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Translational research: Rett syndrome and Tuberous Sclerosis Complex

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Abstract

Purpose of review—Rare genetic diseases that affect behavior and cognition provide a unique opportunity to study the mechanisms of neurodevelopmental disorders through the examination of animal models, which can lead to development of hypotheses and treatments testable in human beings. Rett syndrome (RTT) and Tuberous sclerosis complex (TSC) are both Mendelian disorders that present with autism, epilepsy, and intellectual disability where animal model work has been directly translated into clinical treatment trials currently underway. Here we review recent advances in our understanding of RTT and TSC pathogenesis and signaling pathways that may be targeted for novel treatments.

Recent findings—Animal models generated by engineering mutant forms of the mouse homologs of human genes involved in RTT and TSC has allowed dissection of the molecular pathology. They have further acted as *in vivo* assays of potential therapeutic strategies that have translated to human clinical trials.

Summary—Single gene disorders associated with neurodevelopmental disorders provide powerful model systems to study the roles of individual molecules and associated signaling pathways in the genesis of autism, epilepsy, cognitive impairment and neuropsychiatric symptoms. These diseases are leading to disease-modifying human therapies that may eventually translate to wider therapeutic strategies for autism.

Keywords

mTOR; MECP2; IGF1; TSC1; TSC2

INTRODUCTION

Autism is one of the most highly heritable neuropsychiatric diseases with up to 90% penetrance, although the underlying genetics are complex. A number of single-gene

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disorders confer elevated risks of autism. Although these diseases account for the minority of cases of autism, the molecular perturbations caused by genetic mutations may serve to identify common pathogenic mechanisms shared by autism spectrum disorders (ASD). Many of these diseases, including TSC, RTT, Angelman syndrome and Fragile X syndrome give rise to perturbations of neuronal development and/or function, particularly in the structure, organization and function of the synapse. Synaptic dysfunction likely underlies some of the co-morbidities seen in ASD such as epilepsy, cognitive impairment and motor dysfunction. These monogenic disorders allow creation of animal models of autism-associated disease and the ability to probe the fundamental neurobiology of these conditions. Such disease models have led to testable hypotheses for pathogenesis and intervention. Pre-clinical advances have led to the identification of rational targets for drug therapies focused on reversing or modifying the underlying neural dysfunction. These animal models have also raised the exciting possibility of disease reversal and rescue of neurocognitive phenotypes in symptomatic adult animals in conditions such as RTT, TSC and Fragile X. Increasingly these preclinical discoveries are being translated directly into human clinical trials. Here we review recent advances in the neurobiology of two single gene disorders that overlap with autism, TSC and RTT, and the promise of translation of this understanding into human therapies.

RETT SYNDROME

RTT (MIM 312750) is an X-linked neurodevelopmental disorder primarily affecting girls. Over 95% of typical RTT cases are due to mutations in the gene encoding the transcriptional modulator methyl-CpG-binding protein 2 (*MECP2*)[1]. In classical RTT children have apparently normal early psychomotor development followed by profound developmental stagnation and regression after the age of 6 months, with loss of fine motor skills and language and acquisition of hand stereotypies and autistic behavior. Postnatal slowing of head growth is common. Following regression children enter a pseudo-stationary phase. During this period other clinical manifestations such as seizures, respiratory dysfunction, gastrointestinal disease, anxiety, sleep disorders, autonomic abnormalities and growth failure become prominent. As RTT individuals age mood disorders, scoliosis, dystonia and Parkinsonism evolves leading to progressive psychomotor deterioration[2–4]. Neuropathological studies demonstrate lower brain weights and smaller, more densely packed neurons with sparse dendritic arborizations but no evidence of degeneration or atrophy, suggesting that RTT is not a primary neurodegenerative disorder[5].

MECP2 structure and function—MeCP2 is a methyl-CpG binding domain (MBD) protein that binds symmetrically methylated CpG sites. It localizes primarily in the nucleus and binds the transcriptional co-repressor Sin3a to recruit the histone deacetylases resulting in chromatin compaction[6]. MeCP2 is abundantly expressed in the brain from fetal life onwards, initially in the brainstem and Cajal-Retzius cells and then in progressively rostral and neo-cortical regions during childhood[7]. The full molecular pathology of RTT is still to be determined but current evidence points to pleiotropic functions of MeCP2 in transcriptional regulation, brain development, neuronal structure, synaptic function and glial-neuronal trophic interactions (well reviewed in [8] and [9]). Transcriptional profiling experiments in *Mecp2* loss-of-function and gain-of-function mice suggest that MeCP2 may act both as a specific transcriptional repressor and activator[10].

