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Using Patient-Derived Induced Pluripotent Stem Cells to Model and Treat Epilepsies

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Abstract

Human induced pluripotent stem cells (iPSCs) are transforming the fields of disease modeling and precision therapy. For the treatment of neurological disorders, iPSCs introduce the possibility for targeted cell-based therapies by deriving patient-specific neural tissue *in vitro* that may ultimately be used for transplantation. We review iPSC technologies and their applications that have already advanced our understanding of neurological disorders, focusing on the epilepsies. We also discuss the application of powerful new tools such as genome editing and multi-well, multielectrode array recording platforms to iPSC disease modeling and therapy development for the epilepsies. Despite some limitations, the field of iPSCs is evolving rapidly and is quickly becoming vital for understanding mechanisms of genetic epilepsies and for future patient-specific therapeutic applications.

Keywords

Epilepsy; induced pluripotent stem cell; cerebral organoid; neural progenitor; seizure disorder

Introduction to iPSCs

Understanding neurological disease mechanisms has traditionally involved the use of animal models or studies of postmortem tissue. For neurogenetic disorders in particular, genetically modified mouse models have been useful, but they often fail to recapitulate critical aspects of the human disease phenotype. Postmortem tissue may be difficult to acquire and generally represents late stages of disease that offers limited mechanistic information in many instances. The advent of the induced pluripotent stem cell (iPSC) method by Yamanaka and colleagues in 2006 [1] redefined the field of translational research by providing access to patient-derived cells for clinical disease studies [2]. Neurological disease modeling is

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Compliance with Ethics Guidelines

Conflicts of Interest

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especially attractive for iPSC applications with the ability to derive patient-specific neurons for *in vitro* studies [3–6], a feat that was nearly impossible during a subject's lifetime. The iPSC approach not only enables the study of neural development and function in neurogenetic disorders using patient-specific neurons, but the neurons also harbor both the mutant gene of interest as well as other potential modifier genes within the patient's specific genomic background. Moreover, iPSCs introduce the possibility of autologous cell-based therapy, an area of research that is actively being developed and tested. For the epilepsies, animal models remain critical for studying behavioral and network aspects of the disorders, but iPSCs are quickly proving to be valuable for studying cellular and molecular pathways *in vitro* and for high-throughput drug screening platforms.

iPSCs are generated by the introduction and forced expression of specific transcription factors — the most common being the four original "Yamanaka" factors, Oct3/4, Klf4, Sox2, and c-Myc — into somatic cells, a process termed 'reprogramming.' The primary somatic cell source used most often is dermal fibroblasts, although recent studies have demonstrated the feasibility of using less invasive sources such as hematopoietic cells or kidney epithelial cells derived from urine [7, 8]. Within 3–5 weeks in culture after reprogramming, a small fraction of the starting somatic cells (0.1 to 1%) is converted to pluripotent stem cell colonies that share remarkable similarities to human embryonic stem cells (hESCs). Initially, Yamanaka and colleagues introduced the transcription factors using retroviral vectors for reprogramming; however, concerns regarding the potentially detrimental consequences of genomic integration, particularly if iPSCs would ultimately be used in a clinical setting, quickly drove the field towards non-integrating approaches for genetic modifications. Currently, most protocols utilize episomal vectors [9] or Sendai viral vectors [10], both of which are non-integrating. After reprogramming, iPSCs are theoretically an infinite source of starting material from which many relevant cell types may be derived and studied. However, the quality of each newly generated iPSC line needs to be rigorously assessed for characteristics such as genomic integrity, hallmarks of pluripotency and differentiation potential. The lines may then be used to study various aspects of pluripotency mechanisms, early human embryonic development or as human disease models by differentiating them into tissue-specific cells relevant to a disease of interest. The latter aspect is particularly exciting for disorders in which the relevant cells are difficult to acquire in large quantities from humans, such as cardiac myocytes, neurons, and hepatic cells. Furthermore, the ability to generate large quantities of tissue-specific cells introduces the possibility of high throughout drug screening and toxicity studies. To date, iPSCs have been generated for over 20 different central nervous system (CNS) disorders, ranging from neurodevelopmental to neurodegenerative diseases (reviewed in [5, 11]), and this number is growing rapidly.

