Progress in the molecular mechanisms of genetic epilepsies using patient-induced pluripotent stem cells

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Summary

Research findings on the molecular mechanisms of epilepsy almost always originate from animal experiments, and the development of induced pluripotent stem cell (iPSC) technology allows the use of human cells with genetic defects for studying the molecular mechanisms of genetic epilepsy (GE) for the first time. With iPSC technology, terminally differentiated cells collected from GE patients with specific genetic etiologies can be differentiated into many relevant cell subtypes that carry all of the GE patient’s genetic information. iPSCs have opened up a new research field involving the pathogenesis of GE. Using this approach, studies have found that gene mutations induce GE by altering the balance between neuronal excitation and inhibition, which is associated, among other factors, with neuronal developmental disturbances, ion channel abnormalities, and synaptic dysfunction. Simultaneously, astrocyte activation, mitochondrial dysfunction, and abnormal signaling pathway activity are also important factors in the molecular mechanisms of GE.

KEY WORDS: Genetic epilepsy, Induced pluripotent stem cells, Synapse, Ion channels.

Genetic epilepsy (GE) is a genetic disease with epileptic seizures as a core symptom that is directly caused by known or presumed genetic defects.1–4 Due to ethical constraints, it is impossible to perform experiments on patients or to obtain their brain tissue for research. Research on the molecular mechanisms of GE has therefore been performed almost exclusively in animal models.5 The differences between humans and animals often prevent animal models from truly reflecting the reasons for the development of epilepsy in humans. Induced pluripotent stem cell (iPSC) technology is creating a new platform for the study of the molecular mechanisms of GE. With this new technology, terminally differentiated cells collected from GE patients who present with specific genetic patterns can be reprogrammed into iPSCs and then differentiated into neuronal subtypes. These cells carry all of a patient’s specific genetic information, including its differences from healthy controls. Thus, iPSCs can provide a reliable platform for the study of the molecular mechanisms of GE.6–25

Studies using iPSC technology to investigate the molecular mechanisms of GE have included studies focusing on Rett syndrome, Dravet syndrome, Phelan-McDermid syndrome (PMDS), and fragile X syndrome (FxS). Classic GE syndromes include childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and epilepsy with generalized tonic–clonic seizures alone (GTCS). A recent study using human genetic screening and animal experiments confirmed that CAE and JAE are associated with multiple combined mutations in T-type calcium channels and γ-aminobutyric acid (GABA)A receptors rather than single-gene mutations.26–28 In
addition, gene mutations in several proteins other than ion channels can also cause seizures through spikes in discharges that create abnormal neural circuits in the cortex and thalamus. Abnormalities in the frontal cortex and thalamus are the major changes that are observed via imaging of JME. Bilateral thalamic neuron dysfunction and a thinner corpus callosum may be markers of structural brain changes in JME patients compared with GTCS patients. However, studies on the pathogeneses of CAE, JAE, JME, and GTCS using iPSC technology have not been performed. Here, we review and summarize several recent studies on the molecular mechanisms of genetic epilepsies in a subset of patients. iPSC models for most of these diseases have demonstrated altered processes of neuronal excitation and inhibition caused by neurodevelopmental disturbances, ion channel abnormalities, and changes in synaptic functions. In addition, astrocyte activations, mitochondrial dysfunctions, and signaling pathway activations have been observed in some of these disease models.

**Key Points**

- This review discusses the progress made in using patient-induced pluripotent stem cells to study the molecular mechanisms of genetic epilepsies in a subset of patients.
- iPSC models for most of these diseases have demonstrated altered processes of neuronal excitation and inhibition caused by neurodevelopmental disturbances, ion channel abnormalities, and changes in synaptic functions.
- In addition, astrocyte activations, mitochondrial dysfunctions, and signaling pathway activations have been observed in some of these disease models.

**Development of iPSC Technology**

In 2006, Takahashi et al. first proposed the concept of iPSCs. The authors introduced 4 transcription factors into fibroblasts obtained from a rat and a human patient and successfully reprogrammed the fibroblasts into iPSCs. Next, Higurashi et al. used this technology to study a patient with Dravet syndrome, which is characterized by refractory seizures. The authors differentiated the iPSCs into neurons and, using electrophysiologic techniques, detected weak action potentials in inhibitory neurons. Subsequently, Livide et al. induced different iPSCs from the cells of a patient with Rett syndrome carrying a methylated CpG-binding protein 2 gene (MECP2) mutation (p.Arg306Cys) and from the cells of 2 patients with Rett syndrome carrying cell cycle–dependent kinase-like protein 5 gene (CDKL5) mutations (p.Gln347Ter and p.Thr288Ile) and from the cells of 2 patients with Rett syndrome carrying mutations (p.Gln347Ter and p.Thr288Ile) and from the cells of 2 patients with Rett syndrome carrying mutations.

**Influence of the transmembrane motion of ions**

The normal transmembrane movement of ions is the main factor that generates and maintains the action potential and the resting membrane potential of neurons. Abnormal ion channels can lead to abnormal transmembrane movement of ions and serve as the basis of seizures.

