Original Research

Isolation and characterization of multipotent progenitor cells from the human fetal aorta wall

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Abstract

Recent evidence indicates that vascular progenitor cells may be the source of smooth muscle cells (SMCs) and endothelial cells (ECs). In the present study we isolated CD105$^+$, CD34$^-$ and fetal liver kinase$^+$ (Flk1$^+$) cells from the human fetal arterial wall and demonstrated that they were vascular progenitors for both ECs and SMCs. *In vitro*, these cells cultured with vascular endothelial growth factor could differentiate into cells that expressed endothelial markers. Meanwhile, cells cultured with platelet-derived growth factor-BB could differentiate into cells that expressed smooth muscle markers. When transplanted into NOD/SCID mice, they contributed to neoangiogenesis *in vivo* during wound healing. These cells could also differentiate into osteogenic and adipogenic lineages *in vitro*. Hence multipotent vascular progenitor cells do exist in the arterial wall and they may have implications in the physical and pathological conditions of the vessel. Because these cells can be expanded in culture without obvious senescence for more than 30 population doublings, they may be an important source of ECs for cellular pro- or anti-angiogenic therapies.

Keywords: mesenchymal stem cells, CD105, vascular progenitor cells, endothelial cells, smooth muscle cells

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Introduction

All blood vessels are lined by endothelium and, except for the capillaries, surrounded by one or more layers of smooth muscle cells (SMCs). These two cell populations are the major components in the normal blood vessel wall. Ontogenetically, blood vessel formation in the embryo starts by differentiation of endothelial cells (ECs) from the splanchnic mesoderm, especially fetal liver kinase 1^+ (Flk1$^+$) mesoderm cells.[1] In general, it is thought that the subendothelial SMCs differentiate from mesenchymal cells, mesoderm, neural crest or epicardial cells and migrate from the vessel wall.[2–5] Mironov *et al.*[6] reported that the aorta of the 5- to 12-week-old embryos consisted of three sublayers differing in cellular composition. Although the majority of embryo aortic cells appeared like SMCs, cells of the outer sublayer resembled fibroblasts or poorly differentiated mesenchymal cells. As both ECs and SMCs may derive from the mesoderm, it is possible that they have a common progenitor. However, difficulty in preparing pure populations of these lineages has hampered dissection of the mechanisms underlying vascular formation in the development of the embryo. Recently, a common vascular progenitor was proposed by Yamashita *et al.*[7] They reported that Flk1$^+$ cells derived from embryonic stem cells can differentiate into both ECs and mural cells and can reproduce the vascular organization process.

Mesenchymal stem cells (MSCs) were originally isolated from bone marrow that has the potential to differentiate into multiple lineages, including bone, cartilage, adipose and muscle.[8–10] In recent years, others and we have demonstrated that subpopulations of MSCs from bone marrow could also differentiate into ECs under specific conditions.[11–13] In our study, the typical phenotype of these progenitor cells is CD105$^+$, Flk1$^+$ and CD34$^-$. CD105, or endoglin, is a homodimeric membrane glycoprotein primarily expressed on ECs. In association with transforming growth factor (TGF)-β receptors I and II, it can bind TGF-β1 and -β3 and form a functional receptor complex.[14] Interestingly, except on ECs and adventitial fibroblasts, CD105 is also found on a minority of medial SMCs in normal coronary arteries.[15]

In an effort to study the distribution of CD105$^+$ cells in fetal tissues by immunohistochemistry, we found that

$CD105^+$ cells also exist in a variety of tissues, including arterial walls (data not shown). The fact that CD105 is reportedly expressed in both ECs and SMCs, and $CD105^+$ cells from bone marrow are also positive for Flk1, a marker for putative vascular progenitor cells,[7] prompted us to isolate $CD105^+$ cells from the arterial walls and investigate their differentiation potential. Here, we describe for the first time the isolation and *ex vivo* expansion of $Flk1^+CD105^+CD31^-$ αSMA^- vascular progenitor cells from the human fetal aorta wall that can differentiate at the single-cell level not only into ECs but also into SMCs, adipocytes and osteocytes.

Methods

Cell preparation

All human tissues used in this study were procured under approval of the Institutional Review Board of Chinese Academy of Medical Sciences & Peking Union Medical College and were in compliance with the Helsinki Declaration. Fetal aortas were obtained from 20 to 22 week gestation fetuses. The fetal aorta walls were excised, washed with D-Hank's solution and carefully depleted of connective tissues, and ECs were scraped off. The remaining arterial wall was cut and digested for 45 min at 37°C with type II collagenase (0.2%; Worthington Biochemical Corp, Lakewood, NJ, USA), followed by digestion with 0.1% trypsin. The tissues were triturated vigorously and passed through a 70-μm filter, and the cells were collected by centrifugation. The cells were resuspended and plated in expansion medium. Expansion medium consisted of 58% D-MEM/F-12 (Gibco Life Technologies, Paisley, UK), 40% MCDB-201 medium (Sigma, St Louis, MO, USA), 2% fetal calf serum (FCS, Gibco Life Technologies), 10^{-8} mol/L dexamethasone (Sigma), 10^{-4} mol/L ascorbic acid 2-phosphate (Sigma), 10 ng/mL epidermal growth factor (Sigma), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco) at 37°C and a 5% CO_2 humidified atmosphere. When the cells reached 75% confluency, they were harvested by trypsinization and $CD105^+$ cells were sorted by means of micromagnetic beads (Miltenyi Biotec, Sunnyvale, CA, USA). Flow cytometry analysis showed that 99.5% of the eluted cells were $CD105^+$. Then the sorted cells were cultured at a concentration of one single cell per well in 96-well plates pre-coated with extracellular matrix (ECM) gel (Sigma) supplemented with 3% FCS, 5 ng/mL interleukin-6 (Sigma), 30 ng/mL stem cell factor (Sigma) and 30 ng/mL bone morphogenetic protein-4 (BMP-4; Sigma). This is referred to as the BMP-4 culture system in this paper. After 10 days, single-cell clones were picked up and expanded in expansion media supplemented with 10 ng/mL interleukin-6 and 1% lenolenic acid bovine serum albumin (Gibco). Culture-expanded clonal cells were used in the following experiments. All the experiments were performed with the 20th passage and the 35th passage; the results presented in this article are all with the 35th passage.

