1. Introduction

1.1 Oligodendrocytes biology and function

Oligodendrocytes (OLG) produce and maintain CNS myelin. There are five basic phases of the OLG lineage: generation, migration, proliferation, differentiation and myelination. Four of these five phases are dependent on the successful migration of the precursor cells away from their site of generation, to their site of proliferation and differentiation prior to successful myelination. OLG originate in the sub-ventricular zone (SVZ) as oligodendrocyte progenitors cells (OPCs)(Miller 1996). OPCs migrate extensively through the developing CNS to populate developing white matter tracts (Levison et al. 1993). (Fig.1.1). Previously, a widely accepted hypothesis was that OPCs migrate along a chemotactic gradient, however, recent studies provide evidence in support of an alternative explanation (Jarjour et al. 2003; Tsai et al. 2003; Spassky et al. 2002). Rather than moving away from the SVZ in response to attractive cues present in a concentration gradient, negative cues repel OPCs away from the SVZ. Several studies show that SVZ expression of Netrin-1 repels OPC away from the SVZ in the spinal cord (Tsai et al. 2003; Jarjour et al. 2003).

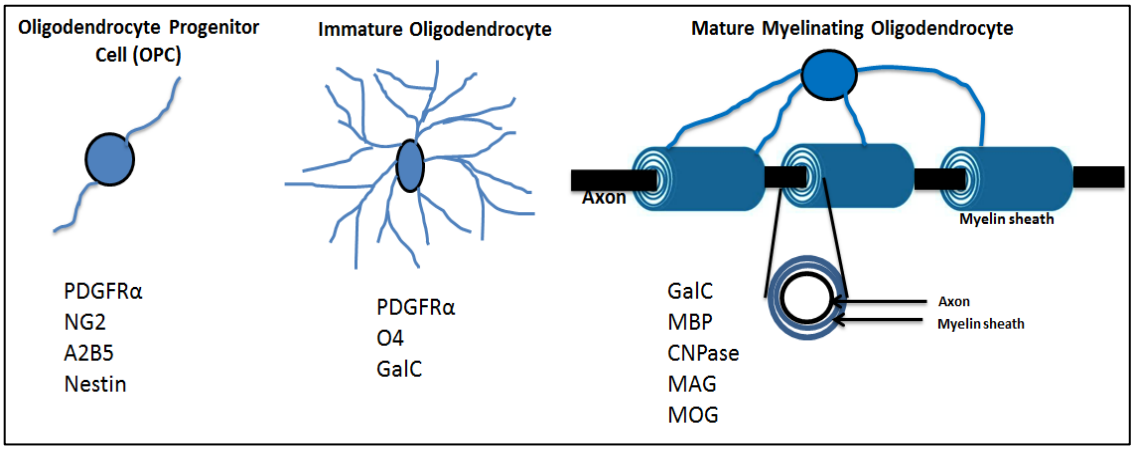

Fig.1.1 Different stages of oligodendrocyte maturation.

Specific markers allow to identify the differentiation status of cells of the oligodendrocyte lineage. Oligodendrocyte progenitor cells with simple morphology (PDGFRα, NG2, A2B5 and Nestin). Immature OLGs with extension of intricate process meshworks (PDGFRα,O4 and GalC). Mature myelinating OLGs involve wrapping of axons in multiple layers of myelin

membrane (GalC, PLP, MBP, MAG, MOG and CNPase) (Adapted from (Jackman et al. 2009; Neman and de Vellis 2012).

1.2 Disorders of central nervous system and oligodendrocytes

The most important characteristic of a healthy nervous system is the efficient transduction of nerve impulses along axons, facilitated by the myelin sheath. Myelination is a critical stage of mammalian development. An intact myelin system facilitates efficient saltatory conductance of nerve impulses through the central nervous system (CNS). The importance of myelination to normal brain function is evident from the pathology of numerous disorders of the brain. Several syndromes, including leukodystrophies, Down's Syndrome, and Perlizeus-Merzbacher Disorder (Vlkolinsky et al. 2001), are known to exhibit abnormal brain myelination. Myelin defects also characterize disorders of later life, such as Schizophrenia(Peirce et al. 2006; Carter 2006), major depression and bipolar disorder (Uranova et al. 2001), and age-related cognitive decline (Hinman and Abraham 2007). The demyelinating disease, Multiple Sclerosis (MS) is probably the most well known disease of the adult white matter(Ludwin 2006). More recently, several disorders of childhood brain development have also been identified as having disruptions in the formation of myelin, including autism (Bonilha et al. 2008; Ben Bashat et al. 2007), acute disseminating encephalomyelitis(Callen et al. 2008a), pediatric MS (Callen et al. 2008b; Chitnis 2006) and cerebral palsy(Dammann and Leviton 1998). These disorders of myelin result in extensive medical care requirements, and cost the Health System every year.

