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ORIGINAL RESEARCH PAPER

Comparison of human post-embryonic, multipotent stem cells derived from various tissues

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Abstract Multipotent stem cells were isolated from human fetal heart, liver, muscle, lung, derma, kidney, and adipose tissue, and then analyzed for their characteristics and function. Cells with characteristics similar to bone marrow-derived post-embryonic multipotent stem cells can be selected and cultured from tissues other than bone marrow. This may then help explain the "stem cell plasticity" found in multiple human tissues.

Keywords Fetal heart · Fusion · Plasticity · Stem cells · Transdifferentiation

Introduction

Tissue-specific stem cells from bone marrow (BM), lung, brain, cord blood, synovial membrane and other tissues differentiate into cells of different tissues and may have greater plasticity than previously anticipated (Cao et al. 2005; Hu et al. 2003; Jiang et al. 2002a, b; Park et al. 2006; Polisetty et al. 2008; Qiao et al. 2008; Reyes et al. 2001; Sabatini et al. 2005; Sakaguchi et al. 2005; Shih et al. 2005) but the mechanisms responsible for stem cell plasticity are still not clear.

We suppose that there is a presently undefined subfraction of stem cell populations with greater differentiation potential than expected. They remain in some or all tissues and organs beyond embryonic development and can give rise to different kinds of stem cells for self-repair and self-renewal in case of tissue damage and senescence. They may migrate to other damaged tissue and differentiate under local cues. Results from other laboratories have also suggested that subpopulations of cells within specific tissues might remain unrestricted and multipotent potential that is reminiscent of embryonic stem cells (Clarke et al. 2000; D'Ippolito et al. 2004; Howell et al. 2003; Watt and Hogan 2000). Therefore, the ability to purify, culture and manipulate these post-embryonic stem cells would provide investigators with an invaluable cell source to study cell and organ development. Besides, such cells might have a very broad developmental capacity and may potentially be used to generate a variety of cell types for transplantation in different diseases.

Recently, we identified a rare cell population [$Flk1^{+}CD34^{-}$ post-embryonic, multipotent stem cells (PMSCs)] within human fetal BM mesenchymal stem

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cells (MSCs) cultures that could be expanded for more than 50 population doublings without any obvious senescence or loss of differentiation potential, and these cells could differentiate not only into osteogenic, adipogenic and endothelial lineages, but also hepatocyte-like cells, neuroectoderm and erythroid cells at the single-cell level (Fang et al. 2003; Guo et al. 2003). Furthermore, these cells could not only contribute to hematopoietic reconstitution and endothelium, which were incorporated into foci of neoangiogenesis at high frequency, but also give rise to epithelial cells of the liver, lung, and gastrointestinal tract of irradiated NOD/SCID mice (Fang et al. 2004). These data prompted us to investigate whether there are multipotent stem cells with characteristics similar to BM $Flk1^{+}CD34^{-}$ PMSCs in some other fetal tissues.

Materials and methods

Cell culture

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. Stem cells were isolated as we previously described (Cao et al. 2005; Fang et al. 2004; Guo et al. 2003). Surrounding and connective tissues around the heart, liver, lung, derma, muscle, kidney, and adipose tissue obtained from 14- to 22-weeks gestation fetuses were carefully removed, the remaining tissues were thoroughly minced and then digested at 37°C with 0.2% collagenase (Gibco Life Technologies, Paisley, UK) for 45 min, followed by 0.1% (w/v) trypsin (Gibco) for 45 min. Then the tissues were triturated vigorously and passed through a 70 μm filter, and the cells were collected by centrifugation. The collected cells were then plated in DF_{12} culture containing 40% (v/v) MCDB medium (Sigma, St. Louis, MO, USA), 2% (v/v) fetal calf serum (FCS, Gibco Life Technologies, Paisley, UK), 1× insulin transferrin selenium (Gibco), 10^{-9} M dexamethasone (Sigma), and 10^{-4} M ascorbic acid 2-phosphate (Sigma), 20 ng interleukin-6/ml, 10 ng epidermal growth factor/ml (EGF; Sigma), 10 ng platelet-derived growth factor BB/ml (PDGF-BB; Sigma), 100 units penicillin/ml, and 1,000 units streptomycin/ml (Gibco) at 37°C and a 5% CO_2 humidified atmosphere. Non-adherent cells were discarded 20 h later. The adherent cells were harvested by trypsinization and sorted using MACS CD105 micromagnetic beads (Miltenyi Biotec, Inc., Auburn, CA, USA). Then the sorted cells were cultured respectively at one single cell per well in 96-well plates coated with ECM gel (Sigma) supplemented with 5 ng vascular endothelial growth factor/ml, 30 ng stem cell factor/ml, and 3% (v/v) FCS. After 10 days, single cell clones were picked up and expanded in above expanded culture.

