Genes & Diseases (2024) 11, 101021

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REVIEW ARTICLE

The molecular genetics of PI3K/PTEN/AKT/mTOR pathway in the malformations of cortical development

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Received 7 December 2022; received in revised form 7 April 2023; accepted 30 April 2023
Available online 16 July 2023

KEYWORDS
Epilepsy;
Gene mutation;
Genetics;
Malformations of cortical development;
PI3K/PTEN/AKT/mTOR pathway

Abstract Malformations of cortical development (MCD) are a group of developmental disorders characterized by abnormal cortical structures caused by genetic or harmful environmental factors. Many kinds of MCD are caused by genetic variation. MCD is the common cause of intellectual disability and intractable epilepsy. With rapid advances in imaging and sequencing technologies, the diagnostic rate of MCD has been increasing, and many potential genes causing MCD have been successively identified. However, the high genetic heterogeneity of MCD makes it challenging to understand the molecular pathogenesis of MCD and to identify effective targeted drugs. Thus, in this review, we outline important events of cortical development. Then we illustrate the progress of molecular genetic studies about MCD focusing on the PI3K/PTEN/AKT/mTOR pathway. Finally, we briefly discuss the diagnostic methods, disease models, and therapeutic strategies for MCD. The information will facilitate further research on MCD. Understanding the role of the PI3K/PTEN/AKT/mTOR pathway in MCD could lead to a novel strategy for treating MCD-related diseases.

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Peer review under responsibility of Chongqing Medical University.
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https://doi.org/10.1016/j.gendis.2023.04.041

Introduction

The cerebral cortex is responsible for receiving and processing information and issuing instructions and is undertaking higher functions such as sensory, cognition, controlling emotions, and generating consciousness, which is extremely critical to the normal functioning of the organism.[1] Cortical development is a highly sophisticated and well-organized process, including neural stem cell proliferation and differentiation, neuron migration and maturation, and the production of glial cells. Abnormalities can occur in any part of these processes, which may lead to the malformations of cortical development (MCD). MCD is the principal cause of intellectual disability, autism, epilepsy, and cerebral palsy.[2] Further, the treatment for MCD is not satisfactory, and the prognosis is dire. Understanding the mechanism of MCD is fundamentally essential and may lead to novel approaches to curing MCD in clinical settings.

Over the last decade, increasing evidence has shown that the dysregulation of the PI3K/PTEN/AKT/mTOR pathway is one of the critical causes of MCD.[3–7] By regulating protein synthesis, the PI3K/PTEN/AKT/mTOR signaling pathway can modulate cell growth, proliferation, differentiation, and apoptosis.[8] Furthermore, in the central nervous system, it can modulate cortical developmental processes by regulating the proliferation of neural stem cells, neuron development, gliogenesis, synaptic plasticity, and the assembly and maintenance of circuits.[9,10] Recently, evidence has shown that genetic events can cause the dysregulation of the pathway and further lead to impairment of cortex development. Thus, revealing the molecular genetic events in the PI3K/PTEN/AKT/mTOR pathway may contribute to diagnosing and treating MCD. Many drugs that target specific genes of this pathway have been developed, such as the mTOR inhibitor rapamycin, which can effectively inhibit neuronal overgrowth and epileptic phenotype in mice with MCD.[11–18] This review mainly discusses the molecular genetics and pathogenic mechanisms of MCD by focusing on the PI3K/PTEN/AKT/mTOR pathway and briefly introduces related disease models and targeted therapeutic advances.

Developmental processes and diseases of the cerebral cortex

The development of the central nervous system originates from the neural tube composed of monolayer pseudostratified neuroepithelial cells (NEs). With the closure of the neural tube, three brain vesicles (prosencephalon, mesencephalon, and rhombencephalon) take shape in the anterior part of the neural tube, and the cephalic end of the prosencephalon expands to the sides to form the left and right telencephalon. The NEs in the telencephalic wall proliferate, differentiate, and migrate outward to evolve into the cerebral cortex. We summarize the key time points and events in cerebral cortex development (Fig. 1).

Neural stem cell pool expansion

During postconceptional week 4 (pcw4) in humans or embryonic day 9 (E9) to E10 in mice, cerebral cortex development initiates. NEs are attached to the ventricular surface at the apex and the leptomeningeal surface at the base. The area where their cell bodies are positioned is called the ventricular zone (VZ), the most inferior part of the cortex. NEs increase the number of neural stem cells and expand the VZ by symmetrical proliferative division.[19,20] Since pcw7 (E10), the expression of the Hes family and Fgf10 induces the transformation of NEs to radial glial cells (RGCs). These molecules both reserve some epithelial properties and express glial cell markers, such as brain-lipid-binding protein, glutamate-aspartate transporter, and glial fibrillary acidic protein.[20–22] RGCs also increase the number of neural stem cells by symmetrical proliferative divisions as NEs. After neurogenesis, the expansion of the VZ also continues to rely on the division of RGCs. By E12, most NEs convert to RGCs, which have become the predominant neural stem cells.[23]

Neurogenesis

At pcw7–8 (E10.5), the first neurons, mainly Cajal-Retzius cells,[24,25] are generated by asymmetric division of RGCs and secrete reelin to guide neuron migration and promote neuron differentiation. Cajal-Retzius cells migrate radially between the VZ and the leptomeninges, constituting the preplate (PP).[20,26,27] At pcw9–10 (E11.5), cortical projection neurons begin to be generated and migrate to the PP to form the cortical plate (CP), which divides the PP into two regions, the marginal zone in the superficial layer and the subplate (SP) in the deep layer.[24,28] The marginal zone is mainly occupied by Cajal-Retzius cells, also known as layer I. At approximately E13, RGCs proliferate continuously and accumulate in the basal side of VZ, deriving another proliferation zone, the subventricular zone (SVZ). Since primates have more neural progenitors, SVZ can be subdivided into inner SVZ and outer SVZ.[23] While the stem cell pool expands, individual RGC divide by asymmetric self-renewal to produce an intermediate progenitor (IP) and a neuron, termed direct neurogenesis. IPs are mainly located in the SVZ and form the intermediate zone by massive accumulation. IPs are essential for producing numerous neurons during cortical development, and neurons can be created by the symmetric consumptive division of IPs, namely indirect neurogenesis.[23,28,29]

Neuronal migration and cortical lamination

Neurons migrate to the CP layer through four stages. In the first stage, the neurons migrate radially to the SVZ/intermediate zone after arising from the VZ or SVZ. In the second stage, the neurons stay in the SVZ/intermediate zone for up to 24 h and exist in a multipolar pattern. Since the neurons have not yet established the tight junction with the nerve fibers of RGCs at this stage, there are lateral movements. In the third stage, the neuronal somas move backward toward the ventricle or one of the dendrites of neurons extends toward the ventricle. A portion of the neurons, which can escape this stage, directly proceed to the fourth stage: the neurons invert the multipolar state, extending axons that directly attach to the leptomeninges and migrating to the particular position of the CP with the appearance of bipolar neurons.[30] At pcw8–24 (E12–E16.5),

