



Role of PDGF-A-Activated ERK Signaling Mediated FAK-Paxillin Interaction in Oligodendrocyte Progenitor Cell Migration

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Received: 5 December 2018 / Accepted: 3 January 2019 / Published online: 16 January 2019
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Abstract

Oligodendrocyte progenitor cells (OPCs) originate from the sub-ventricular zone of the developing brain. They migrate and proliferate to occupy the white matter tracts of the central nervous system and transform into myelinating oligodendrocytes. Along their route of migration, OPCs are guided and controlled by several growth factors and chemokines. PDGF-A (platelet-derived growth factor), a growth factor, serves as a monogenic and mitogenic cue during the process and activates intracellular signaling pathways inside the cell. Activation of extracellular signal regulated kinase (ERK) signaling is one of the mechanisms by which PDGF-A induces the migration of OPCs. However, the mechanisms governing the PDGF-A-induced ERK-driven OPCs migration are still unclear. In the current study, we investigated further the role of PDGF-A-induced ERK signaling in OPC migration. First, we confirmed the role of PDGF-A-activated ERK signaling in OPC migration using the pharmacological inhibitor U0126, or siRNA-mediated suppression of ERK expression. Then, we demonstrated that PDGF-A-induced actin reorganization and interaction of focal adhesion kinase (FAK), Paxillin, and pERK signals are impaired in OPCs treated with the MEK inhibitor U0126. Thus, our findings demonstrated that PDGF-A induces OPC migration in an ERK-dependent mechanism via regulation of actin reorganization and FAK-Paxillin interaction.

Keywords Oligodendrocyte progenitor cells (OPCs) · PDGF-A · MAPK-ERK1/2 · Oligodendrocyte migration · Cytoskeletal reorganization

Introduction

Oligodendrocytes progenitor cells (OPCs) originate from the sub-ventricular zone of the developing brain and migrate to populate the white matter of the central nervous system (CNS) (Miller 1996; Kakita and Goldman 1999). OPCs show bipolar processes and are marked by expression of platelet-derived growth factor receptor alpha (PDGFR α), Nkx2. 2, and NG2 (Baumann and Pham-Dinh 2001; Bradl and Lassmann 2010). OPC migration is guided by many soluble and membrane bound cues (Milner et al. 1997; Kessaris et al. 2006) to ensure that cells reach their final destination. Secreted molecules involved in the regulation of OPC migration are growth factors like platelet-derived growth factor (PDGF), fibroblastic

growth factor (FGF), chemotropic molecules such as netrins and secreted semaphorins, and the chemokines CXCL1 (Fig. 1). PDGF-A, a chemotactic cue generated by astrocytic glial cells in the CNS, serves as a major potent motogen for OPCs (Calver et al. 1998; Frost et al. 2009). Recently, vascular endothelial growth factor (VEGF-A) has been demonstrated to promote OPC migration by regulating the actin reorganization and FAK-Paxillin interaction (Hayakawa et al. 2011).

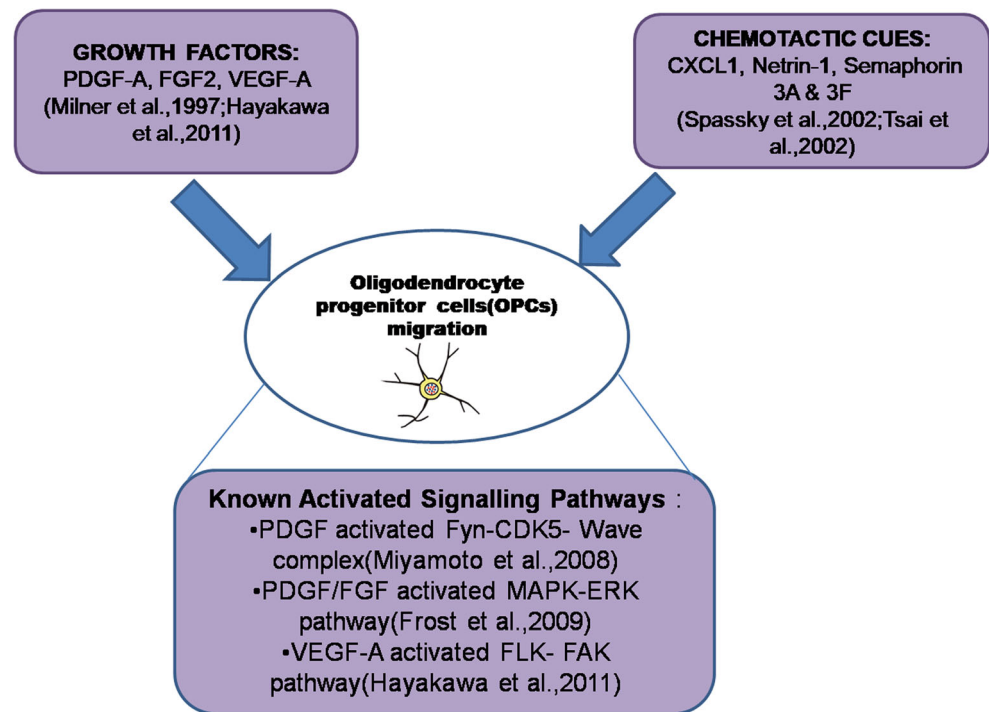
Activation of PDGFR α receptors, by PDGF-A, enhances OPC migration (Armstrong et al. 1990; Frost et al. 2009) and proliferation (Pringle et al. 1989; Fruttiger et al. 1999). PDGFR α is a tyrosine kinase receptor which activates numerous signaling pathways including Phosphoinositide 3-kinase (PI3K) (Ebner et al. 2000), Mitogen-activated protein kinase (MAPK) (Bhatt and Zhang 1996; Vora et al. 2011), and phospholipase C γ (PLC γ) (Heldin et al. 1998). Extracellular regulated kinase (ERK) is one of three major groups of MAPKs which has various functions in cells, and is involved in the proliferation, differentiation, and survival of neurons during development (Cobb 1999; Weber et al. 1997). Recently, PDGF-A in combination with fibronectin has been shown to

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Fig. 1 Growth factors, chemorepellents, and different signaling pathways during OPC migration



augment OPC migration in an ERK-dependent manner (Tripathi et al. 2017). However, the downstream targets of ERK1/2 signalling which cause rearrangement of the cytoskeleton and adaptor proteins leading to OPC migration largely remain unknown.

