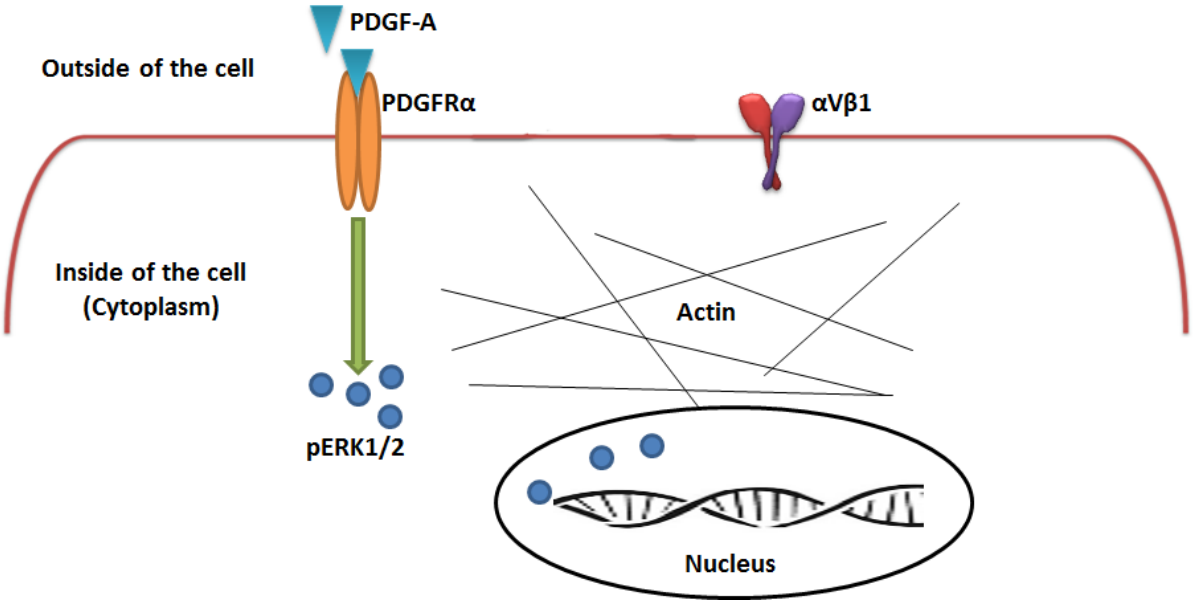
Summary

White matter dysfunction is an important part of many CNS disorders including multiple sclerosis (MS) and vascular dementia. Within injured areas, myelin loss and oligodendrocyte death may trigger endogenous attempts at regeneration. However, remyelination fail due to impaired OPC survival, migration and differentiation during disease progression. *Understanding the regulatory mechanisms of migration of OP is crucial to being able to dissect out the subsequent processes that culminate in myelination.* Studies have shown novel discoveries about the regulatory mechanisms underlying the initiation of OPC migration. However, there are many events regulating the complex process of cell migration, including reorganization of the cytoskeleton, and the formation of focal complexes. These focal complexes are precursors to focal adhesions, which link the cell's actin cytoskeleton to the extracellular matrix (ECM) and/or the axonal membranes. The focus of the present thesis is to better understand regulatory mechanisms involved in the migration/recruitment of OPCs.

A number of different signals are involved in the migration of these cells, although they can be divided fundamentally into two groups: adhesion molecules and secretable molecules. PDGFRα and integrin signalling pathways have well-established roles in OPC migration. However, how these pathways integrate to regulate OPC migration is unknown. In the present study, it was demonstrated that PDGF-A and FN collectively enhanced filopodia formation prior to OPC migration. In OPCs, F-actin was found in filopodia extending from the distal point of the leading edge process. The better understanding of the various actin-associated proteins during the initiation and elongation of filopodia will provide an insight

into the mechanisms of filopodia formation in oligodendroctes. The focal restoration of filopodia bearing OPC numbers would enhance the OPC migration potential in chronic lesions that may be therapeutically beneficial in MS. Finally, the present study also demonstrates a critical role for pERK1/2 in OPC migration and cytoskeleton organization, which was confirmed by using U0126 inhibitor studies. This work has been published (Tripathi et al., 2017).