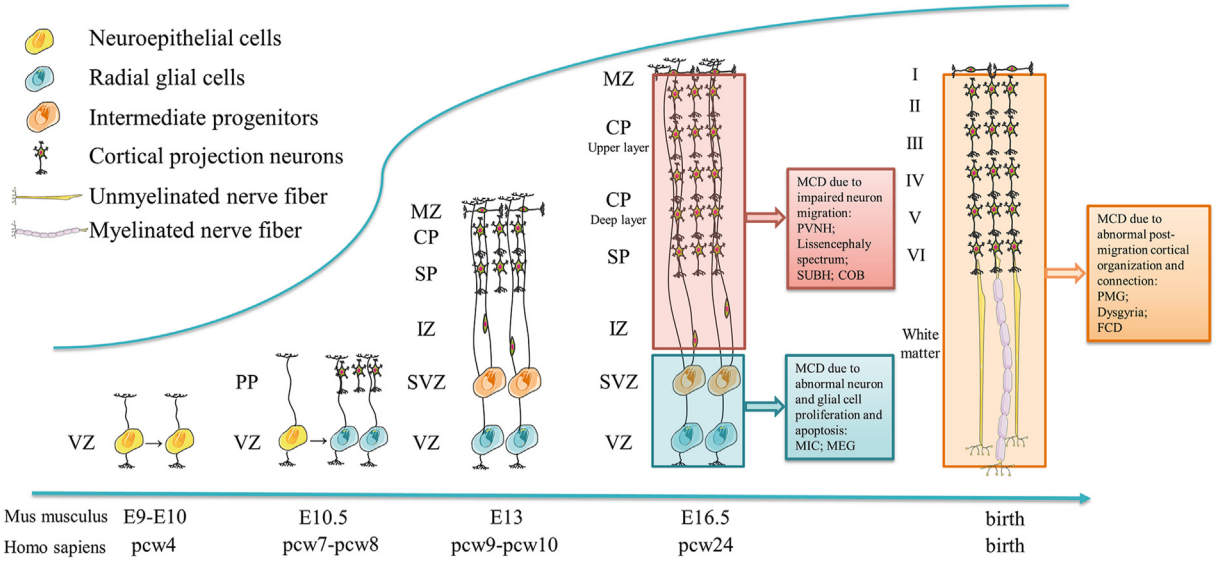

Figure 1 Schematic overview of cortical development in the embryonic brain and MCD types. During the early stages of cortical development, neural stem cells undergo symmetrical proliferative division to expand the VZ pool, producing neurons by direct or indirect neurogenesis. The immature neurons migrate to specific cortical layers in the "inside-out" pattern. Subsequently, neurons mature, glial cells are generated, and the cerebral cortex is organized and connected. The abnormal cortical development process can lead to MCD, whose types are illustrated in the corresponding box. COB, cobblestone malformation; CP, cortical plate; FCD, focal cortical dysplasia; IZ, intermediate zone; MCD, malformations of cortical development; MEG, megalencephaly; MIC, microcephaly; MZ, marginal zone; PMG, polymicrogyria; PP, preplate; PVNH, paraventricular nodular heterotopia; SP, subplate; SUBH, subcortical heterotopia; SVZ, subventricular zone; VZ, ventricular zone.

following the "inside-out" developmental pattern, the CP layer emerges, which consists of six layers of neuronal cells arranged in an orderly manner.[24,31] Neurons produced earlier migrate through the basal neurites of the RGCs to the deeper layer of CP, and neurons subsequently produced can cross over the neuronal layers constituted by neurons previously produced to reach the location closer to the meninges and form new neuronal layers. The layer VI neurons begin to be produced at E12.5, the layer V at E13.5, and then until E16.5 when the last batch of neurons are produced to form layer II of the cortex.[32,33] Once neurons reach their final position within the cortex, dendrites and axons grow, and synapses develop. Neurons located in the marginal zone and SP are the first to shape synapses and mature in morphology, contributing to the migration of CP neurons, the development of synapses, and the formation of afferent and efferent nerves.[34]

Gliogenesis

After pcw24 (E17), the proliferation of RGCs and cortical neurogenesis gradually decrease, while RGCs transform into glial cell progenitors, giving rise to astrocytes, oligodendrocytes, and ependymal. Oligodendrocytes (OLs) generate myelin through the spiral wrapping of their plasma membranes around axons. The myelin can accelerate neurotransmission, provide trophic support to axons, and refine neural circuits.[35,36] The brain continues to undergo dramatic changes even after birth, with neuronal morphology maturation, axonal myelination, synaptogenesis, and neural network formation and remodeling throughout the early life span.[37,38]

Malformations of cortical development

The development of the cerebral cortex is intricate and sophisticated, depending on three basic processes: neuron progenitor proliferation, neuron migration, and cerebral cortex organization. Disruption of any of these steps by inherited factors like mutations of related genes or harmful external environmental factors, including alcohol, radiation, and viral or bacterial infections can lead to MCD. Based on the implicated cortical developmental processes, MCD can be divided into three categories[39]: (i) MCD due to abnormal neuron and glial cell proliferation and apoptosis, such as microcephaly (MIC) and megalencephaly (MEG). MIC is defined as a head circumference measurement smaller than a certain value for babies of the same age and sex, and vice versa. MIC can present with a normal or simplified gyral pattern or with more complex brain abnormalities, which are often associated with epilepsy, autism, and other birth defects. MEG may associate with underlying neurodevelopmental or generalized overgrowth disorders.[40] (ii) MCD due to impaired neuron migration, such as paraventricular nodular heterotopia (PVNH), lissencephaly spectrum, subcortical heterotopia, and cobblestone malformation (COB). Neuronal heterotopia means groups of neurons in an abnormal location, lissencephaly refers to the abnormal gyral pattern, and COB exhibits irregular and pebbled-like lesions in the cortical surface resulting from the over-migration from the CP into the leptomeninges.[2] (iii) MCD due to abnormal post-migration cortical organization and connection, such as polymicrogyria (PMG), dysgyria, and focal cortical dysplasia (FCD). PMG is characterized by excessively small

cerebral gyri with an irregular, pebbled cortical surface and a stippled grey-white matter boundary, which is difficult to be distinguished from COB.[41] FCD is characterized by deranged neurons in white matter, dyslamination, and abnormal balloon cells, leading to a higher risk of seizures and possible intellectual impairment.[42] According to the International League Against Epilepsy (ILAE), FCD I has abnormal lamination, FCD II is associated with aberrant cytology, and FCD III occurs alongside another lesion, including hippocampal sclerosis or tumors.[39,43] It is worth noting that FCDI and FCD III belong to the class II MCD. Hemimegalencephaly (HME), FCD II, and tuberous sclerosis complex (TSC), which share similar genetic etiologies and histopathological features, are all classified as class III MCD.[44–46] HME is usually severe because of the larger lesions.[12] However, TSC and FCD are not the same disorder. TSC shows unique clinical manifestations, including hamartomas, cortical tubers, impaired myelination of the lesional white matter, neuronal migration lines (NML), and tumor-like lesions, such as subependymal nodules (SEN) and subependymal giant cell astrocytoma (SEGA).[47,48] With the development of molecular genetics, the classification of MCD based on gene mutations and signaling pathways can complement and extend histopathological classification.[39]

