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Bone Marrow-Derived Regenerated Smooth Muscle Cells Have Ion Channels and Properties Characteristic of Vascular Smooth Muscle Cells

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Abstract

Rationale: Numerous reports, including our own, have recently suggested the presence of putative smooth muscle progenitor cells in the bone marrow (BM) and those smooth muscle-like cells may be differentiated from BM stromal cells (BMSCs). However, few studies have addressed whether the differentiated cells also possess the functional properties of smooth muscle cells (SMCs). Contractility is the primary function of native vascular SMCs.

Objective: The aim of this electrophysiological study was to characterize BM-derived SMCs using the patch-clamp technique and Ca^{2+} imaging with fura-2.

Methods and results: To investigate whether BM-derived SMCs exhibit functional vascular SMC properties, we measured Ca^{2+} and K^+ currents in BM-derived SMCs using the whole-cell patch-clamp method. The cells showed L-type and T-type Ca^{2+} channel currents, Ca^{2+}-activated K^+ channel (K_{Ca}) currents, and delayed rectifier K^+ channel (K_V) currents. We also measured agonist-evoked $[Ca^{2+}]_i$ transients in BM-derived SMCs using fura-2 imaging. Such $[Ca^{2+}]_i$ transients were observed in response to the vascular SMC-specific agonists, bradykinin (10^{-6} M) and angiotensin II (10^{-7} M).

Conclusions: BM-derived SMCs displayed contractile activity and expressed several ion channels critical for contractile behavior in a manner compatible with native vascular SMCs. BMSC-derived cells thus have the potential to differentiate into functional vascular SMCs, suggesting bone marrow stromal tissue as a useful source of cells for the treatment of injured arteries and to construct tissue-engineered grafts for adult arterial revascularization.

Keywords: Smooth muscle cells; Bone marrow-derived; Vascular grafts; Arterial pressure; Contractility; Ion channels; Tissue engineering

Introduction

The construction of stable blood vessels is a fundamental challenge for tissue engineering in regenerative medicine. One of the most important issues in vascular tissue engineering remains the availability of reliable and expandable cell sources for constructing tissue-engineered vascular grafts for use in adult arterial revascularization [1], and particularly smooth muscle cell (SMC) sources [2]. We and others previously demonstrated that smooth muscle-like cells can be derived from bone marrow (BM) cells (BMC) both *in vitro* [3] and *in vivo* [4]. However, the functional property of these cells such as force generation or contractile activities were not investigated and therefore, it remains unclear whether these cells are functionally equivalent to mature SMCs.

The present study investigated the physiological characteristics of BM-derived SMCs, including contractile activity and Ca^{2+}/K^+ currents. Electrophysiological experiments using patch clamping and Ca^{2+} imaging with fura-2 were conducted to characterize the BM-derived cells, with differentiated SM-like cells exhibiting a contractile function similar to that of native smooth muscle. The BM-derived SMCs also expressed four types of ion-channel currents that are observed in native SMCs. Our study thus describes a source of bone marrow cells for smooth muscle applications, provides a reliable smooth muscle cell source for vascular-tissue engineering, and describes an in vitro system to test the functional capacity of BM-derived SMCs to contract and relax in response to common pharmacological agents.

Materials and Methods

Cell culture and plasmid construction

Mononuclear cells obtained by the Ficoll-Paque protocol [5] from murine (C57BL6 mouse) bone marrow (2 x 10^5 nuclear cells/ml) were plated in 100-mm sterile flasks and cultured in Dexter-type culture medium [6] containing 10% heat-inactivated horse serum (Sigma). BM-derived adhesion cells appeared within a week (early-phase adhesion cells, E-ad cells) and gradually differentiated into BMSCs over the next 3-7 weeks.A 480-base fragment of the human SM22α promoter [7] was obtained by PCR from human genomic DNA using the following primers: forward 5'-GGATCCCATGTCCCATCAGA-3' and reverse 5'-GGGGCGCTGGCTGGGTGAGG-3'. The fragment was integrated into a promoterless GFP vector (pd2EGFP, Clontech, Mountain View, CA), and then transfected by lipofection (10 μg /100 mm flask; Invitrogen, Carlsbad, CA) into the E-ad cells on the fifth day after seeding of the mononuclear cells. The efficiency of lipofection was 18-32% in the study. Individual clones were successfully obtained using cloning cylinders (Sigma, St. Louis, MO) and G418 (500 μg/ml, Gibco BRL, Grand Island, NY) selection. GFP-positive clones (<1% of total cells) were subsequently cultured for immunochemical staining and

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physiological studies. The smooth muscle cell line of A7r5 cells were used for immunostaining against SMC-specific antibodies.