Based upon increasing knowledge of the molecular cues that underlie embryonic brain development, various protocols have been developed to direct pluripotent stem cells towards specific neuronal cell fates. Many neural and brain-related cell subtypes have been generated thus far from human iPSCs (Table 1). The ability to neurally differentiate iPSCs allows one to study the development of patient-derived cells over time as they mature and manifest neurological disease phenotypes. These "disease-in-a-dish" models provide the unique opportunity to understand the progression of pathology and gain insight into the prevention

of clinical disease onset. Despite the rapid development of protocols for the derivation of multiple neuronal subtypes, however, it is important to keep in mind that most culture conditions generate a mixed population of neurons. Further advances are therefore needed to enrich for specific subtypes.

Recently, several groups have reported generating neurons directly from somatic cells by the forced expression of specific transcription factors, a process termed 'direct reprogramming' that completely bypasses the iPSC stage. One advantage of direct reprogramming for potential therapy of CNS injury is that, if done *in vivo*, this approach may allow for the conversion of cells within a glial scar to functional neurons [12]. Additionally, direct reprogramming avoids some challenges such as tumorigenesis that may arise with the clinical use of iPSCs. The direct reprogramming strategy has distinct disadvantages for studying disease mechanisms *in vitro*, however, as it does not offer a limitless supply of starting materials as with iPSCs. Some groups are now trying to circumvent this issue by direct reprogramming to a neural stem cell, rather than a post-mitotic neuron, that can then be expanded and passaged [13]. Unlike the iPSC method, moreover, direct reprogramming is more laborious for generating multiple different cell types, typically involves the use of integrating vectors, and it is not yet clear what specific neuronal subtypes may be generated with direct reprogramming techniques.

For the remainder of this review, we will focus on the use of patient-specific iPSCs, which hold extraordinary promise for the field of neurological research in that neural cells can be readily obtained and their cellular and molecular properties studied *in vitro*. It is important to note that even in the absence of patient-derived materials, new gene editing technologies, such as the clustered regularly interspaced short palindromic repeat (CRISPR) system with CRISPR-associated protein-9 nuclease (Cas9), now allow for the generation of 'virtual patients' to study neurogenetic disorders. This gene editing system relies on an elegant interplay of proteins and short guide RNAs to insert, delete or substitute base pairs or short DNA segments at specific genetic loci. Using this approach to generate a disease-specific mutation onto an otherwise unaffected human iPSC line, for example, yields isogenic 'patient' and control lines that differ only by the presence or absence of the gene mutation (which can be a base pair substitution, insertion or deletion). Alternatively, CRISPR/Cas9 can be used to correct neurological disease-causing gene mutations from patients, thereby deriving isogenic control lines that contain the patient's genomic background. This technology is a powerful tool for assaying how specific mutations affect cellular function.

iPSC Modeling of Epilepsies: Pros and Cons

Epilepsy is one of the most common neurological disorders with a prevalence amongst the general population of about 7.1 per 1000 persons. The hallmark of the vast array of epileptic syndromes is spontaneous recurrent seizures that may be focal or generalized in nature. Unfortunately, despite its high prevalence, approximately 30–40% of patients have medically refractory epilepsy that is poorly managed with anti-epileptic drugs (AEDs). A small proportion of these patients may be eligible for resective surgery but most are never evaluated. The constellation of epileptic syndromes can be either acquired or genetic in nature, although the etiology cannot always be clearly defined. Unfortunately, the mainstay

of treatment for epilepsy patients is targeted towards controlling the seizures and no therapies exist for preventing epilepsy after a neurological insult or from a genetic predisposition. Animal models exist for both acquired and genetic forms of epilepsy with most studies utilizing rodents, although models using species with less CNS complexity, such as Drosophila and zebrafish, are becoming more prevalent.