PI3K/PTEN/AKT/mTOR pathway

Normal physiological PI3K/PTEN/AKT/mTOR pathway

The PI3K/PTEN/AKT/mTOR pathway plays key roles in cortical development, participating in several processes, including neuron proliferation, axon and dendrite growth, dendritic branching, and synapse formation. This pathway also facilitates the establishment of polarity in immature neurons and the maintenance of polarity in mature neurons (Fig. 2).[49–52] Phosphatidylinositol-3-kinase (PI3K) is the necessary component for activating this pathway. The main downstream target of PI3K is AKT serine/threonine kinase (AKT), which is vitally important among a wide range of biological processes such as cell growth, survival, and metabolism.[53] AKT, also known as protein kinase B, which can be phosphorylated and activated by various cellular components, could directly or indirectly regulate the mechanistic target of rapamycin kinase (mTOR) which is the downstream master effector molecule.[54] AKT also directly acts on the downstream GSK-3β (glycogen synthase kinase-3β)/CCND2 signaling pathway to regulate protein synthesis.[55] mTOR functions by forming mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) with related proteins. mTORC1 mainly regulates two signaling pathways, ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E (eIF4E)-binding proteins (4E-BPs) pathway, which engages in the assembly of translation initiation complex and protein synthesis[56,57]; mTORC2 can regulate the pathway activation state by phosphorylating AKT.[58] In addition, the PI3K/PTEN/AKT/mTOR pathway also maintains homeostasis by the activation of negative regulators. The phosphatase and tensin homolog (PTEN) act on PIP3 to dephosphorylate it to PIP2, reducing activated AKT levels.[59] The TSC complex subunit 1/2 (TSC1/2) inhibits mTOR activation mediated by RHEB (Ras homolog mTORC1 binding). mTORC1 indirectly inhibits AKT activity by antagonizing mTORC2, thereby inhibiting the activity of the PI3K/AKT/mTOR pathway.[58]

PI3K/PTEN/AKT/mTOR pathway in abnormal cortical developmental states

PI3K

PI3K, a member of the lipid kinase family, can be classified according to substrate preference and sequence homology. Class IA PI3K is predominantly expressed in the brain and is the heterodimer consisting of regulatory subunits encoded by *PIK3R1*, *PIK3R2*, and *PIK3R3* and catalytic subunits encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD*. In response to extracellular signals, such as fibroblast growth factor, the class IA PI3K regulatory subunit is phosphorylated via receptor tyrosine kinases (RTKs) to activate p110, encoding by *PIK3CA*, thereby activating the PI3K/AKT pathway.[60,61] Activated PI3K is recruited to the cell membrane and preferentially acts on the substrate phosphatidylinositol-4,5-bisphosphate (PIP2) to generate the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3).[62] PIP3 interacts directly with the AKT PH domain, aggregating inactivated AKT in the cell membrane and causing the conformational change that makes it susceptible to be phosphorylated at Thr308 by phosphoinositide-dependent kinase 1 (PDK1).[63] Additionally, RAB39B can interact with PI3K components. Deficiency of RAB39B can induce abnormalities of the PI3K/AKT/mTOR pathway in the mouse cortex and cerebral organoid.[64]

PIK3CA and *PIK3R2* encode the catalytic subunit p110α and the regulatory subunit p85β of PI3K, respectively. *PIK3CA* mutations are distributed throughout the entire coding sequence,[65] and the hotspot mutation regions may be helical and kinase domains.[66] Mutations at different domains may induce PI3K hyperactivation by different molecular mechanisms. Mutations in the C2 domain may alter charge or conformation, enhancing recruitment of the mutant-p110α to cell membranes; mutations in the helical domain may affect the interaction with other proteins; mutations in the kinase domain, near the hinge of the activation loop, may alter the position of the activation loop.[67] Several *PIK3R2* mutations can disrupt the inactive conformation of the PI3K dimer and hyperactivate catalytic subunit,[68,69] like mutations in the conserved residues located at the binding site of p110α and p85β and the SH2 domain, can cause sustained activation of the catalytic subunit by affecting the binding of the two subunits.[70,71] *PIK3CA* or *PIK3R2* mutations result in increased PI3K activity. PI3K with enhanced activity aggregates towards the cell membrane, more PIP3 is generated and induces PDK1 to locate at the cell membrane, and the phosphorylation of AKT increases, resulting in the entire pathway in the hyperactivated state.[65,67–69] The above reasons lead to abnormalities in the downstream signaling pathways, affecting the growth and development of neurons and synaptic plasticity, resulting in neuronal hypertrophy, as well as abnormalities in the morphology, location, and

