CHAPTER 6

SUMMARY

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Epigenetic programming is the process through which an environmental stimulus during development alters the gene expression by changing the epigenetic marks (e.g., DNA methylation, histone modifications, etc.) to produce a persistent phenotypic effects. Epigenetics also plays a fundamental role in CNS development and function. MeCP2 is a global transcriptional regulator of the CNS, which functions as a gene-specific transcriptional silencer bybinding throughout the genome as well as transcriptional activator due to its involvement in the transcriptional modulation of active genes.The X chromosome linked *MECP2* gene is associated with several neurological disorders, like Rett syndrome etc.Additionally, MeCP2 has also been proposed to directly affect splicing, miRNA biogenesis andcentrosomal functions. It is the highly disordered structure of MeCP2 along with its post-translational modifications that generate and regulate this functional versatility and make MeCP2 a multifaceted protein.

Undoubtedly, several studies havereported that differential phosphorylation of MeCP2 is a key mechanism by which the methyl-binding protein modulates its affinity for its partners, gene expression and cellular adaptations to stimuli and neuronal plasticity.The addition of a negatively charged phosphate group can dramatically impact protein functions. In neurons, MeCP2 phosphorylation events occur under basal conditions and/or in response to neuronal activity and certain developmental stimuli. Additionally, it has been shown that MeCP2 phosphorylation affects the dendritic arborization, spine maturation and thereby controlling the development and function of the nervous system. However, there are only few reports on the MeCP2 phosphorylation in the glial cells. Understanding the function and regulation of MeCP2 phosphorylation in glial cellsis essential for advancing our comprehension of RTT and *MECP2* related disorders.The present study focused on the regulation of phosphorylation sites that were evolutionally conserved or have been found to be mutated in patients.

The study mainly focused on the regulation of MeCP2 phosphorylation in glial cells of CNS mainly oligodendrocytes and astrocytes. There is large number of developmental stimuli which affects the overall development of these glial cells in CNS. However, no information exists for these factors regulating MeCP2 phosphorylation in glial cells. The

data from the present study is the first report to conclusively demonstrate the involvement of growth factor (BDNF) and ECM (LN) in MeCP2 phosphorylation, which is essential for proper development of the glial cells of CNS.In addition to this, the study reported for the first time that the reactive astrocytes after LPS exposure show the up-regulation of MeCP2 phosphorylation thereby affecting its sub-cellular localization.

In mature OLGs, LN treatment leads to dephosphoryation of MeCP2at S80 thereby allowing the expression of various genes. This was further confirmed by blocking α6β1 integrin which is the only receptor for LN. This resulted in increase in the pS80MeCP2 expression. ECM demonstrated significant role than growth factors in the regulation of MeCP2 phosphorylation in oligodendrocytes. Moreover, the study demonstrated that in mature OLGs, LN treatment increases the levels of MBP which is the direct target of MeCP2. In oligodendrocytes, pS80MeCP2 may affect myelination indirectly through the regulation of MBP expression in oligodendrocytes (Fig-6.1). Importantly, the study showed that, although MeCP2 is expressed in all the cells of CNS, it appears that pS421MeCP2 is not expressed in OLGs. This is probably due to the fact that the phosphorylation of MeCP2 is dependent on both the cell type and the stimuli and thus each cell will not consist of all possible modifications. This work has been published (Parikh et al., 2017)