Flow cytometry analysis

For immunophenotype analysis of the expanded clonal cells, cells were detached and stained sequentially with primary antibodies and appropriate fluorescein isothiocyanate (FITC)-conjugated secondary antibodies. Working concentrations for primary antibodies against human CD105 (PharMingen), CD31, CD34, CD45, Flk1 (Santa Cruz Biotechnology, Inc, CA, USA), HLA-DR (Mountain View, CA, USA), α-smooth muscle actin (αSMA; Dako Corp, Carpenteria, CA, USA), smooth muscle myosin heavy chain (MHC; Dako) and calponin (Dako) were 10–20 ng/mL. For the detection of intracellular protein Flk1, cells were permeabilized with methanol/phosphate-buffered saline (PBS) for two minutes at −20°C. We used same-species, same-isotype irrelevant antibody as negative control. After three washes with PBS containing 0.5% bovine serum albumin, cells were resuspended in PBS and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Endothelial differentiation

The culture-expanded clonal cells were plated on fibronectin at $2 \times 10^4/cm^2$ in EGM-2 medium (Clonetics Inc, San Diego, CA, USA) with 10 ng/mL vascular endothelial growth factor (VEGF; Clonetics Inc) and cultured for 15–20 days.[16] After differentiation induction, cells were fixed with 4% paraformaldehyde and subjected to immunofluorescence analysis for CD31, Tek (Santa Cruz) and von Willebrand factor (vWF) by confocal fluorescence microscopy. To demonstrate endothelial differentiation, fluorescence-activated cell sorting (FACS) and Western blot methods were also used. For Western blotting, protein extracts were prepared from logarithmically growing cells (both before and after differentiation) and analyzed as previously described.[13]

SMC differentiation

A total of 2×10^4 cells/cm^2 were plated in EGM-2 medium supplemented with 50 ng/mL platelet-derived growth factor-BB (PDGF-BB, R&D Systems, MN, USA) with medium exchanges every three days for about 15–20 days. After differentiation induction, cells were fixed with 4% paraformaldehyde and subjected to immunofluorescence analysis for αSMA, MHC and calponin. Cells were evaluated by confocal fluorescence microscopy. Results were further confirmed by FACS analysis and Western blot.

For Western blotting, protein extracts were prepared from logarithmically growing cells (both before and after differentiation) by lysis in buffer (25 mmol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl, 1% Nonidet P-40, 1 mmol/L EDTA and 2 mmol/L EGTA). Then the protein lysates were separated on a sodium dodecyl sulfate 7.5% polyacrylamide gel (Bio-Rad, Hercules, CA, USA) and transferred onto nitrocellulose membrane. After blocking, blots were incubated with monoclonal antibodies to αSMA, human smooth muscle MHC, calponin and β-actin at 1:100 in the blocking solution for one hour at room temperature. The blots were washed and incubated with a sheep anti-mouse horseradish peroxidase-conjugated IgG (E I du Pont de Nemours, MA, USA) at 1:5000 for one hour. Immunodetection using the

enhanced chemiluminescence method (ECL kit; Amersham) was performed according to the manufacturer's instructions.

Adipogenic differentiation

The culture-expanded clonal cells at $2 \times 10^4/cm^2$ were induced for three weeks in Dulbecco's modified Eagle medium supplemented with 1% FCS, 1×10^{-7} mol/L dexamethasone and 1.0×10^{-9} mol/L insulin. At the end of the culture, the cells were fixed in 10% formalin for 10 min and stained with fresh oil red-O solution (Sigma) to show lipid droplets in induced cells. Reverse transcriptase–polymerase chain reaction (RT–PCR) was also performed for the detection of adipocyte-specific gene lipoprotein lipase.[13]

Osteogenic differentiation

The culture-expanded clonal cells at $2 \times 10^4/cm^2$ were induced in osteogenic medium (1.0×10^{-8} mol/L dexamethasone, 2.0×10^{-4} mol/L ascorbic acid, 7.0×10^{-3} mol/L β-glycerolphosphate; Sigma) for two to three weeks. Then the cells were stained with Von Kossa to reveal osteogenic differentiation. RT–PCR was also performed for the detection of bone cell-specific gene osteopontin.[13]

Transplantation of cells to animal models for wound neoangiogenesis

We used an established wound-healing model to investigate the differentiation of cells into vascular beds during angiogenesis.[17] All animal handling and experimental procedures were approved by the Animal Care and Use Committee of Chinese Academy of Medical Sciences & Peking Union Medical College. Female NOD/SCID mice (6- to 8-week-old) were bred and maintained under defined conditions in individually ventilated, HEPA filtered cages (Techniplast, Milan, Italy). Mouse skin was cleaned with 70% alcohol and a full-thickness wound was made by pinching a fold of flank skin and punching through the two layers of skin with 4-mm punch biopsy. Four hours later, the culture-expanded cells (10^6 cells per mouse) were injected via the tail vein. Wound tissues were harvested together with normal skin tissue from the opposite flank 10 days later. Incorporation of donor-derived cells into wound vasculature was analyzed by three-color immunofluorescence with mAbs against mouse vWF (Cy5-coupled), human αSMA (FITC-coupled) and human vWF (phycoerythrin [PE]-coupled).