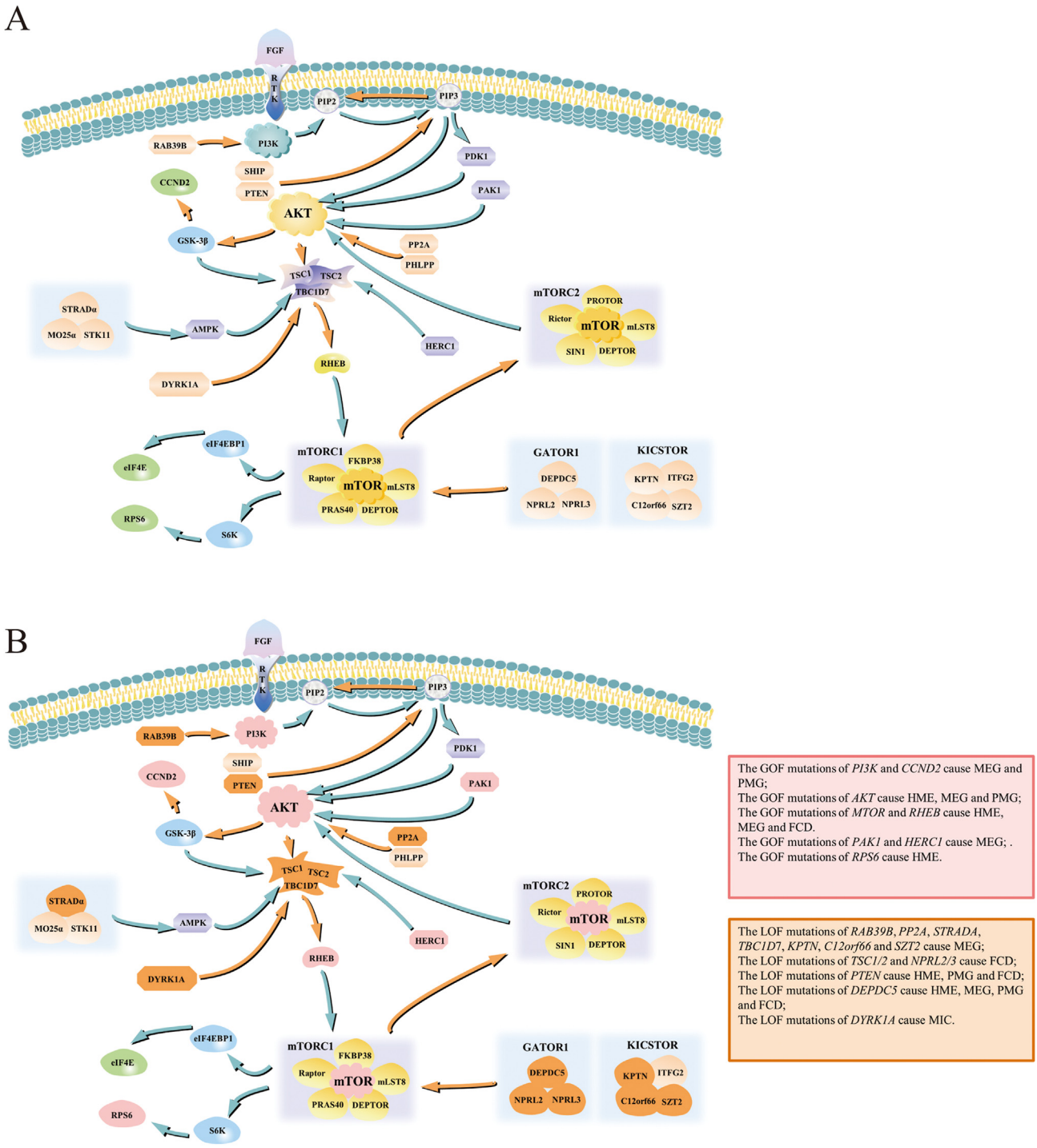

Figure 2 PI3K-PTEN-AKT-mTOR pathway and MCD. **(A)** The PI3K-AKT-mTOR pathway in the normal state. The activated effects of genes on the downstream signaling components are represented by canal blue arrows, and the inhibitory effects are represented by orange arrows. **(B)** The PI3K-AKT-mTOR pathway in the abnormal pathological state. The genes with GOF mutations leading to MCD are colored in light pink, and the genes with LOF mutations leading to MCD are colored in orange. The specific MCD diseases caused by gene mutations are organized in the corresponding boxes. FCD, focal cortical dysplasia; HME, hemimegalencephaly; MEG, megalencephaly; MIC, microcephaly; PMG, polymicrogyria.

number of synapses and neurites.[72,73] Megalencephaly-capillary malformation syndrome (MCAP), HME, and other disorders associated with cellular overgrowth due to mutations in *PIK3CA* are now generically referred to by the term "PIK3CA-related overgrowth spectrum".[74]

PIK3CA mutations cause diseases such as MCAP, HME, and MEG. The most common mutation is the somatic heterozygous mutation.[75,76] Swarr et al reported that MCAP fetuses carrying the heterozygous mutation (p.R115P) in *PIK3CA* developed pleural effusions and fetal hydrops.

Thus, MCAP syndrome should be considered when a fetus suffered non-immune hydrops.[77] *PIK3CA* mutant mice with MEG and hypoglycemia phenotypes were discovered in 2015,[78] and then four MCAP children with *PIK3CA* mutation showed hypoglycemia symptoms in 2018.[79,80] Subsequently, nine MCAP children with *PIK3CA* mutation exhibited growth hormone deficiency. Therefore, the researchers recommend assessing MCAP children's glucose levels and performing early endocrine surveillance for them.[79,80] Moreover, an MCAP girl with a recurrent *PIK3CA* somatic mutation (p.Glu453Lys) had elevated glycated hemoglobin levels and pancreatic steatosis. This case indicates that pancreatic screening is critical for patients with PIK3CA-related disease.[81]

Both germline and somatic mutations of *PIK3R2* can cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) and bilateral perisylvian polymicrogyria.[70,71,82]

PTEN

PTEN, a lipid and protein phosphatase, dephosphorylates PIP3, thereby inhibiting the accumulation of PIP3 in cells and suppressing the PI3K/AKT pathway. Experiments *in vitro* and *in vivo* showed that deficiency of *PTEN* can increase phosphorylated AKT and S6[83,84] and over-activate PI3K/AKT pathway, leading to cortical developmental disorders.[84,85]

PTEN mutations cause cortical developmental disorders such as FCD and MEG. Schick et al reported a somatic missense mutation in *PTEN* in FCD patients.[86] Several *de novo* germline missense and frameshift mutations in the *PTEN* were found in patients with autism spectrum disorder (ASD), and MEG was the typical phenotype of these ASD patients.[87–89]

PTEN haploinsufficiency mice can exhibit increased cortical neuronal cell soma volume and dendritic complexity, axons arising from cortical neurons are overgrown, and the mice develop MEG phenotype and exhibit behavioral abnormalities.[90–92] The conditional *PTEN* knockout mice may show disrupted migration, enhanced neural stem cell proliferation and differentiation, hypertrophic neurons, and altered neuronal membrane properties.[93,94] The mice can exhibit progressive MEG, spontaneous seizures, abnormal electroencephalography activity, and ASD-related behavioral abnormalities.[95,96]

AKT

AKT is vital for regulating cell growth. AKT1 and AKT3 are the main forms of AKT in the cerebral cortex. AKT3 is enriched in the RGCs and acts as a key protein for cortex development.[97,98] AKT is phosphorylated on two key residues. One residue is the prementioned Thr308 phosphorylated by PDK1. The other is Ser473 phosphorylated by mTORC2, which may further increase AKT activity. AKT occupies the central position in the pathway, transmitting the upstream signal to the downstream, and is essential for the proper activation of the entire signaling pathway.

