

ABSTRACT

Astrocytes are one of the most abundant and essential glial cells in the central nervous system performing diverse roles in maintaining the homeostatic scaffold associated with maintaining the functional environment. Astrocytes regulate the uptake and release of neurotransmitters, blood flow and the blood brain barrier. They also modulate synaptic plasticity and are the main source of energy in the brain in addition to being key players in neuroprotection as well as information processing, learning and memory. Many of the physiological functions performed by the astrocytes are governed by the secreted factors which include cytokines, trophic factors, neurotrophins and many more, which greatly influence the surrounding neurons, oligodendrocytes as well as astrocytes. Astrocytes work in close association with neurons and oligodendrocytes and also support their pathology and physiology. Recently, research has garnered interest in investigating the involvement of astrocytes in regulating central nervous system myelination.

Methyl-CpG-Binding Protein 2 (MeCP2) is an epigenetic protein that binds to CpG islands in the genome and induces multiple gene regulatory functions by conforming changes in the chromatin structure and resulting in cell specific gene expression. Mutations of MeCP2 cause neurodevelopmental disorder known as Rett syndrome. MeCP2 affects the neuronal survival and morphology by disrupting the dendritic arborisation and dendritic outgrowth in neurons. In addition, MeCP2 deficient astrocytes have been linked with abnormal neuronal functions and show dysregulation in certain synaptic genes. MeCP2 is also a known regulator of BDNF which is a well-known pro-myelinating factor secreted by astrocytes. However, role of astrocytic MeCP2 in central nervous system myelination is largely not known.

The data from the current study indicates altered levels of astrocytes secreted factors such as LIF, CNTF (myelination promoting factors), PDGF, CXCL10 (myelination inhibiting factors) which are well studied factors involved in CNS myelination regulation. Astrocytic MeCP2 also influences neurotrophins' (NGF and BDNF) secretome pattern, transcript levels and protein expression. Further, astrocytic MeCP2 inhibited oligodendrocyte precursor cell survival and proliferation, both of which are a pre-requisite for myelination in the brain. Oligodendrocyte differentiation was hampered in response to MeCP2 deficient astrocytes as evident by the elevated

expression of PDGFR α , a marker of oligodendrocyte precursor cells (OPCs); which suggests that astrocytic MeCP2 maintains the progenitor state of oligodendrocytes. In addition, myelin basic protein expression was also found to be down-regulated in mature oligodendrocytes. Current study established a unique *in vitro* triple culture of astrocytes-DRG neurons and oligodendrocyte to replicate an *in vivo* condition of myelination. In these triple cultures, axo-glial interaction genes namely Caspr, Notch1, NF155 and Nrg1 were found to be under the regulation of astrocytic MeCP2 along with key myelin transcript and protein expression of MBP, PLP, MAG, MOG. Total myelin protein was also reduced in MeCP2 deficient astrocytes condition.

Overall, the findings from the present study conclusively reveals the role of astrocytic MeCP2 in regulating CNS myelination.