Fig-6.1(A)



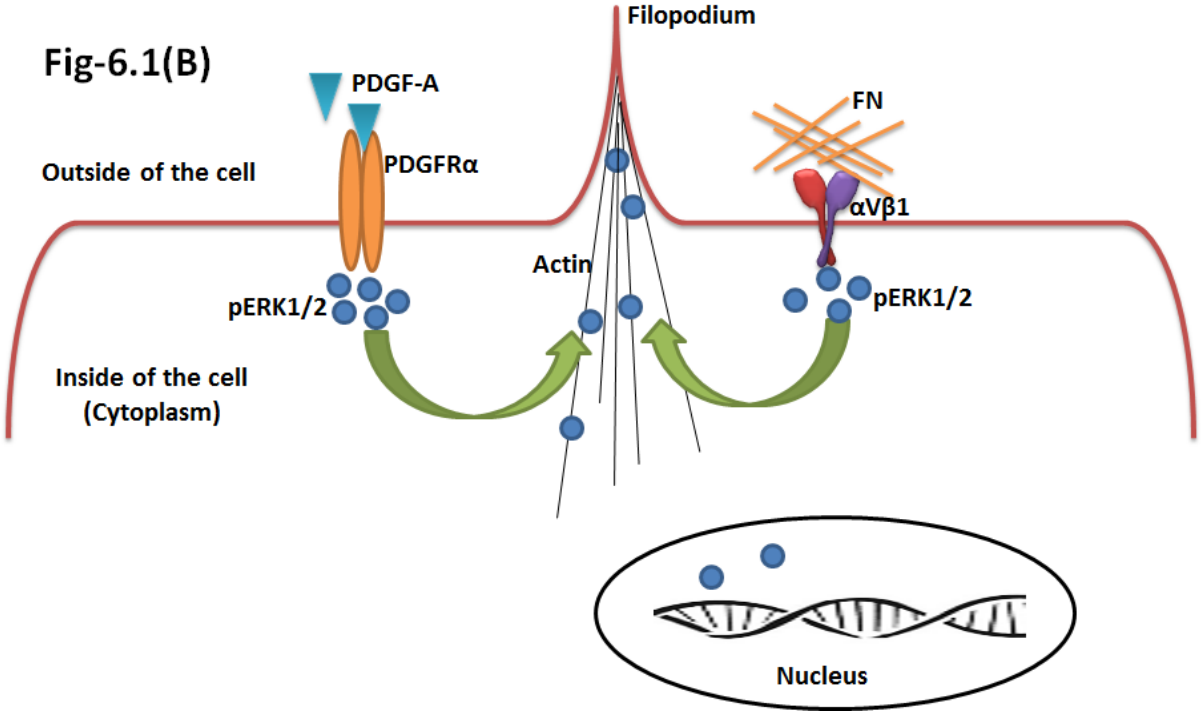


Fig-6.1: Diagrammatic representation of the combined effects of PDGF-A (1ng/ml) and FN.

(A) *Represents the individual effect of PDGF-A (1ng/ml) concentration and* ***(B)*** *Represents the combined effects of PDGF-A (1ng/ml) concentration and FN. In the present study the effects of PDGF-A and FN have been examined to further interpret the distribution and organization of microfilaments in OPCs prior to migration. Combined treatment [FN + PDGF-A(1ng/ml)] resulted in the enhanced microfilaments in the cell process In the present study, it was demonstrated that PDGF-A and FN collectively enhanced filopodia formation prior to OPC migration. Finally, the present study also demonstrates a critical role for pERK1/2 in OPC migration and cytoskeleton organization, which was confirmed by using U0126 inhibitor studies*

