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Original Article

Post-transcriptional regulation of PDGFα-receptor in O-2A progenitor cells

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Abstract: Platelet-derived growth factor alpha-receptor (PDGFαR) mediated signaling plays a key role in the development of glial cells of the central nervous system. In vivo and in vitro studies show that PDGFαR is actively expressed in proliferative and motile oligodendrocyte type-2 astrocyte (O-2A) glial progenitor cells. However, PDGFαR expression is barely detectable in mature glial cells. The exact mechanism underlying the loss of PDGFαR expression is unknown. In this study, we employed a rat brain-derived O-2A glial progenitor cell line, CG4 as a culture model to investigate signals capable of inhibiting PDGFαR gene expression. PDGFαR mRNA levels decreased significantly as CG4 cells differentiated into both oligodendrocyte and astrocyte lineages. We showed that inhibition of PDGFαR expression was promoted by prostaglandin E2 via protein kinase A activation. Both cAMP analogs (db-cAMP and 8'bromo-cAMP) and adenylate cyclase activator (forskolin) were potent suppressors of PDGFαR expression in CG4 cells. This inhibitory effect resulted from an increased destabilization of PDGFαR mRNA instead of a decreased PDGFαR gene transcription. Importantly, db-cAMP failed to reduce PDGFαR mRNA levels in several PDGFαR over-expressing human glioma cell lines. Together, these results suggest that cAMP-dependent pathway played a key regulatory role in controlling PDGFαR mRNA levels during normal glial development, and that a breakdown in the cross talk between cAMP and PDGF pathways may underlie the uncontrolled proliferation and immature differentiation state in the glial tumors.

Keywords: PDGF, cyclic AMP, mRNA turnover, glioma

Introduction

Platelet-derived growth factor (PDGF) is a potent mitotic factor acting principally on mesoderm-derived cells. Biologically active PDGF consists of selective homo- or hetero-dimers of four subunits, A, B, C, and D [1-5]. The most common dimeric subtypes detected in vivo are PDGF-AA, -AB, –BB, -CC, and –DD. PDGF-AA, -AB, and -BB dimers are active upon dimerization, whereas PDGF-CC and –DD dimers are inactive until cleaved by extracellular proteases. PDGF dimers elicit their biological activities by binding to specific cell surface membrane receptors. Unlike the ligands, only two receptor subunits, the α- and the β-, have been identified [6-9]. The α-receptor subunit can interact with all four PDGF subunits, whereas the β-receptor subunit only interacts with PDGF B and D subunits. Like the ligands, biologically active PDGF receptors can be homo- or hetero-dimers of the α- and β-receptor subunits. The recognition specificity of the PDGF receptors leads to responses that are specific for different PDGF isoforms, which account, in part, for the tissue-specificity and the control of the distinct biological functions of PDGF action.

While cells of mesenchymal origin are the first known targets of PDGF, subsequent studies have demonstrated that PDGF also regulates a restricted set of ectoderm-derived cell types [10, 11]. The best-characterized ectodermal model is in the study of gliogenesis in the central nervous system [12-16]. PDGFA and α-receptor are the major ligand and receptor subunits expressed in embryonic brain tissues [17-19]. The major source of PDGFA in developing brain is thought to be Type I astrocytes and neurons [14, 16, 20]. By contrast, PDGFαR expres-

sion is first detected in the sub-ventricular zone (SVZ) where glial progenitor cells originate. Their subsequent migration towards neurons to form mature myelin forming oligodendrocytes is thought to be in response to the PDGF secreted from neurons and/or type 1 astrocytes. Hyperproliferation of glial progenitor cells is observed in transgenic mice that overexpress PDGF A in neurons [21] whereas glial cell differentiation is severely impaired in PDGFA null mice [22]. Collectively, these data support the importance of the PDGF signal pathway in the regulatory communication between the differentiated neurogenic cells and the undifferentiated glial progenitor cells.

The bipolar motile oligodendrocyte and type-2 astrocyte (O-2A) progenitor cells represent the major glial precursor type that expresses PDGFαR in the central nervous system. It has been proposed that PDGF-mediated signaling controls an array of biological activities including proliferation, motility, survival, and timed differentiation of the O-2A progenitor cells. The in vivo level of PDGFαR expression peaks around birth when the O-2A progenitors cells are rapidly dividing [13, 14, 23, 24]. As these precursor cells begin to differentiate, they become non-responsive to PDGF stimulation, a physiological change that coincides with the disappearance of PDGFαR expression in mature glial cells. The underlying cause for the loss of PDGFαR gene expression during glial cell differentiation remains unknown. In this study, we show that elevated cAMP level inhibits the transcript expression of PDGFαR in O-2A progenitor cells, thus establishing a role for a cross talk between cAMP- and PDGF signaling pathways in glial cell differentiation. We also found a breakdown of this cross talk in human glioma cells, providing an explanation for the continued expression of PDGFαR and the uncontrolled growth in these cancer cells.

