Platelet-Derived Growth Factor-BB–Induced Suppression of Smooth Muscle Cell Differentiation

Bethany J. Holycross, Randal S. Blank, Maria M. Thompson, Michael J. Peach†, and Gary K. Owens

Previously, we demonstrated that treatment of postconfluent quiescent rat aortic smooth muscle cells (SMCs) with platelet-derived growth factor (PDGF)-BB dramatically reduced smooth muscle (SM) α-actin synthesis. In the present studies, we focused on the expression of two other SM-specific proteins, SM myosin heavy chain (SM-MHC) and SM α-tropomyosin (SM-αTM), to determine whether the actions of PDGF-BB were specific to SM α-actin or represented a global ability of PDGF-BB to inhibit expression of cell-specific proteins characteristic of differentiated SMCs. SM-MHC and SM-αTM expression were assessed by one- or two-dimensional gel electrophoretic analysis of proteins from cells labeled with [35S]methionine, as well as by Northern analysis of mRNA levels. Synthesis of both SM-specific proteins was decreased by 50–70% in PDGF-BB–treated cells as compared with cells treated with PDGF vehicle. Treatment of cells with 10% fetal bovine serum, which produced a mitogenic effect equivalent to that of PDGF-BB, decreased SM-MHC synthesis by 40% but increased SM-αTM synthesis. SM-MHC and SM-αTM mRNA expression was decreased by 80% at 24 hours in PDGF-BB–treated postconfluent SMCs, whereas treatment with 10% fetal bovine serum did not decrease the expression of SM-αTM mRNA but did inhibit SM-MHC mRNA expression by 36%. Consistent with the absence of detectable PDGF α-receptors on these cells, PDGF-AA had no effect on either mitogenesis or expression of SM-MHC or SM-αTM. These findings indicate that proliferation, per se, does not coordinately reduce SM-specific protein expression and suggest that circulating or locally produced PDGF-BB may play a generalized role in the modulation of the SMC phenotype to a less differentiated state, a characteristic of SMCs in atherosclerotic blood vessels. (Circulation Research 1992;71:1525–1532)

KEY WORDS • fetal bovine serum • α-tropomyosin • myosin heavy chain • cell culture • rat aortic smooth muscle

Accelerated smooth muscle cell (SMC) growth is known to play an integral role in atherosclerotic lesion formation and is a characteristic feature in arteries of hypertensive patients and animals. The proliferating SMCs within atherosclerotic lesions are phenotypically altered with respect to normal medial SMCs and it has been suggested that the phenotypic state of a vascular SMC may have an important influence on the growth properties of the cell. Therefore, it is of fundamental importance to understand the factors that regulate SMC growth and phenotypic state in order to understand vascular pathobiology. Our recent work has focused on the ability of platelet-derived growth factor (PDGF) and 10% fetal bovine serum (FBS) to induce changes in the expression of the smooth muscle (SM)–specific contractile protein SM α-actin as a marker for change in SM phenotype. Interestingly, although 10% FBS was as mitogenic as PDGF, FBS did not alter the expression of SM α-actin mRNA, whereas PDGF suppressed the expression of SM α-actin mRNA by 70–80%. Additional studies demonstrated that continuous exposure of SMCs to PDGF maintained an 80% inhibition of SM α-actin synthesis but did not maintain cells in a proliferative state, as measured by thymidine labeling indexes. These studies suggest that PDGF plays a major role in control of SMC phenotype and that these effects may be distinct from its role as a mitogen.

SMC differentiation, however, involves coordinate regulation of a repertoire of SM-specific proteins, not solely SM α-actin. Thus, it is possible that the marked suppression of SM α-actin expression after PDGF-BB treatment reflects a specific effect on SM α-actin expression rather than an overall effect on SMC differentiation and maturation. Given that the principal function of
differentiated SMC is contraction, the contractile proteins represent logical candidates to assess the differentiated state of SMCs. Whereas SMC differentiation has been described using a two-state model in which cells exist in either a "contractile" or "synthetic" state, it is important that SMC differentiation be considered as a continuum of SMC phenotypes ranging from that of a committed, but as yet undifferentiated, SMC in the embryo to the mature fully differentiated SMC in a blood vessel. As such, quantitative and qualitative measurements of SM-specific gene products can be used to assess the relative position of cells on the continuum as well as the effects of positive and negative regulators of SM phenotype.