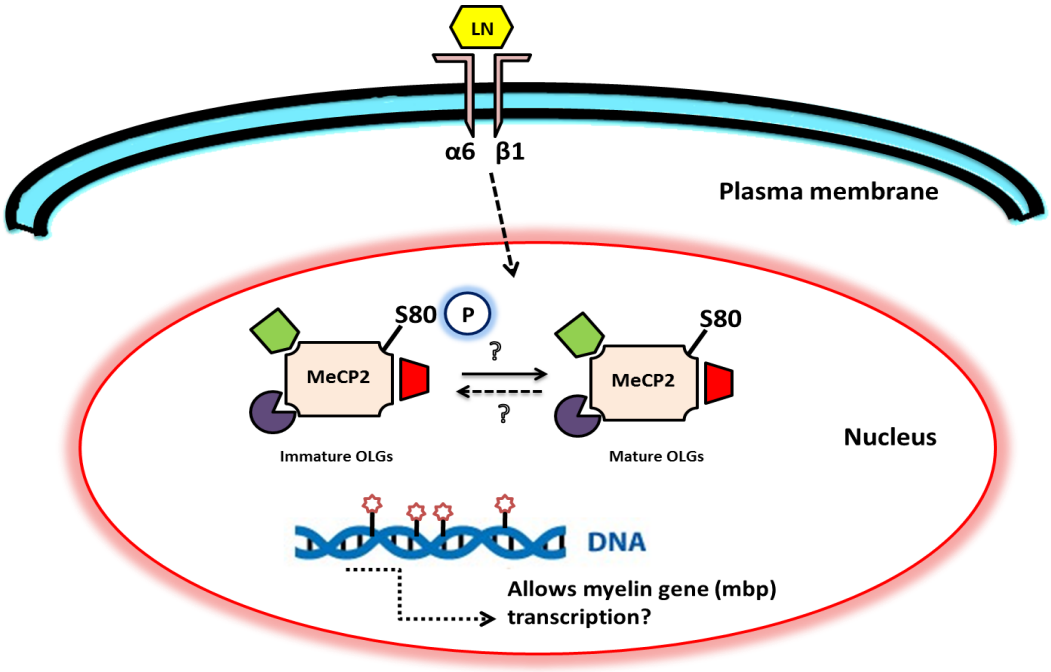

Fig-6.1 Diagrammatic representation of the effect of Laminin (LN) on differential phosphorylation of MeCP2 at S80 in oligodendrocytes: *LN induced phosphorylation and dephosphorylation of pS80MeCP2 in immature and mature OLGs respectively. Stimulation of LN receptor α6β1 activates unknown kinases and phosphatases may modify the phosphorylation and dephosphorylation status of MeCP2. Phosphorylation of S80, together with dephosphorylation of S80, may modulate the binding of MeCP2 to the promoters of myelinspecific genes (mbp).*

Moreover, in this study the factors affecting MeCP2 phosphorylation in astrocytes were also assessed in which it was found that BDNF increased the pS421MeCP2 expression levels.The results showed that upon BDNF treatment, the pS421MeCP2 was localized into the nuclei from the cytoplasm (Fig-6.2).Moreover immunocytochemistry results demonstrated the sub-nuclear localization of pS421MeCP2 in the BDNF treated astrocytes and itwas shown to be associated with the euchromatin region.By inhibiting the CamKII signaling by KN93, there was decrease in the level of pS421MeCP2 and the association of pS421MeCP2 with the euchromatin also decreased drastically. BDNF is known to increase the S100βwhich is a direct target of MeCP2 in astrocytes. In conjunction with these reports it can be speculated that increased pS421MeCP2 may

regulate the expression of S100β stimulated by BDNF. The underlying molecular mechanisms need to be investigated.