Materials and methods

Cell culture

CG4, a rat brain-derived O-2A progenitor cell line was kindly provided by Dr. Randy McKinnon (UMDNJ-RWJ Medical School, New Jersey). All experiments were carried out using early passage cells (<12 passages). CG4 cells were maintained as proliferating progenitors in serum -free growth medium (GM) containing a mixture of 80% Sato's medium (Dulbecco modified Eagle's high glucose medium DMEM containing 200 units/ml penicillin, 50 μg/ml streptomycin, 1 mM glutamine, 200 μg/ml sodium pyruvate, 120 ng/ml progesterone, 5 μg/ml insulin, 50 μg/ml transferrin, 60 ng/ml triiodothyronine, and 1 mM sodium selenium) and 20% conditioned medium collected from B104 neuroblastoma cells. To prepare B104 conditioned medium, 4 X 10^6 B104 cells were plated onto 150 mm culture dish in DMEM containing 10% fetal calf serum (FCS). On the next day, the medium was changed to serum-free DMEM containing 200 units/ml penicillin, 50 μg/ml streptomycin, 1 mM glutamine, 200 μg/ml sodium pyruvate, 120 ng/ml progesterone, 200 ng/ml insulin, and 0.5 μg/ml BSA. B104 conditioned medium was collected after 72 hours incubation, clarified by slow speed centrifugation, and filtered through 0.45 mM membrane. Aliquots were stored at –20°C until use.

CG4 cells were induced to differentiate into oligodendrocytes when cultured in 100% Sato's medium (referred to as DM) with changes every two days. Under this condition, cells with oligodendrocyte morphology could be observed between 48 to 72 hours. Expression of myelin basic protein was used as the molecular probe in Western analysis to confirm oligodendrocyte differentiation. CG4 cells were induced to differentiate into astrocytes when cultured in 90% Sato's medium supplemented with 10% FCS. Expression of glial fibrillary acidic protein was used as the molecular probe in Western analysis to confirm astrocyte differentiation.

RNA analysis

Total RNAs were prepared from cells by Trizol-RNA extraction method according to the manufacturer's (InVitrogen) recommendations. For Northern blot analysis, 10 μg total RNA isolated from cells was denatured, electrophoresed on a 0.8% formaldehyde-style agarose gel, and transferred to nylon membrane. Nucleic acids were covalently linked to the nylon membrane by baking at 80°C for 2 hours. Membrane was preincubated at 42°C overnight in buffer containing 50% formamide, 0.1% sodium dudocyl sulphate, 5X Denhardt, 6X SSC, and 100 μg/ml heat denatured salmon sperm DNA before the addition of ^{32}P-radiolabeled DNA probe. DNA probes were radiolabeled using nick-translation kit (Invitrogen) under condition recommended

by the manufacturer. Membrane was washed twice at room temperature at low stringency (2 X SSC and 0.1% SDS; SSC= 0.15 M NaCl and 0.015 M sodium citrate) followed by two additional washes at high stringency at 42°C (0.2 X SSC and 0.1 % SDS) before exposed to X-films for autoradiography or to phosphoimager cassettes for quantitative analysis. For normalizing sample loading, membrane was stripped with boiling water and re-hybridized with radiolabeled GAPDH cDNA.

Nuclear run-on

The rate of gene transcription was measured by nuclear run-on assay under conditions previously described [25]. In brief, nuclei were prepared from a total of five 150 mm tissue culture dishes per condition tested. Transcription was initiated and propagated by incubating nuclei with a mixture of ribonucleotides containing ^{32}P-UTP to allow incorporation of radiolabel into newly synthesized RNA. After incubation, nuclei were treated with DNase I and Proteinase K. RNA was isolated by extraction with phenol-chloroform-isoamyl alcohol followed by isopropanol precipitation. Equal amount of radioactivity corresponding to labeled nascent RNA from each run-on reaction was used in hybridization with nylon membrane strips that contained 10 μg of linearized DNA samples of interest for 48 hours at 65ºC. Membrane strips were washed three times in 2X SSC at 65ºC and once in the presence of 10 μg/ml RNase A at 37ºC before exposure to X-ray film for autoradiography.

Transient transfection and CAT assays

CG4 cells were seeded at 70% confluent a day prior to transfection. Transient transfection was carried out using DNA-CaPO4 precipitate method. Beta-galactosidase (LacZ) DNA driven by CMV promoter was co-transfected along with the CAT reporter construct to monitor transfection efficiency. After removing DNA-CaPO4, cells were replenished with GM with or without 1 mM db-cAMP for 48 hours before harvest. Cell lysates for lacZ and CAT assays were prepared as previously described [26]. The amount of cell lysate used in CAT assay was standardized by the lacZ activity. Quantitation of CAT activity was determined by scintillation counting.

