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Review

The use of iPSC technology for modeling Autism Spectrum Disorders

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ABSTRACT

Autism Spectrum Disorders (ASDs) are a group of neurodevelopmental disorders that influence social skills, involving communication, interaction, and behavior, usually with repetitive and restrictive manners. Due to the variety of genes involved in ASDs and several possible environmental factors influence, there is still no answer to what really causes syndromic and non-syndromic types of ASDs, usually affecting each individual in a unique way. However, we know that the mechanism underlying ASDs involves brain functioning. The human brain is a complex structure composed of close to 100 billion cells, which is a big challenge to study counting just with post mortem tissue investigation or genetic approaches. Therefore, human induced pluripotent stem cells (iPSC) technology has been used as a tool to produce viable cells for understanding a working brain. Taking advantage of patient-derived stem cells, researchers are now able to generate neurons, glial cells and brain organoids in vitro to model ASDs. In this review we report data from different studies showing how iPSCs have been a critical tool to study the different phenotypes of ASDs.

1. Introduction

Autism Spectrum Disorders (ASDs) are neurodevelopmental lifelong disorders, usually identified in early childhood (Ben-Itzchak et al., 2013; Wasilewska and Klukowski, 2015; Quaak et al., 2013). ASDs affected individuals display deficits in social communication and interactions, also exhibiting restrictive and repetitive behaviors (Baio et al., 2018; Ben-Itzchak et al., 2013; Brito et al., 2018; De La Torre-Ubieta et al., 2016). Sensory deficits and reliance to do routine activities can be likewise related. Differences in clinical phenotypes are frequently observed, being associated with strong genetic basis and comorbidities (Clarke et al., 2016; Hoang et al., 2017). The most frequently observed comorbidities displayed in ASDs individuals includes: ADHD (Attention Deficit Hyperactivity Disorder), OCD (Obsessive Compulsive Disorder), Schizophrenia, Epilepsy, Motor and Intellectual Disability (ID), Sleeping and Gastrointestinal Disorders (Bauman, 2010; De La Torre-Ubieta et al., 2016; Muskens et al., 2017).

Since the 1940's many theories around ASD's etiology were speculated, but nowadays, they are associated with the interplay between genetics and environmental factors (De Rubeis et al., 2014; De La Torre-Ubieta et al., 2016; Vorstman et al., 2017; Hoang et al., 2017; Ng et al., 2017). Strong evidences regarding the genetic impact around ASDs suggest that more than one thousand genes can be implicated in the etiology of this disorder (SFARI gene, 2018). Other cases of autism or any other neurological disorder in the same family may be considered as genetic risk factors for ASDs, being the heritability estimated around 83% (Sandin et al., 2014; Geschwind and State, 2015; Iossifov et al., 2014; Karimi et al., 2017; Sealey et al., 2016). In addition, exposition to some environmental factors during pregnancy, such as the use of medication, active or passive smoking, pollution, consumption of drugs and alcohol, mother infections and parental age have been considered as risk factors for ASDs (Karimi et al., 2017; Modabbernia et al., 2017; Sealey et al., 2016).

ASDs prevalence studies suggest that 1–2% of the world population has some degree of autism, with an incidence increasing around the years probably related to a better understanding towards ASDs and diagnostic abilities (Cawthorpe, 2016; Fombonne et al., 2009). In the United States the last report with the ratio of people diagnosed with ASDs in North America was 1:59 (Baio et al., 2018).

Aiming to unveil the unknown etiology of ASDs and a better understanding of the interaction between genetics and environmental factors, several groups of scientists have been currently employing iPSC (induced Pluripotent Stem Cells) technology in order to grasp the mechanisms underlying autism and its complexity, as well as other

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Table 1
Types of ASD modeled by iPSC

