Synopsis of the thesis on

Regulation of MeCP2 phosphorylation in CNS glial cells

Submitted to

The Maharaja Sayajirao University of Baroda

For the degree of

Doctor of Philosophy(Ph.D.) in Zoology

By

Zalak Parikh

Under the supervision of

Dr.Prakash P.Pillai

January 2018

Division of Neurobiology
Department of Zoology, Faculty of Science,
The Maharaja Sayajirao University of Baroda,
Vadodara – 390 002,
Gujarat

Introduction:

Oligodendrocytes (OL) are glial cells that produce and maintain myelin, the lipid enriched axon-ensheathing membrane, which is essential for saltatory conduction of action potentials in the central nervous system (CNS), whereas astrocytes are composed of at least one half of human brain tissue volume and are also excitable cells and play important roles in information processing and modulation of neuronal activity (Perea and Araque, 2005; Haydon and Carmignoto, 2006; Wang et al., 2006; Winship et al., 2007;Yin et al., 2006). Astrocytes are known to significantly modulate synaptogenesis during development and play diverse and active roles in synaptic physiology in the adult brain. There are five basic phases of the OL lineage: generation, migration, proliferation, differentiation and myelination. Furthermore, extensive studies have reported various transcription factors essential for oligodendrocyte specification as well as growth factors that influence oligodendrocyte proliferation and differentiation (Lu et al., 2000, 2002; Orentas and Miller, 1996; Takebayashi et al., 2000). Many different growth factors (GFs), such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF); ECM proteins, such as Laminin (Ln), and Fibronectin (FN) and neurotrophins, such as neuronal growth factor (NGF), and brain derived neurotrophic factor (BDNF) have been shown to play an important role in glial cell development(Bogler et al. 1990; Baron et al. 2005; Milner et al. 1997; Novak and Kaye 2000; Frost et al. 2009; Vora et al. 2011; O'Meara et al. 2011). Oligodendrocyte progenitor cell (OPC) migration is critical not only during normal myelination but also during remyelination (Keough and Yong 2013). Moreover, studies have shown the altered levels of GFs and ECM in the demyelinating lesions (Hughes et al. 2013). One critical aspect of these biological processes is the mechanism by which OLG transcription factors/regulators integrate the external stimuli to trigger changes in gene expression. It has been studied that environmental factors can regulate the gene expression by epigenetic modifications (Vigetti et al. 2014).

Methyl-CpG-binding protein 2 (MeCP2) in recent times have been shown to be a well-known link between epigenetics and human disease; mutations in which lead to the neurological disorders, such as Rett syndrome(Xu et al. 2014; Chahrour et al. 2008; Zachariah and Rastegar 2012). MeCP2 is present in normal brain and found not only in neurons, but also in all types of glia, including astrocytes, oligodendrocyte progenitor cells (OPCs), and oligodendrocytes (OLGs) (Ballas et al., 2009). Importantly, MeCP2-deficient astrocytes isolated from Mecp2-/y mice cannot support neuronal dendritic growth, indicating a critical role of MeCP2 in glial function (Maezawa et al., 2010). Brain magnetic resonance study in Mecp2-/y mice detected a significant reduction in the thickness of the corpus callosum which shows that white matter isaffected in Rett syndrome (Saywell

et al. 2006). Furthermore, it has been shown that myelin gene expressions are altered in MeCP2-null mice (Nguyen et al. 2013; Vora et al. 2011).