In addition to OPCs migrating during development of the CNS, damage to myelin, initiates the movement of OPCs into the lesioned area to effect repair(Wolswijk 2000). Once the OPCs have reached their final destination, they stop migrating in response to a localised concentration of the chemokine CXCL1(Tsai et al. 2002) , which also induces proliferation of the OPCs (Robinson 1998) .The molecular mechanisms underlying OPC differentiation prior to the myelin membrane extension and axonal wrapping remain unclear. OLGs develop from the proliferative population of OPCs. There are ~5% OPCs present amongst the total CNS cells in the adulthood (Dawson et al. 2003), which is a continual source of new OLGs that produce myelin (Young et al. 2013).

Earlier studies have shown the role of newly generated OLGs in the remyelination (Kang et al. 2010; Tripathi et al. 2010; Zawadzka et al. 2010) with no involvement of preexsiting OLGs (Gensert and Goldman 1997; Keirstead and Blakemore 1997; Fancy et al. 2004; Crawford et al. 2016). However the center of the lesioned area where preexisting OLGs do not survive newly generated OLGs do the remyelination. This suggests that myelin generated solely by preexisting OLGs is unlikely to be sufficient for complete remyelination and that any strategies should be designed to harness the potential of newly generated OLGs (Fig.1.2).

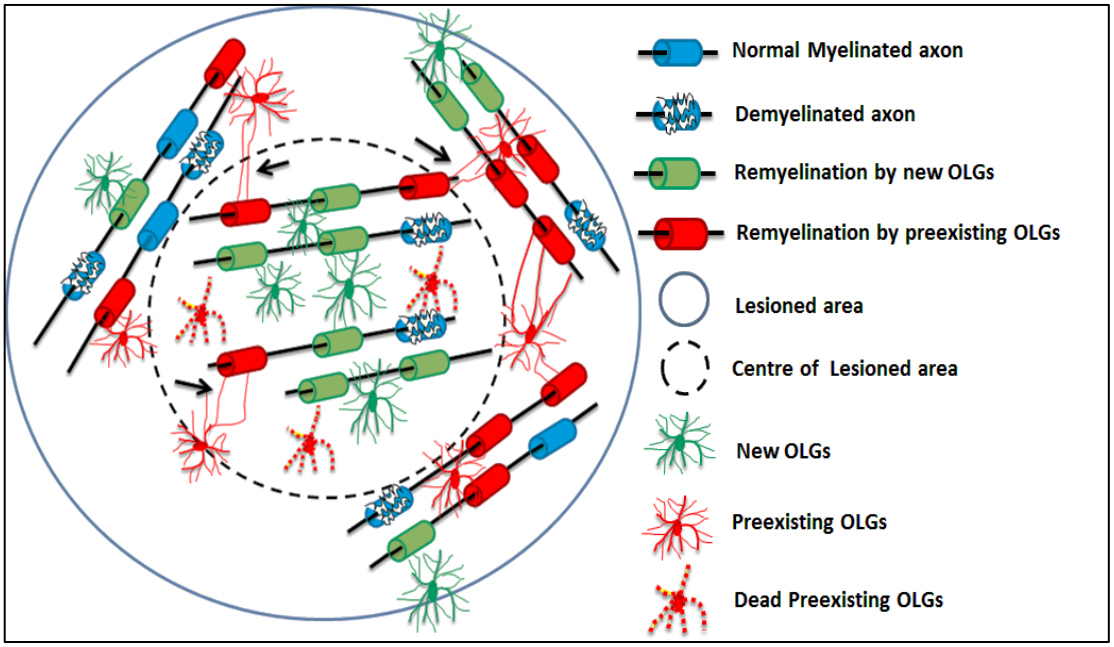

Fig.1.2: Role of newly generated OLGs in remyelination

In the center of the lesioned area where preexisting OLGs do not survive, demonstrating the role of newly generated OLGs in the remyelination. Preexisting OLGs can extend processes into lesioned areas (black arrows) and contribute to myelin repair by wrapping axons. This shows the importance of OPC migration towards the damaged neurons during remyelination.

Understanding the regulatory mechanisms of migration of OPC is crucial to being able to dissect out the subsequent processes that culminate in myelination. There are 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 (Ridley et al. 2003). 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.

