Brain Research 1656 (2017) 49–54

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Review

Modeling autism spectrum disorders with human neurons

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ARTICLE INFO

Article history:
Accepted 29 January 2016
Available online 5 February 2016

Keywords:
Autism spectrum disorders
Disease modeling
Human induced pluripotent stem cells
Human neurons

ABSTRACT

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impaired social communication and interactions and by restricted and repetitive behaviors. Although ASD is suspected to have a heritable or sporadic genetic basis, its underlying etiology and pathogenesis are not well understood. Therefore, viable human neurons and glial cells produced using induced pluripotent stem cells (iPSC) to reprogram cells from individuals affected with ASD provide an unprecedented opportunity to elucidate the pathophysiology of these disorders, providing novel insights regarding ASD and a potential platform to develop and test therapeutic compounds. Herein, we discuss the state of art with regards to ASD modeling, including limitations of this technology, as well as potential future directions.

This article is part of a Special Issue entitled SI: Exploiting human neurons.

Contents

1. Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disability with a complex etiology, generally diagnosed based on criteria that include deficits in social communication and social interaction, as well as restricted, repetitive patterns of behavior, interests, or activities. Typical signs and symptoms are usually manifest in the early developmental period, although social deficits or behavior are often not apparent until later, when

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http://dx.doi.org/10.1016/j.brainres.2016.01.057
0006-8993/

the child has difficulty meeting social or educational demands. In the United States, ASD is diagnosed in children as young as 53 months (regardless of gender or ethnicity). The prevalence is approximately 1:68 children, affecting four times more males than females (Wingate et al., 2014). Both early and long-term interventions are recommended; although those interventions can reduce symptoms of autism in children, responses are quite variable among individuals (Pierce et al., 2011; Warren et al., 2011), suggesting that better diagnostic tools are needed. Although the exact etiology of ASD remains unknown, a genetic component is likely (Geschwind, 2013; State and Levitt, 2011). Therefore, identifying genetic signatures and biological markers could facilitate diagnosis of autism in young children (Courchesne et al., 2015).

There are two categories of ASD, namely monogenic autism (due to a mutated gene) and complex/multigenic or idiopathic autism (uncertain genetic background). Monogenic forms of ASD include the following distinct genetic disorders: Fragile X syndrome, Rett syndrome, Timothy syndrome, Tuberous sclerosis, Joubert's syndrome, Angelman syndrome, and Phelan–McDermid syndrome (each accounts for not more than 1% of all ASD cases, with the entire group accounting for approximately 10% (Abrahams and Geschwind, 2008; Freitag et al., 2010; Geschwind, 2008)). Therefore, most ASD individuals are idiopathic, with evidence of *de novo* mutations (especially for simplex families or hereditary mutations), or inheritance of common polymorphisms contributing to autism risk in multiplex families (Abrahams and Geschwind, 2008; Iossifov et al., 2014; Jiang et al., 2013; O'Roak et al., 2012). There are many chromosomal loci and genetic alterations implicated in ASD pathophysiology, consistent with the inherent heterogeneity of the disease (Geschwind, 2013). Complex ASD seems to be a combination of several genetic abnormalities that cause pathway damage (Geschwind, 2008). Consequently, for the vast majority of ASD cases, understanding pathogenetic mechanisms underlying ASD phenotypic behavior remains a challenge. Some mutations are related to synapse-associated molecules (Südhof, 2008), whereas for some other cases, perhaps there is an imbalance among excitatory/inhibitory neuronal circuitry (Mariani et al., 2015; Rubenstein, 2010). Notwithstanding, pathogenic mechanisms underlying autistic behavior remain unknown for the majority of ASD individuals.

