Synopsis of the thesis on

Role of PDGF-A activated intracellular signaling in Oligodendrocyte progenitor migration

Submitted to
The Maharaja Sayajirao University of Baroda

For the degree of
Doctor of Philosophy (Ph.D.) in Zoology

By
Juhi Singh

Under supervision of

Dr. Prakash P. Pillai

JULY 2017

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

Role of PDGF-A activated intracellular signalling in Oligodendrocyte progenitor migration

INTRODUCTION

Oligodendrocytes, myelin forming glial cells, are counted among the most vulnerable cells of the central nervous system due to their complex differentiation and unique metabolism or physiology. Oligodendrocyte progenitor cells (OPCs) originate from embryonic telecephalon, ganglionic eminences and sub-ventricular zone (SVZ) of the brain, from where they migrate to colonize the entire CNS(Rakic et al., 2003; Kessaris et al.,2006) Thus, OPC migration is a pre-requisite for myelination in the brain.

Several growth factors, chemokines and chemotropic factors regulate oligodendrocyte progenitor behaviour which includes Platelet derived growth factor A (PDGF-A) (Vora et al. 2011), Fibroblast growth factor 2 (FGF2) (Baron et al. 2000), VEGF, CXCL1, Netrin and Semophorins. PDGF-A has been shown to be critical for the formation of normal myelin during development (Soriano 1997). PDGF-A has also been known to modulate several different OPCbehaviors, including proliferation (Baron et al. 2000, 2002; Frost et al. 2003), migration (Armstrong et al. 1991; Frost et al. 1996, 2009), and survival (Barres et al. 1992; Ebner et al. 2000). Further, PDGF-A induced oligodendrocyte differentiation are mediated by the extracellular signal-regulated kinases 1 and 2 (ERK1/2) which are protein kinases activated when PDGF-A acts on the PDGFRα receptors (Hu et al.2008).

PDGFRα is the marker of Oligodendrocyte precursors which upon PDGF-A ligand binding, autophosphorylates and dimerizes to activate further several signalling cues and protein kinases (Soriano et al., 2003). Some signalling pathways have been reported to guide the process of migration which includes MAPK-ERK, CDK5 etc. CDK5 (Cyclin dependent kinase 5) has been reported to regulate Oligodendrocyte migration by phosphorylation of WAVE2 through non receptor tyrosine kinase Fyn (Miyamato et al., 2008).Mitogen-activated protein kinases (MAPKs) are a family of serine- threonine kinases that contribute to the regulation of various cellular processes, including cell proliferation, differentiation, and apoptosis. PDGF-A regulates OPC migration via the extracellular regulated kinase (ERK) signalling pathway (Frost et al., 2009,Vora et al., 2012). Parallely, scientists have stated that ERK regulates cell migration during wound healing in vitro by modulating the phosphorylation of FAK(focal adhesion kinase) and Paxillin and the consequent formation of focal adhesions (Teranishi et al., 2009).In another study, Hayakawa and colleagues has demonstrated that VEGF-A induced OPC migration by regulating actin reorganisation and binding of FAK with Paxillin (Hayakawa et al.,2011). The FAK (focal adhesion kinase) and Paxillin are major membrane proteins involved in the cell migration(Deakinet

al.,2008;Parsons et al.,2003). Paxillin interacts with ERK and unfolds a series of cell morphological changes. Further, the activation and phosphorylation of ERK lead to its localization to the cell periphery where there is a direct binding of the ERK to the actin filaments(Chuderland et al., 2005;Leinweber et al.,1999). The phosphorylated ERK (pERK) remains associated with focal adhesions in its active form (Fincham et al., 2003).

