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Invited Mini Review

mTOR signalling pathway - A root cause for idiopathic autism?

Harsha Ganesan[1], Venkatesh Balasubramanian[1], Mahalaxmi Iyer[2], Anila Venugopal[1], Mohana Devi Subramaniam[3], Ssang-Goo Cho[4] & Balachandar Vellingiri[1,]*

[1]Human Molecular Cytogenetics and Stem Cell Laboratory, Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore 641046, Tamil Nadu, [2]Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641043, Tamil Nadu, [3]Department of Genetics and Molecular Biology, Vision Research Foundation, Sankara Nethralaya, Chennai 600006, Tamil Nadu, India, [4]Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul 05029, Korea

Autism spectrum disorder (ASD) is a complex neurodevelopmental monogenic disorder with a strong genetic influence. Idiopathic autism could be defined as a type of autism that does not have a specific causative agent. Among signalling cascades, mTOR signalling pathway plays a pivotal role not only in cell cycle, but also in protein synthesis and regulation of brain homeostasis in ASD patients. The present review highlights, underlying mechanism of mTOR and its role in altered signalling cascades as a triggering factor in the onset of idiopathic autism. Further, this review discusses how distorted mTOR signalling pathway stimulates truncated translation in neuronal cells and leads to downregulation of protein synthesis at dendritic spines of the brain. This review concludes by suggesting downstream regulators such as p70S6K, eIF4B, eIF4E of mTOR signalling pathway as promising therapeutic targets for idiopathic autistic individuals. [BMB Reports 2019; 52(7): 424-433]

INTRODUCTION

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder categorized by concomitant manifestation of diminished social communication, restricted/perseverative/stereotypical behaviour and repetitive pattern of activities. The incidence rate of ASD was 58.7 per 10,000 individuals in 2013 (1). However, the number of reported cases with mild to severe autism has gradually increased over the years in various countries. In the United States alone, 1 in 68 children has ASD (2). ASD can be differentiated into four different disorders based on the Diagnostic and Statistical Manual of Mental Disorders IV (DSM − IV): Autistic disorder, Asperger's disorder, Childhood disintegrative disorder and Pervasive developmental disorder (PDD) (3-6). According to DSM − V manual, autism is broadly classified into syndromic and non−syndromic types (7). Syndromic autism comprises of disorders with known causative agents, including monogenic disorders (8) such as Phelan-McDermid syndrome, Noonan syndrome, Rett-syndrome, Timothy syndrome, Tuberous sclerosis complex (TSC), TSC associated Poly hydramnios megalencephaly and symptomatic epilepsy syndrome (PMSE), Phosphatase and tensin homolog (PTEN), Fragile X Syndrome (FXS), Neurofibromatosis, and a number of developmental disorders covered by ASD (3, 9). Idiopathic autism is the non−syndromic type with no known genetic or epigenetic cause for manifestation of the disease (9). Probable causative factors for idiopathic autism include environmental factors (10) such as toxins, pesticides, infection, and in utero exposure to drugs like valproic acid (9-14). There has been various hypotheses, mutational targets, and pathways about idiopathic autism (9, 12). However, none of them could explain the exact reason behind the onset and expression of this disorder.

Signalling pathways are important for the regulation of specific molecular mechanism after activation through a target molecule. Alteration in some signalling pathways can lead to expression of certain features in the human brain, including megalocephaly, axonal misregulation, alteration in neuron size, connectivity of neuronal circuits, proliferation of cerebral cells, protein synthesis and dendritic spine density variation at different regions of the brain (15, 16). Among various pathways, mechanistic target of rapamycin (mTOR) plays a centralized role as a signalling hub that can lead to regulation of certain physiological features of autism. Specifically, in the brain, mTOR pathway is involved in the regulation of synaptogenesis, corticogenesis, and associated functions of neurons (2). Several studies (9, 13, 16) have indicated that the Akt/mTOR pathway which regulates translation at dendritic spines is a potential molecular substrate of autism. Indeed, mutations in genes encoding Akt-mTOR cascade components cause disorders with higher rates of autistic characteristics. Nicolini *et al*. (9) highlighted that decreased mTOR could adversely affect spines by disregulating cortical circuits

