Chondroitin sulfate A chains enhance platelet derived growth factor-mediated signalling in fibrosarcoma cells

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Abstract

Platelet derived growth factor is involved in the autocrine growth stimulation of malignant cells, the stimulation of angiogenesis and the recruitment and regulation of tumor fibroblasts. PDGF has been shown to physically interact with glycosaminoglycans which are abundant in the fibrosarcoma cell microenvironment. Aim of the present study was to examine the effects of glycosaminoglycans on the mitogenic function of platelet derived growth factor in two human fibrosarcoma cell lines (B6FS, HT1080). For this purpose exogenously added glycosaminoglycans, regulators of endogenous glycosaminoglycan synthesis (sodium chlorate as selective inhibitor and β-D-xylloside as a stimulator) and specific glycosidases to cleave cell-associated glycosaminoglycans, were utilized. Platelet derived growth factor demonstrated a growth stimulating effect on B6FS, whereas no effect was evident on HT1080 fibrosarcoma cells. β-D-Xyloside had no effect on the basal level or the platelet derived growth factor-induced cell proliferation, whereas sodium chlorate severely reduced the basal level of proliferation in both cell lines. Significant co-stimulatory effects of chondroitin sulfate A in combination with platelet derived growth factor BB on the growth of HT1080 and B6FS cells were found. The co-stimulatory effect of chondroitin sulfate A was not due to transcriptional up regulation of platelet derived growth factor receptors genes, but rather to more efficient signalling of tyrosine kinase receptors. In conclusion, this study shows that chondroitin sulfate A can enhance the mitogenic activity of platelet-derived growth factor in fibrosarcoma cells utilizing a pathway which involves tyrosine kinases. This result introduces a new modulating role for chondroitin sulfate in signalling pathways critical for cancer growth.

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1. Introduction

Fibrosarcoma is an uncommon soft tissue tumor, defined as a malignant tumor composed of fibroblasts and is associated with abnormal deposition of collagen and other extracellular matrix components. Platelet-derived growth factor (PDGF) is a major polypeptide mitogen for mesenchymal cells, and it can be produced by fibrosarcoma cells as well as by stroma cells. PDGF is suggested to deliver a survival signal, by inhibiting cell apoptosis and promoting cell proliferation (Kelly, Raines, Ross, & Murray, 1985). It has also been considered to participate in malignant processes including autocrine transformation and human oncogenesis.
PDGF is formed by heterodimerization of two distinct but highly homologous polypeptides, A and B. Two additional isoforms, PDGF-C and D, have recently been described (Li & Eriksson, 2003). Both A and B chains can be produced as long and short isoforms through the alternative splicing of exon 6. The short isoforms are the most prevalent in the extracellular matrix and have been shown to be more stable and active than the long isoforms which are secreted in small quantities. It is suggested that the long isoforms are immobilized on the cell surface through the retention motif and are destined to serve autocrine purposes (Andersson, Ostman, Westermark, & Heldin, 1994; Pollock & Richardson, 1992). The retention motif is a carboxyterminal stretch of highly basic amino acids which can interact with negatively charged biomolecules such as the glycosaminoglycans (GAGs).

PDGF signalling is mediated through binding to the high affinity receptors α and β, which possess tyrosine kinase properties. PDGFRα can bind to A, B and C PDGF chains while PDGFRβ binds B and D chains (Li & Eriksson, 2003). Growth factor signalling has been previously shown to be significantly assisted by the interaction with proteoglycans. Basic FGF interacts with heparan sulfate proteoglycans through the glycosaminoglycan chains which stabilize its interaction with the FGF receptor and thus enhance its signal delivery (Rapraeger, Krufka, & Olwin, 1991). Likewise, PDGF interacts with proteoglycans mainly through the GAG chains. Binding to GAGs is mediated by electrostatic interaction of the negatively charged GAGs with the positively charged peptides in the PDGF sequence as well as with the retention motif of the long PDGF isoforms (Fager et al., 1995). The physical interaction of the PDGF isoforms with matrix and cell associated GAGs has been studied in detail. Both the long and the short isoforms of PDGF can bind GAGs, the former with 10–100-fold greater affinity (Lustig et al., 1999). Specifically, PDGF-BB short (PDGF-BB) was obtained from R&D diagnostics. Chondroitin sulfate type A (CSA), dermatan sulfate (DS), heparin, sodium chloride, β-D-xyloside and genistein were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Chondroitinase ABC and Heparitinase were also purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Cell culture reagents were obtained from Biochrom KG (Berlin, Germany). [Methyl-3H] thymidine was purchased from (Moravek Biochemicals, USA). Polyclonal antibodies against the protein core of PDGFRα, PDGFRβ, p-PDGFRβ and actin as well as anti-rabbit and anti-goat HRP conjugated secondary antibodies were purchased from Santa Cruz Biochemicals (USA).

