Chapter 7
Summary

Neuronal signal propagation or action potential in vertebrates is speeded up by the electrical insulation of axons with an ensheathing, specialized glial plasma membrane: myelin sheath. Wrapping of this myelin sheath around axon called "Myelination" is done by oligodendrocytes in the CNS. Myelination is a very important event for the proper functioning of the CNS and so the development of oligodendrocytes and myelination of axons is a highly regulated process and controlled by several intrinsic and extrinsic factors. The present study focused on neurotrophins (NGF and BDNF) in regulating CNS myelination through oligodendrocytes differentiation from NSCs, OPC migration, proliferation and remyelination in cytokine affected myelination and action of NGF and BDNF mediated by TrkA and TrkB receptors respectively. In addition, Estradiol mediated neurotrophins expression in cortical astrocytes emphasized on the importance of Astrocytic NGF and BDNF in regulation of CNS myelination. Thus overall results suggested that NGF and BDNF can be considered as therapeutic agents for demyelination diseases such as Multiple sclerosis, Alzheimer, Devic's disease.

In the present study, we show the involvement of NGF in the survival and proliferation of NSCs while on the other hand; BDNF regulates only cell viability while exhibiting no effect on the proliferation capacity of NSCs. Both neurotrophins significantly lead to the up-regulation of Notch, a key molecule for neuron-glial switching. Elevated levels of Notch by NGF-BDNF suggested potential role of neurotrophins in driving differentiation towards gliogenesis lineage. Our findings show up-regulation of MBP in both transcript and protein levels dose dependently neurotrophins treated groups thus suggesting essential role of NGF and BDNF in activation of oligodendrocyte specific lineage from NSCs. Pharmacological inhibitors studies revealed that both neurotrophin receptors-TrkA and TrkB resulted in significant down-regulation of MBP which advocate that the action of NGF and BDNF in oligodendrocytes differentiation is through Trk receptors. ERK is one of the main downstream signaling pathways for NGF and BDNF, involved in NSCs differentiation. Phosphorylated ERK (p-ERK) levels were down-regulated in TrkA and TrkB inhibited groups suggesting that ERK act as a major neurotrophin signaling pathway to direct NSCs into oligodendroglial lineage. Morphometric analysis of

neurospheres in the current study observed considerable increase in the size and number of NSCs treated with neurotrophins via both Trk receptors (TrkA and TrkB) thus indicating towards an important role in NSC niche regulation through neurotrophins. NGF and BDNF, along with their receptors, also play a crucial role in the regulation of oligodendrocyte lineage. In the present study, BDNF was observed to regulate OPCs migration and proliferation; both of which were significantly hampered in TrkB inhibition groups; thus confirming that the action of BDNF was specifically through TrkB receptor. Similar observations were recorded in NGF induced OPC proliferation, migration and differentiation via the NGF specific TrkA receptor. Overall, BDNF and NGF were involved in the regulation of oligodendrocyte lineage from migration to proliferation and differentiation via their specific Trk receptors (TrkB and TrkA respectively).

CNS myelin is the multi-layered membrane that surrounds most axons and is produced by oligodendrocytes. Here, in current study, for understanding myelination mechanism and myelin sheath biochemistry we established myelinating culture in which myelinating event start at DIV (day *in-vitro)* 15 and by DIV 23, all axons get myelinated. Establishment of myelinating cultures was confirmed by the Immunocytochemical as well as biochemical presence of Neurofilament (NF) and Myelin basic protein (MBP) expression, markers of neurons and myelinating oligodendrocytes respectively. TNF-α, an inflammatory cytokine, is one of the molecules for disturbing the CNS myelination. In the present study 20ng/mL TNF-α dose was ideal for induction of demyelination event but did not induce cell apoptosis. TNFR1 receptor was involved in the TNF-α induced demyelination in myelinating spinal cord culture. Decrease levels of myelin basic protein confirmed the establishment of *in-vitro* demyelination model. Levels of both, NGF and BDNF were down-regulated in TNF-α induced demyelination, thus establishing a link between TNF-α and these neurotrophins in the regulation of CNS myelination. On the other hand, in experimental groups with combine treatment of neurotrophins and TNF-α, myelin protein expression was maintained which strongly suggests of neurotrophins potential to improve myelination in cytokine induced demyelination. In a nutshell, BDNF and NGF, both neurotrophins were involved in the regulation of myelin basic protein

(MBP) concentration and also for maintain and improving CNS myelinating during inflammation condition. This pivotal role of BDNF and NGF can lead to therapeutic target in demyelination diseases such as Multiple sclerosis.