The gain of function (GOF) mutations at the PH domain, catalytic kinase domain, and the C-terminus of AKT can lead to ongoing activation of AKT and the PI3K/PTEN/AKT/mTOR pathway, causing MCD associated with cortex overgrowth.[99]

The GOF mutations of the *AKT3* are associated with a wide spectrum of brain involvement ranging from focal or segmental brain malformations (such as HME and PMG) to diffuse bilateral cortical malformations, MEG, and heterotopia.[99] *AKT3* gene dosage imbalances are involved in the control of brain growth.[100] For example, people with *AKT3* duplication mutations or tetraploids suffer from MEG and related syndromes (MCAP and MPPH).[68,70,101] *AKT3* somatic GOF mutation can also lead to MCD, such as HME, resulting from AKT3 hyperactivation.[75,98] AKT overactivation can increase glucose utilization. Thus, MCD patients with GOF mutations of *AKT*, such as *AKT3* mutation (p.Val183Asp), may be accompanied by persistent hypoglycemic symptoms.[102] Also, growth hormone deficiency and hypoglycemia occurred in the children suffering from MCD due to *AKT3* germline mutation (p.Glu40Lys).[103] Besides *AKT3*, Cowden syndrome patients with *AKT1* germline mutation and Proteus syndrome patients with somatic GOF mutation of *AKT1* also showed MEG and HME.[67,104,105]

On the other hand, loss of function (LOF) mutations of *AKT3* can cause MIC.[106,107] Ciaccio et al proposed the term "Mirror syndromes" characterized by MIC, assuming a mechanism contrary to the overactivation of the PI3K/PTEN/AKT/mTOR pathway.[108] Moreover, in contrast to the enlarged brain size in mice with *AKT3*-activated mutations (p.D219V), *AKT3* knockout mice show that brain size decreases selectively due to the reduction of neuron number and size.[109] However, verifying this hypothesis by identifying more MIC cases resulting from the LOF mutations of other genes in this pathway is still necessary.

When the genes that encode AKT positive or negative regulators are mutated, sustained activation of the PI3K pathway and MCD occur.[110] Protein phosphatase 2 regulatory subunit B′ delta (PPP2R5D) is the component of PP2A. Its mutants interact with AKT, leading to continuous activation of the pathway and triggering cell size increase and cell growth dysfunction,[111] which causes MEG eventually.[112] Alternatively, P21-activated kinase 1 (PAK1) is a positive regulator of AKT. PAK1 mutants increase AKT phosphorylation levels, leading to sustained pathway activation and causing MEG.[113]

In addition, GSK-3β is a critical molecule downstream of AKT.[114] Activated AKT directly phosphorylates GSK-3β to make it inactive and deliver the signals against apoptosis.[115] AKT is a crucial regulator of neural crest induction because the inhibition of GSK-3β can stabilize its substrates (β-catenin and Snai1), which are essential for neural crest and cranial bone development.[116] Also, GSK-3β phosphorylates cyclin D2 (CCND2) and makes CCND2 more sensitive to the degradation mediated by ubiquitin or protease,[117] regulating neural progenitor proliferation and differentiation.[118,119] It seems that these factors can regulate brain shape and head size at the same time.

GSK-3β is a critical factor for establishing neuronal polarity, and the mutations of key genes in this pathway may directly regulate axon formation by affecting GSK-3β function.[120] For example, the mutations of *DDX3X* and *RAC1*, which are the upstream genes of *AKT*, can make GSK-3β fail to stabilize β-catenin and Snai1, leading to craniofacial disorders and MCD, such as MIC, MEG, and PMG.[121,122] The GSK-3β is inhibited when the pathway is overactivated, causing CCND2 not to be phosphorylated and degraded,[55,68]

the neural progenitor cell cycle decreases, and the numbers of RGCs and IPs increase, leading to MCD such as MEG.[102,117]

The GOF mutations of *CCND2* may alter the structure of CCND2 phosphorylation sites, whereas unphosphorylated CCND2 resists degradation and accumulates, disrupting the normal progression of the neural progenitor cell cycle.[55,117] Therefore, the migration of cortical neurons that GSK-3β participates in is aberrated, leading to MCD-like MEG and polymicrogyria (PMG).[55] The hotspot mutation region of *CCND2* in MPPH patients is around its conserved residue that can be phosphorylated by GSK-3β.[55] Interestingly, the incidence of polydactyly in MPPH patients with *CCND2* mutations is significantly higher than in MPPH patients carrying mutations of other genes in the pathway. However, the specific mechanism is unknown.[55,123]

TSC1/2

The TSC complex, the negative regulator of the PI3K/PTEN/AKT/mTOR pathway, has three subunits: TSC1, TSC2, and Tre2-Bub2-Cdc16-1 domain family member 7 (TBC1D7).[124] TSC2 contains the GTPase-activating protein domain for a small G-protein Rheb (Ras homologue enriched in the brain), TSC1 acts as a scaffold that binds and stabilizes both TSC2 and TBC1D7[124,125]; TBC1D7 binds to and is stabilized by TSC1, and knockdown of TBC1D7 can decrease the association of TSC1 and TSC2.[124] The TSC complex inhibits mTORC1 through its GAP (Gtpase-activating protein) activity of Rheb.[125] If the TSC complex is disrupted, Rheb will not shift from the active form (GTP-bound Rheb) to the inactive form (GDP-bound Rheb) and fail to inhibit mTORC1.[124,126] AKT can directly phosphorylate TSC2, which destabilizes TSC2 and disrupts its interaction with TSC1.[127] Also, the phosphorylation of TSC2 by GSK-3β may be relevant for inhibiting the TSC1/TSC2 complex.[128] In addition, serine/threonine kinase 11 combines with STE20 related adaptor-α (STRADα) and mouse protein 25-α (MO25α) to form a trimeric complex that can activate the TSC complex by activating protein kinase AMP-activated catalytic subunit alpha 2 (AMPK) to phosphorylate TSC2.[129,130]

TSC1/2 somatic mutations and germline heterozygous mutations are sufficient to disrupt the structures and functions of the TSC complex,[131,132] making it unable to inhibit mTOR activation. Similar to the mechanism of *PTEN*, *TSC* mutants disrupt the balance of the activation state of the PI3K/PTEN/AKT/mTOR pathway, leading to dysregulation of neuronal growth and ultimately causing MCD, such as FCDII, TSC, and HME.[131,133,134]

In mice, mosaic knockout of *Tsc1* and *Tsc2* via CRISPR-Cas9 application is sufficient to cause spontaneous behavioral seizures, cytomegalic neurons, and defective neuronal migration.[131] FCDII, TSC patients, and animal models showed cortical dysplasia accompanied by increased OLs progenitor proliferation, reduced myelin, and impaired white matter.[135–139] For *TSC1/2*, a two-hit mutational mechanism is assumed to cause FCD, TSC, and HME lesions,[105,140–143] as shown in cancer according to Knudson's two-hit model.[144]

Besides, the LOF or GOF mutations of the genes that encode TSC complex regulators can abnormalize the activation of the pathway, causing MCD. For example, a study revealed that overexpression of dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) increased phosphorylation of both TSC1 and TSC2,[145,146] the LOF mutants of *DYRK1A* can trigger MIC.[147] HECT and RLD domain containing E3 ubiquitin-protein ligase family member 1 (HERC1) can interact with TSC2, and GOF mutations of *HERC1* can increase the activity of mTORC1 and persistent activation of the pathway, eliciting MEG.[148,149]