MeCP2 is a multifaceted protein as it functions as a transcriptional repressor or activator via binding to methylated CpGs in the promoters and nearby regions of target genes (Tao et al., 2009). MeCP2 undergoes various post-translational modifications (PTMs), such as phosphorylation, ubiquitination, acetylation, and sumoylation(Bellini et al. 2014). However, the functional roles of these PTMs are largely unexplored. Recent studies have shown that phosphorylation has extremely important effects on the modulation of MeCP2 function (Damen and Heumann 2013). Accumulated evidences suggest that MeCP2 could be phosphorylated at multiple sites, of which the well-documented MeCP2 phosphorylation sites are S421 and S80; phosphorylation of which modulates the transcription in opposite manners leading to gene silencing or activation (Tao et al. 2009; Zhou et al. 2006; Chao and Zoghbi 2009; Rutlin and Nelson 2011).Activity-dependent phosphorylation of MeCP2 at serine 421 leads to decreased binding of MeCP2 from the promoter of Bdnf and increases the expression of BDNF in neurons (Zhou et al., 2006). Whereas, pMeCP2 (phosphorylated MeCP2) S80 under resting conditions in neurons, promotes the binding of MeCP2 to the promoters of target genes to repress gene transcription (Tao et al., 2009). Strikingly, other studies have further shown that the transcriptional roles of pS421 MeCP2 and pS80 MeCP2 vary according to their specific target genes, and that pS421 MeCP2 can even bind across the genome without selectivity (Cohen et al., 2011). Animal model studies have further shown that even the loss of a specific phosphorylation site of MeCP2 (S80, S421) disturbs normal maturation of the mammalian brain (Tao et al. 2009). Earlier, reports have also shown that, unlike S421 phosphorylation, S80 phosphorylation not only occurs in neuronal cells but also seen in non-neuronal cells such as Hela cells and human fibroblasts (Bracaglia et al. 2009; Tao et al. 2009). Tao et al(2009) found that S80 phosphorylation is the highly conserved phosphorylation site of MeCP2. The expression and function of MeCP2 phosphorylation at S80 (pS80MeCP2) has been well studied in neuron (Gonzales et al. 2012). It has been shown that the expression of pS292 MeCP2, a newly-reported phospho-MeCP2 (pMeCP2), is induced in reactive astrocytes in the ischemic adult brain (Liu et al., 2015), but its function is not yet clear.Recently, a study on spatio-temporal expression of pS80 MeCP2, pS421 MeCP2, and pS292 MeCP2 in the postnatal rat brain was carried out and explored the function of MeCP2 in the developing cerebellum via shRNA-mediated MeCP2 deficiency (Liu et al. 2017). However, there is no data so far available in OLGs regarding MeCP2 phosphorylation status. Notably, diverse pMeCP2s can interact with distinct co-factors to initiate disparate transcriptional pathways (Gonzales et al., 2012), indicating that the dynamic balance between phosphorylation and dephosphorylation is crucial for MeCP2 function.

Nevertheless, phosphorylation of MeCP2 is essential for proper protein function, and how distinct patterns of phosphorylation alter MeCP2 binding to the chromatin remains to be investigated in glial cells. Summarizing, most data suggest that MeCP2 is a multifunctional protein that “transiently” performs its function(s) depending on its differential phosphorylation. Almost no information is available so far on the phosphorylation and their regulation in glial cells. Therefore present study focuses on the role of developmental stimuli [growth factors (GFs), neurotrophins, and extracellular matrix (ECM)]in regulating MeCP2 phosphorylation in glial cells.

Hypothesis

We hypothesize that 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.

Key questions

1. What are the expression and distribution patterns of MeCP2 phosphorylation?
2. What are the factors regulating MeCP2 phosphorylation**?**
3. How MeCP2 phosphorylation regulates its chromatin affinity and association?

Significance of the Current Study:

Elucidating how post-translational modifications may affect MeCP2 function in glial cells and will advance our understanding of MeCP2 normal function and the etiology of MeCP2 related disorders.

Specific Objectives:

1. **To study the expression and sub-cellular localization of MeCP2 in glial cells**
2. **To study the factors regulating MeCP2 phosphorylation**
3. **To study the underlying molecular mechanisms regulating MeCP2 phosphorylation and its chromatin association.**

Summary of the Work Done:

Objective 1:*To study the expression and sub-cellular localization of MeCP2 in glial cells*

To date, most studies have reported pMeCP2s exclusively in neurons (Tao et al., 2009 & Li et al., 2011), except for the observation of astrocytic pS292 MeCP2 (Liu et al., 2015). Moreover, the precise levels of MeCP2 are vital for brain development and its abnormal expression causes Rett syndrome (RTT), a genetic neurological disorder (Amir et al., 1999). Therefore, in this study we set out to identify the expression and distribution pattern of the two most important sites of MeCP2 phosphorylation pS80 MeCP2 and pS421 MeCP2 along with total MeCP2 in the glial cells of CNS. It is known that MeCP2 is predominantly present in the nucleus, but it is also found to be expressed in the cytoplasm and postsynaptic compartment of neurons at a relatively low level (Miyake et al., 2007).Hence, we investigated the sub-cellular localization of pMeCP2s in the rat glial cells of CNS to assist further understanding of MeCP2 function.