1.3 Regulation of oligodendrocyte behaviour

Many different growth factors, such as platelet-derived growth factor A (PDGF) and fibroblast growth factor-2 (FGF2); chemokines, such as Chemokine (CXC-motif) Ligand1 (CXCL1); and neurotrophins, such as neuronal growth factor, and brain derived neurotrophic factor have been shown to play an important role in normal myelination (Tsai et al. 2002; Frost et al. 1996; Frost et al. 2003; Baumann and Pham-Dinh 2001) In addition ECM proteins, such as Laminin (Ln) (Sasaki et al. 2002), Netrin (Nn)(Jarjour et al. 2003; Tsai et al. 2003), and Fibronectin (Fn)(Sheppard et al. 1995) are known to play a critical role in the dispersal of OPCs during brain development. However, despite the abundance of literature, there is no consensus on the regulation of myelination. Rather, much of the literature is contradictory, and consequently, the events leading to normal myelination remain unclear.

1.4 Growth Factor receptor regulation of OPC behaviour

There are a vast number of extracellular cues in the developing brain environment. Many of which regulate cell behaviours via differential expression of receptors on the cell surface. Two growth factors generally considered to play significant roles in developmental myelination are PDGF and FGF2. PDGF is known to play a significant role in the regulation of both the migration and proliferation of OPCs(Frost et al. 1996; Ellison et al. 1996; Calver et al. 1998; Fruttiger et al. 1999; Milner et al. 1997; Armstrong et al. 1991). The significance of PDGF in development of the CNS is clearly demonstrated in the knockout mouse (Calver et al. 1998; Li et al. 1996) . Total ablation of PDGF Receptor alpha (PDGFRα) is embryonic lethal, however, the heterozygous knockouts show significant reduction in myelination with significantly reduced numbers of OLG throughout the spinal cord (Li et al. 1996; Soriano 1997). We have shown that PDGF plays a significant role in regulating the dispersal of OPC throughout the developing brain (Frost et al. 2009). Recently, the role of PDGF-A has been shown in the tissue repair after spinal cord injury (Yao et al. 2017).

FGF2 is known to be functionally redundant for developmental myelination (Murtie et al. 2005), however, studies of knockout mice show that FGF2 plays a significant role for remyelination of the spinal cord (Messersmith et al. 2000). There are few studies involving FGFR knockout transgenic animals, however, there has been no study of OLG lineage cells in these models (Colvin et al. 1996; Ilona et al. 2006) There are, however, several studies investigating the role of the different FGFRs in the regulation of OLG lineage progression(Messersmith et al. 2000) (Fortin et al. 2005; Bansal et al. 2002,2003). OL lineage cells are known to express only 3 of the four receptor subtypes, FGFR1-3. FGFR1 is expressed throughout the lineage, FGFR3 is down-regulated upon OLG differentiation, FGFR2 is reciprocally up-regulated, and FGFR4 is not expressed by OLG lineage cells at all (Bryant et al. 2008). There are 22 members of the FGF family, and each interacts with the different receptors with different affinities, however, FGF2 is the most well studied in the regulation of OLG lineage progression (Frost et al. 2003; Wolswijk and Noble 1992) (Murtie et al. 2005; Miller et al. 2000; Raballo et al. 2000).

1.5 The role of Integrins in regulating OLG behavior

Integrins are the largest family of ECM receptors. They are non-covalently associated, heterodimeric receptors consisting of an α and a β subunit, the combination of which determines their ligand specificity. Each of the 24 different integrins has a specific and non-redundant function in developmen. Integrins play a significant role in cell adhesion to ECM and also in cell-cell adhesion, signaling across the cell membrane in both directions (Hynes 2002). OPC express a limited set of integrins, which change as the cell progresses through its lineage (**Fig.1.3**) (Milner and Ffrench-Constant 1994). Studies from the ffrench-Constant lab have revealed a role for integrins in the regulation of various OLG behaviours (55-61) (Baron et al. 2005; Blaschuk et al. 2000; Buttery and ffrench-Constant 1999; Frost et al. 1999; Milner et al. 1997; Milner et al. 1999). For example, they showed that αvβ1 plays a role in regulating OPC migration (Milner et al. 1996), αvβ3 plays a role in the regulation of OP proliferation (Baron et al. 2002), αvβ5 plays a role in regulating OPC differentiation (Blaschuk et al., 2000; Buttery and ffrench-Constant 1999) and α6β1 plays a role in the regulation of axo-OLG interactions (Frost et al. 1999). Known functions for OPC integrins indicate that switches in integrin expression may result in cell behaviour changes (Baron et al. 2005; Blaschuk et al. 2000). For example, Blaschuk et al.(2000) showed that inhibition of the αvβ5 integrin inhibited OL differentiation, suggesting that OPC require αvβ5 integrin expression in order to differentiate. In that same study, we showed that over expression of the αvβ3 integrin enhanced OPC proliferation (Blaschuk et al. 2000). Our findings were backed-up by a subsequent study demonstrating the role for αvβ3 integrin in regulating PDGF induced OPC proliferation (Baron et al. 2002; 2005). Our previous studies have also shown that interactions with axons cause OLG integrin switches(Milner et al. 1997). Thus we developed the hypothesis that integrin switching causes changes in OLG lineage cell behaviours. Interestingly, Baron et al. (2002) have shown that OPCs require αvβ3 integrin association with PDGFRα in order to proliferate. Downstream pathways activated by the association of PDGFRα with αvβ3 integrin are the PI3K and protein kinase C (PKC) pathways, which are known to regulate OPC proliferation(Ebner et al. 2000; McKinnon et al. 2005). There is evidence that OPC migration occurs in the absence of growth factors *in vitro* (Frost et