*Corresponding author. Tel: +91-4222428514; Fax: +91-422-2422 387; E-mail: geneticbala@yahoo.co.in; geneticbala@buc.edu.in

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implicated in higher cognitive functions and behaviour, thus causing autistic phenotypes. Findings of Hutsler and Zhang (16) also prove the hypothesis that disruptions in the mTOR pathway can negatively affect spines and contribute to autism neuropathology in patients. Hence, in this review, we will highlight the importance of mTOR signalling pathway in idiopathic autism. We will also discuss certain mTOR regulators that play a vital role in the disruption of translation initiation and protein synthesis as a triggering factor for the expression of autistic phenotypes. Finally, we suggest that mTOR signalling pathway will be revolutionary in the field of translation research as a promising strategy to discover remedies for idiopathic autism.

COMMON FEATURES OF IDIOPATHIC AUTISM BRAIN

Neurodevelopment comprises of certain crosslinked molecular mechanisms including neurogenesis, corticogenesis, and synaptogenesis. Adult neurogenesis is the development of new neurons in specific regions of the brain such as the hippocampus and olfactory bulb. This process is particularly important for the conversion of Neural Stem Cells (NSCs) and neural precursors into functional neurons (17). Corticogenesis that leads to mammalian neurocortex development is a part of embryonic neurogenesis. It is required for the origin of six layers of the cortex where neuronal migration begins at the ventricular zone and proceeds towards their final position in the brain (18, 19). Synaptogenesis is the concluding step in neural development which comprises of initiation and linkage of pre- and post-synaptic domains in target neurons as well as regulation of synaptic development through mTOR and Wnt signalling pathways. Following synaptogenesis, ratio of excitatory to inhibitory synapses (E/I ratio) is also important for neural circuit assembly and it analysis the synaptic output (20, 21). Alterations in these key regulatory processes has been reported in neurodegenerative and developmental disorders such as ASD, Parkinson's disease, Alzheimer's disease, Huntington's disease, Amyotrophic Lateral Sclerosis and Schizophrenia (17). During corticogenesis, neuronal migration and laminar formation are critical for brain function. Their disruption due to overexpression of certain glycoproteins such as reelin secreted by cajal-retzius cells and GABAergic interneurons can lead to ASD, bipolar disorder, and schizophrenia (22). Oblak *et al*. (23) have linked decreased GABA receptor signalling to behavioural abnormalities seen in ASD patients. They confirmed this with autoradiography techniques where densities of GABA receptors were monitored in fusiform gyrus and cingulated cortex regions of the brain involved in corticogenesis. Cortical development, the chief course of action in corticogenesis, requires activation and placement of neuronal progenitor cells at radial and angential axis regions of the cerebral cortex in the brain. One major gene involved in corticogenesis is T-Box Brain 1 (TBR1) gene that is also associated with ASD, in which there is a disrupted localization of *TBR1* (24). TBR1 protein can interact with kinase CASK to induce autism (25). Later during synaptogenesis, molecules specific to scaffolding and cell adhesion such as neurexins, neuroligin, cadherin, and Shank3 can alter neuronal activity in ASD and idiopathic autism individuals (21). Specifically, interaction of neuroligins with neurexins is known to regulate postsynaptic activity of both excitatory and inhibitory synapses (20, 26). Mutations in these molecules have been reported in various cases of autism, leading to inhibition, receptor downregulation, and altered synaptic protein placement at the cingulated cortex and fusiform gyrus (25, 27). The importance of β-catenin, a Wnt signalling molecule, in both corticogenesis and synaptogenesis was reviewed by Williams and Casanova (28). They noted that depleted intelligence level of autistic individuals was related to increased total brain volume (TBV) that was also observed in megalencephaly (28, 29). Megalencephaly is also observed in TSC categorised under syndromic autism. This was proven by the deletion of TSC specific gene through Cre-Lox system knockout in mice, leading to megalencephaly with characteristically enlarged soma and nucleus, decreased to absent astroglial activity, mislamination, ectopic placement of neurons, and mTOR hyperactivity (30). Additionally, syndromic autism has increased E/I ratios in various interrelated systems such as emotional, social and sensory systems of the brain. This imbalance in E/I ratio can lead to hyperexcitation and hypoinhibition at cortical circuits in FXS, a type of ASD (20). E/I imbalance could also disrupt N-methyl-D-aspartic acid receptor (NMDAR) dependent cascade which negatively regulates mTOR pathway (31). Among other factors involved in manifestation of autism etiology, amyloid-β precursor protein (APP) shows elevated levels at synapses, leading to overgrowth of cranial neurons and further alteration of (PI3K/Akt/mTOR) signalling pathways. These alterations are due to *PTEN* mutations that cause brain tumours and macrocephaly in autism, proving that abnormal cell proliferation could also be associated with mTOR imbalance (32). Furthermore, Osborne (19) has linked hyperactivation of mTOR to PMSE, showing that disruption of corticogenesis is associated with mTOR. Megalencephaly is not only observed in PMSE, but also found in idiopathic autism patient. It is a common feature of idiopathic autism patient where there is an increase of cell growth that could be an outcome of increased protein synthesis due to mTOR hyperactivation (19). Idiopathic autism patients exhibit a loss in socio-emotional and face recognition ability. Such loss is linked to changes in the anterior, posterior cingulated cortex and the fusiform gyrus in the cerebral cortex where GABA receptor binding ability is decreased (23). Alteration in mTOR pathway can lead to heterotrophia and dysplasia where the structure of the cortex is distorted by changing the cortical circuitry (19). Accelerated mTOR activity at late corticogenesis in idiopathic autism individuals can result in misplacement of neurons, leading to alteration of neural identity. These disadvantages could be