To study the growth pattern of the expanded cells, the culture-expanded cells from each single colony were seeded at a concentration of 5,000 cells/well in a 24-well plate. The adherent cells were trypsinized, harvested and counted by 0.4% (w/v) trypan blue exclusion method at different time points.

Immunophenotypic analysis (Guo et al. 2003)

For analyzing immunophenotypes, the culture-expanded clonal cells were washed with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA, Sigma), and incubated with primary antibodies for 30 min at 4°C. To detect intracellular antigens, we fixed cells in 2% (v/v) paraformaldehyde for 15 min at 4°C, and then permeabilized them with 0.1% saponin (Sigma) for 1 h at room temperature. Working concentrations for primary antibodies against human CD44 (NeoMarkers Fremont, CA), CD34, CD45, Flk1, (Santa Cruz Biotechnology, Inc., CA, USA), GlyA (Maxim Biotech, Inc., South San Francisco, CA, USA), HLA Class I and HLA-DR (Mountain View, CA, USA) were 10–20 ng/ml. We used same-species, same-isotype irrelevant antibody as negative control. Then cells were washed with PBS containing 0.5% (w/v) BSA and incubated with FITC-conjugated secondary antibodies for 30 min at 4°C. After three washes, cells were resuspended in PBS and analyzed using a fluorescence-activated cell sortor (FACS Vantage SE; BD).

The culture-expanded cells differentiate into adipocytes (Fang et al. 2004)

The culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were induced respectively at 2×10^4 cm^{-2} in Dulbecco's

modified Eagle medium supplemented with 1% (v/v) FCS, 10^{-7} M dexamethasone, and 10^{-9} M insulin. At the end of the culture, the cells were fixed in 10% (v/v) formalin for 10 min and stained with Oil Red O (Sigma) to show lipid droplets in induced cells. To demonstrate adipogenic differentiation, RT-PCR methods were used to analyze the expression of lipoprotein lipase (LPL).

Differentiation of culture-expanded cells into osteocytes (Fang et al. 2004)

The culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were induced respectively at 2×10^4 cm^{-2} in osteogenic medium (10^{-8} M dexamethasone, 2×10^{-4} M ascorbic acid, 7×10^{-3} M β-glycerolphosphate; Sigma). Then the cells were stained with Von Kossa to reveal osteogenic differentiation. Briefly, cells were fixed with formaldehyde at 4°C for 1 h, treated with 2% (w/v) silver nitrate (Sigma) at 37°C for 10 min, and then placed directly under a 60 W lamp for 1 h. To demonstrate osteogenic differentiation, RT-PCR was performed to detect the expression of osteopontin (OPN).

Differentiation of culture-expanded cells into endothelial cells (Fang et al. 2004)

The culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were plated on fibronectin at 2×10^4 cm^{-2} in EGM-2 medium (Clonetics Inc., San Diego, CA, USA) with 10 ng vascular endothelial growth factor/ml (VEGF; Clonetics Inc) and cultured for 15–21 days. After differentiation induction, cells were fixed with 4% (v/v) paraformaldehyde and subject to immunofluorescence analysis for CD31, Tek, and vWF by confocal fluorescence microscopy. To demonstrate endothelial differentiation, 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 (Fang et al. 2004).

Matrigel culture system (Guo et al. 2003)

The culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were plated on fibronectin at 2×10^4 cm^{-2} in 96-well plate pre-coated with Matrigel (Gibco). Culture media was M199 (Gibco) supplemented with FCS, VEGF and bFGF for the formation of capillary-like structure.

Differentiation of culture-expanded cells into hepatocyte-like cells (Kakinuma et al. 2003; Schwartz et al. 2002)

The culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were plated at 10^4 cells cm^{-2} on 1% matrigel-coated glass slides in the expansion medium without IL-6, EGF, and PDGF-BB. After 8–12 h, media were removed, and cells washed twice with PBS were cultured in serum-free medium supplemented with 30 ng hepatocyte growth factor/ml (HGF, R&D Systems Inc., Minnesota, USA) and 20 ng fibroblast growth factor-4/ml (FGF-4, R&D Systems Inc.) for 14–21 days. To demonstrate hepatocyte differentiation, immunofluorescence and RT-PCR methods were used to analyze the expression of albumin (ALB), cytokeratin 18 (CK18), and HNF-1α.

