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Pluripotent stem cell-based models of spinal muscular atrophy

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ABSTRACT

Motor neuron diseases, as the vast majority of neurodegenerative disorders in humans, are incurable conditions that are challenging to study in vitro, owing to the obstacles in obtaining the cell types majorly involved in the pathogenesis. Recent advances in stem cell research, especially in the development of induced pluripotent stem cell (iPSC) technology, have opened up the possibility of generating a substantial amount of disease-specific neuronal cells, including motor neurons and glial cells. The present review analyzes the practical implications of iPSCs, generated from fibroblasts of patients affected by spinal muscular atrophy (SMA), and discusses the challenges in the development and optimization of in vitro disease models. Research on patient-derived disease-specific cells may shed light on the pathological processes behind neuronal dysfunction and death in SMA, thus providing new insights for the development of novel effective therapies.

Contents

1. Stem cells as a model for neurodegenerative diseases

Neurodegenerative disorders, such as spinal muscular atrophy and other motor neuron diseases, do not benefit from any current therapy. One of the reasons of the lack of an effective cure is that the development of reliable disease models represents a demanding challenge. Indeed, the fundamental inaccessibility to the human neural cell types specifically affected in neurodegenerative disorders prevents their isolation for in vitro studies on pathogenic mechanisms behind cell death and for drug screening efforts. However, the ability to differentiate stem cells into neurons and neuronal subtypes may now provide new perspectives for a satisfactory solution of this issue.

Two main types of stem cells can be described: adult tissue stem cells, dedicated to the repairment and maintenance of cell populations in their own tissues; and pluripotent stem cells, such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), whose peculiar feature is that, as well as making copies of themselves, they can become any of the different cell types in the human body.

For decades, the only available source of pluripotent stem cells has been represented by hESCs, isolated from the inner cell mass of a pre-implantation blastocyst. hESCs are able to give rise to any cell belonging to the three germinal layers, including neurons and motor neurons that show chemical and electrophysiological properties of mature neuronal cells. Despite these undeniable advantages, hESCs present ethical constraints due to the necessity of blastocyst's destruction for their

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generation. In addition, the potential employment of hESCs in transplantation experiments would correspond to heterologous cell injection, thereby requiring immunosuppressant therapies and long-term issues if this strategy were translated into a clinical setting.

Differentiation of cells from precursors was thought to be an irreversible process, until Yamanaka (Takahashi and Yamanaka, 2006) developed a method to obtain stem cells from differentiated cells by combining four factors (Oct3, Sox2, Klf4 and c-Myc) that normally act together in embryonic stem cells to guarantee the maintenance of self-renewal and pluripotency (Hochedlinger and Plath, 2009). After being inserted in skin cells, these factors bind to genes encoding hESC proteins, that overwhelm the competing message from the skin genes and redirect the cell towards an embryonic fate. By replicating, these newly re-programmed cells, termed iPSCs, become indistinguishable from hESCs both in morphologic features and in their ability to self-renew. iPSCs are also able to differentiate into cells belonging to all the three germ layers, both in vitro and in vivo, where they form embryoid bodies and teratomas, respectively.

Compared to hESCs, iPSCs offer relevant advantages under several aspects, which bestow great interest on this groundbreaking approach to stem cells. Indeed, since iPSCs can be obtained from adult skin cells, there is no need for a fertilized embryo, thus relieving important ethical concerns. Besides, iPSCs can be generated from individual patients, thus providing the basis for the development of replacement tissues that would be a perfect match to the patient and would not be rejected by the immune system because of genetic identity. Furthermore, since iPSCs can be differentiated into human disease-specific neuronal and glial cells under the effect of identified factors, they represent unprecedented tools for the study and elucidation of the pathogenesis of neurological disorders. Moreover, iPSCs and their cell derivatives may create further opportunities to identify and screen potentially therapeutic compounds and speed up the process by which drugs come through. Overall, iPSCs appear to have the potential to revolutionize the approach to the study and the treatment of human diseases (Rubin and Haston, 2011).

2. Spinal muscular atrophy: features of the disease

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by homozygous deletions or mutations in the survival of motor neuron 1 gene (*SMN1*) at the locus 5q11.2–q13.3 (OMIM 600354) (Lefebvre et al., 1995). With an incidence of 1 in 6000–10,000 live births (Prior et al., 2010), SMA represents the first genetic cause of mortality in infants (Lorson et al., 2010; Lunn and Wang, 2008) and the second most common autosomal recessive disease in Caucasians after cystic fibrosis (Wirth, 2000). The pathogenesis of SMA lies in the selective degeneration of lower α-motor neurons in the ventral horns of the spinal cord, resulting in denervation-dependent muscular atrophy, generalized muscle weakness, paralysis and precocious death (Crawford and Pardo, 1996; Lefebvre et al., 1997). The mechanisms by which the reduction in the levels of SMN protein, encoded by the ubiquitously expressed *SMN1* gene, leads to the selective degeneration of motor neurons are not fully understood yet.

