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# Sp1, Acetylated Histone-3 and p300 Regulate TRAIL Transcription: Mechanisms of PDGF-BB-Mediated VSMC Proliferation and Migration

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## ABSTRACT

We recently reported that TNF-related apoptosis-inducing ligand (TRAIL) is important in atherogenesis, since it can induce vascular smooth muscle cell (VSMC) proliferation and arterial thickening following injury. Here we show the first demonstrate that TRAIL siRNA reduces platelet-derived growth factor-BB (PDGF-BB)-stimulated VSMC proliferation and migration. PDGF-BB-inducible VSMC proliferation was completely inhibited in VSMCs isolated from aortas of TRAIL$^{-/-}$ mice; whereas inducible migration was blocked compared to control VSMCs. TRAIL transcriptional control mediating this response is not established. TRAIL mRNA, protein and promoter activity was increased by PDGF-BB and subsequently inhibited by dominant-negative Sp1, suggesting that the transcription factor Sp1 plays a role. Sp1 bound multiple Sp1 sites on the TRAIL promoter, including two established (Sp1-1 and -2) and two novel Sp1-5/6 and -7 sites. PDGF-BB-inducible TRAIL promoter activity by Sp1 was mediated through these sites, since transverse mutations to each abolished inducible activity. PDGF-BB stimulation increased acetylation of histone-3 (ac-H3) and expression of the transcriptional co-activator p300, implicating chromatin remodelling. p300 overexpression increased TRAIL promoter activity, which was blocked by dominant-negative Sp1. Furthermore, PDGF-BB treatment increased the physical interaction of Sp1, p300 and ac-H3, while chromatin immunoprecipitation studies revealed Sp1, p300 and ac-H3 enrichment on the TRAIL promoter. Taken together, our studies demonstrate for the first time that PDGF-BB-induced TRAIL transcriptional activity requires the cooperation of Sp1, ac-H3 and p300, mediating increased expression of TRAIL which is important for VSMC proliferation and migration. Our findings have the promising potential for targeting TRAIL as a new therapeutic for vascular proliferative disorders. J. Cell. Biochem. 113: 2597–2606, 2012. 

**KEY WORDS:** TRAIL; TRANSCRIPTION; VSMC; PROLIFERATION AND MIGRATION

Atherosclerosis can lead to heart disease and stroke, and is the most common cause of mortality in the Western world [Lusis, 2000]. Migration of vascular smooth muscle cells (VSMCs) from the media to the intima, and subsequent VSMC proliferation are important events contributing to atherosclerotic lesion development [Ross and Glomset, 1973; Ross, 1995]. VSMC proliferation is also associated with several clinical conditions such as in-stent restenosis, transplant vasculopathy and vein bypass graft failure [Dzau et al., 2002]. Growth factors and cytokines have been detected in atherosclerotic lesions, as well as injury-induced vascular diseases, and these factors can influence proliferation, migration and extracelluar matrix synthesis in VSMCs [Ross, 1993; Chan et al., 2010].

The platelet-derived growth factor (PDGF) family is composed of PDGF-A, -B, -C and -D. PDGF's can interact with their receptors, PDGFR-α and PDGFR-β, in a ligand-dependent and cell specific manner, and are key drivers of VSMC proliferation and migration [Raines, 2004]. Upon binding its receptors, PDGFs can activate

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multiple pathways that regulate VSMC growth such as MAPK, ERK and PI3K [Tallquist and Kazlauskas, 2004]. Although all PDGFs can form functional homodimers, only PDGF-A and PDGF-B can form functional heterodimers [Raines, 2004]. PDGF-BB can bind homodimers and heterodimers of PDGFR-α and -β, whereas PDGF-AA is selective and only binds to PDGFR-α homodimers [Seifert et al., 1989]. PDGF-BB and PDGFR-β are important in atherosclerosis, since PDGF-B is expressed in macrophages, and both PDGF-B and PDGFR-β are expressed in VSMCs within atherosclerotic lesions [Rubin et al., 1988; Evanko et al., 1998]. Inhibition of PDGF-B and PDGFR-β reduced VSMC accumulation in the rat carotid injury model [Leppanen et al., 2000; Noiseux et al., 2000] and inhibition of PDGFR-β but not PDGFR-α blocked atherosclerotic lesion size and number of intimal VSMCs [Sano et al., 2001].

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of ligands known to regulate apoptotic and non-apoptotic functions of cells [Kavurma and Bennett, 2008]. In humans, TRAIL protein is abundantly expressed in VSMC-rich regions of failed saphenous vein bypass grafts, co-localising with proliferating cell nuclear antigen, a marker for proliferation [Kavurma et al., 2008a]. We have shown that TRAIL plays an important role in VSMC proliferation in part, by regulating the expression of insulin-like growth factor-1 receptor (IGF1R) [Kavurma et al., 2008a], a potent mitogen for VSMCs. Using TRAIL$^{-/-}$ and wild-type mice, we recently showed that TRAIL induction after vascular injury involves fibroblast growth factor-2 (FGF-2), phosphorylation of the transcription factor Sp1, and its interaction with NFκB on the TRAIL promoter; this led to VSMC proliferation and intimal hyperplasia [Chan et al., 2010]. Regardless of this new information, the transcriptional control of TRAIL is not fully established. Our findings raised the possibility that other potent growth factors may also regulate TRAIL expression. Here we demonstrate that PDGF-BB's proliferative and migratory effects on VSMCs, is mediated in part by TRAIL, and that PDGF-BB-induced TRAIL transcriptional activity involves Sp1, p300 and acteylation of histone-3 (ac-H3). Thus, understanding the responses of growth factors and cytokines to VSMC proliferation, may lead to better strategies in inhibiting intimal thickening in the vessel wall.

## MATERIALS AND METHODS

### PLASMID CONSTRUCTS

pGL-1523Luc has been described before and kindly obtained from Mark Evers (Human Biological Chemistry and Genetics, University of Texas Medical Branch, Texas) [Wang et al., 2000]. pEBG-NLS and pEBG-Sp1 were obtained from Gerald Thiel (Institute for Genetics, University of Cologne, Cologne, France). pCMVβ and pCMVβ-p300 have been described [Zhang et al., 2003]. mSp1-1 and mSp1-2 pGL-1523Luc have also been described [Chan et al., 2010]. mSp1-5/6 and mSp1-7 pGL-1523Luc were generated using the QuickChange Site-directed Mutagenesis kit (Stratagene, La Jolla, CA) using oligos; mSp1-5/6: 5′-GGGAAAAGCAAAGAAAATCAATCAAATCCTTGG-CTGAGGACAT TATCAAAAGG-3′, mSp1-7: 5′-GGAGAGGAGCT-TCTTTCAGTTTAAATAA TTTCCAACGACTACTTTGAGACAAGAGC-3′ (mutation in bold and underlined).

### CELL CULTURE AND REAGENTS

WKY12-22 pup-rat VSMCs were cultured in Waymouth's medium (Invitrogen, Carlsbad, CA), containing 10% fetal bovine serum (FBS). Primary human VSMCs were cultured in Waymouth's medium (Invitrogen). Additional supplements to cells included 10 μg/ml streptomycin, 10 U/ml penicillin and 1 mM L-glutamine. Cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$. Primary VSMCs were not used beyond passage 10. Unless specified, WKY12-22 pup-rat VSMCs were used in all transfection and luciferase assays. Rat, mouse and human recombinant PDGF-BB were purchased from Sigma–Aldrich (St Louis, MO). Recombinant Sp1 was purchased from Promega (Madison, WI). Suberoylanilide hydroxamic acid (SAHA) was obtained from Cayman Chemicals (Ann Arbor, MI).

### MICE

C57Bl6 wild type (WT) mice were purchased from ARC (Perth, Australia). TRAIL$^{-/-}$ mice were originally sourced from AMGEN and described previously [Chan et al., 2010]. Male mice 6–12 week of age weighing approx. 20 g were used in specific pathogen-free conditions, according to the Animal Care and Ethics Committee guidelines at UNSW (Sydney, Australia). Primary VSMC isolation from TRAIL$^{-/-}$ and WT mice was carried out as described previously [Lynn Ray et al., 2001; Chan et al., 2010], and cultured in high glucose DMEM medium containing 20% FBS, 10 μg/ml streptomycin, 10 U/ml penicillin and 1 mM L-glutamine. Cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ and not used beyond passage 10.