SMCs express a number of isoforms of contractile proteins that are either selective or unique to SM, including SM \( \alpha \)-actin, SM myosin heavy chain (SM-HMC), and SM \( \alpha \)-tropomyosin (SM-\( \alpha \)TM). Previous studies in our laboratory demonstrated that vascular SMCs express two isoforms of myosin heavy chain (MHC), referred to as SM1 (204 kd) and SM2 (200 kd), which are unique to SM, as well as a nonmuscle MHC common to a variety of cell types. SM1 and SM2 isoforms are produced by differential RNA splicing of the 3' region of a single MHC gene, resulting in two RNA species of approximately 6,500 nucleotides. Cultured vascular SMCs express several tropomyosin (TM) isoforms, two of which are found in all mammalian SM tissues examined, with minor species variations. SM-\( \alpha \)TM mRNA is a product of differential mRNA splicing of a single TM gene and is distinct from striated and nonmuscle TM isoforms by virtue of the expression of exon 2. The SM-specific expression of the SM isoforms of MHC and TM makes them appropriate indexes with which to monitor changes in the differentiated state of SMCs.

The principal aim of the present study was to determine whether PDGF affects SM \( \alpha \)-actin selectively or whether it also suppresses expression of other SM-specific proteins such as SM-MHC and SM-\( \alpha \)TM. Additional studies deal with the relation between growth and cytodifferentiation in cultured SMCs by comparing the effects of serum- versus PDGF-BB-induced growth on expression of these SM-specific contractile proteins. Finally, we investigated whether PDGF-BB or PDGF-AA exert differential effects on the expression of SM-specific contractile proteins.

Materials and Methods

Cell Culture

Rat thoracic aortic SMCs were isolated and cultured as described previously. Cells were grown for 5 days after initial confluence in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, N.Y.) and Ham's F12 (GIBCO) containing 10% FBS (Hyclone Laboratories Inc., Logan, Utah), 200 \( \mu \)g/ml L-glutamine, 100 units/ml penicillin (GIBCO), and 100 \( \mu \)g/ml streptomycin (GIBCO). Cells were switched to a defined serum-free medium containing equal parts of Dulbecco's modified Eagle's medium and Ham's F12 supplemented with 1 \( \mu \)M insulin (Sigma Chemical Co., St. Louis, Mo.), 5 \( \mu \)g/ml transferrin (Sigma), 200 \( \mu \)g/ml L-glutamine, 0.2 mM ascorbate (Sigma), 6.25 ng/ml sodium selenite (GIBCO), 100 units/ml penicillin, and 100 \( \mu \)g/ml streptomycin for 5 days to induce quiescence and to promote expression of SM-specific contractile proteins. Phenotypic modulation of SMCs is a sine qua non of cell culture and undoubtedly begins when cells are placed in vitro. However, the culture system used in these studies has been previously described and is widely used for the culture of vascular SMCs, which continue to express SM-specific genes including SM-MHC, SM myosin light chain, SM-\( \alpha \)TM, SM \( \alpha \)-actin, and a variety of other markers appropriate for vascular SMCs and exhibit agonist-induced phosphorylation of myosin light chain and agonist-induced shape changes consistent with contraction (References 9 and 14–16 and authors' unpublished observations). Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO\(_2\)-95% air with media changes every 2–3 days.

RNA Preparation

For the RNA studies, cells were treated for 24 hours with 0.1, 1, 10, or 20 ng/ml PDGF-AA or PDGF-BB (Upstate Biotechnology, Inc.), 10% FBS (Hyclone), or PDGF vehicle (10 mM acetic acid, 2 mg/ml bovine serum albumin) in serum-free medium. Cells were rinsed with cold phosphate-buffered saline, and RNA was extracted by the guanidinium isothiocyanate/CsCl method. Extracted RNA was dissolved in sterile water, and concentration was measured spectrophotometrically. The samples were stored at −70°C until used.