The glial cells of CNS were isolated using the standard protocol by Chen et al., 2007. The glial cells isolated were characterized using cell specific markers. Oligodendrocytes were characterized using PDGFRα, O4 and MBP for showing OPC, Immature and mature stages of OLGs respectively. GFAP was used as a marker for astrocytes. The characterization study was done using Immunofluorescence and Western Blot analysis. In the present study, we used Western blot analysis tofirst checkthe expression levels of total and phosphorylated MeCP2 (pS80 & pS421) in immature and mature OLGs.Brain lysate was taken as a positive control. There was a significant increase in the levels of MeCP2 and pS80 MeCP2 expression with the OLG maturation. However, we could not detect any expression of pS421 in oligodendrocytes. This may be due to its selective expression in certain cell types. We further studied its sub-cellular localization using Western blot analysis of the sub-cellular fractions. The oligodendrocytes were subjected to sub-cellular fraction using NE-PER Kit (Thermo Fisher). Nuclear and Cytosolic fractions were isolated using this kit. The purity of the fractions was confirmed using GAPDH and Histone (H3) antibodies. GAPDH was used for the cytosolic fraction and H3 was used to check the nuclear fraction. Further, using Western blot analysis, the expression levels of MeCP2 and pS80 MeCP2 were checked in the immature and Mature OLGs in both nuclear and cytosolic fractions. The MeCP2 and pS80 MeCP2 were mainly expressed in the nuclear compartment. This result was confirmed by Confocal imaging which showed the nuclear localization of MeCP2 and pS80 MeCP2 in immature and mature oligodendrocytes. Similar study was performed to study the expression and localization of MeCP2, pS80MeCP2 and pS421 MeCP2 in primary cortical astrocytes derived from rat brain and C6 rat glial tumour cell line. We found that MeCP2 and pS80 MeCP2 were expressed in the nuclear

compartment whereas pS421 MeCP2 in astrocytes was also expressed in the cytoplasmic compartment. This was further confirmed with the Confocal imaging that pS421 was also found in the cytoplasmic region of Astrocytes. The above work was presented at *31st Annual Conference of Indian Academy of Neuroscience Allahabad (Oct 25th-27th 2013)*. Together, our data demonstrate that MeCP2 and pMeCP2 have distinct features of differential expression during the glial cell development. The patterns of expression and distribution of MeCP2 and pMeCP2s are distinct and vary with the cell types.Moreover, MeCP2 and pS80 MeCP2 are concentrated in the nucleus in astrocytes as well as in oligodendrocytes, while pS421 MeCP2 was also found to be localized in the cytoplasm in astrocytes and C6 glioma cell line. However, we could not detect the expression of pS421 MeCP2 in oligodendrocytes.

Objective 2-To study the factors regulating MeCP2 phosphorylation

During normal glial cell development in the CNS, both GFs and ECM coexist in the cellular microenvironment, but the underlying mechanism by which they regulate the glial cell behavior is poorly understood. Studies have shown the altered levels of GFs and ECM in the demyelinating lesions where glial cells are the majority of cells in CNS that provide support and protection for neurons (Silver and Miller, 2004; Hughes et al. 2013). During development, extrinsic and intrinsic stimuli create epigenetic marks (Delcuve et al. 2009). These epigenetic modifications thereby alter the chromatin structure and modulate the transcription capability (Olynik and Rastegar 2012).Hence, in the present study, we have investigated the individual effects of PDGF-A (10ng/ml), FGF (10ng/ml), BDNF (50ng/ml), FN (10µg/ml), and LN (10µg/ml) on the MeCP2 phosphorylation at pS80 in OLGs. PLL was used as control matrix. Our data shows that immature OLGs exposed to LN resulted in enhancedexpression of pS80MeCP2 compared to other treatment groups. The data shows the importance of ECMmore than GFs in the regulation of MeCP2 phosphorylation at S80 site.Morphological analysis of OLGs showed that LN significantly increased the differentiation of immature OLGs to mature OLGs. Hence, we examined the effects of FN and LN on mature N19 OLGs.Western blot analysis showed significant decrease in pS80MeCP2 expression levels in the mature OLGs exposed to LN compared to those exposed to FN and PLL (control). Our data also confirmed that LN enhanced the expression of MBP in mature OLGs.