al. 1996; Fruttiger et al. 1999), but for this cells must be plated on a permissive substratum(Frost et al. 1996; Kakita and Goldman 1999). Providing further evidence that cross-talk between intracellular signaling pathways regulates OP behaviour.

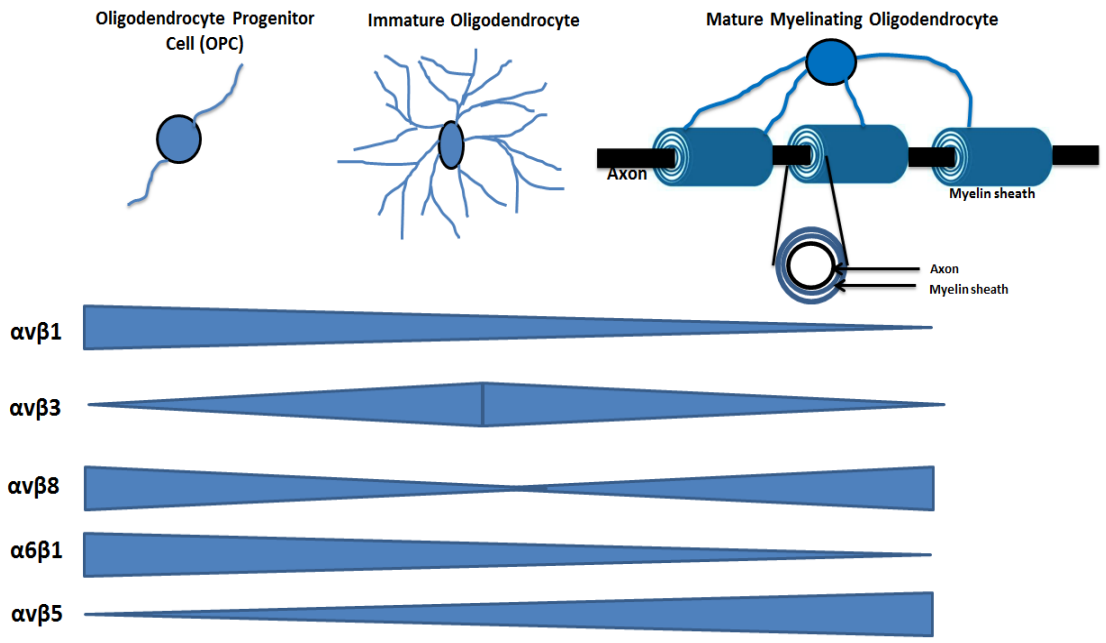

Fig.1.3 Representation of integrin receptor expression over the course of oligodendrocyte development. *Integrin receptors are differentially expressed during oligodendrocyte maturation. αvβ1-integrin and α6β1-integrin are strongly expressed in the oligodendrocyte precursor phase, whereas αvβ5-integrin is strongly expressed in late stages of development. αvβ8 is principally expressed in early and late stages of oligodendrocyte maturation, while αvβ3-integrin is expressed strongly in intermediate stages (Adapted from Milner and Ffrench-Constant 1994; O'meara et al., 2011)*

1.6 Receptor Tyrosine Kinases (RTK) activated intracellular signaling cascades

PDGF and FGF2 are potent mitogens (i.e. inducers of proliferation) for OPC(Frost et al. 1996; Baron et al. 2000; Bogler et al. 1990; Noble et al. 1988), as well as potent motogens (i.e. inducers of migration) (Frost et al. 1996; Armstrong et al. 1990). There are two different PDGF receptors, α and the β (Heldin et al. 1998), and four different FGF receptors(Szebenyi and Fallon 1999). The PDGFRα is known to be critical for