Incorporation of transplanted cells in neoangiogenesis in irradiated NOD/SCID mice

Our previous studies provided evidence that extensive microvascular injury is a critical lesion in the gastrointestinal tract syndrome after whole-body radiation.[18] Mice were sublethally irradiated (300 cGy) with a cesium source (MDS Nordion; Gammacell, Ottawa, CA, USA) prior to transplantation of 10^6 cultured cells via the tail vein. Mice were killed two months later. Incorporation of donor-derived cells into small intestine vasculature was analyzed by the above three-color immunofluorescence method.

Results

Single-cell origin of CD105$^+$ cells from the arterial wall

Previously we found that CD105$^+$ cells could be detected beneath the endothelium of the arterial arch, including the intima, media and adventitia of the vessel, by immunochemistry. Here we isolated CD105$^+$ cells from the fetal aorta walls. To prove single-cell derivation of the differentiated cell types, the sorted CD105$^+$ cells were cultured at a concentration of one cell per well in 96-well plates precoated with ECM gel in the BMP-4 culture system. Wells with single adherent cells were identified during the first 24 h. The appearance of cell colonies was checked daily. Wells that appeared to contain more than one colony were discarded. After 20 days, a total of 49 colonies were found, but only four were successfully passaged. Cell phenotype analysis showed that 99% of the cells expressed Flk1 and CD105, but did not express CD34, CD45, CD11a, CD11b, CD31 and vWF. They also did not express vascular smooth muscle marker αSMA, MHC and calponin (Figure 1). The cells were fibroblast shaped or polygonal.

Multiple differential potentials of cultured-expanded clonal cells

Until now, there has been no evidence that cells from human vascular walls could differentiate into cells with endothelial characteristics. In the present study, cell clones were harvested, expanded and divided into portions for different cell differentiation induction (Figures 2a and b). When clonal cells were cultured in endothelium-specific induction media (EGM-2 media supplemented with VEGF) for three weeks, cells adopted a cobblestone-like morphology, typical of ECs (Figure 2c). Figure 2d showed the cells cultured in vascular smooth muscle-specific induction media (see below for details).

We then evaluated these progenies for markers of endothelial differentiation. Undifferentiated cells did not express CD31, Tek and vWF. Following treatment with VEGF for 21 days, about 70% of cells expressed CD31, Tek and vWF (Figure 3a). Parallel experiments with human umbilical vascular ECs served as positive control. Results were further confirmed by FACS analysis (Figure 3b) and Western blot (Figure 3c).

When the clonal cells were cultured in vascular smooth muscle-specific induction media (EGM-2 media supplemented with PDGF-BB) for three weeks, they assumed a typical 'hill and valley' morphology (Figure 2d). Undifferentiated cells did not express α-SMA, MHC and calponin. Following treatment with PDGF-BB for 21 days, about 80% of cells expressed αSMA, MHC and calponin (Figure 4a). Parallel experiments with human vascular SMCs (hVSMCs) served as positive control. Results were further confirmed by FACS analysis (Figure 4b) and Western blot (Figure 4c). As these cells could differentiate