Mouse models are invaluable for studying various behavioral, histological, and electrophysiological properties of human epilepsies. Many genetically modified mice have been generated to study a host of human epilepsies and other CNS disorders in which seizures are a manifestation. Rodent models have been crucial in uncovering pathological changes after seizures and have aided the field in understanding key molecular mechanisms that drive epileptogenesis. However, important drawbacks exist for studying human disorders with rodents. For one, different strains of mice have different seizure susceptibility thresholds to both chemoconvulsants and to genetic modifications. For instance, C57BL/6 mice that are heterozygous for a mutation in *Scn1a*, a causal gene for Dravet Syndrome, develop spontaneous seizures within the first few weeks of life while 129S6/SvEvTac mice with an identical mutation do not develop an overt phenotype [14]. Using patient-derived iPSCs avoids this genetic background issue as the cells harbor the patient's genome to faithfully model the disease *in vitro.* Furthermore, many epileptic syndromes are developmental in origin yet rodent brain development is very different from that of the human brain. Neurons in the developing rodent cortex are generated almost exclusively from the neural stem cell population in the ventricular zone, the radial glia (RG) cells. These cells produce subventricular zone intermediate progenitors that then divide and migrate to generate the rodent cortex. In contrast, human cortical development also includes a vastly expanded subventricular zone which contains outer radial glia (oRG) and intermediate progenitor cells that contribute to a much more complex cortex. The oRG are sparse in rodents but can be readily identified in neurons differentiated from iPSCs. Furthermore, the oRG-like cells that can be found in human iPSC-derived neural cultures, and most recently in human cerebral organoid cultures (described below), undergo mitotic somal translocation in a similar manner to that of oRGs found *in vivo* [15].

It is important to note that despite certain advantages over rodents, human iPSC disease models have their own disadvantages. Patient iPSCs have a limited, if any, role for the investigation of acquired epilepsies, such as those caused by traumatic brain injury or stroke. In these instances, animal models with intact three-dimensional networks and in which *in vivo* insults can be recapitulated are the preferred system. Neurons derived from iPSCs fail to mimic the expansive three-dimensional architecture of the normal neuronal circuitry and are missing other crucial elements such as vasculature and microglia. These obstacles may be overcome, at least in part, by transplanting iPSC-derived neural progenitors *in vivo* into rodent brains or by growing them as cerebral organoids (which will be discussed later in this review). Another critical issue is the difficulty of generating fully mature cell types, including neurons, from iPSCs. Functional studies of iPSC-derived neurons demonstrate an immature electrophysiological profile that more closely mimics the neurons within the developing embryonic brain rather than those of the adult brain. This hurdle is significant particularly for studies of late-onset neurological disorders such as Parkinson's and Alzheimer's diseases. However, many groups are working to overcome this obstacle by

creative measures such as prematurely aging cells [16] as well as deriving new culture conditions to promote neuronal maturation and the acquisition of mature electrophysiological properties [17]. Lastly, concerns exist regarding variability when using female iPSCs as a result of erosion of X-chromosome inactivation (XCI). Studies have demonstrated that derivation conditions, passage number, and feeder cells all heavily impact the XCI status of iPSCs [18, 19]. Work in the field has shown that the inactive X-chromosome can reactivate in female iPSCs and remain active despite neuronal differentiation, leading cells to aberrantly express some genes through active transcription from both X-chromosomes [20]. These findings point to a need to carefully monitor the XCI status in female iPSCs and their terminally differentiated progeny, particularly in X-linked disorders.

