripotent stem cell modeling has shown exciting promise in shedding light on human neurodegenerative disease mechanisms, there are significant practical challenges to be overcome before these models robustly represent *bona fide* disease progression. One of the most significant challenges we currently face is to recapitulate, within the practical boundaries of *in vitro* experimentation, diseases that take years to decades to present in patients.

We review here the progress that has been made in the development of iPSC models of motor neuron disease (MND), and the role that aging plays in these disorders. In addition, we discuss the importance of aging iPSC-derived motor neuron cultures for the establishment of comprehensive and robust *in vitro* models.

Stem cell modeling

Pluripotent stem cells

Embryonic stem cells (ESCs) have the unlimited capability both to self-renew and to differentiate into every tissue that constitutes an organism. ESCs are derived from the inner cell mass of blastocyst stage embryos and, when maintained in precisely defined culture conditions, can maintain their unlimited proliferation potential and pluripotent state [8]. Human ESCs have proved to be an excellent tool to study early events in human embryonic development, as well as providing an unlimited source of tissue for the purposes of disease modeling, drug screening, and even cellular transplantation (Figure 1) [9].

Seminal experiments of the past century, including somatic cell nuclear transfer (SCNT) in which adult nuclei are transplanted into enucleated oocytes [10,11], and cell fusion experiments between somatic cells and ESCs [12–14], have demonstrated that somatic cells maintain the ability to regain the pluripotent and self-renewing capabilities of embryonic stem cells throughout differentiation. Forced expression of select transcription factors, including Oct4 (Pou5f1), Sox2, Klf4, and c-Myc, that are responsible for maintaining pluripotency in ESCs, was later discovered to induce pluripotency in somatic cells, which are then referred to as iPSCs [15,16]. By all verified measures, including gene activation, differentiation potential, capacity for self-renewal, and epigenetic landscape, iPSCs appear to be highly similar, if not indistinguishable, to ESCs after complete reprogramming has been accomplished

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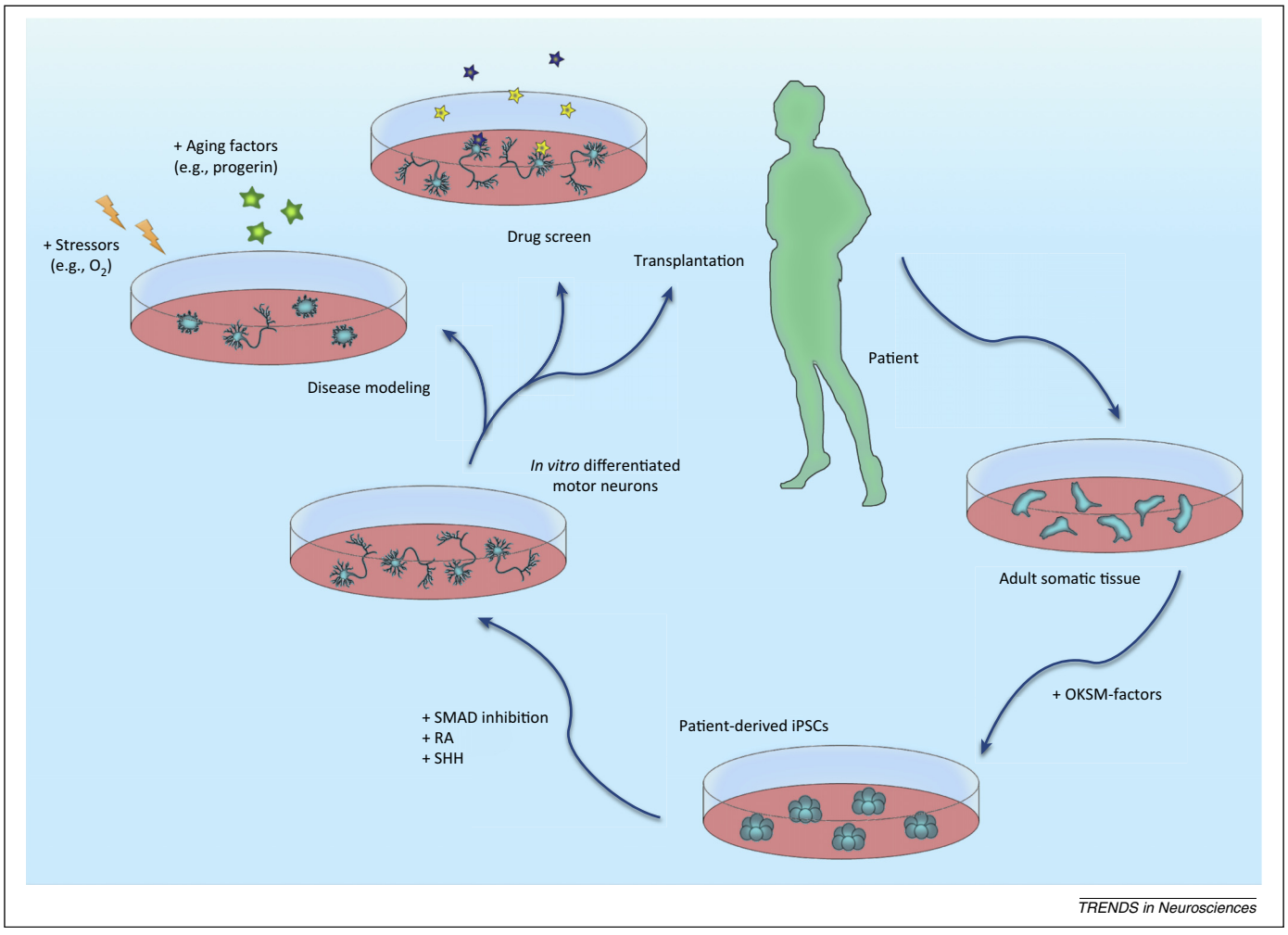