fetal viability(Calver et al. 1998), whereas FGFR knockout mice are viable(Colvin et al. 1996; Ilona Klejbor et al. 2006). FGF and PDGF receptors are RTKs. Activation of RTKs results in activation of several different intracellular signaling pathways, including Phosphoinositide 3-Kinase (PI3K) (Ebner et al. 200), mitogen activated protein kinase (MAPK)(Bhat and Zhang 1996) and phospholipase Cγ (PLCγ) (Heldin et al. 1998). McKinnon et al. (2005) have previously shown that the PI3K cascade regulates OPC proliferation. Previous studies have shown that PI3K is not involved in the regulation of OPC migration (Frost et al. 2009). There is evidence that OPC proliferation and migration are mutually exclusive behaviours. We have shown that inhibition of OPC proliferation with AMPA(Gallo et al. 1996) does not prevent the inhibition of migration by CXCL1(Tsai et al. 2002). The present study is focused on to dissect out the regulation of RTK induced OPC migration.

There have been several previous studies that have shown that RTK/Ligand binding duration is critical for the activation of different downstream signaling pathways (Heldin et al. 1998, 1999, 2001; Stork 2002). Earlier study supports this hypothesis, with transient activation of the PDGFRα resulting in migration but not proliferation of OPC(Frost et al. 2009). Other studies provide further support for the role of MAPK in the regulation of OPC distribution in the developing cortex(Kato et al. 2005). Extracellular regulated kinase (**ERK**) is one of three major groups of MAPK. ERK has various functions, and is involved in the proliferation, differentiation and survival of neurons during development.

ERK1/2 regulation of cell behaviour - Previous study has shown that PDGFRα activation of ERK1/2 signaling regulates OPC migration(Frost et al. 2009) (Frost et al. 2009b) (Frost et al. 2009b) Further, it has been shown that activation of ERK1/2 by transient exposure to 10ng/ml PDGF, is sufficient to sustain OPC migration for up to 72 hours, however, at 1ng/ml, PDGF treatment for up to 30 minutes is not sufficient to induce OPC migration (Frost et al., 2009; Vora *et al.*, 2011). PDGF activation of the cell cycle, requires sustained PDGF signaling(Frost et al. 2009b)(Frost et al. 2009b) (Frost et al. 2009). Interestingly, FGF2 requires sustained exposure to induce OPC migration, even at 10ng/ml. FGF2 induces ERK activation within 5 minutes, and to the same extent at PDGF-A. However, OPC migration is not induced by transient exposure

to FGF2 (Vora *et al.*, 2011). This indicates that PDGF requires interaction with a secondary signaling pathway in order to induce OPC migration. Previous studies have shown that OPCs migration is regulated by β1 integrins (Milner et al. 1996). Integrin-linked kinase play key role in OPC migration (O'Meara et al. 2016)

1.7 Integrin activated intracellular signaling cascades

There are many integrin-associated actin-binding proteins, the majority of which interact with the β integrin subunit. Talin was the first cytoplasmic protein identified as an integrin binding protein. It colocalizes with integrins at the sites of cell-substratum contact (Liu et al. 2000). Talin binds to the β integrin subunit upon interaction with the ECM. Talin forms a weak link to actin, which is reinforced by the recruitment of vinculin, which binds both talin and actin (Liu et al. 2000). Talin accumulation is an early event during the formation of focal adhesion complexes (**FACs**). Focal adhesions (**FAs**) connect the extracellular matrix to the internal cytoskeleton. FACs assemble at the point of cell adhesion, triggering further downstream signaling events leading to cytoskeletal reorganization. Key signaling kinases coupled to the transmembrane and extracellular domains of integrins include ERK. Integrin signaling is transduced through a series of proteins and protein complexes, including Fyn and Paxillin (**Pax**). Fyn, a tyrosine kinase, interacts with Shc and links integrins directly to the Ras/Raf/ERK signaling pathway (Roberts et al. 2003). Pax is a member of the FA protein family. Once Pax has been activated by integrin binding, it interacts directly with ERKs (Chuderland et al. 2005). Pax interaction with ERK is essential for changes in cell morphology. Further, pERK is recruited to actin filaments, where it binds directly (Leinweber et al. 1999). Cell adhesion is necessary for mitogen activation of ERK, although adhesion mediated activation of ERK can also occur in the absence of mitogenic stimulation. pERK is transported to the periphery of the cell, where it targets newly formed FAs. pERK remains associated with the FAs in its active form(Fincham et al. 2000).

