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Review

Stem cells and modeling of autism spectrum disorders

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ABSTRACT

Human neurons, generated from reprogrammed somatic cells isolated from live patients, bring a new perspective on the understanding of Autism Spectrum Disorders (ASD). The new technology can nicely complement other models for basic research and the development of therapeutic compounds aiming to revert or ameliorate the condition. Here, we discuss recent advances on the use of stem cells and other models to study ASDs, as well as their limitations, implications and future perspectives.

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Autism

Autism is a developmental disorder that affects the brain's normal development of social and communication skills, with symptoms appearing in the first three years of life (A.D.A.M. Medical Encyclopedia, 2005). Since many different etiologies can generate this same behavioral outcome, the many disorders with autistic features, such as classical autism, pervasive developmental disorder not otherwise specified (PDD-NOS, also called atypical autism), and Asperger's syndrome are grouped under Autism Spectrum Disorders (ASD) (Berg and Geschwind, 2012). The ASD group also comprises Rett syndrome

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(RTT) and childhood disintegrative disorder (CDD—initially termed childhood schizophrenia). In these two conditions the child is born with what appears to be normal development, but around the age of 3 for RTT and 10 for CDD, the acquired skills learned are lost (for example, language and coordination), and the autistic features manifest. Importantly, RTT is primarily a mutation on the MeCP2 gene, which categorizes it as one of the few autistic disorders with a known genetic cause. Disorders such as fragile X (FX), Angelman, Prader–Willi and Timothy (TS) syndromes are caused by specific chromosomal aberrations that also present neurodevelopmental and speech delays that can result in an autistic phenotype. Although they are not grouped under the ASD, these disorders as well as schizophrenia, can be studied along with ASD to provide new insights about the development and networking in the nervous system of the autistic phenotype. The exact number of autistic children born worldwide is difficult to estimate, either because this number is increasing with the improvement and availability of the diagnosis or simply due to an increase in the rate of affected newborns (Hertz-Picciotto and Delwiche, 2009; King and Bearman, 2009). Although the prevalence rate is predicted to be 1 in 15,000 in the current Diagnostic and Statistical Manual (DSM-IV-TR) by the American Psychiatric Association (2000), most research reports identify the prevalence to be much higher (Fombonne, 2005; Kogan et al., 2009). In 2008 in the United States, the Center for Disease Control estimated that 1 in 88 live births result in ASD, an increase of 78% from 2002 to 2008. Interestingly, it has been found that among children born with ASD, boys are five times more prone to be affected than girls (1 in 54 for boys and 1 in 252 for girls) (Centers for Disease Control and Prevention, 2012). In 63% of children with ASD, diagnosis occurs during the first 3 years of age when intellectual disability is not observed, as described in 14 sites in the United States in 2008 (Centers for Disease Control and Prevention, 2012). Even with the increasing efforts to improve the identification of ASD, there is still no medical test to diagnose ASD, and families rely on specialized professionals to conduct psychological and behavioral evaluations. The advances in clinical diagnostics alongside new genetic discoveries suggest that the number of children with ASD could be highly underestimated. An increase in the identification of autistic children directly affects the cost involved in the caretaking of these children. Furthermore, the cost is mainly shouldered by the family since most communities are not prepared to meet ASD needs. The annual medical expenses per child can range from $2,100 to $11,200, and with medical interventions this cost can increase to an average of $50,000 per year in the United States. Their more specialized education can cost up to $13,000 per year. As these children mature into autistic adults, the majority of them, do not live independently (Bruder et al., in press). The need for better diagnosis and treatment of ASD is therefore a concern that is increasing not only among scientists and physicians, but from an economic standpoint as well (Kogan et al., 2008).