Immunocytochemistry

Immunocytochemistry was performed using antibodies recognizing platelet-derived growth factor β receptor (PDGF-β, Santa Cruz Biotechnology, Santa Cruz, CA, diluted 1:250), Flk-1 (Santa Cruz, diluted 1:200), smooth muscle myosin heavy chain (SM) embryo, (SMemb, marker of immature SMC, diluted 1:400), calponin h1 (Santa Cruz, diluted 1:100), alpha-smooth muscle actin (α-SM actin, Biomega, diluted 1:200), and smooth muscle myosin heavy chain-1 (SM-1, marker of mature SMC [8], diluted 1:400). The SMemb and SM-1 antibodies were kindly provided by Dr M. Periasamy.

Northern blot analysis

RNAs were prepared using an RNeasy total RNA purification kit (Qiagen, Hilden, Germany) and then 20 μg of RNA was transferred to nylon membrane (hybond-N+, Amersham Pharmacia, Uppsala, Sweden). The membranes were hybridized with the following probes: a 783-bp murine SM22α cDNA [7], a 841-bp murine calponin cDNA [9], and a 513-bp β actin cDNA [10].

Measurement of intracellular free calcium concentration

Cells plated on glass-bottom dishes were loaded with 3 μM fura-2 acetoxymethylester (AM) in the dark for 30 min. The loaded cells were rinsed three times in normal Tyrode's (NT) solution [11] containing (in mM) 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5 HEPES, and 10 glucose (pH was adjusted to 7.4 with NaOH), and then incubated in NT solution for an additional 30 min in the dark at 37°C in 5% CO_2/95% air. Solutions were superfused at a rate of 2 ml/min: 10^{-6} M of bradykinin, 10^{-7} M of angiotensin II, or 60 mM of KCl was applied for 30-60 seconds. Data acquisition and analysis were carried out using AquaCosmos 2.0 (Hamamatsu, Japan).

Electrophysiological measurement

Cells were plated on coverslips for patch clamping using pipettes that were pulled fresh from plain capillary tubes made of soda-lime glass (Chase Instruments, USA); resistance was 2-4 MΩ when pipettes were filled with solution. Membrane currents were recorded using an appropriate patch-clamp amplifier (Axopatch 200A, Axon Instruments, Palo Alto, CA). Data acquisition and analysis were carried out using pClamp software (Axon Instruments). Current signals were filtered at 1 kHz and sampled at a frequency of 5 kHz. Cells were voltage clamped [12] at a holding potential of -80 mV, and currents were evoked by +10 mV-increment depolarizing steps of 500 ms to +80 mV. Ca^{2+} currents were recorded in Cs Tyrode (CsT) solution or high Ca^{2+} solution. CsT solution contained (in mM) 135 NaCl, 5.4 CsCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5 HEPES, and 10 glucose; pH was adjusted to 7.4 with NaOH. The high Ca^{2+} solution contained (in mM) 135 NaCl, 5.4 KCl, 3.6 $CaCl_2$, 1.0 $MgCl_2$, 5 HEPES, and 10 glucose; pH was adjusted to 7.4 with NaOH. The pipette solution used for recording Ca^{2+} currents contained (in mM) 125 CsCl, 1.0 $MgCl_2$, 5 HEPES, 3 Mg-ATP, and 5 Cs-BAPTA; pH was adjusted to 7.3 with CsOH. K^+ currents were recorded in NT solution or Ca^{2+}-free solution, which contained (in mM) 135 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 5 HEPES, and 10 glucose; pH was adjusted to 7.4 with NaOH. The pipette solution used for recording K^+ currents contained (in mM) 140 KCl, 1.0 $MgCl_2$, 10 HEPES, 5 Mg-ATP, and 10 EGTA; pH was adjusted to 7.3 with KOH.

Contractile activity study

Cells plated on glass-bottom dishes were washed in PBS and then incubated in NT solution for 30 minutes. Cells were imaged on an ECLIPSE TE 300 microscope (Nikon, Japan) every 5 min from 0-45 min after exposure to 10^{-6} M of bradykinin, 10^{-7} M of angiotensin II, or 60 mM of KCl, for analysis of cell shape using ACT-2U software (Nikon, Japan).

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). For a single comparison, unpaired Student's t-test was used to evaluate statistical significance. For multiple comparisons, statistical significance was evaluated by one-way ANOVA followed by Tukey's post hoc test. Differences were considered to be significant when $P<0.05$.

Results

Isolation of smooth muscle-like cells from bone marrow

Five days after transfection of the SM22α promoter/GFP plasmid into the BM-derived E-ad cells, GFP was detected in a cell population (Ad(G) cells: BM-derived smooth muscle progenitor cell: BM-SMPC) that expressed neither mature (calponin) nor immature (SMemb) SMC markers at that time (Figure 1A and 1B). Over the next 3-5 weeks, these BM-SMPCs grew into individual clones, the cells of which had a flattened appearance and gradually gained phenotypes consistent with mature smooth muscle lineage cells. Immunocytochemistry revealed individual clones/BM-derived SM-like cells (BM-derived SMCs) that were clearly

