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CRITICAL REVIEW AND INVITED COMMENTARY

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

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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

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.[29–31] Abnormalities in the frontal cortex and thalamus are the major changes that are observed via imaging of JME.[32] Bilateral thalamic neuron dysfunction and a thinner corpus callosum may be markers of structural brain changes in JME patients compared with GTCS patients.[33,34] 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 GE, with a particular focus on some newly identified mutated genes, such as *MTTK* (thymidine kinase 2, mitochondrial), *PIGA* (phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A), *STXBP1* (syntaxin-binding protein 1), *ARHGEF9* (Rho guanine nucleotide exchange factor 9), and *LIS1* (lissencephaly-1) (Table 1).

Development of iPSC Technology

In 2006, Takahashi et al.[35] 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.[36] 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.[37] 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 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.[38,39] Some subsequent studies have suggested that an *MECP2* mutation decreases neuron size and synapse number and affects the differentiation of glutamatergic neurons.[40–42] 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).[43] Thus, iPSC technology has created favorable conditions for the direct study of the molecular mechanisms of human GE.

iPSC Studies on the Molecular Mechanisms of GE

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.[44] 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.[45,46] 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.[47] Ten percent of *SCN1A* mutations on chromosome 2 are associated with generalized epilepsy and febrile seizures plus (GEFS+).[48–50] *SCN1A* mutations cause approximately 85% of Dravet syndrome cases, which are characterized by intractable infantile seizures and cognitive impairment.[51] Using an iPSC technique, Jiao et al.[52] 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

Table 1. Summary of induced pluripotent stem cell studies of genetic epilepsies

Gene	Protein	Disease	Findings	Publication(s)
SCN1A	Nav1.1	Dravet syndrome GEFS+ICEGTC SMEI	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)[36] Liu et al. (2016)[21] Sun et al. (2016)[53]
CACNA1C	Cav1.2	Timothy syndrome	Defects in calcium signaling, limited neuronal differentiation	Pasca et al. (2011)[58] Krey et al. (2013)[59]
MTTK	tRNALys	MERRF syndrome	Enhanced mitochondrial autophagy, reduced growth	Chou et al. (2016)[24] Hamalainen et al. (2013)[62]
STXBP1	STXBP1	Ohtahara syndrome	Impairment in synaptic transmission and neurite outgrowth	Patzke et al. (2016)[66] Yamashita et al. (2016)[67]
SHANK3	SHANK3	PMDS	Defects in excitatory synaptic transmission, insulin-like growth factor 1 (IGF1) can be rescued in some phenotypes	Shcheglovitov et al. (2013)[69] Holder et al. (2016)[70]
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	Livide et al. (2015)[37]
FMR1	FMR1	FxS	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	Telias et al. (2013)[79] Doers et al. (2014)[80] de Esch et al. (2014)[81] Halevy et al. (2015)[83] Lu et al. (2016)[82] Li et al. (2017)[111] Achuta et al. (2018)[84]
UBE3A	UBE3A	Angelman syndrome, PWS	Altered resting membrane potential, neuron-specific long noncoding RNA (lncRNA) - in the silenced paternal UBE3A	Chamberlain et al. (2010)[86] Okunoetal. (2017)[87] Fink et al. (2017)[90] Chen et al. (2016)[90] Stanurova et al. (2016)[88]
17p13.3		Miller-Dieker syndrome	Increased horizontal cell divisions causing a cell migration defect, disturbance of the N-cadherin/β-catenin signaling axis	Bershteyn et al. (2017)[13] Iefremova et al. (2017)[96]
GFAP	GFAP	Alexander disease	Increased glutamate neurotransmitter release, nerve activity alterations, neuronal maturation promoted by mature glial cells	Tang et al. (2013)[103] Williams et al. (2014)[104] Odawata et al. (2014)[102] Kondo et al. (2016)[99] Ishii et al. (2017)[101] Lischka et al. (2018)[105]
ARHGEF9	CB	X-linked intellectual disability with epilepsy	Disinhibited mTORC1 signaling contributes to the pathologic process	Machado et al. (2016)[20]
TSC1/TSC2	Hamartin	Tuberous sclerosis	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]

AMPA, α-amino-3-hydroxy-5-methyl-4-boxazolepropionate 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.

interneurons.[21,26,53] 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.[5] 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.[58–60]

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 α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and *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,[74] 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.[75] 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.[76] Patient-derived neurons display defective neurite initiation and extension, reduced synaptic function, reduced synapse number, and reduced expression of the excitatory postsynaptic marker PSD95.[77–80] Subsequently, de Esch et al.[81] 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 *NNAT*).[82] The inhibition of RE-1 silencing transcription factor (REST) induces the upregulation of many of these related genes.[83] 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.[84] 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.[85] 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.[86,87] 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.[88] In the late stage of neuronal development and maturation, the resting membrane potential exhibits depolarization, decreased spontaneous excitatory postsynaptic action potentials, decreased neuron 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.[89] Chen et al.[90] 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.[91,92] 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.[13,93] Some studies have demonstrated that the quality and proliferation properties of outer radial glial cells are critical to the process of cortical neuron migration.[94,95] 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.[96] These studies indicated that migration disorders play an important role in the pathogenesis of GE.[97] 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*),

develop a leukoencephalopathy with macrocephaly, seizures, and psychomotor retardation, which usually leads to death within the first decade.[98] Kondo et al.[99] 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.[100] Recently, Ishii et al.[101] 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[102] and increase their high-frequency spontaneous discharges.[103] 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.[104] A recent study found that only mature, differentiated glial cells could maintain spontaneous neuronal electrical activity.[105] Studies have demonstrated that glial cell disorders caused by genetic mutations play an important role in unbalanced excitability in the nervous circuit.[106]

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.[107] 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.[108] Machado et al.[20] 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.[109] The mitochondrial autophagy observed in these mutated cells caused decreased mitochondrial axonal and global turnover and impaired mitochondrial metabolism.[110] Decreased expression of synaptic receptors and synaptic proteins has been observed.[111] A recent study revealed that the gene mutation resulted in synaptic dysfunction, a decrease in glutamate receptor delta 2 (GRID2), and hypoexcitability of Purkinje cells derived from iPSCs from TSC patients. These dysfunctions were rescued with the mTORC1 pathway inhibitor rapamycin.[112] These results suggest that the hyperactivity of this signaling pathway may impair mitochondrial metabolism and neuronal development and alter nerve excitability.[113]

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.

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