### TRANSIENT TRANSFECTION ANALYSIS

WKY12-22 pup-rat VSMCs were seeded into six-well titre plates (WTPs) and at 40% confluence, cells were starved for 24 h. Cells were then transfected with indicated constructs using FuGENE6 transfection reagent (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions, and 24 h later, rat PDGF-BB (50 ng/ml) was added. Cells were harvested after 24 h, and luciferase activity measured using the Luciferase assay system (Promega). For RNA expression studies, pup-rat VSMCs were seeded into 100 mm plates. Following serum withdrawal (24 h), cells were transfected with pCMVβ or pCMVβ-p300. Cells were harvested for RNA 24 h later.

### PROLIFERATION ASSAYS

Wild type and TRAIL$^{-/-}$ VSMCs were seeded into a 96-WTP (6,000 cells/well). Forty-eight hours later cells were serum arrested. The following day, mouse PDGF-BB (50 ng/ml) was added and total cell numbers were counted using a Coulter counter after 72 h. Twenty percent FBS was used as a positive control. For TRAIL knockdown studies, human VSMCs (6,000 cells/well) were seeded into a 96-WTP. After 48 h, cells were serum-arrested. Twenty four hours later, growth quiescent cells were transfected with 200 nM small interfering RNA (siRNA) targeting human TRAIL (Santa Cruz Biotechnology, Santa Cruz, CA; sc-36719: a pool of three target-specific 19–25 nt siRNAs) or AllStar control siRNA (Qiagen, Valencia, CA) using FuGENE6 for 3 h prior to addition of human

PDGF-BB (50 ng/ml). After 72 h, cells were washed with PBS, trypsinised and total cell counts measured using a Coulter Counter.

### IN VITRO SCRATCH ASSAY

Human, WT and TRAIL$^{-/-}$ VSMCs were seeded into 6-WTPs. Cells were serum-arrested at confluence for 24 h. Growth quiescent human VSMCs were transfected with 200 nM TRAIL siRNA or AllStar control siRNA for 3 h, prior to scraping injury as previously described [Kavurma and Khachigian, 2003a]. The medium was replaced followed by the addition of human PDGF-BB (50 ng/ml). For WT and TRAIL$^{-/-}$ VSMCs, mouse PDGF-BB (50 ng/ml) was added following scraping injury. Cell growth in the denuded area was assessed after 48 h; Eight images from each sample were taken (4X magnification). Percentage of cellular regrowth in the denuded zone was determined by morphometric analysis using Adobe Photoshop Software.

### RNA EXTRACTION AND REAL-TIME QUANTITATIVE PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) or TRI reagent (Sigma–Aldrich), and cDNA generated using Superscript II (Invitrogen) or iSCRIPT (Bio-Rad, Hercules, CA). Taqman gene expression assay system (Applied Biosystems) probes for rat TRAIL and rat β-actin were used, and mRNA expression analysed using the Rotor-Gene 3000 (Qiagen, Doncaster, Victoria, Australia). Values were normalised to β-actin. For human TRAIL and GAPDH expression, SYBR Green (Bioline, Alexandria, NSW, Australia) was used. Human TRAIL forward primer; 5′-ACCAACGAGCT-GAAGCAGAT-3′, reverse primer; 5′-CAAGTGCAAGTTGCTCAGGA-3′, human GAPDH forward primer; 5′-GAAGGCTGGGGCTCATTT-3′, reverse primer; 5′-CAGGAGGCATTGCTGATGAT-3′. Values were normalised to GAPDH. Thermal cycling conditions were as follows: 94°C for 10 min followed by 50 cycles at 94°C for 20 s, 60°C for 45 s and 72°C for 20 s.

### ELECTROPHORETIC MOBILITY SHIFT ASSAYS (EMSAs)

Recombinant Sp1 was incubated in binding buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.2 mM DTT, 1 mM EDTA, 2.5% glycerol, 0.2% Nonidet P-40, 1 mg/ml poly(dIdC)) and radiolabelled probe. Bound complexes were resolved by 8% non-denaturing PAGE, gels were vacuum-dried, exposed to X-ray film, and visualised by autoradiography. 5′ oligo sequences are as follows: Oligo Sp1-1 and Oligo Sp1-2 have been described [Chan et al., 2010], Oligo Sp1-3: 5′-ACATCTGATAGTG**GGGAG**ATTTGGGGCTGG-3′ (Sp1-3 site underlined), Oligo Sp1-4: 5′-ACTTCCTACCT**GTCC**AGCCTAACACA-CAGG-3′ (Sp1-4 site underlined), Oligo Sp1-5/6: 5′-GGGAAAAG-CAAAGAAAATC**CCTCCCCTCC**TTGGCTGAGGACATTATCAAAAGG-3′ (Sp1-5/6 site bold and underlined), Oligo Sp1-7: 5′-TTCTT-TCAGTTT**CCCTCC**TTTCCAACGACT-3′ (Sp1-7 site underlined). Sequence of mutant oligos are: mSp1-5/6: 5′-GGGAAAAGCAA-AGAAAATC**AA**TC**AAA**TCCTTGGCTGAGGACATTATCAAAAGG-3′, mSp1-7: 5′-TTCTTTCAGTTT**AAA**T**AA**TTTCCAACGACT-3′ (mutation in bold and underlined).

### CHROMATIN IMMUNOPRECIPITATION (ChIP)

Chromatin immunoprecipitation (ChIP) was performed as described [Nelson et al., 2006] using human VSMCs treated with PDGF-BB (50 ng/ml) for 24 h. Antibodies (0.5 μg) used include: Sp1 (Santa Cruz Biotechnology), p300 (Santa Cruz Biotechnology), ac-H3 (Cell Signalling, Baverly, MD), and rabbit IgG (Millipore, Billerica, MA). The human TRAIL promoter was amplified by real time PCR using SYBR Green (Applied Biosystems, Foster City, CA) as previously described [Chan et al., 2010]. Primers sequences were; Sp1-5/6 site, 5′-AAACAGGCCTTGTGCCTATG-3′ (forward), 5′-ATCCATGCACC-CCTTATCTG-3′ (reverse), Sp1-1/-2/-7, 5′-GGAGAGCAAGAAAGA-GAAGAGAGA-3′ (forward), 5′-GTGAGGAAATGAAAGCGAATG-3′ (reverse). Thermal cycling conditions were as follows: 95°C for 10 min followed by 60 cycles at 95°C for 10 s, 60°C for 15 s and 72°C for 20 s.

### Co–IMMUNOPRECIPITATION (Co–IP)

Whole cell lysates (300–500 μg) from PDGF-BB-treated human VSMCs were used in Co-IP analysis. Immunoprecipitation was performed using Reversible Catch and Release Immunoprecipitation System® v2.0 (Millipore) according to manufacturer's instructions. Briefly, whole cell lysates were incubated with 10 μg of rabbit polyclonal Sp1 antibody (Santa Cruz Biotechnology) and antibody capture affinity ligand at 4°C with gentle rotating. Bound immunoprecipitates were washed and eluted. The eluted samples were separated on 6% and 12% SDS-PAGE or gradient SDS PAGE (4–15%), followed by Western blotting.

### WESTERN BLOTTING

WKY12-22 pup-rat VSMCs and/or human VSMCs were serum-arrested for 24 h prior to PDGF-BB or SAHA exposure for another 24 h. Total protein was harvested as described [Kavurma and Khachigian, 2003b]. Soluble TRAIL protein was detected after concentrating the culture medium (Amicon Ultra-4 Centrifugal Filter Units, Milipore). Protein were resolved on 6, 12 and 14% SDS-PAGE or gradient SDS PAGE (4–15%), and transferred onto Immobilon-P PVDF membranes (Milipore). Membranes were blocked with 5% skim milk, followed with primary antibody incubation overnight at 4°C or 1 h at RT. Secondary antibodies conjugated with horseradish peroxidase were used. Proteins were detected by chemiluminescence (ECL™ Western Blotting Detection Reagent, GE Healthcare, Chalfont St Giles, Buckinghamshire, UK). Antibodies used include: rabbit polyclonal Sp1 (1:500; Santa Cruz Biotechnology), rabbit polyclonal TRAIL (1:200; Abcam, Bambridge, UK), rabbit polyclonal ac-H3 (1:1,000; Cell Signalling), rabbit polyclonal H3 (1:1,000; Cell Signalling), rabbit polyclonal p300 (1:1,000, Santa Cruz Biotechnology), mouse monoclonal β-actin (1:30,000, Sigma–Aldrich), and mouse monoclonal α-tubulin (1:2,000, Sigma–Aldrich).