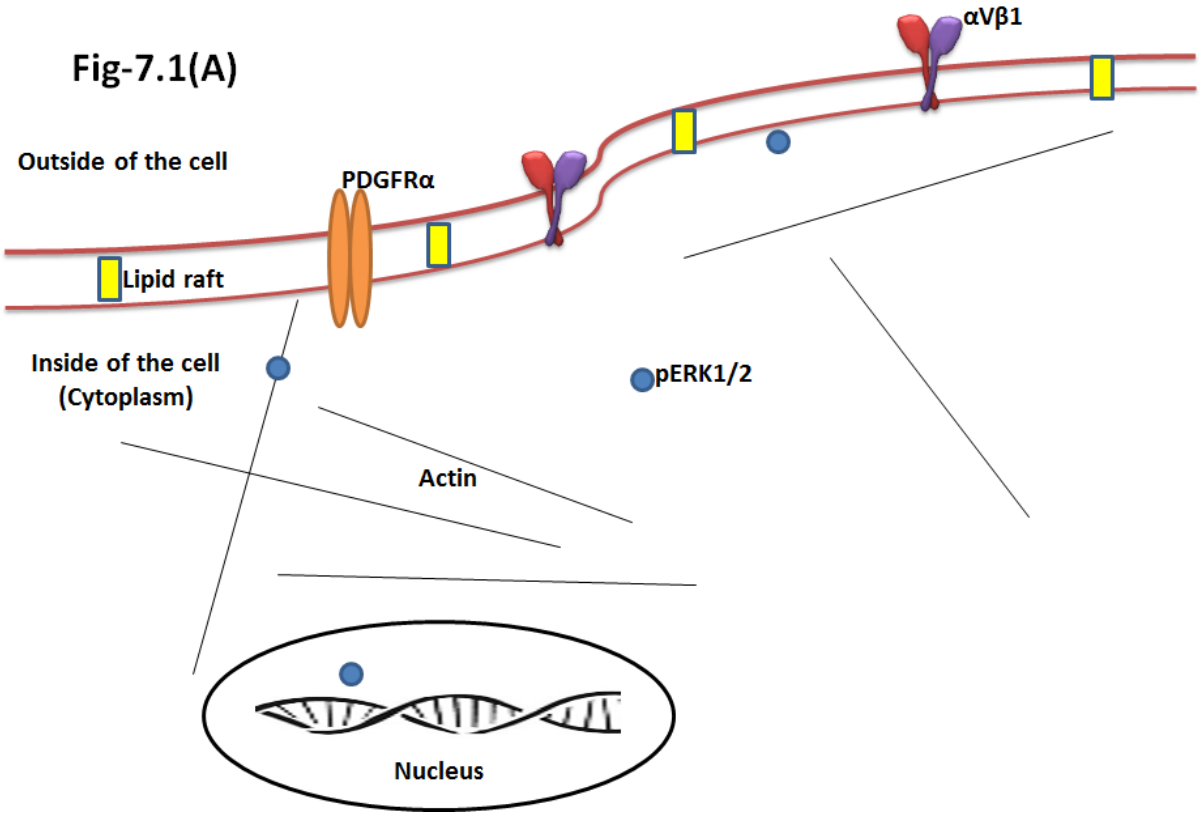
However the higher concentration of PDGF-A (10ng/ml) didn't require FN engagement to enhance the OPC migration. Higher concentration of PDGF-A alone was able to increase the pERK1/2 levels. Moreover, F-actin staining and filopodia formation by the [FN+PDGF-A(10ng/ml)] was not significantly higher than [PLL+PDGF-A(10ng/ml)] group. It is very interesting to see how the higher concentration of PDGF-A (10ng/ml) can modulate the OPC migration machinery without involvement of ECM. For that we have hypothesized that *PDGFRα activation causes integrin switching, lipid raft microenvironment formation and the recruitment of integrins prior to cytoskeletal rearrangement.*

PDGFRα and integrins can exchange or amplify their signaling pathways via both "Outside-in" and "Inside-out" signaling. Studies on OPCs have shown that PDGF-A is not only a mitogen (inducing proliferation) but also a potent motogen (inducing migration)). However, there have been no reports regarding the relationship between PDGFRα activation mediated integrin expressions in OPCs. The present data, along with the others have shown that the higher concentration of PDGF-A (10ng/ml) significantly enhanced the OPC migration without engagement of ECM. So our next question was to understand how higher concentration of PDGF-A modulate integrins to enhance OPC migration? For that we have hypothesized that dose and time dependent PDGFRα activation causes switches in integrin expression. Here dose means concentration of PDGF-A and time represents transient/continuous exposure of PDGF-A. The present study demonstrates the role of PDGFRα activation on integrin expression. With increase in PDGF-A concentration, the integrin expression increases. Not only concentration of PDGF-A but also the time

