Human hair follicle stem cell differentiation into contractile smooth muscle cells is induced by transforming growth factor-β1 and platelet-derived growth factor BB

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Abstract. Smooth muscle cells (SMCs) are important in vascular homeostasis and disease and thus, are critical elements in vascular tissue engineering. Although adult SMCs have been used as seed cells, such mature differentiated cells suffer from limited proliferation potential and cultural senescence, particularly when originating from older donors. By comparison, human hair follicle stem cells (hHFSCs) are a reliable source of stem cells with multi-differentiation potential. The aim of the present study, was to develop an efficient strategy to derive functional SMCs from hHFSCs. hHFSCs were obtained from scalp tissues of healthy adult patients undergoing cosmetic plastic surgery. The hHFSCs were expanded to passage 2 and induced by the administration of transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor BB (PDGF-BB) in combination with culture medium. Expression levels of SMC-related markers, including α-smooth muscle actin (α-SMA), α-calponin and smooth muscle myosin heavy chain (SM-MHC), were detected by immunofluorescence staining, flow cytometry analysis and reverse transcription-polymerase chain reaction (RT-PCR). When exposed to differentiation medium, hHFSCs expressed early, mid and late markers (α-SMA, α-calponin and SM-MHC, respectively) that were similar to the markers expressed by human umbilical artery SMCs. Notably, when entrapped inside a collagen matrix lattice, these SM differentiated cells showed a contractile function. Therefore, the present study developed an efficient strategy for differentiating hHFSCs into contractile SMCs by stimulation with TGF-β1 and PDGF-BB. The high yield of derivation suggests that this strategy facilitates the acquisition of the large numbers of cells that are required for blood vessel engineering and the study of vascular disease pathophysiology.

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Introduction

Smooth muscle cells (SMCs) comprise the muscular wall of the blood vasculature, where they provide a contractile function and are critical in predominant human diseases, such as atherosclerosis, hypertension and asthma (1). As a result, SMCs are critical for blood vessel construction during tissue engineering (2). Although it is possible to isolate SMCs from existing blood vessels, the process is invasive, requires major surgery and injures the donor site (3). In addition, the limited replicative life span of human adult SMCs and their slow rate of collagenous matrix production *in vitro* present important hurdles in the engineering of mechanically robust and biologically functional grafts (4,5). Due to recent advances in the stem cell field, studies now have access to numerous novel cell source alternatives for vascular engineering, which exhibit the potential to provide large numbers of autologous cells with vast differentiation capacity (6).

Although bone marrow (7-9) and adipose tissues (10-12) have been extensively studied as sources of mesenchymal stem cells (MSCs), recent studies have indicated that the hair follicle is a rich source of multipotent adult stem cells and may be an easily accessible alternative source of autologous SMCs (13,14). Hair follicle stem cells (HFSCs) exhibit a broad potential to differentiate into adipogenic, osteogenic, chondrogenic, neurogenic and myogenic lineages under appropriate conditions (15-18). When compared with bone marrow-derived stem cells, HFSCs are easier to obtain as acquisition is less invasive with a lower risk of morbidity at the donor sites and provide a higher yield at harvest (14). Thus, HFSCs may be a preferred novel cell source for blood vessel engineering.

TGF-β1 is an important cytokine that is involved in vessel formation and assembly. TGF-β1 knockout was fatal in mice as a result of defective haematopoiesis and vasculogenesis due to a decrease in endothelial-mesenchymal cell contact (19). TGF-β1 has also been shown to affect SMC differentiation *in vitro* (20) and in a rat artery injury model *in vivo* (21). In

previous studies, several cells were induced by TGF-β1 to differentiate into cells with an SMC-like phenotype and function (22-24).

The platelet-derived growth factor (PDGF) family is composed of disulphide-bonded homodimers of four polypeptide chains, PDGF-AA, -BB, -CC and -DD, and one heterodimeric protein, PDGF-AB (25). PDGF-BB is critical for the proliferation and migration of vascular smooth muscle cells. Genetic studies in mice showed that the loss of PDGF-BB resulted in a severe deficiency in vascular cell recruitment, leading to vascular leakage and haemorrhage (26,27).

The aim of this study was to investigate the potential of human HFSCs (hHFSCs) to differentiate into the SMC phenotype upon induction by TGF-β1 and PDGF-BB in low-serum medium. The expression of characteristic SMC markers in the resulting hHFSCs was analysed at the protein and gene levels. Moreover, the contractile function of induced hHFSCs, represented by the ability of cells to contract collagen gel, was also demonstrated.