Types of autism	Associated mutations	Models used	Number of patient's cell lines	Associated cellular phenotype	References
Non-syndromic ASD	Over 1.000 genes	Brain organoids	4	Increased GABAergic progenitor cells and neurotransmitter GABA	Mariani et al. (2015)
		Neurons	1	Reduced total length and dendritic arborization, fewer synapses and impaired calcium dynamics	Griesi-Oliveira et al. (2015)
		NPC and neurons	8	Increase in cell proliferation, upregulation of GABAergic genes in NPCs. Reduced density of excitatory synapses and inhibitory/excitatory neurotransmitters	Marchetto et al. (2017)
		Brain organoids	1	Alterations in DLX6-AS1 and DLX1 gene expression	Wang et al. (2017)
		NPC	6	Abnormal interplay between Reelin–DAB1 and mTORC1 networks	Sánchez-Sánchez et al. (2018)
		Neurons and astrocytes	3	Reduced synapses, less arborization, glial dysfunctions and IL6 increased level	Russo et al. (2018)
Phelan McDermid	Deletion of 22q13.3 or a pathogenic variant in SHANK3	Neurons	2	Significant deficits in excitatory synaptic transmission	Shcheglovitov et al. (2013)
		Neurons	2	Deficits in excitatory synaptic transmission	Darville et al. (2016)
		Pyramidal neurons	4	Altered spinogenesis, decrease in spine densities	Gouder et al. (2018)
Fragile X	FMR1 gene mutation (X chromosome)	Neurons	3	Abnormal neuronal differentiation	Sheridan et al. (2011)
		Neurons	1	Altered synaptic, transcriptional and chromatin genes	Liu et al. (2012)
		Neurons	3	Show defects in initial neurite outgrowth	Doers et al. (2014)
Timothy syndrome	A point mutation in an alternatively spliced exon of CACNA1C, the gene that encodes the α1 subunit of Cav1.2	Neurons	2	Deficits in calcium signaling, mutant channels exhibiting dendritic retraction activity-dependent, deficits in neuronal differentiation and abnormal expression of tyrosine hydroxylase	Pasca et al. (2011)
		Neurons	2	Dendritic retraction	Krey et al. (2013)
Rett syndrome	Mutation of one allele of MeCP2 gene	Neurons	2	Fewer synapses, reduced spine density, smaller soma size, altered calcium signaling and electrophysiological defects	Marchetto et al. (2010)
		NPC and neurons	1	Increased frequency of de novo LINE-1 retrotransposon	Muotri et al. (2010)
		NPC	3	Altered expression of neural progenitor genes	Nageshappa et al. (2016)
		Neurons and astrocytes	3	Morphologic defects in neurons. Mutant astrocytes induce abnormalities in neurons	Williams et al. (2014)
CDKL5	Mutations in the X-linked CDKL5 gene	Neurons	2	Decrease in density of dendritic spines	Ricciardi et al. (2012)
		Neurons	2	Reduced number of excitatory synapses and decrease in density of dendritic spines	Amenduni et al. (2011)
		Neurons	3	Altered microtubule dynamics	Baltussen et al. (2018)
Tuberous sclerosis complex	Mutation in TSC1 and in TSC2	Neurons	3	Increased soma size, decreased neurite length and abnormal connections	Li et al. (2017)
		Neurons	2	Delayed neuronal differentiation	Zucco et al. (2018)
		Cortical neurons and oligodendrocyte (OLs)	2	Cellular hypertrophy and increased axonal density	Nadadhur et al. (2019)
Timothy syndrome	de-novo genetic mutation in Cav1.2	NPC and cortical neurons	5	Altered calcium signaling, dendritic retraction and neuronal differentiation deficits	Pasca et al. (2011)
		Neurons	2	Deficiency in calcium signaling, altered cellular structure and less complexity	Krey et al. (2013)
		Brain organoids	3	Abnormal migration of interneurons	Birey et al. (2017)
Angelman syndrome	Loss of function of UBE3A, a gene located at 15q11-q13 region from maternal chromosome	Neurons	3	No phenotype alterations	Chamberlain et al. (2010)
		Neurons	3	Reduction of synaptic activity and plasticity	Fink et al. (2017)

neurological disorders (Fink et al., 2017; Griesi-Oliveira et al., 2015; Marchetto et al., 2017; Marchetto et al., 2010; Mariani et al., 2015; Russo et al., 2018; Tian et al., 2014; Vicidomini et al., 2017). Here, we will describe how iPSC technology can help to understand ASDs pathophysiology, uncovering the mechanisms of CNS cells while keeping the genetic background of individuals with ASDs in a dish. This technology not only may help us to understand neurodevelopmental disorders, but also enables to test drugs in a safe environment with human relevance and background (Brito et al., 2018).

1.1. Non-syndromic and syndromic forms of ASDs

Non-syndromic forms of autism are considered as having a multifactorial inheritance, in which genetic and environmental factors may play a role leading to different degrees or types of autism (Hoang et al., 2017). Although some progress has been made to understand ASDs genetic etiology, only 1% or fewer ASDs individuals have a known genetic culprit (Fernandez and Scherer, 2017). While syndromic ASDs have an established genetic cause and somatic abnormalities, it can also manifest typical ASDs behavior and phenotype (Fernandez and Scherer, 2017) (Table 1). Below, we will briefly present some syndromes already recognized and established as being part of syndromic ASDs.

1.1.1. Rett syndrome

Rett Syndrome (RTT) is a neurodevelopmental disorder with a higher prevalence in females, being sometimes lethal in males and it was the first form of syndromic autism with a very defined genetic cause associated (Amir et al., 1999). The majority of RTT patients (95%) have a mutation in the X-linked transcriptional regulator MethylCpG-binding Protein 2 (*MECP2*). In the remaining 5% of cases, individuals affected by RTT or RTT-like features may present mutations in other genes, such as the Cyclin-dependent kinase-like 5 gene (*CDKL5)* (Neul, 2012) and Forkhead box protein G1 gene (*FOXG1*) (Ariani et al., 2008).