Complicating this scenario is the possibility that MeCP2 may also act at a more global level as a histone linker in neurons. In the absence of MeCP2, histone H1 levels increase to those seen in non-neuronal cells with a global rise in acetylation and de-repression of transcription of repetitive elements[11]. The relationship between specific regulation of brain-specific transcripts and more global transcriptional repression through deacetylation is unknown.

Despite the complexity of the role of MeCP2 as a transcriptional regulator, it is clear that some genes fundamental to brain development and function consistently show expression changes in the absence of MeCP2. BDNF is a key growth factor in neurogenesis, neuronal survival and maturation and synaptic plasticity. In culture, MeCP2 binds to the BDNF promoter IV and represses transcription. MeCP2 is released following membrane depolarization allowing activity-dependent BDNF transcription[12]. BDNF is currently one of the best-characterized targets of MeCP2 transcriptional regulation, directly linking MeCP2 to activity-dependent synaptic plasticity as well as more homeostatic regulation of neuronal structure and function.

Animal models of RTT—The development of animal models has been critical to understanding the functional consequences of MeCP2 loss-of-function and gain-of-function, as well as critical cell lineages and cell-specific and non-cell autonomous consequences of MeCP2 loss in both neurons and, intriguingly, in glia. These models, generally based on Cre-LoxP tissue-specific and time-specific conditional deletions, have been generated and recapitulate many symptoms of the human phenotype. These include abnormal gait, limb stereotypies, seizures, cardiorespiratory irregularities, reduced social interactions, anxiety, learning and memory deficits, growth failure and early death (reviewed comprehensively in [13].) RTT animal models also show electrophysiological and neuronal and synaptic morphological anomalies analogous to human RTT. MeCP2 plays crucial role in experience-dependent refinement of synaptic circuits [14,15]. Studies in animal models have demonstrated that loss of MeCP2 in forebrain neurons alone can give rise to the disease while loss of MeCP2 in other neuronal populations (e.g. hypothalamus, dopaminergic, serotonergic, GABAergic) identify potential neural substrates for different aspects of the RTT phenotype including autistic behaviors[16].

Recent studies have demonstrated that loss of MeCP2 from glia negatively influences neurons in a non-cell autonomous fashion, inhibiting dendritic arborization. In globally MeCP2-deficient mice, preferential re-expression of MeCP2 in astrocytes caused significant phenotypic improvements as well as restoration of normal dendritic morphology, suggesting a non-cell autonomous role for glia in the pathogenesis of RTT[17]. Animal studies have also critically informed our understanding of the role of MeCP2 through the life span. Deletion of *Mecp2* in fully mature adult mice develop symptoms similar to germ line null mice suggesting that MeCP2 effects in gene expression and neuronal function appear soon after deletion and are independent of any specific pre or postnatal developmental stage[9]. It appears rather that neuronal function is dependent on normal MeCP2 expression and function throughout postnatal life.

Translational studies and therapeutics—From a translational standpoint, by far the most compelling studies from RTT animal models have been the demonstration of potential reversibility of the disorder. Re-expression of MeCP2 in symptomatic mice that lack *Mecp2* rescues most aspects of the disease[18]. Critically however, other studies have demonstrated that MeCP2 overexpression is detrimental, suggested clinically by the syndrome of severe mental retardation associated with *MECP2* duplication[19]. These studies suggest that expression of MeCP2 at optimum levels in as many cells as possible in the brain is critical to restoration of normal brain function. About 35% of pathogenic *MECP2* mutations are nonsense mutations that may be amenable to pharmacologically induced “read through” of premature stop codons. Recent *ex vivo* culture studies from human and mouse fibroblasts carrying one of the more common nonsense RTT mutations (R168X) demonstrated read through by aminoglycosides including gentamicin, capable of suppressing translation termination[20,21].

Attention has also focused on potential downstream targets of MeCP2. Increasing BDNF levels in *Mecp2*-deficient mice can improve survival and partially reverse the RTT-like phenotype[22]. This has led to potential therapeutic strategies with ampakines that can increase BDNF expression (exogenous BDNF itself does not cross the blood-brain barrier well) and BDNF mimetics. Treatment of *Mecp2*-null mice with the ampakine CX546 that elevates central BDNF levels improved respiratory function[23].