**Voltage-gated sodium channel abnormalities**

Many subtypes of voltage-gated sodium channels (Nav) have been identified, and each channel consists of a large α subunit and 2 small β subunits. Mutations in the genes SCN1A (encoding Nav1.1), SCN2A (encoding Nav1.2), SCN3A (encoding Nav1.3), SCN8A (encoding Nav1.6), SCN9A (encoding Nav1.7), and SCN1B (encoding Navβ1) produce abnormal voltage-gated sodium channels and are associated with epilepsy. Nav1.1 is expressed at high levels in the central nervous system, and SCN1A mutations, of which there are more than 1,257 types, cause various types of epilepsy. Ten percent of SCN1A mutations on chromosome 2 are associated with generalized epilepsy and febrile seizures plus (GEFS+). SCN1A mutations cause approximately 85% of Dravet syndrome cases, which are characterized by intractable infantile seizures and cognitive impairment. Using an iPSC technique, Jiao et al. studied Dravet syndrome patients with SCN1A (F1415I) mutations and GEFS+ patients with SCN1A (Q1923R) mutations and found that the glutamatergic neurons derived from both types of patients exhibited hyperexcitability. The sodium currents and action potentials in the glutamatergic neurons from the Dravet syndrome patients were stronger than those from the GEFS+ patients, which is consistent with the severity of the clinical seizures in the 2 conditions. However, several subsequent studies using iPSCs combined with CRISPR/Cas9 gene repair techniques, and neuron-specific fluorescence labeling techniques confirmed that SCN1A gene mutations are more likely to cause reduced inhibition throughout the neural network by weakening the activity of one healthy control. These researchers found that these mutations downregulated the ionic glutamate receptor D1 (GluD1), which is known as a synaptic adhesion molecule that maintains normal presynaptic and postsynaptic membrane stabilities. Some subsequent studies have suggested that an MECP2 mutation decreases neuron size and synapse number and affects the differentiation of glutamatergic neurons. These abnormal phenotypes can be alleviated by using insulin-like growth factor 1 (IGF1), which interacts with IGF1/IGF1R and thyroid hormone receptor (TRalpha 3). Thus, iPSC technology has created favorable conditions for the direct study of the molecular mechanisms of human GE.
| Gene     | Protein | Disease                  | Findings                                                                                                                                  | Publication(s)                      |
|----------|---------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| SCN1A    | Nav1.1  | Dravet syndrome          | Deficits in sodium currents and action potential firing, increased excitatory level of spontaneous postsynaptic activity | Liu et al. (2013)\(^{54}\), Jiao et al. (2013)\(^{52}\), Higurashi et al. (2013)\(^{26}\), Liu et al. (2016)\(^{21}\), Sun et al. (2016)\(^{53}\) |
| GEFS+    | ICEGTC  | SMEI                     |                                                                                                                                         | Liu et al. (2016)\(^{21}\)          |
| CACNA1C  | Cav1.2  | Timothy syndrome         | Defects in calcium signaling, limited neuronal differentiation                                                                            | Pasca et al. (2011)\(^{58}\), Krey et al. (2013)\(^{59}\), Chou et al. (2016)\(^{24}\), Hamalainen et al. (2013)\(^{62}\) |
| MTK      | tRNALys | MERRF syndrome           | Enhanced mitochondrial autophagy, reduced growth                                                                                         | Yamashita et al. (2016)\(^{67}\)    |
| STXBP1   | STXBP1  | Ohtahara syndrome        | Impairment in synaptic transmission and neurite outgrowth                                                                               | Patzke et al. (2016)\(^{66}\)       |
| SHANK3   | SHANK3  | PMDS                     | Defects in excitatory synaptic transmission, insulin-like growth factor I (IGF1) can be rescued in some phenotypes                  | Shcheglovitov et al. (2013)\(^{55}\) |
| PIGA     | PIGA    | MCAHS2                   | Decreased proliferation, abnormal membrane depolarization                                                                              | Yuan et al. (2017)\(^{75}\)         |
| MECP2    | MECP2   | Rett syndrome            | Reduces GABAergic neurotransmission, fewer synapses, reduced dendritic arborization and reduced spine density; IGF1 causes neurite improvement that may be related to the thyroid hormone receptor | Chen et al. (2018)\(^{41}\), Yoo et al. (2017)\(^{40}\), de Souza et al. (2017)\(^{43}\), Chin et al. (2016)\(^{42}\) |
| CDKL5    | CDKL5   | Rett syndrome            | Phosphorylated MECP2, aberrant dendritic spines Impaired methylation of the FMR1 promoter region, defective neurite initiation and extension, increased differentiation of CP-AMPAR and N-methyl-D-aspartate (NMDA) receptor-coexpressing cells lacking GluA2 | Livide et al. (2015)\(^{37}\), Tellas et al. (2013)\(^{79}\), de Souza et al. (2017)\(^{43}\), Li et al. (2017)\(^{21}\) |
| FMR1     | FMR1    | FxS                      |                                                                                                                                         | Fink et al. (2017)\(^{70}\), Okunoe et al. (2017)\(^{57}\), Stanurova et al. (2016)\(^{88}\), Bershteyn et al. (2017)\(^{13}\), Iefremova et al. (2017)\(^{96}\) |
| UBE3A    | UBE3A   | Angelman syndrome, PWS   | Altered resting membrane potential, neuron-specific long noncoding RNA (lncRNA) - in the silenced paternal UBE3A                  | Achuta et al. (2018)\(^{84}\), Chamberlain et al. (2010)\(^{86}\), Okunoe et al. (2017)\(^{57}\), Fink et al. (2017)\(^{70}\), Chen et al. (2016)\(^{90}\), Stanurova et al. (2016)\(^{88}\), Iefremova et al. (2017)\(^{96}\) |
| I7p13.3  |         |                         |                                                                                                                                         | Tang et al. (2013)\(^{103}\), Williams et al. (2014)\(^{104}\), Odawara et al. (2014)\(^{102}\), Kondo et al. (2016)\(^{99}\), Ishii et al. (2017)\(^{101}\), Lischka et al. (2018)\(^{105}\), Machado et al. (2016)\(^{20}\) |
| GFAP     | GFAP    | Alexander disease        | Increased glutamate neurotransmitter release, nerve activity alterations, neuronal maturation promoted by mature glial cells        |                                                                                       |
| ARHGEF9  | CB      | X-linked intellectual disability with epilepsy | Disinhibited mTORC1 signaling contributes to the pathologic process mTORC1 pathway hyper activation, defects in neuronal differentiation, hypoexcitability, and reduced synaptic activity | Sundberg et al. (2018)\(^{112}\), Li et al. (2017)\(^{111}\), Ebrahimi-Fakhari et al. (2016)\(^{110}\), Ebrahimi-Fakhari et al. (2015)\(^{109}\) |
| TSC1/TSC2| Hamartin| Tuberous sclerosis       |                                                                                                                                         |                                                                                       |