Materials and methods

Isolation and culture of hHFSCs. hHFSCs were obtained from human scalp tissues from healthy adult patients (average age, 30 years) undergoing cosmetic plastic surgery. All protocols of human tissue handling were approved by the Research Ethical Committee of the Shanghai 9th People's Hospital (Shanghai, China) and written informed consent was obtained from the patients. Briefly, two to three pieces of scalp debris (4-9 mm) were postoperatively collected and washed two to three times with phosphate-buffered saline (PBS). The fat tissue was carefully removed and the scalp was cut into smaller sections. The scalp sections were then transferred to a 15-ml centrifuge tube and washed three times to remove any remaining hair or blood clots. Washed tissue pieces were digested with 1 mg/ml collagenase type I (Invitrogen Life Technologies, Carlsbad, CA, USA) at 37˚C with occasional agitation. After 2 h of digestion, single-hair follicles were released from the full-thickness skin, filtered through a 40-mm cell strainer (BD Biosciences, San Jose, CA, USA) and washed extensively in PBS. Subsequent to this, each hair follicle was placed in one well of a 96-well plate (BD Biosciences), to allow for cell migration onto the tissue culture plastic, and was cultured in 100 μl low-glucose Dulbecco's modified Eagle's medium (LG-DMEM; Gibco, Grand Island, NY, USA) containing 10% foetal bovine serum (FBS; HyClone, Logan, UT, USA). Cells that originated from the bulge region were visually identified as epidermal keratinocytes, while cells migrating from the dermal sheath or papilla exhibited the morphological appearance of mesenchymal cells. The wells populated with cells originating from the dermal sheath or papilla were selected, pooled and expanded. The cells were then plated in 100-mm culture dishes (Falcon, Oxnard, CA, USA) at a density of $4x10^4$ cells/cm^2 and cultured in LG-DMEM (Gibco) supplemented with 10% FBS, 100 U/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA), and 100 mg/ml streptomycin (Sigma-Aldrich) (defined as growth medium). The medium was changed twice a week. Cells were passaged when they had reached 70-80% confluence and second passage hHFSCs were used in the subsequent experiments. The characterisation of hHFSCs was determined by the CD marker profile and the ability to differentiate into osteogenic, adipogenic and chondrogenic lineages, as previously described (data not shown) (15,16).

Induction of SM differentiation. To evaluate the effects of TGF-β1 and PDGF-BB on the differentiation of hHFSCs into SMCs, hHFSCs reaching subconfluence were cultured in DMEM supplemented with 5 ng/ml recombinant human TGF-β1 (R&D Systems, Minneapolis, MN, USA) and 10 ng/ml recombinant human PDGF-BB with 1% FBS. DMEM supplemented with 1% FBS was defined as the basal medium (BM). Human umbilical artery SMCs (hUASMCs) served as a positive control. The culture media were changed every two days. Cell characterisation and functional evaluation were performed subsequent to eight days of culture.

Immunofluorescence staining. The hHFSCs were harvested, resuspended in PBS, fixed with 4% paraformaldehyde (Sigma-Aldrich) for 15 min and permeabilised with 0.1% Triton X-100 (Sigma-Aldrich) for 10 min. Subsequent to washing with PBS, the cells were blocked with 3% bovine serum albumin (BSA, Sigma-Aldrich) for 30 min and incubated with the following primary antibodies: Mouse monoclonal anti-α-smooth muscle actin (α-SMA, C6198, Sigma-Aldrich), rabbit monoclonal anti-α-calponin (ab46794; Abcam, Cambridge, UK) and mouse monoclonal anti-smooth muscle myosin heavy chain (SM-MHC, M7786, Sigma-Aldrich). Following incubation with the primary antibodies for 60 min at room temperature (RT), the cultures were washed with PBS three times. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit secondary antibody (Millipore, Billerica, MA, USA) was used to detect the localisation of anti-α-calponin antibodies. FITC-conjugated goat anti-mouse secondary antibody (Millipore) was used to detect the localisation of anti-α-SMA and anti-SM-MHC antibodies. In addition, cell nuclei were stained with propidium iodide. Control samples consisted of cells without primary antibody and were used to assess background fluorescence. The images were viewed by a fluorescence microscope (Eclipse E400 epi-fluorescence microscope; Nikon, Tokyo, Japan).

Flow cytometry analysis. Cells were trypsinised, centrifuged at 500 x g for 5 min (Allegra 64R; Beckman Coulter, Brea, CA, USA), resuspended in PBS/1% BSA and incubated with anti-α-SMA (dilution, 1:100; Sigma-Aldrich), anti-α-calponin (dilution, 1:100; Abcam) and anti-SM-MHC (dilution 1:100; Sigma-Aldrich) for 30 min at RT on a shaking plate (Lab Rotors; Thermo Scientific, Logan UT, USA). The cells were then washed, resuspended in PBS/1% BSA with FITC-conjugated secondary antibody and incubated for 30 min at RT on a shaking plate. Subsequent to this, cells were washed and fixed. FITC-conjugated isotype-matching immunoglobulins were used to determine non-specific staining. Fluorescence was determined using a flow cytometer (FACSCalibur; Becton-Dickinson, San Jose, CA, USA) and the data were analysed using CellQuest software (Becton-Dickinson).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR). Expression levels of SMC-specific markers (α-SMA, α-calponin and SM-MHC) were identified

Table I. Primers for polymerase chain reaction analysis.