Another growth factor that, like BDNF, is widely expressed in the CNS is Insulin-like growth factor 1 (IGF1). IGF1 strongly promotes neuronal cell survival and synaptic maturation via the PI3K and MAPK signaling, similar to BDNF. MeCP2 may potentially regulate IGF1 expression by binding to its promoter region. In addition, both RTT mice and patients express aberrantly high levels of the IGF binding protein 3. IGF1, particularly in its tri-peptide form, crosses the blood brain barrier and neuronal activity drives localized transport of serum IGF1 into the CNS. These factors suggest IGF1 as an attractive candidate for treatment of RTT. Exogenous administration of IGF1 partially reverses RTT symptoms in MeCP2 mutant mice[24]. This work has led directly to Phase 2 studies of recombinant human IGF-1 (mecasermin, Increlex®) in girls aged 2–12 years.

TUBEROUS SCLEROSIS COMPLEX

TSC is a multisystem genetic disorder, in which 90–95% of the affected individuals have CNS manifestation. TSC can present with intellectual disability, autism spectrum disorders (ASD), and seizures [25]. Epilepsy occurs in 80% to 90% of all patients and is often medically refractory. Approximately 45% of patients have mild-to-profound intellectual disabilities and ASD occurs in up to 50% of patients [25,26]. The neuropathological findings in the brain usually take the form of (1) subependymal nodules (SENs), (2) subependymal giant cell astrocytomas (SEGAs) and (3) cortical tubers [27]. Accumulating evidence suggests that TSC patients have non-tuber abnormalities that contribute to the development of the neurological phenotype – in particular, disorganization of axon tracts, aberrant myelination and synaptic plasticity. These findings have markedly expanded the window of therapy and the potential treatment options.

Proteins encoded by *TSC1* and TSC2 genes, also known as hamartin and tuberin respectively, bind to each other to form a GTPase activating protein (GAP) complex that plays a critical role in the regulation of protein synthesis, controlling cell growth and size [28]. Active TSC2 inhibits Ras family GTPase Rheb, which in turn positively regulates the serine-threonine kinase mammalian target of rapamycin (mTOR), a central regulator of protein synthesis. mTOR kinase exists in two distinct functional complexes, mTOR Complex 1 and mTOR Complex 2, defined by two groups of binding partners. mTORC1 is bound strongly and is quickly inhibited by rapamycin, while mTORC2 inhibition requires prolonged rapamycin treatment, which blocks mTORC2 assembly [29]. Phosphorylation by mTORC1 activates ribosomal S6 kinases (S6K1 and S6K2) and inhibits the translational regulator 4E-BP1 – both events that positively regulate translation of 5′capped mRNAs [30,31]. Thus, without the functional TSC complex, mTORC1 is hyperactive, resulting in constitutively phosphorylated S6 protein, disinhibited protein synthesis, and subsequent cell growth [32,33].

Roles of TSC1/2 protein complex in neuronal development and function—

Studies performed both *in vitro* and *in vivo* using mouse models demonstrated that Tsc1 and Tsc2 proteins play crucial roles not only in cell growth, but also in axon specification, guidance, myelination, dendritic morphology, synaptic plasticity and function, similar to what was described for MeCP2 above. During early development, TSC pathway components are expressed in neurons in a polarized manner, much higher in nascent axons than dendrites [34–36]. In the CNS, almost all neurons have a single axon and multiple

dendrites. Overexpression of Tsc1 and Tsc2 suppresses axon formation while loss of Tsc1 or Tsc2 function leads to increased axon number [34]. Furthermore, haploinsufficiency of Tsc2 is sufficient to produce aberrant neuronal projections. Axons of retinal ganglion cells find their synaptic targets in the thalamus by interacting with a group of repulsive axon guidance molecules called ephrins, which bind to cell surface receptors called Eph receptors. *Tsc2*+/− axons display abnormal growth cone collapse in response to ephrins [37]. Finally, neuronal Tsc1 or Tsc2 expression appears to be crucial for proper myelination of axons. Loss of *Tsc1* in neurons causes a lack of induction of myelination, consistent with crucial developmental interaction between neurons and oligodendrocytes [38].