AMPA, a-amino-3-hydroxy-5-methyl-4-boxazolespropane acid; FxS, fragile X syndrome; GEFS+, mild inherited disorder generalized epilepsy with febrile seizures plus; GFAP, glial fibrillary acidic protein; ICEGTC, intractable childhood epilepsy with generalized tonic–clonic seizures; MERRF, myoclonic epilepsy associated with ragged-red fibers; MCAHS2, multiple congenital anomalies-hypotonia-seizure syndrome 2; NMDA, N-methyl-D-aspartic acid receptor; PMDS, Phelan-McDermid syndrome; PWS, Prader-Willi syndrome; SMEI, severe myoclonic epilepsy in infancy; tRNALys, mitochondrial transfer RNA for lysine.
Moreover, until a later period of differentiation, the sodium ion currents, action potential thresholds, and spontaneous discharge frequencies in these 2 conditions were demonstrated to differ from those of healthy controls. The overactivation of sodium channels leads to enhanced neuronal activity, which leads to network hyperexcitability.54

Voltage-gated calcium channel abnormalities

Niemann-Pick type C (NPC1) is a rare progressive neurodegenerative disease that presents with cerebellar atrophy, decreased cognitive abilities, and severe epilepsy. Children generally die within 1–2 years after onset.55 Electrophysiologic examinations have revealed attenuated calcium-mediated currents in induced neurons and reduced voltage-gated ion channel activity.56

A mutation in the fragile X mental retardation 1 gene (FMR1), which is on the X chromosome and encodes a multifunctional polyribosome-associated RNA-binding protein (FMRP), is found in FxS patients with mental retardation and seizures. Liu et al.57 detected fewer synapses, reduced expression of the excitatory postsynaptic marker PSD95, reduced synaptic puncta density and neurite length, and increased frequencies and amplitudes of the evoked currents in these differentiated neurons, all of which could be rescued by a calcium channel blocker. These findings indicate that calcium channel abnormalities are the cause of the abnormal morphologies of neurons derived from iPSCs with mutations in FMR1.

Mutations in the CACNA1C gene encoding the L-type voltage-gated calcium channel (Cav1.2) leads to Timothy syndrome, which is characterized by long-QT syndrome (LQTS), epilepsy, and autism.58 Compared with normal controls, abnormal current signals induced by calcium ions in iPSCs derived from these patients have been observed, and neuronal differentiation is limited. The abnormal discharge activity of the neurons and RhoA signaling pathway hyperactivity could be rescued by the L-type calcium channel blocker nimodipine. Channel abnormalities may be one of the important pathogenic mechanisms that lead to nervous system disease with epilepsy.59–60

Mutations affecting mitochondrial function

Mitochondrial DNA mutations are a common cause of nervous system diseases, but the molecular mechanisms are unclear. In 1991, Noer et al.61 found that mutations in the mitochondrial gene MTTK, which encodes the mitochondrial transfer RNA for lysine (tRNAlys), cause the genetic disease myoclonic epilepsy associated with ragged-red fibers (MERRF) syndrome, which occurs in children aged 5–15 years and is characterized by muscle spasms, seizures, and ataxia. Chou et al.24 studied the possible mechanisms of this disease and found that MTTK mutations can lead to decreased oxygen consumption, increased reactive oxygen species (ROS) generation, slower cell growth, and abnormal mitochondrial morphology in iPSCs derived from patients with MERRF syndrome. Furthermore, derived neural precursor cells also display mitochondrial dysfunction, increased ROS generation, and upregulated antioxidant gene expression levels. Hamalainen et al.62 further explored the mechanism of epilepsy caused by abnormal mitochondrial function. These authors found that respiratory chain complex I was degraded by autophagy mediated by PTEN kinase 1 (PINK1) and Parkin. Mitochondrial autophagy has been suggested to play an important role in the pathogenesis of mitochondrial encephalopathy complicating GE.

Mutations affecting neuronal development

Neuronal development includes the maturation of neurites and neuronal migration.63 Structural changes in and dysfunctions of neurites and abnormal migration during the neuronal development period will generate dysplastic neurons,64 and this process is an important factor in the generation of epilepsy.