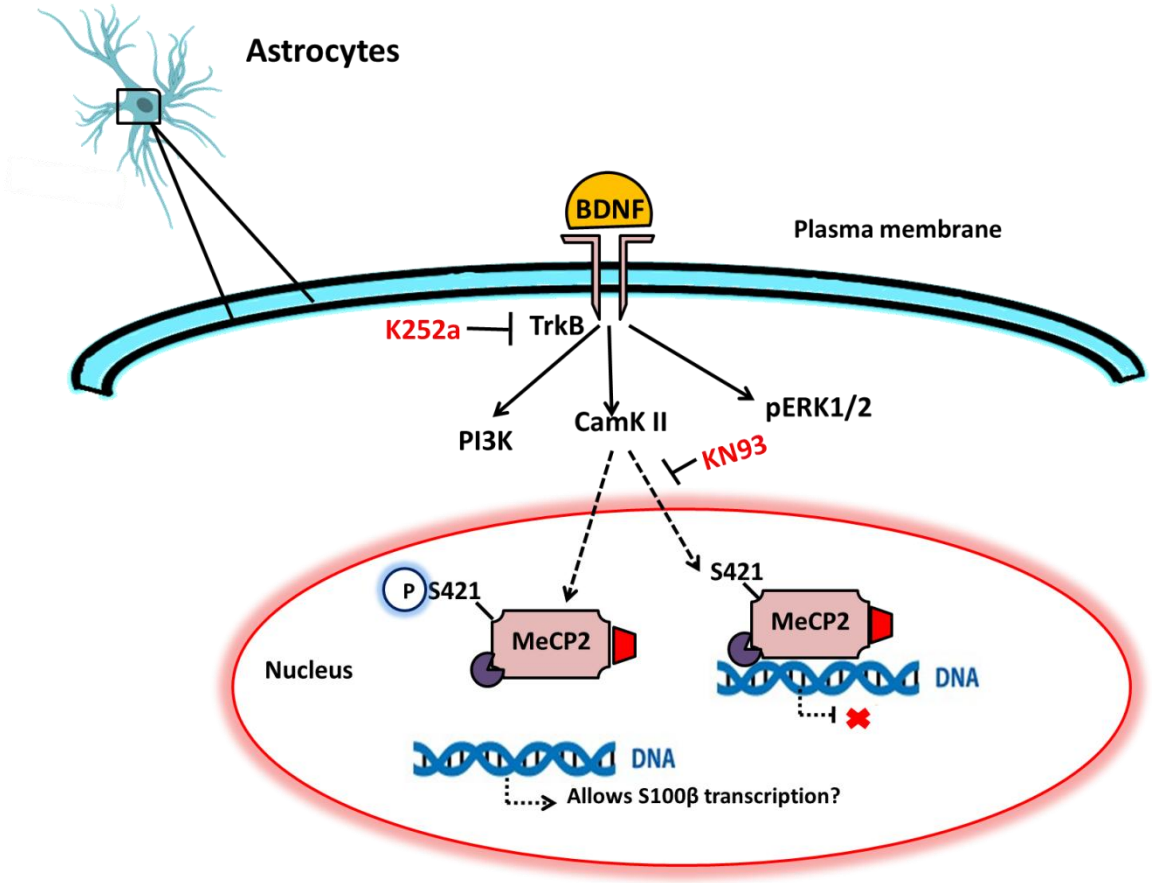

Fig-6.2 Schematicrepresentation of the effect of BDNF on MeCP2 S421 phosphorylation in astrocytes: *BDNF induced MeCP2 phosphorylation at S421 in astrocytes. BDNF treatment also leads to the nuclear localization of pS421MeCP2 in astrocytes. Stimulation of BDNF receptor TrkB activates PI3K, pERK1/2 and CamKII pathways. Inhibition of CamKII by KN93 leads to dephosphorylation of pS421MeCP2 which may modulate the binding of MeCP2 to the promoters of specific genes (S100β).*

To further study the effects of BDNF on MeCP2 phosphorylation and its chromatin association C6glioma cell line was used as a model system. MeCP2 not only play a role in neurodevelopmental disorders but it has been a substantial epigenetic regulator in many cancers like prostrate, lung, liver, breast cancers etc. However, there are no reports on MeCP2 phosphorylation in this glial cell derived cancer. In the current work,increased levels of pS80MeCP2 and pS421MeCP2 were observed when C6 glioma cell line was treated with BDNF and this phosphorylation was mediated by

TrkBreceptor[Fig-6.3 (A)]. Stimulation of BDNF receptor TrkB activates PI3K, pERK1/2 and CamKII pathways. Inhibition of any of these pathways had no effect on MeCP2 phosphorylation. Moreover, inhibition of TrkB receptor by K252a leads to decrease in MeCP2 phosphorylation (S80 & S421).