RNA	Primer sequences (nucleotides)	Fragment size (bp)
α-SMA	Forward: GGTGATGGTGGGAATGGG (18) Reverse: GCAGGGTGGGATGCTCTT (18)	188
Calponin	Forward: GGCGAAGACGAAAAGGAAA (19) Reverse: GGGTACTCGGGAGTCAGACAG (21)	447
SM-MHC	Forward: TGCTTTCGCTCGTCTTCC (18) Reverse: CGGCAACTCGTGTCCAAC (18)	516

α-SMA, α-smooth muscle actin; SM-MHC, smooth muscle myosin heavy chain.

by isolating the total RNA from cells using the RNeasy total RNA isolation kit (Qiagen, Inc., Valencia, CA, USA) and cDNA was synthesised using the SuperScript First-strand Synthesis system (Life Technologies, Barcelona, Spain). Specific genes were amplified by PCR using the Fast-Run Taq Master kit (Protech Technologies Inc., Taipei, Taiwan). The primer sequences designed using the Primer Express software are listed in Table I. The cDNA product was amplified by PCR using standard methods (35 cycles of 95˚C for 30 sec, 56˚C for 30 sec and 72˚C for 60 sec). Gel electrophoresis was then performed on a 2% agarose gel treated with ethidium bromide and bands were visualised using a UV light box. hUASMCs and human chondrocyte cells (hCs) served as positive and negative controls, respectively.

Collagen gel lattice contraction assay. The contractility of cells was measured as previously described (28). Briefly, cells were trypsinised by treatment with trypsin-ethylenediaminetetraacetic acid, counted and resuspended in serum-free DMEM at a density of $1x10^6$ cells/ml. The prepared cell suspension was added to a collagen gel solution to achieve a final concentration of 3 mg collagen/ml and a density of $4x10^5$ cells/ml. The cell collagen mixture was poured into 12-well culture plates for 1 h to polymerise the collagen cell lattices. The lattices were mechanically released from the bottom of the tissue culture dishes by gently pipetting medium at the lattice-dish interface to initiate collagen gel contraction. The extent of gel contraction of each group at different time points was calculated by measuring the dimensions of the gel lattices viewed by a digital camera. The area of the gel lattices was analysed using NIH ImageJ software (National Institutes of Health, Bethesda, MD, USA). The relative lattice area was obtained by dividing the area at each time point by the initial area of the lattice.

Statistical analysis. Each experiment was repeated at least three times. The results are presented as the mean ± SD. Comparisons between groups were performed by a paired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Culture of hHFSCs. The total quantity of cells isolated from each scalp tissue sample ranged from $5x10^4$ to $1x10^5$ cells. Approximately 0.5-2% of the isolated tissue cells were identified to be the adherent stem cells. Subsequent to 1-2 days of culture, the hHFSCs were observed to be elongated (Fig. 1A). Within another 3-4 days, the cells reached confluency and were subsequently passaged onto a novel plate.

TGF-β1 and PDGF-BB induce differentiation of hHFSCs into SMCs. At the second passage, 5 ng/ml TGF-β1 and 10 ng/ml PDGF-BB were selected to induce the differentiation of hHFSCs into the SMC lineage. hHFSCs treated with TGF-β1 and PDGF-BB for eight days acquired a spindle morphology and grew in a hill and valley pattern similar to that previously observed in primary isolated hUASMCs. No obvious change was identified in the hHFSCs cultured in BM (Fig. 1B-D). Fifth passage hHFSCs appeared to be a relatively homogenous population that exhibited a fibroblast-like spindle morphology.

Expression of SMC-specific markers in hHFSCs treated with TGF-β1 and PDGF-BB. To determine whether TGF-β1 and PDGF-BB induced the differentiation of hHFSCs into the SMC phenotype, SM-specific proteins (α-SMA, α-calponin and SM-MHC) were detected by immunofluorescence staining. In parallel, the three markers in hUASMCs were also analysed as a positive control. As shown in Fig. 2, there was a baseline expression of α-SMA, but marginal expression of α-calponin and SM-MHC in undifferentiated hHFSCs cultured in BM. When cultured in DMEM supplemented with TGF-β1 and PDGF-BB, expression levels of α-SMA and α-calponin were identified to be enhanced. Furthermore, expression of SM-MHC was markedly increased, reaching a similar level to that in the hUASMCs.

To determine the percentage of SM differentiated cells in the hHFSCs, the expression levels of α-SMA, α-calponin and SM-MHC were analysed by flow cytometry. As shown in Fig. 3, the α-SMA was detected in 17.41±0.78%, 45.32±1.21% and 89.46±0.83% of undifferentiated hHFSCs, induced hHFSCs and hUASMCs, respectively. By comparison, marginal expression of α-calponin (3.47±0.82%) and SM-MHC (2.78±0.63%) was observed in the undifferentiated hHFSCs, while their expression levels reached 57.24±2.08% and 70.71±1.78%, respectively, in the induced hHFSCs, which was closer to the expression observed in hUASMCs.

The gene expression profile analysed by RT-PCR further confirmed the differentiation of hHFSCs into SMCs. As shown