1.1.2. CDKL5

CDKL5 is a very rare neurodevelopmental disorder, caused by mutations in *CDKL5* gene (Ricciardi et al., 2012; Trazzi et al., 2018) and it was initially included in an atypical form of RTT. CDKL5 affected patients display clinical features related to RTT, including motor impairment, intellectual disability, visual deficits, early-onset epilepsy and autism. However, due to the very early seizures variants not found in *MECP2* mutated patients, CDKL5 condition is considered a distinct clinical entity rather than a variant of RTT (Archer et al., 2006).

1.1.3. Phelan-McDermid syndrome

Phelan-McDermid Syndrome (PMS) displays a deletion in the *SHANK3* gene (22q13). PMS is a neurodevelopmental disorder and affected individuals manifest autistic features (around 80%), intellectual disability, delayed or absent speech and hypotonia. Correlations between genotype-phenotype analysis have shown that the larger the deletion is in the *SHANK3* gene, the more apparent are the dysmorphic features and the impairments related to autism (Soorya et al., 2013).

1.1.4. Fragile X syndrome

Fragile X Syndrome (FXS) is correlated to an unstable expansion of the CGG trinucleotide repeat within Fragile X Mental Retardation (*FMR1*) gene, located in the X chromosome. Approximately one-third of people with FXS also have ASDs (Hernandez et al., 2009; U.S. National Library of Medicine, 2018a).

1.1.5. Tuberous sclerosis complex

Tuberous Sclerosis Complex (TSC) is considered a rare neurodevelopmental disorder resulting from mutations in the TSC1 and TSC2 genes (Nadadhur et al., 2019). TSC patients can present irregularity in lungs, kidneys, skin, eyes, heart, and brain. TSC affected individuals also may exhibit behavioral, psychiatric, psychosocial and intellectual disabilities (Vignoli et al., 2015). ASDs are the most common psychiatric problem in TSC, affecting over 61% of individuals, whereas the mechanism remains unknown (Gipson, 2013; Vignoli et al., 2015).

1.1.6. Timothy syndrome

Timothy Syndrome (TS) is a multisystem rare disorder in which individuals present autism features, different cardiac problems, syndactyly, dysmorphic facial features, immune deficiency and neurological dysfunction (Bett et al., 2012). A common de novo genetic mutation in Cav1.2 gene is responsible for the TS phenotypic abnormalities in different and multiple organ systems (Splawski et al., 2004). TS shares some of the features of ASDs, like deficit in communication and socialization skills and in developing language and speech. Intellectual disability and seizures, other common features present in people with ASDs, are also present in individuals with TS (U.S. National Library of Medicine, 2018b).

1.1.7. Angelman syndrome

Angelman Syndrome (AS) is a neurodevelopmental disorder caused by the loss of function of UBE3A, a gene located at 15q11-q13 region from maternal chromosome (Peters et al., 2004). AS affected individuals present profound mental retardation, ataxia, continual outbursts of laughter, severe language and speech delay, abnormal electroencephalogram and seizures. Besides the core symptoms described above, they also present hyperactivity, stereotypic behavior and impulsivity, phenotypes related to ASDs (Lossie et al., 2001; Peters et al., 2004; Trillingsgaard and Østergaard, 2004). Mutations in the same chromosome region 15q11-q13, but from the paternal homologue, cause Prader-Willi Syndrome (PWS), a complex neurodevelopmental disorder presenting a great variability of symptoms, such as mild to moderate intellectual disability, hypotonia, irritability, social dysfunction, compulsivity, hyperphagia, rigidity, growth hormone deficiencies, feeding problems, hypogonadism and, in some cases, psychosis (Dykens et al., 2007; Elena et al., 2012). A limited percentage of individuals with ASDs may present mutation in chromosome 15q11.2-q3 and there are not enough data on the rates and characteristics of ASDs in PWS.

2. iPSC to model ASDs

Pluripotent stem cells (PSC), including embryonic stem cells (ESC) and induced-PSC (iPSC), are undifferentiated cells with self-renewal capability and the potential to differentiate into most cell types of the body, providing the possibility to model human cells and tissues in vitro (Centeno et al., 2018; Romito and Cobellis, 2016). The use of iPSC offers opportunities for analyzing brain development and the consequences of its dysfunctions in neurodevelopmental disorders, without the ethical issues of embryonic lines or limited durability of primary cultures. It provides limitless in vitro CNS models for disease modeling, still keeping the genetic background of patients, which is very useful, both to study monogenic diseases as well as more complex polygenic diseases (Liu et al., 2018). Additionally, the enthusiasm of using iPSC for patient-specific autologous therapy or drug screening gives rise to a novel design of medicine practice, the personalized medicine.