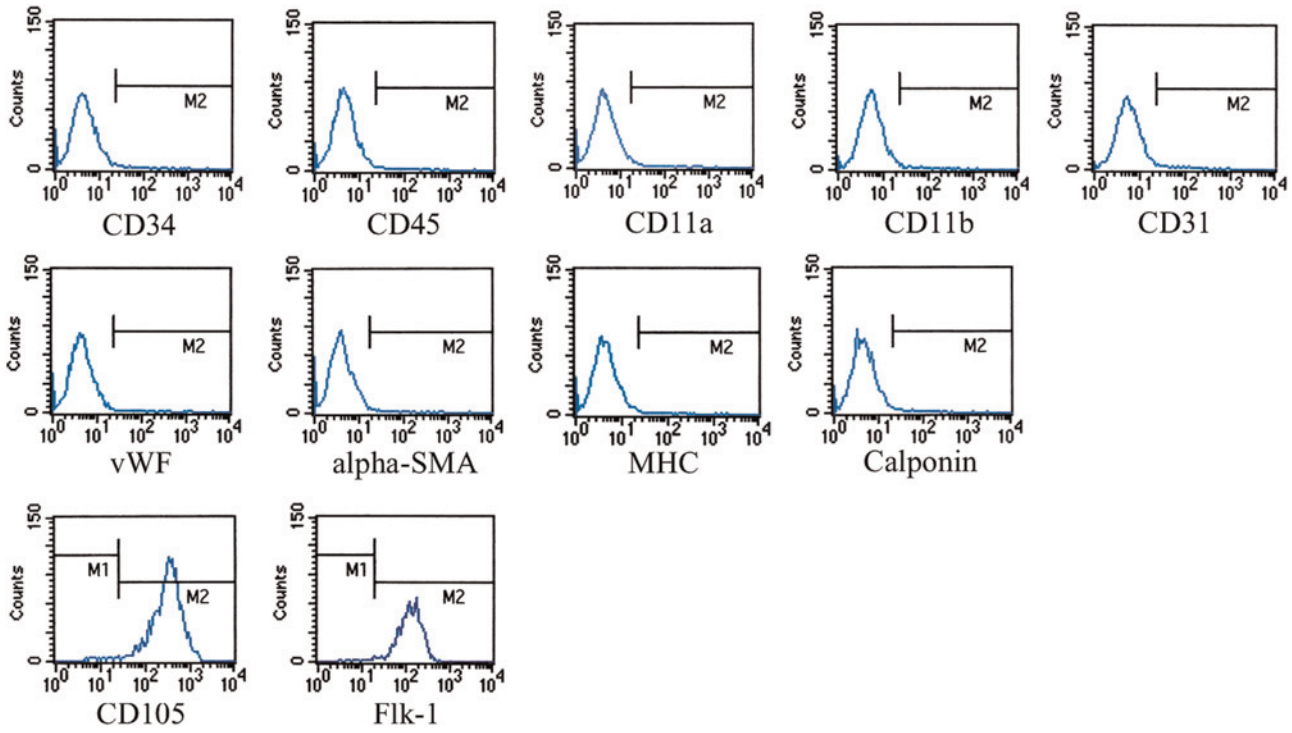

Figure 1 Immunophenotype of the single cell-derived clonal cells from the human fetal aorta wall. (A colour version of this figure is available in the online journal)

into ECs and SMCs, we term these cells multipotent vascular progenitor cells (MVPCs).

When clonal MVPCs were cultured in adipocyte-specific induction media for two weeks, more than 90% of cells differentiated into lipid-laden cells that stained by oil-red O (Figures 5a–d). Results were further confirmed by RT–PCR analysis for expression of adipocyte-specific gene lipoprotein lipase (Figure 5e).

When clonal MVPCs were cultured in osteogenic medium for two to three weeks, more than 90% of cells could be induced into osteogenic lineage as demonstrated by Von Kossa staining (Figures 6a and b). Results were further

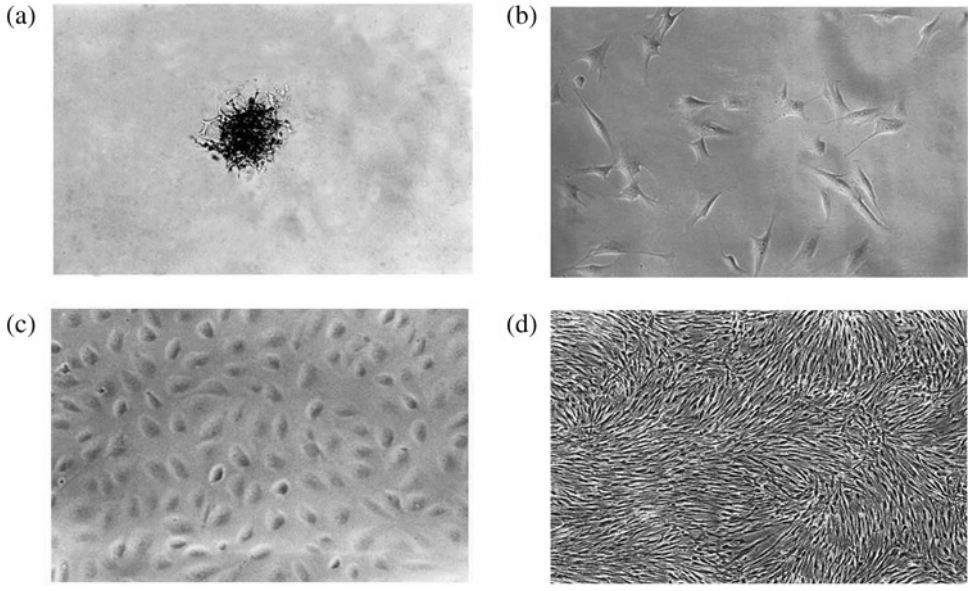

Figure 2 Growth and differentiation of human fetal aorta wall-derived primitive stem cells *in vitro*. (a) Diluted cells from the aorta wall were seeded in the bone morphogenetic protein-4 system and a single colony was observed at the second week. (b) Undifferentiated culture-expanded cells contained polygonal and fibroblast-like cells. (c) When cultured in EGM-2 medium with vascular endothelial growth factor, a confluent monolayer of endothelial-like outgrowth cells with cobblestone appearance was observed. (d) When cultured in EGM-2 medium with platelet-derived growth factor-BB, confluent differentiated cells (vascular smooth muscle cells) with 'hill and valley' morphology were observed. Original magnification, ×100. A representative example of four experiments is shown