2. Materials and methods

2.1. Materials

PDGF-BB short (PDGF-BB) was obtained from R&D diagnostics. Chondroitin sulfate type A (CSA), dermatan sulfate (DS), heparin, sodium chloride, β-D-xyloside and genistein were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Chondroitinase ABC and Heparitinase were also purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Cell culture reagents were obtained from Biochrom KG (Berlin, Germany). [Methyl-3H] thymidine was purchased from (Moravek Biochemicals, USA). Polyclonal antibodies against the protein core of PDGFRα, PDGFRβ, p-PDGFRβ and actin as well as anti-rabbit and anti-goat HRP conjugated secondary antibodies were purchased from Santa Cruz Biochemicals (USA).

2.2. Cell culture

HT1080 human fibrosarcoma cells (obtained from AATC) were grown in DMEM (GIBCO) (HT1080 and DLF) supplemented with 10% fetal bovine serum (FBS) (GIBCO) and B6FS human fibrosarcoma cell line (Thurzo et al., 1976) were grown in RPMI (GIBCO) supplemented with 10% FBS. Prior to stimulation with growth factors the cells were incubated in 2% FBS medium for 24 h. PDGF-BB (final concentration 10 ng/ml which was selected after dose response experiments) were added in 2% FBS medium. Prior to RNA extraction the cells were treated with each factor for 24 h.

2.3. RNA isolation and real-time PCR

Total ribonucleic acid was isolated with the TRIzol method (GibcoBRL) according to the manufacturer’s
instructions. Five micrograms of total RNA was used for cDNA synthesis using the ThermoScript™ RT-PCR System (Invitrogen). The primers were mRNA specific to avoid misleading results from traces of DNA contamination PDGFRα primer forward 5'-CCTGGCTGAAAAACATCTGACT-3', PDGFRα primer reverse 5'-CAGCTTTATTTGCTGCCATCCTT-3', PDGFRβ forward 5'-GTCACCACATCTCCTCCATCA-3', PDGFRβ reverse 5'-CTCATATGCTTTGAGCA-AGAAGCC-3', GAPDh forward 5'-GGAAGGTGAAGGTCGGAGTCA-3' and GAPDh reverse 5'-GT-CATGATGGCAACAATATCCACT-3'. For the real-time PCR reaction we utilized the QuantiTech SYBR Green master mix (Qiagen) in a total volume of 20 μl. Standard curves were run in each optimized assay which produced a linear plot of threshold cycle (Ct) against log (dilution). The real-time PCR reactions were carried out in an ABI 7000 cycler. The amount of each target was quantified based on the concentration of the standard curve and was presented as arbitrary units. The quantity of each target was normalised against the quantity of GAPDh.

2.4. Western blot

Cells were incubated in 2% FBS supplemented medium for 72 h. The cells were lysed with Tris/HCl 50 mM, EDTA 0.5 M, Triton-X100 1%, NaCl 0.1% and protease inhibitors (PMSF 1 mM, NEM 5 mM, benzamidin 5 mM). The cell lysates were electrophoresed on 8% polyacrylamide Tris/Glycine gels and transferred to nitrocellulose membranes in 10 mM CAPS, pH 11, and containing 10% methanol. The membranes were blocked for 12 h with PBS containing 0.1% Tween-20 (PBS-T) and 5% (w/v) low-fat milk powder. The membranes were incubated for 1 h at room temperature with the following primary antibodies: rabbit anti-PDGFRα (1:200), rabbit anti-PDGFRβ (1:800) or goat anti-Actin (1:400) (all from Santa Cruz Biotechnology) in PBS containing 0.1% Tween-20 (PBS-T) and 1% (w/v) low-fat milk powder. The immune complexes were detected after incubation with peroxidase-conjugated anti-rabbit or anti-goat antibody (Santa Cruz Biotechnology), diluted (1:4000) in PBS-T, 1% low-fat milk, with the SuperSignal West Pico Chemiluminescent substrate (Pierce), according to the manufacturer’s instructions.