Currently, iPSC have been largely used to model diseases that affect the CNS, especially because the difficulty to access the brain. Most iPSC disease modeling studies use the conventional 2D monolayer culture platform recreating well defined neural cell types disease-relevant (Fig. 1) (Russo et al., 2015). Different phenotypes in neural precursor cells, neurons and glial cells derived from iPSC were already reported for syndromic and non-syndromic autistic individuals (Griesi-Oliveira et al., 2015; Marchetto et al., 2017; Marchetto et al., 2010; Russo et al., 2018; Shcheglovitov et al., 2013). In addition, more complex 3D models were also developed (Lancaster et al., 2013; Lancaster and Knoblich, 2014) and could bring new insights to ASDs understanding (Fig. 1).

Here, we describe the findings obtained using conventional 2D

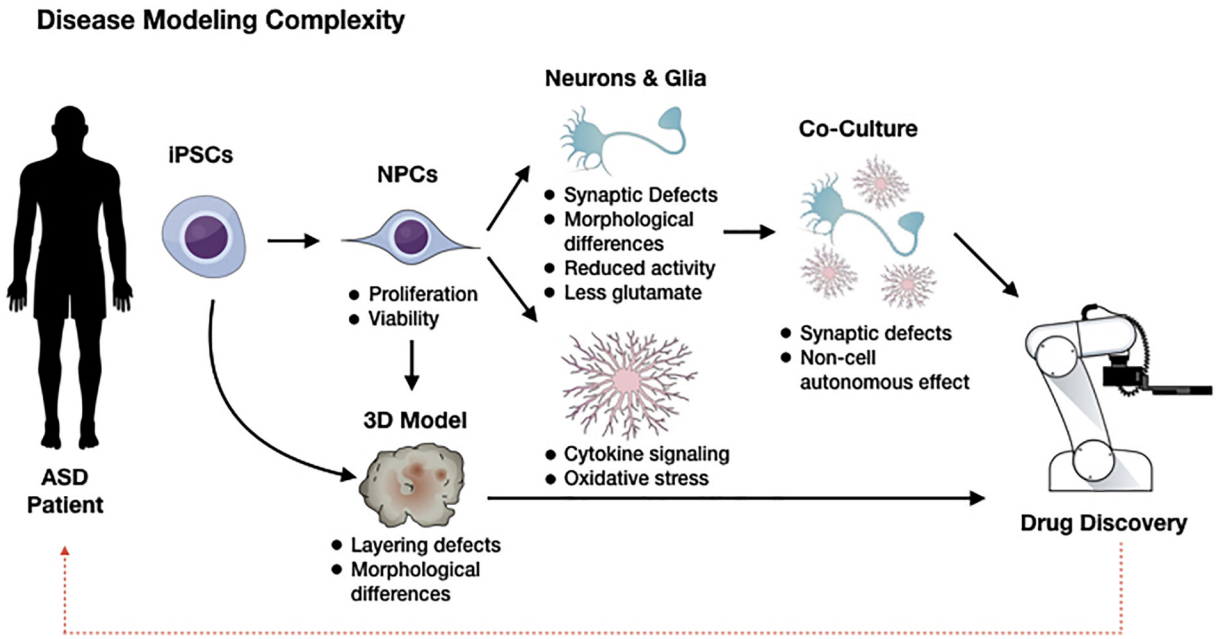

Fig. 1. Diagram of disease modeling and drug development with iPSC. Specific iPSC can be derived from individuals with ASDs. Human iPSC could be further differentiated into the affected neuronal and glial subtypes for disease modeling in vitro. The identified phenotypes could be used as readouts for drug discovery.

models in a dish.

2.1. Neural progenitor cells

Neural Progenitor Cells (NPC) are multipotent and self-renewing neural stem cells (Van Praag et al., 2002). Dysfunction of NPC has been associated with many neurological disorders, such as non-syndromic autism (Griesi-Oliveira et al., 2015; Marchetto et al., 2017) and syndromic autism, including RTT (Marchetto et al., 2010) and TS (Pasca et al., 2011).

NPC from non-syndromic autistic individuals mutants for TRPC6 gene did not show changes in proliferation (Griesi-Oliveira et al., 2015). In contrast, NPC from non-syndromic autistic individuals presenting macrocephaly revealed an increase in cell proliferation and an upregulation of GABAergic genes (Marchetto et al., 2017). An abnormal interplay between Reelin–DAB1 and mTORC1 networks was also reported in iPSC-derived NPC from non-syndromic ASDs individuals (Sánchez-Sánchez et al., 2018).

In regard of RTT syndrome, NPCs derived from RTT-iPSC were able to underwent X-inactivation when differentiating into functional neurons (Marchetto et al., 2010). Another report demonstrated that NPC derived from RTT-iPSC increased long interspersed nuclear element-1 retrotransposon (LINE-1), showing that MECP2 regulates these events (Muotri et al., 2010). Regarding NPC derived from iPSC models with MECP2 duplication, an altered expression of neural progenitor genes was reported (Nageshappa et al., 2016).