iPSC Modeling of Genetic Epilepsies

In the 25 years since the first gene linked to a clinical seizure phenotype was discovered [21], there has been an explosion in the number of genetic loci that are reported to be important in epilepsy. To date, over 500 genes are listed [22] and this number is rising rapidly. Human iPSC models of genetic epilepsies are increasingly being developed to link the causative gene mutation with altered neuronal function and to explore seizure mechanisms. Using iPSCs to model genetic epilepsies with early childhood or infantile onset is currently favored by many groups owing to the ease of modeling early development with iPSCs. Disorders with complete or near complete epilepsy penetrance that have been modeled to date include Dravet syndrome [23–25], Angelman syndrome [26], and a Rett syndrome variant caused by cyclin-dependent kinase-like 5 (*CDKL5*) mutations [27, 28]. Due to space limitations, only the Dravet syndrome (DS) studies will be discussed here. DS is an epileptic encephalopathy with onset in the first year of life that manifests with intractable seizures followed by cognitive regression. Most Dravet syndrome cases are caused by *de novo* loss-of-function mutations in the *SCN1A* gene that encodes for the voltage-gated sodium channel, $Na_V1.1$. Initial findings from mouse Dravet syndrome models suggested loss of sodium channel function selectively in cortical GABAergic interneurons, particularly in parvalbumin- and somatostatin-expressing interneurons, with no loss of sodium currents in excitatory pyramidal neurons [29–31]. These studies led to the hypothesis that the hyperexcitability in Dravet syndrome was due to a loss of network inhibition. Indeed, another study described similar findings in differentiated GABAergic neurons from one patient with Dravet syndrome that showed decreased action potential generation [23]. Despite these results pointing to interneuron dysfunction, however, other groups have reported distinctly different findings. Liu *et al.* examined iPSCs derived from two Dravet syndrome patients differentiated into mixed populations of forebrain-like GABAergic and glutamatergic neurons [25]. The group found increased sodium current in both excitatory and inhibitory neurons as compared with control neurons, in addition to a decreased threshold for action potential generation and spontaneous bursting. The hyperexcitability findings were recapitulated by another group using both DS-iPSC-derived neurons as well as induced neurons directly reprogrammed from DS patient fibroblasts [24]. Subsequent studies of a second DS *Scn1a* knockout mouse model, using developmental time points not studied previously, reported increased sodium currents and excitability of

hippocampal pyramidal neurons [32], supporting the mechanisms of excessive excitation and reinforcing the value of studying patient-derived neurons. Taken together, these findings suggest that seizure-generating network dysfunction in DS most likely arises from complex alterations involving both inhibitory and excitatory circuitry.

Beyond “pure” genetic epilepsy syndromes, several other neurodevelopmental disorders with seizures as a manifestation have been studied using iPSCs, including Rett syndrome, fragile X syndrome (FXS), Timothy Syndrome, 15q11.2 deletion syndrome, and Phelan-McDermid syndrome (PMDS). These studies have led to key insight into disease mechanisms. For instance, Rett syndrome iPSC-derived neurons demonstrate decreases in excitatory synaptic numbers and dendritic spine density [33–36] while FXS iPSC-derived neurons have abnormalities in maturation and synaptic transmission [37–41]. Another powerful aspect of the iPSC approach is that it can be used to generate multiple tissue types to study disorders such as Timothy syndrome, an autism-spectrum disorder caused by a calcium channel mutation that affects both the heart and the brain. Studies of Timothy syndrome patient-iPSCs demonstrate that both cardiomyocytes and neurons have abnormalities such as altered cardiac transmission and contraction, and impaired neuronal differentiation [42–44]. Finally, Yoon *et al.* demonstrate that iPSC disease modeling can inform *in vivo* animal studies when they discovered that neural rosettes derived from 15q11.2 deletion patient-iPSCs have defects in adherens junctions and apical polarity, findings that were recapitulated in a mouse model of the disorder [45].

PMDS is caused by loss-of-function mutations in the *SHANK3* gene and manifests as an autism spectrum disorder with intellectual disability that often is accompanied by seizures. Mouse models of PMDS exist but demonstrate key phenotypic differences from that of the human disease, likely to due to the large number of isoforms that exist for the SHANK3 protein. Shcheglovitov *et al.* generated iPSCs from two different PMDS patients with *SHANK3* deletions and subsequently differentiated the iPSCs into cortical-like neurons [46]. The group found that PMDS patient excitatory neurons had defects in excitatory synaptic function as a result of decreased excitatory neurotransmitter receptors as well as fewer excitatory synapses. This phenotype was rescued by the expression of SHANK3 protein or treatment with insulin-like growth factor 1 (IGF1). These results demonstrate that iPSCs are able to capture aspects of human disease that are not manifested in rodent models and may inform future development of therapeutics.