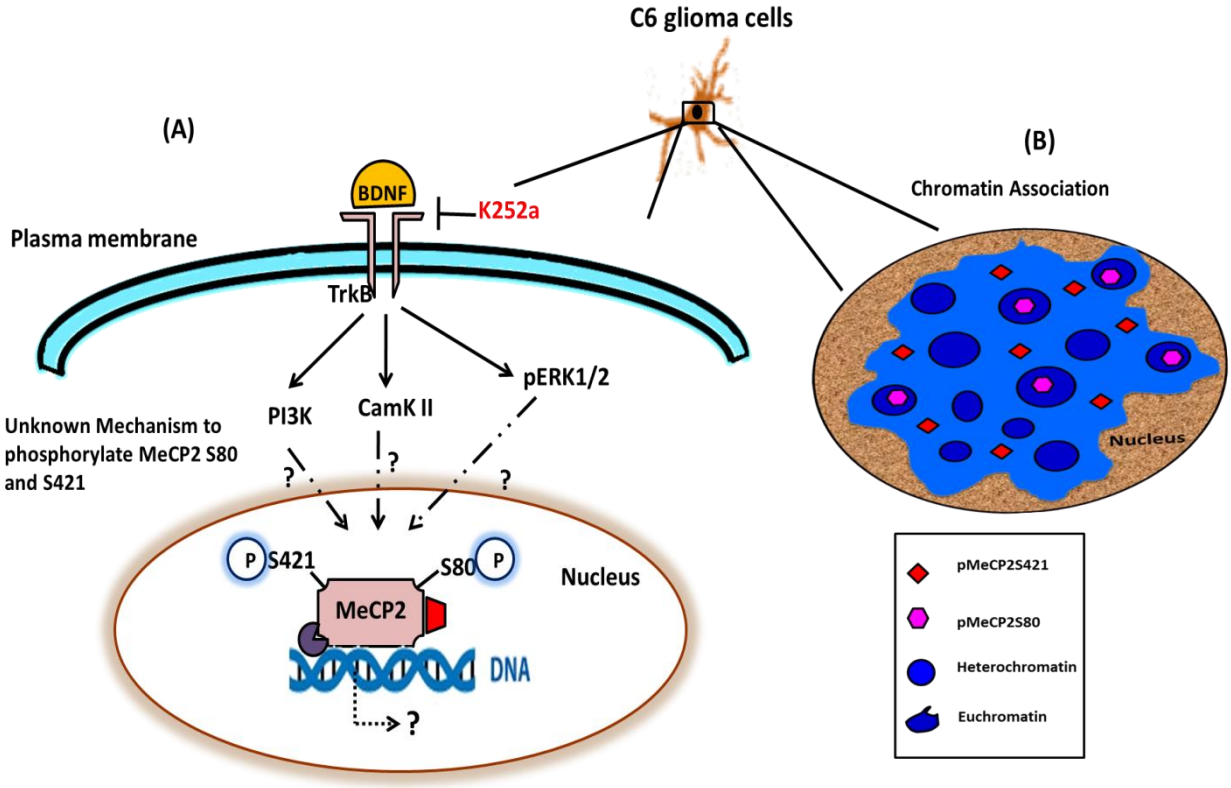

Fig-6.3 Graphical representation showing the effect of BDNF on MeCP2 phosphorylation at S80 and S421 in C6 glioma cells: (A) *BDNF induced MeCP2 phosphorylation at S80 andS421 in C6 glioma. BDNF treatment also leads to the nuclear localization of pS421MeCP2 in astrocytes. Stimulation of BDNF receptor TrkB activates PI3K, pERK1/2 and CamKII pathways. Inhibition of any of these pathways had no effect on MeCP2 phosphorylation. Moreover, inhibition of TrkB receptor by K252a leads to decrease in MeCP2 phosphorylation (S80 & S421).**(B)**Pattern of chromatin association of pS80 and pS421MeCP2 upon BDNF treatment.pS80MeCP2 was found to be strongly associated with the heterochromatin represented by chromocenters and pS421MeCP2 was found to be associated with euchromatin*

Moreover, when the chromatin associationstudies were done by immunofluorescence and biochemical fractionation of the nuclei, it was observed that pS80MeCP2 on BDNF treatment is found to be associated with heterochromatin and on the contrary pS421MeCP2 is associated with euchromatin [Fig-6.3 (B)].This study makes it appealing to speculate a particular type of association of phosphoMeCP2s to regions of the chromocenters whose molecular explanation remains to be explored.These studies demonstrate that pS80MeCP2 and pS421MeCP2 phosphoryationaffects its sub-nuclear localization and chromatin affinity. These observations suggest that pS80MeCP2 might play a role in transcription repression while pS421MeCP2 play a role in transcription activation and further suggests that MeCP2 intensely changes its affinity for chromatin which is in turn regulated by stimuli such as BDNF.