Measurement of Protein Content and Synthesis

Sample preparation, polycrylamide gel electrophoresis, and \(^{35}\)Smethionine incorporation into proteins as a measure of protein synthesis were performed as described previously, with the exception that TM isoforms were resolved on a pH 4.5–5.4 gradient. Whereas no definitive studies have identified the TM isoform encoded by exon 2, we have assigned the designation SM-\( \alpha \)TM to the more basic TM subunit according to the following criteria: It has an apparent molecular mass of approximately 40 kd, shows reduced electrophoretic mobility in the presence of 5 M urea, and is an isoform not detected in any other non-SM tissue examined (authors' unpublished observations). The more acidic TM observed in mammalian cultured SMC and SM tissues, designated TM-1, was found to comigrate with a TM isoform expressed at low levels by human platelets, as well as showing great similarity with a nonmuscle TM reported in a rat embryonic cell line. In addition, cultured SMCs express at least two other nonmuscle TM isoforms, consistent with the nonmuscle TM described by Yamawaki-Kataoka al, with molecular masses of approximately 30 kd, which comigrate with TM isoforms found in platelets and endothelial cells. These are designated TM-4 and TM-5 for the sake of consistency with previous studies.

Densitometric analysis of MHC synthesis was performed on a model FB934 densitometer (Fisher BioTech). Background absorbance was subtracted from each lane. Densitometric analysis of TM gels was performed using the BioImage System (Millipore Corp., Bedford, Mass.) with appropriate background subtraction. Analyses were limited to bands (MHC) or spots (\( \alpha \)TM) with densities in the linear range of the autoradiographic film.
RNA Blots and Hybridization

For gel electrophoresis, 10 μg total RNA was denatured by heating for 10 minutes at 65°C in a solution of 4.4 M formaldehyde and 5% formamide and resolved on a 1% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to a nylon membrane (Micron Separations Inc., Westboro, Mass.) by Northern blotting. The resulting blot was heated to 80°C under vacuum for 2 hours and subsequently exposed to an ultraviolet transilluminator (254 nm, 500 μW/cm²) for 1 minute, air-dried, and stored until used.

Rat SM-MHC mRNA was identified using a 373-bp cDNA probe corresponding to SM2-type MHC (provided by Drs. P. Babij and M. Periasamy, University of Vermont). This cDNA probe recognizes both the SM1 and SM2 isoforms of SM-MHC but not nonmuscle MHC. SM-αTM was identified using a 128-bp cDNA probe specific to exon 2 of the rat SM-αTM gene. The exon 2–specific probe was isolated by the polymerase chain reaction from a cDNA containing exons 1, 2, and 4 of SM-αTM (provided by Drs. M. Taubman and B. Nadal-Ginard, Harvard Medical School, Boston). Exon 2 was subcloned into pGEM 3zf(−), and the linearized plasmid containing the insert was used in random priming reactions. All probes were labeled with [α-32P]dCTP using Prime-a-Gene reagents (Promega Corp., Madison, Wis.). All blots were hybridized and washed using the method of Church and Gilbert. The blots were dried and exposed to Kodak X-Omat AR or K film at −70°C in the presence of an intensifying screen. The optical densities of hybridization signals on x-ray films were measured using a model FB934 densitometer (Fisher Biotech).

Mitogenic Studies

Relative rates of DNA synthesis were assessed by determination of [3H]thymidine incorporation into trichloroacetic acid–precipitable material as previously described. Cells were pulsed for 6 hours (18–24 hours after agonist treatment) with [3H]thymidine (2 μCi/ml) and then washed once with cold phosphate-buffered saline. This was followed by one 10-minute wash with 10% (wt/vol) cold trichloroacetic acid at 4°C and one 10-minute wash with 10% trichloroacetic acid at 22°C. Cells were then dissolved in 1N NaOH, placed in scintillation fluid, and counted. Quadruplicate dishes were analyzed per sample.

Statistical Analysis

Multiple means were first analyzed by analysis of variance (ANOVA). When ANOVA led to rejection of the null hypothesis, means were compared using Dunnett’s tests, with p<0.05 considered to be statistically significant.

Results

SM-MHC and SM-αTM Protein Synthesis Was Decreased by PDGF-BB, Not Affected by PDGF-AA, and Differentially Affected by FBS

Our initial aim was to determine whether PDGF isoforms or 10% FBS altered the expression of SM-MHC or SM-αTM in cultured rat aortic SMCs. SMCs maintained in defined serum-free medium synthesized both the SM2 and nonmuscle isoforms of MHCs.