In the current study, we addressed the role of PDGF-A-activated ERK signaling leading to oligodendrocyte migration and cytoskeletal reorganization. We confirmed previous findings that the transient exposure of PDGF-A enhances the OPC migration without inducing the OPC proliferation. PDGF-A-induced ERK activation in promoting OPC migration was confirmed using pharmacological inhibitor and siRNA-mediated suppression of ERK. Further, PDGF-A regulates the actin reorganization and interaction of FAK-Paxillin in ERK-dependent manner. Thus, the present study demonstrates that PDGF-A-activated ERK can promote the migration by regulating the cytoskeleton arrangement.

Material and Methods

Oligodendrocyte Culture and Isolation

OPCs were isolated from mixed glial cultures generated from the 1–2 day old rat pups(Charles Foster) as described previously (Sharma et al. 2015; Chen et al. 2007). Mixed glial cultures were maintained at 5% CO₂ and 37 °C in DMEM (Gibco, NY, USA) supplemented with 10% FBS (Gibco, NY, USA) and 1% Penicillin and Streptomycin (Himedia, Mumbai, India) on PLL (Poly-L-

Lysine) (Sigma, St Louis, USA)-coated tissue culture flasks for 7–10 days. OPCs were removed by shaking mixed glial flasks overnight in an orbital shaker. The supernatant, containing cells shaken off the monolayer, is placed in a non-tissue culture plastic petri dish to allow the differential adhesion of microglial cells. After the “subtraction” process, the cells obtained are greater than 95% OPCs which were used for further experiments (Milner et al. 1997). Purified OPCs were plated in DMEM/F12 containing B27 supplement (Gibco), and 1% penicillin/streptomycin (Gibco) on PLL (Sigma)-coated dishes or glass coverslips and were serum starved overnight prior any treatment.

Agarose Drop Assay

Migration was assessed by agarose drop migration assay as described previously (Frost et al. 2000). Approximately 35,000–50,000 cells were resuspended in a small drop (1.5 µl) of low melting point agarose and plated onto a 24-well plate. After 24 h, drops were exposed to PDGF-A (Sigma, St Louis, USA) 10 ng/ml (30 min) or 10 µM MEK inhibitor U0126 (2 h) (Santa Cruz Biotech, CA, USA). The migratory properties of cells were evaluated by their ability to migrate out of the agarose drop. Migration away from the edge of the drop was measured in micrometer using NIS Element Software (Nikon, Tokyo, Japan) on four sides of the drop. Each day, the tissue culture plate was oriented on the microscope platform in the same position, and the four points of the compass were used to determine the sides of the drop. Each

experiment had four replicates for the treatment groups and the data was quantified from 3 to 4 independent experiments (*N*).

BrdU Staining Assay

OPCs were treated with 10 μ M BrdU (SRL, Mumbai, India) for 4 h prior to fixation with ice-cold methanol for 10 min at 4 °C. Then in order to denature DNA, cells were treated with 2 M HCL for 60 min at 37 °C. BrdU incorporation was visualized by immunocytochemistry using a mouse anti-BrdU primary antibody (Thermo Fisher Scientific, MA, USA) followed by anti-mouse FITC (Bangalore Genei, Bangalore, India). Also, nuclei were stained with DAPI (4', 6-diamidino-2-phenylindole) (Sigma-Aldrich, St. Louis, USA) (Sharma et al. 2018). Fluorescent staining was observed and photographed using the Flouid cell imaging system (Thermo fisher scientific, MA, USA). All coverslips were photographed around four corners and about 100 cells were counted per field. The plate was always oriented the same way on the microscope platform, and the four compass points were used as the four sides. BrdU-positive cells were calculated as percentage of total cells (labeled by DAPI).

Small Interfering RNA (siRNA) Transfection

OPCs were transfected with siRNA duplexes (predesigned and synthesized by Sigma-Aldrich, St. Louis, USA) specific for rat ERK1 (siRNA ID: SASI_Rn02_00261822), ERK2 (siRNA ID: SASI_Rn01_00107865), or with a universal negative control siRNA (SIC001), using Hiperfect transfection reagent (Qiagen, MA, USA) following the manufacturers protocol. Gene silencing was observed after 24 h of transfection.

Western Blotting

Cells were lysed in Laemmli buffer and stored at -20 °C. Protein concentration was determined by Qubit protein assay kit (Invitrogen, MA, USA) in Qubit 2.0 fluorometer (Invitrogen, MA, USA). Cell lysates with equal protein loads (40 μ g) were resolved by SDS polyacrylamide gel electrophoresis (10%) and transferred to nitrocellulose membrane. Membrane was blocked in 3% BSA in Tris buffered saline (TBS) containing Tween 20 (0.2%), followed by overnight incubation with primary antibody at 4 °C. The following primary antibodies were used: anti-pERK1/2 (R&D Systems, MN, USA), anti-ERK1/2 (Sigma, St. Louis, MO, USA), anti- β actin (Thermo Pierce, MA, USA), anti-FAK (Santa Cruz, TX, USA), and anti-paxillin (Santa Cruz, TX, USA). Bands were visualized using corresponding horseradish peroxidase-conjugated secondary antibodies (Sigma, St. Louis, MO, USA). Specific immunoreactivity was visualized using an ECL kit (Invitrogen, MA, USA). Images of specific

protein bands on X-ray films were digitally scanned and intensity was calculated using Image J software (NIH, MD, USA).