ERK 1/2 is one of the common pathways between PDGFRα and integrin activation. Role of ERK1/2 has been confirmed in OPC migration (Frost et al. 2009a). Providing

further evidence that *ERK is an essential regulatory molecule in the complex process of cell migration* (***Fig.1.4***)

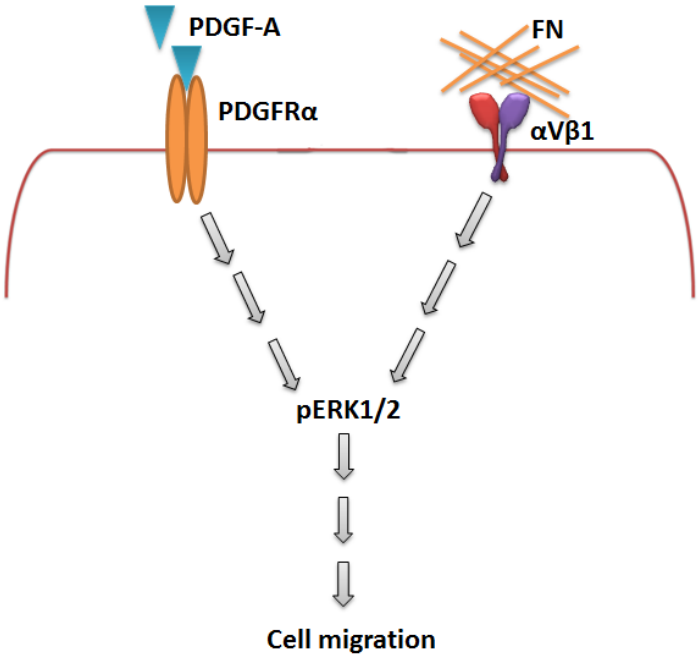

Fig.1.4*- Intracellular signaling pathway network activated by PDGF receptor activation. Receptor ligand binding results in receptor dimerization, which in turn leads to autophosphorylation of the cytoplasmic tail of the receptor. Downstream pathways activated include Ras-Raf-ERK. Integrin activation leads to cytoskeletal reorganization by activation of extracellular regulated kinase (ERK or MAPK). ERK is one of the common activated pathways in PDGF receptor and integrin activation, which leads to cytoskeletal reorganization (Huang et al. 2004; Kim et al. 2010; Frost et al. 2009a).*

1.8 Role of Lipid Rafts in the Stabilization of PDGFRα and Integrins interaction

Structurally, lipid rafts are defined as dynamic membrane microdomains enriched in cholesterol and sphingolipids, in which proteins and lipids are orderly assembled (Kramer et al. 1997; Gielen et al. 2006; Hoetzl et al. 2007; Simons and Gerl 2010). These structures are more organized than the typical bilayer, but are free floating within it. Lipid rafts ensure that signaling cascade constituents are within appropriate molecular distance to facilitate signal transduction by raft-bound receptors (Lingwood

and Simons, 2010). Functionally, small rafts coalesce together into domains ranging from 70 nm to 1 μm in diameter, depending on the stimulus and the cell type (Gupta and DeFranco, 2003), through additional lipid-lipid or lipid-protein specific interactions that also modulate their thickness (Castro et al., 2014; Lingwood and Simons, 2010; Silva et al., 2007). LINGO-1 suppresses OLG differentiation by ErB2 translocation and activation in lipid rafts (Lee et al. 2014)

1.9 Cytoskeleton of oligodendrocytes

The cytoskeleton of oligodendrocytes consists of microfilaments and microtubules but it is devoid of intermediate filaments (Song et al. 2001; Simpson and Armstrong 1999). The actin microfilaments are structured into cytoarchitectural meshworks which then generates mechanical forces which further helps a cell to migrate (Fukui 1993; Gavin 1997; Brandt 1998) and the same is true for oligodendrocytes (Simpson and Armstrong 1999). Recently it has been shown that electric signals regulate directional OPC migration by β1 integrin associated with actin cytoskeleton (Zhu et al. 2016). There are significant changes in OPC morphology during its migration, but mechanism by which they change their shape, extend their processes and regulate their movement is still not clear.

The overall goal of this study was to identify the molecular mechanisms underlying PDGFRα-integrin interaction mediated OPC migration.

1.10 Glioblastoma multiforme

Another cell model system used in the present study was C6 glioma. In that we have studied the PDGFRα and integrin interactions in ECM detached condition. Glioblastoma multiforme (GBM) is the most common brain tumor with very poor prognosis despite recent progress in chemotherapy and immunotherapy. The poor prognosis of malignant gliomas is related to the ability of tumor cells to infiltrate the surrounding tissue and making the tumor unresectable (Sul and Fine 2010; Henriksson et al. 2011; Charles et al. 2011; Tarassishin et al. 2014). It has a very aggressive course and the few therapeutic options available – neurosurgery, radiotherapy and methylating agents like temozolomide, – have failed. The targeted therapy approach