The autistic brain

From the perspective of a neurodevelopmental scientist, a primary goal is to better understand the complexities of the human brain by examining its course of development. Ideally, the fate of a cell could be traced by placing specific color markers in live tissue, starting at the time of its generation from progenitors, until maturation. This would lead to the discovery of the cell defects based on their live phenotype, maturation dynamics, networking profile, and laminar distribution. If this information could be correlated with genetic and proteomic data, there would be substantial and highly provocative information available to use towards developing a cure. Tracing cells through development is impossible to carry out in humans, however; and most of the live imaging techniques available provide only a fraction of the information needed to better evaluate brain disorders of developmental origin. A major challenge for studies of the autistic brain is the large spectrum of diseases that are classified as ASD, since they present so many differences on the phenotype of the disorder. For example, a child diagnosed with Asperger's syndrome most often demonstrates normal speech capacity, IQ score, and is able to live a normal and independent life. In contrast, males born with a defect in the MeCP2 gene are severely affected by the gene disruption and in the majority of cases, cannot walk or communicate, making these individuals completely dependent on the care of others. These children often die at a young age. When conducting studies on autistic patients, it is also important to understand that the outcome of the disease is largely affected by the specific type of mutation. Taken together, it is somewhat expected that an individual incapable of speaking, walking, or performing tasks individually would present with disparities in the brain when compared to an individual that can easily execute such tasks. However, since few studies have compared different autistic subgroups with each other, the most significant differences may yet be unknown (Lotspeich et al., 2004; Yu et al., 2011). Another issue involved in autistic brain research is that most live imaging brain techniques, such as magnetic resonance imaging (MRI), require the individual to remain still during the exam, which is a difficult task for children and more severely affected adults. Furthermore, a large number of patients must be included that meet Diagnostic and Statistical Manual-IV (DSM-IV) diagnostic criteria as assessed by a specialized clinician in order for results to be considered significant. Factors such as age and gender should also be taken into consideration when performing brain imaging studies, since both have been shown to affect brain size during development (Aylward et al., 2002; Courchesne et al., 2011a); in regards to gender, there are also differences in verbal and spatial domains (Beacher et al., 2012). Although the MRI procedures present the issues mentioned above, many studies have shown interesting results and provided many contributions to our understanding of the autistic brain. In 1988 Courchesne et al. first identified that the cerebellum is smaller in autism, suggesting a developmental hypoplasia (Courchesne et al., 1988). However, there were no differences in pathways to the cerebellum and midbrain in autistic individuals versus controls (Hsu et al., 1991). These cerebellum findings were later challenged by another group that was unable to find significant differences in this area using the same method (Piven et al., 1992). In RTT, the group led by Courchesne identified that these patients have a global hypoplasia of the brain, and present a progressive cerebellar atrophy that increases with age (Murakami et al., 1992). The differences found in RTT patients were significant. Later, MeCP2 was identified as one of the primary causes of RTT in these infants, and so knowledge about the type of mutation can indicate an even more significant brain difference. The importance of the correlation between genotype and phenotype can be demonstrated from RTT studies performed as emphasized by another author (Carter et al., 2008). Other structural disparities in the brains of autistic patients have been observed in the amygdala–hippocampal complex, which was found to be significantly smaller in size (Abell et al., 1999; Saitoh et al., 2001). Another large study, with conflicting results, shows that the amygdala is enlarged in autistic children, a difference not seen in teenagers. The same study found the hippocampus to be enlarged in all ages (Schumann et al., 2004). In addition, a decrease in white matter (McAlonan et al., 2005) and a significant increase in gray matter (Ecker et al., 2012) were identified in autistic versus control individuals. Many other specific brain regions were accessed by live imaging for detailed review in other studies (Anagnostou and Taylor, 2011; Stigler et al., 2011). In summary, the volumetric distinctions found in the brain anatomy of autistic patients suggest a cell population variability, and such changes can only be studied by a taking closer look at the post-mortem brain or through *in vitro* modeling, in parallel to genetic analysis. Neuropathology studies are common when evaluating the differences in cell morphology and distribution, in addition to live imaging techniques. Some findings in MRI correlate directly with post-mortem analysis, such as a weight (size) increase in the autistic brain versus control at early ages, showed initially by Kemper and Bauman (1998). In this study, abnormalities in the neocortex