Next we tested the above mentioned GFs and ECM factors in primary cortical astrocytesto study the MeCP2 phosphorylation patterns.The protein levels of MeCP2, pS80MeCP2 and pS421MeCP2 were checked using Western Blot analysis. There was significant increase in pS421 MeCP2 in the BDNF treated group of Astrocytes. There was no significant difference in the levels of total MeCP2. Moreover there wasdecrease in pS80 MeCP2 levels in BDNF treated astrocytes. The sub-cellular localization of pS421MeCP2 in the BDNF and NGF treated group of astrocytes was

studied. Interestingly the pS421 MeCP2 level significantly increased in nucleus compared to cytoplasm. pS421 MeCP2 was found to be localized in the BDNF and NGF treated group. These results provide us the basis to conclude that neurotrophin treatment in astrocytes lead to nuclear localization of pS421 MeCP2.Similar effects of BDNF and NGF was observed on MeCP2 phosphorylation in C6 glioma cell line.

Additionally, we also analyzed the effect of Lipopolysaccharide (LPS) on MeCP2 phosphorylation in Astrocytes. The protein levels of MeCP2, pS80MeCP2 and pS421MeCP2 were analyzed using Western Blot analysis.There was significant increase in pS421 MeCP2 and total MeCP2 in the LPS treated group of Astrocytes.There was no significant difference in the levels pS80 MeCP2.The sub-cellular localization of pS421MeCP2 in the LPS treated group of astrocytes was studied. Excitingly we got the pS421 MeCP2 levelsignificantly morecompared to the control group in the cytoplasmic fraction.

Objective 3- To study the underlying molecular mechanisms regulating MeCP2 phosphorylation and its chromatin association.

We next aimed to identify the molecular mechanisms by which different growth factors regulate the MeCP2 phosphorylation in glial cells. The results show that LN significantly increased the pS80expression in immature N19 OLGs and decreased pS80expression in mature N19 OLGs.LN is in the extracellular surface of the neurons, and OLGscontact neuronal LN through α6β1 integrin (O'Meara et al.2011; Simons and Trajkovic 2006).Morphological analysis showed that LN significantlyincreased the differentiation of immature N19OLGs to mature N19 OLGs. We further verified the LN-dependent pS80MeCP2 phosphorylationby blocking LN receptor, α6β1-integrin, the only known LN receptor (Baron et al. 2003). The expressionof α6β1-integrin in immature and mature N19 OLGs was also checked by co-immunoprecipitation studies.Blocking α6β1 significantly increased the expression ofpS80MeCP2. We further confirmed these observationsby pS80MeCP2 nuclear localization studies usingconfocal microscopy imaging. The data showed significantdecrease in the LN-dependent nuclear pS80MeCP2 expressionand co-localization as compared to the PLL control.However, blocking the LN receptor (α6β1) significantly increased the LN-dependent decrease in the nuclearpS80MeCP2 expression and colocalization.Nuclear co-localization of pS80 MeCP2 was further confirmed by the study ofthe mean fluorescence intensity. Our datashows that LN exposure increased the pS80 MeCP2 expressionin the immature N19 OLGs but decreased expression levels in the matureN19 OLGs. Collectively, the data shows LN-dependent differential regulationofthe pS80 MeCP2 in OLG lineage progression. *This part of the study is published in the Journal of Molecular Neuroscience (Parikh et al., 2017).*

In Astrocytes and C6 glioma cell line, we next assessed whether MeCP2 is phosphorylated by any specific kinase. To study this first we assessed the pathways activated by different stimuli which are phosphorylating MeCP2. We evaluated the expression of pERK1/2 and pAKT in the astrocytes treated with neurotrophins (BDNF & NGF) using Western blot analysis. These are already known pathways activated by neurotrophins. Thereafter, we treated astrocytes with the specific kinase inhibitors of these pathways. We used pharmacological inhibitors for ERK1/2 and PI3K pathways from Calbiochem. We used UO126(10µM) and LY294002 (50µM) to inhibit ERK and PI3Kpathways respectively.We also blocked CaMKII activity using KN93,a potent and specific endogenous protein inhibitor ofCaMKII (Chang et al., 1998). To identify the kinase(s) that phosphorylate MeCP2, the corticalastrocytes were incubated with above mentioned kinase inhibitors;the cells were then treated with BDNF and NGF and assessedfor the induction of MeCP2 S421 phosphorylation.The CaMKIIinhibitor KN-93(5µM)effectivelyblocked the neurotrophin induced MeCP2 phosphorylation at S421compared to other inhibitors.