Figure 1. Schematic of the experimental setup. Patient-derived somatic cells are cultured and reprogrammed to a pluripotent state by overexpression of pluripotency factors. Patient specific iPSCs are expanded and differentiated towards spinal motor neurons by engaging SMAD [from *Caenorhabditis elegans* sma (small body) and *Drosophila* mothers against decapentaplegic (MAD)], retinoic acid (RA), and sonic hedgehog (SHH) signaling pathways. Terminally differentiated motor neurons can be used for various purposes, including as a high-throughput platform for drug-screening, and to study disease onset and progression *in vitro* – which may be expedited with the help of aging factors and cellular stressors – and potentially for cellular transplantation to help to reconstitute damaged tissue. Abbreviation: OKSM, Oct4, Klf4, Sox2, and c-Myc.

[17–19]. Intriguingly, iPSCs lose nearly all epigenetic marks that were once acquired throughout the differentiation and maturation of the somatic cells from which they are derived, generating once again a fully immature cell state. By harnessing the pluripotent differentiation capacity and ability for self-renewal held by ESCs derived from preimplantation stage embryos, iPSC technology provides researchers with an unlimited resource of patient-specific cells of any cell type. This may have a great impact on research of human disease and development as well as the future of personalized regenerative medicine.

In vitro *motor neuron differentiation*

Several protocols have been established to differentiate pluripotent stem cells towards neural lineages. Such protocols have been developed by studying natural differentiation of ESCs towards these cell types [20–22] followed by stages of empirical optimization of protein and small-molecule concentrations to activate and inhibit the appropriate pathways in a way similar to the processes taking place during embryonic development [2,23–25]. With these techniques we are able to generate large numbers of neural cells of various types *in vitro*.

Motor neuron differentiation from ESCs starts with derivation of early neuroectoderm, which is usually achieved by dual SMAD [from *Caenorhabditis elegans* sma (small body) and *Drosophila* mothers against decapentaplegic (MAD)] inhibition using small molecules to inhibit transforming growth factor β (TGFβ) and bone morphogenetic protein (BMP) signaling [24,26]. Addition of retinoic acid steers differentiation towards the caudal cell types of the spinal cord, and activation of the sonic hedgehog pathway coaxes the neural stem cells towards ventral motor lineages [20]. Markers specific to end-stage embryonic motor neurons (e.g., Hb9, Islet1, choline acetyltransferase, synapsin), their phenotypic characteristics (e.g., morphology of cell body and neurites), and electrophysiology to measure neural excitability and firing patterns, are used to verify the presence of the desired cell type in the differentiated population [26–28]. These populations are usually highly heterogeneous, and the relative abundance of various subpopulations generated by such protocols may vary between cell lines.

Although stem cell motor neuron differentiation is a relatively efficient means to generate cells expressing key generic motor neuron markers such as Hb9 and Islet1,