controlled by cap-dependant translation that targets 4E-Binding Proteins (4E-BPs) and prevents ectopic neuron placement and mislamination due to accelerated downstream mTOR signalling (33). Lin *et al*. (33) have proven that knockdown of 4E-BP can lead to various changes in the neural architecture, including elevated soma size in the neuron, dendritic development, and changes of synaptic potential and spine density. These findings reveal that mTOR signalling pathway alterations could be contributory mediators of idiopathic autism.

SIGNALLING PATHWAYS AS MODULATORS OF SYNDROMIC AND IDIOPATHIC AUTISM

Autism is linked to certain signalling pathways such as Wnt pathway, calcium and calmodulin pathway and PI3K/mTOR signalling pathway. Wnt pathway gives a better understanding of cellular differentiation, polarity, and proliferation in different tissues. Its deregulation leads to various types of cancers and certain cases of ASD as reported by Oron and Elliot (34). Maturation and development of brain are associated with the canonical Wnt pathway. In individuals with ASD, the Wnt signalling pathway is linked to cortical patterning, upregulation of dendritic spine density, and alteration of spine morphology (35). Other than Wnt signalling, calcium concentrations and calmodulin binding capacity can also affect various components of the Central Nervous System (CNS), specifically targeting the pre- and post-synaptic responses of neurons (36). Wen *et al*. (37) have linked MAPK signalling pathways to 20 other functional pathways and 22 ASD associated genes. They also mentioned the role of calcium and MAPK signalling pathways as interacting hubs for various other interrelated pathways in ASD. Another targeted candidate is nerve growth factor (NGF) induced signalling that leads to significant decrease in 4E-BP1 protein and eukaryotic translation initiation factor 4E (eIF4E) that induces oxidative stress in autistic patients (38). Both 4E-BP1 and eIF4E are major components of the mTOR signalling pathway. Nicolini *et al*. (9) have reported a deregulation of 4E-BP1 in valproic acid (VPA) exposed mice model for idiopathic autism. Hence, we can correlate NGF signalling pathway and mTOR signalling pathway with oxidative stress related ASD. In this review, we will focus on mTOR pathway which has received wide attention owing to its important role in activating target genes in syndromic autism.