To study the effect of the stimuli/growth factor on MeCP2 chromatin association we performed biochemical fractionation of nuclei and examined the differences in sub-nuclear distributions of total and phosphorylatedMeCP2. Micrococcal nuclease fractionation of purified nucleigenerates three distinct nuclear fractions, S1, S2, and P. The S1 and P fractions primarily contain actively expressedgenes while the S2 fraction consistslargely of constitutively silenced genes. The majority of pS421MeCP2 BDNF and NGF treated astrocytes was found in the nuclease-sensitive S1 and S2 fractions.The presence of MeCP2 in the sub-nuclear fraction containing actively expressed genes and silenced genes suggesting that it may regulateexpression of those genes in both positive and negativemanner.Further,the studies to identify the mechanism regulating the MeCP2 phosphorylation in the reactive astrocytes by LPSarein progress, we next assessed the pathways activated by LPS. We evaluated the expression of pERK1/2 in the reactive astrocytes treated with LPS using Western blot analysis. This is already known pathway by LPS in astrocytes. We used pharmacological inhibitors for ERK1/2 and PI3K pathways from Calbiochem. We used UO126 (10µM) to inhibit ERK pathway. The treatment with UO126 effectively blocked the LPS induced MeCP2 phosphorylation at S421compared to LPS treated and control. Further studies on mechanisms regulating MeCP2 phosphorylation and its chromatin association by LPS are in progress.

Conclusion:

Overall, the current study supports the hypothesis that MeCP2 in glial cells is functionally regulated by stimuli mediated phosphorylation. Taken together, the data provide the evidence thatthe patterns of expression and distribution of MeCP2 anddiverse pMeCP2s are discrete and is stimuli dependent in glial cells. In oligodendrocytes the data also pinpointsthe importance of ECMmore than

GFs in the regulationof MeCP2 phosphorylation at S80 site. However, further effortsto better understand the mechanisms by which S80 phosphorylationregulates MeCP2 function in OLGs may furtherour understanding of how mutations in MeCP2 lead to the neurological phenotype of RTT. However, in Astrocytes neurotrophins lead to increase in the pS421 MeCP2 expression and the CamKII kinases are involved in regulating this phosphorylation.Moreover, MeCP2 and pS80 MeCP2are found to be localized in the nucleus, while pS421 MeCP2 is also found to be localized in the cytoplasm. A better understanding of the expressive patterns and exact roles of MeCP2 and the pMeCP2sin astrocytes and oligodendrocytes is still needed and this will provide further insights into the pathogenesis of RTT.

Refrences:

Ballas N, Lioy DT, Grunseich C, Mandel G (2009) Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Nat Neurosci 12(3):311 317

Baron W, Colognato H, Ffrench-Constant C (2005) Integrin-growth factor interactions as regulators of oligodendroglial development and function.Glia 49(4):467–479

Bellini E. Pavesi, G.; Barbiero, I.; Bergo, A.; Chandola, C.; Nawaz, M.S.; Rusconi, L.; Stefanelli, G.; Strollo, M.; Valente, M.M.; et al.(2014).MeCP2 post-translational modifications: a mechanism to control its involvement in synaptic plasticity and homeostasis? Front. Cell Neurosci. 8, 236.

Bogler O, Wren D, Barnett SC, Land H, Noble M (1990) Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. ProcNatlAcadSci USA 87(16):6368–6372

Bracaglia, G.; Conca, B.; Bergo, A.; Rusconi, L.; Zhou, Z.; Greenberg, M.E.; Landsberger, N.; Soddu, S.; Kilstrup-Nielsen, C.(2009). Methyl-CpG-binding protein 2 is phosphorylated by homeodomain-interacting protein kinase 2 and contributes to apoptosis. EMBO Rep10: 1327–1333.

Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320(5880):1224–1229

Chao HT, Zoghbi HY (2009) The yin and yang of MeCP2 phosphorylation. ProcNatlAcadSci U S A 106(12):4577–4578

Cohen S, Gabel HW, Hemberg M, Hutchinson AN, Sadacca LA, Ebert DH, et al. Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. Neuron 2011, 72: 72–85.