Also, members of the Rho family of small GTP-binding proteins, including Rho, Rac, and Cdc42, have been shown to participate in cell growth differentiation and motility (Miyamato et al., 2008).Recently, studieshave demonstrated the role of ROCK in the induction of oligodendrocyte differentiation and formation of myelinwhich suggests that it is a potential therapeutic targetfor the development of remyelination-promoting therapy (Pedraza et al.,2014; Kippert et al.,2009).Further, Inhibition of Rock signalling have been shown to cause apical F-actin accumulation and adherens junction disturbance in the neuroepithelium(Esciun et al.,2015).The involvement of the Rho and Rho-associated protein kinase (ROCK) pathway, along with MAPK, has been investigated in Glioblastoma multiforme cell migration and proliferation (Zohrabian et al.,2009).

Rho via ROCK can stimulate myosin light chain (MLC) phosphorylation through inactivation of MLC phosphatase and also probably through direct phosphorylation of MLC(Chen et al.,2002). Moreover, ERK phosphorylates MLCK and causes some increase in MLCK activity (Klemke et al. 1997, Webb etal.,2004). MLCK activity is necessary for myosin light chain (MLC) phosphorylation, which induces myosin contractility, driving integrin clustering and focal adhesion assembly (Burridge et al., 1997). However, as it has been observed that the phosphorylation of myosin light chain kinase (MLCK) occurs downstream the MAPK/ERK activity, several investigations have linked the Rho and ERK signalling pathways. Thus, the possibility of cross talk of ERK-Rock pathway can be elucidated in OPC migration.

Most of the studies on oligodendrocytes till date have focused on the proliferation and differential aspect which is governed by different molecular mechanisms. Migration of OPC is one of limiting factor in demyelinating diseases such as multiple sclerosis and Leukodystrophies. Understanding the regulatory mechanisms of oligodendrocytes progenitor migration is crucial to being able to dissect out the subsequent processes that culminate in myelination. A little has been known about the molecular mechanism which may help the migration of OPCs from the SVZ to white matter regions of the Brain. *This study unravels the pathways and molecular mechanism behind the OPC migration.*

Further, Autocrine and paracrine PDGFR (Platelet derived growth factor receptor) signalling in brain tumors and gliomas are one of the primary causes of its aberrant invasion, growth and migration (Lokker et al., 2002; McLendon et al., 2008). Migration, adhesion and invasion to the other brain tissue contribute

to glioma metastasis which makes it difficult and resistance to chemotherapy and surgeries (Gritsenko et al., 2012; Grobben et al., 2002; Hoelzinger et al., 2007).

In present study, we focused on the role of PDGF-A activated intracellular signalling pathways during the process of migration in Oligodendrocytes precursors cells and Rat C6 glioma. C6 glioma cell line as experimental model system is well- characterised by the presence of a PDGF autocrine loop. Blocking the PDGF receptors in glioma cell lines causes the inhibition of survival or mitogenic pathway widening the chances of its use as a therapeutic strategy in treating glioblastomas (Lokker et al., 2002)

Thus the understanding of the molecular mechanism of PDGF which regulate glioma cell migration is very important in order to develop potential target for treatment that can limit the glioma progression.

Hence we hypothesize that:

PDGF-A induced activation of ERK1/2 lead to activation of downstream molecules involved in cytoskeleton reorganization and thus migration of oligodendrocytes progenitor cells and glioma. Thus, the objectives of the present study were defined as follow:

Objectives:

- **To determine the role of ERK1/2 in the regulation of OPCs migration and cytoskeletal reorganisation.**
- **To determine the crosstalk of ERK1/2-ROCK signalling in OPCs.**
- **To determine the role of ERK and ROCK signalling in PDGFR inhibition mediated intracellular signalling in C6 glioma growth and migration.**

1. To determine the role of ERK1/2 in the regulation of OPCs migration and cytoskeletal reorganisation.

In the present study it was hypothesized that OPC migration is regulated through transient activation of the ERK1/2 signalling pathway. It was confirmed that transient exposure (30 mins) of PDGF-A (10 ng/ml) was sufficient to drive OPC migration up to 72 hrs in agarose drop and scratch migration assays.