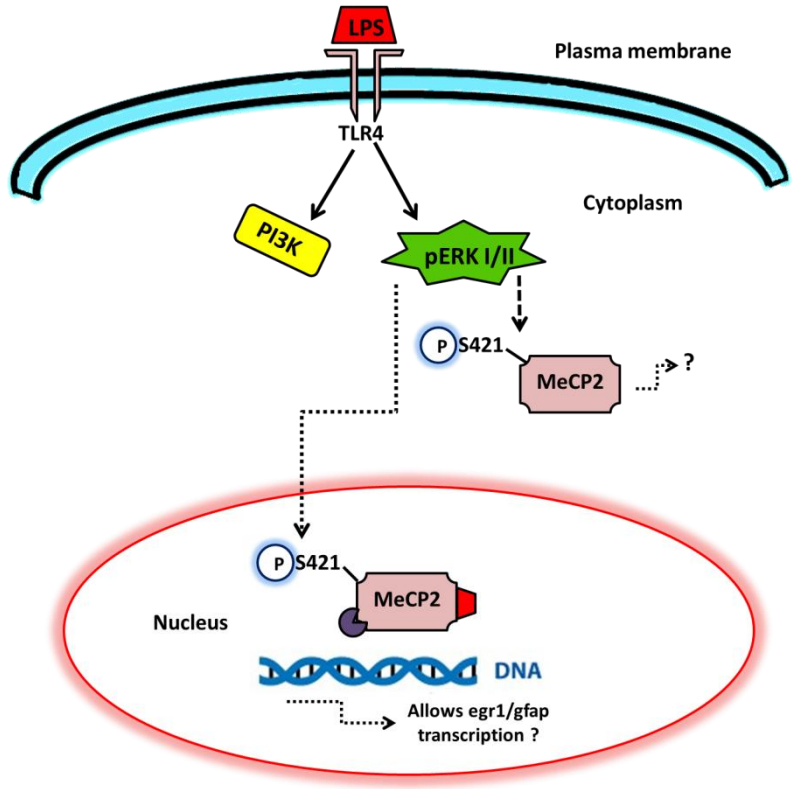

Fig-6.4 Pictorial representation showing the effect of LPS on phosphorylation of MeCP2 at S421 in astrocytes: *LPS treatment leads to increase in pS421MeCP2 which mostly found to be localized in the cytoplasm than the nuclear region. Phosphorylation of pS421MeCP2 may modulate the binding of MeCP2 to the promoters of specific genes (egr-1,gfap,etc). Moreover, the pS421MeCP2 might play a role in cytoplasm in the reactive astrocytes.*

LPS stimulation leads to a largenumber of rapid post-translational and transcriptionalchanges in the astrocytes. In this context, understating the mechanism by which astrocyte plays a rolein CNS immune modulation following a variety of infectiousor inflammatory insults has been a key issue. There are reports suggesting that in the peripheral nervous system (PNS), MeCP2 S421 phosphorylation was first observed in the superficial dorsal horn neurons in the spinal cord after induction of peripheral joint inflammationLPS stimulation leads to increase in phosphorylation of the transcriptional regulator MeCP2 at S421 site in the rat derived primary astrocytes.The present study is the first to report that LPS stimulation leads to increase in phosphorylation of the transcriptional regulator MeCP2 at S421 site in the rat derived primary astrocytes. In addition to this there was increase in GFAP expression after LPS treatment. Hence, LPS induced pS421MeCP2 might regulate the GFAP expression.Blocking the signaling pathways activated by LPS stimulation mainly PI3K and ERK1/2 pathways using pharmacological inhibitors showed decreased in the expression levels of pS421MeCP2 in UO126 (ERK1/2 inhibitor) group(Fig-6.4). Moreover, there was no change in the expression of pS80MeCP2 expression in the LPS treated astrocytes suggesting that pS80MeCP2 might not play an important role in LPS stimulated astrocytes. In contrast to the pS80MeCP2 localization, pS421MeCP2 was found to be localized in cytoplasm compared to the control. Therefore, pS421MeCP2 might play a role in cytoplasm in the reactive astrocytes. Reports have shown that the inhibition of pERK1/2 withU0126 reduced the expression of Egr-1. It is known that Egr-1 is an important component in transcriptional regulation involved in gliosis after ischemic injury. Interestingly, Egr-1 gene is a target of MeCP2 reported in astrocytes. Therefore, LPS mediated MeCP2 phosphorylation may control the gene expression of Egr-1 gene in astrocytes (Fig-6.4).

In conclusion, MeCP2 in glial cells is functionally regulated by stimuli mediated phosphorylation which would allow MeCP2 to bind to distinct sites with an effect on chromatin affinity through sub-cellular localization thereby providing regulatory specificity during CNS myelination. Instead, the phosphorylation events appear to

interact with the signaling networks activated by different environmental stimuli which may determine the functional activity of MeCP2. So far there is no studies pertaining to glial cellswhich demonstrates the effect of MeCP2 phosphorylation on chromatin binding and sub-cellular localization. Future studies need an in vivo approach to reveal the functional role of pS80MeCP2and pS421MeCP2 in glial cells of CNS by studying the MeCP2 S421A and MeCP2 S80A mice. Moreover, the phenotypes observed in these knock-in mice provide an in vivo evidence that the growth factor/ECM dependent modification of MeCP2 is required for glial cell development and function which will not only help to extendthe understanding of stimulusdependent MeCP2 phosphorylation and chromatin association, but may also provide therapeutic insights into RTT and other neuropsychiatric disorders.