Immunocytochemistry

Cells were seeded on PLL-coated glass coverslips and treated with PDGF-A (10 ng/ml) for 30 min or MEK inhibitor U0126 (10 μ M) for 2 h. Further, cells were fixed with 4% (wt/vol) paraformaldehyde, permeabilized with 0.25% (vol/vol) Triton X-100 in PBS for 10 min followed by blocking in 1% (wt/vol) BSA in PBST for 30 min. Cells were incubated overnight at 4 °C with primary anti-FAK (Santa Cruz, TX, USA) and anti-paxillin (Santa Cruz, TX, USA) antibodies. After washing, cells were stained with corresponding fluorescent conjugated secondary antibody for 1 h at room temperature. For actin staining, cells were incubated with Alexa-Fluor 488 conjugated phalloidin (Life technologies, CA, USA) for 20 min to visualize F-actin cytoskeleton. Fluorescence signals were detected using confocal microscopic imaging system (Carl Zeiss, Germany, Model LSM-710).

Quantitative Image Analysis

To measure fluorescence intensities of FAK and paxillin antibody labelling, confocal images of the cells were acquired after different treatments. A region of interest was drawn around each cell to be measured for the fluorescence intensity, and the same size region was drawn in an area without fluorescent objects to be used for background subtraction. The fluorescence intensities collected from the cell bodies and cell processes were measured by using Image J. Representative data from three independent experiments is presented.

Immunoprecipitation

Cells were grown in 90-mm plates in presence or absence of PDGF-A (10 ng/ml) for 30 min or U0126 (10 μ M) for 2 h. For co-immunoprecipitation, cells were lysed in RIPA buffer (0.1% SDS, 0.5% sodium deoxycholate, 1% NP40, 50 mM Tris-HCL (pH 8.0), 150 mM sodium chloride) supplemented with protease inhibitor cocktail (Sigma, St Louis, USA) and phosphatase inhibitor (Roche, Mannheim, Germany). Immunoprecipitation was carried with Anti-FAK (Santa Cruz) antibody using Dynabeads Protein G immunoprecipitation Kit (Life Technologies, Oslo, Norway) following manufacturer's protocol. Immunoprecipitated protein was resolved by SDS-PAGE followed by western blot analysis using the following antibodies: Anti-Paxillin (Santa Cruz, TX, USA), anti-pERK (R&D Systems, MN, USA), and anti-FAK (Santa Cruz, TX, USA).

Statistical Analysis

The data from more than two groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc analysis of multiple comparisons. Data from two groups were statistically analyzed using two-tailed Student's *t* tests. Further, comparative analysis of the fluorescence intensities of cell body and cell processes were analyzed using two-way ANOVAs and post hoc analysis of multiple comparisons with respect to control. Results are expressed as mean standard error mean (SEM). A value of $P < 0.05$ was considered statistically significant ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$). The statistical software used for the studies was PRISM 6.

Results

Reduction of ERK Expression by U0126 or siRNA Inhibits the PDGF-A-Induced OPC Migration

In order to clarify that PDGF-A induces OPC migration through ERK activation, we first analyzed pERK levels in cells treated with or without PDGF-A (10 ng for 30 min) and U0126 (10 μ M for 2 h) or in combination. The levels of pERK were found significantly reduced in cell treated with U0126 alone or with PDGF-A compared to control and PDGF-A exposed OPCs (Fig. 2).

To examine the effect of PDGF-A-regulated ERK signaling in OPC migration, cells were transiently treated with PDGF-A (10 ng/ml) for 30 min, U0126 (10 μ M) for 2 h alone or in combination with PDGF-A followed by analysis of cell migration by agarose drop assay. It was found that PDGF-A exposure for 30 min significantly enhance the migration of OPCs compared to control and U0126 with or without PDGF-A groups (Fig. 3).

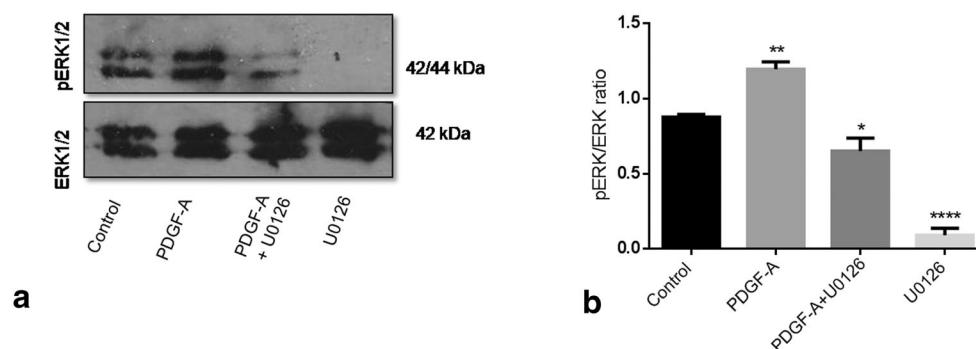


Fig. 2 PDGF-A activates ERK signaling in OPC. **a** Western blot analysis of ERK phosphorylation in OPC cells lane 1: control (No PDGF), lane 2: OP cells harvested immediately after 30-min exposure to 10 ng/ml PDGF-A, lane 3: OP cell harvested immediately 2-h exposure to 10 μ M U0126 and then 30-min exposure to 10 ng/ml PDGF-A, and

lane 4: OP cells harvested after 2-h exposure to U0126. **b** Relative density bands for pERK1/2 protein and total ERK protein obtained. Values were normalized to the control. ($N = 3$) $*P < 0.05$; $**P < 0.01$ compared to control, one-way ANOVA followed by Dunnett test for multiple comparison

To further confirm the role of ERK signaling in OPC migration, we suppressed the ERK1/2 expression in OPCs using specific siRNAs. ERK1/2 knockdown in OPCs was confirmed by western blot and found a significant reduction in the ERK1/2 protein levels in ERK1/2 siRNAs-treated OPCs, compared to negative control (Fig. 4). Next, migration was assessed in ERK1/2 knock down OPCs in presence or absence of PDGF-A. We found significant decrease in ERK1/2 knock down OPCs compared to negative control or PDGF-A-treated groups (Fig. 4). Additionally, no difference was observed in ERK activation between untreated control and the negative control siRNA condition.