A distinctive feature of the human genome is that *Homo sapiens* hosts two *SMN* genes, that exhibit unique alternative splicing: the telomeric *SMN1*, which encodes the functional full-length SMN protein; and its inverted centromeric homologue *SMN2*, which differs from *SMN1* for 5 base pair changes. Of these variations, the C to T substitution at +6 of exon 7 excludes exon 7 from approximately 90% of *SMN2* transcripts, thus resulting in the production of only 10% of the full-length protein and high levels of a truncated, non-functional protein (SMNΔ7) (Lorson et al., 1999; Monani et al., 1999). For this reason, the number of *SMN2* copies that are retained in the patient's genome is the most important modulator of the disease phenotype and leads to a SMN dose-dependent classification of SMA pathology. In particular, based on the degree of severity, SMA can be classified into four types: SMA type I (Werdnig–Hoffman disease) is the most severe form with less copies of *SMN2*, characterized by clinical onset in the early months of life and a rapid course that leads to death within 2 years of age; patients affected by SMA type II (Dubowitz disease) become symptomatic within 18 months of age and usually die before age 40; SMA types III (Wohlfart–Kugelberg–Welander disease) and IV are the mildest forms with more copies of *SMN2*, characterized by an adult-onset and a lifespan that is not generally affected.

It has been hypothesized that SMN protein plays a housekeeping role in RNA processing, participating in the assembly and biogenesis of small nuclear ribonuclear proteins (snRNPs), which are involved in the splicing of introns from pre-mRNA (Bebee et al., 2010; Burghes et al., 1994; Gabanella et al., 2007; Jablonka et al., 2000; Lefebvre et al., 1995; Fischer et al., 1997; Pellizzoni, 2007; Pellizzoni et al., 1998, 2002; Chari et al., 2008; Kolb et al., 2007). In particular, SMN seems to be relevant for U12-depending splicing, linked to motor neuron functionality, which could partially explain the major susceptibility of this cell type (Lotti et al., 2012). More recently, SMN has also been shown to accumulate in dendrites and axons of neurons, where it modulates β-actin, a major component of outgrowing axons, and traffic to neuronal processes of motor neurons (Glinka et al., 2010; Akten et al., 2011; Fallini et al., 2011; Hubers et al., 2011; Tadesse et al., 2008; Rossoll et al., 2003; Fan and Simard, 2002; Carrel et al., 2006; Zhang et al., 2003, 2006).

3. SMA pre-clinical studies: the mouse model

In order to study the molecular processes that are involved in SMA, many experimental models have been developed. An adequate pre-clinical model of the disease can be furnished by mice: indeed, murine genome is relatively easy to manipulate and retains a homologue of the human *SMN* gene (*Smn*), while lacking of the rescue *SMN2* copy.

SMN1 knockout mice and transgenic mouse lines expressing human *SMN2* have been generated in order to establish an animal model with molecular and pathological characteristics that closely mimic human SMA (Monani et al., 2003; Hsieh-Li et al., 2000; Michaud et al., 2010). Studies on such models showed that the expression of *SMN2* on the *Smn*-null background modifies the clinical phenotype, with a higher copy number of *SMN2* being the major determinant of disease severity (Hsieh-Li et al., 2000; Monani et al., 2000).

However, murine models may not be the best substrate to recapitulate the human disease, mainly due to important differences in physiologic and anatomic features between the species. In this respect, generating iPSCs from adult human fibroblasts is standing up as a great opportunity to produce more reliable models of SMA for the translation of pre-clinical studies into human clinical trials (Mattis and Svendsen, 2011).

4. SMA pre-clinical studies: iPSC-based cellular model

The great disproportion between the impact of neurological disorders on public health, which account for the 6.3% of the global burden of disease (World Health Organization, 2008), and the few effective drug therapies available for their treatment, lies in the lack of reliable models for the study of such diseases. Concerning these issues, a growing interest has been developing for fibroblasts-derived iPSCs as models for human neurodegenerative disorders. The major advantages of this model compared to others are represented by the ease of obtaining fibroblasts from skin biopsy samples and growing and maintaining them in culture. Moreover, iPSCs allow the production of physiologically relevant, pathological cells that are available in limitless amounts (Saporta et al., 2011). For genetic diseases, like SMA, further advantages are related to the presence of the mutation in starting cells, which display the molecular phenotype of patient-specific cells. Over the years, progresses in reprogramming techniques by using Yamanaka's factors