RHEB

RHEB, as a gene in the TSC1/2 downstream, is a potent activator of mTORC1.[150] FKBP38 is an endogenous inhibitor of mTOR, which can bind directly to mTOR and downregulate mTORC1 activity. GTP-bound RHEB can antagonize FKBP38 by interacting with it and preventing its association with mTOR.[151,152]

RHEB mutations are associated with FCD, HME, and MEG. The patients also presented intellectual disability with hypotonia, epilepsy, and autism spectrum disorder.[142,153–156] A study in Rheb knockout mice showed up to 75% reduction in mTOR signaling.[157] A GOF mutation of *RHEB* (p.Pro37Leu) is resistant to the inhibitory action of the TSC complex, resulting in increasing mTORC1 activity.[158] The RHEB mutant proteins caused neuronal migration defects and cytological abnormalities, with dysmorphic appearance and enlarged soma size, and induced aberrant axonal development *in vivo* and *in vitro*.[154,158] The Rheb mutated mice exhibited TSC, FCD, and seizures.[13,154,158,159] In the zebrafish model, Rheb mutant proteins significantly increased head size.[154] In addition, rapamycin administration released heterotopic nodules and seizures in Rheb-mutated mice,[158,159] and an ongoing clinical trial used the mTOR inhibitor everolimus to treat patients who carried RHEB mutations.[156]

mTOR

mTOR is a serine/threonine protein kinase that involves in cell growth and regulates cell proliferation. mTOR can phosphorylate its downstream translational control targets, 4E-BPs, and S6Ks.[160] 4E-BPs is composed of 4E-BP1, 4E-BP2, and 4E-BP3 in mammals, and 4E-BP2 is the most abundant isoform in the brain.[161,162] Hypophosphorylated 4E-BPs have a high affinity for eIF4E; while hyperphosphorylated 4E-BPs can separate from eIF4E, which promotes eIF4F complex formation to initiate the translation of relevant mRNAs.[114,163] S6Ks consist of S6K1 and S6K2 in mammals, and all phosphorylation sites are conserved between the two proteins.[164] Ribosome protein S6 (RPS6), the direct downstream effector of S6K, is a component of the 40S ribosome, whose phosphorylation promotes the translation of relevant mRNAs.[165] The mTOR pathway activation affects cell size, number, and synaptic plasticity of neurons.[166] Recently, mTOR was found involved in myelin formation. mTORC1 has been implicated as a key signal for myelination. It can promote the differentiation of OLs from oligodendrocyte progenitor cells and positively regulate myelin growth.[36] In addition, GAP activity towards rags complex 1 (GATOR1) and a four-membered protein complex (KICSTOR) can function as the inhibitor of the mTORC1 pathway in response to amino acids level.[3,167] GATOR1, composed of DEPDC5, NPRL2, and NPRL3, can be implicated in the inhibition of Rag GTPase, which is involved in mTORC1 recruitment at the lysosome membrane, where

RHEB stimulates its kinase activity.[54,168] KICSTOR consists of four proteins, namely, KPTN (kaptin, actin-binding protein), integrin alpha FG-GAP repeat containing 2, C12orf66, and SZT2. KICSTOR can combine with GATOR1 and recruit it to the lysosome, inhibiting mTORC1 and affecting cortical development.[167,169]

mTOR mutations enhance its activity in various ways. Mutations at the FAT structural domain, which can limit the kinase activity of mTOR, may destabilize mTOR.[170] Mutations at the kinase domain may alter the mTOR helix conformation and decrease its activated threshold.[75,171] Some other mutations may inhibit mTOR binding to Deptor, an mTOR endogenous inhibitor.[172] Excessive mTOR activation directly triggers MCD such as FCD, HME, Smith-Kingsmore syndrome associated MEG, and epilepsy.[15,75,76,173] Also, MCD cases with mutations in GATOR1 and KICSTOR genes are reported.[3,174–180] Notably, a germline LOF mutation together with a somatic "second-hit" mutation in *DEPDC5* might be responsible for FCD's development.[181]

In short, in the PI3K/PTEN/AKT/mTOR signaling pathway, both the GOF mutations of the genes encoded positive regulators and the LOF mutations of the genes encoded negative regulators can lead to hyperactivation of this pathway. The dysregulation of the mTOR pathway can cause abnormal RGC and OL proliferation, disturbed neuron migration, and impaired myelination. It leads to MCD with common phenotypic characteristics, such as abnormal neuronal morphology, disorganized cortical lamination, impaired myelin formation, neuronal hyperexcitability, and clinical epilepsy.[169,182] Conveniently, MCD with the above characteristics caused by dysregulation of the pathway can be collectively referred to mTORpathies.[45,183,184] The mutations of genes or related genes in the PI3K/PTEN/AKT/mTOR pathway caused mTORpathies are summarized in Table 1. In summary, mutations in related genes can regulate the pathway activation, affecting cortical development and leading to MCD. We can conclude that class I MCD (MEG and related syndromes (MCAP, MPPH)) is most likely related to these pathway mutations. The second related category is class III MCD, like FCDII, HME, and PMG. Some mutations also could lead to classIIMCD, like heterotopia.

Diagnosis, modeling, and therapy of MCD with PI3K/PTEN/AKT/mTOR pathway-related genetic information

Recent studies have led to a further understanding of MCD. Effective diagnosis and therapy strategies based on molecular genetics and imaging have been developing. The PI3K/PTEN/AKT/mTOR pathway is critical in MCD. To investigate the pathophysiology relationship between PI3K/PTEN/AKT/mTOR pathway and MCD, it is necessary to apply multiple disease models to simulate the clinical phenotype of MCD and to explore the best disease management strategies.

Disease diagnosis

MCD should be considered for patients with intellectual disability or epilepsy. Magnetic resonance imaging (MRI) is critical for clinical diagnosis[209] because MRI has multidirectional and multiparametric imaging features and unique imaging advantages for abnormal cortex lesions. In addition, since many cases are detected after neurological symptoms, such as epilepsy and developmental delay, it is vital to diagnose during the fetal period. However, the detection of MCD by prenatal ultrasonography is difficult. For accuracy and sensitivity, MRI is superior to ultrasonography for diagnosing such diseases, and the application of MRI in prenatal fetal diagnosis is becoming increasingly widespread due to its high resolution.[210]

MIC, paraventricular nodular heterotopia, and other MCD diseases may also be associated with structural variations, such as copy number variations, so chromosomal microarray analysis can significantly improve the diagnostic rate.[211,212] Given the high genetic heterogeneity of MCD and the still-growing number of known causative genes and variants, targeted evaluation of MCD-associated genes in combination with high-throughput sequencing technology is recommended. If the causative variant is still not filtered, the trio exome analysis can be taken to improve the diagnosis rate after appropriate genetic counseling. Since many mutations are not detected using diagnostic MCD panels, more emphasis should give to mosaicism, especially the issue of low-allele frequencies. Analysis of resected brain tissue using deep sequencing and single-cell techniques is recommended.[44]