mTOR - THE SIGNALLING HUB

mTOR plays a centralised role in inducing and activating various interlinked pathways. Thus, it acts as a signalling hub and regulates certain physiological features of the cell. Various intracellular and extracellular processes including protein synthesis, RNA biogenesis, autophagy, cell growth, and survival (39) are pictorially presented in Fig. 1. mTOR is also associated with various diseases including various cancers (40, 41) such as prostate cancer (42), kidney cancer (43), and breast cancer (44) due to mutations and misregulations in the mTOR pathway. It is also associated with other diseases including cardiovascular disease (45), renal diseases (46), pulmonary fibrosis (47), and diabetes mellitus (48). Associations of mTOR signalling pathway with normal and abnormal brain, neurite development, synapse formation and associated functions have been discussed in detail. mTOR signalling pathway is also associated with abnormal developmental features of the brain including Megalencephaly (49, 50), hemimegalencephaly (51), pigmentary mosaicism (50), glioblastoma multiforme (52), astrocytoma (52), and focal cortical dysplasia (53). These defects are mainly associated with malfunction of mTOR associated cells either upstream or downstream of the signalling cascade (50, 54). Specifically, in the brain, mTOR is involved in the regulation of synaptogenesis, corticogenesis, and associated functions of neurons. mTOR is also related to a number of neuropsychatric and neurodevelopmental disorders such as Alzheimer's disease, ASD, and idiopathic autism (55). Altered mTORC1 activity due to mutations in TSC has been linked to Alzheimer's disease characterised by accumulation of cellular amyloid-β proteins, decreased soma size, and decreased activities of mTORC1 and mTORC2 (56). Activation and phosphorylation of Akt, a protein kinase, play a major role in the onset of mTOR pathway because Akt targets TSC and

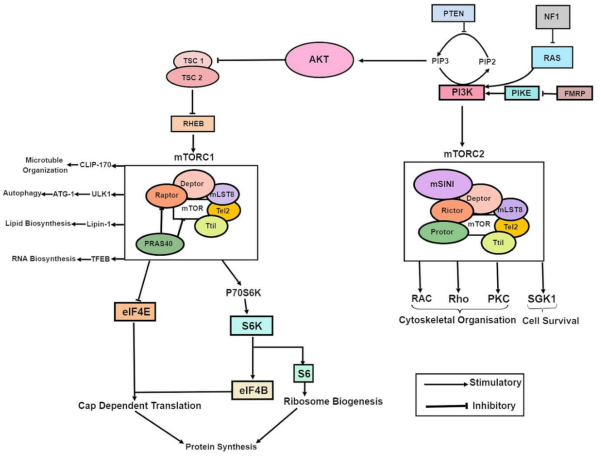

Fig. 1. Schematic representation of various molecular inhibitors and stimulators in mTOR signalling pathway. PTEN and NF1 could inhibit the activation of AKT. AKT when mutated could inhibit the activation of TSC 1 and TSC 2 that are precursors of mTORC1. FMRP inhibition could lead to deactivation of mTORC2. When stimulated, mTORC1 is involved in various cellular processes including microtubule organisation, autophagy, lipid biosynthesis, RNA biosynthesis, and protein synthesis using precursors of CLIP-170, ULK1, Lipin-1, TFEB, and P70S6K, respectively. Stimulation of mTORC2 leads to proper cytoskeletal organisation through RAC, Rho, and PKC. Cell survival is achieved through SGK1.

regulates mTORC1 activity (57). Mutations in TSC1 and TSC2 can lead to alteration in the activity and size of brain, leading to megalencephaly and dysmorphisms of developing neurons, glia, and progenitor cells (58). In contrary to Alzheimer's where altered soma size is the main characteristic feature, hyperactivity of mTOR signalling in ASD leads to megalencephaly, overconnectivity of neurons and increases the spine growth (15).