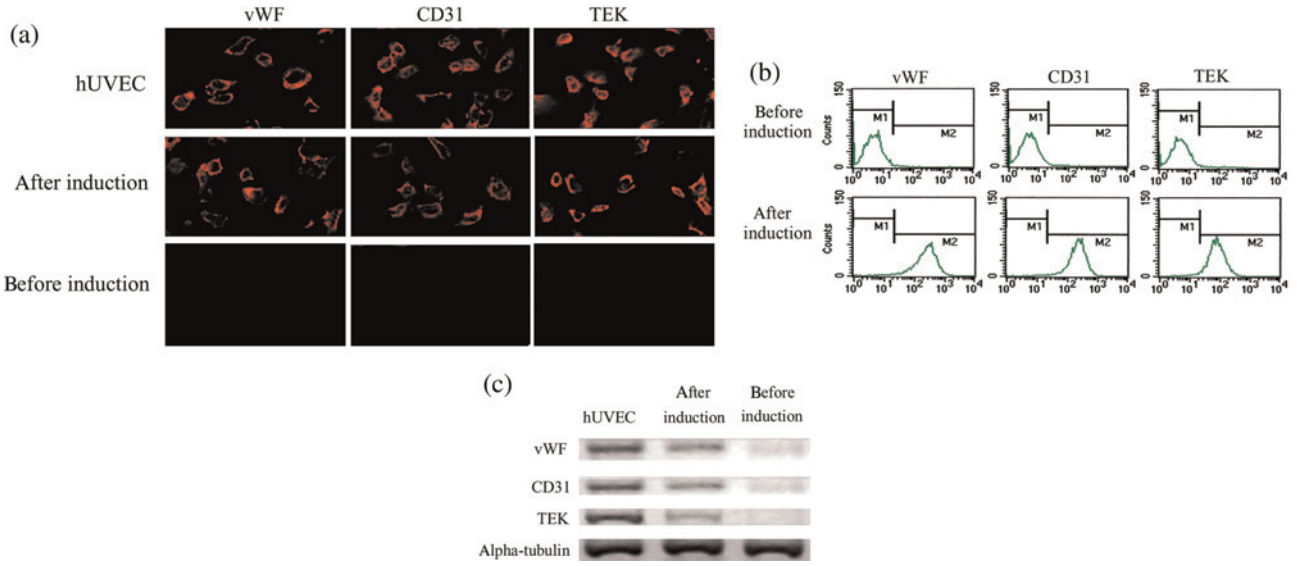

Figure 3 Endothelial differentiation of clonal cells *in vitro*. (a) Differentiation into endothelial cells was evaluated by immunofluorescence microscopy for CD31, Tek and vWF. Human umbilical vascular endothelial cells served as positive control. Results were further confirmed by fluorescence-activated cell sorting (b) and Western blot for von Willebrand factor, Tek and CD31 (c). Original magnification: (a) ×200. A representative example of four experiments is shown. (A color version of this figure is available in the online journal)

confirmed by RT–PCR analysis for expression of bone cell-specific gene osteopontin (Figure 6c).

MVPCs contribute to angiogenesis

To determine whether MVPCs could contribute to angiogenesis of the injured gastrointestinal tract and in the skin wound model, triple-color immunofluorescence was used to observe ECs and SMCs in the same tissue sections. As expected, in mice that had received MVPC transplantation, cells expressing human EC marker vWF were seen in the skin injury section. Among them were cells positive for SMC marker αSMA (Figures 7A and B). No staining was seen in non-transplanted animals (data not shown). In small intestine tissue sections, cells stained by antibody against human vWF, but not human αSMA, were seen (Figures 7C and D).

Discussion

To our knowledge, Sainz *et al.*[19] provided the first evidence that the normal arterial wall harbors side population cells with

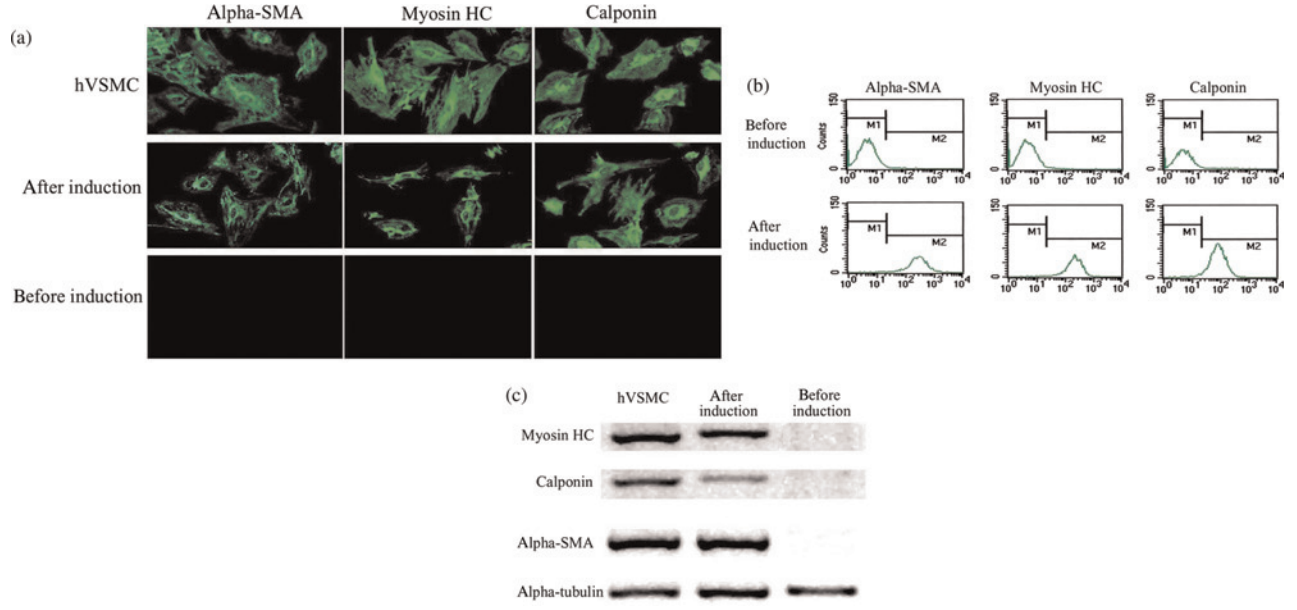

Figure 4 *In vitro* differentiation of clonal cells to vascular smooth muscle cells. (a) Human smooth muscle cells were used as positive control; (b) Differentiation of cells to vascular smooth muscle cells in EGM-2 supplemented with platelet-derived growth factor-BB for three weeks was evaluated by immunohistochemistry for α-smooth muscle actin, myosin heavy chain and calponin; undifferentiated clonal cells served as negative control. (c) Results were further confirmed by fluorescence-activated cell sorting and Western blot. Original magnification: (a) ×400. Results are a representative example of four experiments. (A color version of this figure is available in the online journal)