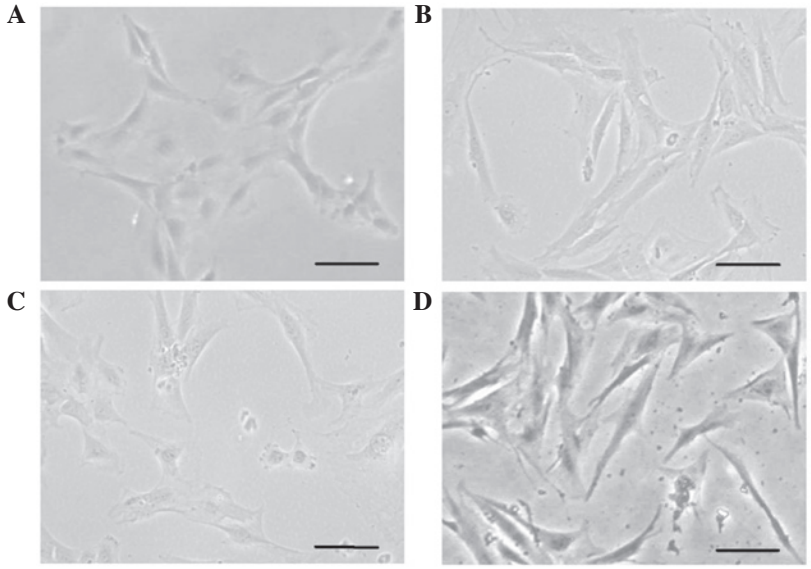

Figure 1 The effect of transforming growth factor (TGF)-β1 and platelet derived growth factor BB (PDGF-BB) on human hair follicle stem cell (hHFSC) morphology. (A) Phase contrast image analysis of cultured hHFSCs. (B) hHFSCs treated with TGF-β1 and PDGF-BB for eight days. (C) Negative control, hHFSCs treated with basal medium only. (D) Positive control, primary human umbilical artery smooth muscle cells (hUASMCs). In the presence of TGF-β1 and PDGF-BB, hHFSCs exhibited a spindle morphology similar to that of hUASMCs. Scale bar, 100 μm.

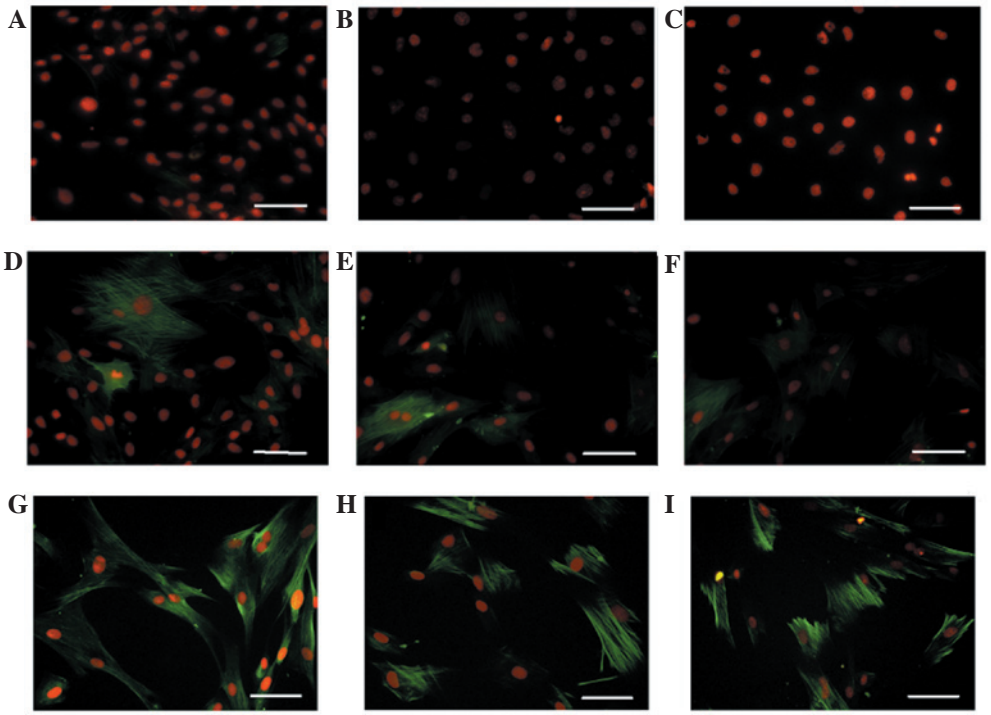

Figure 2 Expression of smooth muscle cell-specific proteins [α-smooth muscle actin (α-SMA), α-calponin and smooth muscle myosin heavy chain (SM-MHC)] under different conditions by immunofluorescence staining. There was limited expression of α-SMA (A) and marginal expression of α-calponin (B) and SM-MHC (C) in undifferentiated human hair follicle stem cells cultured in basal medium (BM). Expression of α-SMA (D), α-calponin (E) and SM-MHC (F) was identified to be enhanced when cultured in BM supplemented with transforming growth factor-β1 and platelet derived growth factor BB, reaching a level similar to that of human umbilical artery smooth muscle cells (G, H and I). Scale bar, 100 μm.

in Fig. 4, undifferentiated hHFSCs expressed low levels of α-SMA mRNA and did not express α-calponin or SM-MHC, which was similar to that observed in the hCs. By contrast, these factors were upregulated in induced hHFSCs at a similar level to that in hUASMCs. These data support the hypothesis that hHFSCs are capable of differentiating into SMCs when induced by TGF-β1 and PDGF-BB.

Characterisation of contractility of SMCs differentiated from hHFSCs. To evaluate whether SMCs, differentiated from hHFSCs by TGF-β1 and PDGF-BB induction, exhibit the ability to contract and generate force, the contractility of the smooth muscle-like cells was analysed using a collagen lattice gel contraction assay. The shrinkage of gels embedded with differentiated SMCs occurred in a time-dependent manner.