Sudden unexpected death in epilepsy (SUDEP) is the most devastating consequence of epilepsy. It is estimated that SUDEP accounts for as many as 15% of all epilepsy-related deaths [47] and the risk of sudden death is 24-fold higher in patients with epilepsy than the general population [48]. The mechanisms of SUDEP remain unknown although growing evidence suggests that cardiac, autonomic, and respiratory dysfunction, alone or in combination, are involved to varying degrees in any given case. Patients with DS appear to have among the highest risk of all epilepsies for developing SUDEP, suggesting that the channelopathies, disorders where multiple cell types are affected by an ion channel gene mutation, may be particularly informative epilepsies for understanding SUDEP. Mutations in genes encoding for ion channels are increasingly being identified as a genetic basis for epilepsies and much work has been done to understand mechanisms behind their

pathogenesis [49]. These same ion channels that were once thought to only be involved in neural excitability are being increasingly identified in other regions such as the heart, and many are expressed in brainstem and autonomic neurons in addition to the forebrain. Protocols already exist or are being developed to derive cardiomyocytes [50, 51], autonomic neurons [52–54], and brainstem serotonergic neurons, the latter which are responsible for carbon dioxide sensing in the brainstem, an important component of normal respiratory function. Using iPSCs from patients with channelopathies such as the previously mentioned DS, potential cellular mechanisms of SUDEP and risk-related biomarkers may be studied by differentiating iPSCs into specific tissue cell types involved in regulating cardiorespiratory function.

Cell therapy for epilepsies

The advent of iPSC methods has transformed the field of regenerative medicine because it introduces the possibility of personalized cell therapy. There are considerable advantages in being able to graft neural cells derived from a subject's own cells back into them for neural repair. Transplantation of human iPSC-derived neural stem cells into human brains has yet to be achieved as numerous safety issues need to be addressed and great strides remain to be made to understand the mechanisms and improve upon the therapeutic feasibility and benefits behind iPSC-derived neural transplantation. Despite the exceptional skills of many groups at generating neural stem cells of multiple lineages *in vitro*, integration of these stem cells when introduced into a brain, particularly a human brain, remains a critical obstacle for the field.

For experimental studies of transplantation applications, iPSCs are differentiated *in vitro* into neural progenitors and the progenitors are injected into the brain of animal models and allowed to reach maturity *in vivo*. Initial studies involve exploring how well the neural progenitors survive, migrate, and integrate into the environment to ultimately provide therapeutic benefits. One obstacle is that the injected cells are generally not pure and represent a mixture of post-mitotic neurons and progenitors that can lead to aberrant growth or migration, as well as teratoma formation if any pluripotent stem cells remain in the cultures. Recently, Tornero *et al.* demonstrated that human iPSC-derived cortical-like progenitors engrafted into the rat cortex after focal ischemic injury were able to integrate and function [55]. These implanted progenitors ultimately matured into functional cortical neurons with no tumor formation, and they also modestly improved behavioral recovery from stroke in the host animal. These results are promising but the *in vivo* maturation of the progenitors is slow, following the prolonged human embryonic developmental time course rather than that of the host, and the rodent brain, while informative in these studies, does not possess the challenges of size and complexity present in the human brain. However, the latter issues are being addressed by recent work showing that autologous transplantation of rhesus monkey iPSC-derived neural stem cells into the monkey brain led to differentiation and integration of the cells with very little inflammation, overgrowth, or tumor formation [56]. These results demonstrate that autologous transplantation in nonhuman primates is feasible and may help inform future attempts at engraftment in the human brain.

For therapeutic transplantation in epilepsy, generating inhibitory interneurons is the obvious strategy to ameliorate seizures. Advances in developmental biology over the past two decades have identified the MGE as the ideal source of interneuron progenitor subtypes that will strengthen inhibitory tone in cortical circuits [57]. A recent, elegant study demonstrated that transplantation of mouse MGE-derived inhibitory interneuron progenitors into the epileptic adult mouse brain significantly reduces the occurrence of seizures [58]. This finding heralds tremendous possibilities in the therapeutic potential of using human pluripotent stem cell-derived inhibitory interneurons for the treatment of epilepsy. Knowledge of key developmental pathways by which MGE progenitors and their cortical interneuron progeny are generated has yielded successful recent efforts to differentiate MGE-like progenitors and cortical interneurons from human PSCs [59, 60]. While inhibitory interneuron progenitors have great capacity for migration that is ideal for cell therapy in the human brain, a major obstacle in their feasibility for human studies is their long maturation timeline. It is difficult to attain the full repertoire of cortical inhibitory interneurons; for instance, most differentiation protocols successfully generate somatostatin-expressing interneurons but parvalbumin-expressing interneurons are rare and appear much later in the differentiation period. These different interneuron subtypes have unique and important roles in modulating network excitability and their slow developmental timeline remains a significant challenge. Nonetheless, a recent study demonstrated that implanting hESC-derived GABAergic interneuron progenitors into the epileptic mouse brain was able to greatly attenuate, if not completely eliminate, seizure activity. These grafted hESC-derived neurons were able to integrate into the mouse hippocampus and demonstrated normal physiological hallmarks of GABAergic interneurons. Furthermore, the progenitors matured into GABAergic interneurons that expressed somatostatin, parvalbumin, calretinin, neuropeptide Y, and calbindin [61]. These and other studies demonstrate great promise in utilizing patient iPSC-derived interneurons for the treatment of the epilepsies.