Disease modeling

The complexity of the human brain and the limitation of obtaining lesion tissue make it challenging to study MCD. Genetically modified mouse models can be an essential strategy with strengths in neuropsychiatric and behavioral studies.[213,214] Animal models with defects in PI3K/PTEN/AKT/mTOR pathway can partially mimic cortical neuron abnormalities, behavior abnormalities, electroencephalography abnormalities, and epilepsy. It is important for exploring the histopathological alterations, and pathophysiological mechanisms of MCD, and screening clinical intervention strategies.[13,15,64,215–223] However, the human brain is more advanced and complex in evolution. Species differences between human and animal brain tissues make animals not fully applicable for studying human brain development and neurological disorders.[224]

Traditional two-dimensional monolayer cells originating from humans can eliminate species effects. For example, the primitive neural stem cells from a patient carrying TSC2 mutations have higher proliferative activity and neurons differentiated from such cells show dysmorphic morphology and abnormal connections among cells.[225] A recent study demonstrates that the OLs can make neuronal defects more apparent by generating TSC patient induced pluripotent stem cell (iPSC) derived cortical neuronal and oligodendrocyte cultures.[226] It is helpful to explore neurodevelopmental diseases through cell culture, but the cells can hardly mimic the actual biological processes *in vivo*, as lacking tissue organization and complexity.

Human three-dimensional brain organoids are novel models for studying human neurodevelopmental process diseases. Cerebral organoids can recapitulate at least some aspects of human cortical development; RGCs and neurons

Table 1 PI3K-PTEN-AKT-mTOR pathway mutations in cortical developmental diseases.

Gene symbol	OMIM No.	Mutation type	Inheritance	Effect of the mutation	Phenotype	Related syndrome
PIK3CA	*171834	Germline, somatic	AD	GOF	MEG, PMG[185] FCDII[84] HME[76]	MCAP, MPPH[68,186]
PIK3R2	*603157	Germline	AD	GOF	MEG, PMG[82]	MPPH[186]
RAB39B	*300774	Germline, somatic	XL	LOF	MEG[187]	ASD, ID[187,188]
PTEN	*601728	Germline, somatic	AD	LOF	HME,[84,189] MEG,[89] FCD,[190] PMG[191]	ASD,[89] Cowden syndrome[192]
AKT1	*164730	Germline, somatic	AD	GOF	HME,[105] MEG[104]	Cowden syndrome,[67] Proteus syndrome[104]
AKT3	*611223	Germline, somatic	AD	GOF	HME,[98] MEG,[68] PMG,[70] FCDII[142]	MPPH, MCAP[68,186]
				LOF	MIC[106,107]	\
PPP2R5D	*601646	Germline	AD	LOF	MEG[193]	ID[194]
PAK1	*602590	Germline	AD	GOF	MEG[113]	IDDMSSD[113]
CCND2	*123833	Germline	AD	GOF	MEG,[79] PMG[55]	MCAP, MPPH[55,79]
TSC1	*605284	Germline, somatic	AD	LOF	FCD,[142] TSC,[195,196] HME[142,196]	focal epilepsy[142]
TSC2	*191092	Germline, somatic	AD	LOF	FCD,[131,142] TSC,[131,142,195] HME[196]	focal epilepsy[142]
STRADA	*608626	Germline	AR	LOF	MEG[197]	PMSE[197]
TBC1D7	*612655	Germline	AR	LOF	MEG[198,199]	MGCPH, ID[198,199]
RHEB	*601293	Somatic	AD	GOF	HME,[142] FCDII,[153]	focal epilepsy,[142] ASD[154]
DYRK1A	*600855	Germline	AD	LOF	MIC[87,147]	ID,[147] ASD[87]
HERC1	*605109	Germline	AR	GOF	MEG[148]	MDFPMR[148]
MTOR	*601231	Somatic germline	AD	GOF	MEG,[200] PMG,[201] FCDII,[202] HME[75]	Smith-Kingsmore syndrome[200]
RPS6	*180460	Somatic	\	GOF	HME[203]	HME with intractable epilepsy[203]
DEPDC5	*614191	Germline, somatic	AD	LOF	HME,[76] PMG,[204] FCDI,[205] FCDII[205]	focal epilepsy,[205] FFEVF1
NPRL3	*600928	Germline, somatic	AD	LOF	HME,[206] PMG,[204] FCDII[207]	FFEVF3[204]
NPRL2	*607072	Germline, somatic	AD	LOF	FCD,[204,208] PMG[204]	FFEVF2[204]
KPTN	*615620	Germline	AR	LOF	MEG[178,179]	Familial intellectual disability-macrocephaly syndrome[178,179]
SZT2	*615463	Germline	AR	LOF	MEG[176,177]	Infantile encephalopathy with epilepsy[176,177]

Table 2 Summary of the mTOR inhibitors.

Drug category	mTOR inhibitors	Dosage	Phase
Antibiotic allosteric mTOR inhibitors	Rapamycin (Sirolimus or Rapamune)	Oral solution and tablet.	FDA approved
	Temsirolimus (Torisel/CCI-779)	Intravenous injection	FDA approved EMEA approved
	Everolimus (Afinitor/RAD001)	Oral tablets	FDA approved
	Ridaforolimus (Deforolimus/ AP23573/MK-8669)	Oral	Phase III[244]
	Umirolimus (Biolimus/Biolimus A9/BA9/TRM-986)	Used in drug eluting stent	Phase IV[245]
	Zotarolimus (ABT-578)	Used in drug-loading stent	Phase IV[246]
	WYE-592	\	Not applicable
	ILS-920	Intravenous injection	Phase I[247]
ATP-competitive mTOR inhibitors	Ku-0063794	\	Not applicable
	AZD8055	Oral solution or tablet	Phase I[248]
	AZD2014 (Vistusertib)	Oral tablets	Phase II[249]
	PP242	Oral	Preclinical Study[250,251]
	PP30	\	Not applicable
	PP487	\	Not applicable
	PP121	\	Not applicable
	Torin 1	\	Preclinical study[252]
	Torin 2	Oral	Preclinical Study[253,254]
	OSI-027 (ASP7486)	Oral	Phase I[255]
	CC-223 (Onatasertib)	Oral	Phase II[256]
	XL388	Oral	Preclinical study[257,258]
mTOR/PI3K dual inhibitors	PI-103	\	Not applicable
	PKI-587 (Gedatolisib/PF-05212384)	Intravenous injection	Phase I[259,260]
	PKI-179	\	Not applicable
	KU-BMCL-200908069-1	\	Not applicable
	PQR309 (Bimiralisib)	Oral	Phase II (available at https://www.hra.nhs.uk/)
	PQR514	Oral	Preclinical study[261]
	WAY-001	\	Not applicable
	WAY-600	\	Not applicable
	WYE-687	\	Not applicable
	WYE-354	\	Not applicable
	WYE-132 (WYE-125132)	Oral	Preclinical study[262]
	Wyeth-BMCL-200910096-27	\	Not applicable
	INK-128 (Sapanisertib/TAK-228/MLN0128)	Oral	Phase I[263,264]
	GDC-0941 (Pctilisib)	Intramuscular injection and oral	Phase II[265,266]
	GDC-0980 (Apitolisib/RG7422)	Intramuscular injection and oral	Phase II[266,267]
	Wyeth-BMCL-200910075-9a-h	\	Not applicable
	GNE-493	\	Not applicable
	GNE-477	\	Not applicable
	GSK2126458 (Omipalisib/ GSK458)	Oral film-coated tablet	Phase I[268,269]
	CMG002	Oral	Preclinical study[270]
	XL765 (Voxtalisib)	Oral	Phase II[271]
	NVP-BEZ235 (Dactolisib/ BEZ235)	Oral	Phase III[272]
	NVP-BGT226 (BGT226)	Oral	Preclinical study[273]
	LY3023414	Oral	Phase II[274,275]