Results and discussion

PGE2 suppresses PDGFαR mRNA expression in O-2A progenitor cells

Studies on the role of PDGF in gliogenesis have been greatly facilitated by the ability to establish oligodendrocyte and type-2 astrocyte (O-2A) progenitor cell cultures from either rat optic nerve or cerebral cortex. The O-2A progenitor cells represent the major glial precursor type that expresses PDGFαR. In this study, we used CG4, a well-characterized O-2A progenitor cell line derived from rat brain to study PDGFαR regulation [27]. CG4 cells can be maintained and propagated for unrestricted periods as undifferentiated progenitors in growth medium (GM) containing serum-free Sato's culture medium supplemented with growth factor-enriched medium conditioned by B104 neuroblastoma cells. Upon removal of the B104 conditioned medium, CG4 cells differentiate into oligodendrocytes or astrocytes, depending on the absence or presence of fetal calf serum, respectively. In both cases, differentiated cells become unresponsive to PDGF with a progressive decline in PDGFαR mRNA levels (**Figure 1A**).

Since PDGF is present at all times in vivo during gliogenesis, it is most likely that other factors are required to block PDGFαR expression. To investigate this, we aimed to identify signaling molecules that could suppress PDGFαR mRNA content in CG4 cells under growth conditions. We first focused on factors that have been shown to play a role in glial cell differentiation or to negatively regulate PDGFαR expression in other cell types. For example, TGFβ is known to down regulate expression of PDGFαR in fibroblasts and smooth muscle cells [28, 29], and to enhance oligodendrocyte differentiation [18]. Responses of PDGFαR mRNA expression to interleukins could either be positive or negative depending on the cell types [30, 31]. In glial progenitor cells, IL-1β is reported to inhibit proliferation and to enhance oligodendrocyte differentiation [32], whereas IL-6 works as a survival factor for oligodendrocytes [33, 34]. As shown in **Figure 1B**, none of these factors affected PDGFαR mRNA levels in growing CG4 cells. Our finding with IL-1β differed from that reported by Vela et al [32], which shows that IL-1β down regulates PDGFαR expression in CG4 cells. We suspect that the cause of this discrepancy is attributed to the different GM used in the two studies. Unlike our study that used a complex medium containing growth factor supplements from B101 cell conditioned medium, Vela' study used a more defined medium containing only