Mutations affecting neurite maturation

Mutations in STXBP1, the gene that encodes syntaxin-binding protein 1 (STXBP1), can lead to various types of seizures.65 Patzke et al.66 used gene knockout combined with Cre/Lox and optogenetic techniques to achieve the targeted knockout of STXBP1 gene expression in neurons from healthy human-derived iPSCs and found that the mutant neurons exhibited a 50% reduction in synaptic transmission. Subsequently, another group studied iPSCs from patients with Ohtahara syndrome with STXBP1 mutations (c.1099C>T; p.R367X), characterized by frequent tonic seizures and burst-suppression electroencephalograms in infancy. The levels of the transcription and protein expression of STXBP1 in the mutated iPSC neurons were reduced by 50% compared with the levels in the induced neurons in the controls, and the growth of neurites was significantly decreased.67 These results suggested that STXBP1 gene mutations affect neurite growth and synaptic transmission.

Phelan-McDermid Syndrome, which is characterized mainly by seizures, mental retardation, and language disorders, can be caused by the deletion mutation 22q13.3 in the SHANK3 gene, which encodes an excitatory postsynaptic scaffolding protein.68 Shcheglovitov et al.69 generated iPSCs from individuals with PMDS and autism and used them to produce functional neurons. These authors found that the SHANK3 mutation could lead not only to decreased synaptic transmission but also to abnormal excitatory synapses with downregulation of N-methyl-D-aspartate (NMDA) receptors. Gene repair or IGF1 interventions can restore excitatory synaptic transmission. It has been suggested that deletions in the SHANK3 gene could possibly mediate the mechanism of epileptic seizures by causing a decrease in excitatory synaptic function.70–73

Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2), characterized by epilepsy, myoclonus,
and mental retardation, can be caused by mutations in another membrane protein gene, the phosphatidylinositol glycan class A gene (PIGA), which encodes a phosphatidylinositol glycan class A protein (PIGA), one of the phospholipid elements of the cell membrane. Yuan et al. found that iPSCs from patients with PIGA mutations (c.1234C>T) exhibit lower proliferative capacity than normal heterozygotes, fewer induced GABAergic neurons with low maturation levels, aberrant synapse formation, and abnormal membrane depolarization. These results suggest that mutations in the PIGA gene may lead to the development of MCAHS2 by reducing inhibitory synaptic function.

The FMR1 gene encodes a ribosome-associated mRNA-binding protein (FMRP) that is involved in regulating the transcriptional efficiency of the target gene, but an expansion of CGG repeats in a region of the gene leads to FMR1 silencing. Patient-derived neurons display defective neurite initiation and extension, reduced synaptic function, reduced synapse number, and reduced expression of the excitatory postsynaptic marker PSD95. Subsequently, de Esch et al. confirmed that hypermethylation of the FMR1 gene promoter leads to the absence of FMRP and that demethylation could significantly rescue neural cell development. FMR1 gene silencing also leads to transcriptional changes in neuronal differentiation-related genes (i.e., WNT1, BMP4, POU3F4, TFAP2C, and PAX3) and synapse-related protein genes (i.e., SHANK1 and NNA7). The inhibition of RE-1 silencing transcription factor (REST) induces the upregulation of many of these related genes. These results demonstrate that FMR1 gene silencing affects various protein molecules that are involved in neurodevelopment, thereby causing disorders in neuronal axon growth and synapse formation. A recent study found that FMR1 mutations lead to decreased expression of GluA2 in AMPA receptors in patient-derived neurons, yielding increased proportions of calcium-permeable AMPAR- and NMDA receptor–coexpressing cells with an abnormal increase in the calcium current. These alterations may be important bases for seizures because FMR1 silencing causes synaptic dysplasia, abnormal excitatory synaptic receptor expression, and neural network hyperactivity.

Angelman syndrome (AS) is a type of maternally inherited disease that is characterized by severe developmental delay, pleasant affect, and epileptic seizures. The causative gene UBE3A, located on chromosome 15q11.2-q13, encodes ubiquitin protein ligase E3. Deletion mutations in the same region of the paternal-origin chromosome lead to Prader-Willi syndrome (PWS). Hypermethylation of chromosome 15q11.2-q13 is present in all patients. Therefore, hypermethylation of the UBE3A gene has previously been considered the main cause of these diseases. However, hypermethylation has not been obvious in iPSC studies. Changes in the UBE3A gene affect the functional maturation of neurons, and the appearance of AMPA receptor–mediated excitatory postsynaptic currents (EPSCs) in patient-derived neurons is significantly delayed. In the late stage of neuronal development and maturation, the resting membrane potential exhibits depolarization, decreased spontaneous excitatory postsynaptic action potentials, decreased neuronal activity, and decreased synaptic plasticity. In combination with the CRISPR/Cas9 gene-editing technique, it was demonstrated that the depolarization of the resting membrane potential induced by the UBE3A mutation might be an important cause of altered cell activity. Chen et al. explored the underlying pathophysiology using iPSC-derived neurons from patients with Angelman syndrome and unaffected controls and found that the long noncoding RNAs (lncRNAs) RNA-binding protein fox-1 homolog (RBFOX1) and RBFOX2 were downregulated in Angelman syndrome neurons. When UBE3A was overexpressed, the differentiation abnormalities in the immature cells were rescued. These results suggested that the deletion of these lncRNAs was a key reason for the abnormal differentiation of immature cells caused by UBE3A gene mutation.

