REVIEW

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MeCP2 in central nervous system glial cells: current updates

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Methyl-CpG binding protein 2 (MeCP2) is an epigenetic regulator, which preferentially binds to methylated CpG dinucleotides in DNA. MeCP2 mutations have been linked to Rett syndrome, a neurodevelopmental disorder characterized by severe intellectual disability in females. Earlier studies indicated that loss of MeCP2 function in neuronal cells was the sole cause of Rett syndrome. Subsequent studies have linked MeCP2 expression in CNS glial cells to Rett syndrome pathogenesis. In this review, we have discussed the role of MeCP2 in glial subtypes, astrocytes, oligodendrocytes and microglia, and how loss of MeCP2 function in these cells has a profound influence on both glial and neuronal function.

Key words: Methyl-CpGbinding protein 2, oligodendrocytes, astrocytes, microglia, Rett syndrome

INTRODUCTION

Epigenetic regulation of genes plays an important role in the development and function of the central nervous system (CNS). Proper regulation of stage-specific gene expression in the nervous system is modulated by epigenetic mechanisms such as DNA methylation, histone modifications, nucleosome/chromatin remodeling, and non-coding RNA-mediated posttranslational regulation (Hsieh and Gage 2005, Wu and Sun 2006). Among these mechanisms, DNA methylation regulates the expression of genes by either inhibiting the binding of transcription factors, or the recruitment of methyl binding domain (MBD) family proteins that includes MeCP2, MBD1, MBD2, MBD4, and Kaiso protein (Bird 2002, Guoping and Hutnick 2005). MeCP2 is a member of the MBD protein family that binds to 5-methylcytosine (5mC) (Meehan et al. 1992). MeCP2 requires an A/T run of four or more base pairs adjacent to the methyl-CpG for efficient DNA binding (Klose et al. 2005). Interestingly, disruption of the AT-hook domain in the transcription repression domain (TRD) of MeCP2 leads to defects in chromatin organization, and thus changes the Rett syndrome phenotype (Baker et al. 2013). MeCP2 also interacts with hydroxyl methyl-CpG (5hmC), which is enriched within active genes (Mellén et al. 2012). In addition to the classic dinucleotide methyl-CG(mCG) MeCP2 has been recently reported to bind to cytosine methylated trinucleotide (mCAC) (Lagger et al. 2017).

MECP2 is an X-linked gene, mutation of which results in the multiple phenotypes that fall under the umbrella of Rett syndrome. Rett syndrome is a neurodevelopmental disorder which occurs mostly in females (Amir et al. 1999) and has been reported to have an incidence of approximately 1/10,000 live female births. Rett syndrome is characterized by normal post-natal development up to 6 months, followed by a subsequent loss of acquired speech and motor skills. The most pronounced changes occur at 12-18 months of age. Rett patients may also develop mental retardation, stereotyped hand movements, ataxia, seizures (after the age of 2) microcephaly, autism and respiratory dysfunctions (Hagberg et al. 1983, Nomura 2005). There is currently no cure for Rett, and most girls live for many years, even decades, requiring continuous supervision and assistance.

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Glial cells in the CNS consist of astrocytes, oligodendrocytes, and microglia. Astrocytes maintain and regulate the level of ions and neurotransmitters at the synapse, provide nutrients and structural support around synapses, and contribute to the integrity of the blood-brain barrier (Wang and Bordey 2008). On the other hand, oligodendrocytes (OL) produce and maintain the myelin sheath around the axons, allowing the rapid conduction of nerve impulses. OL also provides trophic support to axons. In addition to providing insulation and trophic support to neurons, myelinating glia are critical for the proper functioning of the nervous system, supporting the structural and electrical properties of axons by controlling their diameter, as well as the spacing and clustering of ion channels at nodes and paranodes (Baumann and Pham-Dinh 2001). Lastly, microglia are the resident inflammatory cells of the brain and are involved in the regulation of synaptic function (Tremblay et al. 2011). Early studies suggested that MeCP2 is expressed only in neurons where its expression correlates with neuronal maturation. It was thought to be absent in glial cells (Jung et al. 2003, Kishi and Macklis 2004, Shahbazian et al. 2002). In 2010, we showed the MeCP2 expression in OL (Vora et al. 2010). Subsequent studies demonstrate MeCP2 expression in all glial cells including astrocytes, and microglia (Ballas et al. 2009, Maezawa and Jin 2010, Sharma et al. 2015, Tochiki et al. 2012, Vora et al. 2010). Rett syndrome has primarily been associated with functional loss of MeCP2 solely in neuronal cells. However, increasing evidence has demonstrated that genetic rescue of MeCP2 in glial cells also significantly improves the disease phenotypes, which indicate that glial cells, like neurons, are integral to Rett syndrome pathogenesis (Cronk et al. 2015, Lioy et al. 2011, Nguyen et al. 2013). This review elaborates and emphasizes the role of MeCP2 in CNS glial cells, how its loss in CNS glia affects cell functioning, and the possible implications in Rett syndrome pathology. We first discuss the current knowledge of MeCP2 structure and function. Subsequently, we examine the contribution of MeCP2 in glial cell function, and the potential implication in Rett syndrome pathology.