Table 1. Effects of Platelet-Derived Growth Factor Isoforms and Fetal Bovine Serum on DNA Synthesis and Smooth Muscle Myosin Heavy Chain and Smooth Muscle α-Actin mRNA Levels in Cultured Rat Aortic Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Thymidine incorporation (n=3)</th>
<th>SM-αTM mRNA (n=6)</th>
<th>SM-MHC mRNA (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ng/ml</td>
<td>1.15±0.20</td>
<td>1.30±0.17</td>
<td>0.77±0.23</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>1.16±0.03</td>
<td>1.55±0.15</td>
<td>1.15±0.26</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>1.50±0.22</td>
<td>1.56±0.18</td>
<td>1.07±0.03</td>
</tr>
<tr>
<td>20 ng/ml</td>
<td>1.31±0.11</td>
<td>1.07±0.13</td>
<td>0.78±0.11</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ng/ml</td>
<td>1.75±0.25</td>
<td>1.44±0.27</td>
<td>1.10±0.58</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>3.27±0.62</td>
<td>1.79±0.57</td>
<td>1.29±0.51</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>9.24±1.99*</td>
<td>0.81±0.25</td>
<td>0.94±0.28</td>
</tr>
<tr>
<td>20 ng/ml</td>
<td>11.92±2.83*</td>
<td>0.27±0.75†</td>
<td>0.26±0.08†</td>
</tr>
<tr>
<td>10% FBS</td>
<td>12.99±2.11*</td>
<td>1.28±0.32</td>
<td>0.64±0.28†</td>
</tr>
</tbody>
</table>

SM, smooth muscle; αTM, α-tropomyosin; MHC, myosin heavy chain; PDGF, platelet-derived growth factor; FBS, fetal bovine serum.

*Significantly greater than vehicle treatment by Dunnett’s test (p<0.05).

**Significantly less than vehicle treatment by Dunnett’s test (p<0.05).

PDGF-BB treatment elicited a dose-dependent increase in [3H]thymidine incorporation (Table 1). At a dose of 20 ng/ml, PDGF-BB caused a 12-fold increase in [3H]thymidine incorporation, decreased the synthesis of SM-MHC.
of the SM-specific isoform of MHC by 53% (p<0.05), and increased the synthesis of nonmuscle MHC by 20% (p<0.05) (Figure 1). PDGF-BB (20 ng/ml) decreased the synthesis of SM-αTM by 82% (p<0.05) but did not alter the synthesis of two of the nonmuscle isoforms of TM (TM-1 and TM-4) (Figure 2). In contrast, treatment of cells with 10% FBS elicited a 14-fold increase in [3H]thymidine incorporation, similar in magnitude to the PDGF-BB effect (20 ng/ml) (Table 1), but increased the synthesis of all forms of TM (TM-1 by 450%, SM-αTM by 350%, and nonmuscle TM-4 by 530%, p<0.05) (Figure 2). Treatment with 10% FBS decreased SM-MHC expression by 40% (p<0.05) and increased nonmuscle MHC expression by 20% (p<0.05). PDGF-AA had no effect on [3H]thymidine incorporation or on expression of any of the isoforms of SM-MHC or SM-αTM, consistent with the observation that the SMC cultures used in these studies had no detectable PDGF α-receptor subtypes according to [125I]PDGF-AA binding studies (authors' unpublished observations).

**SM-MHC and SM-αTM mRNA Expression Was Decreased by PDGF-BB, Not Affected by PDGF-AA, and Differentially Affected by FBS**

Northern analyses were performed to determine whether PDGF-BB-induced decreases in synthesis of SM-αTM and SM-MHC were accompanied by corresponding decreases in steady-state mRNA levels. Initially, we ascertained the specificity of the SM-αTM probe, which consisted of the cDNA sequence of exon 2 amplified by the polymerase chain reaction. Skeletal muscle mRNA transcripts (1.25 kb), nonmuscle transcripts (1.8 kb), and SM-αTM transcripts (1.8 kb) were detected by a cDNA probe, consisting of exons 1, 2, and 4, which detected all of the differentially spliced transcripts of the αTM gene (data not shown). The SM-specific cDNA probe encoding exon 2 detected a single transcript in uterus, intestine, and SMC, corresponding in size to SM-αTM (Figure 3).