FUNCTION OF mTOR SIGNALLING PATHWAY IN ASD

Overlaps among cellular phenotypes, genes, and molecular and biochemical pathways have led to the classification of a number of monogenetic syndromes as part of the broader spectrum of ASD as depicted in Fig. 2 (7). Mutations in mTOR signalling including TSC1, TSC2, PTEN, and Phosphoinositide 3-kinases (PI3K) components have also led to expression of these disorders (59) as explained in detail in Table 1. One of the major upstream signalling components that activate mTORC1 is Ras homolog enriched in brain (RHEB) in brain cells. It could be blocked by mutated TSC, leading to tumorous outgrowth in the brain. TSC complex inhibits RHEB and mTORC. When this upstream process is altered, there is hyperactivation of mTORC1. Another regulating molecule is PTEN which is involved in Akt activation and lipid signalling

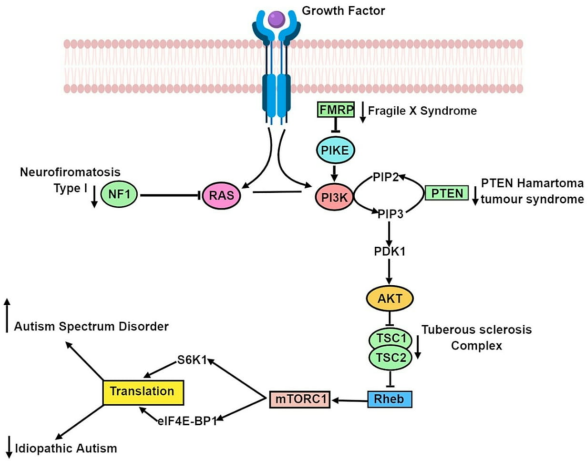

Fig. 2. Cell signalling dysfunction in mTOR signalling pathway leading to syndromic and idiopathic autism. Pictorial illustration of mutations in specific genes FMRP, NF1, PTEN, and TSC1/TSC2 that lead to loss/ reduction of function causing syndromic autism of fragile X syndrome, neurofibromatosis type 1, PTEN Hamartoma tumour syndrome, and tuberous sclerosis complex, respectively. mTORC1 stimulation leads to activation of 4EBP which hinders the translation or activation of P70S6K that triggers translation initiation. Truncated protein synthesis by these down regulators during translation leads to hyperactivation, causing autism spectrum disorder or hypoactivation of protein synthesis that leads to idiopathic autism.

by regulating PI3K levels. Misregulation of PI3K leads to hyperactivity of not just mTORC1, but also Akt pathway (60). Another not well explored aspect of mTOR pathway is the fragile X mental retardation protein (FMRP) that plays a role in activating fragile X syndrome (FXS). It is known that 50% of FXS individuals have ASD and mutation in the *Fmr1* gene that codes for FMRP protein which in turn is involved in PI3K regulation (61, 62). Although the contribution of mTOR to this disorder has not been well understood yet, it is known that PI3K subunit has an elevated amount in knockout mouse models of FXS (61, 62). Enlarged murine cerebellum and hippocampus is also a characteristic of Angelman syndrome where there is a deletion in Ube3a gene that causes reduced activation of mTORC2 and hyperactivation of mTORC1 signalling (63). *Mecp2* mutation causes Rett syndrome that has decreased protein synthesis and mTORC1 activity (59). These features are due to distorted cell signalling and biochemical pathways, leading to brain enlargement in autism (64). Altered signalling in the brain includes changes in growth factor signalling such as brain derived neurotrophic factor (BDNF), NGF, MAP kinase, ERK signalling systems, protein synthesis, and mTOR pathway (15). Hyper expansion of cortical surface area, etiological heterogeneity, and alteration in corticogenesis are some of prodromal features of an autistic individual. The chief process affected downstream of this signalling cascade is the protein synthesis. S6 protein kinases (S6K) and 4E-BPs are two major substrates that govern mRNA translation. Phosphorylation of ribosomal protein S6 and suppression of methylated cap binding between eIF4E and 4E-BPs can lead to inhibition of translation. Hence, mTORC1 activation is mandatory for protein synthesis (65). Although abnormal mTOR function has been connected to syndromic autism by various studies as mentioned above, its role in idiopathic autism has been scarcely explored.