Structure and Function of MeCP2

The MeCP2 protein comprises of structural domains: N-terminal domain (NTD), MBD, the intervening domain (ID), TRD and the C-terminal domain (CTD) (Fig. 1). Among these, the MBD domain of MeCP2 is essential for its binding to methyl CpG dinucleotides, which is linked with most of the disease causing mutations. TRD is required for its association with co-repressor proteins for transcriptional repression. Pathogenic mutations in the ID and CTD domains suggest their

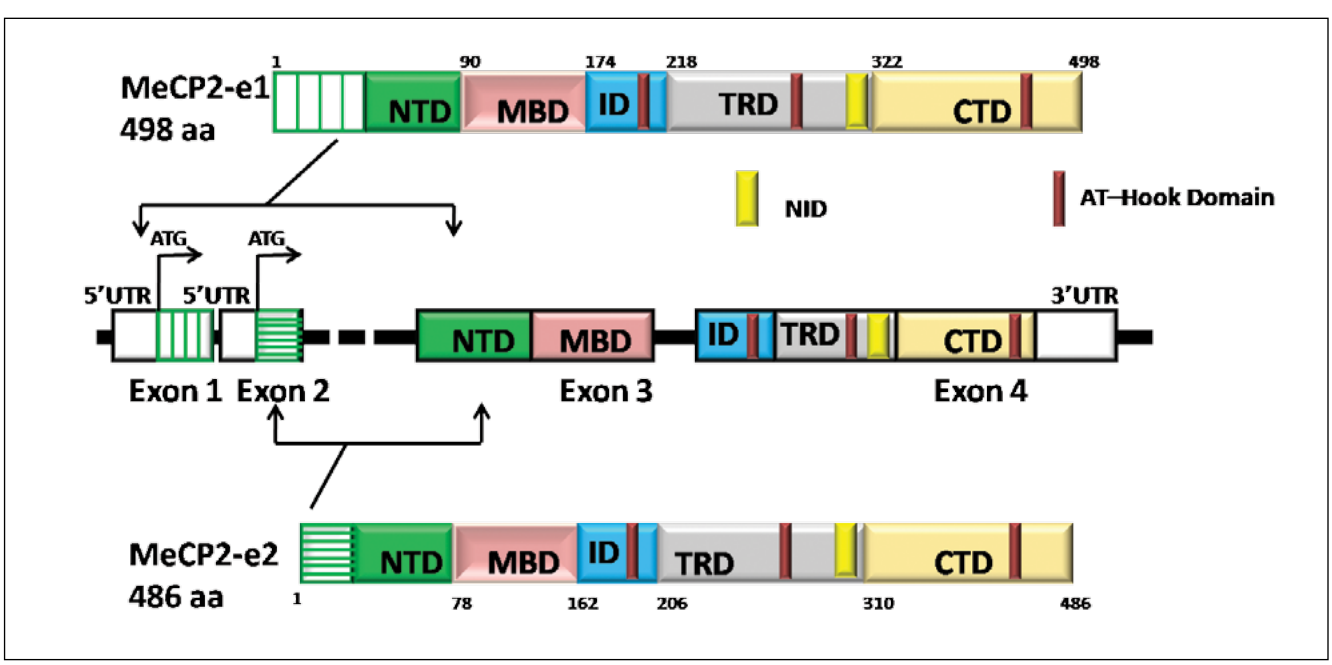

Fig. 1. Structure and Splicing of MeCP2. MeCP2 upon alternative splicing form two different isoform (MeCP2-e1 and MeCP2-e2). MeCP2-e1 is 498 amino acids long and has 21 unique N-terminal residues (vertical green stripes). MeCP2-e2 is 486 amino acids long and has 9 unique residues (horizontal green stripes). The remaining protein sequence is common to both isoforms and can be divided in following domains: the NTD (N-terminal domain), the MBD (methyl-CpG binding domain), the ID (intervening domain), the TRD (transcription repression domain), the CTD (C-terminal domain), the AT-Hook domain, the NCOR--SMRT interaction domain (NID).

importance in MeCP2 dysfunction (Bedogni et al. 2014, Hansen et al. 2010, Lewis 1992, Yusufzai and Wolffe 2000). In addition, there are NCoR/SMRT interaction domain (NID) and AT-hook like domains that have been mapped in MeCP2. Mutation in the NID and AT-hook domains disrupts interactions with partner proteins and results in Rett syndrome (Baker et al. 2013, Leonard et al. 2017, Lyst et al. 2013).