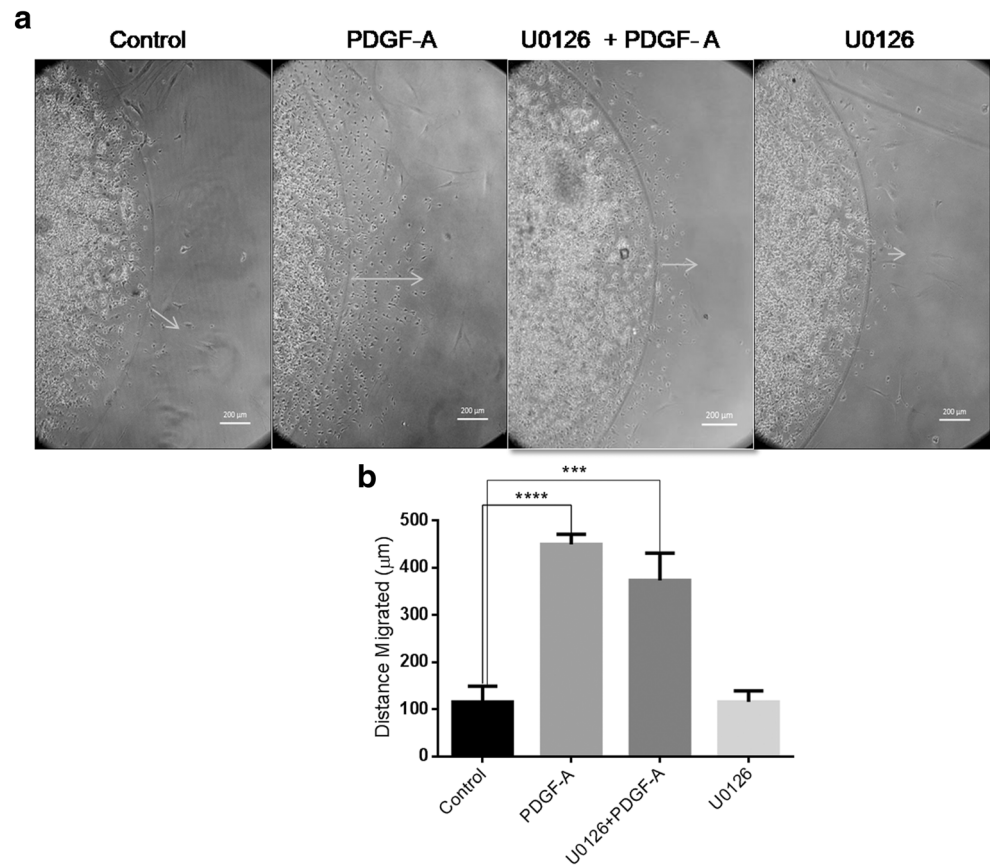
In both the ERK silencing and inhibition experiments, the PDGF-A-mediated migration was not completely blocked as compared to the control. This can be due to additional signaling pathways affecting OPC migration other than ERK1/2. Apart from this, there are positive feedback loops within the ERK cascade that may play a role in the maintained signaling seen after transient activation of the receptor (Frost et al. 2009; Bhalla et al. 2002).

Next, since the PDGF-A is also a mitogen for OPCs, in order to confirm that transient exposure enhance the migration but not proliferation, BrdU assay was performed with OPCs exposed with PDGF-A transiently (30 min) or continuously (24 h). There were significant increase in number of BrdU-positive cells in OPCs treated with PDGF-A for 24 h compared to control and PDGF-A (30 min) (Fig. 5). There was no difference in the number of BrdU-positive cells that were observed between control and PDGF-A (30 min) which suggest that OPCs proliferation requires continuous exposure of PDGF-A (Frost et al. 2009).

PDGF-A Leads to Actin Accumulation

OPC filopodia contain F-actin rich bundles which polymerize and depolymerizes during the process of migration. As shown previously, dynamic changes in actin cytoskeleton is essential

Fig. 3 PDGF-A-activated ERK signaling regulates OPC migration. **a** Migration was analyzed by agarose drop assay in Control, PDGF-A(10 ng/ml) for 30 min,U0126 (10 μ M) for 2 h with PDGF-A (10 ng/ml) for 30 min and U0126 (10 μ M) alone for 2 h ($N = 4$). Micro-pictographs were taken after 72 h after the treatments. Scale bar = 200 μ M (10 \times). **b** Quantitative analysis of distance migrated by OPCs from the corona of agarose drop (μ m). *** $P < 0.001$; **** $P < 0.0001$ compared to control, one-way ANOVA followed by Dunnett test for multiple comparison



for cell migration (Wehrle-Haller and Imhof 2003). Therefore, we next examined the effect of PDGF-A on actin cytoskeletal reorganization in OPCs. Cells were treated with PDGF-A or U0126 followed by immunostaining for actin by Alexa flour-488 conjugated Phalloidin (Fig. 6). We observed that PDGF-A lead to accumulation of actin monomers at the tips of the processes in OPCs. However, treatment of U0126 inhibited the accumulation of actin monomers at the tips of the cell processes. Cells treated with both U0126 and PDGF-A did show few accumulations as compared to the PDGF-A-treated cells.