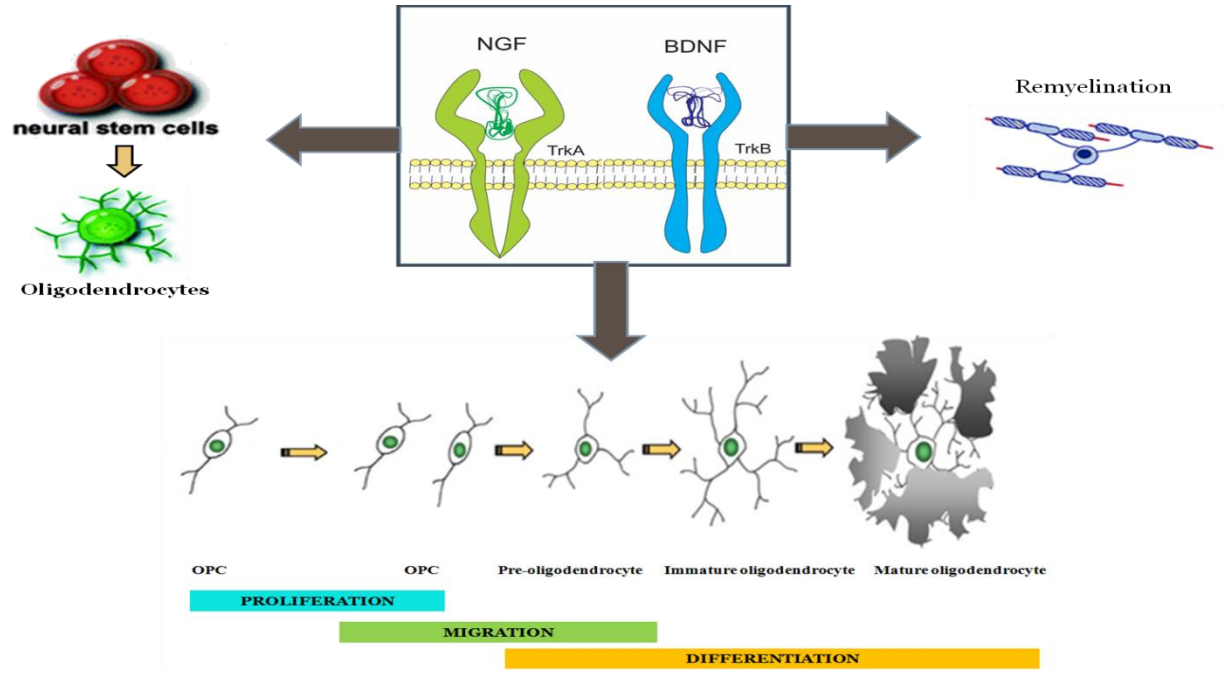

Figure 7.1: Neurotrophins (NGF & BDNF) mediated regulation of CNS myelination

17β-estradiol (E2), a steroid hormone plays a diverse role at cellular and molecular levels in cortical astrocytes cells. Through both the receptors (ERα and ERβ), 17β estradiol contributes to the modulatory levels of neurotrophins (NGF & BDNF). ERβ is majorly involved and responsible for cortical astrocytes cell growth, proliferation and thus play an important role in neuroprotection. E2 induced neurotrophin level in astrocytes was reduced after blocking both estradiol receptors (ERα and ERβ) which confirms the role of estradiol in the modulation of neurotrophin expression. Estradiol induced neurotrophin expression levels following exposures to E2 (6, 12 and 24 hours) and in the case of 24 hrs exposure to E2, neurotrophin expression levels post MEK inhibitor was significantly reduced which indicates ERK might be involved in estradiol signalling pathway through indirect genomic action. Protein analysis shows ERK1/2 act as a secondary molecule for

estradiol mediated neurotrophin modulation, via indirect genomic action and is activated in long term E2 exposure condition for astrocytes survival. Present data strongly indicates ERβ majorly responsible in modulating the level of neurotrophins and thus responsible for astrocytes cell survival compared to ERα.

Overall the data from the present study strongly suggest the involvement of NGF and BDNF in differentiation of NSC-derived oligodendrocytes through specific Trk receptors. Oligodendrocytes lineage (proliferation, migration and differentiation) is also regulated by Trk receptors (TrkA and TrkB) of these two neurotrophins. In addition, during inflammation affected demyelination condition, NGF and BDNF playa very important role to maintain myelin protein levels for regulation of CNS myelination. These findings can be elaborated and extrapolated to look at NGF and BDNF as potential therapeutic targets for several demyelinating disorders such as Multiple Sclerosis.

Unraveling of the complex molecular mechanisms behind the gliogenesis and neurogenesis lineage activation by various signaling pathways like Notch and WNT signaling in Trk knockouts NSCs needs further research and can be a potential future prospect of the current study which will give a detailed understanding of the molecular mechanism by neurotrophins (NGF and BDNF) and their receptors (TrkA and TrkB) during differentiation and maturation of oligodendrocytes and other brain cells.