PDGF-BB (20 ng/ml) treatment significantly decreased SM-MHC (74%, p<0.05) and SM-αTM (73%, p<0.05) mRNA levels (Figures 4 and 5, respectively). No significant effects were observed at lower concentra-

**FIGURE 2.** Left panel: Autoradiographs of two-dimensional gels showing the effects of vehicle (VEH), platelet-derived growth factor (PDGF)-AA (20 ng/ml), PDGF-BB (20 ng/ml), and 10% fetal bovine serum (FBS) on tropomyosin (TM) protein synthesis. Four isoforms of TM were identified in these cells: TM-1, smooth muscle (SM) αTM, and two other forms of nonmuscle TM, designated here as TM-4 and TM-5. Postconfluent quiescent rat aortic smooth muscle cells were labeled with [35S]methionine (50 μCi/ml) between 18 and 24 hours after stimulation with agonist and homogenized in sample buffer. Cell homogenates containing equal amounts of DNA were loaded per gel (2 μg). Counts per minute per gel were the same as in the legend to Figure 1. Right panel: Histogram showing results, after densitometric analyses of gels as illustrated in left panel, of the effects of VEH, PDGF-AA, PDGF-BB, and 10% FBS on TM-1, α-SM, and TM-4 protein synthesis. TM-5 protein synthesis was highly variable between samples and was not analyzed by densitometry. Values represent mean±SEM (n=6–8). *p<0.05 vs. VEH values.

**FIGURE 3.** Autoradiograph of a Northern blot showing the specificity of the exon 2 smooth muscle α-tropomyosin cDNA probe. U, rat uterus RNA; I, rat intestine RNA; SMC, rat aortic smooth muscle cell RNA; SkM, rat skeletal muscle RNA. The cDNA probe was labeled by random priming to a specific activity of 2×10⁶ cpn/μg DNA. Total RNA (10 μg) was loaded per lane.
tions (Table 1). PDGF-AA did not affect SM-MHC and SM-αTM mRNA levels. Interestingly, 10% FBS did not decrease steady-state levels of SM-αTM mRNA but did decrease SM-MHC mRNA levels by 40% (p<0.05) (Figures 4 and 5). These findings are consistent with changes in protein synthesis presented in Figures 1 and 2 and indicate that the effect of PDGF-BB on the synthesis of these proteins is controlled, at least in part, at the mRNA level. The PDGF-BB–induced decrease in SM-MHC and SM-αTM mRNA expression could not be attributed solely to its mitogenic effect, since 10% FBS was equally as efficacious a mitogen for these cells but did not significantly alter SM-αTM mRNA expression (Table 1).

**Ability of PDGF-BB to Decrease SM-Specific Protein Expression Was Not Inhibited by FBS**

It has been suggested that FBS contains significant quantities of PDGF-BB, which may contribute to its mitogenic effect. Our finding that PDGF-BB, but not FBS, downregulates steady-state levels of SM-αTM mRNA suggested that either PDGF-BB was not present in biologically active quantities in the serum used in these studies or that the serum used contained an inhibitor of PDGF-BB action. To test the latter possibility, we coinoculated cells with PDGF-BB and increasing percentages of FBS to determine whether serum inhibited the downregulation of SM-αTM or SM-MHC mRNA levels induced by PDGF-BB (20 ng/ml).

**Figure 4.** Left panel: Autoradiographs of Northern blots depicting the effect of vehicle (VEH), platelet-derived growth factor (PDGF) AA (20 ng/ml), PDGF BB (20 ng/ml), and 10% fetal bovine serum (FBS) on mRNA levels of smooth muscle (SM) myosin heavy chain (MHC). Postconfluent quiescent rat aortic SM cells were treated for 24 hours with agonist. Total RNA (10 µg) was applied per lane, blotted to a nylon membrane, and probed with a SM-specific MHC cDNA probe that was labeled by random priming with [α-32P]dCTP (specific activity, 3×10⁶ cpm/µg DNA). Right panel: Histogram showing the effect of VEH, PDGF AA, PDGF BB, and 10% FBS on SM-myosin heavy chain mRNA levels. Results are from densitometric analysis of Northern blots like those depicted in Figure 3. Values represent mean±SEM (n=6). *p<0.05 vs. VEH value. **p<0.05 vs. VEH and PDGF BB values.