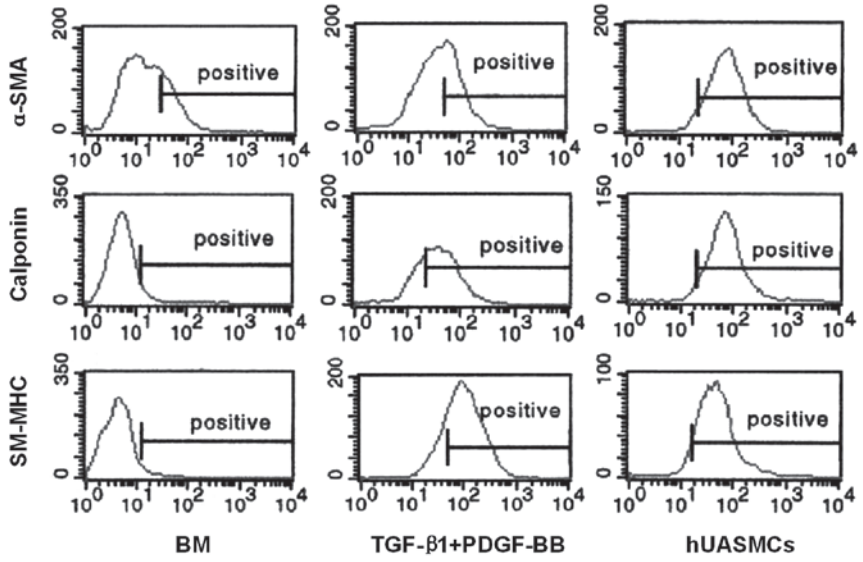

Figure 3 Flow cytometry analysis of smooth muscle cell (SMC)-specific proteins [α-smooth muscle actin (α-SMA), α-calponin and smooth muscle myosin heavy chain (SM-MHC)]. Limited expression of α-SMA and marginal expression of α-calponin, and SM-MHC were observed in undifferentiated human hair follicle stem cells (hHFSCs) cultured in basal medium (BM). The expression was identified to be enhanced in BM supplemented with transforming growth factor-β1 and platelet derived growth factor BB, similar to that in primary human umbilical artery smooth muscle cells (hUASMCs).

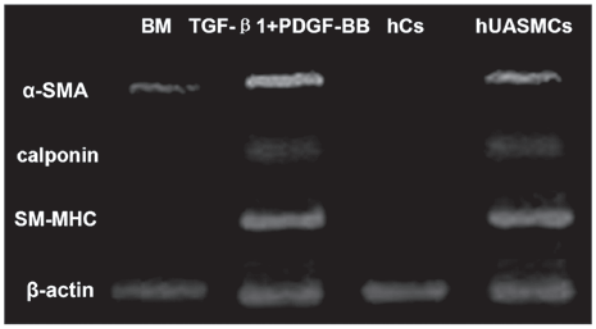

Figure 4. The mRNA levels of α-smooth muscle actin (α-SMA), α-calponin, smooth muscle myosin heavy chain (SM-MHC) and β-actin were determined by reverse transcription-polymerase chain reaction. It was revealed that the expression of α-SMA in human hair follicle stem cells (hHFSCs) stimulated with the combination of transforming growth factor (TGF)-β1 and platelet derived growth factor BB (PDGF-BB) was higher than that of undifferentiated hHFSCs. The expression of α-calponin and SM-MHC was observed in hHFSCs induced by TGF-β1 and PDGF-BB, similar to that in hUASMCs. Human chondrocyte cells served as negative controls.

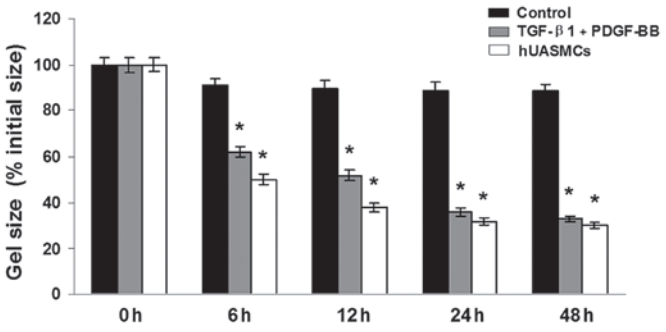

Figure 5. Contractile activity of the smooth muscle-like cells differentiated from hHFSCs. The shrinkage of gels embedded with differentiated SMCs occurred in a time-dependent manner. Collagen gel matrix containing undifferentiated human hair follicle stem cells contracted to a lesser extent. Data are presented as the mean ± SD (n>4). *P<0.05 versus control (0 h) analysed by Student's t-test.

After 48 h, the area of gel lattice was markedly decreased. However, collagen gel matrix containing undifferentiated hHFSCs contracted to a lesser extent. Compared with the gel containing undifferentiated cells, the area of the collagen lattice with differentiated hHFSCs was significantly diminished after 6 h (Fig. 5). Thus, these results showed that with the combined induction by TGF-β1 and PDGF-BB, the hHFSCs differentiated into the SMC phenotype and acquired the contractile ability observed in hUASMCs.

Discussion

Although there has been promising progress towards the development of biological vascular grafts in treating vascular diseases, the cell source remains a predominant obstacle in vascular tissue engineering. Adult somatic SMCs have been shown to exhibit limited replicative capacity and, therefore, are not an optimal source of seed cells for constructing tissue-engineered blood vessels, as previously suggested (4,29-32). Although SMCs have been derived from multipotent adult stem cells (9,10,16,19, 33-35), HFSCs have been investigated less frequently.