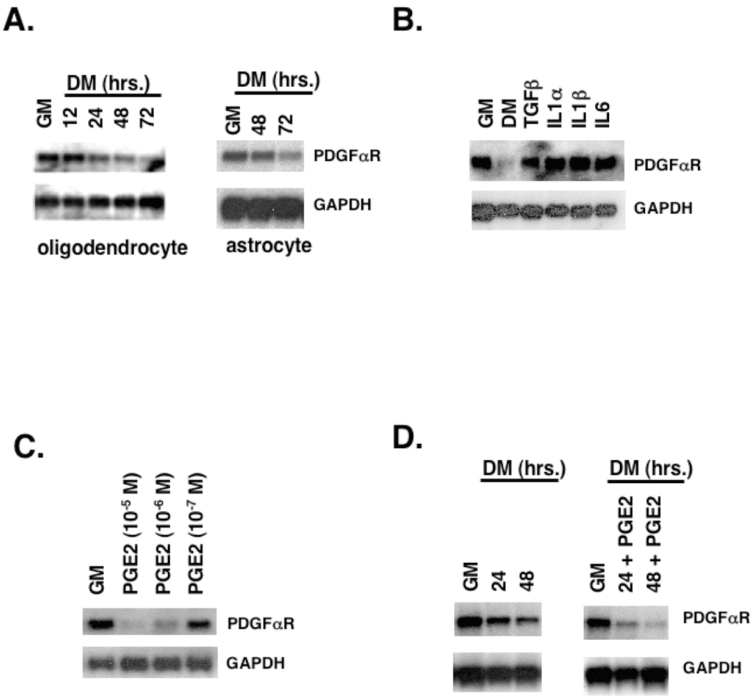

Figure 1. Regulation of PDGFαR mRNA levels in O-2A progenitor cells under growth (GM) and differentiation (DM) conditions. (A) CG4 cells were induced to differentiation into oligodendrocytes (left panel) or into astrocytes (right panel) under the condition described in Materials and Methods. Total RNA was prepared from CG4 cells at indicated times of differentiation. (B) Growth factors and chemokines known to promote oligodendrocyte differentiation did not affect PDGFαR mRNA levels in O-2A progenitor cells. CG4 cells grown at 70% confluence in GM (lane 1) either were switched to DM (lane 2) or were treated in GM with TGFβ (10 ng/ml, lane 3), IL-1α (10 ng/ml, lane 4), IL-1β (10 ng/ml, lane 5), and IL-6 (10 ng/ml, lane 6) for 48 hours before harvesting for RNA preparation and analysis. (C) PGE2 suppressed PDGFαR mRNA levels in a concentration-dependent manner. Proliferating CG4 cells in GM were treated with the indicated concentrations of PGE2 for 48 hours. RNA was collected and analyzed. (D) PGE2 accelerated the rate of decline of PDGFαR mRNA levels in differentiating oligodendrocytes. CG4 cells grown in oligodendrocyte-inducing DM were treated with (right panel) or without (left panel) PGE2 ($1x10^{-6}$ M) for the time indicated. RNA was collected and analyzed. Northern blot analysis was used to assess the steady state level of PDGFαR mRNA expressed from samples collected under specified conditions. GAPDH mRNA was used as an internal control for normalizing sample loading.

PDGF and FGF as growth factor supplement to maintain CG4 cell proliferation. The B104 cell derived conditioned medium in our test system likely contained many more "growth" promoting factors that counteracted IL-1β activity. Despite the higher complexity in our GM, we were able to identify one bioactive material, prostaglandin E2 (PGE2), that overcame the growth promoting activities from the B104 cells and reduced PDGFαR mRNA expression in CG4 cells (**Figure 1C**). When PGE2 was tested in CG4 cells grown in oligodendrocyte differentiation medium, we found that PGE2 accelerated the decline of PDGFαR mRNA (**Figure 1D**). The action of PGE2 was effective under either growth or differentiation conditions, suggesting that it triggered glial cell differentiation independent of growth factor signaling.

PDGFαR expression is inhibited by agents that elevates cAMP level and activates protein kinase A

In many cell types, PGE_2 exerts its effects through G protein–coupled prostaglandin receptors that principally lead to cAMP- or IP3/DAG-dependent activation of protein kinase A (PKA) or protein kinase C (PKC) pathway, respectively [35]. In CG4 cells, we found that H-89, an inhibitor of PKA, blocked the reduction in PDGFαR mRNA induced by PGE2 (**Figure 2A**), suggesting a role for the PKA pathway in suppressing PDGFαR expression during O-2A cell differentiation. In support of this idea, we found that PDGFαR mRNA level was reduced in cells treated with forskolin that increases endogenous cAMP levels through activation of adenylate cyclase (**Figure 2B**, lane 3). We also showed that cAMP analogs such as non-metabolizable db-cAMP and 8'bromo-cAMP, reduced PDGFαR mRNA in proliferating CG4 cells (**Figure 2B**, lanes 2 and 4). The effect of db-cAMP on PDGFαR mRNA levels was both time- and dose-dependent (**Figure 2C-D**). At 1 mM, a standard concentration used by others in this system, the PDGFαR mRNA level was maximally reduced between 18-24 hours (**Figure 2C**). At a fixed 24 hour-treatment, a significant reduction of PDGFαR mRNA was observed at 0.25 mM db-cAMP (**Figure 2D**). A maximal reduction in PDGFαR mRNA level at