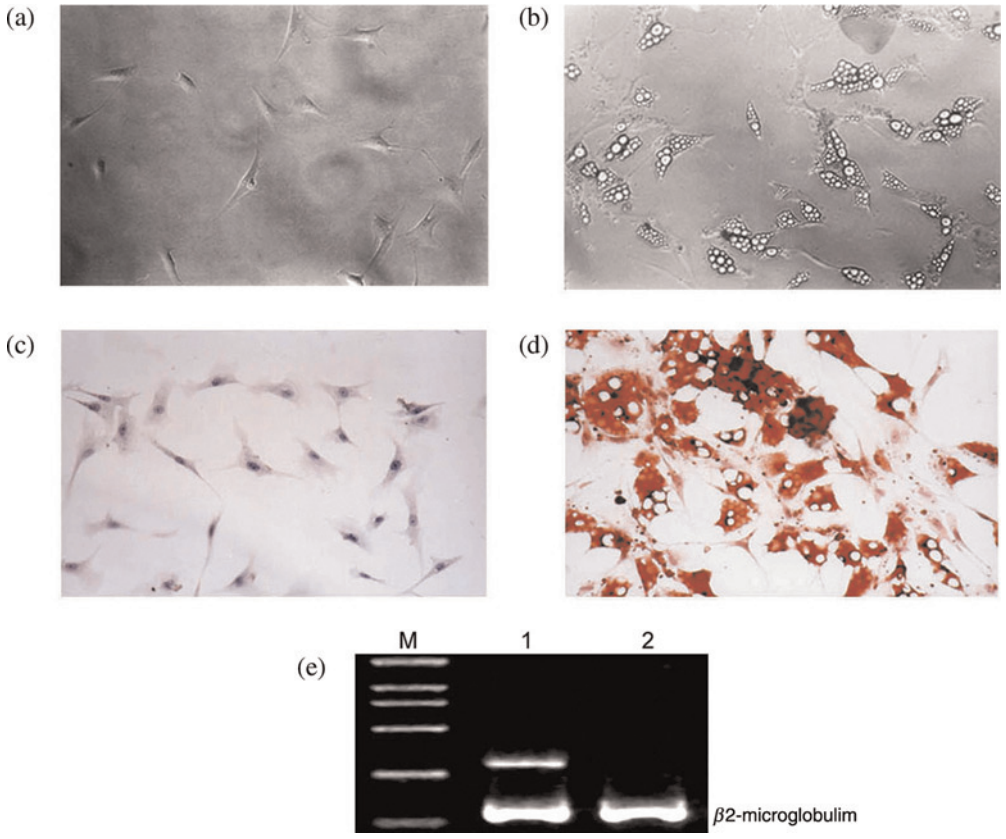

Figure 5 *In vitro* differentiation of clonal cells to adipocytes. (a, b) Morphology of clonal cells before and after adipogenic induction. Compared with cells before induction (c), cells after adipogenic induction were full of lipid droplets stained by oil red-O (d). (e) Results were further confirmed by expression of lipoprotein lipase (298 bp, lane 1). Expression of β2-microglobulin served as internal control (115 bp). Lane 2: uninduced cells. Original magnification: (a–d) $\times$100. Results are a representative example of four experiments. (A color version of this figure is available in the online journal)

vascular progenitor properties in adult mice. We describe here for the first time the isolation and *ex vivo* culture of MVPCs from the human fetal vascular wall that can, at the single-cell level, generate mesenchymal (osteoblasts, adipocytes and smooth muscle) and visceral mesodermal (endothelial) cell types. After transplantation, these MVPCs could contribute to injury-induced vascular regeneration. These findings may have many implications physically and pathologically.

Ontogenetically, blood vessel formation in the embryo starts by differentiation of ECs from the splanchnic