For TS, NPC and also cortical neurons showed phenotypes linked to the mutated channel malfunction, defects in calcium signaling, and gene expression-dependent activity (Pasca et al., 2011).

2.2. Neurons

Neurons process and transmit information through electrical and chemical signal via synapses. Abnormal formation of neuronal networks and synaptogenesis were previously associated with ASDs in postmortem brain tissues studies (Sajdel-Sulkowska et al., 2011). Human postmortem analysis have revealed neuronal cellular phenotypes such as altered neuronal morphology, reduced soma size, fewer dendritic spines, and reduced dendritic arborization (Armstrong et al., 1998; Belichenko et al., 1997), in which similar findings were also reported using iPSC to model non-syndromic ASDs individuals. In one non-syndromic autistic individual carrying a mutation in the TRPC6 gene, iPSC-derived neurons revealed changes in neuronal morphology, including reduced total length and dendritic arborization, as well as in functionality such as fewer synapses and impaired calcium dynamics. In addition, hyperforin and insulin-like growth factor 1 (IGF-1) were able to rescue the neuronal abnormalities in vitro (Griesi-Oliveira et al., 2015).

Regarding neuronal functionality, the concept of excitatory and inhibitory balance is considered a key point during neurodevelopment. In ASDs, the imbalance between excitatory and inhibitory synapses were reported before, especially for some gene mutations related to synaptic function, leading to a disrupted social behavior and cognitive deficits (Rubenstein and Merzenich, 2003; Rubenstein, 2010; Hensch, 2005). Some previous work also reported that the modular arrangement of neocortex minicolumns in the brain of ASDs is narrower, with a defect in GABAergic fibers, leading to an increase in seizure episodes (Casanova et al., 2003). On the other hand, iPSC-derived neurons from FOXG1 mutated patients showed an overproduction of GABAergic neurons (Mariani et al., 2015). Other study using iPSC-derived neurons from a cohort of ASDs-macrocephalic individuals revealed significant reduction in both inhibitory and excitatory neurotransmitters and a reduced density of excitatory synapses, with functionality rescued by IGF-1 treatment (Marchetto et al., 2017). Recently, our group modeled a cohort of non-syndromic ASDs individuals, matching clinical criteria, including stereotyping, language impairment, nonaggressive behavior, sleep disturbance, with no history of epileptic seizures, not macrocephalic at birth and without genetic variants in common. Neurons derived from these cohort presented a significant decrease in excitatory neurotransmitters, synaptic events and spontaneous spike rate, thus revealing reduced activity of neurons (Russo et al., 2018).

Human iPSC technology has also been used to study syndromic form of ASDs. iPSC-derived neurons from RTT patients exhibited increased frequency of de novo LINE 1 retrotransposon, as also reported for NPC (Muotri et al., 2010). In addition, neurons displayed reduced dendritic spine density, smaller soma size, fewer synapses, alterations in $Ca2+$ influx, and altered electrophysiology. Also, in this study the treatment using IGF-1 was able to rescue the synaptic number (Marchetto et al.,

2010). IGF1 is currently in clinical trials for RTT patients. In contrast, a duplication on MeCP2 gene produced an increase in the synaptogenesis and dendritic complexity in cortical neurons, with an alteration in neuronal network synchronization (Nageshappa et al., 2016). For the CDKL5, an atypical form of RTT, two groups taking advantage of iPSC technology, produced post mitotic neurons from patients with the referred gene mutation. However, the results didn't find consent, varying from none phenotypical differences and reduction on the number of excitatory synapses and decrease in density of dendritic spines (Amenduni et al., 2011; Ricciardi et al., 2012). More recently, CDKL5 KO neurons revealed altered microtubule dynamics (Baltussen et al., 2018).

Defects in the development, maintenance, and plasticity of neuronal network connectivity are implicated in FXS. iPSC-derived neurons from FXS showed epigenetic differences on FMR1 gene expression, resulting in abnormal neuronal differentiation (Sheridan et al., 2011). In addition, a reduced postsynaptic and synaptic puncta density, less neurite length, and elevated calcium influx were reported in iPSC-derived neurons (Doers et al., 2014; Liu et al., 2012).

Human iPSC-derived neurons from PMDS revealed significant deficits in excitatory synaptic transmission. These deficits were recovered by the expression of SHANK3 and by the pharmacological treatment with IGF-1. According to the results presented, there is a strong link between the SHANK3 gene and the synaptic abnormalities, which can be treated both pharmacologically and genetically (Shcheglovitov et al., 2013). In addition, SHANK3 protein levels and its recruitment to the glutamatergic synapses was tested using iPSC-derived platform (SA001 hESC line). Aripripazole, BWB 70C, Fendiline HCI, Fluoxetine, Lithium and Valproic Acid were tested, the last three being able to increase SHANK3 levels in neurons (Darville et al., 2016). More recently, a model using iPSC-derived pyramidal neurons showed alterations in synaptogenesis and spine densities (Gouder et al., 2018).