### STATISTICAL ANALYSIS

Data were analysed using Microsoft Excel and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). All results were expressed as mean ± SEM. Where appropriate, statistical comparisons were performed using Student's *t*-test, or ANOVA (one or two-way) using Bonferroni's multiple comparison test. $P < 0.05$ was considered significant.

## RESULTS

### TRAIL IS INVOLVED IN PDGF-BB-INDUCED VSMC PROLIFERATION AND MIGRATION

Our recent demonstration that TRAIL plays a pro-proliferative and pro-survival role for VSMCs both in vitro and in vivo [Kavurma et al., 2008a; Chan et al., 2010], provides new insight into how proliferation and other important processes related to growth function are regulated by TRAIL. However, whether TRAIL plays a role in PDGF-BB-induced proliferation and migration is unclear. PDGF-BB significantly induced human VSMC proliferation (Fig. 1A). TRAIL siRNA significantly inhibited PDGF-BB-induced proliferation, whereas the AllStar control siRNA had no effect (Fig. 1A). In addition to proliferation, PDGF-BB can mediate VSMC migration. We next assessed the role of TRAIL in PDGF-BB-stimulated migration of human VSMCs. TRAIL siRNA reduced PDGF-BB-stimulated migration following scratch injury, as compared with the control siRNA (Fig. 1B). This is the first demonstration implicating TRAIL in the migration of VSMCs. TRAIL knockdown by TRAIL siRNA was confirmed at the level of RNA ($47.1 \pm 1.3\%$ inhibition, $P < 0.0001$) and protein (Fig. 1C,D). To further validate a role for TRAIL in PDGF-BB-mediated VSMC proliferation and migration, we used VSMCs isolated from WT and TRAIL$^{-/-}$ mice. WT VSMCs proliferation and migration was significantly induced by PDGF-BB (Fig. 1E,F). In contrast, TRAIL$^{-/-}$ VSMC response to PDGF-BB was significantly impaired (Fig. 1E,F). In proliferation assays TRAIL$^{-/-}$ VSMCs did not even respond to PDGF-BB (Fig. 1E). In contrast, PDGF-BB-induced migration was blocked in the TRAIL-deficient cells (Fig. 1F). These findings suggest that TRAIL regulates PDGF-BB-inducible proliferation and in part, PDGF-BB-inducible migration of VSMCs.

### PDGF-BB INDUCES TRAIL GENE EXPRESSION AND PROMOTER ACTIVITY IN VSMCs

Whether PDGF-BB can directly regulate TRAIL transcription and gene expression is unknown. To assess this, transient transfections were performed in pup-rat VSMCs using pGL-1523Luc, a luciferase

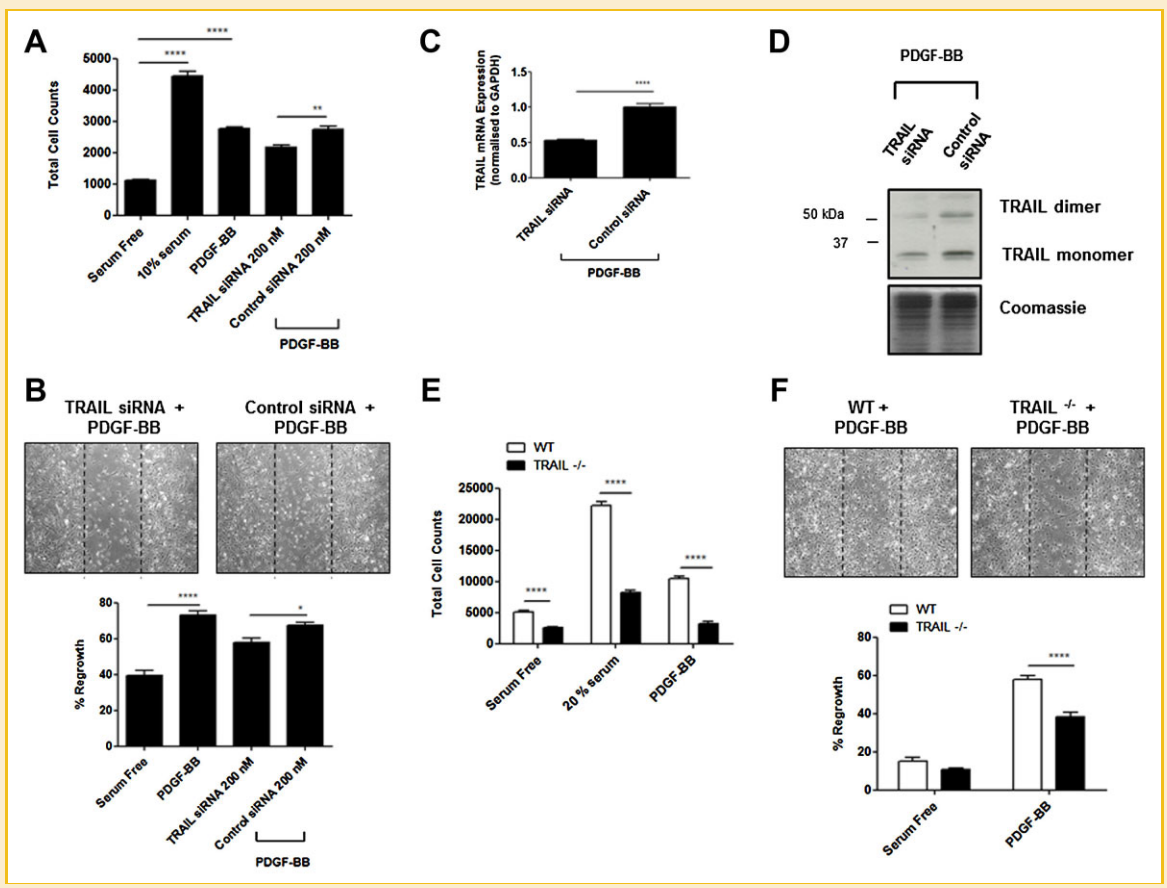


Fig. 1. PDGF-BB-induced VSMC proliferation and migration involves TRAIL. A: TRAIL siRNA inhibits PDGF-BB-induced human VSMC proliferation (four replicates/treatment). B: PDGF-BB-induced human VSMC migration is inhibited by TRAIL siRNA (2 replicates/treatment). Top panel: Representative images (4X magnification) demonstrating reduced VSMC migration in the presence of TRAIL siRNA compared with control siRNA. Bottom panel: Cellular growth in the denuded zone was assessed 48 h later by morphometry. C: TRAIL mRNA as assessed by qPCR (four replicates/treatment) and (D) protein expression demonstrates knockdown of TRAIL in human VSMCs transfected with TRAIL siRNA for 24 h. Coomassie demonstrates unbiased loading. E: PDGF-BB-induced VSMC proliferation is inhibited in TRAIL$^{-/-}$ versus WT VSMCs (four replicates/treatment). F: VSMC migration induced by PDGF-BB is inhibited in TRAIL$^{-/-}$ VSMCs (two replicates/treatment). Figures are representative of 3–4 independent experiments; Results are mean $\pm$ SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