Damen D, Heumann R. MeCP2 phosphorylation in the brain: from transcription to behavior. BiolChem 2013, 394: 1595–1605

Frost E, Zhou Z, Krasnesky K, Armstrong R (2009) Initiation of oligodendrocyte progenitor cell migration by a PDGF-A activated extracellular regulated kinase (ERK) signaling pathway. Neurochem Res 34(1):169–181

G. Perea, A. Araque(2005)Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytesJ. Neurosci., 25: 2192-2203

Gonzales ML, Adams S, Dunaway KW, LaSalle JM. Phosphorylation of distinct sites in MeCP2 modifies cofactor associations and the dynamics of transcriptional regulation. Mol Cell Biol 2012, 32: 2894–2903

Haydon and Carmignoto (2006) Astrocyte control of synaptic transmission and neurovascular couplingPhysiol. Rev., 86 :1009-1031

Keough MB, Yong VW (2013) Remyelination therapy for multiple sclerosis. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 10(1):44–54. doi:10.1007/ s13311-012-0152-7

Liu F, Ni JJ, Huang JJ, Kou ZW, Sun FY. VEGF overexpression enhances the accumulation of phospho-S292 MeCP2 in reactive astrocytes in the adult rat striatum following cerebral ischemia. Brain Res 2015, 1599: 32–43.

Liu F, Ni JJ, Sun FY (2017) Expression of phospho-MeCP2s in the developing rat brain and function of postnatal MeCP2 in cerebellar neural cell development. Neurosci Bull 33(1):1–16. doi:10.1007/ s12264-016-0086-x

Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH 2002: *Common developmental requirement for oligfunction indicates a motor neuron/oligodendrocyte connection*. Cell 109:75– 86.

Lu QR, Yuk D-I, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH 2000: *Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system*. Neuron 25:317–329.

M. Rutlin, S.B. Nelson. (2011)MeCP2: phosphorylated locally, acting globally Neuron, 72: 3-5

Maezawa I, Jin LW. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. J Neurosci 2010, 30: 5346–5356.

Milner R, Anderson HJ, Rippon RF, McKay JS, Franklin RJM, Marchionni MA, Reynolds R, Ffrench-Constant C (1997) Contrasting effects of mitogenic growth factors on oligodendrocyte precursor cell migration. Glia 19(1):85–90

Miyake K, Nagai K. Phosphorylation of methyl-CpG binding protein 2 (MeCP2) regulates the intracellular localization during neuronal cell differentiation. NeurochemInt 2007, 50: 264–270.

Nguyen MV, Felice CA, Du F, Covey MV, Robinson JK, Mandel G, Ballas N (2013) Oligodendrocyte lineage cells contribute unique features to Rett syndrome neuropathology. The Journal of neuroscience : the official journal of the Society for Neuroscience 33(48): 18764–18774. doi:10.1523/JNEUROSCI.2657-13.2013

Novak U, Kaye AH (2000) Extracellular matrix and the brain: components and function. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia 7(4):280–290. doi:10. 1054/jocn.1999.0212

O'Meara RW, Michalski JP, Kothary R (2011) Integrin signaling in oligodendrocytes and its importance in CNS myelination. J Signal Transduct 2011:354091

Orentas DM, Miller RH 1996: *A novel form of migration of glial precursors*. Glia 16:27–39

Saywell, V. et al. Brain magnetic resonance study of Mecp2 deletion effects on anatomy and metabolism. (2006) Biochem. Biophys. Res. Commun. 340:776–783.

Takebayashi H, Yoshida S, Sugimori M, Kosako H, Kominami R, Nakafuku M, Nabeshima Y 2000: *Dynamic expression of basic helix-loop-helix Olig family members: implication of Olig2 in neuron and oligodendrocyte differentiation and identification of a new member, Olig3*. MechDev 99:143–148

Tao J, Hu K, Chang Q, Wu H, Sherman NE, Martinowich K, Klose RJ, Schanen C, JaenischR,WangW, Sun YE (2009) Phosphorylation of MeCP2 at Serine 80 regulates its chromatin association and neurological function. ProcNatlAcadSci U S A 106(12):4882–4887

Vigetti D, Viola M, Karousou E, Deleonibus S, Karamanou K, De Luca G, Passi A (2014) Epigenetics in extracellular matrix remodelling and hyaluronan metabolism. FEBS J 281(22):4980–4992. doi:10. 1111/febs.12938

Vora P, Pillai PP, Zhu W, Mustapha J, Namaka MP, Frost EE (2011) Differential effects of growth factors on oligodendrocyte progenitor migration. Eur J Cell Biol 90(8):649–656

Wang, X., Lou, N., Xu, Q., Tian, G.-F.,Peng, W. G., Han, X., et al. (2006).Astrocytic Ca2+ signaling evoked bysensory stimulation in vivo. Nat.Neurosci. 9, 816–823.