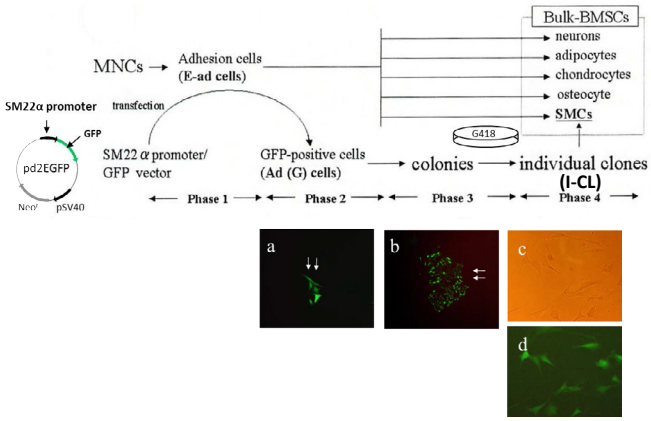

Figure 1A: Chart for illustrating the course used to acquire samples. Bone marrow mononuclear cells (MNCs) dispersed in culture dishes, part of them changed to early phase adhesion cells (E-ad cells) within 1 week. For the next 3-7 weeks, the adhesion cell fraction was grown into BMSCs that had been characterized as a mesenchymal cell population with various cell lineages including SMCs. The dashed-line quadrangle indicates that SMCs coexist with many other cell types among bulk-BMSCs. E-ad cells were transfected with the SM22α promoter/GFP construct. In the transfected E-ad cells, GFP-positive cells (Ad (G) cells) appeared 5 days after the transfection and expressed neither mature (calponin) nor immature (SMemb) SMC-specific marker proteins at that time (see section B). After G418 selection, they were grown into individual clones (I-CL) that exhibited phenotypes consistent with smooth muscle lineage cells (see section C). Thus, Ad (G) cells eventually differentiated into SMCs that were presumed to be bulk-BMSCs. To clarify longitudinal transition of the expression of SMC-specific proteins, the total course is conveniently divided from phase 1 to phase 4. As shown in lower panel, Ad(G) cells came to be detectable (lower panel a) at phase 2, and grew into colonies at phase 3 (lower panel b). In phase 4, the colonies were picked up and investigated as individual clones (I-CL, lower panel c) with stable GFP expression (lower panel d).

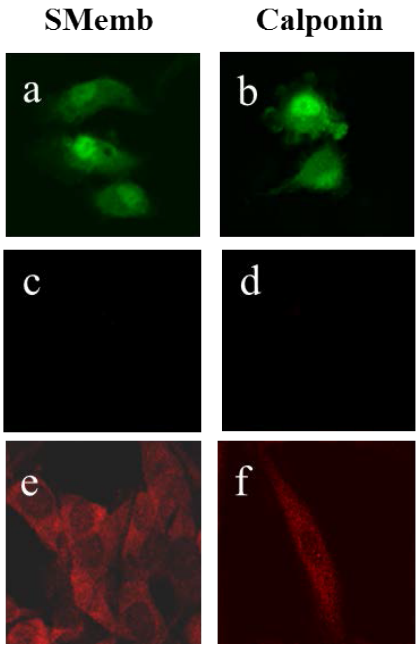

Figure 1B: Confocal photomicrographs of Ad (G) cells. Ad (G) cells (panel a and b) were not stained by the anti-SMemb (panel c, diluted 1:1000) or anti-calponin antibodies (panel d, diluted 1:100). Thus, Ad (G) cells expressed neither mature (calponin) nor immature (SMemb) SMC-specific proteins at this time. Magnification, x 100. Immunostaining of NIH3T3 fibroblast cells against the SMemb antibody and native smooth muscle cells against calponin antibody were shown as positive controls (panel e and f, respectively).

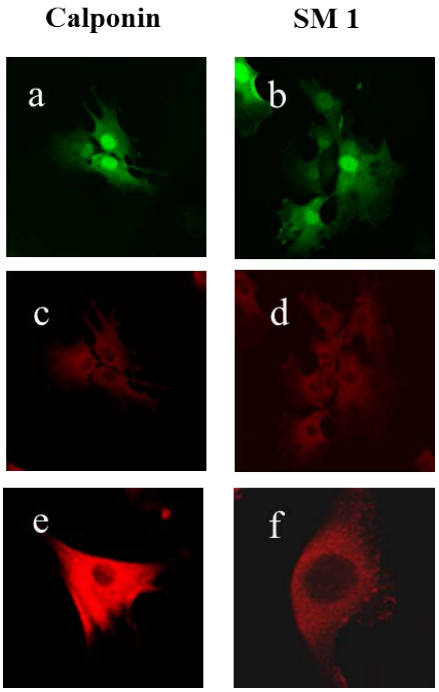

Figure 1C: Confocal photomicrographs of individual clones (I-CL) cells immunostained for calponin and SM-1. The clusters of I-CL cells (panel a and b) were clearly immunopositive for the SMC-specific proteins, calponin and SM-1 (panel c and d). Magnification, x 100. Immunostaining of A7r5 smooth muscle cells against the SMC-specific proteins' antibodies were shown as positive controls (panel e and f).

stained for the SMC markers, calponin and SM-1 (Figure 1C).