TSC iPSC-derived neurons showed increased soma size, decreased neurite length and abnormal connections (Li et al., 2017). Delayed neuronal differentiation related to phosphatidylinositol 3-kinase/AKT was also reported in iPSC-derived neurons (Zucco et al., 2018). More recently, cortical neurons and oligodendrocyte (OLs) cultures were generated to investigate neuron-glia interactions (Nadadhur et al., 2019). The authors showed an increased network activity and dendritic branching in mono cultures of neurons. However, in co-cultures with OLs, neuronal alterations became more apparent, presenting cellular hypertrophy and increased axonal density. Pharmacological treatment using mTOR regulator rapamycin suppressed these defects (Nadadhur et al., 2019).

In TS, neurons derived from iPSC demonstrated deficits in calcium signaling, with mutant channels exhibiting dendritic retraction activity-dependence, deficits in neuronal differentiation and abnormal expression of tyrosine hydroxylase (TH). The abnormal expression of TH was reversed by roscovitine treatment (Pasca et al., 2011). Another study observed an increase in intracellular calcium in human iPSC-derived neurons and in rodents after membrane depolarization suggesting that neurons present deficiency in calcium signaling. It has also been seen that some changes in the membrane potential have occurred, in which they regulate the signaling by controlling the arborization and decreasing the complexity of the cellular structure (Krey et al., 2013).

The first study modeling AS and PWS from iPSC derived patients did not reveal any phenotypic differences between AS and control neurons, but that UBE3A imprinting occurred during neuronal differentiation in AS cells (Chamberlain et al., 2010). Induction of SNHG14 expression was observed behind silencing of the paternal UBE3A gene, suggesting that this is a late event during neuronal differentiation (Stanurova et al., 2016). In addition, another study that generated iPSC from AS patients with large deletion of 15q11-q13 chromosome, and differentiated into functional neurons showed a decrease of synaptic activity and reduction of synaptic plasticity (Fink et al., 2017).

2.3. Glial cells

Glial cells are the most abundant cell type in the CNS and play essential roles in maintaining brain homeostasis providing support and protection for neurons (Barres and Raff, 1993; Kettenmann and Verkhratsky, 2008; Zhang, 2001; Zheng et al., 2018). More specifically, astrocytes support neuronal networks, axonal guidance, give nourishment of neurons, respond to inflammation and form along with other cells the blood brain barrier. In addition, astrocytes contribute to neuronal morphology and synaptogenesis (Eroglu and Barres, 2010; Johnson et al., 2007; Pekny et al., 2014; Ullian et al., 2004; Zhang and Barres, 2010). Current applications of iPSC technologies in patient-specific models tend to focus on neuronal phenotypes. However non-neuronal cell types of CNS have been shown to play a very important role in several pathologies, including ASDs. Glial alterations have also been described in postmortem ASDs samples (Edmonson et al., 2014). Generation of glia cells from patient-derived iPSC could help understanding the mechanisms involved in ASDs.

Astrocytes-derived from iPSC revealed their role in RTT patient's cell model. Mutant astrocytes could induce abnormalities and affect the neurons, but using IGF-1 and GPE (a peptide containing the first 3 amino acids of IGF-1) morphologic defects were rescued (Williams et al., 2014).

Our group was the first to describe an iPSC model of non-syndromic autism focusing on the interplay between neurons and astrocytes. Based on co-culture experiments, we observed that ASDs-derived astrocytes interfered with proper neuronal development. In contrast, we observed an improvement in ASDs neuronal morphology and synaptogenesis combining these neurons with control astrocytes. Furthermore, after identifying an increased level of interleukin-6 (IL-6) secretion from astrocytes in individuals with ASDs as a possible culprit for neural defects, we were able to increase synaptogenesis by blocking IL-6 levels (Russo et al., 2018). IL-6, one of the most important neuroimmune factors, is normally expressed at relatively low levels and increases under pathological conditions (Gadient and Otten, 1997; Jüttler et al., 2002; Spooren et al., 2011). Previous studies already reported an increasing of IL-6 in plasma (Ashwood et al., 2008; Emanuele et al., 2010; Malik et al., 2011), peripheral blood cells (Enstrom et al., 2011), postmortem brain (Li et al., 2009; Vargas et al., 2005; Wei et al., 2011) and cerebrospinal fluid in autism (Vargas et al., 2005). Our work demonstrated that glial dysfunctions could contribute to non-syndromic autism pathophysiology generating novel insights and providing a platform for drug discovery and future potential therapies (Russo et al., 2018).