Challenges and future directions

The field of stem cell disease modeling and regenerative therapy is progressing at a rapid rate and exciting strides are continuously being made to build upon current technologies. A recent landmark paper from Lancaster *et al.* demonstrated that human PSCs, including both ESCs and iPSCs, have powerful self-organizing capacities and could be grown as 3-dimensional neuronal structures known as cerebral organoids [62]. The organoids share many features of the human brain including progenitor zones that variably recapitulate the dorsal cortical ventricular zones, laminar cortical structures, primitive regional specifications, as well as an enlarged subventricular zone with oRGs (Figure 1). The group was able to show that organoids grown from iPSCs derived from a patient with autosomal recessive primary microcephaly due to a truncation mutation of cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (*CDK5RAP2*) have premature neuronal maturation as compared with control organoids, a phenotype that was partially rescued by restoring the missing protein. Cerebral organoids are a valuable tool towards modeling and understanding the epilepsies because they not only allow for the study of the entire brain in a more physiological, 3-dimensional structure, but also generate many of the cell types relevant for human brain diseases in parallel. Many current protocols generate pure populations of

specific neuronal cell types that are later artificially mixed with other cell types which may not mimic the development of these cells *in vivo*. Cerebral organoids overcome this hurdle and allow for many different cell types to develop together, which is critical for studying epileptogenic disorders that are developmental in origin.

Another promising direction related to epilepsy patient-derived iPSCs is in drug screening. Treating patients with epilepsy usually involves empirically administering AEDs, but not all AEDs work for every patient or every type of epilepsy. Not only are some AEDs ineffective for some patients but, importantly, certain AEDs may increase seizures in certain types of epilepsies. Rodent models are commonly used for pre-clinical testing of new drugs, but many promising drugs have not panned out in clinical trials despite improving symptoms in rodent models. Furthermore, the size and expense of rodents make them difficult to use for high-throughput drug screening. iPSCs are currently being explored for use in high throughput drug screening as they offer the unique opportunity to develop and test therapies on patient-specific neurons. In conjunction with the recently developed multi-well, multielectrode array (MEA) platform, iPSCs give the field of epilepsy research a unique opportunity to rapidly screen large drug libraries on patient-derived neurons. Although not directly related to epilepsy, a recent study by Wainger *et al.* utilized iPSC technology with MEAs to demonstrate that motor neurons generated from ALS patient-derived iPSCs are hyperexcitable due to a reduction in the delayed-rectifier potassium current. They ameliorated the hyperexcitability and motor neuron death using the drug retigabine, a potassium channel activater, which is already FDA approved in the United States as an anticonvulsant. This work has led to a recent phase 1 clinical trial to test the utility of retigabine in the treatment of human ALS. This same approach holds great promise for abnormalities of excitability caused by epilepsy and suggests that the ultimate goal of patient-specific precision therapy is attainable.