of exposure, differentially regulated the integrin expression profile in OPCs. Continuous exposure of PDGF-A significantly enhanced the expression of integrin αVβ3 as compared to the transient exposure. This finding fits very well with the knowledge that the αVβ3 integrin is required for PDGF induced OPC proliferation. However, there was no difference in the αVβ1 expression levels, which fits with our findings that there was no significant difference in migration of OPCs treated transiently or continuously with higher dose (10ng/ml) of PDGF-A. Next, the effect of transient and continuous exposure of PDGF-A on PDGFRα internalization was studied. It was observed that PDGFRα internalized significantly increased in the continuous exposure. In context to integrin switching and PDGFRα internalization, the present study supports that OPCs are differentially regulated spatially by PDGF-A in dose and time dependent manner. While this is highly speculative at present, it is an exciting prospect that should stimulate a lot of further work on the dual role of PDGF-A in OPC migration and proliferation. As the data from the chapter-3 shows that PDGF-A (10ng/ml) can significantly increase the activation of ERK1/2 and actin cytoskeleton. Next question was to access the role of pERK1/2 and actin cytoskeleton in PDGFRα activation mediated integrin expression. To address this, ERK1/2 and actin cytoskeleton inhibition studies was done using U0126 and AG1295, respectively. Inhibiting ERk1/2 and actin cytoskeleton significantly decreased the PDGFRα activation mediated expression of αVβ1 and αVβ3 integrins. Our data confirms that PDGFRα activation causes switches in integrin expression, which involves the activation of ERK1/2 and actin cytoskeleton.

Next question we addressed was the role of membrane microenvironment in PDGFRα and integrin interaction during OPC migration? Lipid rafts are specialized microdomains within the plasma membrane which are enriched in specific lipid components such as cholesterol and sphingolipids. These function as signaling platforms in which interactions between receptor proteins, adapter molecules, and effectors are tightly regulated to modulate signal generation.

Data from the present study shows that PDGFRα activation not only increased lipid raft in OPCs but also the lipid raft-actin cytoskeleton interaction. Lipid rafts were found aggregated after PDGF-A treatment. In Chapter-4 PDGF-A (10ng/ml) dependent integrin αvβ1 aggregation was shown. Here the role of lipid rafts was investigate in PDGFRα activation mediated αvβ1 aggregation. Data shows that integrin αvβ1 was found co-localized with lipid rafts after PDGF-A (10ng/ml) treatment. Inhibition of PDGFRα and lipid rafts significantly reduced the αvβ1 aggregation and lipid raft localization. Although extensive research has been done on the role of rafts in signal transduction, many of the studies utilize fairly indirect approaches, such as cholesterol depletion, to implicate rafts in signaling. Truly unequivocal experiments are rare. Thus, while the data are consistent with a role for rafts, no unifying model of exactly how rafts function in signal transduction has yet evolved. Further progress in defining the role of rafts in cell signaling will require the development of new tools to visualize lipid rafts more effectively and to isolate and study distinct populations of these domains.