MeCP2 has two isoforms (*MeCP2_E1* and *MeCP2_E2*) produced by alternative splicing of mRNA (Fig. 1). In the brain, the *MeCP2_E1* isoform is expressed at 10 times to the level of MeCP2_E2 (Mnatzakanian et al. 2004). The expression of *MeCP2_E1* is markedly higher in primary neurons, as compared to primary astrocytes (Kaddoum et al. 2013, Zachariah et al. 2012). Dastidar and colleagues showed that the two isoforms are functionally different (Dastidar et al. 2012). They showed that elevated expression of *MeCP2_E2* isoform promotes neuronal death by suppressing the expression of FoxG1. FoxG1 is a protein that promotes neuronal survival by inhibiting MeCP2_E2 activated cell death. Mutations in *FoxG1*are also found in Rett syndrome (Dastidar et al. 2012). *MeCP2_E2* is functionally redundant for development of the CNS, but is involved in embryo viability and placenta development. Further, mutations in the *MeCP2_e2* gene confer a survival disadvantage for carriers of the maternal allele (Itoh et al. 2012). Although *MeCP2_e2* is thought to not be involved in Rett syndrome development, it may result in a phenotype that is not currently classified under the Rett umbrella. Mutation of *MeCP2_E1* results in classic Rett syndrome (Itoh et al. 2012). DNA methylation pattern at the regulatory elements of MeCP2 may regulate the differential expression of its two isoforms in specific brain regions (Olson et al. 2014).

MeCP2 has a highly disorganized structure that acquires local secondary structure upon binding to other macromolecules, thereby inviting multiple protein-protein interactions. Post-translation modification of MeCP2 also regulates its activity and interaction with other proteins (Bedogni et al. 2014). Neuronal activity, specifically release of glutamate at the synapse, regulates MeCP2 action by dephosphorylation and phosphorylation at serine 80 (S80) and 421 (S421) respectively, which regulates transcription of target genes. Phosphorylation of MeCP2 at S421 leads to the transcriptional induction of *Bdnf* gene (Zhou et al. 2006). Mutation of *Mecp2* at S421A in mice results in defective synaptic development and abnormal behavior. Contrary to S421, S80 is dephosphorylated by neuronal activity, which weakens its binding to chromatin (Tao et al. 2009). Interestingly, chromatin immunoprecipitation (ChIP) sequencing shows that phosphorylation of S421does not regulate gene expression, it appears that MeCP2 functions as histone-like factor, when phosphorylated at S241. In this way, it facilitates a genome-wide response of chromatin to neuronal activity (Cohen et al. 2011). Further to this, MeCP2 deletion results in a genome-wide increase in acetylated histone 3 and histone 1.

Recent studies have shown that MeCP2 phosphorylation at three sites (S86, S274, and T308) is induced differentially by neuronal activity, BDNF and other agents that increase the intracellular cAMP levels. Phosphorylation at T308 site inhibits its interaction with NCoR co-repressor complex, and thus suppresses the MeCP2 mediated transcriptional repression. These findings suggest that loss of MeCP2 and NCoR / SMRT co-repressor interaction leads to Rett syndrome (Ebert et al. 2013, Lyst et al. 2013). MeCP2 regulation of target gene expression is mediated through its interaction with other partner proteins, including co-repressors mSIN3a (Nan et al. 1998), cSki (Kokura et al. 2001), transcription factor Ying Yang 1 (YY1), which is involved in activation as well as repression (Forlani et al. 2010), YB1 (RNA interacting protein) (Young et al. 2005), Swi/Snf family ATPase α-thalassemia/mental retardation syndrome X linked (Atrx) (Nan et al. 2007), nuclear receptor co-repressor (NcoR) and DNA methyl transferase 1 (Dnmt1) (Kokura et al. 2001). In addition, MeCP2 also binds to the corepressor of RE1 silencing transcription factor (CoREST) (Ballas et al. 2005), the transcriptional activator cAMP response element binding protein (CREB) (Chahrour et al. 2008) and the tri-thorax-related protein Brahma (Harikrishnan et al. 2005). Further, recent studies have shown that MeCP2 binds to GC-rich sequences in correlation with nucleosome binding (Rube et al. 2016). It is believed that the genome wide binding, post-translational modification and differential phosphorylation states contribute to the multifunctional properties of MeCP2.

Although a ubiquitous protein in nature, MeCP2 is most predominantly expressed in the brain, and is highly expressed in neuronal cells compared to other cell types. Its expression level correlates with the maturation of neurons which suggests the noteworthiness of MeCP2 in neuronal maturation and maintenance (Kishi and Macklis 2004, Shahbazian et al. 2002). MeCP2 density in neuronal nuclei was found to be as abundant as histone octamer. Neuronal loss of MeCP2 results in changes in chromatin organization mediated by the increase in histone acetylation and doubling of histone H1 (Skene et al. 2010). Further, it also regulates the nuclear size and RNA synthesis levels in neurons during their maturation (Yazdani et al. 2012). This implicates MeCP2 as a global regulator of chromatin states instead of gene specific regulator. In dorsal horn neurons, post inflammation, MeCP2 phosphorylation

leads to increased expression of target genes such as the serum- and glucocorticoid- regulated kinase (*Sgk1*): FK 506 binding protein 5 (*Fkbp5*), a glucocorticoid receptor-regulating co-chaperone of hsp-90, and the sulfotransferase family 1A, phenol-preferring, member 1 (*Sult1A1*). SGK1 supports the induction of ankle joint inflammation. Moreover, MeCP2, DNMT and histone deacetylase (HDAC) levels are modulated within the superficial dorsal horn during the maintenance phase of persistent pain states, indicating a prominent role of MeCP2 in chromatin organization during the maintenance phase of chronic pain (Géranton et al. 2007, Tochiki et al. 2012).