THE SPARSELY EXPLORED TYPE OF AUTISM: IDIOPATHIC AUTISM

Despite there are increased knowledge about pathways that create havoc in syndromic autism, there are far fewer researches related to the physiology and manifestation of idiopathic autism. VPA (66) induced knockout mouse models and induced Pluripotent Stem Cells (iPSC) (67) are the widely used models to study idiopathic autism. Similar to knockout mice models, BTBR mice can serve a model organism for studying ASD and idiopathic autism. Meyza and Blanchard (68) have highlighted the importance of BTBR $T^{+}Itpr3^{tf}/J$ mouse for studying idiopathic autism as convergence of multiple signalling pathways and exhibition of distinct neuroanatomical features.

mTOR is involved in various neurodevelopmental processes, including neuronal differentiation, axon guidance, cell migration, and neural region patterning. These processes are altered in idiopathic autism. Roles of dendritic spine

Table 1. Effect of various mTOR associated signalling molecules autism spectrum disorders

mTOR involved molecule (gene/ precursor/ receptor)	Affected region in brain	Effect of mTOR associated molecule/ Results	Model system	Process involved	Disease induced	Analytical methods	Reference
PTEN	Hippocampus, Cerebral cortex	Macrocephaly, Neurohypertrophy Increased length and thickness in dendritic arbors Variation in response to sensory stimuli, learning and anxiety.	Mouse	AKT/mTOR pathway activation and Gsk3β inactivation	-	Cre mediated recombination in mice Behavioural testing of mutant mice Immunohistochemical staining Cell counting Golgi staining Electron microscopy EEG/EMG recording	86
PTEN	Hippocampus, Cerebral cortex	Macrocephaly, Neurohypertrophy, Increased seizures, Decreased adaptability to environmental stimuli, Pten's effect on PI3K cascade, inturn affects the circadian rhythm.	Mouse	Circadian rhythm	-	EEG/EMG recording Wheel running test Statistical analysis	87
Tsc1 Rheb	Neural progenitor cells in Sub Ventricular Zone	Heterotropia in RMS and OB Enlarged microglia Cell migration disrupted by Rheb with Mash1 cells at the RMS Neuronal migration speed decreased Increased mTOR signalling does not affect the action potential	Mouse	Increased mTOR signaling	Tuberous Sclerosis	Electrophoration of plasmid Migration, morphometric, micronodule assays Immunostaining Confocal microscopy	88
Tsc1 Tsc2	Hippocampal pyramidal neurons	Increased phosphorylation of S6 Absence of Tsc1 leads to increase in soma size but decrease, decrease in dendritic spines, increased synaptic current	Mouse Rat	Tsc up/down regulation	Tuberous sclerosis	Immunostaining Two-photon laser scanning microscopy Electrophysiology	89
Rictor	Central Nervous System Purkinje cells	mTORC2 affects cell size, Neuronal morphology and function Only RiPuKO mice had synaptic functional changes mTORC2 plays key role in synaptic homeostasis	Mouse	Rictor deficiency	-	Immunohistochemical analysis Electrophysiological analysis Biochemical analysis RT-PCR Mouse behaviour analysis	90
Tsc1 Pten	Hippocampal neurons	Increase in action potential, dendrite length and soma size Tsc1 loss does not affect the excitatory neurons, unlike pten which increases it Excitation to inhibition ration in the neural network is altered	Mouse	Excitatory and inhibitory synaptic transmission	-	Electrophysiological analysis Immunocytochemistry	91
Tsc1 Tsc2	Axon	Tsc1/2 plays an important role in axon formation, neural polarity Tsc2 inhibition leads to mTOR activation Tsc/mTOR pathway leads to axonal regeneration	Mouse	Polarised activation and inactivation of Tsc pathway	Tuberous sclerosis	Transfection Lentiviral infection Immunocytochemistry Immunohistochemistry	92