PDGF-A Induce pERK1/2-Paxillin-FAK Complex Formation in OPCs

OPCs migrate by the process of filopodia formation and protrusion from its distal ends and regular arrangement and disarrangement of focal adhesions (Song et al. 2001; Simpson and Armstrong 1999). FAK and paxillin are among the members of proteins associated with focal adhesions which are essential for cell spreading and migration (Furuta et al. 1995; Schaller 2001). Interaction between these two proteins is critical for the activation of signaling cascades associated with migration (Turner 2000; Subauste et al. 2004). Moreover, studies have shown that HGF stimulates the association of

paxillin and FAK in a MAPK-dependent and inhibition of which leads to reduced cell migration and spreading (Liu et al. 2002; Ishibe et al. 2004).

Therefore, we assessed whether PDGF-A-induced ERK1/2 regulates focal adhesion formation by regulating the FAK-Paxillin interaction. OPCs were treated with PDGF-A or U0126 followed by immunostaining with FAK and paxillin antibodies (Fig. 7). Fluorescence intensities were measured in the cell bodies and cellular processes of the OPCs to analyze the individual FAK and Paxillin protein levels in all treatments groups. In immunocytochemical studies, PDGF-A was found to induce the colocalization of FAK and paxillin and accumulation of these proteins in both the cell bodies and cellular processes of OPCs as compared to control and U0126-treated cells. Also, FAK-paxillin was found to be colocalized at these processes due to presence of a merged yellow fluorescence as compared to U0126 and control. The U0126 + PDGF-A-treated cells did not show the colocalization of the proteins as compared to the PDGF-A alone-treated cells.

Further, we confirmed the FAK-paxillin and pERK interaction in OPCs in response to PDGF-A. Interaction of pERK with focal adhesion proteins FAK and paxillin has been shown to play an important role in cell migration. OPCs were exposed to PDGF-A or U0126 followed by immunoprecipitation with anti-FAK antibody and subjected to western blot

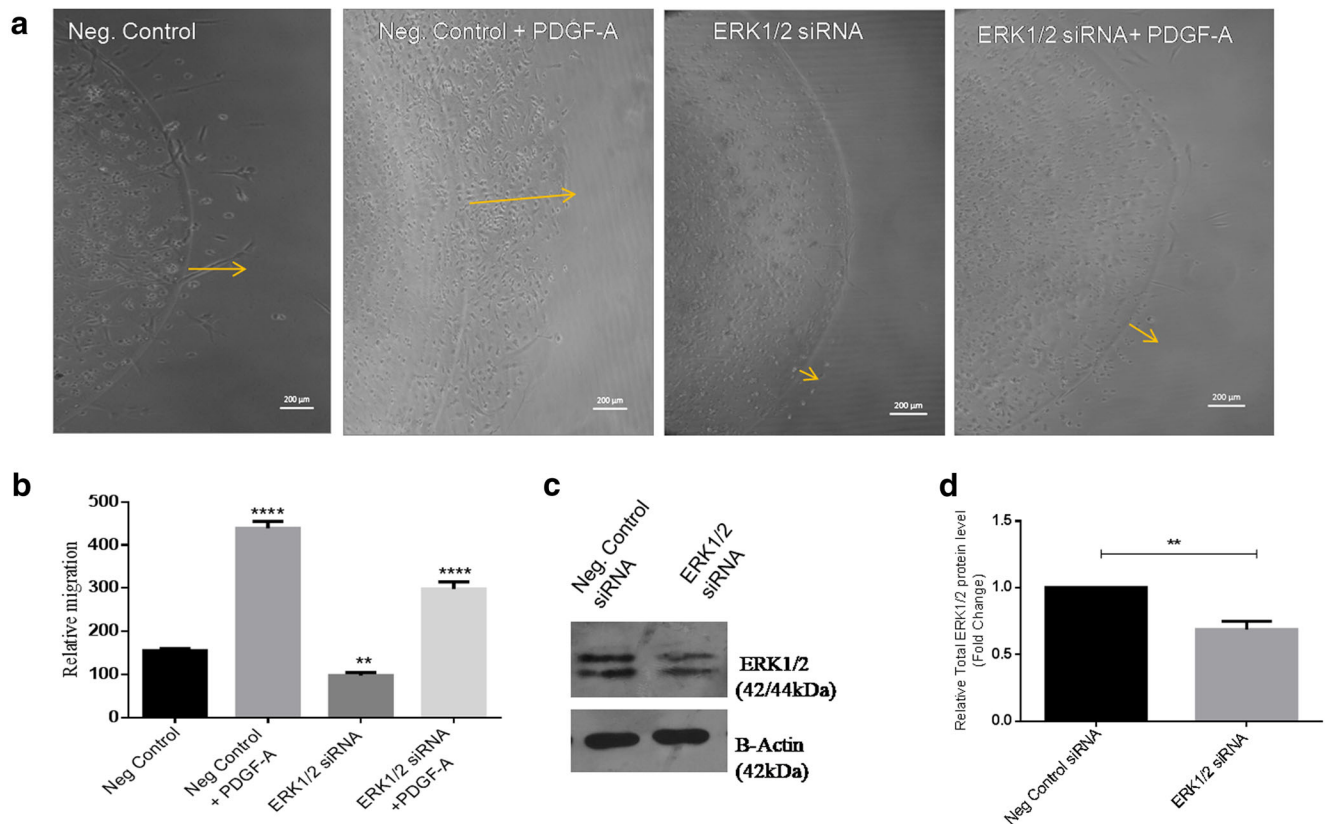


Fig. 4 Knockdown of ERK in cultured oligodendrocyte progenitor cells (OPCs). **a** OPC migration was analyzed by agarose drop assay in negative control siRNA, negative control siRNA with PDGF-A(10gn/ml) for 30 min, ERK1/2 siRNA (10 nmol) and ERK1/2 siRNA with PDGF-A (10 ng/ml) for 30 min. (Scale bar: 200 μ m). **b** Quantitative analysis of

distance migrated by OPCs from the corona of agarose drop (μ m). **c** Representative western blot of negative control and ERK 1/2 siRNA knockdown. **d** Relative change in the ERK1/2 protein expression. Values represent mean \pm SEM from 3 to 4 samples. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ compared to control

analysis of with antibodies for paxillin, pERK, and FAK. The total ERK levels in Fig. 2 were analyzed from this same experiment samples. We demonstrated that PDGF-A augments the pERK-FAK-paxillin interaction in OPCs, compared to control or U0126 (Fig. 8) which suggests that PDGF-A-induced ERK1/2 signaling promotes OPC migration by regulating FAK and paxillin interaction.