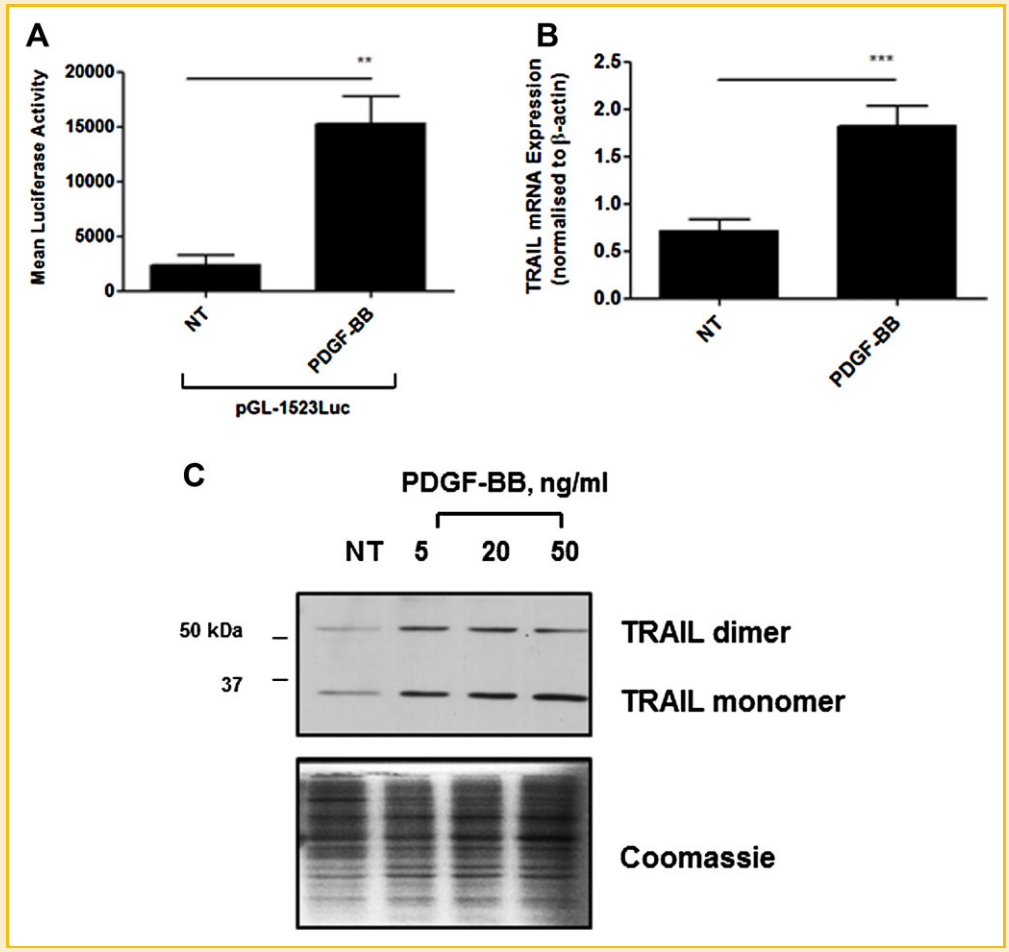


Fig. 2. PDGF-BB regulates TRAIL gene expression in VSMCs. A: PDGF-BB activates the human TRAIL promoter. Pup-rat VSMCs were transfected with 2 μg pGL-1523Luc for 24 h before addition of rat PDGF-BB (50 ng/ml) for another 24 h. Cells were harvested and luciferase activity was measured (three replicates/treatment). B: Serum starved pup-rat VSMCs were treated with rat PDGF-BB (50 ng/ml) for 24 h. TRAIL mRNA expression was determined by real-time PCR. Data were normalised to β-actin (three replicates/treatment). C: Western blotting for soluble TRAIL protein in pup-rat VSMCs exposed to PDGF-BB as described in the methods. Concentrated media was resolved on 12% SDS-PAGE. Coomassie demonstrates unbiased loading. Figures are representative of 2–3 independent experiments; Results are expressed as mean ± SEM. NT, no treatment; $^{**}P < 0.01$, $^{***}P < 0.001$.

reporter construct driven by 1,500 bp of the human TRAIL promoter [Wang et al., 2000]. PDGF-BB treatment for 24 h increased TRAIL luciferase activity (Fig. 2A) and mRNA (Fig. 2B). PDGF-BB also increased protein levels of TRAIL (Fig. 2C). This is the first demonstration that TRAIL is positively regulated by the potent growth factor PDGF-BB in VSMCs.

### Sp1 IS REQUIRED FOR PDGF-BB-INDUCIBLE TRAIL TRANSCRIPTION

We previously demonstrated that TRAIL transcription following vascular injury and FGF-2 treatment, is mediated via two Sp1-binding sites (Sp1-1 and Sp1-2) [Chan et al., 2010]. To determine whether activation of TRAIL transcription by PDGF-BB is also under the positive influence of Sp1, transient transfection and luciferase assays were performed using a dominant-negative Sp1 (pEBG-Sp1) expression vector and its backbone (pEBG-NLS). The Sp1 protein generated from pEBG-Sp1 has an intact DNA binding domain, however, it does not have an activation domain, and hence is unable to "transactivate," competing with endogenous Sp1 [Petersohn and Thiel, 1996]. In our system, basal and PDGF-BB-inducible TRAIL promoter activity was blocked in the presence of pEBG-Sp1 (Fig. 3A). Upon careful inspection of the human TRAIL promoter we identified additional Sp1 binding elements (Fig. 3B; Sp1-3, Sp1-4, -5/6 and -7) and performed EMSA using recombinant Sp1. Consistent with our previous findings [Chan et al., 2010], recombinant Sp1 bound Sp1-1 and Sp1-2 (Fig. 3C). Sp1-5/6 and Sp1-7 also bound Sp1 in a specific manner, since mutation to the GC-rich Sp1 element(s) abolished binding (Fig. 3D). No binding was observed for sites Sp1-3 or -4 (Fig. 3C).

We next generated transverse mutations in pGL-1523Luc to assess the functionality of these Sp1-sites able to bind recombinant Sp1 (5/6 and 7; Fig. 3D). Mutation in Sp1-2 significantly reduced basal activity whereas mutation in Sp1-1, -5/6 and -7 did not significantly reduce basal activity. Mutation in Sp1-1 reduced PDGF-BB-inducible TRAIL promoter activity whereas mutation in Sp1-2 completely inhibited luciferase activity, suggesting that this site is essential for TRAIL transcription (Fig. 3E). Mutation in sites