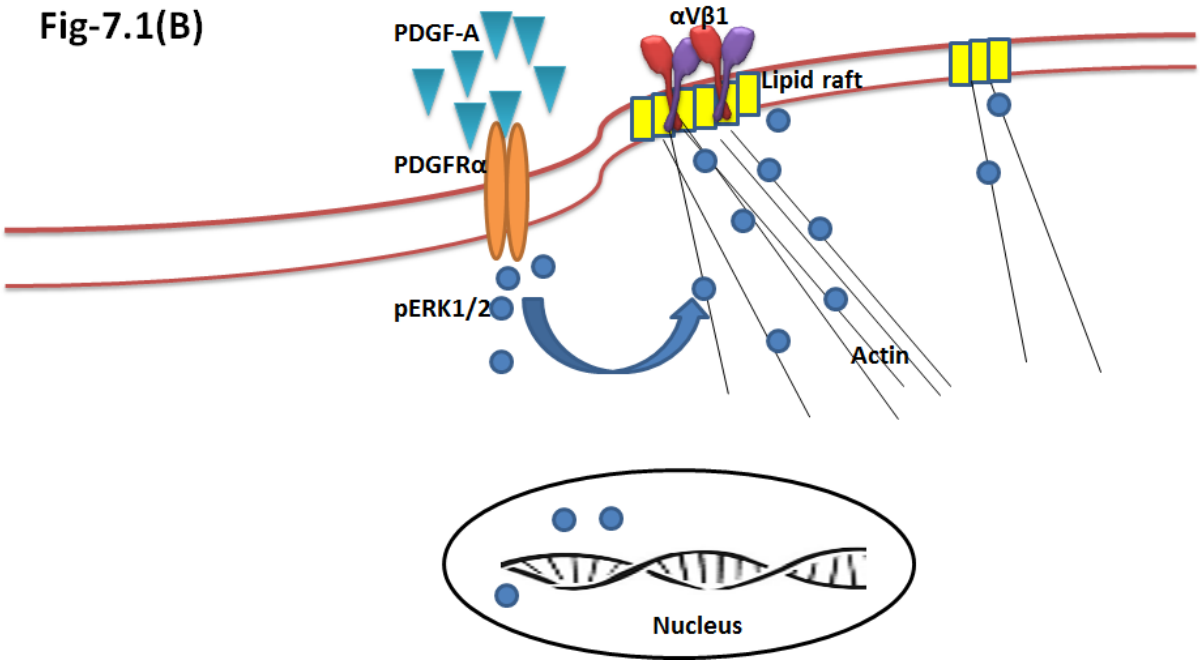


Fig-6.2: Diagrammatic representation of the effects of higher concentration of PDGF-A (10ng/ml).

(A) Represents the arrangement of pERK1/2, actin cytoskeleton, αVβ1 and lipid rafts in OPCs without PDGF-A exposure ***(B)*** *Represents the effects of PDGF-A (10ng/ml) the arrangement of pERK1/2, actin cytoskeleton, αVβ1 and lipid rafts in OPCs. Data shows that integrin αvβ1 was found co-localized with lipid rafts after PDGF-A (10ng/ml) treatment. PDGF-A (10ng/ml) significantly enhanced the pERK1/2 and actin cytoskeleton interaction.*

Till now the study was focused on the cell-ECM interaction. In all the studies OPCs were either attached to the FN or PLL. In that we have studied the molecular mechanisms underlying PDGFRα activation on OPC migration. Next, the role PDGFRα activation was studied in the cell system (C6 glioma) which can survive in the ECM detached condition. Cell–cell adhesion and cell–ECM adhesion is important for tissues and regulates cell growth and differentiation in a strict manner to maintain tissue integrity. Only a few cell types, such as blood cells, do not require cell–ECM adhesion for their survival and growth. Cells that lack adhesion to the ECM usually cease proliferating and undergo cell death, also known as anoikis (Frisch and Screaton; 2001). Invasive and metastatic cancer cells usually acquire anoikis resistance and thereby reduce their dependency on cell–ECM adhesion; these cells can grow in suspension and metastasize from the primary tumor. The detailed mechanisms of anoikis resistance are not fully understood and anoikis resistance targeting drugs are not currently available. The present study was focused on the role of PDGFRα inhibition on the modulation of integrin expression during anoikis resistance in C6 glioma cells. It was observed that in suspended C6 glioma, integrin αVβ1 was significantly increased and integrin αVβ3 was significantly decreased in the PDGFRα inhibited condition. Further, it was observed that PDGFRα and αVβ3 integrin

uncouples during anoikis resistance. They were not interacting when C6 cells were in suspension as compared to attached groups. Moreover, the PDGFRα phosphorylation status of suspended C6 glioma was significantly increased as compared to attached condition. Next, the role of lipid raft was examined during the PDGFRα-αVβ3 interaction in C6 glioma anoikis resistance condition. The data confirmed that lipid raft internalization regulate PDGFRα-αVβ3 interaction in C6 glioma cells. As anoikis resistance is unnecessary for the maintenance of organs by normal cells, this phenomenon is characteristic of malignant cancer cells and therefore constitutes a possible therapeutic target.

Overall the present data shows that protein-protein interactions initiate OPC migration, which is amplified and stabilized by protein-lipid raft interactions. By understanding the above interactions, regulation of the ECM secretion and the controlled release of GFs at the sites of demyelinated lesion may serve as a driving force for the directional OPC migration in the demyelinating diseases. Moreover, data from the C6 gloma study could be useful for developing new therapies against the anoikis resistance to treat the malignant cancers.