2.5. Proliferation assay

Growing cells from non-confluent cultures were harvested and seeded in 24 well plates (Costar, USA) at a density of 20 x 10^3 cells per well in 1 ml of DMEM or RPMI (10% FBS). The cells were allowed to rest overnight. Prior to stimulation the cells were incubated in medium with 2% FBS for 24 h. The medium was replaced with fresh supplemented with heparin, CSA or DS at concentrations of 1, 10, 30 and 100 μg/ml. In the cases where chondroitinase ABC or heparitinase treatment was necessary, the medium was replaced with fresh supplemented with chondroitinase ABC (0.1 u/ml) or heparitinase (0.001 u/ml). After 1 h of enzymatic treatment, PDGF-BB (10 ng/ml) was added in fresh medium with 2% FBS. After 32 h of incubation [methyl]-3H thymidine (Moravek Biochemicals, USA) was added up to the final concentration of 0.2 μCi/ml and the cells were incubated for further 16 h. Radioactivity was measured in a liquid scintillation spectrometer (Beckman Instruments, USA).

2.6. Statistical analysis

The statistical significance was evaluated using the t-test and the one way completely randomized variance analysis (ANOVA) using the Microcal Origin (version 5.0) software.

3. Results

3.1. Expression of PDGF receptors and effect of PDGF-BB on fibrosarcoma cell growth

Two poorly differentiated human fibrosarcoma cell lines, predominantly consisting of fibroblastoid cells (B6FS) (Fig. 1A) (Thurzo et al., 1976) and rounded/elongated cells (HT1080) (Fig. 1B) (Rasheed, Nelson-Rees, Toth, Arnstein, & Gardner, 1974) were utilized in this study. Both cell lines exhibit a malignant phenotype and have a high metastatic capacity. HT1080 cells have been extensively studied, whereas B6FS cells are less known. It is a standard practice in studies searching for the effects of growth factors to utilize starvation conditions so that the growth factor effect can be clearly measurable. Initially a pilot experiment was designed in order to establish that the cell lines utilized possessed both PDGFRs at the conditions the main experiment would be carried out. PDGFRα and β receptors as well as PDGF-BB were detected at the level of mRNA and protein in both cell lines, when cultured with 10% FBS (not shown). It should be noted that after a 48 h starvation period (2% FBS), B6FS receptor expression (mRNA and protein) was constant, while in HT1080 cells PDGFRβ was significantly decreased and PDGFRα mRNA was
undetectable. The relative expression of PDGFRα and β after a 48 h (mRNA) or 72 h (protein) serum starvation (2% FBS) period, in the two cell lines, is presented in Fig. 1C and D. The proliferation of HT1080 cells was not significantly affected by PDGF-BB treatment (Fig. 2A), whereas, B6FS cells were substantially stimulated (Fig. 2B).

3.2. Effects of endogenous GAGs/PGs on the PDGF-BB-induced cell proliferation

To examine the effects of endogenously synthesised GAGs on PDGF-BB function, we utilized β-d-xyloside, which is a well known stimulator of CS chains production. β-d-xyloside alone had no significant effect on the proliferation of either B6FS or HT1080 cells. When combined with PDGF-BB no significant increase of proliferation was observed in both HT1080 and B6FS cells as compared with just the PDGF-BB treatment (Fig. 2A and B).

Sodium chlorate, a known inhibitor of GAG sulfation, is used to effectively reduce the synthesis of all proteoglycans. As shown in Fig. 2, sodium chlorate treatment caused a significant reduction of the basal level prolifer-
3.3. Exogenous GAGs significantly affect the mitogenic effect of PDGF-BB

In order to evaluate the role of GAGs on PDGF activity, CSA, DS and heparin were exogenously added with or without PDGF and the effects on cell proliferation were examined (Fig. 3). DS and heparin showed an inhibitory effect in a dose dependent manner (data not shown). The concentration of 30 µg/ml for CSA, which caused a mild stimulatory effect on both cell lines, was chosen for further studies. None of the GAGs used at the selected concentrations had either cytotoxic effects or caused significant changes in the cell morphology.