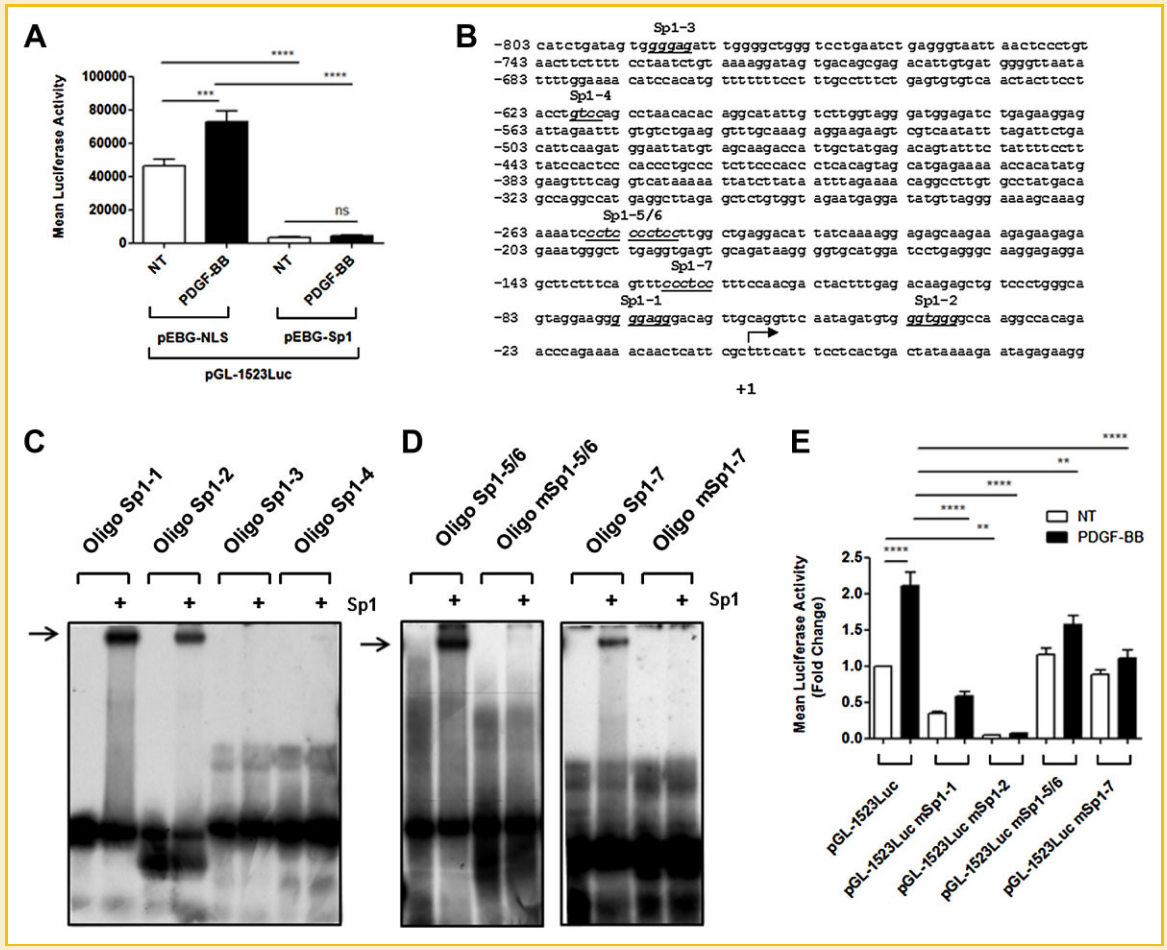


Fig. 3. PDGF-BB activation of the TRAIL promoter is Sp1 dependent. A: TRAIL promoter activity after transfection with dominant-negative Sp1 (pEBG-Sp1). Pup-rat VSMCs were serum-arrested and co-transfected with 2 μg pEBG-Sp1 or control pEBG-NLS together with 2 μg pGL-1523Luc for 24 h followed by rat PDGF-BB (50 ng/ml) exposure for a further 24 h. Luciferase activity was measured (n = 2 experiments). B: The human TRAIL promoter sequence (accession number: AF178756); the transcription start site was described previously [Wang et al., 2000]. Sp1 binding sites are indicated in **bold**, *italics* and underlined. C: EMSA with $^{32}$P-labelled oligonucleotides to Sp1-1, Sp1-2, Sp1-3, and Sp1-4, and recombinant human Sp1. D: EMSA with $^{32}$P-labelled oligonucleotides to Sp1-5/6, Sp1-7 and mutant oligos, mSp1-5/6 and oligo mSp1-7, together with recombinant human Sp1. E: TRAIL promoter activity with mutations to Sp1-1, -2, -5/6 and -7 in pGL-1523Luc. Serum arrested pup-rat VSMCs were transfected with 2 μg pGL-1523Luc or pGL-1523Luc bearing mutated Sp1-binding sites for 24 h followed by PDGF-BB (50 ng/ml) treatment for another 24 h. Luciferase activity was measured (n = 3 experiments). Figure C and D are representative of 2–3 independent experiments; Results are mean ± SEM. NT, no treatment; $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Sp1-5/6 and Sp1-7 also inhibited PDGF-BB-inducible TRAIL transcription (Fig. 3E). These findings demonstrate that mutation to any one of the four Sp1 binding elements (Sp1-1, -2, -5/6 and -7) reduces transcriptional activity, and that each element is critical for PDGF-BB-inducible TRAIL transcription.

### PDGF-BB MODULATES CHROMATIN BY ENHANCING ac-H3 AND p300 PROTEIN EXPRESSION IN VSMCs

Regulation of gene expression involves interactions between chromatin remodelling factors and recruitment of transcription factors [Davie et al., 2008], including histone acetyltransferases (HATs) and histone deacetylases (HDAC). HATs induce hyperacetylation of histone resulting in open chromatin structure, and in turn, activating transcription. Conversely, HDACs mediate gene repression by catalyzing the removal of acetyl groups, resulting in closed chromatin [Cairns, 2001; Selvi and Kundu, 2009]. We next used the HDAC inhibitor, SAHA, as a model to modulate changes to chromatin. HDAC inhibition by SAHA led to increased acetylated histone-3 (ac-H3) expression without altering total H3 protein levels (Fig. 4A). SAHA also activated TRAIL transcription and protein expression (not shown). Recent studies have implicated PDGF-BB in chromatin remodelling events [Yoshida et al., 2007]. Serum-starved human VSMCs were exposed to various concentrations of PDGF-BB for 24 h, followed by Western blotting. Like SAHA (Fig. 4A), PDGF-BB exposure increased ac-H3, without affecting total H3 (Fig. 4B), suggesting that PDGF-BB can also modulate chromatin modifying proteins in VSMCs. p300 is a transcriptional co-activator with intrinsic HAT activity [Ogryzko et al., 1996]. Interestingly, PDGF-BB also increased expression of p300 (Fig. 4C), and overexpression of p300 increased TRAIL promoter activity and mRNA expression (Fig. 4D,E). Taken together, these findings suggest that TRAIL

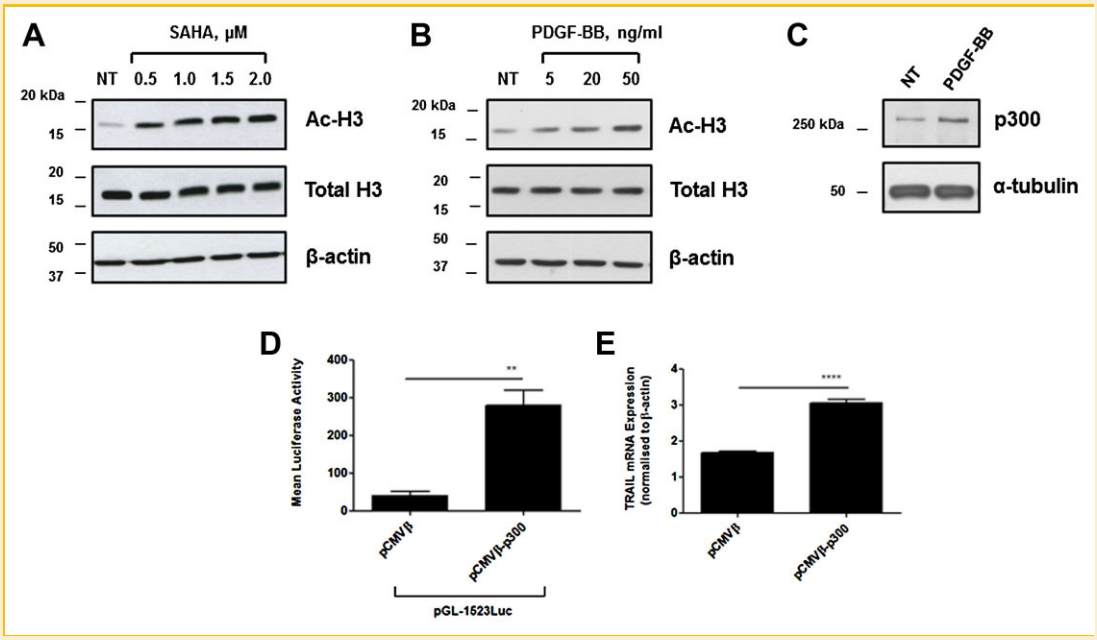


Fig. 4. PDGF-BB increases ac-H3 and p300 levels in VSMCs. A: Western blotting for acetylated-H3 (ac-H3) and total H3 in human VSMCs treated with SAHA for 24 h. β-actin demonstrates unbiased loading. B: Western blotting for ac-H3 and total H3 in human VSMCs treated with indicated concentrations of PDGF-BB for 24 h. β-actin demonstrates unbiased loading. C: Western blotting demonstrating that PDGF-BB increases p300 protein expression in human VSMCs. α-tubulin demonstrates unbiased loading. D: p300 overexpression (pCMVβ-p300, 6 μg) increases TRAIL transcriptional activity in pup-rat VSMCs compared to the backbone control (pCMVβ) (three replicates/treatment). E: pCMVβ-p300 overexpression (6 μg) in pup-rat VSMCs, increases TRAIL mRNA (normalised to β-actin) (four replicates/treatment). Figures are representative of 2–4 independent experiments; results are mean ± SEM. NT, no treatment; $^{**}P < 0.01$, $^{****}P < 0.0001$.

transcription and expression may be regulated by modification to chromatin induced by PDGF-BB.