Mutations affecting neuronal migration

Miller-Dieker syndrome, characterized by the clinical manifestations of mental retardation and intractable epilepsy, is associated with 17p13.3 heterozygous deletion mutations. This 17p13.3 region includes the gene LIS1, which participates in neural migration, and the gene YWHAE, which binds with a phosphoserine on another interacting protein. Loss of 17p13.3 leads to increased iPSC apoptosis, delayed cell migration, and abnormal mitosis of the primate-specific outer radial glial cells, which decreases the expression of the normal properties of radial glial cells during cortical development. Some studies have demonstrated that the quality and proliferation properties of outer radial glial cells are critical to the process of cortical neuron migration. Recent studies have demonstrated that asymmetrical cell division and the proliferation of radial glial cells cause cortical neuron migration disturbances that might be related to changes in the structure of microtubule networks and the destruction of cortical marginal structures found in morphologic observations of pathologic patient-derived brain tissue. Regulating the dysfunction of outer radial glial cells could rescue the mitotic abnormalities and neural cortex development disorders. These studies indicated that migration disorders play an important role in the pathogenesis of GE. The abnormalities in the primate-specific outer radial glial cells that may play a key role in the pathogenesis of Miller-Dieker syndrome cannot be observed in animal models. Therefore, iPSC technology may provide a reliable platform for direct investigations of the neuronal migration disorder mechanisms associated with the pathogenesis of human GE.

Mutations affecting glial cell activation

Patients with Alexander disease (AxD), associated with mutations in the glial fibrillary acidic protein gene (GFAP),

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develop a leukoencephalopathy with macrocephaly, seizures, and psychomotor retardation, which usually leads to death within the first decade. Kondo et al. found that GFAP accumulates locally in patient-derived glial cells, the Rosenthal-fiber-like structure in the intracellular area becomes entangled, and there are increases in N-cadherin expression, mammalian target of rapamycin (mTOR) signaling pathway activity, and the release of cytokine and glutamate neurotransmitters. Recently, Ishii et al. used iPSCs combined with single-cell RNA transcription analysis technology and found that iPSC-induced glial cells can enhance the activities of AMPA and NMDA receptors in excitatory neurons and increase their high-frequency spontaneous discharges. Other studies also found that glial cells can increase the excitatory field potentials and spontaneous synaptic discharge frequencies of neurons that are differentiated from co-cultured iPSCs. Thus, enhanced glial cell activity may be involved in epileptic seizures by increasing intracellular signal activation and glial-neural network excitability. A similar phenomenon was found in iPSC-derived astrocytes from Rett syndrome patients; patient-derived astrocytes were able to induce abnormal differentiation and reduce the axon lengths and synapse numbers of co-cultured interneurons. Simultaneously, healthy-control–derived glial cells could effectively improve the axon length and synapse number in co-cultured interneurons derived from patients with Rett syndrome, which suggests that glial cells may affect synapse formation in neurons through non–cell-autonomous effects from astrocytes. A recent study found that only mature, differentiated glial cells could maintain spontaneous neuronal electrical activity. Studies have demonstrated that glial cell disorders caused by genetic mutations play an important role in unbalanced excitability in the nervous circuit.

Signaling pathway abnormalities

The mammalian target of rapamycin complex-1 (mTORC1) signaling pathway plays an important role in the development and maintenance of normal nervous system function. Activation of the mTORC1 signaling pathway results in a decrease in the aggregation of inhibitory GABA_A receptors on the postsynaptic membrane and a decrease in the number of inhibitory synapses. Mutation of the collybistin protein (CB) gene (ARHGEF9) reduces GABAergic neurotransmission, affects synaptic plasticity, and results in several nervous system diseases, such as epilepsy, anxiety disorders, and autism. Machado et al. collected fibroblasts from patients with X-linked intellectual disability with epilepsy due to mutations in the CB gene and reprogrammed them into iPSCs that were differentiated into neural precursor cells. These researchers found that the CB protein forms a complex with mTOR under normal circumstances and negatively regulates the activity of the mTORC1 signaling pathway. Neurons derived from cells with ARHGEF9 mutations showed abnormal phosphorylation and increased activity of the mTOR-signaling pathway. Aberrant activation of the mTOR-signaling pathway has been detected in differentiated neural cells and astrocytes derived from patients with mutations in tuberous sclerosis complex genes (TSC1 or TSC2), which encode tuberous sclerosis complex proteins, causing tuberous sclerosis. The mitochondrial autophagy observed in these mutated cells caused decreased mitochondrial axonal and global turnover and impaired mitochondrial metabolism. Decreased expression of synaptic receptors and synaptic proteins has been observed. A recent study revealed that the gene mutation resulted in synaptic dysfunction, a decrease in glutamate receptor delta 2 (GRID2), and hypoxcitability of Purkinje cells derived from iPSCs from TSC patients. These dysfunctions were rescued with the mTORC1 pathway inhibitor rapamycin. These results suggest that the hyperactivity of this signaling pathway may impair mitochondrial metabolism and neuronal development and alter nerve excitability.