formation, function, and neural plasticity at the fusiform gyrus in idiopathic autism have been studied by Nicolini *et al*. (9). They targeted protein synthesis regulation of mTOR and noted decreased protein expression levels of various isoforms of P13K, p85, Akt, mTOR, p-mTOR, p70S6K, and eIF4B in idiopathic autism. Variance of tropomyosin receptor kinase B (TrKB), a BDNF that monitors the trafficking of postsynaptic density protein-95 (PSD-95) through regulation of P13K/Akt, is also observed in idiopathic autism. TrKB is also expressed at various levels in different regions of the brain (9). TrKB-FL isoforms in neurons are necessary for the activation of TrKB at the glia. Truncation of these molecules leads to alteration of Akt/mTOR pathway which in turn causes adverse effects in dendritic spines and cortical circuits (66-68). Onore *et al*. (69) have performed similar protein analysis in cells of CNS and immune system. They also compared phosphorylated and total protein levels and found higher total protein levels of IRS1 and RSP6 with higher phosphorylated protein levels of PTEN, TSC2, and mTOR in autistic individuals. De Rosa *et al*. (70) have analyzed cortical neurogenesis by determining transcriptional changes at two different time points in the developing brain to monitor differentially expressed genes. They reported dysregulations of synaptic activity, calcium signalling, and cell migration in idiopathic autism. Their work showed a gradual decrease of spiking activity in the brain of affected individual (70), further confirming that downregulation of signalling molecules could lead to the onset of idiopathic autism.

mTOR REGULATORS AS POTENTIAL THERAPEUTIC TARGETS

Novel therapeutic target identification and implementation against diseases have become mandatory for various neurodevelopmental conditions, especially ASD and idiopathic autism since the number of individuals affected by them is increasing globally (3). mTOR is involved in the regulation of various intracellular processes as explained in Fig. 1. However, its key role in ASD and idiopathic autism is the maintenance of amino acid pool by regulating protein translation at dendritic spines in the brain (71).

mTOR-S6K is also involved in the regulation of translation by eIF4F, a protein composed of eukaryotic initiation factors eIF4E and eIF4A (71, 72). Inhibition of cap binding to eIF4F by 4EBPs as a major rate altering process could be prevented when mTORC1 phosphorylates 4EBPs which hinders the inhibitory role of this molecule, thereby activating the translation initiation at specific regions of the brain such as dendritic spines (73). Phosphorylation of specific subunits of mTOR involved in translational initiation is necessary to regulate various cellular processes as shown in Fig. 1. These molecules include eIF4B which activates eIF4A, S6 ribosomal subunit (74), PDCD4 that inhibits eIF4A translation by phosphorylation, and various mRNA splicing factors (75). Presence of certain postsynaptic proteins including postsynaptic density protein 95 kDa (PSD-95), NMDA, metabotropic glutamate receptor subtype 5 (mGlu R5) that take part in translational initiation is important for structural organisation of prefrontal cortex of the brain (76). Phosphorylation of these downstream regulators has been studied by Jernigan *et al*. (76). They found that levels mTOR, p70S6K, eIF4B proteins were decreased in major depressive disorder (MDD). They reported a uniform dysregulation of mTOR/p70S6K/eIF4B pathway that leads to changes in neuronal architecture, thereby causing encephalopathy, dendritic spine alteration, and cancer in some cases (9, 76, 77). Abnormal spine density has been noted in various forms of ASD and idiopathic autism associated with upregulation or downregulation of mTOR pathway at dendritic spines (68). Roles of p70S6K/eIF4B and 4E-BP1/eIF4E in dendritic spines are crucial as they are involved in excitatory postsynapses observed in fusiform gyrus of ASD post-mortem brain samples (9). Nicolini *et al*. (66) have also reported decreased levels of PSD-95 at the fusiform gyrus of idiopathic autism subjects. Furthermore, imbalances of BDNF and TrkB have been widely studied in relation to autism but the change in PSD-95 (postsynaptic marker) protein level could be used as a therapeutic target in treating neural circuitry defects of autism, especially in idiopathic (3, 9, 15, 66). Shott *et al*. (78) have analysed expression levels of Akt1/p70S6K/eIF4B proteins by kinomics using multiplex assay and noted a 6% change in cytoplasmic protein expression level in prion disease. Other postsynaptic proteins associated with autism include neuroligins whose levels are increased after translation in 4EBP in *4E-BP2*-KO and eIF4E overexpressed mice that show significant alteration in E/I ratio. These results further confirm the importance of these downstream regulators of mTOR signalling pathway in circadian regulation and ASD respectively (79, 80). Dennis *et al*. (81) have studied phosphorylated protein translation in 4E-BP1/2 double knockout (DKO) mice and found higher p70S6K1 protein levels in the liver. They also compared γ-form of 4E-BP1 to other forms ($\alpha + \beta + \gamma$). They further demonstrate that eIF4E-eIF4G complex interaction is critical in cap-dependent mRNA translation. Ribosomal protein kinase p70S6K is another rate altering molecule downstream of mTOR signalling pathway (82, 83). P70S6K has been proposed as a molecular marker for lymphoid cancer (82) and ovarian cancer (84) as it is involved in the regulation of protein synthesis in these particular diseases (81, 85). However, its role as a novel therapeutic target for idiopathic autism has been seldom explored. Effect of p70S6K signalling pathway on murine frontal cortex through protein translation has been studied by treating rats with MK-801 which modulates p70S6K-S6/eIF4B pathway in developing rat brain. A significant decrease in phosphorylation of 40S ribosomal subunit S6 by p70S6K has been observed in insular, cingulated, and prefrontal cortex of rat brain (85). This decrease in phosphorylation results in altered protein expression in an idiopathic autism brain as pictorially represented in Fig. 3,