Further, In order to confirm that PDGF-A induce the activation of ERK pathway, pERK1/2 protein level was analyzed in the presence or absence of MEK inhibitor (10μM U0126) and PDGF-A (10ng/ml). It was found that PDGF-A significantly increase the levels of pERK1/2 as compared to the control or U0126 with or without PDGF-A. Moreover, to rule out the possibility that transient exposure (30 mins) of PDGF-A does not induce the OPCs proliferation, BrdU staining was carried out in OPCs exposed to PDGF-A transiently (30 mins) or continuously (24 hrs) and found that there was no significant difference observed in BrdU positive cells between control and transiently exposed group. However, continuous exposure of PDGF-A for 24 hrs was found to induce significant proliferation of OPCs compared to control.

Further, to confirm the role of ERK in OPC migration, siRNA mediated knock down of ERK1/2 was carried out in OPCs. ERK1/2 knock down in OPCs was confirmed at protein level by western blotting. Next, migration of ERK1/2 knock down OPCs were analyzed by agarose drop assay in presence or absence of PDGF-A. It was observed that ERK1/2 knockdown significantly reduce the migration of OPCs in presence or absence of PDGF-A.

The present study hypothesized the PDGF-A activated ERK is translocated to the cell periphery where it regulates focal adhesion disassembly prior to OPC migration. ERK influences the FAK-Paxillin interactions by phosphorylation of FAK or Paxillin which leads to regulation of focal adhesion dynamics (Huang et al., 2004; T Igishi et al., 1999). Alteration of FAK and Paxillin dynamics results in decreased migration by reduction in focal adhesion turnover and lamellipodia formation (Deramaudt et al., 2011). ERK modulates disassembly of FAK-Paxillin complex which suggests its role in assembly and disassembly of focal adhesions (Hunger-Glaser et al., 2003). In present study, PDGF-A induce the ERK-Paxillin-FAK interaction confirmed by co-immunoprecipitation and Immunocytochemistry which was consistent with the above studies elucidating the role of ERK-Paxillin-FAK complex in focal adhesion formation and further migration. Our lab (Tripathi et al.,2015) has previously demonstrated that PDGF-A and FN collectively increased the pERK1/2-F-actin interaction and enhanced the filopodia formation prior to OPC migration. In the present study, PDGF-A led to the polymerisation of F-actin filaments atOPC leading edges as compared to control or U0126 treated cells. Further, pERK1/2 expression was seen to be localised with the F-Actin at these leading edges of OPCs in the PDGF-A treated OPCs as compared to the Control and U0126 treated OPCs which supports our previous studies.

2. To determine the crosstalk of ERK1/2-ROCK signalling in OPCs.

To study the role of PDGF-A activated Rock signalling pathway during the OPC migration, OPCs were treated with ROCK inhibitor (Y-27632) with or without stimulation by PDGF-A. Migration was assessed

by agarose drop. A significant decrease in the migration was observed in ROCK treated group in presence or absence of PDGF-A as compared to the control. Further, To determine the crosstalk of ERK and ROCK signalling in OPCs, the levels of pERK were checked in OPCs treated with ROCK inhibitor in presence or absence of PDGF-A. There was no significant change in the pERK levels observed in OPCs treated with ROCK inhibitor in presence or absence of PDGF-A suggests that ROCK mediated regulation of OPCs migration does not involve ERK signalling pathway.

Moreover, effect of inhibition of Rock signalling on OPC proliferation was studied and found that it does not lead to OPC proliferation. BrdU staining was carried out in OPCs exposed Y-27632 in the presence and absence of PDGF-A which states that there was no significant difference in BrdU positive cells between control and Y-27632 exposed group.Next, to understand the effect of ROCK inhibition on the FAK-Paxillininteraction in OPCs, immunocytochemistry was done in presence or absence of Y-27632 with or without PDGF-A. It was found that there was no difference in FAK-Paxillin interactions inY-27632 treated cells compared to control.