BM-derived SMCs expressed α-SM actin

Neither the BM-bulk-stromal cells nor the BM-SMPC populations showed staining for α-SM actin. In contrast, the BM-derived SMCs clearly expressed the α-SM actin protein (Figure 2A). Northern blot analyses also showed abundant expression of calponin and SM22α at the mRNA level (Figure 2B).

BM-derived SMCs exhibit four types of ion channel currents

We used whole-cell patch clamping to detect ion channel currents in the BM-derived SMCs. Two types of Ca^{2+} channel currents were defined (n=22): low voltage-activated Ca^{2+} current (LVA) and high voltage-activated Ca^{2+} current (HVA), with both LVA and HVA also expressed simultaneously (Figure 3A, left panel). On the other hand, the bulk-stromal cells (n=5) showed no inward currents (Figure 3A, right panel), while BM-SMPCs (n=20) showed LVA inward current (10%) or no inward current (90%) (data not shown). The LVA and HVA inward currents were enhanced by switching from CsT solution

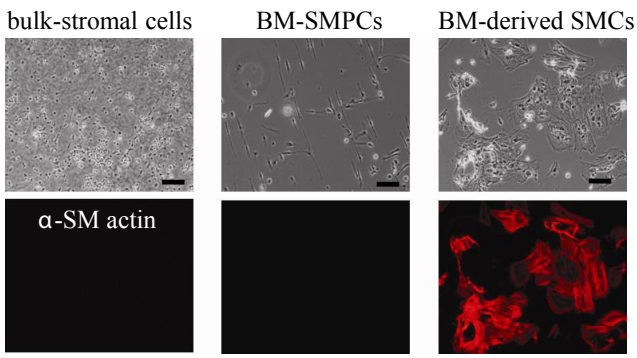

Figure 2A: No Neither BM bulk-stromal cells or Ad (G) cells/BM-SMPCs showed staining with the anti-α-SM actin antibodies. The clusters of I-CL cells (right panels) were clearly stained for α-SM actin (right lower panel). Bars, 100 mm.

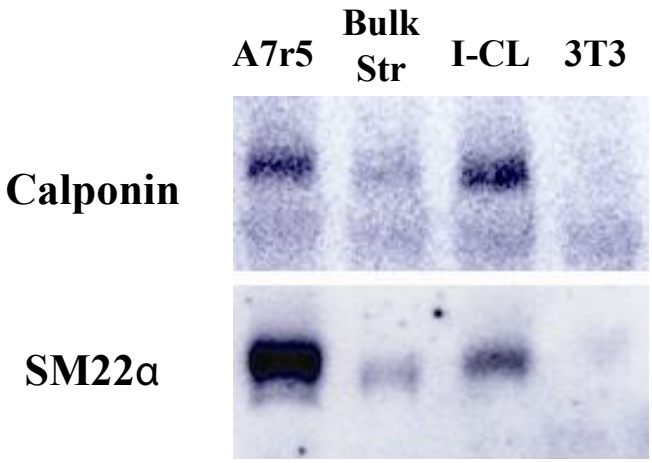

Figure 2B: mRNA levels of calponin and SM22α in A7r5 cells, bulk-BMSCs, I-CL cells, and 3T3 cells. Each sample lane was loaded with 20 μg of total RNA, with β-actin expression in each sample used as an intensity control. I-CL cells expressed both calponin and SM22α at the mRNA level; while bulk-stromal cells expressed calponin mRNA at < 1% of the I-CL cell intensity (quantified with a BAS 2500 Bioimage Analyzer). The SMC line A7r5 was used as a positive control.

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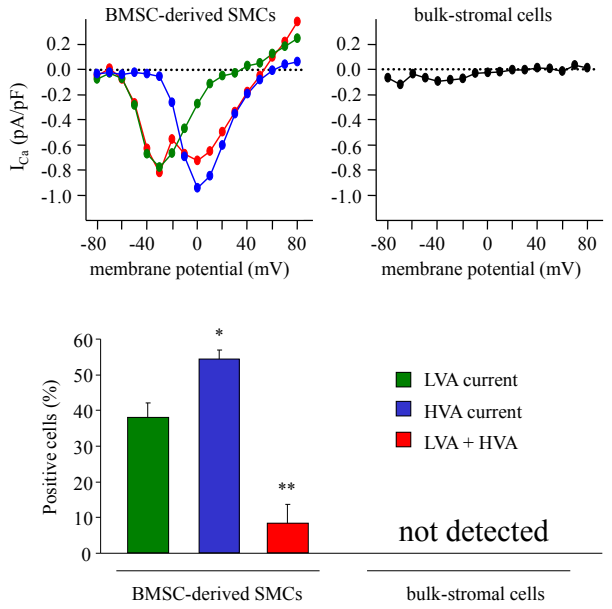