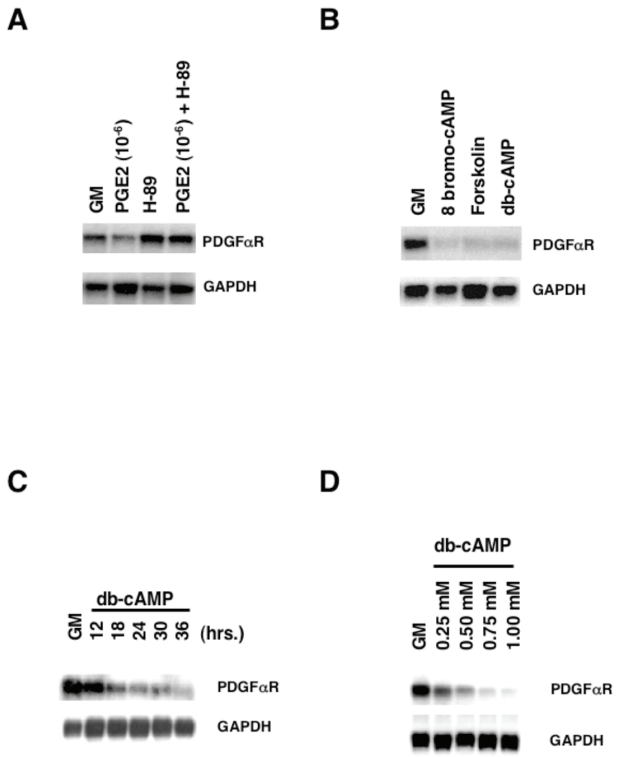

Figure 2. Suppression of PDGFαR gene expression in CG4 cells involved cAMP-dependent protein kinase A pathway. (A) Inhibition of protein kinase A abrogated PGE2-induced decrease in PDGFαR mRNA content. CG4 cells grown in GM were treated without (lanes 1 and 3) or with PGE2 (lanes 2 and 4) in the absence (lanes 1 and 2) and presence (lanes 3 and 4) of 10 μM protein kinase A inhibitor H-89 for 48 hours before harvesting for RNA preparation. (B) Cyclic AMP analogs and forskolin suppressed PDGFαR expression in O-2A progenitor cells. CG4 cells grown in GM were treated without (lane 1) or with 1 mM 8'bromo-cAMP (lane 2), 50 μM forskolin (lane 3), or 1 mM db-cAMP (lane 4) for 48 hours before harvesting for RNA preparation. Time (C) and concentration (D) dependent regulation of PDGFαR gene expression by db-cAMP. For determining dose dependence of db-cAMP on PDGFαR mRNA content, CG4 cells in GM were treated with the indicated concentration of db-cAMP for 24 hours. For determining time dependence, CG4 cells were treated with 1 mM db-cAMP for the indicated times.

0.25 mM db-cAMP was reached after 72 hours treatment (data not shown). This rate was similar to the rate of decline observed during standard oligodendrocyte differentiation procedure (see **Figure 1A**).

Oligodendrocyte differentiation of freshly isolated glial cells is reported to be accelerated by cAMP [36], although, it remains unclear what factors or mechanisms are involved in the response to cAMP. PGE2 is one of the major prostaglandins produced in the brain and is thought to play critical roles in CNS development and the regulation of many neural properties including synaptic activity and plasticity. Based on our finding, we propose that PGE2 might be a biological ligand for promoting gliogenesis in vivo through cAMP-dependent down regulation of PDGFαR expression.

Cyclic-AMP regulates mRNA stability, not transcription of the PDGFαR in O-2A progenitor cells

The effects of PGE2 (or cAMP) on the steady state level of PDGFαR mRNA could result from changes in synthesis or degradation rate of the PDGFαR mRNA, or both. To investigate this, we carried out experiments to differentiate between the two mechanisms. In the first set of experiments, we used promoter analysis and nuclear run-on assay to measure PDGFαR gene transcription in CG4 cells with or without db-cAMP treatment (**Figure 3**). We tested two PDGFαR promoter-reporter constructs: pGEM0.9αCAT and pGEM6.0αCAT that contained 912-bp and 6-kb of the PDGFαR promoter, respectively. We have previously shown that the 912-bp sequence contains a retinoic acid responsive element (RARE) that responds to co-treatment with a combination of retinoic acid and cAMP in F9 teratocarcinoma cells [37], and that the 6kb sequence contains key elements for driving spatial- and temporal-specific PDGFαR expression in transgenic mice [38]. As shown in **Figure 3A**, both constructs showed strong promoter activity in proliferating CG4 cells, but neither responded to db-cAMP regulation, indicating that transcriptional regulation was not the primary cause for the reduced PDGFαR mRNA content induced by db-cAMP. In support of this idea, we used a nuclear run-on assay (**Figure 3B**) to show that only minimal change (<10% reduction) in the transcription rate of the PDGFαR gene was detected after 24 hours of 1 mM db-cAMP treatment as compared to several control genes (c-kit, c-fos and beta-actin) whose steady state expression was not