To further understand how rock signalling governs the cytoskeletal dynamics of OPCs, pMLC and F-actin staining was done with ROCK inhibitor in presence or absence of PDGF-A. Inhibition of ROCK was characterized by the extension of long, thin processes and multiple branches which suggest the onset of differentiation and inhibition of migration.Similarly,pMLC level was seen to be reduced and abnormally extensive after Y27632 treatment as compared to the control and PDGF-A treated OPCs.

3. To determine the role of ERK and ROCK signalling in PDGFR inhibition mediated intracellular signalling in C6 glioma growth and migration.

To examine PDGFR downstream signaling involved in C6 glioma migration, AG1295, PDGFR inhibitor was used. It was found that PDGFR inhibition reduces the migration of C6 glioma compared to control and PDGF-A treated cells. Further, to assess involvement of PDGFR mediated MAPK-ERK and ROCK signaling in C6 glioma migration, U0126 and Y-27632 were used. There were significant reduction of migration in cells treated with U0126 or Y-27632 was observed compared to control and PDGF-A alone. To confirm that ERK and ROCK signalling are downstream of PDGFR, the levels of pERK and pMLC were determined by western blotting which were found to be decreased in cells treated with PDGFR inhibitor compared to control suggesting that PDGFR inhibition regulates the migration by modulating the downstream ERK and ROCK signalling. To evaluate whether PDGFR, ERK and ROCK signalling regulates proliferation in C6 glioma, BrdU proliferation assay was performed and found a significant

reduction in the percentage of BrdU incorporation in cells treated with AG1295 or U0126 compared to control and PDGF-A treated OPCs.

Next, Tumorigenic potential of C6 glioma after PDGFR inhibition was checked through soft agar assay, adhesion assay and invasion assays and it was found that PDGFR inhibition in C6 glioma impairs the tumorigenic characteristics includes sphere forming ability, anchorage independent growth, invasion and adhesion.

Further, Investigation was done on the impact of PDGFR signalling inhibition on expression of GFAP, FAK, BDNF and MeCP2 which are known to play important role in regulation of C6 glioma pathology. It was found that PDGFR inhibition significantly induced GFAP (1.5-fold), BDNF (3.92-fold) and MeCP2 (2.46-fold) transcript level in C6 cells. Increase in GFAP level was also found to correlate at protein level and marked the differentiation of C6 glioma. Next,the FAK(focal adhesion kinase) protein level which is found to be elevated in many cancers was determined (Gabarra-Niecko et al.,2003).The data showed that PDGFR inhibition reduces the FAK level in C6 glioma, which may lead to inhibition of the migration. FAK has been shown to promote glioblastoma cell proliferation, survival and migration in vitro(Natarajan et al., 2003) Next, In order to study that whether PDGFR inhibition in C6 glioma regulates cytoskeleton reorganization, cells were treated with PDGF-A or AG1295 or Y-27632 followed by immunostaining for actin and pMLC. The stress fibres formation was reduced by PDGFR inhibitor and ROCK inhibitor compared to control and PDGF-A treated group. Also, pMLC was reduced and mostly concentrated around the nucleus in both the PDGFR inhibitor and Rock inhibitor treated as compared to control and PDGF-A treated cells. Next, immunoprecipitation was done to investigate the effect of PDGFR blocking on pERK-FAK-Paxillin interaction in C6 cells. Reduction in pERK-FAK-Paxillin interaction in cells exposed to PDGFR inhibitor, compared to control suggests that ERK influences the FAK-Paxillin interactions which lead to regulation of focal adhesion dynamics.

Further, to understand the role of ERK1 in C6 glioma migration, ERK1 overexpression and knockdown studies are being carried out.