Figure 3A: The graph represents current-voltage (I-V) curves. Cells were voltage clamped at a holding potential of -80 mV, and currents were evoked by +10 mV increment depolarizing steps to +80 mV. In BMSC-derived SMCs, low voltage-activated Ca^{2+} current (LVA, ●), high voltage-activated Ca^{2+} current (HVA, ●), or both LVA and HVA (●) were recorded. In bulk-stromal cells, Ca^{2+} current was not detected (●). The percentages of showing LVA or HVA in cells are shown in lower panel. Data are expressed as the mean ± SEM (*P<0.05, ** P<0.01 vs. LVA current of BMSC-derived SMCs). n=22 (BMSC-derived SMCs), n=5 (bulk-stromal cells).

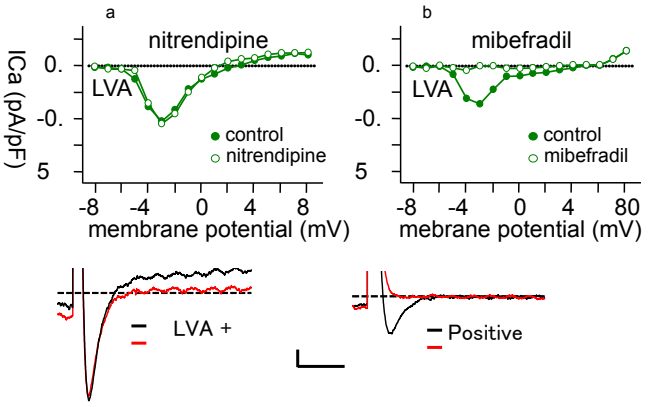

Figure 3B: The graph (panels a and b) represents I-V curves for BMSC-derived SMCs with (○) and without (●) inhibitor. Typical traces (panels c and d) show I_{Ca} elicited by depolarization to -30 mV from -80 mV with (-) or without (-) inhibitor. A selective L-type Ca^{2+} channel blocker nitrendipine did not inhibit LVA current, but it was inhibited by a selective T-type Ca^{2+} channel blocker mibefradil.

to high Ca^{2+} solution, indicating these as T-type Ca^{2+} channel currents (Figure 3B) and L-type Ca^{2+} channel currents (Figure 3C), respectively. We also defined two types of K^+ channel currents in the BM-derived SMCs when switched from NT solution to Ca^{2+}-free solution (n=4): decreased K^+ currents (K_{Ca} current) and resting K^+ currents (K_V current) (Figure 4, left panel). On the other hand, the bulk-stromal cells (n=4) showed K_V current, but no K_{Ca} current (Figure 4, right panel).

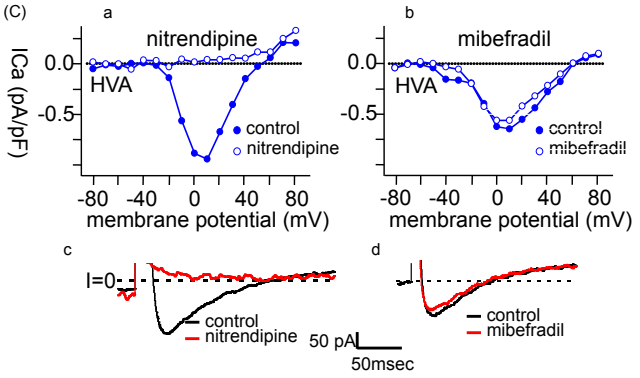

Figure 3C: The graph (panels a and b) represents I-V curves for BMSC-derived SMCs with (○) and without (●) inhibitor. Typical traces (panels c and d) show I_{Ca} elicited by depolarization to +10 mV from -80 mV with (-) or without (-) inhibitor. A selective L-type Ca^{2+} channel blocker nitrendipine inhibited HVA current, but it was not inhibited by a selective T-type Ca^{2+} channel blocker mibefradil.

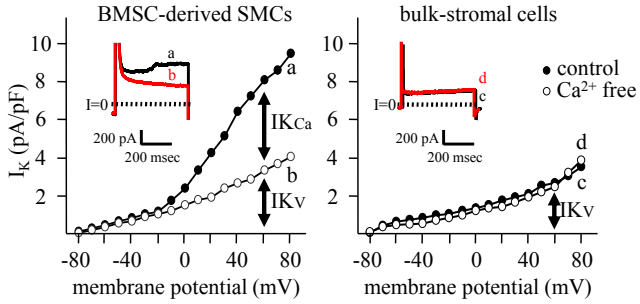

Figure 4: BM-derived SMCs show two types of K^+ channel currents. The graph represents I-V curves for the BM-derived SMCs and bulk-stromal cells. Ten mV-increment depolarizing voltage steps were applied from a holding potential of -80 mV to +80 mV. In BMSC-derived SMCs, switching from control solution (1.8 mM Ca^{2+}, ●) to Ca^{2+}-free solution (○) decreased K^+ channel currents, indicating at least two types of K^+ channels present: decreased K^+ current (K_{Ca} current) and resting K^+ current (K_V current). The bulk-stromal cells showed K_V current, but not K_{Ca} current.