Conclusion

Using induced pluripotent stem cells (iPSCs), we have reviewed ongoing research to characterize disease mechanisms in genetic epilepsies. In general, genetic dysfunctions can be divided into several categories, including changes in neuronal morphology and neural-electrophysiologic dysfunction. In addition, mitochondrial autophagy and disturbances to signaling pathways can also be found in some of these disease models. Consequently, these dysfunctions are suggested to affect the excitation/inhibition balance in the neural network. However, many of these studies, such as those addressing the genes PIGA and ARHGEF9, are single studies and need to be replicated. Given the astonishing pace of advances in the iPSC field since its introduction, use of iPSCs for better understanding the mechanisms of human genetic epilepsies, identifying novel drugs, and developing cell-based epilepsy therapies seems viable.

Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Kwan P, Arzimanoglou A, Berg AT, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 2010;6:1069–1077.
2. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010;51:676–685.
3. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:512–521.
4. Tekgul H, Gencpinar P, Cavusoglu D, et al. The efficacy, tolerability and safety of levetiracetam therapy in a pediatric population. Seizure 2016;36:16–21.

5. Tidball AM, Parent JM. Concise review: exciting cells: modeling excitability and safety of levetiracetam therapy in a pediatric population. Seizure 2016;36:16–21.

6. Helmstaedter C, Loer B, Wohlfarth R, et al. The effects of cognitive rehabilitation on memory outcome after temporal lobe epilepsy surgery. Epilepsy Behav 2008;3:402–409.

7. Chen T, Giri M, Xiao Z, et al. Genetic and epigenetic mechanisms of epilepsy: a review. Neuropsychiatr Dis Treat 2017;13:1841–1859.

8. Guella I, McKenzie MB, Evans DM, et al. De novo mutations in YWHAG cause early-onset epilepsy. Ann J Hum Genet 2017:2:300–310.

9. Howard MA, Barragan EV, et al. Model systems for study and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

10. Upadhyia D, Hattiangady B, Shetty GA, et al. Neural stem cell or human induced pluripotent stem cell-derived GABA-ergic progenitor cell grafting in an animal model of chronic temporal lobe epilepsy. Cont Prot Stem Cell Biol 2016;8:2D 71–2D 747.

11. Shi Z, Zhang J, Chen S, et al. Conversion of fibroblasts to parvalbu- min neurons by one transcription factor, Ascl1, and the chemical compound forskolin. J Biol Chem 2016:6:e434.

12. Schutte SS, Schutte RJ, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Hum Genet 2016;4:1755–1766.

13. Bershteyn M, Nowakowski TJ, Pollen AA, et al. Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

14. Compagnucci C, Piermarini E, Sferra A, et al. Cytoskeletal dynamics and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

15. Shi Z, Zhang J, Chen S, et al. Conversion of fibroblasts to parvalbu- min neurons by one transcription factor, Ascl1, and the chemical compound forskolin. J Biol Chem 2016:6:e434.

16. Tidball AM, Parent JM. Concise review: exciting cells: modeling excitability and safety of levetiracetam therapy in a pediatric population. Seizure 2016;36:16–21.

17. Maljevic S, Reid CA, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Neurochem 2017;143:30–48.

18. Bersheyn M, Nowakowski TJ, Pollen AA, et al. Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

19. Upadhyia D, Hattiangady B, Shetty GA, et al. Neural stem cell or human induced pluripotent stem cell-derived GABA-ergic progenitor cell grafting in an animal model of chronic temporal lobe epilepsy. Cont Prot Stem Cell Biol 2016;8:2D 71–2D 747.

20. Shi Z, Zhang J, Chen S, et al. Conversion of fibroblasts to parvalbu- min neurons by one transcription factor, Ascl1, and the chemical compound forskolin. J Biol Chem 2016:6:e434.

21. Liu J, Gao C, Chen W, et al. CRISPR/Cas9 facilitates investigation of neuronal circuit disease using human iPSCs at cytokinin B432–437.

22. Schutte SS, Schutte RJ, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Hum Genet 2016;4:1755–1766.

23. Howard MA, Barragan EV, et al. Model systems for study and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

24. Compagnucci C, Piermarini E, Sferra A, et al. Cytoskeletal dynamics and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

25. Shi Z, Zhang J, Chen S, et al. Conversion of fibroblasts to parvalbu- min neurons by one transcription factor, Ascl1, and the chemical compound forskolin. J Biol Chem 2016:6:e434.

26. Tidball AM, Parent JM. Concise review: exciting cells: modeling excitability and safety of levetiracetam therapy in a pediatric population. Seizure 2016;36:16–21.

27. Maljevic S, Reid CA, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Neurochem 2017;143:30–48.

28. Bersheyn M, Nowakowski TJ, Pollen AA, et al. Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

29. Upadhyia D, Hattiangady B, Shetty GA, et al. Neural stem cell or human induced pluripotent stem cell-derived GABA-ergic progenitor cell grafting in an animal model of chronic temporal lobe epilepsy. Cont Prot Stem Cell Biol 2016;8:2D 71–2D 747.

30. Shi Z, Zhang J, Chen S, et al. Conversion of fibroblasts to parvalbu- min neurons by one transcription factor, Ascl1, and the chemical compound forskolin. J Biol Chem 2016:6:e434.

31. Compagnucci C, Piermarini E, Sferra A, et al. Cytoskeletal dynamics and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

32. Liu J, Gao C, Chen W, et al. CRISPR/Cas9 facilitates investigation of neuronal circuit disease using human iPSCs at cytokinin B432–437.

33. Schutte SS, Schutte RJ, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Hum Genet 2016;4:1755–1766.

34. Howard MA, Barragan EV, et al. Model systems for study and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.

35. Liu J, Gao C, Chen W, et al. CRISPR/Cas9 facilitates investigation of neuronal circuit disease using human iPSCs at cytokinin B432–437.