The TSC/mTOR pathway also regulates dendritic arborization and spine morphogenesis [39–41]. Activation of PI3K and Akt both increase cell size and dendritic complexity while inhibition of endogenous PI3K and Akt reduce cell size and dendritic branching [39,40]. These effects appear to be mediated through mTOR, as treatment with rapamycin or mTOR RNAi decreased dendritic branching [39,40]. Tsc1 or Tsc2 loss increases spine length and head width and decreases the density of dendritic spines in hippocampal slice cultures [41] and *in vivo* [42]. In addition to structural changes in dendrites, the mTOR pathway plays a role in postsynaptic AMPA receptor expression [43]. In Tsc1 deficient hippocampal neurons, the AMPA/NMDA receptor current ratio was significantly increased relative to that in controls, suggesting an aberrant relative enhancement of synaptic AMPA receptors [41]. Furthermore, loss of Tsc1 in the hippocampus abolishes mGluR-dependent long-term depression [44]. Finally, staining of cortical tubers from TSC patients has demonstrated a reduction in GluR2 and NR2A staining in giant cells and dysplastic neuron cell bodies [45]. Together, the pre- and post-synaptic roles that the TSC/mTOR pathway plays strongly indicate that abnormalities in this pathway are likely to result in defects in synapse formation and plasticity, likely correlating with the neurocognitive and behavioural symptoms of the disease.

CNS Mouse models of TSC—There are several mouse models of TSC, and although none manifest all of the precise manifestations of the CNS phenotype in humans – cortical tubers, subependymal nodules or SEGAs – each has provided valuable insight. The first heterozygous mouse models of TSC established that haploinsufficiency of either *Tsc1* or *Tsc2* causes neurocognitive deficits such as impaired hippocampal-dependent learning, social behavior, synaptic plasticity, learning and memory [46,47]. *Tsc2*+/− mice also exhibit abnormal mother-pup interaction as measured by ultrasonic vocalizations (USV), establishing these mice as potential models for certain aspects of autism [48]. These neuropsychiatric abnormalities are present without obvious concomitant neuropathological alterations, prompting more rigorous investigation of subtle molecular and circuitry level changes. Heterozygous models exhibit no clear anatomic abnormality under gross pathologic evaluation, although $Tsc2^{+/-}$ neurons display abnormal axon guidance and synaptic plasticity [37,46], providing further support for the hypothesis that abnormal neuronal connectivity may underlie the neurological symptoms in TSC disease.

Neuron-specific conditional knockout of *Tsc1* has been generated using *Cre* recombinase under the *Synapsin-1* promoter (*SynI-Cre*) [38]. $Tsc1^{flox/flox}$*;SynICre* mice are viable perinatally, but develop seizures and die starting approximately 4–6 weeks postnatally. These mice exhibit several neuropathological abnormalities similar to those seen in TSC patients including enlarged dysplastic neurons throughout the cortex. mTORC1 inhibitor treatment reversed some of the pathological abnormalities, including neuron size, and lead to improvements in body weight, clasping behavior, tremor, seizures and kyphosis [39]. Deletion of *Tsc2* from radial glial precursors cells using *hGFAP-Cre* transgenic mice results in lamination defects, cortical enlargement, astrogliosis as well as myelination defects [49]. $Tsc2^{flox/flox}$*; hGFAP-Cre* mice exhibited severe compromise in survival and profound

seizure episodes, suggesting again that cortical tubers are not necessary for the observed phenotypes. In fact, none of the genetic crosses using mouse models of TSC have been able to reproduce cortical tubers. However, a recent study inducing Tsc1 loss in a small subset of cortical progenitors by *in utero* electroporation demonstrated tuber-like structures [50].

When *Tsc1* is specifically inactivated in astrocytes, mice demonstrate a marked increase in GFAP-immunoreactive cells and subsequent enlargement of some cortical regions such as the hippocampus accompanied by alterations in neuronal organization [51]. Additionally, these mice developed electroencephalographically confirmed seizures by two months of age. The neuropathological phenotypes in these mice were mTORC1-dependent and treatable, as mTOR inhibitor treatment prevented development of progressive astrogliosis, abnormal neuronal organization, development of epilepsy and premature death in these mice [52]. Epileptogenesis in these mice was attributed to the increases in extracellular glutamate levels due to decreased astrocytic GLT-1 and GLAST glutamate transporters, and treatment with ceftriaxone to increase the transporter expressions in presymptomatic mice decreased excitotoxic neuronal death and severity of epilepsy [53,54].

Another glial pathology in TSC disease is the presence of SENs and SEGAs. SEN-like structures in mice were only recently achieved by loss of Tsc1 specifically in postnatal neural stem/progenitor cells [55]. However, huge progress has been achieved in testing the efficacy of mTOR inhibitors on SEGA volume in patients. In fact, based on clinical trials [56], the U.S. Food and Drug Administration approved an mTOR inhibitor, everolimus, in November 2010 for treatment of SEGAs in TSC patients, who are not candidates for surgical resection.