MECP2 duplication, or over expression, leads to MECP2 duplication syndrome, which is specific in males but can also be found in females. This syndrome may initially resemble Rett syndrome, but the disorder is more clinically severe, and presents differently from classic Rett syndrome (Ramocki et al. 2010). MBD and TRD are responsible for toxicity in MECP2 duplication syndrome (Heckman et al. 2014). Furthermore, the over expression of MeCP2 impairs chick embryo neural tube formation by provoking premature differentiation of proliferating progenitors (Petazzi et al. 2014). The severity of neurological symptoms in Rett patients also depends on X-chromosome inactivation (XCI) patterns (Braunschweig et al. 2004). Most of the Rett syndrome female patients are heterozygous for *MECP2* mutation due to random XCI. Deficiency of MeCP2 in the *Mecp2* mosaic mouse model may not affect primary XCI in early development, but does affect the proportion of neurons expressing the wild-type *Mecp2* allele in both a region- and age-dependent manner (Smrt et al. 2011).

The classical function of MeCP2 is to silence the gene expression through the recruitment of corepressors comprising HDAC and mSIN3 (Nan et al. 1998). *Bdnf* is the archetypal target gene, whose expression is modulated by MeCP2, and has been shown to be involved in neuronal survival, differentiation, and synaptic plasticity. Previous work has shown that MeCP2 represses the expression of BDNF, and is regulated by neuronal membrane depolarization. Neuronal membrane depolarization phosphorylates MeCP2 causing its dissociation from the *Bdnf* promoter (Chen et al. 2003, Martinowich et al. 2003, Zhou et al. 2006). Further, it was demonstrated that BDNF protein levels are lower in the brain of *Mecp2* knockout mice (Chang et al. 2006, Li et al. 2012) and increased in mice over expressing *Mecp2* (Chahrour et al. 2008). These observations imply an important role for altered BDNF level in Rett syndrome disease progression. In contrast to earlier studies, BDNF protein levels were seen to be enhanced in both MeCP2 knock out and MeCP2 over expressing cultured hippocampal neurons (Larimore et al. 2009). These findings suggest that the control of BDNF expression by MeCP2 can dynamically switch between repression and activation. Epigenetic studies have established that MeCP2 acts as a transcriptional repressor, but a recent report by Zoghbi and colleagues demonstrates that MeCP2 activates expression of target genes in the hypothalamus and cerebellum in mice over expressing MeCP2. MeCP2 is associated with transcriptional activator CREB1 at the promoter region of an activated target but not at a repressed target (Ben-Shachar et al. 2009, Chahrour et al. 2008).

Astrocytes and MeCP2

The expression of several glial genes, including α B-crystallin, glial fibrillary acidic protein (*Gfap*), glial excitatory amino acid transporter 1 (*Eaat1*) and *S100A13* were found to be increased in Rett syndrome brain (Colantuoni et al. 2001). Apart from neuronal MeCP2, several studies have shown the non-cell autonomous influence of astrocytic MeCP2 on neuronal cells and its contribution to Rett syndrome pathogenesis. In fact, astrocytes from Rett syndrome mouse model fail to support normal neuronal growth and cause dendritic abnormalities, further supporting a significant role for MeCP2 in neuronal maturation. Moreover, conditioned media from *Mecp2* null astrocytes results in neuronal damage, suggesting the aberrant secretion of toxic factors by mutant astrocytes (Ballas et al. 2009). In a similar line of studies, MeCP2 deficiency in astrocytes has also been correlated with abnormal growth and alterations in BDNF expression levels. MeCP2 also regulates astrocyte immune response by altering the expression of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6) and p38MAPK (Maezawa et al. 2009). The astroglial marker GFAP and S100β expression levels are suppressed in MeCP2 siRNA treated amygdala of female rat brain (Forbes-Lorman et al. 2014), suggesting negative regulation by MeCP2.

MeCP2 deficient astrocytes cause abnormal dendritic arborization of wild neuron in co-culture, which is a prominent neurological feature of Rett syndrome brain. Mutant astrocytes differentiated from isogenic induced pluripotent stem cell (iPSC) lines from human Rett syndrome patients also adversely affect the morphology and function of wild type neurons from mouse and human and such non-cell autonomous effects are partially mediated by secreted factors (Williams et al. 2014). These negative effects on neurons could be mediated by either the presence or absence of growth promoting or inhibiting factors respectively, in the conditioned media of mutant Rett syndrome astrocytes. Further studies are

needed to fully identify these factors in order to develop an effective gene-therapy for Rett syndrome.