BM-derived SMCs showed changes in $[Ca^{2+}]_i$ in response to SMC agonists

We assessed transient $[Ca^{2+}]_i$ changes in the BM-derived SMCs in response to SMC agonists using fura-2. Resting $[Ca^{2+}]_i$ was approximately 250 nM in both the BM-derived SMCs and bulk-stromal cells (Figure 5A). Exposure to bradykinin induced a rapid increase in $[Ca^{2+}]_i$ in about 40% of BM-derived SMCs (n = 14) (see panel b in Figure 5B), but had no such effect of the bulk-stromal cells (n=30) (Figure 5A). The response was diminished after removal of bradykinin. Similarly, exposure to angiotensin II (Figure 5, lower panel) or high KCl induced an increase in $[Ca^{2+}]_i$ in BM-derived SMCs (4w), but not in bulk-stromal cells.

BM-derived SMCs displayed significant contractile activity in response to SMC agonists

The defining property of mature SMC is their ability to contract and generate force in response to vasoactive agonists. To examine whether the BM-derived SMCs generated in this study showed such functional properties, cells were imaged before and after exposure

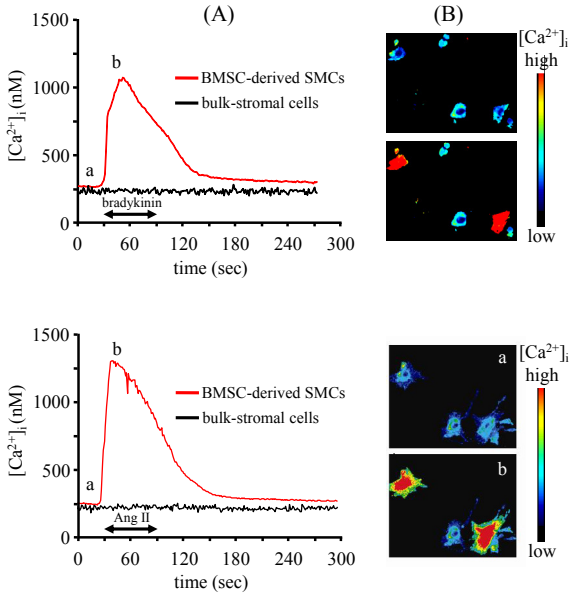

Figure 5: BM-derived SMCs showed a $[Ca^{2+}]_i$ response to bradykinin. (A) Graph represents typical time course of $[Ca^{2+}]_i$ in cells. Exposure to bradykinin induced a rapid increase in $[Ca^{2+}]_i$ in BMSC-derived SMCs (red line), but not in bulk-stromal cells (black line). (B) Representative pseudocolor images, where red and yellow represent high and blue and black represent low $[Ca^{2+}]_i$ concentrations. The pseudocolor images in fura-2-AM-loaded BMSC-derived SMCs (a and b) were acquired at the time corresponding to panels a and b in (A), respectively. As shown in lower panel, BM-derived SMCs showed the same $[Ca^{2+}]_i$ response to angiotensin II.

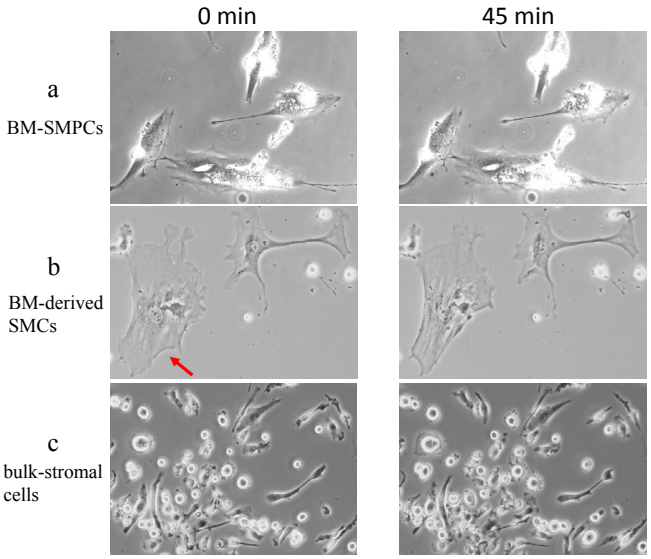

Figure 6A: The pictures represent cells before and 45 min after exposure to 10^{-6} M of bradykinin. BMSC-derived SMCs showed changes in shape and sequential directional movement (panel b arrow). However, BM-SMPCs and bulk-stromal cells showed only non-directional movement (panels a and c). Bars, 100 mm.

to bradykinin. About 40% of the BM-derived SMCs (n=20) showed changes in shapes and sequential directional movement (panel b in Figure 6A) that were consistent with contractile activity. Similar changes were recorded in response to other SMC agonists, angiotensin II or high KCl (Figure 6B and 6C). On the other hand, the bulk-stromal cells (n=30) and BM-SMPCs (n=21) showed only non-directional movement in response to bradykinin (Figure 6A), angiotensin II, or high KCl (Figure 6B and 6C).