has brought new hope of finding more effective strategies to increase the life expectancy of glioma patients. Molecular pathways involved in the progression of other cancer types have been studied in the hope of finding effective targets but the results have not produced relevant benefits for patients. The rapid proliferation rate and highly infiltrative behaviour of glioma still pose insuperable obstacles to the success of these therapies. Integrins have been studied for their role in tumor progression and metastasis because their expression variesduring the transition from a non-neoplastic to a neoplastic state, suggesting that alterations in the adhesion properties of cancer cells may be involved in the early steps of metastasis formation (Seguin et al. 2015). Driven by biological studies, chemical research has found the field of integrin antagonists particularly attractive and a number offamilies of interesting molecules have seen the light following thesynthesis in 1995 of the prototype Cilengitide (Mas-Moruno et al. 2010), opening newroads to research in the field of GBM and cancer in general. Moreover, it has been shown that inhibition of PDGFRβ increased the efficiency of chemotherapy (Falcon et al. 2011).

1.11 Anoikis resistance in glioma

Loss of ECM contact of adherent cells by disruption of integrin ligation promotes death by anoikis, and this form of cell death can be prevented by integrin mediated adhesion. Anoikis is deregulated in various pathological conditions and anchorage-independent growth is a hallmark of tumorigenic transformation of cells favouring metastasis (Paoli et al. 2013). Integrins, the receptors of ECM are upregulated in gliomas. Integrins αvβ3, α5β1 and αvβ6 are frequently upregulated in several tumor types and associated with inferior outcome. In gliomas, the expression of integrins avb3, avb5 and avb8 increases with the WHO grade of malignancy (Desgrosellier and Cheresh 2010; Taddei et al. 2012; Roth et al. 2013). Cancer cells disseminated from their primary location can invade surrounding tissue and form distant metastases; anoikis prevents the persistence of such cells. Therefore, 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 (Buchheit et al. 2014; Slattum and Rosenblatt 2014; Paoli et al. 2013; Douma et al. 2004). 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. *However, the detailed mechanisms of anoikis resistance are not fully understood and anoikis resistance-targeting drugs are not currently available (Yoshino et al. 2016).*

1.12 Role of PDGFRα in glioma

Growth factors can promote survival downstream of integrin signaling under a stress condition such as anoikis. PDGFR has been found to overexpress in the malignant gliomas. In human glioma, PDGF a-receptor-positive cells are found in all grades, although they occur at higher densities in high-grade tumors. Expression of PDGF-A increases from low grade to high grade tumors, this suggests its role in tumor progression (Fleming et al. 1992; Hermanson et al. 1996; Heldin and Westermark 1999; Cenciarelli et al. 2016).

1.13 Role of lipid rafts in glioma

Lipid rafts/caveolae are membrane platforms for signalling molecules that regulate various cellular functions, including cell survival. Growth factor receptors, T-cell receptors, and the tumor necrosis factor receptor superfamily have been shown to interact with rafts/caveolae, and some intracellular signaling molecules are redistributed to rafts/caveolae after the activation of those receptors(Waugh et al. 2001; Harder 2004; Muppidi and Siegel 2004; Gniadecki 2004; Carpenter 2000). Moreover, lipid rafts are found elevated in the cancers and are sensitive to raft depletion (Li et al. 2006).

Understanding the regulatory mechanisms of anoikis resistance of gliomas is crucial to being able to dissect out the subsequent processes that culminate in invasion/metastasis. Our findings will reveal correlation between integrin switching and the lipid raft modulation in anoikis resistance instructed by the PDGFRα/PDGF-AA regulatory axis.

1.14 Rationale of the study

Previous studies have shown significant roles for integrins in OLG lineage behaviours, as described in Chapter-1. Exposure to growth factors changes the way that the OPC respond to the growth factors (Frost et al., 2009). In addition, OPCs respond to growth factors at different concentrations in different ways (Vora et al., 2011). Earlier studies have shown PDGF-A as a potent motogen (i.e. inducers of migration) and a role for the β1 integrin in regulating OPC migration (Armstrong et al., 1990; Frost et al., 1996; Milner et al., 1996). Further, a recent study shows that PDGF activated Fyn plays a regulatory role in OPC migration (Miyamoto et al., 2008), providing evidence that PDGFRα/integrin interactions play an important role in regulating OPC migration. At physiological concentration of PDGF-A (1ng/ml), PDGFRα interacts with αvβ3 during OPC proliferation in lipid rafts, however, at higher concentration of PDGF-A (10ng/ml), PDGFRα and αvβ3 interaction is not required for OPC proliferation(Baron et al., 2002). Transient exposure of higher concentration of PDGF-A is sufficient to significantly enhance migration of OPCs, which is comparable to continuous exposure (Vora et al., 2011). However, PDGFRα activation mediated integrin expression and switching is not known in regards to OPC migration.

