CHAPTER SIX

SUMMARY

OPC migration is an important prerequisite for the proper myelination of the oligodendrocytes in the central nervous system that is regulated by several intrinsic and extrinsic factors. Short term exposure of PDGF-A to OPCs results in the activation of a self regulating feedback loop of the ERK signaling pathway which is involved in the sustained migration of OPCs. However, the process by which activated ERK regulate the OPC migration is yet unclear. Mechanistic studies have shown that cell migration is regulated through ERK-dependent focal adhesion disassembly. Focal adhesion disassembly is required for the acute decrease in cell adhesion to the substratum prior to membrane reorganization and cell movement. Focal adhesion kinase (FAK) and Paxillin are both adhesion associated proteins that transmit external signals into changes in cell motility. Paxillin works as a platform for protein tyrosine kinase such as FAK and also binds to proteins that reorganize actin cytoskeleton during VEGF- induced cell migration. pERK is transported to the periphery of the cell, where it targets newly formed focal adhesions. pERK remains associated with the focal adhesions in its active form.

The present study demonstrated and further confirmed the role of ERK signaling in PDGF-A induced OPC migration using MEK inhibitor and siRNA mediated suppression of ERK. Further, this study reports for the first time that PDGF-A induced OPC migration, through ERK signaling, forms pERK-FAK-paxillin complex at the cell periphery which signals the OPCs for focal adhesion disassembly. In addition, immunoprecipitation further confirm that PDGF-A induced pERK-FAK-paxillin interaction in OPCs. Further, PDGF-A activation activated ERK (pERK) is recruited to actin filaments, where it binds directly to the actin filaments leading changes and reorganization of actin filaments.

Several signaling pathways have been known to be involved in the process of cell migration. In addition of ERK-MAPK signaling pathway, the present study showed the involvement of Rho/ROCK signaling during OPC migration. PDGF-A activated Rho/ROCK was found to regulate the OPC migration through modulation of actin-myosin reorganization. These findings gives an insight in an alternative signaling pathway governing the cellular migration process of OPC irrespective of the well-established CDK5-WAVE signaling known till date. However, how multiple

signaling pathways govern the OPC migration is still an interesting direction for future studies. In multiple sclerosis, OPCs are unable to migrate to the neurodegenerated lesions of CNS. Therefore, unraveling the mechanisms of how PDGF-A-OPC signaling leads to migration may help us explore new therapeutic avenues for this CNS disease.