In the present study, the effect of TGF-β1 and PDGF-BB on the differentiation of hHFSCs toward the SMC lineage was investigated. Considerable variation was observed in the expression of the SMC markers, α-SMA, α-calponin and SM-MHC, at the gene and protein levels. α-SMA is often considered to be an early marker of developing smooth muscle cells (12). SM-MHC and α-calponin are widely accepted to be late markers of SMC differentiation and are more specific to an SMC lineage (36). It was demonstrated that TGF-β1 and PDGF-BB induced the expression of these three proteins in hHFSCs. Furthermore, the induction time was extended to ≥21 days and it was demonstrated that the expression of the three markers remained detectable in differentiated hHFSCs by immunofluorescence staining (data not shown). These

results suggest that the combination of TGF-β1 and PDGF-BB fully stimulates the differentiation of hHFSCs towards the SMC phenotype.

TGF-β1 is important in regulating SMC proliferation and differentiation, and in the maturation of blood vessels in vasculogenesis (37). It has been demonstrated that TGF-β1 enhances the expression and organisation of α-SMA, SM-MHC and smooth muscle protein 22-α in SMC lines as well as primary rat and human SMC cultures (38-42). Furthermore, in rodent models, the level of TGF-β1 in the neointima and damaged media of injured vessels is decreased and is correlated with a decrease in α-SMA, type IV collagen and SM-MHC (24). In addition, TGF-β1 is shown to stabilise elastin mRNA (43,44); increase the mRNA level and enzymatic activity of lysyl oxidase (45); enhance the mechanical strength (30,46) and vascular reactivity of fibrin-based V-SMC tissue equivalents (47); and promote the differentiation of embryonic stem cells (48), bone marrow mesenchymal stem cells (49,50), and bone marrow multipotent adult progenitor cells (34) into mature contractile SMCs.

PDGF-BB released by endothelial cells (ECs) has been demonstrated to stimulate the proliferation of cocultured mesenchymal cells (51). PDGF-BB mediates mesenchymal differentiation and acts as a mitogen and chemoattractant for mesenchymal cells (52). PDGF-BB specifically exerts a paracrine effect on undifferentiated mesenchymal cells. Holmgren *et al* (53) observed the expression of PDGF ligand and receptors in forming blood vessels within the human placenta. It was demonstrated that ECs of developing blood vessels express the PDGF-BB mRNA and protein but not the PDGF-β receptor, whereas mRNA of the PDGF-β receptor was detectable in the SMCs surrounding intermediate and large blood vessels.

The mechanism of SMC differentiation of hHFSCs induced by PDGF-BB and TGF-β1 remains unclear. *In vivo*, TGF-β1 is secreted as a complex with latency-associated propeptide and is targeted to specific locations in the extracellular matrix through binding to latent TGF-β binding protein. TGF-β modulates SMC differentiation by directly binding to the type-I receptor and thereafter activating downstream signals of Smad proteins (54-56). In its C-terminal domain, PDGF-BB contains a stretch of basic amino acids that confer binding to heparan sulphate proteoglycans (HSPGs). It is assumed that HSPG binding generates growth-factor gradients that provide positional information and spatial control of cellular responses (57). Therefore, it was speculated that cross-talk between PDGF-BB and TGF-β1 pathways may contribute to the differentiation of hHFSCs into SMCs; however, the detailed underlying mechanism requires further investigation.

To ascertain a differentiated SMC phenotype, demonstration of the quintessential functional property of contraction is required. The results of the present study showed that hHFSC differentiation was induced by TGF-β1 and PDGF-BB and these cells elicited noteworthy contraction of the collagen gel lattice, a characteristic of differentiated SMCs. The data suggest that hHFSCs are capable of being induced to differentiate into an SMC phenotype with contractile function when stimulated by a combination of TGF-β1 and PDGF-BB. Thus, hHFSCs may be a source of functional SMCs for tissue engineering of blood vessels, as well as other SMC-containing tissues, including bladder, urethral and intestinal tissues.

In conclusion, it was demonstrated that expanded hHFSCs were able to be induced to differentiate along an SMC pathway by TGF-β1 and PDGF-BB stimulation for eight days *in vitro*, as determined by the expression of SMC-specific transcripts and proteins, including α-SMA, SM-MHC and SM-MHC. When embedded in a collagen lattice, the differentiated hHFSCs contracted. These results substantiate the possibility of using hHFSCs as a candidate for cardiovascular tissue engineering and regenerative medicine.