### PDGF-BB ENHANCES Sp1, ac-H3 AND p300 INTERACTION IN VSMCs

Since we found that Sp1 is important for PDGF-BB-inducible TRAIL promoter activity, and that PDGF-BB increases p300, this suggested that Sp1 and p300 may interact. To address this, we performed co-transfection studies using pEBG-Sp1 and pCMVβ-p300. p300-inducible TRAIL promoter activity was inhibited by dominant-negative Sp1 in a dose-dependent manner (Fig. 5A). Furthermore, Co-IP experiments revealed that the physical interaction of Sp1 and p300 was enhanced in the presence of PDGF-BB (Fig. 5B). Sp1's interaction with ac-H3 was also enhanced after PDGF-BB treatment in VSMCs (Fig. 5C). Indeed Sp1, p300 and ac-H3 were enriched on the TRAIL promoter after PDGF-BB treatment (Fig. 5D). Taken together, these findings suggest that Sp1, ac-H3 and p300 interact on the TRAIL promoter to stimulate transcription and TRAIL gene expression.

## DISCUSSION

PDGF-BB is a potent growth factor for VSMCs and has been strongly implicated in the development of atherosclerosis. Our studies provide new insights into mechanisms for PDGF-BB-induced VSMC proliferation and migration, by revealing a novel role for TRAIL in this process. TRAIL, was originally identified in 1995, as a member of the TNF family having the ability to induce apoptosis [Wiley et al., 1995]. Upon binding to its receptors, TRAIL can also initiate proliferation and migration, dependent on ligand concentration, receptor expression and cell type [Kavurma and Bennett, 2008; Kavurma et al., 2008b]. In this study we show for the first time that PDGF-BB-stimulated VSMC proliferation and migration involves TRAIL. This study is the first to also implicate PDGF-BB in the regulation of TRAIL transcription and expression in VSMCs; our studies validate the mitogenic and migratory actions of TRAIL observed in VSMC biology.

TRAIL is regulated by the interferon family member cytokines [Kavurma and Bennett, 2008], insulin [Corallini et al., 2007], TNFα [Fu et al., 2003] and transcription factors STAT [Choi et al., 2003], IRF-1/3/7 [Kirshner et al., 2005; Romieu-Mourez et al., 2006; Papageorgiou et al., 2007], NFκB [Rivera-Walsh et al., 2001], Foxo3a [Ghaffari et al., 2003] and p53 [Kuribayashi et al., 2008]. Although mechanisms for TRAIL transcriptional activity and expression have not been fully elucidated in VSMCs, our recent studies showed that injury and FGF-2 treatment increased TRAIL transcription via Sp1 binding to two functional Sp1-binding elements, Sp1-1 and Sp1-2, on the proximal TRAIL promoter [Chan et al., 2010]. The present study revealed the novel finding of two additional Sp1 binding elements (Sp1-5/6 and Sp1-7) responsible for PDGF-BB-inducible TRAIL transcription. Here we show that PDGF-BB-inducible TRAIL transcription is blocked with mutation to any of the four Sp1-elements (Sp1-1, -2, 5/6 and -7). Furthermore,

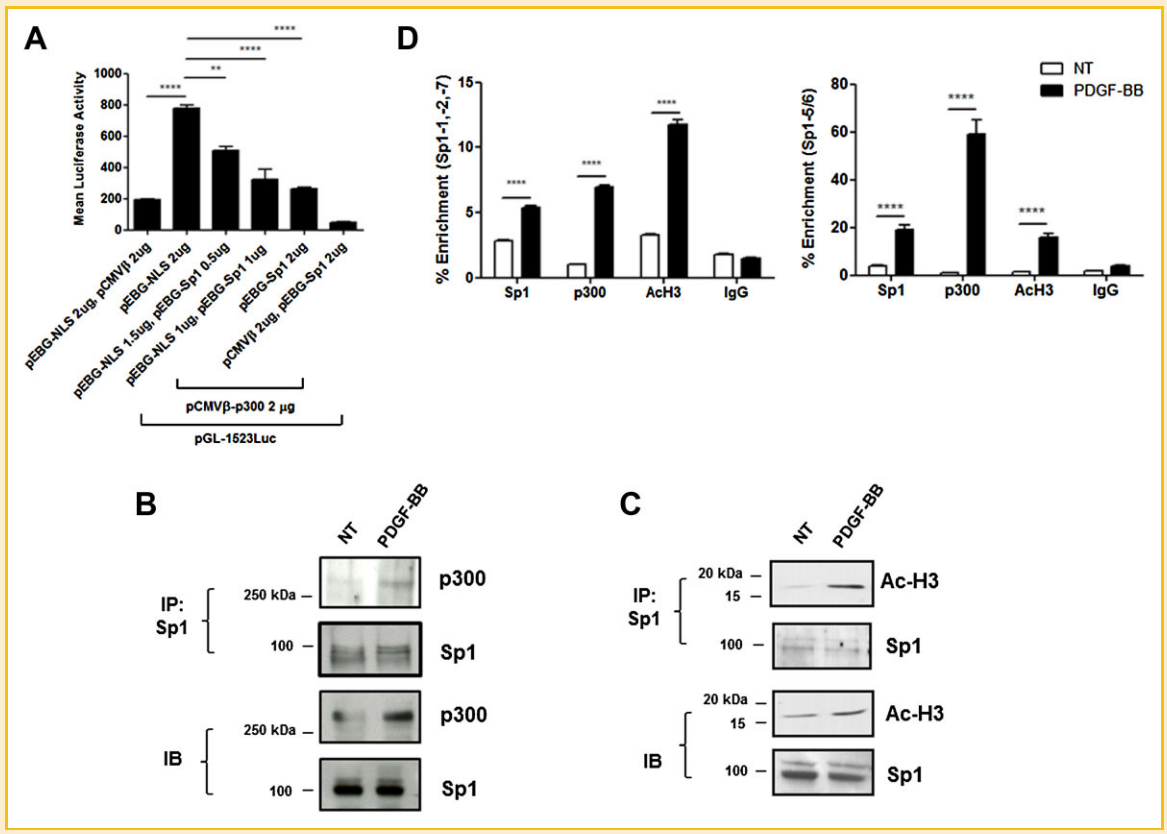


Fig. 5. Sp1, ac-H3 and p300 interact following PDGF-BB treatment in VSMCs. A: Dominant-negative Sp1 (pEBG-Sp1) dose-dependently inhibits p300-inducible TRAIL transcription. Starved pup-rat VSMCs were transfected with 1 μg pGL-1523Luc together with pEBG-NLS, pEBG-Sp1, pCMV-β or pCMVβ-p300, as indicated. Luciferase activity was determined 24 h later (2–3 replicates/treatment). B: Protein lysates from human VSMCs treated with PDGF-BB (50 ng/ml) were immunoprecipitated (IP) with Sp1 antibody, followed by Western blotting for p300 and Sp1. Immunoblotting (IB) for p300 and Sp1 using total lysates (loading control). C: Protein lysates from human VSMCs treated with PDGF-BB (50 ng/ml) were IP with Sp1 antibody, followed by Western blotting for ac-H3 and Sp1. IB for ac-H3 and Sp1 serve as loading controls. D: Sp1, ac-H3 and p300 are enriched on the TRAIL promoter after PDGF-BB treatment (50 ng/ml) in human VSMCs, as assessed by ChIP (3–4 replicates/treatment). TI: total input. IgG was used as negative control. These findings are representative of 2–3 independent experiments. Results are mean ± SEM. NT, no treatment; $^{**}P < 0.01$, $^{****}P < 0.0001$.