**Figure 5.** Left panel: Autoradiographs of a Northern blot showing the effect of vehicle (VEH), platelet-derived growth factor (PDGF) AA (20 ng/ml), PDGF BB (20 ng/ml), and 10% fetal bovine serum (FBS) on smooth muscle (SM) α-tropomyosin (αTM) mRNA levels. Postconfluent quiescent rat aortic smooth muscle cells were treated for 24 hours with agonist. Total RNA (10 µg) was applied per lane, blotted to a nylon membrane, and probed with an SM αTM probe that was labeled by random priming with [α-32P]dCTP (specific activity, 3×10⁶ cpm/µg DNA). Right panel: Histogram of densitometric analysis of autoradiographs of Northern blots depicted in left panel. Values represent mean±SEM (n=6). *p<0.05 vs. VEH value.
PDGF-BB and FBS had additive effects in stimulating \(^{3}H\)thymidine incorporation into DNA (Table 2). However, addition of FBS did not prevent PDGF-BB-induced decreases in either SM-MHC or SM-\(\alpha\)-TM mRNA expression (Figure 6). Therefore, these data indicate that the failure of FBS to reduce SM-\(\alpha\)-TM was not due to a factor present in FBS that inhibits the effects of PDGF-BB on SMC differentiation.

**Discussion**

The results reported in this article demonstrate that PDGF-BB markedly decreased the expression of two SM-specific contractile proteins, SM-\(\alpha\)-TM and SM-MHC. These results, in addition to previously reported effects of PDGF-BB on SM \(\alpha\)-actin,\(^{7,26}\) are consistent with the hypothesis that PDGF-BB elicits a global suppression of expression of cell-specific proteins characteristic of differentiated SMCs. The ability of PDGF-BB to enhance proliferation and suppress differentiation in SMCs is similar to the effect of PDGF-BB and another growth factor, basic fibroblast growth factor, in skeletal muscle. Treatment with these growth factors stimulates myoblast proliferation and concomitantly decreases cell fusion and expression of skeletal muscle myosin.\(^{24,27}\) Although these results are consistent with the dogma of an inverse relation between cell growth and cytodifferentiation, the nature of this relation in SM remains controversial. \(\alpha\)-Tropomyosin in SM-\(\alpha\)-TM, but not SM \(\alpha\)-actin mRNA,\(^{7}\) is downregulated during logarithmic growth of SMCs in serum-containing medium. Additionally, the ability of heparin to upregulate SM \(\alpha\)-actin expression appears to be correlated

**TABLE 2. Effects of Coadministration of Platelet-Derived Growth Factor-BB and Fetal Bovine Serum on DNA Synthesis in Cultured Rat Aortic Smooth Muscle Cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fold increase over control treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-BB</td>
<td>[(^{3}H)Thymidine incorporation (n=3)]</td>
</tr>
<tr>
<td>20 ng/ml</td>
<td>7.98±0.81*</td>
</tr>
<tr>
<td>20 ng/ml+1% FBS</td>
<td>20.01±4.38*</td>
</tr>
<tr>
<td>20 ng/ml+10% FBS</td>
<td>21.70±3.09*</td>
</tr>
<tr>
<td>20 ng/ml+20% FBS</td>
<td>23.39±2.39*</td>
</tr>
<tr>
<td>1% FBS</td>
<td>7.37±0.60†</td>
</tr>
<tr>
<td>10% FBS</td>
<td>12.19±3.39</td>
</tr>
<tr>
<td>20% FBS</td>
<td>18.07±0.90*</td>
</tr>
</tbody>
</table>

PDGF, platelet-derived growth factor; FBS, fetal bovine serum.

*Significantly greater than PDGF-BB (20 ng/ml) treatment by Dunnett’s test (p<0.05).