Combination of CSA (30 µg/ml) with PDGF-BB (10 ng/ml) resulted in a significant co-stimulatory effect, which was more pronounced in HT1080 cells (Fig. 3A and B). The inhibitory effects of DS (30 µg/ml) and heparin (10 µg/ml) in HT1080 and B6FS cells were not affected by the combined presence of PDGF-BB.

3.4. Effects of exogenous GAGs on the transcription of PDGFRα and PDGFRβ

To determine whether the CS co-stimulatory effects on the PDGF-BB induced cell proliferation were achieved by modulation of PDGF receptor levels, GAGs were exogenously added and in continuation PDGFRα and β mRNA changes were measured by real-time PCR. PDGFRβ, which is expressed at very low levels in HT1080 cells, were not significantly affected by the addition of GAGs or PDGF-BB (Fig. 4A). In B6FS cells, which express both PDGFRα and β at higher levels, PDGFRβ was negatively affected by heparin, DS and PDGF-BB (Fig. 4B). PDGFRα was negatively affected by DS (Fig. 4C).
3.5. Effects of cell surface GAG removal on PDGF-BB function

The possible participation of different cell-associated GAGs/PGs to PDGF-BB function was examined by treating the cells with chondroitinase ABC or heparitinase. The enzymic treatment of the cells with chondroitinase ABC aimed at the preferential removal of CS and DS chains, while that with heparitinase targeted at the removal of HS chains. Chondroitinase ABC treatment had no significant effect on the basal level of proliferation in HT1080 and B6FS cells (Fig. 5). Removal of cell associated heparan sulfate by heparitinase treatment had no significant effect on HT1080 cells, while in B6FS it reduced the basal level of cell growth (Fig. 5). In both cell lines the removal of chondroitin and dermatan sulfates had minor effects on PDGF-BB function. On the other hand the removal of heparan sulfate demonstrated an inhibitory effect on PDGF function in B6FS cells.

3.6. Inhibition of tyrosine kinase completely blocks the CSA-induced co-stimulatory effect on PDGF signalling

Genistein (10 μg/ml) was used to evaluate whether the CSA-PDGF-BB induced increase on cell growth in HT1080 cells, as above demonstrated (Fig. 3), was mediated through signalling of tyrosine kinases. HT1080 cells were selected for the inhibition test since they demonstrated the most prominent co-stimulatory CSA-PDGF effect. As shown in Fig. 6A, genistein treatment was able to reduce the phosphorylation of PDGFR-β. It is worth noticing that genistein although it had no effect on the basal level of proliferation in HT1080 cells, it inhibited the stimulatory effect of CSA on PDGF-BB treated cells demonstrating that the increased co-stimulatory activity is mediated through a tyrosine kinase signalling pathway (Fig. 6B).

4. Discussion

In this study the effects of GAGs on PDGF-BB signalling in fibrosarcoma cells were examined. The effects were studied using exogenously added GAGs, regulators of endogenous GAGs synthesis and specific glycosidase digestions. A significant co-stimulatory effect of CSA in combination with PDGF-BB was observed on the growth of HT1080 and B6FS cells. This effect of CSA was not due to transcriptional up regulation of the PDGF receptors but rather due to their more efficient signalling.
PDGF family consists of five different disulide linked isoforms PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, PDGF-DD (Li & Eriksson, 2003) which act through two structurally similar tyrosine kinase receptors denoted PDGFRα and PDGFRβ. The phosphorylation of PDGF receptor triggers downstream a number of signalling enzymes including Src, phosphatidylinositol 3 kinase (P3K), phospholipase Cγ (PLCγ) and Ras (Tallquist & Kazlauskas, 2004). PDGF is a strong mitogen and chemoattractant in mesenchymal cells, including fibroblasts, myofibroblasts and smooth muscle cells. Furthermore, PDGF isoforms and their receptors are involved in autocrine growth stimulation of malignant cells, stimulation of angiogenesis and recruitment and regulation of tumor fibroblasts (Board & Jayson, 2005). Importantly, recent studies demonstrated an involvement of autocrine PDGFR signalling in soft tissue sarcomas (Ostman, 2004). PDGF is suggested to play a key role in dermatofibrosarcoma protuberans (DFSP) and giant cell fibroblastoma (GCF) development through mutational activation of PDGF-B chain or PDGF receptors (Tallquist & Kazlauskas, 2004). DFSP and GCF tumors are associated with translocations that fuse the collagen 1A1 gene with the PDGF-B chain leading to constitutive production of fusion proteins which are processed to mature PDGF-BB and autocrine PDGF receptor stimulation (Pietras, Sjoblom, Rubin, Heldin, & Ostman, 2003; O’Brien et al., 1998; Shimizu et al., 1999; Simon et al., 1997). Also, PDGF is proposed to be a strong mitogen for neurofibrosarcoma-derived schwann cells (Badache & De Vries, 1998).