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References

1. Owens GK, Kumar MS and Wamhoff BR: Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 84: 767-801, 2004.
2. Sundaram S and Niklason LE: Smooth muscle and other cell sources for human blood vessel engineering. Cells Tissues Organs 195: 15-25, 2012.
3. Xu ZC, Zhang WJ, Li H, Cui L, Cen L, Zhou GD, Liu W and Cao Y: Engineering of an elastic large muscular vessel wall with pulsatile stimulation in bioreactor. Biomaterials 29:1464-1472, 2008.
4. Poh M, Boyer M, Solan A, Dahl SL, Pedrotty D, Banik SS, McKee JA, Klinger RY, Counter CM and Niklason LE: Blood vessels engineered from human cells. Lancet 365: 2122-2124, 2005.
5. McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, Niklason LE and Counter CM: Human arteries engineered in vitro. EMBO Rep 4: 633-638, 2003.
6. Bajpai VK and Andreadis ST: Stem cell sources for vascular tissue engineering and regeneration. Tissue Eng Part B Rev 18: 405-425, 2012.
7. Matsumura G, Miyagawa-Tomita S, Shin'oka T, Ikada Y and Kurosawa H: First evidence that bone marrow cells contribute to the construction of tissue-engineered vascular autografts in vivo. Circulation 108: 1729-1934, 2003.
8. Shin'oka T, Matsumura G, Hibino N, Naito Y, Watanabe M, Konuma T, Sakamoto T, Nagatsu M and Kurosawa H: Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. J Thorac Cardiovasc Surg 129: 1330-1338, 2005.
9. Gong Z and Niklason LE. Small-diameter human vessel wall engineered from bone marrow-derived mesenchymal stem cells (hMSCs). FASEB J 22: 1635-1648, 2008.
10. Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, Heydarkhan S, Xu Y, Zuk PA, MacLellan WR and Beygui RE: Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. Cells Tissues Organs 187: 263-274, 2008.
11. Wang C, Cen L, Yin S, Liu Q, Liu W, Cao Y and Cui L: A small diameter elastic blood vessel wall prepared under pulsatile conditions from polyglycolic acid mesh and smooth muscle cells differentiated from adipose-derived stem cells. Biomaterials 31: 621-630, 2010.
12. Harris LJ, Abdollahi H, Zhang P, McIlhenny S, Tulenko TN and DiMuzio PJ: Differentiation of adult stem cells into smooth muscle for vascular tissue engineering. J Surg Res 168: 306-314, 2011.
13. Hsu YC, Pasolli HA and Fuchs E: Dynamics between stem cells, niche, and progeny in the hair follicle. Cell 144: 92-105, 2011.
14. Mistriotis P and Andreadis ST: Hair Follicle: a novel source of multipotent stem cells for tissue engineering and regenerative medicine. Tissue Eng Part B Rev 19: 265-278, 2013.
15. Jahoda CA, Whitehouse J, Reynolds AJ and Hole N: Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. Exp Dermatol 12: 849-859, 2003.

16. Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G, Herlyn M and Xu X: Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol 168: 1879-1888, 2006.
17. Drewa T, Joachimiak R, Kaznica A, Sarafian V and Pokrywczynska M: Hair stem cells for bladder regeneration in rats: preliminary results. Transplant Proc 41: 4345-4351, 2009.
18. Lin H, Liu F, Zhang C, Zhang Z, Kong Z, Zhang X and Hoffman RM: Characterization of nerve conduits seeded with neurons and Schwann cells derived from hair follicle neural crest stem cells. Tissue Eng Part A 17: 1691-1698, 2011.
19. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S and Akhurst RJ: Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. Development 121: 1845-1854, 1995.
20. Shah NM, Groves AK and Anderson DJ: Alternative neural crest cell fates are instructively promoted by TGFbeta superfamily members. Cell 85:331-343, 1996.
21. Grainger DJ, Metcalfe JC, Grace AA and Mosedale DE: Transforming growth factor-beta dynamically regulates vascular smooth muscle differentiation in vivo. J Cell Sci 111: 2977-2988, 1998.
22. Hirschi KK, Rohovsky SA and D'Amore PA: PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. J Cell Biol 141: 805-814, 1998.
23. Chen S, and Lechleider RJ: Transforming growth factor-beta-induced differentiation of smooth muscle from a neural crest stem cell line. Circ Res 94: 1195-1202, 2004.
24. Hirschi KK, Burt JM, Hirschi KD and Dai C: Gap junction communication mediates transforming growth factor-beta activation and endothelial-induced mural cell differentiation. Circ Res 93: 429-437, 2003.
25. Ross JJ, Hong Z, Willenbring B, Zeng L, Isenberg B, Lee EH, Reyes M, Keirstead SA, Weir EK, Tranquillo RT and Verfaillie CM: Cytokine-induced differentiation of multipotent adult progenitor cells into functional smooth muscle cells. J Clin Invest 116: 3139-3149, 2006.
26. Lindahl P, Johansson BR, Levéen P and Betsholtz C: Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277: 242-245, 1997.
27. Hellström M, Kalén M, Lindahl P, Abramsson A and Betsholtz C: Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126: 3047-3055, 1999.
28. Kim YM, Jeon ES, Kim MR, Jho SK, Ryu SW and Kim JH: Angiotensin II-induced differentiation of adipose tissue-derived mesenchymal stem cells to smooth muscle-like cells. Int J Biochem Cell Biol 40: 2482-2491, 2008.
29. McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, Niklason LE and Counter CM: Human arteries engineered in vitro. EMBO Rep 4: 633-638, 2003.
30. Long JL and Tranquillo RT: Elastic fiber production in cardiovascular tissue-equivalents. Matrix Biol 22: 339-350, 2003.
31. Gong Z and Niklason LE: Blood vessels engineered from human cells. Trends Cardiovasc Med 16: 153-156, 2006.
32. Isenberg BC, Williams C and Tranquillo RT: Small-diameter artificial arteries engineered in vitro. Circ Res 98: 25-35, 2006.
33. Cho SW, Lim SH, Kim IK, Hong YS, Kim SS, Yoo KJ, Park HY, Jang Y, Chang BC, Choi CY, *et al*: Small-diameter blood vessels engineered with bone marrow-derived cells. Ann Surg 241: 506-515, 2005.
34. Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B and Ignarro LJ: Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc Natl Acad Sci USA 103: 12167-12172, 2006.
35. Beltrami AP, Cesselli D, Bergamin N, Marcon P, Rigo S, Puppato E, D'Aurizio F, Verardo R, Piazza S, Pignatelli A, *et al*: Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow). Blood 110: 3438-3446, 2007.
36. Miano JM: Mammalian smooth muscle differentiation: origins, markers and transcriptional control. Results Probl Cell Differ 38: 39-59, 2002.
37. Grainger DJ: Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. Arterioscler Thromb Vasc Biol 24: 399-404, 2004.
38. Björkerud S: Effects of transforming growth factor-beta 1 on human arterial smooth muscle cells in vitro. Arterioscler Thromb 11: 892-902, 1991.
39. Deaton RA, Su C, Valencia TG and Grant SR: Transforming growth factor-beta 1-induced expression of smooth muscle marker genes involves activation of PKN and p38 MAPK. J Biol Chem 280: 31172-31181, 2005.
40. Hautmann MB, Madsen CS and Owens GK: A transforming growth factor beta (TGFbeta) control element drives TGFbeta-induced stimulation of smooth muscle alpha-actin gene expression in concert with two CarG elements. J Biol Chem 272: 10948-10956, 1997.
41. Kawai-Kowase K, Sato H, Oyama Y, Kanai H, Sato M, Doi H and Kurabayashi M: Basic fibroblast growth factor antagonizes transforming growth factor-beta1-induced smooth muscle gene expression through extracellular signal-regulated kinase 1/2 signaling pathway activation. Arterioscler Thromb Vasc Biol 24:1384-1390, 2004.
42. Papetti M, Shujath J, Riley KN and Herman IM: FGF-2 antagonizes the TGF-beta1-mediated induction of pericyte alpha-smooth muscle actin expression: a role for myf-5 and Smad-mediated signaling pathways. Invest Ophthalmol Vis Sci 44: 4994-5005, 2003.
43. Kucich U, Rosenbloom JC, Abrams WR, Bashir MM and Rosenbloom J: Stabilization of elastin mRNA by TGF-beta: initial characterization of signaling pathway. Am J Respir Cell Mol Biol 17: 10-16, 1997.
44. Kucich U, Rosenbloom JC, Abrams WR and Rosenbloom J: Transforming growth factor-beta stabilizes elastin mRNA by a pathway requiring active Smads, protein kinase C-delta, and p38. Am J Respir Cell Mol Biol 26: 183-188, 2002.
45. Hong HH, Uzel MI, Duan C, Sheff MC and Trackman PC: Regulation of lysyl oxidase, collagen, and connective tissue growth factor by TGF-beta1 and detection in human gingiva. Lab Invest 79: 1655-1667, 1999.
46. Ross JJ and Tranquillo RT: ECM gene expression correlates with in vitro tissue growth and development in fibrin gel remodeled by neonatal smooth muscle cells. Matrix Biol 22: 477-490, 2003.
47. Yao L, Swartz DD, Gugino SF, Russell JA and Andreadis ST: Fibrin-based tissue-engineered blood vessels: differential effects of biomaterial and culture parameters on mechanical strength and vascular reactivity. Tissue Eng 11: 991-1003, 2005.
48. Sinha S, Hoofnagle MH, Kingston PA, McCanna ME and Owens GK: Transforming growth factor-beta1 signaling contributes to development of smooth muscle cells from embryonic stem cells. Am J Physiol Cell Physiol 287: C1560-C1568, 2004.
49. Kinner B, Zaleskas JM and Spector M: Regulation of smooth muscle actin expression and contraction in adult human mesenchymal stem cells. Exp Cell Res 278: 72-83, 2002.
50. Wang D, Park JS, Chu JS, Krakowski A, Luo K, Chen DJ and Li S: Proteomic profiling of bone marrow mesenchymal stem cells upon transforming growth factor beta1 stimulation. J Biol Chem 279: 43725-43734, 2004.
51. Collins T, Pober JS, Grimbone MA Jr, Hammacher A, Betsholtz C, Westermark B and Heldin CH: Cultured human endothelial cells express platelet-derived growth factor A chain. Am J Pathol 126: 7-12, 1987.
52. Westermark B, Siegbahn A, Heldin CH and Claesson-Welsh L: B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein-tyrosine kinase. Proc Natl Acad Sci USA 87: 128-132, 1990.
53. Holmgren L, Glaser A, Pfeifer-Ohlsson S and Ohlsson R: Angiogenesis during human extraembryonic development involves the spatiotemporal control of PDGF ligand and receptor gene expression. Development 113: 749-754, 1991.
54. Hyytiäinen M, Penttinen C and Keski-Oja J: Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. Crit Rev Clin Lab Sci 41: 233-264, 2004.
55. Derynck R and Akhurst RJ: Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat Cell Biol 9: 1000-1004, 2007.
56. Liang MS and Andreadis ST: Engineering fibrin-binding TGF-β1 for sustained signaling and contractile function of MSC based vascular constructs. Biomaterials 32: 8684-8693, 2011.
57. Gallagher JT: Heparan sulfate: growth control with a restricted menu. J Clin Invest 108: 357-361, 2001.