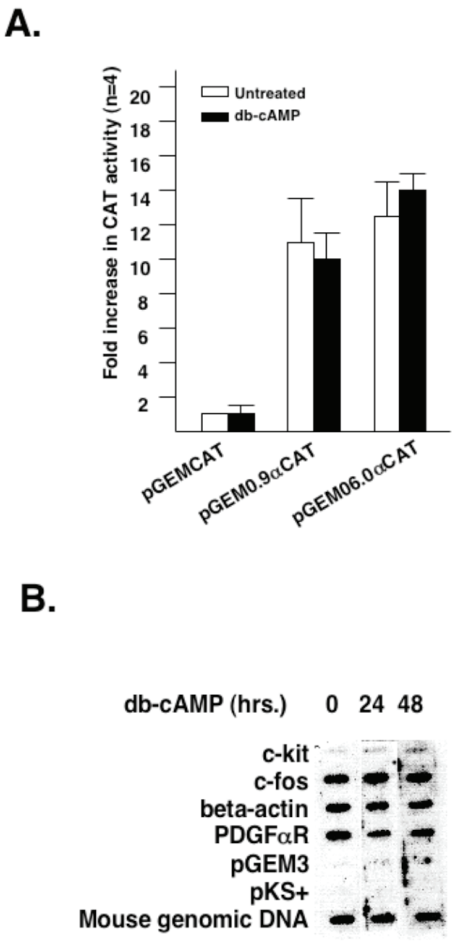

Figure 3. Cyclic-AMP treatment did not affect the rate of PDGFαR gene transcription in O-2A progenitor cells as determined by promoter (A) and nuclear run-on (B) analyses. (A) CG4 cells were transiently transfected in duplicates, each with a total of 10 μg DNA containing 8 μg CAT reporter DNA containing without promoter sequence (pGEMCAT) or with PDGFαR promoter sequences (pGEM0.9αCAT and pGEM6.0αCAT) and 2 μg of CMV-lacZ. After transfection, cells were treated with or without 1 mM db-AMP for 48 hours before harvesting for LacZ and CAT assays. LacZ assay was used to correct for variable transfection efficiency. Fold induction in CAT activity was defined as the level of PDGFαR promoter-CAT activity over that of pGEM-CAT promoterless construct measured in the absence of 1 mM db-cAMP. The pGEMCAT activity from untreated cells was given an arbitrary value of 1. Values were calculated based on minimum of three experiments. (B) Nuclei were prepared from CG4 cells with or without treatment of 1 mM db-cAMP for 24 hours. Equal amount of radioactivity corresponding to labeled nascent RNA from each transcription reaction was hybridized to the nylon strip containing indicated DNA templates. The strips were processed under condition described in Materials and methods.

affected by db-cAMP. Thus, the modest change in transcription of the PDGFαR gene could not account for the large decrease in the steady state levels of PDGFαR mRNA induced by db-cAMP.

In the second set of experiments, we determined the half-life of PDGFαR mRNA by inhibiting transcription with actinomycin D, and measuring PDGFαR mRNA levels at various time points. As shown in **Figure 4A**, the half-life of PDGFαR mRNA decreased drastically from ~16-18 hours in proliferating cells to less than 6 hours in db-cAMP-treated cells. The experiments were repeated at two additional actinomycin D concentrations, 1 μg/ml and 15 μg/ml, and in each case, the half-life of PDGFαR mRNA was measured to ~4-6 hours in the presence of db-cAMP (data not shown). The half-life of GADPH was rather long (>24 hours) and stayed relatively unchanged irrespective of the db-cAMP treatment. It should be noted that there was some cell death (~10-15%) after 24 hours of actinomycin D treatment, especially at the highest concentration in both untreated and db-cAMP treated cells. However, since there was no dramatic difference the turnover rate of GADPH mRNA under these conditions, the nearly 3-fold reduction of the PDGFαR mRNA half-life in the presence of db-cAMP was unrelated to cell death. We used protein synthesis inhibitor, cycloheximide, to determine if on-going protein synthesis was required for the cAMP effect on PDGFαR mRNA degradation (**Figure 4B**). CG4 cells were treated with cycloheximide up to 18 hours since significant toxicity was noted with longer exposure to the drug. At 18 hours of cycloheximide treatment, about 85% of cells were viable as determined by trypan blue staining. Although there was some cell death during the course of this experiment, the treatment itself did not affect the basal level of PDGFαR gene expression in untreated cells (**Figure 4B**, lane 3). We found that cycloheximide did not block the inhibitory effect of db-cAMP, suggesting that cAMP-induced degradation of PDGFαR mRNA did not require on-going protein synthesis.

We hypothesize that the destabilization process may be promoted through mechanisms involving two general modes of action: cAMP destabilizes PDGFαR mRNA by either inactivating a stabilizing factor(s) or activating a destabilizing factor(s). The stabilizing and destabilizing fac-