Conclusion:

Overall, the current study support the hypothesis that PDGF-A induced activation of ERK1/2 lead to activation of downstream molecules involved in cytoskeleton reorganization and thus induce the migration of oligodendrocytes progenitor cells. The data shows that PDGF-Ainduced OPC migration was found to be reducedin presence of MEK inhibitor and ERK knock down conditions which confirms involvement of ERK signaling in OPC migration.Moreover, PDGF-A induced OPCs migration involve

ERK dependent regulation of pERK-Paxillin-FAK complex formation and actin reorganization.Further, along with MAPK-ERK signalling pathway, rock signalling to be involved in regulating OPC migration. The present study suggests an important role of PDGFR signalling in glioma progression and inhibition of PDGFR signaling reduced tumorigenic potential of C6 glioma. PDGFR inhibition regulatesthe migration and proliferation of C6 glioma by modulating the ERK and ROCK signaling. Thus, present study suggests that PDGFR signalling is crucial for growth and migration of both the Oligodendrocyte progenitor cells and C6 glioma.

References:

1. Armstrong, R. C. (1998). Isolation and characterization of immature oligodendrocyte lineage cells. *Methods (San Diego, Calif.), 16*, 282–292.
2. Armstrong, R., Friedrich, V. L., Holmes, K. V., & Dubois-Dalcq, M. (1990). In vitro analysis of the oligodendrocyte lineage in mice during demyelination and remyelination. *Journal of Cell Biology*, *111*(3), 1183–1195.
3. Baron, W., Shattil, S. J., &Ffrench-Constant, C. (2002). The oligodendrocyte precursor mitogen PDGF stimulates proliferation by activation of ??v??3 integrins. *EMBO Journal*, *21*(8), 1957–1966.
4. Barres, B. A., Koroshetz, W. J., Swartz, K. J., Chun, L. L. Y., & Corey, D. P. (1990). Ion channel expression by white matter glia: the O-2A glial progenitor cell. *Neuron*, *4*(4), 507–24.
5. Burridge, K., Chrzanowska-Wodnicka, M., &Zhong, C. (1997). Focal adhesion assembly. *Trends in Cell Biology*, *7*(9), 342–347.
6. Chen, B. H., Tzen, J. T. C., Bresnick, A. R., & Chen, H. (2002). Roles of Rho-associated kinase and myosin light chain kinase in morphological and migratory defects of focal adhesion kinase-null cells. *Journal of Biological Chemistry*, *277*(37), 33857–33863.
7. Chuderland, D. and R. Seger, *Protein-protein interactions in the regulation of the extracellular signal-regulated kinase.*MolBiotechnol, 2005. **29**(1): p. 57-74
8. Deakin, N. O., & Turner, C. E. (2008). Paxillin comes of age. *Journal of cell science*, *121*(15), 2435-2444.
9. Deramaudt, T. B., Dujardin, D., Noulet, F., Martin, S., Vauchelles, R., Takeda, K., &Ronde, P. (2014). Altering FAK-paxillin interactions reduces adhesion, migration and invasion processes. *PLoS ONE*, *9*(3).
10. Ebner, S., Dunbar, M., & McKinnon, R. D. (2000). Distinct roles for PI3K in proliferation and survival of oligodendrocyte progenitor cells. *Journal of Neuroscience Research*, *62*(3), 336–345. http://doi.org/10.1002/1097-4547(20001101)62:3<336::AID-JNR3>3.0.CO;2-H
11. Escuin, S., Vernay, B., Savery, D., Gurniak, C. B., Witke, W., Greene, N. D. E., &Copp, A. J. (2015). Rho-kinase-dependent actin turnover and actomyosin disassembly are necessary for mouse spinal neural tube closure. *Journal of Cell Science*, *128*(14), 2468–248.
12. Fincham, V.J., M. James, M.C. Frame, and S.J. Winder, *Active ERK/MAP kinase is targeted to newly forming cell-matrix adhesions by integrin engagement and v-Src.*Embo J, 2000. **19**(12): p. 2911-23.
13. 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. *Neurochemical Research*, *34*(1), 169–181.