Differentiation of culture-expanded cells into neuroectoderm-like cells (Jiang et al. 2002a, b)

To induce neuroectoderm differentiation, undifferentiated culture-expanded clonal cells (after 40 population doublings) from different fetal tissues were seeded respectively at a density of 5,000 cells cm^{-2} on fibronectin-coated glass slides in the expansion medium without PDGF-BB, EGF and interleukin-6, but with 100 ng bFGF/ml. After 10–19 days' culture, we harvested the induced cells and performed immunofluorescence and Western blot analysis to demonstrate neuroectoderm differentiation.

Immunofluorescence

After hepatocyte and neuroectoderm cell differentiation induction, cells were rinsed briefly in PBS, fixed with 2% (v/v) paraformaldehyde (in PBS) for 10 min, permeabilized with 0.1% (w/v) Triton X-100 in PBS for 10 min, and treated with 5% (v/v) FCS in PBS for 30 min at room temperature. They then were incubated for 1 h with diluted primary antibodies at

room temperature. Rabbit anti-human ALB antibody (Dako, Carpinteria, CA, USA), rabbit anti-human CK18 antibody (Dako),rabbit anti-human HNF-1α (Santa Cruz Biotechnology, Inc., CA, USA), rabbit anti-human neurofilament (NF; Serotec Inc., Raleigh, NC, USA) antibody, rabbit anti-human galactocerebroside antibody (GalC; Chemicon International Inc., Temecula, CA, USA), and mouse anti-human glial acidic fibrillary protein antibody (GFAP; Serotec Inc.) were used. Samples were washed three times with PBS, then incubated for 30 min at room temperature with anti-rabbit IgG-Cy5 (Sigma), anti-rabbit IgG-PE (Sigma), anti-rabbit IgG-FITC (Jackson Immuno-Research Laboratories Inc., West Grove, PA, USA), or anti-mouse IgG-FITC (Sigma). After these, the slides were observed field by field with confocal fluorescence microscopy (AX70, Olympus Optical Co. Ltd., Tokyo, Japan). To enumerate the number of cells staining positive for a given marker, we evaluated 200–400 cells per culture under low power and enumerated the total number of cells staining positive with a given antibody.

Western blotting

For Western blotting, protein extracts were prepared from logarithmically growing cells (both before and after differentiation) by lysis in buffer (25 mM Tris/HCl, pH 7.4, 0.15 M NaCl, 1% Nonidet P-40, 1 mM EDTA, and 2 mM EGTA). Then the protein lysates were separated by 7.5% SDS-PAGE and transferred onto nitrocellulose membrane. After blocking, blots were incubated with mouse monoclonal antibodies against GalC, NF, and β-actin at 1:100 in the blocking solution for 1 h at room temperature. The blots were washed and incubated with a sheep anti-mouse horseradish peroxidase-conjugated IgG (E. I. du Pont de Nemous, MA, USA) at 1:5,000 for 1 h. Immunodetection using the enhanced chemiluminescence method (ECL kit; Amersham) was performed according to the manufacturer's instructions.

Reverse transcription-polymerase chain reaction (RT-PCR)

Cells used for RT-PCR were the culture-expanded clonal cells from different fetal tissues before and after differentiation induction. Total RNA was prepared with Trizol reagents according to manufacturer's protocol (Gibco) and visualized by UV. The first-strand cDNA was synthesized from 4 μg total RNA primed with oligo-dT(18) primer using First-Strand cDNA Synthesis Kit (Gibco). One fiftieth of RT mixture was amplified in GeneAmp PCR System 2400 thermal cycler (Perkin-Elmer Corp., Boston, MA, USA) in a total reaction volume of 20 μl containing 10 pmol of each primer, 200 μM dNTPs and 2 units Taq polymerase (Gibco). Oligonucleotide primers were: OPN (330 bp), forward, 5′-CTA GGC ATC ACC TGT GCC ATA CC-3′, reverse, 5′-CAG TGA CCA GTT CAT CAG ATT CAT C-3′; LPL (298 bp), forward, 5′-ATG GAG AGC AAA GCC CTG CTC-3′, reverse, 5′-TAC AGG GCG GCC ACA AGT TTT-3′; ALB (350 bp), forward, 5′-TTG GAA AAA TCC CAC TGC AT-3′, reverse, 5′-CTC CAA GCT GCT CAA AAA GC-3′; CK 18 (267 bp), forward, 5′-GAG ATC GAG GCT CTC AAG GA-3′, reverse, 5′-CAA GCT GGC CTT CAG ATT TC-3′; Nanog (142 bp), forward, 5′-CAG CTG TGT GTA CTC AAT GAT AGA TTT-3′, reverse, 5′-CAA CTG GCC GAA GAA TAG CAA TGG TGT-3′; OCT-4 (133 bp), forward, 5′-ACA TCA AAG CTC TGC AGA AAG AAC T-3′, reverse, 5′-CTG AAT ACC TTC CCA AAT AGA ACC C-3′); β2-microglobulin (β2M, 115 bp), forward, 5′-ACC CCC ACT GAA AAA GAT GA-3′, reverse, 5′-ATC TTC AAA CCT CCA TGA TG-3′. PCR conditions consisted of: 10 min at 95°C (hot start), 31–38 cycles (actual number noted below) of: 94°C for 1 min, annealing temperature (Ta, noted below) for 1 min, 72°C for 1 min. A final 10 min extension at 72°C was done at the end. Oligonucleotide-specific conditions were: osteopontin, 30 cycles, Ta, 55°C; lipoprotein lipase, 35 cycles, Ta, 60°C; ALB, 33 cycles, Ta, 57°C; CK18, 38 cycles, Ta, 57°C; Nanog, 35 cycles, Ta, 58°C. OCT-4, 35 cycles, Ta, 57°C. Products were analyzed on 1.5% (w/v) agarose gel and visualized with ethidium bromide staining.