Other type of glial cells in CNS is the microglia, the resident macrophages in the brain and spinal cord. In recent years microglia have aroused more attention as significant modulators of neurogenesis, development, and pathogenesis of the CNS (Zheng et al., 2018). Abnormal microglial function was demonstrated in postmortem tissue from autistic individuals as well as in mouse models of ASDs (Edmonson et al., 2014). For example, ASDs postmortem brain tissue exhibits an increased microglial density in gray matter, activated microglial morphology and altered cytokine profiles (Morgan et al., 2010; Suzuki et al., 2013; Vargas et al., 2005), as well as an increased number of markers in the surface of the microglial cells was reported in the prefrontal cortex of postmortem brain tissue from children with ASDs (Edmonson et al., 2014). Additionally, for RTT, altered microglial function was also described (Derecki et al., 2012; Derecki et al., 2013; Tsai, 2012). Microglia from MECP2 null mice, a model of RTT, produced a conditioned media that damaged synaptic connectivity via a glutamate-excitotoxicity mechanism (Derecki et al., 2012). Interestingly, investigating cells with same pathway of differentiation of microglia, macrophage and monocyte from autistic patients presented higher plasma levels of factors involved in immune system regulation (Goines et al., 2011; Schlegelmilch et al., 2011; Sweeten et al., 2003).

There are some described protocols to produce microglia cells from

iPSC (Douvaras et al., 2017; Pandya et al., 2017; Yanagimachi et al., 2013). However, there are still no specific studies in this field for ASDs. The use of iPSC technology could help understanding the mechanisms involved in this type of CNS cell and help in the autism field.

Oligodendrocytes are the glial cells that produce myelin sheaths around nerve fibers in the CNS and have become the optimal cell source for modeling and treating demyelinating diseases (Zheng et al., 2018). Studies have suggested the possible role of oligodendrocytes in the pathogenesis of autism. Reduced concentrations of *N*-acetyl aspartate (NAA), an amino acid considered very important to support myelination, were found in brain regions in autistic patients, specifically in the left frontal cortex (Corrigan et al., 2013; Kleinhans et al., 2007). Oligodendrocyte progenitor cells (OPCs) isolated from the cortex of TS mice showed a more complex morphology and higher levels of myelin protein expression (Cheli et al., 2018). There are few developed protocols to produce oligodendrocytes from iPSC for disease modeling (Wang et al., 2013). The development of this technology may be the guidance we need to fully understand the mechanisms involved in this cellular type and also in autism pathology.

2.4. 3D model of brain organoids

Organoids could be defined as 3D multicellular aggregates derived from stem cells that differentiate and self-organize, mimicking the whole developing organs, keeping structural features and preserving diverse cell types interactions (Dutta et al., 2017). The progress on iPSC-derived brain organoids provided more accurate models for assessing the pathogenesis of ASDs (Lancaster et al., 2013; Lancaster and Knoblich, 2014; Sasai, 2013; Yin et al., 2016). It is also available increments of brain organoids protocols, leading to a production of specific regions of the brain, like hippocampus and cerebellum, as well as cortical folding (Quadrato et al., 2016).

In addition, the use of this technology will possibly allow us to recreate early stages of corticogenesis, studying prenatal brain organization and function (Birey et al., 2017).

Neuronal phenotypes using brain organoids from non-syndromic ASDs individuals, presenting a mutation in the FOXG1 gene, were investigated and an upregulation of genes involved in neuronal differentiation and synaptic formation was reported. In this work, GABAergic progenitor cells and neurotransmitter GABA were increased in organoid structures suggesting an imbalance in ASDs. In addition, transcriptome analyses of organoids revealed that FOXG1 could be responsible for over-production of the GABAergic neuronal lineage, which could be restored by interfering in FOXG1 expression, revealing that FOXG1 could be related to modulation of brain size (Mariani et al., 2015).

Another study also investigated a role of a single mutation for ASDs using cerebral organoids. Transcriptomic analysis of cerebral organoids carrying a mutation in the CHD8 gene showed that DLX6-AS1 and DLX1, genes involved in interneuron differentiation, are among the top differentially expressed genes in CHD8 knockout organoids (Wang et al., 2017). Additionally, cellular phenotypes of TS were studied using three-dimensional spheroids and an abnormal migration of interneurons was observed for the first time (Birey et al., 2017). Similar studies have been performed in schizophrenia (Ye et al., 2017).

Recently, methods of iPSC derived brain organoids within a physiological tissue environment were developed by using efficient in vivo engraftment mouse models, which can help in drug screening (Basuodan et al., 2018; Mansour et al., 2018). The platform of the brain organoids is the only platform that recreates the 3D architecture of the human brain and recapitulates the process of human neurodevelopment. The main disadvantages of this method include: the variability between protocols that can lead to different cell types generated or variable 3D structure shapes and size, the difficulties to apply to it for high-throughput screening, the time in culture can be sometimes longer than 2D and, specifically in the ASDs studies, there is no synaptic assay such as puncta staining or neurite outgrowth phenotype described in the literature due to technique limitations.