14. Gabarra-Niecko V., Schaller M. D., Dunty J. M. (2003). FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev. 22, 359–374 10.1023/A:1023725029589
15. G Gritsenko, P., Ilina, O., &Friedl, P. (2012). Interstitial guidance of cancer invasion. *The Journal of pathology*, *226*(2), 185-199.
16. Grobben, B., De Deyn, P., &Slegers, H. (2002). Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion. *Cell and tissue research*, *310*(3), 257-270.
17. Hayakawa, K., Pham, L., &Som, A. (2011). Vascular endothelial growth factor regulates the migration of oligodendrocyte precursor cells. *The Journal of Neuroscience*, *31*(29), 10666–10670.
18. Hoch, R. V. (2003). Roles of PDGF in animal development. *Development*, *130*(20), 4769–4784. h
19. Hoelzinger, D. B., Demuth, T., &Berens, M. E. (2007). Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. *Journal of the National Cancer Institute*, *99*(21), 1583-1593.
20. Hu, J.-G., Fu, S.-L., Wang, Y.-X., Li, Y., Jiang, X.-Y., Wang, X.-F., …Xu, X.-M. (2008). Platelet-derived growth factor-AA mediates oligodendrocyte lineage differentiation through activation of extracellular signal-regulated kinase signaling pathway. *Neuroscience*, *151*(1), 138–47.
21. Hu, Y., Lu, S., Szeto, K. W., Sun, J., Wang, Y., Lasheras, J. C., &Chien, S. (2014). FAK and paxillin dynamics at focal adhesions in the protrusions of migrating cells. *Scientific Reports*, *4*, 6024.
22. Huang, C., Jacobson, K., & Schaller, M. D. (2004). MAP kinases and cell migration. *Journal of Cell Science*, *117*(Pt 20), 4619–28.
23. Hunger-Glaser, I., Salazar, E. P., Sinnett-Smith, J., &Rozengurt, E. (2003). Bombesin, lysophosphatidic acid, and epidermal growth factor rapidly stimulate focal adhesion kinase phosphorylation at Ser-910: Requirement for ERK activation. *Journal of Biological Chemistry*, *278*(25), 22631–22643.
24. Igishi, T., Fukuhara, S., Patel, V., Katz, B.-Z., Yamada, K. M., & Gutkind, J. S. (1999). Divergent Signaling Pathways Link Focal Adhesion Kinase to Mitogen-activated Protein Kinase Cascades: Evidence for a role of Paxillin in c-jun NH2-terminal kinase activation. *Journal of Biological Chemistry*, *274*(43), 30738–30746.
25. Kessaris, N., M. Fogarty, P. Iannarelli, M. Grist, M. Wegner, and W.D. Richardson, *Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage.* Nat Neurosci, 2006. **9**(2): p. 173-9
26. Klemke, R. L., Cai, S., Giannini, A. L., Gallagher, P. J., Lanerolle, P. De, &Cheresh, D. A. (1997). Regulation of Cell Motility by Mitogen-activated Protein Kinase. *The Journal of Cell Biology*, *137*(2), 481–492.
27. Leinweber, B.D., P.C. Leavis, Z. Grabarek, C.L. Wang, and K.G. Morgan, *Extracellular regulated kinase (ERK) interaction with actin and the calponin homology (CH) domain of actin-binding proteins.*Biochem J, 1999. 344 Pt 1: p. 117-23
28. Lokker, N. a, Sullivan, C. M., Hollenbach, S. J., Israel, M. a, & Giese, N. a. (2002). Platelet-derived growth factor (PDGF) autocrinesignaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. *Cancer Research*, *62*(13), 3729–35. Retrieved from
29. McLendon, R., Friedman, A., Bigner, D., Van Meir, E. G., Brat, D. J., Mastrogianakis, G. M., ...& Yung, W. A. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*, *455*(7216), 1061-1068
30. Miyamoto, Y., Yamauchi, J., & Tanoue, A. (2008). Cdk5 Phosphorylation of WAVE2 Regulates Oligodendrocyte Precursor Cell Migration through Nonreceptor Tyrosine Kinase Fyn. *Journal of Neuroscience*, *28*(33), 8326–8337.