Discussion

OPC migration is a prerequisite for the proper myelination in the CNS. OPC originate from the restricted regions of ventricular and sub-ventricular zones in the prenatal brain, and migrate extensively to populate white matter regions of the CNS (Milner et al. 1996; Frost et al. 2009). Failure of OPC migration leads to defective myelin, axonal damage and demyelinating diseases such as Perliazeus Merzbacher disease, leukodystrophies, and schizophrenia (Pringle and Richardson 1993; Krämer-Albers et al. 2006; Matalon et al. 2006; Tkachev et al. 2007) Yet, the molecular mechanism governing oligodendrocyte migration is complex and intricate with multiple growth factors and extracellular signals further

activating several intracellular signaling pathways. OPCs are guided and controlled by different growth factors, chemotropic factors and chemokines including FGF2, PDGF-A, VEGF, CXCL1, Netrins, and Semaphorins (Baumann and Pham-Dinh 2001; Hayakawa et al. 2011; Vora et al. 2012; Spassky et al. 2002) (Fig. 8).

Further, a study by Miyamoto and colleagues demonstrates that Cdk5 regulates PDGF-dependent OPC migration through the direct phosphorylation of WASP (Wiskott–Aldrich syndrome protein)-family verprolin homologous protein 2 (WAVE2) at Ser-137. Another study by Frost and colleagues has demonstrated that PDGF-A signaling regulates the OPC migration through the ERK signaling pathway in a dose- and time-dependent manner (Frost et al. 2009; Vora et al. 2011). The effect of PDGF-A on long distance migration of OPCs has been widely discussed and understood (Armstrong et al. 1990; Ellison et al. 1996). Further, short-term exposure (30 min) to PDGF-A (10 ng/ml) has been described to be sufficient enough to transiently induce OPC migration for up to 72 h. But, long-term or continuous exposure of PDGF-A (10 ng/ml) for 24 h led to increased OPC proliferation as compared to the control (Fruttiger et al. 1999; Vora et al. 2011). In the present study, pharmacological

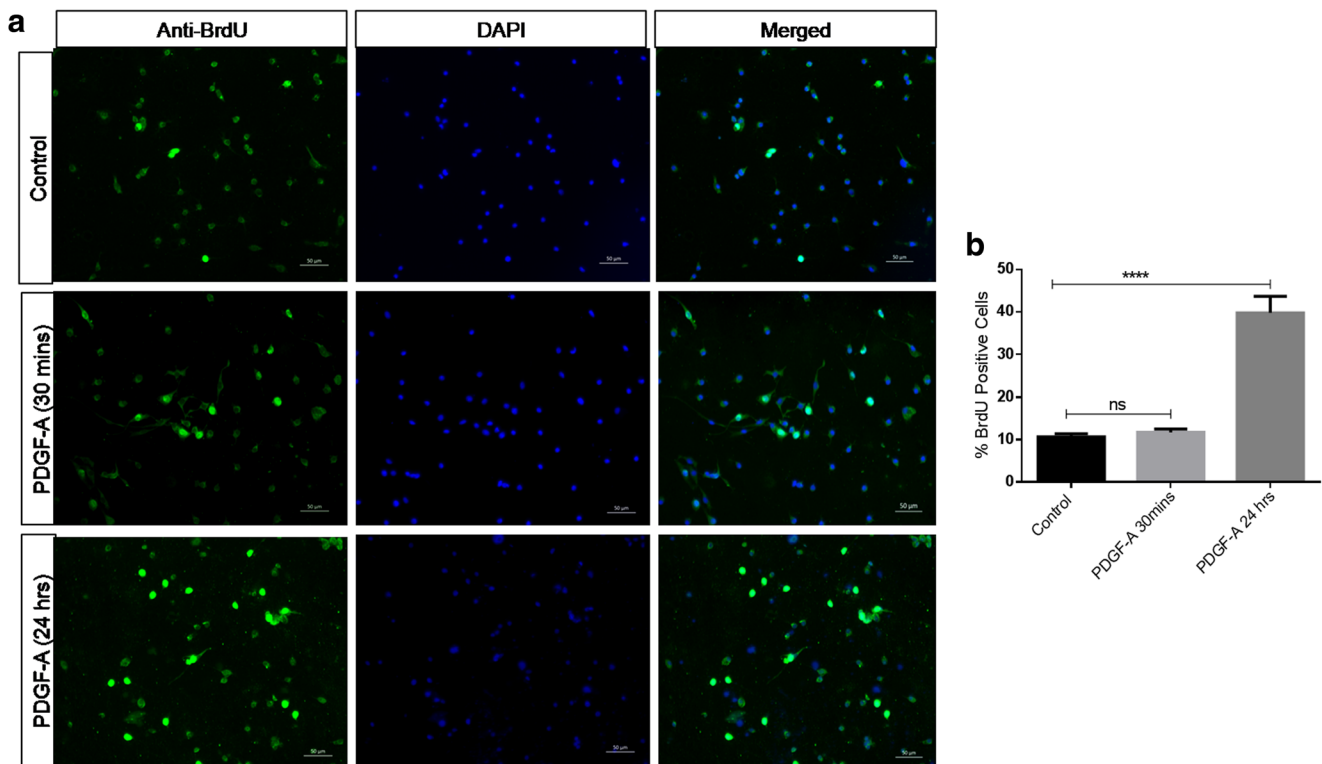


Fig. 5 Continuous exposure PDGF-A for 24 h leads oligodendrocyte progenitor cells proliferation. **a** Representative fluorescence images of BrdU-positive cells (Green-BrdU) in oligodendrocytes progenitors cells pre-treated for 30 min and 24 h with PDGF-A (10 ng/ml) respectively (Blue-DAPI) (scale bar = 100 μm). **b** The statistical data showed percentage BrdU-positive cells calculated from panel A. Every