36. Schutte SS, Schutte RJ, Petrou S. Models for discovery of targeted ther- apy in genetic epileptic encephalopathies. J Hum Genet 2016;4:1755–1766.

37. Howard MA, Barragan EV, et al. Model systems for study and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017;4:435–449 e434.
54. Liu Y, Lopez-Santiago LF, Yuan Y, et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. Ann Neurol 2013;1:128–139.

55. Fusco C, Rusconi A, Galla D, et al. New Niemann-Pick type C1 gene mutation associated with very severe disease course and marked early cerebellar vermis atrophy. J Child Neurol 2013;12:1694–1697.

56. Rabenstein M, Peter F, Joost S, et al. Decreased calcium flux in Niemann-Pick type C1 patient-specific iPSC-derived neurons due to higher amount of calcium-impermeable AMPA receptors. Mol Cell Neurosci 2017;83:27–36.

57. Liu J, Kosieluska KA, Cao Z, et al. Signaling defects in IPSC-derived fragile X premutation neurons. Hum Mol Genet 2012;17:3795–3805.

58. Pasca SP, Portmann T, Voineagu I, et al. Using IPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nat Med 2011;12:1657–1662.

59. Krey JF, Pasca SP, Shcheglovitov A, et al. Timothy syndrome is associated with activity-dependent dendritic retraction in rodent and human neurons. Nat Neurosci 2013;2:201–209.

60. Ali W, Bubolz BA, Nguyen L, et al. Epilepsy is associated with ventricular alterations following convulsive status epilepticus in children. Epilepsia Open 2017;4:432–440.

61. Noer AS, Sudo H, Lerrit P, et al. A tRNA(Lys) mutation in the mtDNA is the causal genetic lesion underlying myoclonic epilepsy and ragged-red fiber (MERRF) syndrome. Am J Hum Genet 1991;4:715–722.

62. Hamalainen RH, Manninen T, Koivumaki H, et al. Tissue- and cell-type-specific manifestations of heteroplasmic mtDNA 3243A>G mutation in human induced pluripotent stem cell-derived disease model. Proc Natl Acad Sci USA 2013;38:E3622–E3630.

63. Barnes AP, Polleux F. Establishment of axon-dendrite polarity in developing neurons. Annu Rev Neurosci 2009;32:347–381.

64. Ohtaka-Maruyama C, Okamoto M, Endo K, et al. Synaptic transmission from subplate neurons controls radial migration of neocortical neurons. Science 2018;363:313–317.

65. Khaki Y, Mercimek-Mahmutoglu S. STXBP1 encephalopathy with epilepsy. In Adam MP, Ardinger HH, Pagon RA, et al. (Eds) GeneReviews®. Seattle, WA: University of Washington; 1993:1993–2018.

66. Patzke C, Sudhof TC. The conditional KO approach: Cre/Lox technology in human neurons. Cell Stem Cell 2010;5:407–413.

67. Benarroch EE. HCN channels: function and clinical implications. Neurology 2013;3:304–310.

68. Biel M, Wahl-Schott C, Michalakis S, et al. Hyperpolarization-activated cation channels: from genes to function. Physiol Rev 2009;89:847–919.

69. Kase D, Imoto K. The role of HCN channels on membrane excitability in the nervous system. J Signal Transduct 2012;2012:619747.

70. Fauth C, Steindl K, Toutain A, et al. A recurrent germline mutation in the PIGA gene causes Simpson-Golabi-Behmel syndrome type 2. Am J Med Genet A 2016;2:392–402.

71. Yuan X, Li Z, Baines AC, et al. A hypomorphic PIGA gene mutation causes severe defects in neuron development and susceptibility to complement-mediated toxicity in a human iPSC model. PLoS ONE 2017;4:e0174074.

72. Gerhardt J. Epigenetic modifications in human fragile X pluripotent stem cells; Implications in fragile X syndrome modeling. Brain Res 2017;1656:55–62.

73. Urbach A, Bar-Nur O, Daley GQ, et al. Differential modeling of fragile X syndromes by human embryonic stem cells and induced pluripotent stem cells. Cell Stem Cell 2010;5:407–411.

74. Sheridan SD, Theriault KM, Reis SA, et al. Epigenetic characterization of the FMR1 gene and aberrant neurodevelopment in human induced pluripotent stem cell models of fragile X syndrome. PLoS ONE 2011;10:e26203.

75. Telias M, Segal M, Ben-Yosef D. Neural differentiation of fragile X human embryonic stem cells reveals abnormal patterns of development despite successful neurogenesis. Dev Biol 2013;1:32–45.

76. Does ME, Masser MT, Nichol R, et al. iPSC-derived forebrain neurons from FXS individuals show defects in initial neurite outgrowth. Stem Cells Dev 2014;15:1777–1787.

77. de Esch CE, Ghazvini M, Loos E, et al. Epigenetic characterization of the FMR1 promoter in induced pluripotent stem cells from human fibroblasts carrying an unmethylated full mutation. Stem Cell Reports 2014;4:548–555.

78. Lu P, Chen X, Feng Y, et al. Integrated transcriptome analysis of human iPS cells derived from a fragile X syndrome patient during neuronal differentiation. Sci China Life Sci 2016;11:1093–1105.