31. Natarajan, M., Hecker, T. P., &Gladson, C. L. (2003). FAK Signaling in Anaplastic Astrocytoma and GlioblastomaTumors. *The Cancer Journal*, *9*(2), 126-133
32. Parsons, J. T. (2003). Focal adhesion kinase: the first ten years. *Journal of cell science*, *116*(8), 1409-1416.
33. Pedraza, C. E., Taylor, C., Pereira, A., Seng, M., Tham, C.-S., Izrael, M., & Webb, M. (2014). Induction of Oligodendrocyte Differentiation and In Vitro Myelination by Inhibition of Rho-Associated Kinase. *ASN Neuro*, *6*(4), 175909141453813. Rakic, S. and N. Zecevic, *Early oligodendrocyte progenitor cells in the human fetal telencephalon.* Glia, 2003. 41(2): p. 117-27.
34. Soriano, P. (1997). The PDGFα receptor is required for neural crest cell development and for normal patterning of the somites. *Development*, *124*(14), 2691–2700.
35. Teranishi, S., Kimura, K., & Nishida, T. (2009). Role of formation of an ERK-FAK-paxillin complex in migration of human corneal epithelial cells during wound closure in vitro. *Investigative Ophthalmology & Visual Science*, *50*(12), 5646–5652. http://doi.org/10.1167/iovs.08-2534
36. Vora, P., Pillai, P. P., Zhu, W., Mustapha, J., Namaka, M. P., & Frost, E. E. (2011). European Journal of Cell Biology Differential effects of growth factors on oligodendrocyte progenitor migration ☆. *European Journal of Cell Biology*, *90*(8), 649–656.
37. Webb, D. J., Donais, K., Whitmore, L. A., Thomas, S. M., Turner, C. E., Parsons, J. T., &Horwitz, A. F. (2004). FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nature Cell Biology*, *6*(2), 154–61.
38. Zohrabian, V., Forzani, B., &Chau, Z. (2009). Rho/ROCK and MAPK signaling pathways are involved in glioblastoma cell migration and proliferation. *Anticancer …*, *124*, 119–123. Retrieved from http://ar.iiarjournals.org/content/29/1/119.short

Workshop/conference/seminar :

- Poster Presentation on "PDGF-A activated signalling pathways and molecular mediators in glioma growth and migration" in 14th Meeting of the Asian-Pacific Society for Neurochemistry APSN 2016 (27th – 30th August 2016) at Kuala Lumpur, Malaysia.

- Poster Presentation on "Role of ERK and Rho associated protein kinase (ROCK) signalling in PDGF-A induced Oligodendrocyte progenitor cells migration and cytoskeleton reorganization" in *"XII European Meeting on Glial Cells in Health and Disease"(13th July-18th July, 2015) at Bilbao, Spain.*

- Poster Presentation on "Role of PDGF-A induced ERK-Paxillin complex in OPC migration" at International conference on *Recent trends in Biomedical Sciences & Annual meeting (2014) of SRBCE at Holy Cross College, Trichy, T.N.*

- Poster Presentation on " The role of extracellular growth kinase (ERK1/2) in oligodendrocyte Migration" *at Advanced school of Axonal Transport and Neurodegenerative Disorders organised at ICTS-TIFR, Colaba, Mumbai(13th Jan-22nd Jan,2013).*

Publications/Awards:

- **Singh, J.,** Sharma, K., Pillai, P. P.(2017) PDGFR inhibition mediated intracellular signalling in C6 glioma growth and migration:Role of ERK and ROCK pathway. (Under review).

- Sharma, K., **Singh, J.,**Pillai, P. P., & Frost, E. E. (2015). Involvement of MeCP2 in Regulation of Myelin-Related Gene Expression in Cultured Rat Oligodendrocytes. *Journal of Molecular Neuroscience*, *57*(2), 176-184.

- Sharma, K., **Singh**, J.,Pillai, P. P(2017) MeCP2 in central nervous system glial cells: Current updates (Under communication)

- DBT-CTEP International Travel Award (2016) to attend Biennial meeting of APSN-2016 at Kuala Lumpur, Malaysia.

Place:Vadodara

Date: 13th July 2017