MeCP2 expression in GFP-labeled wild type astrocytes was reduced in a time-dependent manner when co-cultured with *Mecp2*$^{-/+}$ astrocytes. This non cell autonomous effect is mediated via gap junctions, specifically Cx-43-containing gap junctions. These observations suggest that MeCP2 deficiency spreads through *Mecp2*$^{-/+}$ tissue via the non-cell autonomous transfer of MeCP2 suppressor via gap junctions. These negative regulators may include Ca^{2+}, inositol trisphosphate, glutamate, or small regulatory miRNAs, such as miR132 that post transcriptionally down regulates MeCP2 (Klein et al. 2007, Maezawa et al. 2009).

Preferential re-expression of MeCP2 in the astrocytes of MeCP2 deficient mice restores the Rett syndrome phenotype. Specifically, locomotion, anxiety levels and respiratory abnormalities return to a normal pattern, and the mice live longer. This indicates that glia are an integral component in the neuropathology of Rett syndrome (Lioy et al. 2011). Abnormal glutamate metabolism has been found in Rett syndrome brain. MeCP2 impaired the glutamate clearance rate of *Mecp2* null astrocytes by regulating glutamate transporters and glutamine synthetase, which may further influence the progression of Rett syndrome (Okabe et al. 2012).

Astrocytes from MeCP2 deficient mice show reduced expression of stathmin-like 2 (STMN2) protein that results in altered microtubule assembly and dynamics. STMN2 promotes astrocytic microtubule disassembly and it's down regulation in MeCP2 deficient astrocytes increase microtubule growth. Since astrocytes are also involved in dendritic outgrowth and synaptogenesis, it was thought that STMN2 down regulation in MeCP2 deficient astrocytes may explain the dendritic abnormalities observed in Rett syndrome brain (Nectoux and Floria 2012, Slezak and Pfrieger 2003). However, recent studies have shown that microtubules dynamics and vesicular transport is altered in astrocytes derived from *Mecp2*$^{308/y}$ mice, and human astrocytes derived from iPSC from a Rett syndrome patient with a *MECP2p. Arg294* mutation. Impaired microtubule dependent dynamics and vesicular transport in MeCP2 deficient astrocytes was found to be restored upon treatment of microtubule stabilizing agent EpothiloneD (EpoD). Thus, microtubules may be potential therapeutic target for Rett syndrome (Delépine et al. 2016). MeCP2 deficiency is also found to be associated with reduced sensitivity of medullary astrocytes to changes in PCO2/[H^+] (Turovskyet al. 2015). MeCP2 deficiency alone, in astrocytes, significantly depresses the hypercapnic ventilatory response in mice. These results suggest a role for astrocytes in the regulation of respiratory response to CO_2 and further suggest a role for astrocytic MeCP2 in respiratory abnormalities observed in Rett syndrome (Garg et al. 2015).

In a study, NTera-2, a human embryonal carcinoma cell line, was induced to differentiate into astrocytes (Cheng et al. 2011). It was observed that MeCP2 in association with Sin3A inhibits the differentiation of NTera-2 into astrocyte-like lineage by suppressing GFAP expression. Upon differentiation, the promoter undergoes a conformational change triggered by STAT3 binding that causes the release of Sin3A/MeCP2 complex and *Gfap* gene activation. Moreover, MeCP2 deficiency in Rett syndrome-human iPSC derived neural stem cells resulted in an increase in the number of differentiated astrocytes and dysregulation of GFAP expression (Andoh-Noda et al. 2015). Finally, all the above studies indicate a role for MeCP2 in cellular differentiation and lineage specific gene expression.

A combined approach of gene expression microarray and MeCP2 ChIP-seq in astrocytes found a set of potential MeCP2 target genes whose products are involved in normal astrocytic signaling, cell division and neuronal support functions, the loss of which may contribute to the Rett syndrome phenotype (Yasui et al. 2013). Validation of selected target gene transcripts by qRT PCR revealed that MeCP2 deficiency in astrocytes consistently affects the expression of *Apoc2, Cdon, Csrp* and *Nrep* apolipoprotein C-II (*Apoc2*). Cell adhesion molecule-related/downregulated by oncogenesis (*Cdon*) and cysteine and glycine-rich protein 1 (*Csrp*) expression levels are elevated in MeCP2 deficient mice, while the neuronal regeneration-related protein (*Nrep*) expression levels are reduced (Yasui et al. 2013). Defects in *Apoc2* and *Cdon* expression in astrocytes may be associated with cardiac defects and brain structure abnormalities observed in Rett syndrome. *Csrp1*, which is upregulated in astrocytes in absence of MeCP2, appears to have a role in neuronal regeneration. *Nrep* is an important factor involved in glial mobility and neoplasia. Gene expression profiles of wild-type and mutant astrocytes from *Mecp2*$^{308/y}$ mice demonstrate two interesting genes encoding secreted proteins, chromogranin B (*Chgb*) and lipocalin 2 (*Lcn2*). Both of which are dysregulated in MeCP2 deficient astrocytes and exert negative non-cell autonomous effects on neuronal properties (Delépine et al. 2015). LCN2 secreted by reactive astrocytes is involved in binding and transport of lipids and other hydrophobic molecules. Interestingly, LCN2 plays an important role in the regulation of neuronal excitability and spine morphology (Ferreira et al. 2013). Chgb, a component of exocytosis vesicles was found in the secretory granule cargoes involved in BDNF secretion (Sadakata et al. 2004). Another important molecule nuclear receptor subfamily 2, group