coverslips was photographed around four corners and about 100 cells were counted per field. Values represent mean ± SEM from the independent 3–4 experiments * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ compared to control, one-way ANOVA followed by Dunnett test for multiple comparison

inhibition and siRNA-mediated suppression of ERK1/2 signaling confirmed that transient exposure of PDGF-A induces the migration of OPCs via ERK signaling without promoting proliferation. This concurs with our previous lab reports and forms the basis of our further investigation on ERK1/2 downstream targets and cytoskeletal changes.

OPC migration involves the formation of filopodia, growth cone-like structures, at the leading edges process and substrate detachment at the trailing process (Song et al. 2001; Schmidt et al. 1997) Filopodia are tightly bound actin bundles which

later polymerize and widen to form lamellipodia with the help of microfilament branching (Bauer et al. 2009; Mattila and Lappalainen 2008). Previously, our lab has demonstrated that combined treatment of PDGF-A and fibronectin increased ERK1/2-F-actin interaction and enhanced filopodia formation prior to OPC migration (Tripathi et al. 2017). In the present study, we found that PDGF-A activation alone led to the accumulation of F-actin monomers and polymerization in the tips of the OPC processes as compared to control or U0126-treated cells. Filopodia play a significant role in cell migration

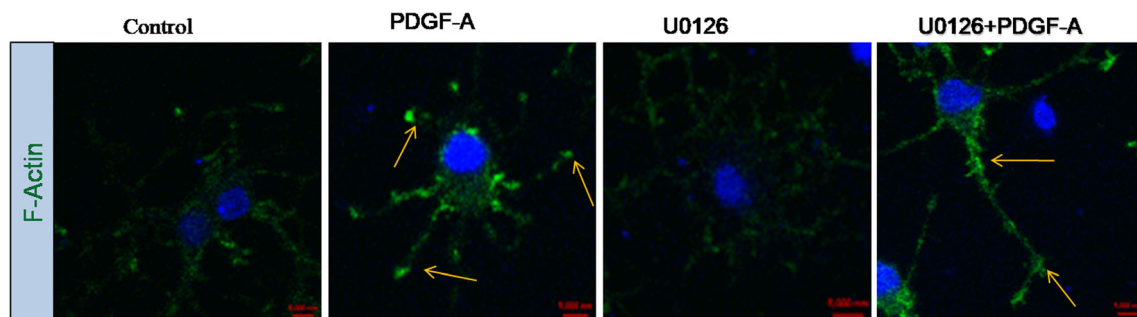


Fig. 6 PDGF-A leads to accumulation of F-actin monomers. Cells were fixed and stained with phalloidin Alexa Flour- 488 (green) to visualize F-actin. And DAPI (blue) to stain nuclei. Compared with control OPC group, PDGF-A-treated cells showed accumulation of F-actin fibers at

the tips of cell processes (scale bar, 1000 nm). Yellow arrows indicate the presence of accumulated actin monomers in the cell processes. The experiment was performed three times with similar results