The fibrosarcoma cell microenvironment is rich in GAGs/PGs and other extracellular matrix components. We selected to work with fibrosarcoma cells which are mildly responsive to PDGF stimulation in culture, to determine whether GAGs can modify the PDGF-induced cellular response. PDGF-BB had a significant stimulatory effect on the proliferation of B6FS, whereas no effect was evident on HT1080 cell proliferation. This result correlates well with the expression of their respective PDGFRs, since B6FS cells expressed both receptors at high levels in contrast to HT1080 cells, which express mainly PDGFRβ at very low levels. In addition, HT1080 cells display a very high basal level of proliferation and possess a mutation in N-Ras, which renders part of the PDGFR pathway constitutively activated (Gupta et al., 2001).

Sodium chlorate treatment is an established method for inhibiting the biosynthesis of all sulfated proteoglycan classes. Specifically sodium chlorate inhibits the sulfation of the glycosaminoglycan chain, which results in the production of aberrantly glycosylated proteoglycans whose secretion is prevented by the quality control mechanisms of the cell (Fannon & Nugent, 1996). Previously, sodium chlorate has been shown to decrease fibroblast responsiveness to growth factors (Fannon & Nugent, 1996) and to significantly affect the mechanisms which control the cell cycle (Keller, Brauer, & Keller, 1989). When B6FS and HT1080 cells were treated with sodium chlorate their basal proliferation level was significantly reduced, demonstrating that sulfated proteoglycans are essential for the growth of both fibrosarcoma cell lines. PDGF-BB in combination with sodium chlorate fully restored the basal proliferation level of HT1080 cells, whereas only partially that of B6FS cells. To study the role of cell-associated GAGs/PGs on PDGF-BB function, the cells were treated with chondroitinase ABC and heparitinase. Specific GAG-degrading lyases on live cells have been routinely used for examining the activities of chondroitin sulfate and heparan sulfate proteoglycans of the cell membrane (Denholm, Cauchon, Poulin, & Silver, 2000; Lyon, Rushton, & Gallagher, 1997; Maeda, Nishiwaki, Shintani, Hamanaka, & Noda, 1996; Milev, Monnerie, Popp, Margolis, & Margolis, 1998; Rapraeger & Yeaman, 1989). The removal of cell surface chondroitin sulfate or heparan sulfate in both HT1080 and B6FS cell lines had minor effects on PDGF-BB function. These results are in agreement with a previous study which showed that chondroitinase ABC treatment did not reduce the binding of PDGF-BB on the membrane of CHO cells (Garcia-Olivas et al., 2003).

The increased endogenous production of GAGs/PGs can affect cell proliferation, as it has been previously shown in several normal and cancerous cell lines treated with β-d-xyloside (Miao et al., 1995). The aglycone derivative of β-d-xyloside used in the present study was p-nitrophenyl, which is known to induce mainly chondroitin/dermatan sulfate synthesis (Lugewma & Esko, 1991). Treatment with β-d-xyloside had no significant effect on the basal level of proliferation in both HT1080 and B6FS cells. Combination of β-d-xyloside with PDGF-BB in B6FS cells resulted in a slight increase of cell proliferation, demonstrating a potential co-stimulatory effect of GAGs on PDGF-BB function. Furthermore, we exogenously applied CSA, DS and heparin preparations which we had previously biochemically characterized (Nikitovic et al., 2005). Treatment of the cells with DS or heparin alone resulted in an inhibitory effect in both cell lines. This was more pronounced in the case of heparin. These results are in agreement with our previously reported data on osteosarcoma (Nikitovic et al., 2005) and mesothelioma cells (Syrokou et al., 1999). Similarly, DS, heparin and heparan sulfate have been found to inhibit the proliferation of
normal fibroblasts (Ferrao & Mason, 1993; Westergren-Thorsson et al., 1993). In this study, treatment with CSA (30 μg/ml) resulted in a mild stimulatory effect in both cell lines. Secreted sulfated chondroitin and heparan sulfate, has been shown to block starvation induced apoptosis in lung fibroblasts. Specifically they can induce phosphorylation of Bcl-associated death protein (BAD) and inhibit caspases 3 and 7 cleavage (Cartel & Post, 2005).