Another cell model system used in the present study is C6 glioma, where, PDGFRα and integrin interactions in ECM detached condition is studied extensively. Glioma is the most common brain tumor with very poor prognosis despite recent progress in chemotherapy and immunotherapy. Expression of the cognate ligand, PDGF A-chain, is dramatically increased from low or undetectable levels in low grade (I and II) to high levels in high grade (III and IV) tumors, suggesting a role in tumor progression (Fleming et al., 1992; Hermanson et al., 1996; Heldin and Westermark, 1999; Cenciarelli et al., 2016). In gliomas, the expression of integrins avb3, avb5 and avb8 increases with the WHO grade of malignancy (Desgrosellier and Cheresh, 2010; Taddei et al., 2012; Roth et al., 2013). 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 (Buchheit et al.,2014; Slattum and Rosenblatt 2014; Paoli et al., 2013; Douma et al., 2004). Moreover, lipid rafts are found elevated in the cancers and are sensitive to raft depletion (Li et al., 2006). However, the detailed mechanisms of glioma anoikis

resistance are not fully understood and moreover anoikis resistance-targeting drugs are not currently available.

1.15 Hypothesis of the study

PDGFRα activation causes integrin switching, lipid raft microenvironment formation and the cytoskeletal rearrangement during OPC migration and glioma anoikis resistance.

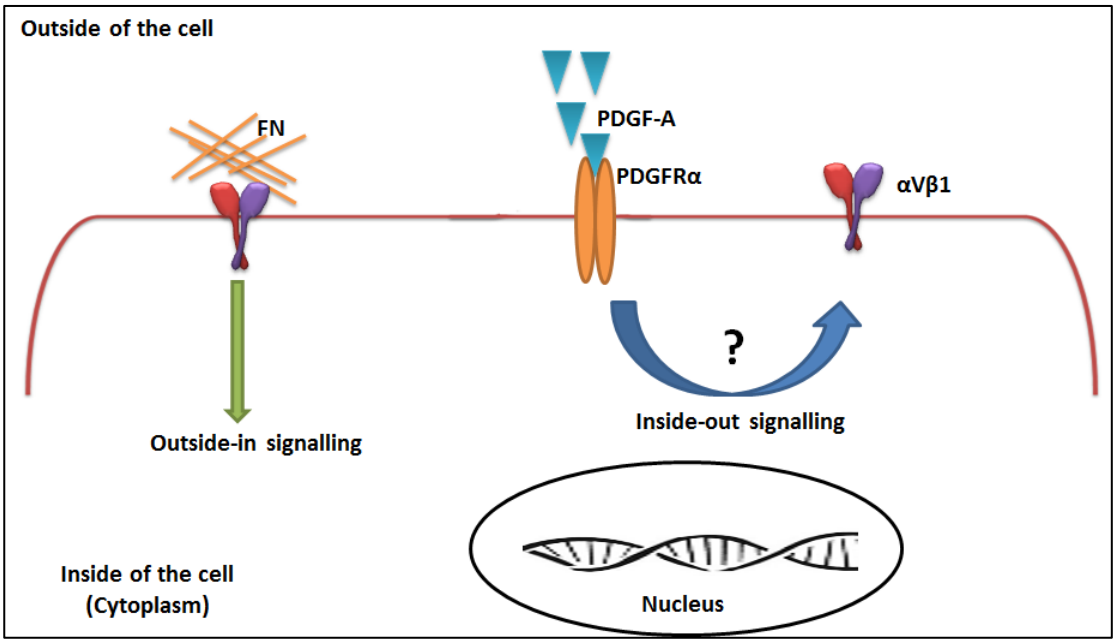

Fig- 2.1 Mechanisms of integrin activation

There are two mechanisms of integrin activation: one is outside-in and another is inside-out. The present study is focused to find out the role of PDGFRα activation mediated integrin modulation by 'inside-out' mechanism.

Based on the above proposed hypothesis, following were the objectives of the present study:

1.16 Objectives of the study

Objective 1-

To study the combinatorial effects of PDGF-A and ECM on OPC migration

Objective 2-

To study the Role of PDGFRα in Integrin switching

Objective 3-

To study PDGFRα and Integrin interaction: role of Lipid rafts

1.17 Significance of current study

Modifying expression level of integrins on oligodendrocyte precursors may increase the migratory capacity of these cells. Therapeutic strategy for transplanting genetically modified oligodendrocyte precursors may be developed to repair widespread lesions in demyelinating disorders like Multiple sclerosis. In addition, the elucidation of the mechanism underlying the unregulated PDGFRα activation and lipid raft mediated regulation of glioma anoikis resistance will provide new insights into the mechanism of invasion/metastasis and also provide new targets for cancer prevention and therapy.