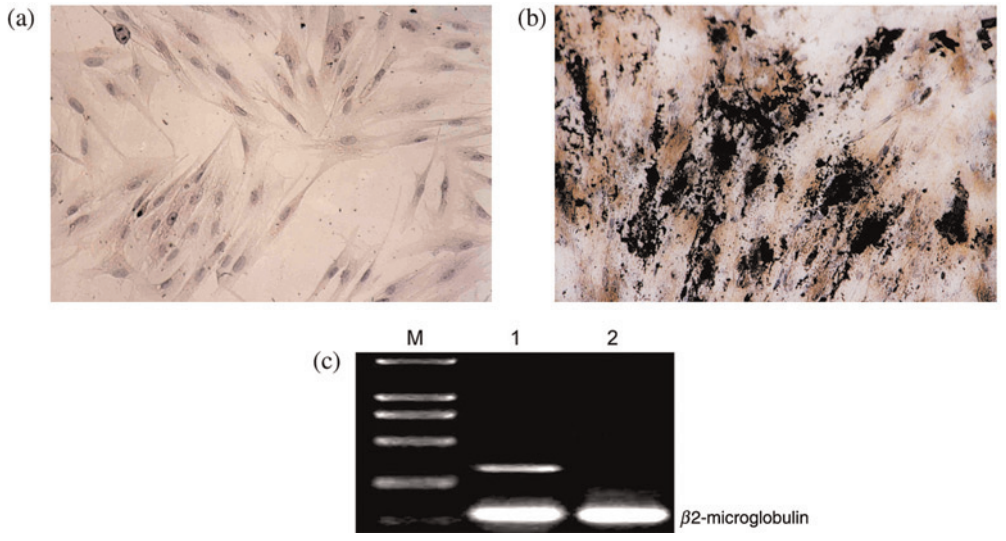

Figure 6 *In vitro* differentiation of clonal cells to osteocytes. Von Kossa staining of clonal cells before (a) and after adipogenic induction (b). (c) Total RNA was analyzed by RT–PCR for mRNA expression of osteopontin (330 bp, lane 1) and β2-microglobulin (115 bp). The uninduced cells serve as control (lane 2). Original magnification: (a) $\times$200; (b) $\times$100. Results are a representative example of four experiments. (A color version of this figure is available in the online journal)

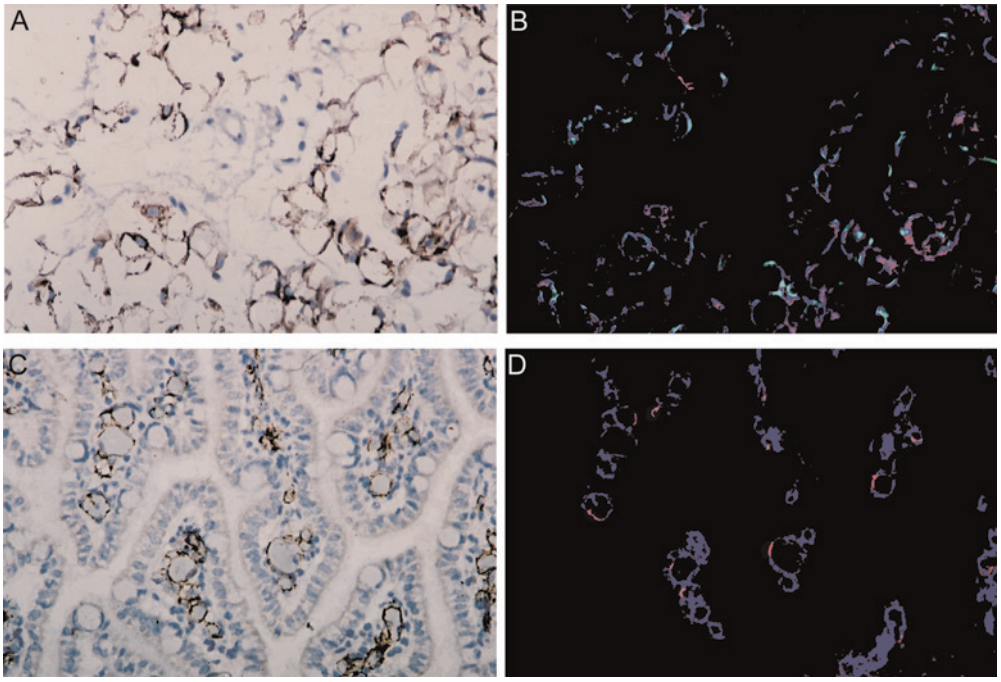

Figure 7 Immunofluorescence staining of human clonal cells-derived endothelial cells and smooth muscle cells during neoangiogenesis. (A) The deparaffinized section of the wound-healing site created in skin was stained with antimouse von Willebrand factor (VWF) antibody. (B) The wound-healing site in skin was stained with antimouse VWF antibody, antihuman VWF antibody or antihuman α-smooth muscle actin (αSMA) antibody. (C) The deparaffinized section of small intestine was stained with antimouse VWF antibody. (D) Small intestine was stained with antimouse VWF antibody, antihuman VWF antibody or antihuman αSMA antibody. Confocal microscopic images of antimouse VWF (blue signal), antihuman αSMA (green signal) and antihuman VWF (red signal) are shown. Original magnification $\times$200. Results are a representative example of four experiments. (A color version of this figure is available in the online journal)

mesoderm, especially Flk1$^+$ mesoderm cells.[1] Postnatal neoangiogenesis, in response to tissue injury and remodeling, was previously thought to occur solely via the proliferation and migration of ECs from pre-existing vasculature. In addition, recent studies suggest that endothelial progenitors located within the peripheral blood and bone marrow, appear to function in neovascularization and angiogenesis, and perhaps also re-endothelialization of injured arteries, and significantly contribute to injury- and pathology-induced neovascularization.[20–22] In the present study, we showed that Flk1$^+$CD105$^+$ MVPCs could differentiate into ECs under the induction of VEGF. To our knowledge, only Hu *et al.*[23] mentioned in the Discussion part of their recent report that endothelial progenitor cells may also reside in the vessel walls.