F, member 2 (*Nr2f2*) was found to be dysregulated in MeCP2 deficient astrocytes and is involved in down and up-regulation of several target genes in astrocytes, such as chemokine (C–C motif) ligand 2 (*Ccl2*): *Lcn2* and *Chgb*. This suggests knowledge of the involvement of Nr2f2 in MeCP2 deficient astrocytes could lead to better understanding of Rett syndrome pathophysiology (Delépine et al. 2015). In addition, the astrocytic glutamate transporter GLAST, the GPCRs mGluR3 and S1P1, along with protein- binding partners Moesin, Merlin, and PTEN were disrupted in transcriptomic or proteomic data from MeCP2 deficient mice. Further, there were reduced expression of markers associated with reactive astrocytes (*Gfap*, *Gap43*, *Vim*, HSPB1, and ANXA3) suggesting decreased astrocytic reactivation in Rett syndrome (Pacheco et al. 2017). In a different study, it was found that ischemia induced the expression of Phospho-S292 MeCP2 in reactive astrocytes accompanied by enhanced expression of vascular endothelial growth factor (VEGF) in the striatum. In addition, over expression of VEGF in ischemia-injured striatum increases the accumulation of pS292 MeCP2 in reactive astrocytes (Liu et al. 2015), suggesting a role for MeCP2 in the modulation of reactive astrocyte function.

Oligodendrocytes and MeCP2

Brain magnetic resonance imaging in *Mecp2-/y* mice detected a significant reduction in the thickness of the corpus callosum (Saywell et al. 2006). Diffusion tensor imaging (DTI) studies in Rett syndrome patients also found a significant reduction in fractional anisotropy (FA) in the corpus callosum (Mahmood et al. 2010). MRI studies have also shown alterations in cerebellar white matter, and corpus callosum of all available MeCP2 mutant mice currently available (Allemang-Grand et al. 2017). These results correlate with white matter impairment seen in human Rett syndrome patients. Myelin-associated oligodendrocytic basic protein (MOBP) is one of the myelin proteins that may be involved in the compaction or stabilization of myelin but the exact function of MOBP is still unclear (Montague et al. 2006). *Mobp* was found to be up-regulated in *Mecp2* null mice, and is directly regulated by MeCP2 binding through its promoter (Urdinguio et al. 2008). Myelin genes such as myelin basic protein (*Mbp*) and myelin associated glycoprotein (*Mag*) have been found to be increased in the corpus callosum of *Mecp2*$^{308/y}$ mouse brain (Vora et al. 2010). Major CNS myelin proteins, including MBP, proteolipid protein (PLP) and MAG play an important role in myelin sheath formation and integrity. PLP and MBP are distributed throughout the myelin sheath and are essential for compaction of myelin. MAG is located in the periaxonal regions and may serve to facilitate cell–cell interactions between oligodendrocytes and axonal membranes during myelination (Baumann and Pham-Dinh 2001, Fulton et al. 2010). The expression of CNPase, another myelin specific protein, is also reduced in the white matter and hippocampus of a Rett syndrome mouse model (Wu et al. 2012). F2-Dihomo-isoprostanes (F2-Dihomo-IsoP), peroxidation products from adrenic acid, a known component of myelin were found to be increased by about two orders of magnitude in patients in stage I of Rett syndrome and decreased in later stages of disease progression. This increased level of F2-Dihomo-isoprostanes in patients suggests early white matter damage in Rett syndrome brain. Some neurological signs in Rett syndrome patients overlap with X-linked adrenoleukodystrophy and supports the theory of white matter damage in Rett syndrome (Durand et al. 2013).