Much of the focus in the field has been on astrocytes and neurons, perhaps because of their direct contributions to epilepsy and tuber formation. Nonetheless, the correlation between the severity of cognitive impairments and degree of hypomyelination has brought to surface that the mTOR pathway is also critical in development and function of oligodendrocytes [57–59]. The exact role of Tsc1/2 in oligodendrocytes has not been elucidated yet, but neuronal loss of Tsc1 leads to hypomyelination in mice [38]. Myelination deficits are commonly observed in TSC patients, both focally within tubers and more diffusely [60–62]. Diffusion tensor imaging studies indicate that TSC patients have occult damage in the normal appearing white matter and that this damage may contribute to neurocognitive disability in these patients [62–65].

Challenges and future directions—In the 20 years since RTT was described widely in the English-speaking literature and identification of genetic causes of TSC and RTT, there has been a staggering explosion in understanding these diseases at a molecular, cellular, functional and clinical level. The generation of mouse models that mirror the human disease closely has been instrumental in identification of rational therapeutic targets and the translation of preclinical discoveries into human trials. Now clinical treatment trials are ongoing for both RTT and TSC, focusing on several aspects of the disease, including cognitive and behavioral outcomes. The landscape of RTT and TSC research has been completely altered by these important steps bringing basic science observations to the clinic.

While clinical trials are ongoing with these pioneering therapeutic agents, the search for other translational targets is also gaining steam. In RTT, one of the more promising has been the identification of micro-RNAs (miRNA) that target MeCP2. Animal studies have also demonstrated that levels of some miRNAs are disrupted in the absence of MeCP2 suggesting further downstream targets for potential therapeutic interventions[66,67]. Characterization of the interaction between miRNAs and the TSC-mTOR pathway will likely provide important insights into TSC as well.

A powerful potential addition to the translational armamentarium in neurodevelopmental disorders is the development of cellular models for high-throughput screening. Recently induced pluripotent stem cells (iPSCs) have been generated from RTT patient fibroblasts. These RTT iPSCs are able to generate functional neurons with a specific morphological and electrophysiological cellular phenotype[68,69]. Furthermore, treatment with IGF1 is able to rescue the morphological phenotype in these iPSC derived neurons. Several laboratories are working on iPSCs for TSC as well. Thus, these iPSCs may represent a significant advance in screening assays for further compounds that may modulate or reverse the neuronal phenotype.

It is important to note that while we set out to identify common pathogenic mechanisms by studying genetic diseases such as RTT and TSC, the cell biological pathways discovered and therapies under study currently are very distinct. Recombinant human IGF-1 (mecasermin, Increlex®) would be expected to upregulate mTOR and increase protein synthesis while rapamycin (sirolimus) and everolimus would inhibit mTOR have the opposite effect on protein translation. These observations based on early clinical trials have several important implications. First, they highlight the importance of designing targeted therapies specific for each genetic disease. Furthermore, they indicate that one type of therapy that relieves neurological symptoms in one disease could potentially be harmful in the other. Finally, they strongly argue that abnormalities that underlie autism and other neurodevelopmental disorders are likely shared at the level of neuronal circuits despite the fact that they may diverge at the level of molecular pathways. By experimentally dissecting the MeCP2 and TSC1/2 signaling using more precise genetic tools, we may be able to uncover the circuits that contribute to the core features of autism.

CONCLUSION

Despite the promise of better understanding of molecular mechanisms through animal models, significant challenges remain. Elucidating the role of MeCP2 and TSC1/2 in normal neuronal function and identification of additional bona fide downstream targets is a crucial step to develop disease-reversing or disease-modulating treatments for patients. Likewise understanding the fundamental requirement for normal MeCP2 and TSC1/2 expression and how best to compensate for their loss is likely be central to understanding the neurobiology of autism and neurocognitive impairment and therapies for these disorders.

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Key Points

- Mendelian disorders, such as RTT and TSC, allow creation of animal models of autism-associated disease and the ability to probe the fundamental neurobiology of these conditions.
- Mouse models have also raised the exciting possibility of disease reversal and rescue of neurocognitive phenotypes in symptomatic adult animals in conditions such as RTT and TSC.
- BDNF and IGF are identified as potential downstream targets of MeCP2 and a Phase 2 clinical trial with recombinant IGF is ongoing in RTT girls.
- mTOR inhibitors can rescue neurological symptoms in mouse models of TSC, have proven effective in TSC-associated astrocytomas and are now being tested for efficacy in neurocognitive outcomes in children with TSC.
- Discovering the fundamental roles of MeCP2 and TSC1/2 in the brain is likely to be central to understanding the neurobiology of autism and developing therapies for neurodevelopmental disorders.