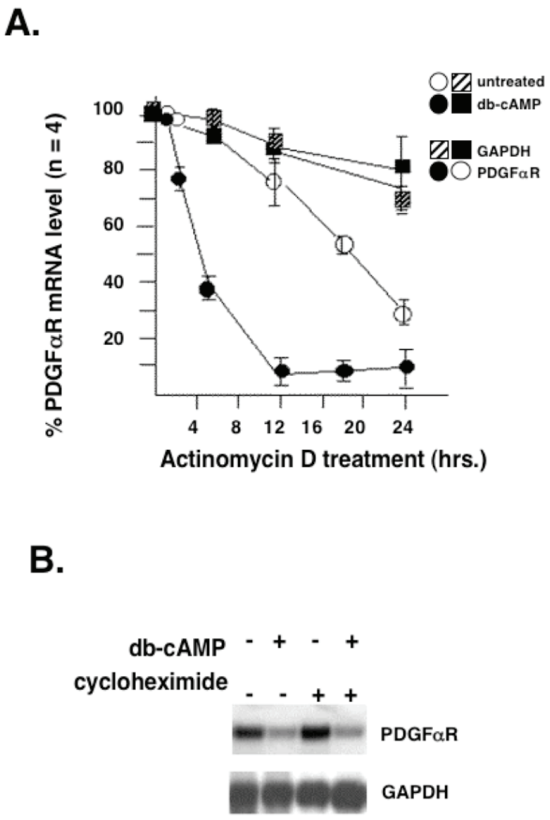

Figure 4. Cyclic-AMP treatment altered the rate of PDGFαR mRNA degradation in O-2A progenitor cells. (A) Effect of db-cAMP on the half-lives of PDGFαR and GADPH mRNAs. Because the db-cAMP effect was not immediate, CG4 cells were pre-treated with 1 mM db-cAMP for 12 hours before the addition of actinomycin -D (5 μg/ml). Total RNA was collected from actinomycin-D treated cells at indicated time intervals and analyzed for PDGFαR and GAPDH expression. Radioactive signals were quantified using phosphoimager. The levels of PDGFαR and GAPDH mRNAs in cells treated with or without db-cAMP prior to actinomycin D addition were assigned an arbitrary number of 100%. (B) The effect of cycloheximide on db-cAMP regulated PDGFαR expression in O-2A progenitor cells. CG4 cells grown in GM were treated without (lanes 1 and 3) or with (lanes 2 and 4) 1 mM db-cAMP in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 5 μg/ml cycloheximide for 18 hours.

tors likely involve direct interaction between the cis-acting element such as sequence-specific primary sequence or secondary structure of PDGFαR mRNA, and the trans-acting factor such as cAMP-regulated RNA binding protein that recognizes the cis-acting element. Such regulation can also be indirect. MicroRNAs could potential serve as mediators for an indirect action of the trans-acting factor. MicroRNAs are small, non-coding RNA molecules expressed in many organisms, which regulate gene expression by post-transcriptional (mRNA degradation) and translational mechanisms. MicroRNAs have been indicated as important regulators for the initiation of oligodendrogenesis [39, 40], and a few microRNAs have recently been shown to down regulate PDGFαR expression during this process. It will be important to examine whether these known microRNAs or other microRNAs with predicted binding sites in PDGFαR transcript can lead to PDGFαR mRNA degradation in O-2A progenitor cells following PEG2 and cAMP stimulation.

Loss of cross talk between cAMP and PDGF signaling in brain tumors

Gliomas are brain tumors derived from oligodendrocytes, astrocytes, and ependymocytes, and are characterized by uncontrolled cell proliferation and infiltrative growth into the brain [41]. The majority of gliomas harbor genetic or molecular alterations in growth factor signaling pathways that provide proliferative advantages to these tumor cells. PDGF signaling is one of the most commonly over-activated growth factor pathways detected in human gliomas. Overexpression of PDGFαR is found in glial cells of both low- and high-grade tumors [42]. Inhibition of PDGF signaling in PDGFαR overexpressing human glioma cell lines has been shown to block their tumorogenicity in animals [43]. These studies support the notion that aberrant PDGFαR signaling is important determinant of gliomagenesis. Although both accumulation of PDGFαR mRNA and enhanced tyrosine protein kinase activity are shown to occur in glioma, the cause of these aberrations in the development of glioma tumors remains undetermined. Amplification and rearrangement in PDGFαR gene have been reported sporadically in some highly aggressive forms of glioma [42-45], but the frequency of these genetic aberrations is so low that additional mechanisms must be involved.

Since cAMP appears to control PDGFαR expression during normal gliogenesis as suggested in this study, we wanted to determine if a defect in the cAMP-signaling might lead to glial cell proliferation and transformation due to PDGFαR over-expression. This is an attractive idea since db-cAMP treatment of human glioma cells does not