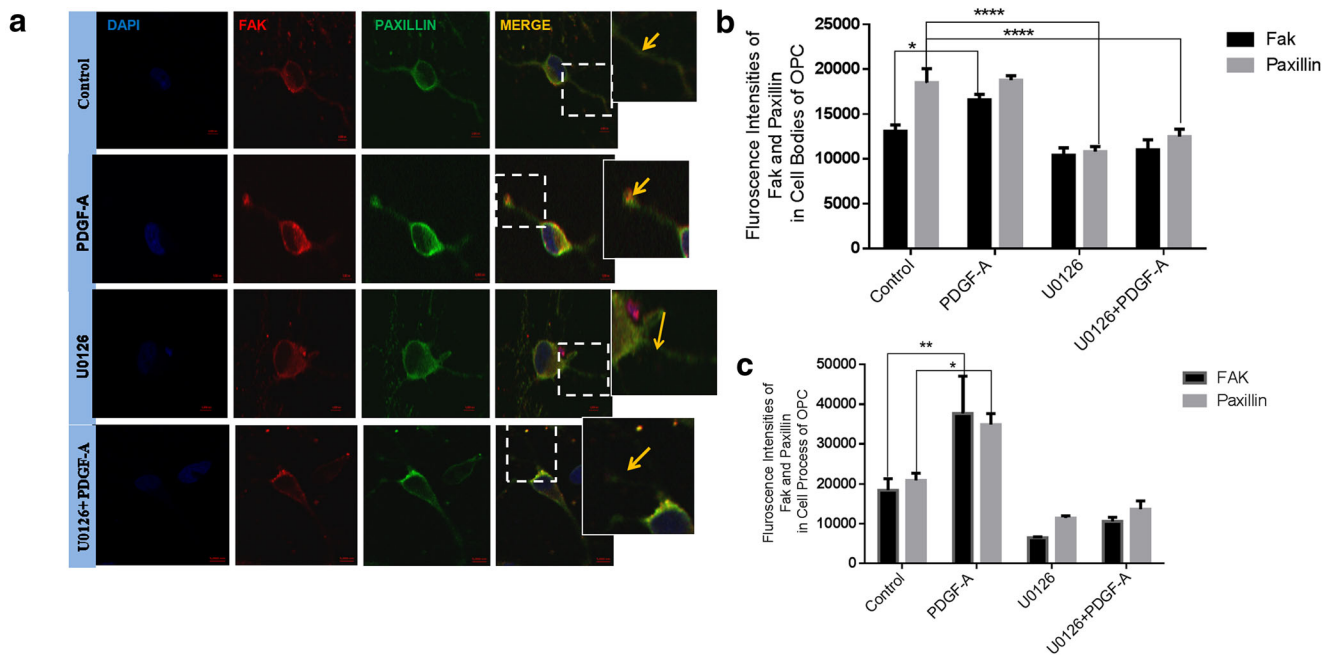


Fig. 7 PDGF-A induced FAK-Paxillin colocalization: **a** Association of FAK with Paxillin OPCs was visualized by immunostaining with anti-FAK (TRITC-Red) and anti-Paxillin (FITC-Green) antibodies in control, PDGF-A (10 ng/ml), U0126 (10 μM) with and without PDGF-A-treated OPCs (scale bar, 1000 nm). For the fluorescence intensities in the cellular process, the boxed areas are enlarged in the images on the right, and then

split into separate channels using ImageJ. Yellow arrows indicate the regions of interest taken for the analysis of the two proteins in the cells processes of the magnified panels. The experiment was performed three times with similar results. **b, c** Quantification and graphical representation of the normalized fluorescence intensities of FAK/ Paxillin proteins in cell body and cell process respectively

and neurite outgrowth, and are known to form prior to cell migration. This study suggests that the actin accumulation at the tips of the cell processes can lead to filopodia formation and OPC migration. However, ERK inhibition did not show any difference in the cellular F-actin accumulation. Hence, it suggests that PDGF-A continues to promote actin polymerization when ERK signaling is inhibited and can be due to the other ERK-independent mechanisms during OPC migration.

The present study also hypothesizes that PDGF-A-activated ERK is recruited to the cell periphery where it regulates focal adhesion disassembly prior to OPC migration. Focal adhesions (FA) are comprised of tightly compact regions of the plasma membrane that are in immediate proximity to the substratum in cultured cells (Lo 2006; Parsons 2003). FA dynamics involve an uninterrupted process of coordination between FA and actin cytoskeleton where numerous cytoskeletal proteins also come into play. Among the different focal adhesion proteins known, FAK and paxillin are known to be prominent cytoskeletal proteins involved in assembly and disassembly of cell adhesion, and thus promote directional cell movement (Hu et al. 2014; Schaller 2001). Both the proteins localize in the dynamic adhesions of the migrating cell which form and rapidly disassemble at the base of protrusions seen in migrating cells (Webb et al. 2004). Our previous lab results have also shown PDGF-A-induced pERK recruitment to the cell peripheral regions and cellular processes during migration (Tripathi et al. 2017). Our current study demonstrates for the first time that PDGF-A induces the pERK-Paxillin-FAK colocalization and complex formation in OPCs, which suggests a novel mechanism of PDGF-A-induced OPCs migration. pERK activates the tyrosine phosphorylation of FAK and paxillin which further promotes the formation of focal adhesions consistent with studies in human corneal epithelial cells (Teranishi et al. 2009). Further, OPC treatment with U0126 reduces the FAK-paxillin interaction in

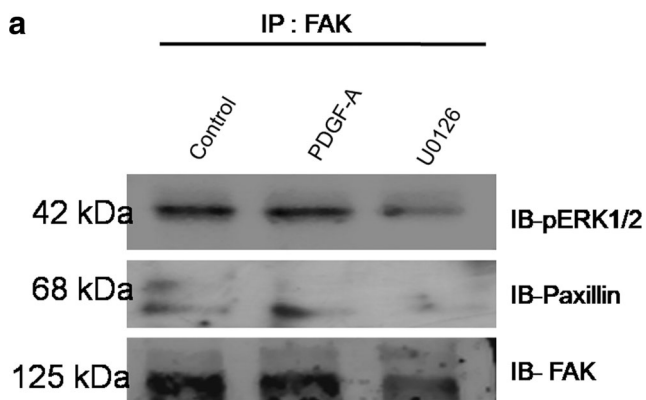


Fig. 8 PDGF-A-induced pERK1/2-FAK-Paxillin interactions. Cells were exposed to PDGF-A (10 ng/ml) and U0126 (10 μM) for 30 min and 2 h respectively. Cell lysates (1 mg protein) were subjected to immunoprecipitation (IP) with anti-FAK antibody conjugated to Dynabeads, and immunoprecipitates were subjected to SDS-PAGE and Western blotting with anti-FAK, anti-pERK1/2, and anti-paxillin antibodies

both the cell bodies and cellular processes. Recently, VEGF-A has also shown to promote the OPC migration by promoting the FAK-paxillin interaction (Hayakawa et al. 2011). Alteration of FAK and paxillin dynamics results in decreased migration by reduction in focal adhesion turnover and lamellipodia formation (Deramandt et al. 2014).

In conclusion, our findings demonstrate that PDGF-A-activated ERK signaling promotes OPC migration by regulating actin cytoskeleton and pERK-FAK-paxillin complex formation. These findings provide 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, what governs the OPCs to switch to different signaling pathway is still an interesting direction for future studies. OPC migration is critical for the normal formation of the CNS. Anything that disrupts this migration will result in incorrect or aberrant myelination, which in turn will lead to some form of brain disorder such as autism spectrum disorder, bipolar, ADHD, dyslexia, leukodystrophies, disappearing white matter disease, and many other as yet unidentified developmental disorders of the brain. Therefore, unraveling the regulatory mechanisms of OPC migration may help us explore new therapeutic avenues for this CNS disease.

Acknowledgements We are thankful to DBT-MSUB-ILSPARE programme for the confocal microscopy at Dr. Vikram Sarabhai Science Block, Faculty of Science, The M. S University of Baroda, Vadodara.

Funding information The financial assistance from University Grant Commission (F. No. 41-148/2012 (SR) dated 13 July 2012), New Delhi, India is highly acknowledged.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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