Studies carried out in our laboratory have shown that MeCP2 knock-down in cultured rat oligodendrocytes results in the increased expression of several myelin genes, including *Mbp*, *Plp*, *Mog*, *Mobp*, *Bdnf* and the transcriptional regulator *Yy1*. These results indicate that MeCP2 acts as negative regulator of myelin gene expression in oligodendrocytes (Sharma et al. 2015). Recently, the Nurit Ballas group has shown a significant contribution of oligodendrocytes in Rett syndrome pathology (Nguyen et al. 2013). Mice lacking oligodendrocyte specific MeCP2 developed a severe hind-limb clasping phenotype. Restoration of oligodendrocyte MeCP2 resulted in significant improvement of some Rett syndrome phenotypes specifically the hind-limb clasping phenotype. Moreover, the loss of MeCP2 in oligodendrocyte lineage cells does not affect the expression pattern of proteins (MBP and PLP) involved in myelination. However, the myelin genes *Mbp* and *Plp* were found to be impaired in *Mecp2*$^{Stop/y}$ mouse brain. MBP expression was reduced while the PLP protein level was increased in *Mecp2*$^{Stop/y}$ mice brain. MBP levels were only partially restored upon expression of MeCP2 in oligodendrocytes while levels of PLP remained unchanged (Nguyen et al. 2013). These data imply MeCP2 expression in oligodendrocytes has little effect on the expression of myelin-related proteins. Further studies are required to clarify these findings. However, one explanation is the non-cell autonomous effect of another cell, either neuronal or glial, on the expression of myelin proteins.

RNA sequencing and proteomics data from MeCP2 deficient mice revealed disruption of the pathways related to morphology, migration and myelination/demyelination of oligodendrocytes (Pacheco et al. 2017). In addition, it was identified that increased expression of MeCP2E1 in an EAE model of multiple sclero-

sis hampers the myelin repair process by repressing the BDNF levels (Khorshid et al. 2017). The regulation and function of MeCP2 phosphorylation in neurons and astrocytes is well known. Currently, our group has demonstrated differential regulation of MeCP2 phosphorylation at S80 in N19 (mouse oligodendroglial cell line) in response to the extracellular matrix component (ECM) laminin (Parikh et al. 2017). Laminin increases the level of pS80 MeCP2 in immature oligodendrocytes, and reduces level in mature oligodendrocytes (Parikh et al. 2017).

Microglia and MeCP2

Microglia primarily function as immune cells in the brain after injury or disease. However, microglia are also involved in normal synaptic function. Studies have also shown that all cell types in the brain, both neuronal and non-neuronal, communicate via release of soluble factors in the mature and aging brain (Tremblay et al. 2011). For example, conditioned media from *Mecp2* null microglia contains high levels of glutamate, which is neurotoxic. Wild type neurons treated with *Mecp2* null microglia conditioned media show abnormal dendritic morphology, signs of microtubule disruption and damage of postsynaptic glutamatergic components (Maezawa and Jin 2010). Previous studies on astrocytes demonstrated that MeCP2 deficient astrocytes detrimentally influence neuronal dendrite formation (Ballas et al. 2009, Maezawa et al. 2009). The Ballas group suggest that *Mecp2* null astrocytes release a soluble neurotoxic factor with slow activity that requires incubation of at least 3 days in culture, but the factor has not been identified. Further, Maezawa and Jin (2010) found that wild type neurons, treated with conditioned media derived from primary mixed glial culture from *Mecp2* null mice, show dendritic damage while conditioned media from the highly pure culture of *Mecp2* null astrocytes show no dendritic abnormalities. They suggest this is due to the microglia present in mixed glial culture releasing the neurotoxic factor. Wild type neurons treated with conditioned media from *Mecp2* null microglia, show robust dendritic abnormalities. Due to an abnormally high level of glutamate released by*Mecp2* null microglia, which causes excitotoxicity that may contribute to dendritic and synaptic abnormalities in Rett syndrome (Maezawa and Jin 2010). Further, it was identified that increased levels of glutaminase and connexin 32 in *Mecp2* null microglia are responsible for excess glutamate production and release, respectively. This explains that microglia, and not astrocytes, are the major source of soluble neurotoxic factors (Maezawa and Jin 2010). Further in the same line, a recent report showed that MeCP2 deficiency leads to mitochondrial dysfunction and neurotoxicity in microglia, by regulating glutamine homeostasis. MeCP2 acts as the repressor of major glutamine transporter sodium-coupled neutral amino acid transporter 1 (SNAT1) in microglia. MeCP2 down regulation or SNAT1 over expression in microglia resulted in mitochondrial dysfunction and neurotoxicity due to overproduction of glutamate (Jin et al. 2015).