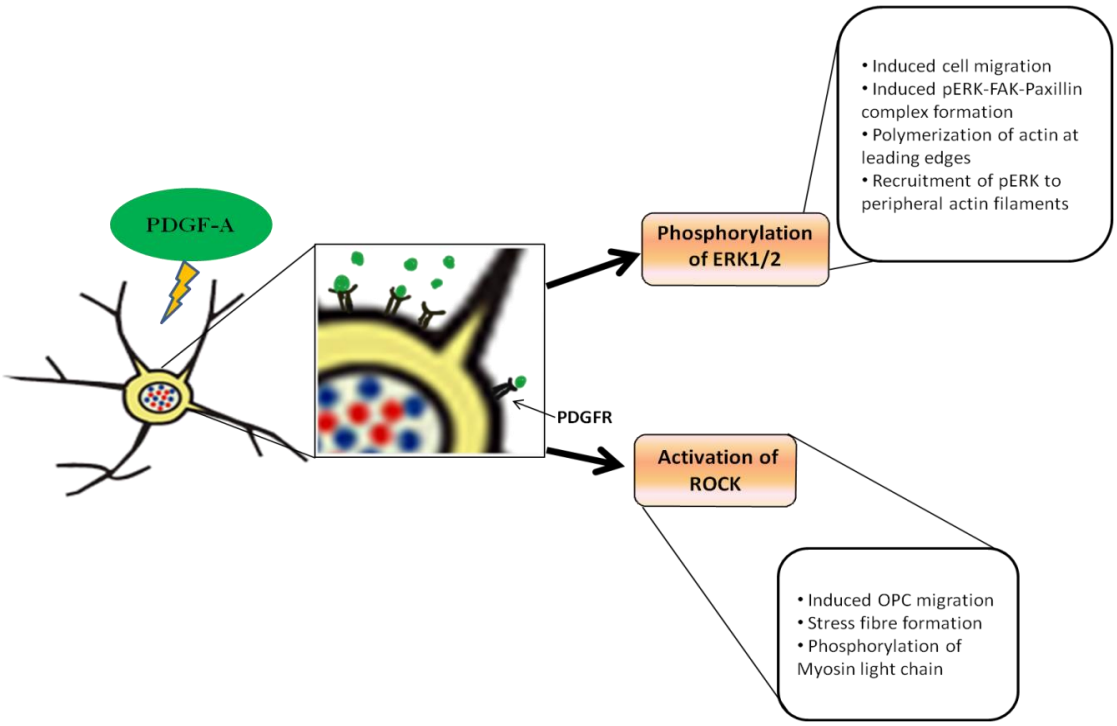

Figure 6.1: Effects of PDGF-A induced ERK signaling pathway in OPCs

Apart from the role in neurodevelopment disorders, defects in PDGFR signaling has been known to be involved to cause specific cancers such as melanoma, glioblastoma, prostate, colorectal, ovarian, breast , acute myeloid leukemia , liver etc. Aberrant PDGFR signaling in gliomas is one of the primary cause of tumor progression. PDGFR autocrine signaling, in particular, affects the survival, proliferation and differentiation of rat C6 glioma. However, the role of PDGFR signaling in glioma migration and progression is still unclear. In present study, using rat C6 glioma as the model system, it is demonstrated that inhibition of site specific PDGFR signalling results in the reduction in migration and tumorigenic properties of glioma such anchorage independent growth, adhesion, invasion etc. Further, PDGFR signalling was found to involve ERK and ROCK signalling for glioma migration and cytoskeletal reorganisation. Similar to OPCs, PDGF signalling in glioma activates ERK and ROCK which further aids in its survival and proliferation. PDGFR

signalling blocking shows reduction in the pERK-FAK-Paxillin complex formation and low levels of acto-myosin, which confirms the novel role PDGFR signalling in glioma migration. In conclusion, the study extended the earlier role of PDGFR inhibitor and demonstrated novel downstream mechanisms of PDGFR signaling inhibition on tumor behavior of C6 glioma which could be targeted for future therapy.

The present study, also, elucidates the specific role of ERK1 in regulating glioma progression and migration. However, more studies are required to confirm and it remains an area for further investigation. Apart from the PDGFR activated ERK and ROCK signalling in glioma, calcium signalling also participates in C6 glioma migration. Inhibition of calcium signalling showed decreased cell migration, cell survival and anchorage independency. Overall, C6 glioma migration was found to be a cumulative effort directed by different signalling pathways simultaneously on stimulation.

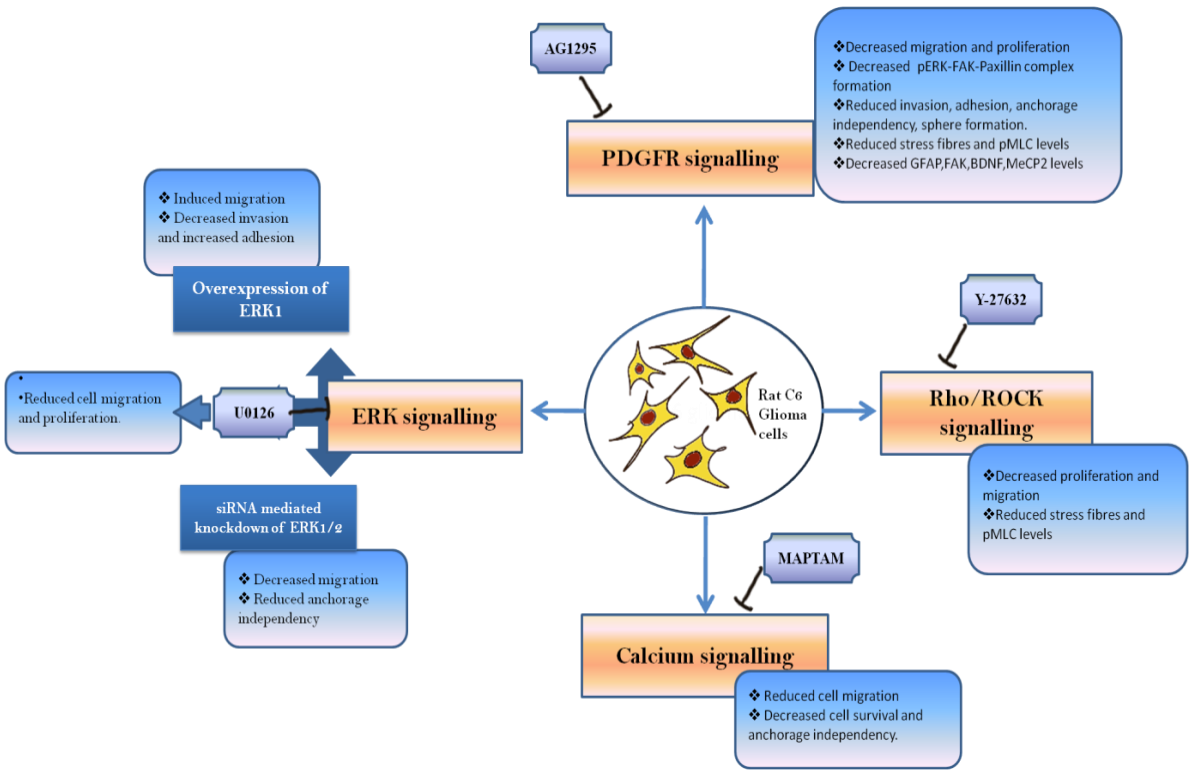

Figure 6.2: Effects of inhibiting signaling pathways in C6 glioma migration