Discussion

The present study used SM22α promoter and fluorescence-activated cell selection to define a proliferative cell population displaying biochemical and physiological characteristics of SMCs. Specifically, these BM-derived SMCs displayed contractile activity and expressed many ion channels critical for their contractile behavior comparable

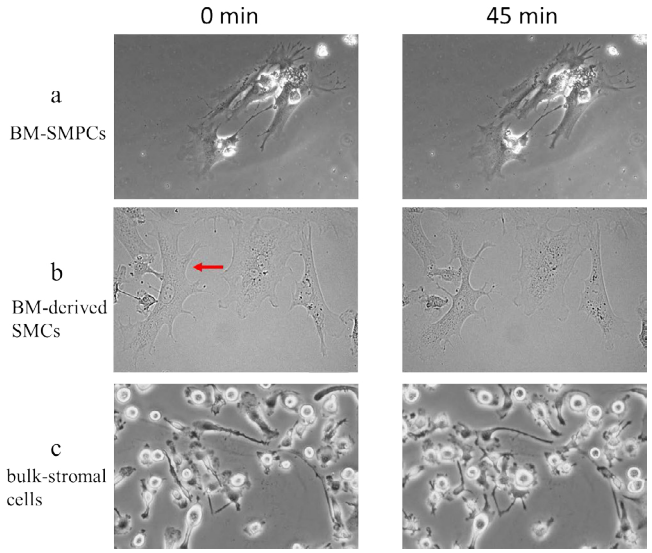

Figure 6B: The pictures represent cells before and 45 min after exposure to 10^{-7} M of angiotensin II. BMSC-derived SMCs showed changes in shape and sequential directional movement (panel b arrow). However, BM-SMPCs and bulk-stromal cells showed only non-directional movement (panels a and c).

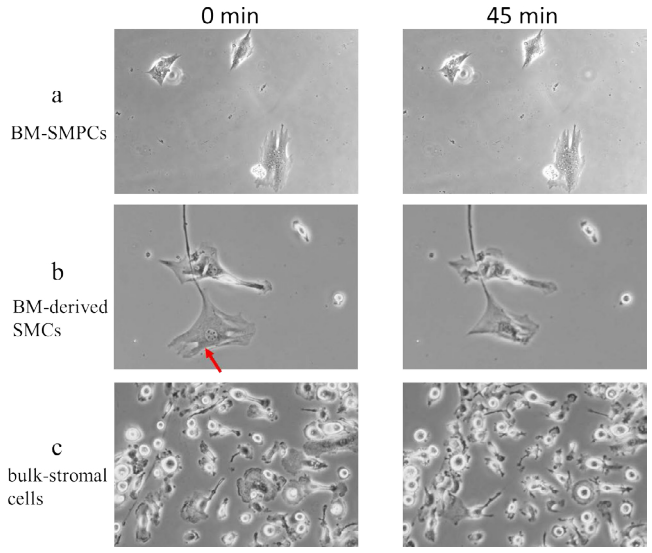

Figure 6C: The pictures represent cells before and 45 min after exposure to 60 mM KCl. BMSC-derived SMCs showed changes in shape and sequential directional movement (panel b arrow). However, BM-SMPCs and bulk-stromal cells showed only non-directional movement (panels a and c).

to those of native vascular SMCs. Importantly, the differentiated BM-derived cells, but not their precursors, exhibited the functional ability to contract and relax in direct response to pharmacological agents.

Assessing stem cell differentiation should involve not only identifying structural proteins and transcription factors consistent with a differentiated cell type, but also the demonstration of differentiated-cell functions. We previously reported and established a SM22α promoter to select for SMCs from BM stromal cells [3]. Putative BM-SMPCs, named Ad (G) cells, showed an active SM22α promoter and expressed neither immature nor mature SMC markers. G418 selection for 21 days produced clones of cells expressing SMC marker proteins and transcripts, suggesting that a fraction of the cells with active SM22α promoter differentiated into smooth muscle like cells. The present study investigated, using the SM22α promoter and fluorescence-activated cell selection, to clarify those BM-SMPCs and differentiated BM-derived SMCs exhibit both biochemical and physiological characteristics of SMC function.