79. Angleman D, Ray P, Brown NC, et al. Angelman Syndrome: A Clinical Review. J Child Neurol 2016;12:1067–1072.

80. Okuno H, Nakabayashi K, Abe K, et al. Changeability of the fully methylated status of the 15q11.2 region in induced pluripotent stem cells derived from a patient with Prader-Willi syndrome. Congenit Anom (Kyoto) 2017;4:96–103.

81. Yuan X, Li Z, Baines AC, et al. A hypomorphic PIGA gene mutation causes Simpson-Golabi-Behmel syndrome type 2. Am J Med Genet A 2016;2:392–402.

82. den Duit IA, Blaauw WW, Hoogendijk R, et al. An organoid-based model for testing the efficacy of antioxidants in Angelman syndrome-derived induced pluripotent stem cells. Stem Cell Reports 2015;1:37–46.

83. Stanurova J, Neureiter A, Hiber M, et al. Angelman syndrome-derived neurons display late onset of paternal UBE3A silencing. Sci Rep 2016;6:30792.

84. Fauth C, Steindl K, Toutain A, et al. The conditional KO approach: Cre/Lox technology in human neurons. Cell Stem Cell 2010;5:407–413.

85. Chen PF, Hsiao JS, Sirois CL, et al. RBFOX1 and RBFOX2 are dispensable in iPSCs and iPSC-derived neurons and do not contribute to neural-specific paternal UBE3A silencing. Sci Rep 2016;6:25368.

86. Okuno H, Nakabayashi K, Abe K, et al. Changeability of the fully methylated status of the 15q11.2 region in induced pluripotent stem cells derived from a patient with Prader-Willi syndrome. Congenit Anom (Kyoto) 2017;4:96–103.

87. Stanurova J, Neureiter A, Hiber M, et al. Angelman syndrome-derived neurons display late onset of paternal UBE3A silencing. Sci Rep 2016;6:30792.

88. Shinomura K, Sugiuara C, Takahashi H, et al. Genetic copy number variations at 17p13.3 and epileptogenesis. Epilepsy Res 2010;2:303–309.

89. De Giorgio R, Carrozzo R, Shen Y, et al. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. Nature 1993;363:717–721.

90. Qian X, Nguen HN, Song MM, et al. Brain-region-specific organoids using mini-bioreactors for modeling Zika virus exposure. Cell 2016;5:128–1285.

91. Stahl R, Walcher TE, De Juan Romero C, et al. Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. Cell 2013;3:535–549.

92. Kondo T, Funayama M, Miyake Y, et al. Erratum to: modeling Alexander disease with patient iPSCs reveals cellular and molecular pathology of astrocyte Acta Neuropathol Commun 2016;1:101.

93. Santello M, Bezzi P, Volttiera A. TNFaGp controls glutamatergic glutotransmission in the hippocampal dentate gyrus. Neuron 2011;5:988–1001.
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101. Ishii MN, Yamamoto K, Shoji M, et al. Human induced pluripotent stem cell (hiPSC)-derived neurons respond to convulsant drugs when co-cultured with hiPSC-derived astrocytes. *Toxicology* 2017;389:130–138.

102. Odawara A, Saitoh Y, Alhebshi AH, et al. Long-term electrophysiological activity and pharmacological response of a human induced pluripotent stem cell-derived neuron and astrocyte co-culture. *Biochem Biophys Res Commun* 2014;41:1176–1181.

103. Tang X, Zhou L, Wagner AM, et al. Astroglial cells regulate the developmental timeline of human neurons differentiated from induced pluripotent stem cells. *Stem Cell Res* 2013;2:743–757.

104. Williams EC, Zhong X, Mohamed A, et al. Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. *Hum Mol Genet* 2014;11:2968–2980.

105. Lischka FW, Efthymiou A, Zhou Q, et al. Neonatal mouse cortical but not isogenic human astrocyte feeder layers enhance the functional maturation of induced pluripotent stem cell-derived neurons in culture. *Glia* 2018;4:725–748.

106. Maezawa I, Swanberg S, Harvey D, et al. Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. *J Neurosci* 2009;16:5051–5061.

107. Shimojima K, Sugawara M, Shichiji M, et al. Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. *J Hum Genet* 2011;8:561–565.

108. Harvey K, Duguid IC, Allbred MJ, et al. The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. *J Neurosci* 2004;25:5816–5826.

109. Ebrahimi-Fakhari D, Sahin M. Autism and the synapse: emerging mechanisms and mechanism-based therapies. *Curr Opin Neurol* 2015;2:91–102.

110. Ebrahimi-Fakhari D, Saffari A, Wahlster L, et al. Impaired mitochondrial dynamics and mitophagy in neuronal models of tuberous sclerosis complex. *Cell Rep* 2016;4:1053–1070.

111. Li Y, Cao J, Chen M, et al. Abnormal neural progenitor cells differentiated from induced pluripotent stem cells partially mimicked development of TSC2 neurological abnormalities. *Stem Cell Reports* 2017;4:883–893.

112. Sundberg M, Tochitsky I, Buchholz DE, et al. Purkinje cells derived from TSC patients display hyperexcitability and synaptic deficits associated with reduced FMRP levels and reversed by rapamycin. *Mol Psychiatry* 2018; https://doi.org/10.1038/s41380-018-0018-4.

113. Parent JM, Anderson SA. Reprogramming patient-derived cells to study the epilepsies. *Nat Neurosci* 2015;3:360–366.