Given the inherent heterogeneity of the genetic background associated with autism, modeling this disease using transgenic animals is inherently difficult. Brain samples collected postmortem from individuals with ASD have long been used to help clarify an autistic phenotype; however, that approach has important limitations, because usually the brain represents terminal stage of the disease; brain cells are dead and the tissue is fixed. Alternatively, an interesting strategy to study disorders that affect central nervous system (CNS) physiology would be to use developments in the burgeoning field of stem cells to produce target cell types for each disease. Using a pluripotent cell, *e.g.* embryonic stem cells (ESC), it is possible to produce, theoretically, any cell *in vitro*, including multiple functional neural cell types. Producing pluripotent cells from somatic cells (termed induced pluripotent stem cells, iPSC) has the potential to generate relevant cell types from genetic disorders. Fortunately, recent advances in cellular reprogramming (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) have provided a breakthrough in human cellular disease modeling, making it possible to recapitulate live brain cells *in vitro*, while preserving the genetic background of individuals. The use of iPSC to generate viable human neurons or other neural cells *in vitro* has provided an outstanding opportunity to study a simplified neuronal network from a neurological disease with human genetic disease background preserved, which is particularly important for complex or multifactorial diseases like ASD (Beltrão-Braga et al., 2013; Marchetto et al., 2011, 2010a, 2010b; Mitne-Neto et al., 2011). Moreover, iPSC facilitates characterization of early developmental time points, giving information potentially useful in early diagnosis (including potential biological markers) and is particularly advantageous for understanding disease development and progress by IPSC-derived organoids (Mariani et al., 2015). These developments, in conjunction with exome and genome-wide sequencing data, would help to elucidate the neurodevelopmental course of autistic phenotypes (Willsey et al., 2013).

This review describes recent efforts related to ASD disease modeling using iPSC as stem cell source for *in vitro* production of neural cells. Based on findings summarized herein, it is clear that ASD disease modeling is already contributing to our understanding of disease etiology. Furthermore, this technology provides an unprecedented opportunity to manipulate ASD neural networks in a controlled environment to test strategies to recover altered neural phenotypes. In addition, these findings could also help us to better understand other neurodevelopmental diseases.

2. Disease modeling

Since iPSC were first described, it has been used to model many diseases (Soldner and Jaenisch, 2012). For neurological diseases, where the raw material is often difficult to access, the use of iPSC to generate neurons (or other neural types) is particularly exciting. The first work to generate neural cells from iPSC was done using cells from a patient with amyotrophic lateral sclerosis (ALS) (Dimos et al., 2008). Although ALS-iPSC were successfully differentiated into motor neurons, cellular phenotype was not described. The first comparison between affected and non-affected cells derived from iPSC was a study published the following year, from a patient with spinal muscular atrophy (SMA). In this study, motor neurons derived from SMA-iPSC patient had low survival compared to motor neurons derived from a non-affected family member (Ebert et al., 2009). Nevertheless, since this first report, many others have been published, giving insights into unprecedented opportunities to study neurological diseases, including novel opportunities to test potential drugs to ameliorate or cure the condition.

Although almost any disorder can be modeled by iPSC, the challenge is identification of a robust and replicable cellular phenotype that is relevant to the target disease; unfortunately, this may be very difficult to achieve (Chailangkarn et al., 2012; Tiscornia et al., 2011). Several, neurodevelopmental disorders are popular targets for disease modeling using iPSCs, include Cockayne syndrome and ASD-related disorders, such as Rett syndrome (RTT), Fragile X syndrome (FXS) and even complex autism to test rare variants (de Sousa Andrade et al., 2012; Griesi-Oliveira et al., 2014; Marchetto et al., 2010a, 2010b; Urbach et al., 2010). Regardless, modeling complex autism is of particular interest, as the genetic background of each individual is preserved and any route involved in pathophysiology of autism could be investigated.

3. Monogenic autism disease modeling

Monogenic autisms are neurodevelopmental disorders, usually with monogenetic causes identified, and whose individuals display clear autistic behaviors. It is noteworthy that some have already been modeled *in vitro* using iPSC technology (Amenduni et al., 2011; Marchetto et al., 2010a, 2010b; Paşca et al., 2011; Urbach et al., 2010). Below and in Table 1 we summarize the main findings of these reports.