3. Current and future scenarios

Induced Pluripotent Stem cells represent an unprecedented tool to investigate disorders that affect the CNS. Thus, taking advantage of iPSC disease modeling sounds like a complementary strategy to better uncover the mechanisms of ASDs, using alive cells and maintaining genetic background of autistic individuals, which is particularly interesting considering that ASDs is polygenic. In the case RTT for example, iPSC technology unveiled cellular and molecular phenotypes regarding MECP2 gene mutation and also provided evidences that addition of IGF-1 could rescue synaptic events on neurons (Marchetto et al., 2010). Furthermore, co-culture models, as illustrated by ASDs-derived neurons cultured on top of astrocytes, provided valuable insights regarding astrocyte's role on ASDs and neuroinflammation (Russo et al., 2018).

Another valuable tool that could be combined with iPSC technology is the production of isogenic cell lines. For syndromic ASDs or single cell mutation, isogenic cell lines could be a valuable tool to verify the role of one gene, connecting it with a clinical phenotype. This could be provided either using genome editing technology, such as clustered regularly interspaced short palindromic repeats (CRISPR) or selecting clones based on X inactivation, for mutated genes located on chromosome X, like RTT for example (Freitas et al., 2019).

However, as any experimental method, iPSC disease modeling for ASDs also presents some limitations that should be run through, like the diversity between iPSC clones, the appropriate experimental methods and validations in order to generate robust phenotypes, which in turns could be directed linked with the genetic background of ASDs individual. Besides, it is important to consider that correction of gene mutated in patient-derived iPSC generating isogenic cell lines could provide ideal control iPSC for use as controls in disease models. Additionally, it is important to consider the number of individuals modeled or, at least, identify clinical endophenotypes to cluster them in order to identify in vitro phenotypes that could be later related to clinical signals of ASDs. Considering that and thinking on ASDs genetic diversity, our group created an initiative to collect dental pulp stem cells from many ASDs individual, using it as cell sources to modeled ASDs. This initiative turns into a biobank in Brazil, called “The Tooth Fairy Project” (Russo et al., 2018). Started in 2009, the project collected teeth from ASDs individuals from all parts of Brazil. Our goal was to give visibility to the project, thus we created a NGO, with a site, a Facebook page and e-mail address, promoting the family's contact and teeth donations. Actually, we have about 300 of stem cells waiting to be modeled.

Regarding the use of iPSC technology to replace a 2D monolayer culture model by 3D brain organoids, this empowered scientists to recreate self-organized cortical layers, with regional specifications, multiple cell interactions and migration, observed during brain development. 3D models bring us closer to human brain development than ever before, contributing to a better understanding of ASDs. Thus, comparing 2D with 3D systems, organoids gave us the possibility to enhance our understanding of the pathobiology of neurological diseases. However, despite some sophistication already available in brain organoid technology, like production of specific regions and folding, there is still much to be improved. For example, human brain contains billions of cells, not only neuronal cell types. Brain organoids in a dish have only a fraction of the different cellular subtypes expected in the brain, missing for example endothelial cells, blood, and microglia. For this last cell, the incorporation of immune and inflammatory cells, will lead brain organoid model to another level of complexity, especially useful to model infectious diseases, but also to investigate neurodevelopmental and neurodegenerative diseases. Additionally, blood vessels are not a feature of in vitro brain organoids, and the lack of vascularization presumably leads to an abnormal distribution of nutrients to the cells. Also, this represent a gap to study interactions of the blood-brain-

barrier (BBB), especially relevant to consider drug delivery to the brain. One way proposed to overcome BBB lack was the engraftment of human brain organoids into mice-brain, as a way to incorporate vasculature, leading the interaction with blood and host physiology (Mansour et al., 2018).

Not only the etiology of ASDs has been uncovered using iPSC, but therapeutic areas are also making progress using this great tool. Based on either mechanism of cellular toxicity or on neurite outgrowth, iPSC-diseases modeling technology is accelerating clinical trials. For example, based on results of IPSC RTT modeling, the IGF-1 is ongoing for clinical trials (Kolevzon et al., 2014; Khwaja et al., 2014; https://ClinicalTrials.gov; protocols numbers: NCT01970345; NCT01894958; NCT01777542; NCT01525901; NCT01253317). Compounds like Luteolin have been used for anti-inflammatory properties in in vitro astrocytes and were already tested in ASDs patients diet with success, proving the ability of iPSCs to address cell-specific compounds (Jeon et al., 2014; Tsilioni et al., 2015; Zuiki et al., 2017).

In summary, iPSC brought a tool to investigate the molecular and functional pathways of brain cells and target drug testing for ASDs treatment, transforming it into clinical trials, improving the quality of life of ASDs individuals and their families.

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Conflict of interest

The authors declare that they have no competing interests.

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