Combination of CSA with PDGF-BB resulted in a significant increase of the cell proliferation in both HT1080 and B6FS cells. In contrast, the combined effect of DS and PDGF-BB was either neutral (in HT1080 cells) or inhibitory (in B6FS cells). Concurring with our results, a previous study showed that PDGF-BB binds with high affinity CSA and DS, whereas only DS can displace PDGF-BB binding on the CHO cell surface (Garcia-Olivas et al., 2003). Heparin co-treatment resulted in an inhibitory effect probably due to the highly antiproliferative effects. Summarizing our results suggested that a large molecular excess of exogenous chondroitin sulfate can be co-stimulatory to the PDGF-BB mitogenic function. Nevertheless, we cannot exclude the possibility that the endogenous chondroitin sulfate as well, cell-bound or secreted, is also co-stimulatory but, due to the small in absolute quantity changes induced in our experimental settings, the actual co-stimulatory effect might be undetectable.

In order to examine whether the co-stimulatory effect of CSA on PDGF-BB function was caused by increased PDGFRα and β expression, we analyzed by real-time PCR the transcript levels of both genes after CSA, DS, heparin and PDGF-BB treatment. The mRNA levels of PDGFRβ receptor in the HT1080 cells were not affected by any of the treatments. In B6FS cells, CSA had no effect on neither of the receptors, DS was found to downregulate both PDGFRα and β, whereas heparin downregulated just PDGFRβ. Therefore, the observed co-stimulatory effect of CSA was not due to increased PDGFR expression. Conversely, the demonstrated inhibitory effects of DS and heparin on PDGF-BB mitogenic function in B6FS cells could be partly explained by the reduction of PDGFR gene expression. These results are in agreement with a previous study which showed that in human smooth muscle cells heparin (Lustig et al., 1996), HS, DS, but not CSA (Fager et al., 1995), prevent the proliferation induced by PDGF. Furthermore, it has been shown that GAG chains bound on decorin significantly affect the PDGF function on SMCs as regards to cell proliferation, migration and collagen synthesis (Nili et al., 2003). Finally, we investigated whether the observed co-stimulatory effect of CSA was due to more efficient signalling of the PDGFR by using genistein, a specific inhibitor of tyrosine kinases (Sarkar & Li, 2002). The co-stimulatory effect of CSA to the PDGF-BB-induced mitogenic function on fibrosarcoma cells was completely blocked by genistein, which demonstrated that this effect is perpetrated through tyrosine kinase signalling.

Although PDGF physical interaction with GAGs has been studied in detail, data on the actual result of this interaction on PDGF function are lacking. In conclusion, our results revealed a significant cooperative action of CSA in combination with PDGF-BB on the proliferation of fibrosarcoma cells. The co-stimulatory effect of CSA and PDGF-BB is not due to the transcriptional activation of PDGF receptors, but rather due to more efficient signalling. A theoretical model explaining the observations of this study would suggest that CSA could bind PDGF-BB and present it to PDGFR potentiating the ligand effect in situations where due to low concentrations this interaction with PDGFR would be submaximal. Indeed the maximal co-stimulatory effect was observed in the cell line with very low expression of PDGFRs. This interaction could potentially lead to activation of multiple signalling pathways downstream of the PDGFR, which eventually could control fibrosarcoma cell apoptosis, proliferation or differentiation. The proposed model can find significant application in fibrosarcoma where the microenvironment rich in GAGs/PGs would be able to modify the cellular response to PDGF-BB signalling. Further studies are needed in order to determine if the co-stimulatory effects of CSA can lead to distinct differences in the intracellular pathway that is utilized in the PDGFB-BB signalling.

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