Winship, I. R., Plaa, N., andMurphy, T. H. (2007). Rapidastrocyte calcium signals correlatewith neuronal activity and onset of the hemodynamic response in vivo. J. Neurosci. 27,6268–6272

YangY, Lewis R, Miller RH(2011) Interactions between oligodendrocyte precursors control the onset of CNS myelination. DevBiol 350(1): 127–138. doi:10.1016/j.ydbio.2010.11.028

Yin X, BaekR.C., KirschnerD.A., PetersonA., FujiiY., NaveK.A., MacklinW.B., Trapp B.D. (2006)Evolution of a neuroprotective function of central nervous system myelin J. Cell Biol., 172 : 469-478

Zachariah RM, RastegarM(2012) Linking epigenetics to human disease and Rett syndrome: the emerging novel and challenging concepts in MeCP2 research. Neural plasticity 2012:415825. doi:10.1155/2012/ 415825

Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JA,Weitz CJ, Greenberg ME (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 52(2):255–269. doi:10.1016/j.neuron.2006.09. 037

Journal Publications:

Parikh ZS, Tripathi A, Pillai PP.*Differential regulation of MeCP2 phosphorylation by laminin in oligodendrocytes* J Mol Neurosci. 2017 Aug; 62(3-4):309-317. doi: 10.1007/s12031-017-0939-4. Epub 2017 Jun 14. PMID: 28616777

Tripathi A, **Parikh ZS**, Vora P, Frost EE, Pillai PP. *pERK1/2 Peripheral Recruitment and Filopodia Protrusion Augment Oligodendrocyte Progenitor Cell Migration: Combined Effects of PDGF-A and Fibronectin.* Cell MolNeurobiol. 2017 Mar;37(2):183-194. doi: 10.1007/s10571-016-0359-y. Epub 2016 Mar 18. PMID: 26993510

Parikh ZS, Tripathi A, Pillai P. P.*Stimuli dependent MeCP2 phosphorylation and chromatin association in cortical astrocytes* (**manuscript under preparation**).

Conference Proceedings:

Abstracts published in meetings/conferences

- **International**

1. PrakashPillai, Kedar Sharma and**Zalak Parikh.***MeCP2: A novel epigenetic regulator of cortical oligodendroycte differentiation.* **40th Annual Meeting of Japan Neuroscience Society at MakuhariMesse, Tokyo, Japan** (July 20-23, 2017). **(Invited Talk to P.P. Pillai) (Role: co-author)**

2. PrakashPillai, Kedar Sharma and**Zalak Parikh.***A novel epigenetic regulator of cortical oligodendroycte differentiation: Possible implications for neurodevelopmental disorders.***EMBO Workshop on Cortical Development in Health and Disease, at The David Lopatie Conference Centre, Weizmann Institute of Science, Rehovot, Israel** (April 26-29, 2015). **(Invited Talk to P.P. Pillai) (Role: co-author)**

3. Prakash P. Pillai, AshutoshTripathi, Juhi Singh, **Zalak Parikh** and Kedar Sharma. *Role of PDGFRα – integrin crosstalk in OPC migration*. **12th Meeting of the Asian- Pacific Society for Neurochemistry (APSN)**, Kaohsiung, Taiwan (August 23rd -26th, 2014) **(Invited Talk to P.P. Pillai) (Role: co-author)**

4. AshutoshTripathi, **Zalak S. Parikh** and Prakash P. Pillai. *Role of extracellular matrix and growth factors in oligodendrocyte cytoskeletal reorganization*at**XI European Meeting on Glial Cells in Health and Disease Berlin** July 3 - 6, 2013. (**Role :Co- author) ICMR International Travel Award,** ICMR Ref No.- 3/2/TG-40/HRD-2012, dtd-24.05.2013

5. *Cross-talk between cytokines and neurotrophins in cortical oligodendrocytes, astrocytes and myelinating spinal-cord cultures***11thBiennial meeting of the Asian Pacific Society for Neurochemistry -The 55th Annual meeting of the Japanese Society for neurochemistry** (**APSN/JSN** Sept 30th-Oct 2nd 2012**(Invited Talk to P.P. Pillai) (Role: co-author)**

