Review

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The Human Model: Changing Focus on Autism Research

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ABSTRACT

The lack of live human brain cells for research has slowed progress toward understanding the mechanisms underlying autism spectrum disorders. A human model using reprogrammed patient somatic cells offers an attractive alternative, as it captures a patient's genome in relevant cell types. Despite the current limitations, the disease-in-a-dish approach allows for progressive time course analyses of target cells, offering a unique opportunity to investigate the cellular and molecular alterations before symptomatic onset. Understanding the current drawbacks of this model is essential for the correct data interpretation and extrapolation of conclusions applicable to the human brain. Innovative strategies for collecting biological material and clinical information from large patient cohorts are important for increasing the statistical power that will allow for the extraction of information from the noise resulting from the variability introduced by reprogramming and differentiation methods. Working with large patient cohorts is also important for understanding how brain cells derived from diverse human genetic backgrounds respond to specific drugs, creating the possibility of personalized medicine for autism spectrum disorders.

Keywords: Autism spectrum disorders, Brain, Drug screening, Human induced pluripotent stem cells, Human neurons, Human-specific disease modeling

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Autism spectrum disorder (ASD) is a lifelong developmental disability that is mainly characterized by difficulties in social communication and the presence of focused repetitive or stereotyped behaviors and appears within the first 3 years of life (1). Because different etiologies can generate a similar behavioral outcome, many disorders with autistic features are grouped under the ASD umbrella.

Family history and twin studies suggest that these disorders share genetic roots in at least some cases (2,3). The mounting evidence suggests that heritable and de novo genetic variations play a significant role, but these studies have also reported striking genetic heterogeneity (4–6). Disorders such as fragile X syndrome (FXS), Rett syndrome (RTT), and Timothy syndrome (TS) are caused by specific genetic alterations that also present neurodevelopmental and speech delays, resulting in an autistic phenotype. Although these syndromic forms are no longer clinically grouped under ASD, these disorders have provided useful insights into sporadic or idiopathic (nonsyndromic) forms of autism. Current genomic efforts to discover novel causative variants have focused on small chromosomal deletions or duplications in the form of copy number variations (CNVs) measured by the genotyping of large numbers of individuals (7,8). CNVs were found in cadherins and protocadherins, implicating the neuronal cell adhesion pathway in ASD, or the ubiquitin-proteasome system, which regulates synaptic attributes such as neurotransmitter release and synaptic vesicle recycling (9). Other studies have found that genes with rare CNV defects interfere with neurodevelopmental pathways by affecting the maturation and function of glutamatergic synapses that may be disrupted in ASD (7,10). Recent studies integrating ASD candidate genes with spatiotemporal co-expression networks have demonstrated that ASD genes converge on the transcriptional regulation in pyramidal (glutamatergic) cortical neurons during mid-fetal human development (11,12).

Neuropathologic imaging has also provided important insights into ASD. Macrocephaly and altered brain development trajectories with early overgrowth and later normalization have been reported in some ASD patients (13). This increase in brain size during the first 3 years of life was shown to precede the first clinical manifestation (14–19). Several pieces of evidence suggest that accelerated brain growth in this ASD population begins prenatally and continues during the first few years of life (14,17,20–22). Some magnetic resonance imaging findings correlate directly with a postmortem analysis, such as a weight (size) increase in ASD brains at early ages, describing the neurons as more packed and a reduced number of Purkinje cells in the cerebellum (23). Subsequent studies have focused on identifying cellular abnormalities, such as increases in the number of neurons (24) and the glial density (25) in the prefrontal cortex.

The prevalence rate of ASD has dramatically risen over the years. The exact reasons for this increase remain unclear; however, the improvement in and availability of diagnosis and a legitimate increase in the rate of affected newborns may be contributing factors (26,27). According to the Centers for Disease Control and Prevention 2014 Autism and Developmental Disabilities Monitoring Network, approximately 1 in 68

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children has been identified with an ASD in the United States. ASD is almost five times more common among male than female individuals.

There is no cure for ASD. ASD treatment requires a strong collaboration among multiple professionals, and the cornerstone of treatment involves individualized educational interventions, including early and intensive behavioral strategies and therapies for better clinical outcomes (28,29). The cost for this type of personalized treatment can be quite high (30). As these children mature into autistic adults, the majority do not live independently (31). Thus, the need for early diagnosis and better treatment of ASD is not only an increasing concern among scientists and physicians but also an increasing concern from an economic perspective (32). However, the human nature of ASD, with its intrinsic heterogeneity and large spectrum of clinical symptoms among patients, is a major challenge for studying ASD.