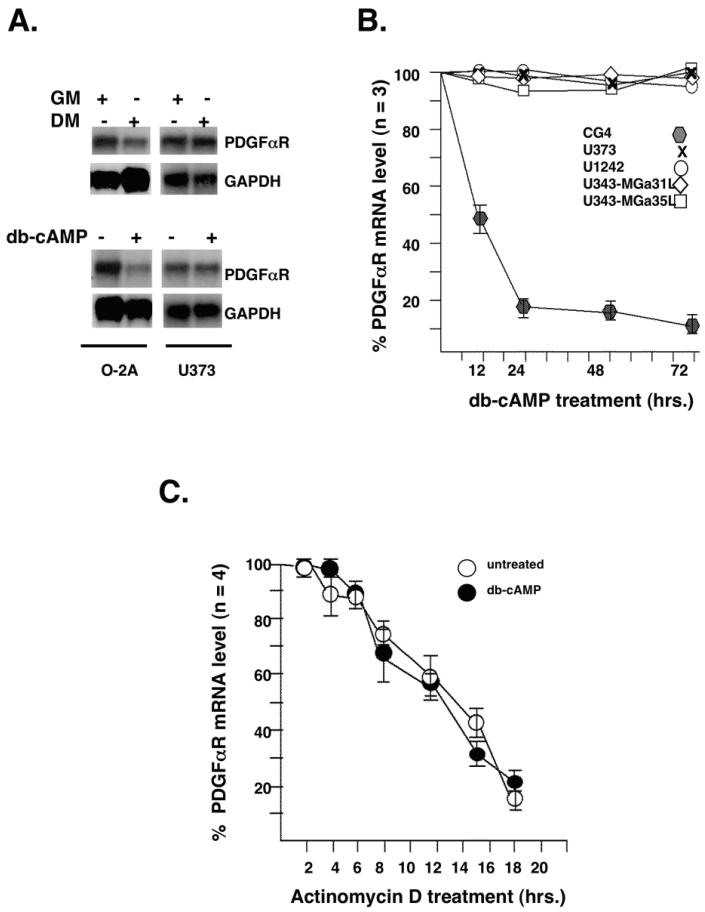

Figure 5. Human glioma cells were resistant to cAMP-induced PDGFαR mRNA degradation. (A) Northern blot analysis on the levels of PDGFαR mRNA in CG4 and a human glioma cell line (U373) upon 24-hour treatment with DM (upper panel) or 1 mM db-cAMP (lower panel). (B) Quantitative analysis of the effect of db-cAMP on PDGFαR mRNA levels in CG4 and four human glioma cell lines (U373, U1242, U343-MGa31L, U343-MGa35L) over a period of 72 hours. The levels of PDGFαR mRNA corrected for the GAPDH mRNA are reported as % PGDFαR mRNA at zero time point (untreated). (C) Actinomycin D treatment did not affect the half-life of PDGFαR mRNA in U373 glioma cell line. The experimental condition was the same as described in Figure 4A legend.

affect their responses to differentiation and mitogens such as PDGF [46], indicating the normal interplay between cAMP and PDGF pathways might be disrupted in the cancer cells. To investigate this, we compared the levels of PDGFαR mRNA between CG4 and human glioma U373 cells in response to 24-hour treatment with DM (**Figure 5A**, top panel) and 1 mM db-cAMP (**Figure 5A**, bottom panel). As expected, the PDGFαR mRNA level was reduced under both culture conditions (**Figure 5A**, left panel) in control CG4 cells whereas the PDGFαR mRNA level was not changed in U373 cells (**Figure 5A**, right panel). To verify the observation in U373 cells, we repeated the experiment in three additional human glioma cell lines (U1242, U343-MGa31L, and U343-MGa35L), all of which over-express PDGFαR in the absence of gene amplification. As shown in **Figure 5B**, we found the level of PDGFαR mRNA in all four glioma cell lines remained the same even after 72 hours of db-cAMP exposure. Under the same experimental condition, the level of PDGFαR mRNA in CG4 cells was reduced by 80% within the first 24 hours. The same result was obtained when the experiment was performed with 5 mM db-cAMP and for up to a week of drug treatment (data not shown). We further demonstrated that the half-life of PDGFαR mRNA in the four tumor cell lines remained the same (~14-16 hours) in the presence or absence of db-cAMP, equivalent to the half-life of PDGFαR mRNA in the proliferating CG4 cells (**Figure 4A**). An example of this result obtained from U373 cell line is presented in **Figure 5C**.

As previously reported, we noticed a marginal reduction in the proliferation rate without promoting differentiation in all four glioma cell lines with prolonged or higher doses of db-cAMP exposure. It has been known for sometime that brain tumors have a much lower level of cAMP compared to the normal counterpart, suggesting an intrinsic defect in cAMP production in brain tumor cells [47. 48]. This study shows that human glioma cells harbor multiple cellular defects in the cAMP pathway as raising cAMP level

was insufficient to arrest growth and promote post-mitotic cell differentiation. It will important in future work to determine what downstream effectors of cAMP are able to restore the normal reduction in PDGFαR expression and how restoring these impaired effectors might enhance cAMP activity in promoting glioma cell differentiation.

Conclusion

Glial cells serve critical roles in nervous system. It is important to understand molecular events that influence glial cell growth and differentiation as defects in either process can result in a variety of pathological disorders. Here, we examine the molecular mechanism involved in the interaction between two key players, PDGFαR and cAMP, in CNS signal transduction pathways. In fact, the crosstalk between PDGF and cAMP signaling pathways might be a more general mechanism throughout the nervous system for controlling normal function and development. For example, it has been previously reported that the PDGFαR expression is regulated by cAMP in the myelin-forming Schwann cells in the peripheral nervous system [49]. This study points to prostaglandin E2 acting through the cAMP-dependent protein kinase A pathway as a key regulator of PDGF activity during normal gliogenesis. Aberrant levels of both molecules have been linked to the etiology of malignant brain tumors. Our data show that a disruption in the communication between these PDGFαR and cAMP signal transduction pathways could be a key trigger for the initiation and development of glioma, the most common malignancy of the adult brain. Thus, Future studies will be needed to identify mechanisms for reduced responses to intracellular cAMP levels in glioma cells and into the dynamic interaction between cAMP and PDGF signaling that could lead to new therapeutic strategies for treating this deadly disease.

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