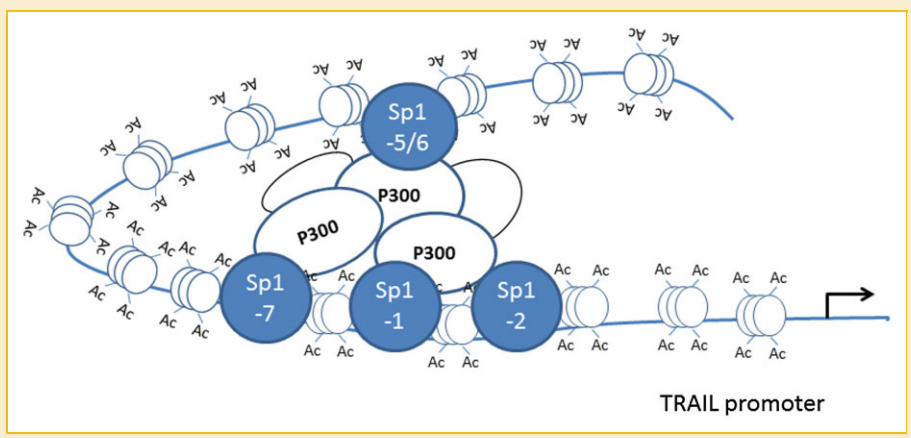


Fig. 6. Model for transcriptional regulation of TRAIL by PDGF-BB. PDGF-BB increases levels of ac-H3 leading to open chromatin and transcriptional activation. TRAIL transcriptional activity is mediated by complex formation between Sp1, ac-H3 and p300. PDGF-BB-inducible TRAIL transcription requires Sp1 to bind all four elements, since mutation to a single functional Sp1-binding site blocks complex formation and PDGF-BB-inducible TRAIL transcription. Arrow indicates transcriptional activation. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

we show direct evidence of PDGF-BB modulating chromatin modifying proteins, and implicate Sp1-ac-H3-p300 interactions in the control of TRAIL transcription (Fig. 6).

Alterations in chromatin structure via histone modification can contribute to the pathogenesis of atherosclerosis [Pons et al., 2009]. For instance, increased histone acetylation by the HDAC inhibitor, trichostatin A (TSA) worsens atherosclerosis in hyperlipidemic mice [Choi et al., 2005]. More recently, PDGF-BB has been implicated in chromatin remodelling events [Yoshida et al., 2007], e.g. PDGF-BB can modify histone by inducing hypoacetylation of histone 4 on the SMα-actin promoter [Yoshida et al., 2007]. We show for the first time that PDGF-BB treatment in VSMCs resulted in greater expression of ac-H3, while total H3 levels were unaltered. A similar finding was also observed following SAHA treatment. This suggests that like SAHA, PDGF-BB can stimulate an open chromatin formation via modification of H3 on the TRAIL promoter. Interestingly, Sp1 and ac-H3 involvement has been reported in other cell types. The methyltransferase inhibitor 5-aza-2′-deoxycytidine treatment resulted in increased ac-H3 and interaction of Sp1 with TGFβ-inducible early gene-1 to the upstream Foxp3 enhancer [Lal et al., 2009]. Furthermore, in human epidermoid carcinoma cells, phorbol 12-myristate 13-acetate-induced deacetylation of Sp1, in turn recruiting p300 to acetylate histone 3, led to increased 12(S)-lipoxygenase expression [Hung et al., 2006]. Whether PDGF-BB results in deacteylation of Sp1 for TRAIL transcriptional activity in VSMCs remains to be determined. While we show enrichment of ac-H3 levels on the endogenous TRAIL promoter in VSMCs, a recent study demonstrated reduced ac-H3 enrichment on SMα-actin promoter after PDGF-BB treatment [Thomas et al., 2009]. PDGF-DD also displayed loss of ac-H3 levels on this promoter [Thomas et al., 2009]. In another study from the same group, PDGF-BB repression of SM22α gene expression occurred via Sp1, although the authors could not detect Sp1 enrichment on the SM22α promoter [Wamhoff et al., 2004].

Association of p300 with transcription factors is important for regulating gene transcription [Kalkhoven, 2004]. p300 and Sp1 have been shown to cooperate. For instance, Sp1 and p300 cooperate to enhance $p21^{WAF1/CIP1}$ transcription in the presence of TSA [Xiao et al., 2000] and also during nerve growth factor-mediated neuronal differentiation [Billon et al., 1999]. Furthermore, epidermal growth factor (EGF)-induced keratin 16 gene expression requires the cooperation and interaction of p300 and Sp1 [Chen et al., 2007]. In the same study, EGF-induced phosphorylation of p300 via ERK led to increased HAT activity and Sp1 interaction [Chen et al., 2007]. Despite the fact that Sp1 and p300 have been shown to cooperate to regulate expression of target gene(s), their cooperation in regulating TRAIL transcription has not been described. We show that Sp1 is required for p300-inducible TRAIL promoter activation, suggesting p300-Sp1 cooperation. We also show enrichment of these factors on the TRAIL promoter following PDGF-BB treatment. Whether a common mechanism exists between PDGF-BB, Sp1, ac-H3 and p300, open or closed chromatin switching between genes remains to be determined.

In summary, here we show that PDGF-BB induced modulation of chromatin, TRAIL transcriptional activity, and VSMC proliferation and migration is TRAIL dependent. Further studies are required to fully understand the complex transcriptional regulation of TRAIL and it's myraid of roles contributing to VSMC biology. Understanding these pathways may lead to better strategies for the treatment of vascular proliferative disorders.

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## REFERENCES

Billon N, Carlisi D, Datto MB, van Grunsven LA, Watt A, Wang XF, Rudkin BB. 1999. Cooperation of Sp1 and p300 in the induction of the CDK inhibitor p21WAF1/CIP1 during NGF-mediated neuronal differentiation. Oncogene 18:2872–2882.

Cairns BR. 2001. Emerging roles for chromatin remodeling in cancer biology. Trends Cell Biol 11:S15–S21.

Chan J, Prado-Lourenco L, Khachigian LM, Bennett MR, Di Bartolo BA, Kavurma MM. 2010. TRAIL promotes VSMC proliferation and neointima formation in a FGF-2-, Sp1 phosphorylation-, and NFkappaB-dependent manner. Circ Res 106:1061–1071.

Chen YJ, Wang YN, Chang WC. 2007. ERK2-mediated C-terminal serine phosphorylation of p300 is vital to the regulation of epidermal growth factor-induced keratin 16 gene expression. J Biol Chem 282:27215–27228.

Choi EA, Lei H, Maron DJ, Wilson JM, Barsoum J, Fraker DL, El-Deiry WS, Spitz FR. 2003. Stat1-dependent induction of tumor necrosis factor-related Apoptosis-inducing ligand and the cell-surface death signaling pathway by interferon β in human cancer cells. Cancer Res 63:5299–5307.

Choi J-H, Nam K-H, Kim J, Baek MW, Park J-E, Park H-Y, Kwon HJ, Kwon O-S, Kim D-Y, Oh GT. 2005. Trichostatin A exacerbates atherosclerosis in low density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol 25:2404–2409.

Corallini F, Celeghini C, Rizzardi C, Pandolfi A, Di Silvestre S, Vaccarezza M, Zauli G. 2007. Insulin down-regulates TRAIL expression in vascular smooth muscle cells both in vivo and in vitro. J Cell Physiol 212:89–95.

Davie JR, He S, Li L, Sekhavat A, Espino P, Drobic B, Dunn KL, Sun J-M, Chen HY, Yu J, Pritchard S, Wang X. 2008. Nuclear organization and chromatin dynamics—Sp1, Sp3 and histone deacetylases. Adv Enzyme Regul 48:189–208.

Dzau VJ, Braun-Dullaeus RC, Sedding DG. 2002. Vascular proliferation and atherosclerosis: New perspectives and therapeutic strategies. Nat Med 8: 1249–1256.

Evanko SP, Raines EW, Ross R, Gold LI, Wight TN. 1998. Proteoglycan distribution in lesions of atherosclerosis depends on lesion severity, structural characteristics, and the proximity of platelet-derived growth factor and transforming growth factor-beta. Am J Pathol 152:533–546.

Fu M, Zhu X, Zhang J, Liang J, Lin Y, Zhao L, Ehrengruber MU, Chen YE. 2003. Egr-1 target genes in human endothelial cells identified by microarray analysis. Gene 315:33–41.