CURRENT ASD MODELS

Studies using several experimental models have improved our understanding of ASD. The best models consider the sophistication of the human brain within the constraints of cost and practicality. The inaccessibility to live neurons, either from postmortem brains or living individuals, has hindered the investigation of the mechanisms underlying ASDs. Other issues associated with postmortem analyses are similar to those associated with live imaging, such as the sample size, gender, age, and heterogeneity of the disorder itself. All of the above-mentioned issues are added to the possible lack of information on the medical or drug use history of the individuals whose brains are being studied and the differences in the methodologies or statistical analyses used among the research groups. Nonetheless, useful information on the ASD pathology has been extracted from gene expression studies using postmortem brain tissues (33,34). Furthermore, we have also gained a significant amount of knowledge of the genetics of ASD by performing genomic analyses on blood samples from affected and nonaffected individuals (8,35,36). However, these are not ideal cell types for neuroscience experimentation because blood cells do not exhibit several of the specialized structures (for example, the synaptic machinery) found in neurons. Fetal primary human progenitor/stem cells represent an acceptable experimental ASD model, but the intrinsic difficulties in their manipulation, expansion, and accessibility restrict their use (37,38).

Finally, the inherent differences between the mouse and human genetic backgrounds (39), immune systems (40), and brain circuits (41) contribute to the challenges of using rodent models of ASD (42). With a relatively shorter evolutionary distance and a more heterogeneous genetic background than inbred laboratory mice, nonhuman primate models have also been used to study ASD (43). Recent efforts have also focused on the targeted genetic manipulation of nonhuman primates to carry alterations found in syndromic forms of ASD, but mechanistic insights from these models have not yet been reported (44,45) and may be inaccurate because of the uniqueness of the brain transcriptional networks in modern humans (34,46). Thus, a new human model with unlimited access to relevant cellular material could nicely complement the efforts from previous approaches.

A HUMAN PLURIPOTENT EXPERIMENTAL MODEL FOR ASD

Despite some ethical controversy, human embryonic stem cell research has become a promising area in developmental research. For the first time, researchers can potentially explore the early stages of human development in vitro using this powerful tool to gain insights into human neurodevelopment (47). However, the progress toward understanding neurodevelopmental diseases has been hampered by the scarce availability of disease-specific human embryonic stem cells carrying ASD genetic alterations in the genome. The generation of induced pluripotent stem cells (iPSCs) using human cells has been accomplished (48,49). This breakthrough involves a relatively simple approach that uses a set of transcription factors to jump-start and reprogram the entire genetic network landscape to a pluripotent stage. In addition to overcoming ethical issues, this new technique has gained attention for its potential to generate disease-specific pluripotent stem cells with unprecedented simplicity. Human iPSCs, which allow researchers to recapitulate an individual's development in the laboratory, are tempting models for understanding complex disorders with heritable and sporadic conditions (50).

Given the uniqueness of human cognition and behavior, an in vitro human neurodevelopmental model capable of recapitulating the early stages of development could reveal specific biochemical and cellular features of our species that are difficult to reproduce in other models (37,51). The iPSC technology also presents the potential opportunity to manipulate phenotypic alterations with candidate drugs, paving the way for future drug-screening platforms (52).

MAJOR ROADBLOCKS TO ESTABLISHING AN IPSC ASD HUMAN MODEL

The introduction of iPSCs has been a breakthrough for studying human diseases. However, understanding the limitations of this approach is important to extract the most relevant data from this model. As with all in vitro models, cells in culture are not in the same environment as the living organism. Additionally, the current culture conditions are not yet fully optimized for deriving enriched populations of disease-relevant neuronal subtypes (53–60) or glial cells (61) from human pluripotent stem cells despite recent efforts.

A recent study comparing primary human fetal progenitor cells, which were isolated directly from the developing brain, with differentiating iPSCs found that although both of these in vitro models recapitulated certain aspects of human corticogenesis and the transcriptional network related to ASD, the iPSC-derived neurons exhibited relatively lower transcriptional overlap with in vivo human development (38). The high variability reported for these experiments clearly demonstrates that different experimental protocols may affect the degree of cortical maturity that iPSC-derived neurons can achieve in culture.

Another relevant issue concerns the use of proper cellular control conditions. Based on previous experiences with mouse models, isogenic cell lines may represent the ultimate control condition. Genome editing allows for rigorous study