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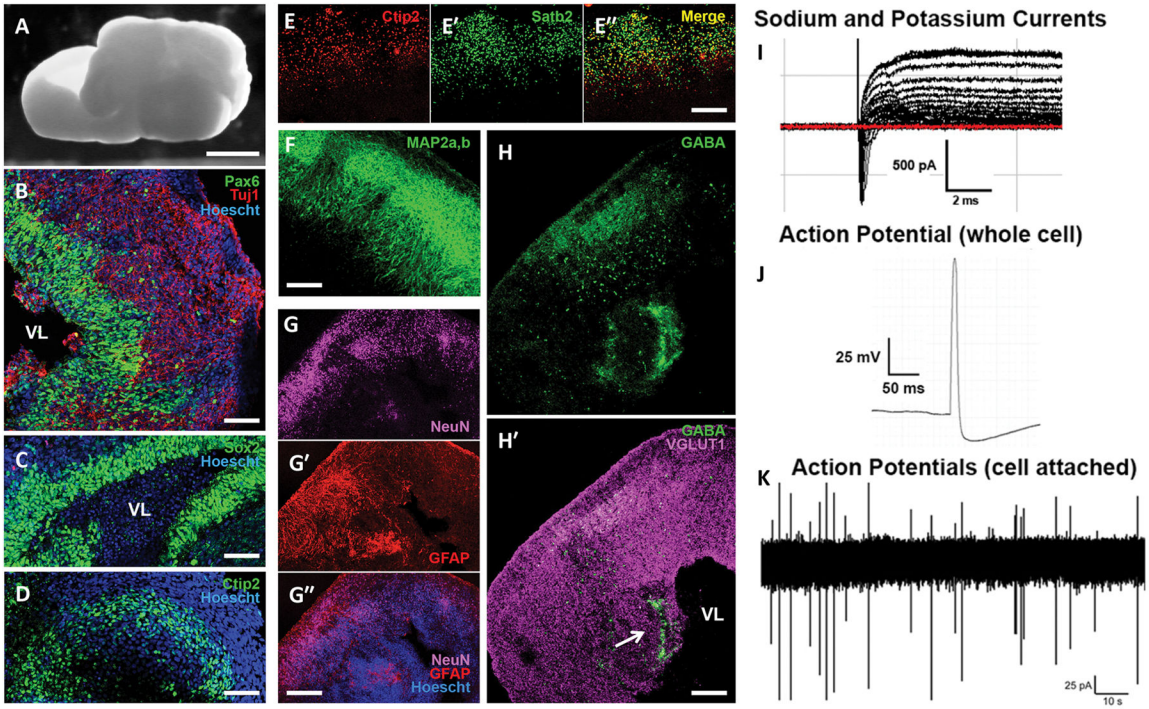

Figure 1.

Cerebral organoids derived from human iPSCs at 2 months (A–D) and 4 months (E–H). **A**) Low-magnification brightfield image of a cerebral organoid at 2 months in culture. **B**) Immunohistochemistry at 2 months shows Pax6+ neural progenitors surrounding a ventricular-like zone (VL) and numerous TuJ1+ immature neurons in the cortical mantle-like structure. **C, D**) Sox2+ neural progenitors cells surround a VL (**C**) dorsal cortical laminar organization appears with cells that express the deep cortical layer marker Ctip2. **E-E″**) At 4 months of age, organoids demonstrate cortical lamination with Ctip2+ deep layer neurons (**E**) and Satb2+ upper layer neurons (**E′**). The merged image is shown in the right panel (**E″**). **F**) Map2a,b immunostaining reveals mature neurons in a structured cortical-like region. **G-G″**) NeuN staining confirms the mature identity of the cortical neurons (**G**) and GFAP+ cells (**G′**) demonstrate the presence of astrocytes within the organoids. Bottom panel shows the merged image (**G″**). **H-H′)** Most neurons within the organoid are glutamatergic neurons (VGLUT1+; magenta in **H′**) with some GABAergic neurons (GABA+) scattered throughout (**H**; green in **H′**), as well as more densely packed in a region reminiscent of the ganglion eminence (arrow in **H′**). **I-K**) Whole-cell patch clamp recordings from human iPSC-derived cerebral organoids at 4 months of age reveal that the neurons within have sodium and potassium currents (**I**) and fire action potentials as seen with whole-cell (**J**) and cell-attached (**K**) configurations. Scale bar = 1 mm for **A**; 100 μm for **B–E**; 200 μm for **F–H.**

Table 1

Neural and other brain-related cell types differentiated from iPSCs.

Cell type	Reference
Cortical-like excitatory neurons	[63, 64]
Dentate granule cell-like neurons	[65]
Cortical-like inhibitory interneurons	[59, 60]
Motor neurons	[66, 67]
Dopaminergic neurons	[68, 69]
Astrocytes	[70, 71]
Oligodendrocytes	[72]
Brain microvascular endothelial cells	[73]