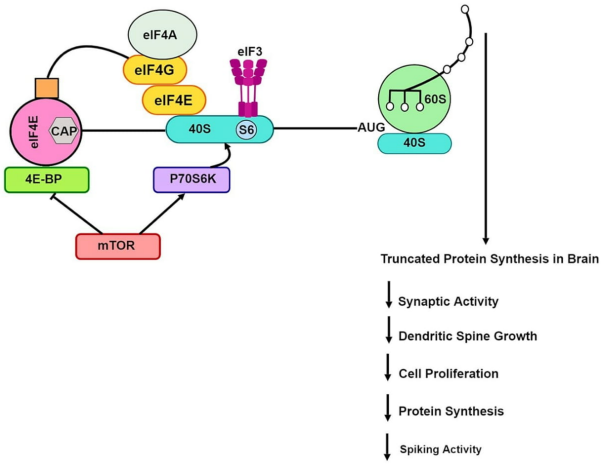

Fig. 3. Role of mTOR signalling pathway during protein translation. Translation initiation at the brain is regulated by various translation initiation factors including eIF3, eIF4A, eIF4B, eIF4G, and cap binding protein eIF4E. Mechanism of mTOR is regulated by either 4E-BP or P70S6K. 4E-BP binds to eIF4E and inhibits the initiation of translation while S6 kinase from P70S6K binds to the 40S ribosomal subunit and leads to truncated protein synthesis in affected regions. Altered translation leads to development of adverse characteristics in the neuronal phenotype of idiopathic autism individuals.

leading to distorted phenotypic expression. Based on these findings, we propose that interaction between p70S6K/eIF4B and 4E-BP1/eIF4E is a benchmark in autism targeted therapy. It would pave way for future research on idiopathic autism.

CONCLUSION

mTOR plays a critical role in not only the regulation of various cellular functions such as cell growth, cell proliferation, lipid synthesis, and protein synthesis, but also plays a critical role in neurodevelopmental processes such as regulation of neurogenesis, corticogenesis, synaptogenesis, axon guidance, and cellular migration (2). Truncation of signalling molecules of mTOR that govern both upstream and downstream processes in the cascade can lead to developmental disorders such as ASD and idiopathic autism. Subjects with idiopathic autism exhibit drastic decreases in synaptic activity, dendritic spine growth, cellular proliferation, protein synthesis, and spiking activity due to significant downregulation of mTOR signalling molecules in the brain. The major process that could alter the downregulation of mTOR signalling cascade is truncated mRNA translation through binding to protein 4E-BP1 and altering 5′ capped mRNA translation initiation by initiation factor eIF4E. eIF4E either up-regulates or decreases p70S6K and eIF4B expression which in turn can modulate mTOR dependent translation (9). As no alteration was reported in 4E-BP1 and eIF4E but a demur in p70S6K signalling was observed in the mTOR pathway, p70S6K could be targeted as a regulator of idiopathic autism. Collectively, we conclude that proper regulation of p70S6K and associated down regulators of mTOR signalling pathway is a critical in the maintenance of brain homeostasis.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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