- **National**

1. **Zalak S. Parikh**, AshutoshTripathi and Prakash P. Pillai*"Extracellular regulation of MeCP2 phosphorylation in glial cells."* at 35th Annual Meeting of The Society for Reproductive Biology and Comparative Endocrinology Department of Animal Biology, University of Hyderabad, Hyderabad -500046, India 9th - 11th February, 2017. (**Role : First author**)

2. **Zalak S. Parikh**, AshutoshTripathi and Prakash P. Pillai*"Phosphorylation Dynamics Of Methyl-Cpg-Binding Protein 2 (Mecp2) In Glial Cells Of Central Nervous System"* at 33rd Annual Conference of Indian Academy of Neuroscience Allahabad (Oct 31st-2nd Nov 2015): (**Role : First author**)

3. **Zalak S. Parikh**, AshutoshTripathi and Prakash P. Pillai*"Expression and distribution pattern of MeCP2 in oligodendrocyte lineage progression"* on ADULT NEUROGENESIS: From Stem Cells to Therapies at Tata Institute of Fundamental Research, Mumbai, India (Feb 6-8th, 2014) (**Role : First author**)

4. AshutoshTripathi, **Zalak S. Parikh** and Prakash P. Pillai. *pERK1/2 Distribution and Actin Cytoskeletal Reorganization in Oligodendrocyte Progenitor Cells: Role of PDGF-A and Fibronectin* on2nd Foundation Day Celebration Dr.Vikram Sarabhai Science Centre, The MSU Baroda, Vadodara, Gujarat, India (30th September, 2014) (Best ORAL presentation award) (**Role:Co- author)**

5. **Zalak S. Parikh**, AshutoshTripathi and Prakash P. Pillai*"Expression and distribution pattern of MeCP2 in glial cells of CNS"* at 31st Annual Conference of Indian Academy of Neuroscience Allahabad (Oct 25th-27th 2013): (**Role : First author**)

6. Prakash P. Pillai, AshutoshTripathi and **ZalakParikh**."*Intracellularsignalling in migration, proliferation, differentiation of oligodendrocyte progenitors during development of the brain"* at 30th Annual Conference of Indian Academy of Neuroscience (Amritsar Oct 27th-30th 2012): (**Invited Talk to P.P. Pillai**) (**Role: co-author)**

Workshops:

- **Attended Science Communication Workshop supported by Wellcome Trust- DBT India Alliance** held on 11th March 2016, MSU Baroda.
- **Attended IBRO-APRC workshop on Emerging Trends in Neuroscience: Molecular and Cellular Approaches,** Varanasi, 19th-23rd Oct, 2013
- **Hands on training on CHIP Assay**, Chennai, 19th Dec-21st, 2012

Achievements:

March 2017	Senior Research Fellowship (SRF): Indian Council of Medical Research (ICMR), New Delhi. project No: 3/1/2/64/Neuro/2015-NCD-I
February 2016	Received International Exchange Fellowship 2016 from Asia-Pacific Regional Committee **(APRC)** of the International Brain Research Organization **(IBRO)** to work in laboratory of **Dr. Kazunori Nakajima, Keio School of Medicine, Tokyo Japan for four months**.

Acknowledgments:

This work was funded partially by various grants - Science and Engineering Research Board **(SERB) (No.-SR/FT/LS-52/2011, 1st May, 2012), New Delhi, India; DBT-RGYI(No.-BT/PR6081/GBD/27/492/2012, 11th Oct, 2013), New Delhi.** We also thank **ICMR(No: 3/1/2/64/Neuro/2015-NCD-I), New Delhi** for the financial assistance in the form of Senior Research Fellowship. We acknowledge Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara for the laboratory facility. We are highly thankful to DBT-MSUB-ILSPARE programme for the confocal microscopy facility at Dr. Vikram Sarabhai Science Block, Faculty of Science, The Maharaja Sayajirao University of Baroda,Vadodara.

Animal ethical statement:

All the mentioned studies were approved by the Institutional Animal Ethics Committee (IAEC), Biochemistry Dept., Faculty of Science, The Maharaja Sayajirao University of Baroda. The protocol numbers are mentioned below:**ZD/13/2014, ZD/28/2014, BC/13/2015, ZD/03/2016, ZD/03/2017.**

Date: **Signature of the candidate**

(Dr. Prakash P Pillai) Guide