Similarly, the progenitor for vascular SMCs remains a controversy. SMCs have different embryonic origins, depending on the segment of the arterial system involved. In some vertebrates, SMCs in the upper portion of the thoracic aorta are derived from a neuroectodermal source, whereas those in the abdominal aorta are derived from a mesenchymal source.[3] Although likely, this has not been confirmed in humans. The SMCs of coronary arteries appear to originate from a third precursor population in the intracardiac mesenchyme.[2] SMCs within the media of large arteries may be heterogeneous, with different proliferative and matrix-producing capabilities.[4] Also, bone marrow cells were demonstrated to be a source of SMCs.[24]

To complicate matters further, transdifferentiation of ECs to SMCs was reported. DeRuiter *et al.*[25] reported that vascular ECs can delaminate, migrate into the subendothelium and transdifferentiate into SMCs. Arciniegas *et al.*[26] reported that adult bovine ECs can transdifferentiate into SM-like cells *in vitro*. They showed TGF-β1 induced α-SM actin expression in aortic ECs, whereas expression of factor VIII-related antigen was lost. While we were preparing this manuscript, a new study by Hu *et al.*[23] showed that Sca-1$^+$ progenitor cells purified from the mouse aortic root can migrate through an irradiated vein graft to the neointima of the vessel and transdifferentiate into SMC-like neointimal cells that expressed the early SMC differentiation marker gene SM22. Thus it seems that progenitors for SMCs may be more heterogeneous. Our study provides new insights into the origin of cells populating the developing neointima.

On the other hand, one of the most important findings of this study is the differentiation of progenitor cells toward adipocytes and osteocytes. Atherosclerosis is a chronic fibroproliferative inflammation of the arterial wall brought about and exacerbated by the dynamic interaction of disordered lipid metabolism and other insults and risk factors with homeostatic mechanisms designed to protect against such injury.[27,28] Fatty-streak formation is a typical pathological change in atherosclerosis. Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. Later they are joined by various numbers of SMCs, foam cells and platelets. As MVPCs could be induced to transdifferentiate into adipocytes *in vitro*, it is reasonable to hypothesize that this process may occur in the development of atherosclerosis.

In the development of atherosclerosis, deposition of lipid is a critical step. The recruitment of circulating monocytes into the vascular intima and their subsequent transformation into macrophages/foam cells are key elements of the initiation of atherosclerosis. In the present study, we found that MVPCs had the potential to differentiate into lipid cells. Whether this happens *in vivo* is an interesting question that deserves further study.

In arterial plaque, there are a number of cell types (among them mononuclear phagocytes, T cells, B cells, natural killer cells and mast cells), all thought to originate from circulating hematopoietic and immune precursors. But two key cell types, ECs and SMCs, are presumed to be resident arterial cells. According to this model, proliferating arterial cells are derived from the clonal expansion of rare resident arterial progenitor cells in response to specific stimuli.[29] These findings even led to the more extreme model that views plaque as a benign SMC tumor in the arterial wall. Identification of putative vascular progenitor cells that give rise to different cells may well fit this benign SMC tumor model. To explain the presence of progenitor cells in the vessel, one possibility is that these cells are reserved during development from embryo to adult, although there is a possibility that they may come from bone marrow.

Calcification is a common feature of advanced atherosclerotic lesions. Atherosclerotic plaque calcification, especially in the coronary arteries, decreases vessel elasticity, augments plaque brittleness, and leads to increased clinical complications such as myocardial infarction, impaired vascular tone, dissection in angioplasty, poor surgical outcome and coronary insufficiency due to loss of aortic recoil.[30,31] Despite its clinical significance, the molecular mechanisms regulating vascular calcification are unclear. Historically, ectopic calcification has been considered a passive process involving spontaneous calcium phosphate mineral precipitation in necrotic tissue. However, several lines of evidence have recently emerged supporting the concept that ectopic calcification, like the mineralization of bones and teeth, is a cell-regulated process. Then what kinds of cells contribute to vascular calcification? Recently, an SMC phenotypic transition associated with vascular calcification has been proposed by several authors. Steitz *et al.*[32] showed that bovine aortic SMCs lost their lineage markers, SM22α and smooth muscle α-actin, within 10 days of being placed under calcifying conditions. Conversely, the cells gained an osteogenic phenotype as indicated by an increase in expression and DNA-binding activity of the transcription factor, core binding factor α1 (Cbfa1). Moreover, genes containing the Cbfa1 binding site, OSE2, including osteopontin, osteocalcin and alkaline phosphatase, were elevated.[32] Shioi *et al.*[33] described increases in osteopontin transcripts and a reduction in SM α-actin protein levels using a β-glycerophosphate-induced bovine vascular SMC calcification model. *In vitro* studies also suggest that vascular pericytes retain the capacity to differentiate into osteoblast-like cells in the artery wall.[34]

These studies all indicate that pathological calcification of blood vessels shares features with normal bone formation. Proteins involved in the regulation of skeletal bone formation were present in human atherosclerotic lesions. These proteins include osteoprotegerin and its ligand, bone sialoprotein, BMP -2 and BMP-4, osteocalcin, osteonectin, matrix Gla protein and osteopontin. Considering the similarities between the calcification of arteries and osteogenesis, it is possible that progenitors of osteoblasts may also exist in the arterial wall. In the present study, we isolated vascular progenitor cells that could be induced to undergo calcification similar to mineralization in skeletal tissue, including the expression of osteopontin. Hence these progenitor cells share many phenotypic features with MSCs isolated from bone marrow.

In conclusion, it seems that there is at least a less studied cell population in the vessel wall. What is the role of this cell population in the physiology and pathology of blood vessels? Do ECs and SMCs convert their phenotype during developmental or pathological processes and give rise to an interim phenotype that is $CD105^{+}$? Here, we describe for the first time the isolation and *ex vivo* expansion of cells from the human fetal aorta wall that can differentiate at the single-cell level not only into osteoblasts, chondrocytes, SMCs and adipocytes, but also into cells of endothelium *in vitro*. When transplanted into mice, they can engraft into new vessels and adopt an endothelial fate. Thus we found a possible common origin for aortic ECs and SMCs. An extension of this finding may imply that vascular progenitors are able to differentiate into all types of mesenchymal cells.

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