It was observed that restoration of MeCP2 in microglia attenuates the symptoms of Rett syndrome in the mouse model (Derecki et al. 2012). Thus, via multiple approaches, wild type *Mecp2*-expressing microglia within the context of *Mecp2* null male mouse altered numerous facets of disease pathology. For example, lifespan was increased, breathing patterns were normalized, apneas were reduced, body weight was increased to near wild type, and locomotor activity was improved. *Mecp2*$^{+/-}$ females also exhibited significant improvements as a result of wild type microglial engraftment (Derecki et al. 2012). In addition, it has been observed that in MeCP2 deficiency condition, microglia are activated, but are lost with disease progression. Peripheral macrophage and monocytes population were also found to be depleted in *Mecp2* null mice. Postnatal re-expression of *Mecp2* using Cx3cr1^{creER}extends the lifespan of *Mecp2* null mice. MeCP2 also regulate glucocorticoid and hypoxia-induced transcripts in MeCP2 deficient microglia and macrophages. Further, MeCP2 modulates the transcription of inflammatory genes in response to TNF stimulation of microglia (Cronk et al. 2015). However, another study completely contradicts the findings of Derecki et al. 2012 that transplantation of wild type microglia reverses the Rett syndrome pathology (Wang et al. 2015). This suggests a direct role for microglia in Rett syndrome pathogenesis is controversial. In addition, it has been demonstrated that microglia may contribute to Rett syndrome pathogenesis by excessively engulfing and eliminating the presynaptic inputs at the end stage of the disease, leading to synapse loss. While the specific loss or gain of *Mecp2* expression in microglia has a modest effect on abovementioned phenotype. This indicates that the effect mediated by microglia is secondary and independent of microglia specific loss of *Mecp2* expression (Schafer et al. 2016). More recently, it has been demonstrated that immune dysregulation and oxidative stress observed in *Mecp2* null microglia in response to LPS could be attenuated by dendrimer based delivery of N-Acetyl cysteine (NAC). This implies a potential for glial cell directed therapy using dendrimer conjugated NAC for Rett syndrome (Nance et al. 2017). Intriguingly, ablation of CX3CR1, a chemokine

receptor in microglia involved in neuron-microglia interaction, improves the Rett syndrome like phenotype, including negative effects on neurons in *Mecp2* knock out mouse. Further suggesting a therapeutic potential for CX3CR1 antagonists (Horiuchi et al. 2016). In another study transcriptomic data of microglia from Rett syndrome mouse models confirmed that MeCP2 deficiency differentially regulates the activation of cellular stress genes that contribute to neurological symptoms (Zhao et al. 2017).

CONCLUSIONS

In summary, we have discussed the importance of glial cellMeCP2 in Rett syndrome pathophysiology and its role in the regulation of neuronal functions (Table I). Glial cells contribute significantly to the pathology of Rett syndrome and could be a potential future therapeutic target. Most of the studies to date have focused only on neuronal MeCP2 function related to Rett syndrome. Since MeCP2 is a genome wide modulator, future studies must be focused to unravel the role of MeCP2 in the regulation of glial cell functions like myelination, axo-glial interaction, immune modulation, synaptic regulation and related diseases. Other neurological disorders have been shown to have MeCP2 involvement of include MeCP2 duplication syndrome, X-linked mental retardation, and autism, Angelman Syndrome, Fetal Alcohol Spectrum Disorder, Schizophrenia and Huntington's disease (Ezeonwuka and Rastegar 2014). Thus, there is an urgent need to explore the role of glial MeCP2 in neurological diseases at the cellular and molecular level.

Table I. Summary of glial cells phenotype affected by MeCP2.

Cell type	Phenotype	MECP2 abnormalities	References
Astrocytes	Failed to support neuronal growth and causes dendritic abnormalities	$Mecp2^{tm1.1Bird}$	Ballas et al. 2009
	Up-regulation of BDNF expression, reduced pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) released		Maezawa et al. 2009
	Increased expression of GFAP and S100β, Decreased glutamate clearance, Up-regulation of glutamine synthatase		Okabe et al. 2012
	Reduced CO_2 sensitivity		Turovsky et al. 2015
	Elevated expression of *Apoc2, Cdon, and Csrp*, reduced expression of *Nrep*		Yasui et al. 2013
	Up-regulation of NR2F2 expression	$Mecp2^{308/y}$	Delepine et al. 2015
	Down-regulation of STMN2 expression, increased microtubule growth, impaired microtubule dynamic, Altered microtubule dependent vesicle transport		Nectoux and Florian 2012; Delépine et al. 2016
	Increased GFAP, S100β and BDNF expression, failed to support neuronal growth in *in vitro*	Mecp2 siRNA	Forbes-Lorman et al. 2014; Maezawa et al. 2009
Oligodendrocytes	Up-regulation of *Mbp, Plp, Mog, Mobp, Bdnf* and *Yy1*	Mecp2 siRNA	Sharma et al. 2015
	Up-regulation of *Mobp* expression	$Mecp2^{tm1.1Bird}$	Urdinguio et al. 2008
	Severe hand claspsing Phenotype	$Mecp2^{loxJ/y}$/NG2Cre	Nguyen et al. 2013
	Down-regulation of MBP and Up-regultion of PLP	$Mecp2^{Stop/y}$	
	Reduced CNPase expression	$Mecp2^{308/y}$	Wu et al. 2012
	Increased expression of *Mbp* and *Mag* in corpus callosum and *Plp* in forebrain		Vora et al. 2010
Microglia	Excess glutamate release, Increased glutaminase and Cx32 expression, damage dendrites and synapse	$Mecp2^{tm1.1Bird}$	Maezawa and Jin 2010
	Up-regulation of SNAT1, Increased ROS and glutamate, Less ATP production,		Jin et al. 2015
	Activated and depletion with disease progression, Incresead expression of glucocorticoid and hypoxia-induced transcripts		Cronk et al. 2015
	Differential expression of activation and cellular stress genes		Zhao et al. 2017

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