The construction of stable blood vessels is a fundamental challenge for tissue engineering in regenerative medicine. Although the use of human cells in low-pressure applications (< 20 mmHg) has achieved significant clinical success in pediatrics [13], the promise of a tissue-engineered graft for adult arterial revascularization remains unrealized [14]. The *in vivo* mechanical functioning of native arteries is mainly governed by elastin and activated SMCs at physiological pressure. McAllister et al. [15] clearly revealed that aneurysms developing in some grafts after implantation were attributable to the selection of fibroblasts as a cell source. It is therefore feasible that vascular grafts biofabricated from fibroblasts would contain insufficient elastin and be prone to aneurysms developing due to collagen fatigue. The one way to overcome these limitations is to select another cell source capable of differentiating into vascular SMCs under optimum perfusion conditions in a bioreactor [1] (such as BM-derived mesenchymal stem cells). Such cells could successfully and sufficiently supply both elastin and collagen. Thus, our established BM-SMPC population seems to be a good candidate for constructing tissue-engineered grafts for adult arterial revascularization.

Contractile activity is the defining property of mature SMC and one of the most important properties of blood vessels. The BM-derived SMCs generated in the present study displayed changes in shape and sequential directional movement in response to vasoconstrictors such as bradykinin, angiotensin II, and high KCl. High KCl concentration induces muscle contraction by opening the L-type [16], slow, calcium potential-dependent channels, while angiotensin II acts through angiotensin II receptors type-1 and type-2, and bradykinin acts through bradykinin receptors. The BM-derived SMCs therefore showed both non-receptor- and receptor-mediated pathways of vascular reactivity. In contrast, the bulk-stromal cells and BM-SMPCs displayed no contractility, apart from some non-directional movement in response to bradykinin, angiotensin II, and high KCl, indicating that BM contains only a small proportion of functional SMCs that retain their biochemical and contractile properties after purification and expansion *in vitro*.

SMCs express many ion channels and membrane receptors critical for contractile behavior mediated through the Ca^{2+}/calmodulin-dependent phosphorylation of myosin light chain [17]. Influx of Ca^{2+} from voltage-activated Ca^{2+} channels and intracellular stores is the major source of Ca^{2+} for contraction. In cardiomyocytes and vascular SMCs, the most important Ca^{2+}-activated channels are the L-type Ca^{2+} channels [17]. Using whole-cell patch clamping, we demonstrated functional L-type channels on the BM-derived SMCs that were not present on either undifferentiated BMSCs or BM-SMPCs selected with SM22α GFP alone. Functional L-type channels could be detected 4 weeks after the GFP selection, at current densities similar to those in rat aortic SMCs. These results are consistent with further maturation of the BM-derived SMCs after a few passages, as previously shown by SM1/2 expression patterns [3].

Recently, murine embryonic stem (ES) cells [18] and different types of primary adult stem/progenitor cells including mesenchymal stem cells [19] (MSCs), BM-derived multipotent stem cells [20], neural stem cells [21], mesoangioblasts [22], and hair-foricle cells [23] have been used to elucidate signals that induce commitment and differentiation of SMCs. The iPS cells [24] also differentiate into all three germ layers including α-SM actin-positive cells. Although most of these studies demonstrated protein expressions consistent with SMCs, few characterized the differentiated cells for functional properties of SMCs. One exceptional study revealed that human amniotic fluid stem cells (hAFSCs) under selective culture conditions are able to give rise to functional SMCs [25]. However, they have shown the presence only of the delayed rectifier and Ca^{2+}-dependent K^+ currents in hAFSCs-derived SMCs. It has been reported that both L-type and T-type Ca^{2+}-channel expression patterns [26] are regulated during SMC development [27]. The current electrophysiological experiments using the patch-clamp technique showed 4 types of channels including both L-type and T-type Ca^{2+}-channel expression in the BM-derived SMCs. The previous study also attempted to culture SMCs from bone marrow hAFSCs by stimulation with cytokines and growth factors such as PDGF-BB and TGF-β [25]. Although soluble factors in the medium can differentiate a fraction of cells toward the SMC lineage, these approaches carry potential clinical risks with respect to overly accelerating cell proliferation and mutagenesis. Moreover, the dilemmas in using hAFSCs for constructing tissue-engineered vascular grafts for adults induced alloreactivity. Thus, our established BM-derived SMC using the SM22α promoter and fluorescence-activated cell selection could be a reliable source of healthy SMCs that can be safely harvested and that require minimal manipulation for constructing tissue-engineered vessels.

In conclusion, we have demonstrated that BM-derived SMCs express SMC-specific proteins and ion channels in a temporally appropriate fashion and display functional attributes similar to primary vascular SMC populations, making them a good candidate stem cell population for developmental studies and tissue engineering. Most importantly, these BM-derived SMCs displayed contractility and expressed many ion channels critical for cellular contractile behavior that are comparable to native matured vascular SMCs. The robust differentiation from BM-SMPCs to nearly homogeneous populations of SMCs described here will allow us to further characterize the molecular processes underlying SMC differentiation. Since BM-derived Ad(G) cells have the potential to differentiate into functional SMCs, BMSC tissue could be a useful source of cells for treating injured tissues where smooth muscle plays an important role, including regenerative tissue-engineered blood vessels for revascularization.

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We dedicate this research article to late Dr Takeshi Nakamura