Table 2 (*continued*)

Drug category	mTOR inhibitors	Dosage	Phase
	PKI-402	\	Preclinical study[276]
	PF-04691502	Oral and intravenous administration	Phase II[277]
	VS-5584 (SB2343)	Oral	Preclinical study[278,279]
	GDC-0084	Oral	Phase I[280]
	Wortmannin	Intraperitoneal administration	Preclinical study[281,282]
Other new mTOR inhibitors	P529 (Palomid 529/RES-529)	Subconjunctival injection	Phase I[283]
	JR-AB2-011	\	Not applicable
	RapaLink-1	Subcutaneous injection	Preclinical study[284]
	RapaLink-2	\	Not applicable
	RapaLink-3	\	Not applicable
Natural products with mTOR inhibitory activity	Curcumin	Oral	Phase IV[285]
	Epigallocatechin gallate (EGCG)	Oral	Phase IV[286]
	Resveratrol (RSV)	Oral	Phase IV[287]
	Sulforaphane (SFN)	Autism spectrum disorder	Phase II[288]
	Cryptotanshinone (CPT)	\	Preclinical study[289,290]
	Genistein	Oral	Phase III[291]
	Caffeine	Oral and intravenous injection	FDA approved
	Salidroside (Rhodioloside)	\	Preclinical study[292,293]
	Oridonin (Rubescensin A)	\	Not applicable
	Capsaicin	Qutenza patch	FDA approved
	Arctigenin (ATG)	Intraperitoneally injection	Preclinical study[294]
	Apigenin	\	Preclinical study[295]
	Vitexin	Oral	Preclinical study[296]
	Andrographolide	\	Preclinical study[297,298]
	Rotundic acid	\	Not applicable
	Gingkolic acid	\	Not applicable
	Afrocyclamin A	\	Not applicable
	Docetaxel	Injection concentrate	FDA approved

exhibit typical morphological and functional features.[227,228] Brain organoids carrying the *PTEN* mutation were constructed, and *PTEN* mutant human organoids grew larger than controls and developed complex surface folds. While the mouse brain is naturally lissencephalic, and *Pten* mutant mouse organoids developed with no surface folds, underscoring the need to develop human model systems.[229,230] Recently, human brain organoids carrying the *TSC2* mutations also successfully showed the TSC phenotype. Furthermore, the study suggested that the hyperproliferation of caudal late interneuron progenitor cells resulted in brain tumors and cortical tubers.[231] Also, human brain organoids derived from pluripotent stem cells originating from patients with developmental or neurodegenerative brain diseases (*e.g.*, MIC, lissencephaly, ASD) have been successfully constructed. They can mimic impaired neuronal migration, severe apoptosis of neural stem cells, and defective division of outer radial glia cells.[227,232,233] It is more beneficial to explore disease pathogenesis and discover potential drug targets.

Based on the characteristics of different models, the preferred models should be carefully considered when studying MCD disease phenotypes and mechanisms.

Disease therapy

With the advance of whole-exome sequencing and imaging technology, research on cortical developmental disorders was greatly accelerated. Exploring safer and more effective intervention strategies has gradually become a priority. Genetic factors associated with the PI3K/PTEN/AKT/mTOR pathway are important pathogenesis of MCD. Doctors will not be restricted by traditional symptomatic treatment,

such as antiepileptic drugs, stereotactic electroencephalography-mediated radiofrequency ablation, multiple subpial transections, bipolar electrocoagulation on functional cortex, neuromodulation techniques, and surgical excision of the lesions,[234–236] but instead focus on the PI3K/PTEN/AKT/mTOR pathway targeting drugs for etiological treatment. For example, the mTOR inhibitor rapamycin is a targeted drug. It can acutely inhibit mTORC1 by binding it directly. Long-term treatment with rapamycin also can inhibit mTORC2. A possible mechanism is that rapamycin inhibits the assembly of the mTORC2 complex. Thereby, rapamycin can inhibit the abnormal hyperactivation of the pathway caused by mutations of related genes.[237,238] Many studies reveal that rapamycin treatment can rescue neuronal migration defect, neuron overgrowth, brain enlargement, epilepsy, and development delay in animal models of MCD, such as *AKT*, *TSC*, *mTOR*, *PTEN*, *DEPDC5* deficiency mice.[15,17,131,239–241] Currently, mTOR inhibitors mainly include antibiotic allosteric inhibitors, ATP-competitive inhibitors, mTOR/PI3K dual inhibitors, new inhibitors, and natural products. We summarize them in Table 2.[242] In addition, BYL719, a PIK3CA inhibitor that blocks the phosphorylation of AKT, may be a better-targeted drug. Since the therapeutic effect of BYL719 may be driven exclusively by blocking AKT, BYL719 may be an additional option for patients who fail to respond to rapamycin. Moreover, BYL719 does not show significant toxic effects and even improves the clinical symptoms of PIK3CA-related overgrowth spectrum patients better than rapamycin.[243]

Conclusions

Although we have much understanding of MCD, the high heterogeneity of MCD and the overlapping clinical phenotypes of different subtypes, in addition to some MCD in the form of syndromes, all together lead to a high rate of clinical underdiagnosis and misdiagnosis. It is necessary to continue to explore better diagnostic methods and to pay attention to genetic counseling and prenatal screening for MCD. There are many challenges ahead in screening and identifying the causative genes of MCD. More suitable disease models are needed to study the molecular mechanisms of known causative genes and related pathways and to design more effective and safe targeted drugs. Only through accurate diagnosis can patients receive optimal treatment, improve prognosis and living quality, and reduce public health costs.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by the Tou-Yan Innovation Team Program of the Heilongjiang Province, China (No. 2019-15).

Acknowledgements

We thank Dr. Dekai Zhang for supporting this work.

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