†Significantly less than PDGF-BB (20 ng/ml)+1% FBS treatment group by Student’s t test (p<0.05).
with its antiproliferative activity.²⁹ Consistent with results of earlier studies suggesting that SM-MHC expression may be growth-regulated,³⁴ we observed decreased SM-MHC expression with both serum- and PDGF-induced mitogenesis. However, the expression of SM-αTM, as well as previous studies demonstrating that serum-induced growth was not associated with decreased expression of SM α-actin mRNA,⁷,³⁰ indicates that growth, per se, is not associated with coordinate downregulation of the entire differentiation program. These findings indicate a fundamental difference between SMCs and skeletal myoblasts, in that SMCs, unlike skeletal myoblasts, are capable of sustained expression of at least some differentiated proteins during cellular proliferation. Furthermore, results of these studies suggest that the dedifferentiation of SMCs, as occurs in atherosclerotic lesions of humans,⁴,³ as well as myointimal lesions in experimental animal models of atherosclerosis,⁵,³¹ may be the consequence of the action of specific differentiation suppressor molecules such as PDGF-BB, which are known to be present within intimal lesions,³²–³⁴ rather than a direct consequence of accelerated SMC proliferation per se. Indeed, at least in cultured SMCs, PDGF of platelet origin appears to be able to maintain suppression of SM α-actin synthesis after thymidine labeling indexes have returned to basal levels,⁸ further supporting a possible role for PDGF-BB as a differentiation suppressor molecule for SMCs.

The failure of FBS to suppress SM α-actin and SM-αTM expression does not appear to be due to a serum factor that partially inhibits PDGF-BB–associated repression, since the PDGF-induced inhibition of expression of SM α-actin protein²⁶ or SM-MHC and SM-αTM mRNAs was not blocked by high concentrations of serum. Interestingly, no other purified growth factor, including insulin (a necessary component of serum-free medium), transforming growth factor-β, basic fibroblast growth factor, or epidermal growth factor, appears to inhibit SM α-actin expression in our SMCs, suggesting a unique role for PDGF-BB in the control of dedifferentiation of SMCs (Reference 26 and authors’ unpublished observations). It was observed, however, that 10% FBS elicited a modest decrease in SM-MHC mRNA expression, suggesting a role for serum factors in SMC differentiation. These results are in contrast to those reported recently by Babij et al,³⁵ who reported that withdrawal of 10% FBS for 2 days did not elicit an increase in SM-MHC mRNA in confluent SMCs. Although the mechanisms that underlie the differences in action among these mitogens are unknown, these studies clearly indicate that the extent of repression of SM-specific protein expression is not a simple function of the extent of cellular proliferation.

To date, PDGF-BB has proven to be capable of suppressing protein and mRNA expression of three SM-specific proteins. The correlation between the decrease in SM-αTM and SM-MHC mRNA levels and protein synthesis would suggest a transcriptional control of expression. However, results of our previous studies indicate that PDGF-BB–induced decreases in SM α-actin expression are not mediated transcriptionally but rather via selective decreases in the stability of the SM α-actin mRNA.³⁶ It remains to be determined whether PDGF-BB–induced changes in SM-αTM and SM-MHC expression are under transcriptional control.

PDGF-induced downregulation of SM-αTM and SM-MHC in the present studies appeared to be mediated via the PDGF β-receptor, since the cultured SMCs used in these studies lacked detectable PDGF α-receptors, according to [¹²⁵I]PDGF-AA binding studies, and failed to undergo a mitogenic response to PDGF-AA (authors’ unpublished observations). These results are consistent with our previous observations showing that repression of SM α-actin by PDGF was not dependent on the PDGF-A chain. It is estimated that at least 15,000–30,000 active α-subunits per cell are required for PDGF-AA–induced mitogenesis.³⁷,³⁸ Since the SMCs used in these studies lacked this number of receptors and failed to respond mitogenically to PDGF-AA, our results cannot be interpreted as evidence for qualitatively different effects of PDGF α- versus β-receptors on SMC differentiation, since it is possible that, under conditions in which SMCs express sufficient α-receptor subunits, PDGF-AA might elicit similar effects.

In summary, results of these and previous studies show that PDGF-BB is an extremely potent and efficacious agent in inducing coordinate downregulation of expression of multiple SM-specific proteins. In contrast, serum failed to downregulate SM α-actin and SM-αTM and only modestly decreased SM-MHC expression. These results suggest that PDGF-BB may play an important role in the regulation of the differentiated state of SMCs and that this role may be distinct from its role as a mitogen.

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References

12. Wieczorek DF, Smith CWJ, Nadal-Ginard B: The rat α-tropomyosin gene generates a minimum of six different mRNAs coding for


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