Ghaffari S, Jagani Z, Kitidis C, Lodish HF, Khosravi-Far R. 2003. Cytokines and BCR-ABL mediate suppression of TRAIL-induced apoptosis through inhibition of forkhead FOXO3a transcription factor. Proc Natl Acad Sci USA 100:6523–6528.

Hung J-J, Wang Y-T, Chang W-C. 2006. Sp1 deacetylation induced by phorbol ester recruits p300 to activate 12(S)-lipoxygenase gene transcription. Mol Cell Biol 26:1770–1785.

Kalkhoven E. 2004. CBP and p300: HATs for different occasions. Biochem Pharmacol 68:1145–1155.

Kavurma MM, Bennett MR. 2008. Expression, regulation and function of trail in atherosclerosis. Biochem Pharmacol 75:1441–1450.

Kavurma MM, Khachigian LM. 2003a. ERK, JNK, and p38 MAP kinases differentially regulate proliferation and migration of phenotypically distinct smooth muscle cell subtypes. J Cell Biochem 89:289–300.

Kavurma MM, Khachigian LM. 2003b. Sp1 inhibits proliferation and induces apoptosis in vascular smooth muscle cells by repressing p21WAF1/Cip1 transcription and cyclin D1-Cdk4-p21WAF1/Cip1 complex formation. J Biol Chem 278:32537–32543.

Kavurma MM, Schoppet M, Bobryshev YV, Khachigian LM, Bennett MR. 2008a. TRAIL stimulates proliferation of vascular smooth muscle cells via activation of NF-kappa B and induction of insulin-like growth factor-1 receptor. J Biol Chem 283:7754–7762.

Kavurma MM, Tan NY, Bennett MR. 2008b. Death receptors and their ligands in atherosclerosis. Arterioscler Thromb Vasc Biol 28:1694–1702.

Kirshner JR, Karpova AY, Kops M, Howley PM. 2005. Identification of TRAIL as an interferon regulatory factor 3 transcriptional target. J Virol 79:9320–9324.

Kuribayashi K, Krigsfeld G, Wang W, Xu J, Mayes PA, Dicker DT, Wu GS, El-Deiry WS. 2008. TNFSF10 (TRAIL), a p53 target gene that mediates p53-dependent cell death. Cancer Biol Ther 7:2034–2038.

Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, Reid SP, Levy DE, Bromberg JS. 2009. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol 182:259–273.

Leppanen O, Janjic N, Carlsson MA, Pietras K, Levin M, Vargeese C, Green LS, Bergqvist D, Ostman A, Heldin CH. 2000. Intimal hyperplasia recurs after removal of PDGF-AB and -BB inhibition in the rat carotid artery injury model. Arterioscler Thromb Vasc Biol 20:E89–E95.

Lusis AJ. 2000. Atherosclerosis. Nature 407:233–241.

Lynn Ray J, Leach R, Herbert J-M, Benson M. 2001. Isolation of vascular smooth muscle cells from a single murine aorta. Methods Cell Sci 23: 185–188.

Nelson JD, Denisenko O, Bomsztyk K. 2006. Protocol for the fast chromatin immunoprecipitation (ChIP) method. Nat Protoc 1:179–185.

Noiseux N, Boucher CH, Cartier R, Sirois MG. 2000. Bolus endovascular PDGFR-beta antisense treatment suppressed intimal hyperplasia in a rat carotid injury model. Circulation 102:1330–1336.

Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953–959.

Papageorgiou A, Dinney CP, McConkey DJ. 2007. Interferon-alpha induces TRAIL expression and cell death via an IRF-1-dependent mechanism in human bladder cancer cells. Cancer Biol Ther 6:872–879.

Petersohn D, Thiel G. 1996. Role of zinc-finger proteins Sp1 and Zif268/egr-1 in transcriptional regulation of the human synaptobrevin II gene. Eur J Biochem 239:827–834.

Pons D, de Vries FR, van den Elsen PJ, Heijmans BT, Quax PHA, Jukema JW. 2009. Epigenetic histone acetylation modifiers in vascular remodelling: New targets for therapy in cardiovascular disease. Eur Heart J 30:266–277.

Raines EW. 2004. PDGF and cardiovascular disease. Cytokine Growth Factor Rev 15:237–254.

Rivera-Walsh I, Waterfield M, Xiao G, Fong A, Sun SC. 2001. NF-kappaB signaling pathway governs TRAIL gene expression and human T-cell leukemia virus-I Tax-induced T-cell death. J Biol Chem 276:40385–40388.

Romieu-Mourez Rl, Solis M, Nardin A, Goubau D, Baron-Bodo Vr, Lin R, Massie B, Salcedo M, Hiscott J. 2006. Distinct roles for IFN regulatory factor (IRF)-3 and IRF-7 in the activation of antitumor properties of human macrophages. Cancer Res 66:10576–10585.

Ross R. 1993. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 362:801–809.

Ross R. 1995. Cell biology of atherosclerosis. Annu Rev Physiol 57:791–804.

Ross R, Glomset JA. 1973. Atherosclerosis and the arterial smooth muscle cell: Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 180:1332–1339.

Rubin K, Tingstrom A, Hansson GK, Larsson E, Ronnstrand L, Klareskog L, Claesson-Welsh L, Heldin CH, Fellstrom B, Terracio L. 1988. Induction of B-type receptors for platelet-derived growth factor in vascular inflammation: Possible implications for development of vascular proliferative lesions. Lancet 1:1353–1356.

Sano H, Sudo T, Yokode M, Murayama T, Kataoka H, Takakura N, Nishikawa S, Nishikawa SI, Kita T. 2001. Functional blockade of platelet-derived growth factor receptor-beta but not of receptor-alpha prevents vascular smooth muscle cell accumulation in fibrous cap lesions in apolipoprotein E-deficient mice. Circulation 103:2955–2960.

Seifert RA, Hart CE, Phillips PE, Forstrom JW, Ross R, Murray MJ, Bowen-Pope DF. 1989. Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. J Biol Chem 264:8771–8778.

Selvi RB, Kundu TK. 2009. Reversible acetylation of chromatin: Implication in regulation of gene expression, disease and therapeutics. Biotechnol J 4: 375–390.

Tallquist M, Kazlauskas A. 2004. PDGF signaling in cells and mice. Cytokine Growth Factor Rev 15:205–213.

Thomas JA, Deaton RA, Hastings NE, Shang Y, Moehle CW, Eriksson U, Topouzis S, Wamhoff BR, Blackman BR, Owens GK. 2009. PDGF-DD, a novel mediator of smooth muscle cell phenotypic modulation, is upregulated in endothelial cells exposed to atherosclerosis-prone flow patterns. Am J Physiol Heart Circ Physiol 296:H442–H452.

Wamhoff BR, Hoofnagle MH, Burns A, Sinha S, McDonald OG, Owens GK. 2004. A G/C element mediates repression of the SM22α promoter within phenotypically modulated smooth muscle cells in experimental atherosclerosis. Circ Res 95:981–988.

Wang QD, Ji YS, Wang XF, Evers BM. 2000. Isolation and molecular characterization of the 5′-upstream region of the human TRAIL gene. Biochem Biophys Res Commun 276:466–471.

Wiley SR, Schooley K, Smolak PJ, Din WS, Huang C-P, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, Goodwin RG. 1995. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3:673–682.

Xiao H, Hasegawa T, Isobe K. 2000. p300 collaborates with Sp1 and Sp3 in p21(waf1/cip1) promoter activation induced by histone deacetylase inhibitor. J Biol Chem 275:1371–1376.

Yoshida T, Gan Q, Shang Y, Owens GK. 2007. Platelet-derived growth factor-BB represses smooth muscle cell marker genes via changes in binding of MKL factors and histone deacetylases to their promoters. Am J Physiol Cell Physiol 292:C886–C895.

Zhang C, Kavurma MM, Lai A, Khachigian LM. 2003. Ets-1 protects vascular smooth muscle cells from undergoing apoptosis by activating p21WAF1/Cip1: ETS-1 regulates basal and inducible p21WAF1/Cip1 transcription via distinct CIS-acting elements in the p21WAF1/Cip1 promoter. J Biol Chem 278:27903–27909.