Transmission electron microscope observation

The induced cells were fixed in 6% (v/v) glutaraldehyde PBS and 1% (w/v) OsO_4. After dehydration in acetone, the cells were embedded in GP-812 resin and cut into ultrathin sections. Sections were double-stained with uranyl acetate and lead citrate before being observed under H-600-4 type electron microscope.

Results

The biological characteristics of the culture-expanded clonal cells from different fetal tissues

By using flow cytometry, as shown in Table 1, the phenotype of the culture-expanded cells from some other fetal tissues was identical to the phenotype described for human fetal BM-derived $Flk1^+CD34^-$ PMSCs: $CD34^-$, $CD45^-$, $GlyA^-$, class-HLA^-, $HLA\text{-}DR^-$, $CD44^+$, and $Flk1^+$. In addition, these cells cultured for more than 40 population doublings showed persistently displayed fibroblast-like morphology (Fig. 1a) and maintained $Flk1^+$, $CD44^+$, $CD34^-$, $CD45^-$, $GlyA^-$, class-HLA^- and $HLA\text{-}DR^-$. The doubling time of all these cell populations was about 30 h (Fig. 1b).

The results of RT-PCR showed that the culture-expanded cells from various fetal tissues did not express Nanog, but persistently expressed OCT-4 even after more than 40 population doublings (Table 1).

The differentiation of the culture-expanded clonal cells from different fetal tissues into adipocytes and osteocytes

The culture-expanded clonal cells after 40 population doublings from different fetal tissues could be induced to differentiate into adipocytes that were demonstrated by Oil Red-O-positive lipid droplets in cytoplasm (Fig. 2a). LPL, a marker for adipogenesis, was also expressed in these cells after adipogenic induction (Fig. 2e). Meanwhile, the osteogenic differentiation of these cells was indicated by calcium deposition stained by silver nitrate (Fig. 2c). RT-PCR analysis also showed bone cell-specific gene, OPN, in the cells after osteogenic induction (Fig. 2e). Under the transmission electron microscope, lipid droplets in induced adipocyte with membrane were observed apparently, and there was small amount of heterochromatin distributing under the karyotheca (Fig. 2b). Ultrastructure of the induced osteocytes under transmission electron microscope was similar to normal osteocytes morphologically: some collagen fibril matrix mineralized or unmineralized with electron dense calcium deposition was observed outside the induced cells (Fig. 2d). In addition, some needle-shaped crystal calcium deposition similar to bone spicules was also observed inside the cytoplasm (Fig. 2d), suggesting that calcium deposition was produced inside the cells and then released outside.

The time courses of lipid droplet accumulation and calcium deposition were different in cells from different sources. In the processes of adipogenesis, it took less time for skeletal muscle- and derma-derived culture-expanded cells to differentiate into adipocytes, with lipid droplets appearing at about day 3. In contrast, lung and adipose tissue-derived culture-expanded cells, as well as BM-derived $Flk1^+CD34^-$ PMSCs needed 5–9 days, and heart-, kidney-, and liver-derived culture-expanded cells needed more than 15 days to differentiate into adipocytes. During the osteogenic differentiation, skeletal muscle-, adipose- and kidney tissue-derived culture-expanded cells needed 1–2 weeks, just as BM-derived $Flk1^+CD34^-$ PMSCs did, but culture-expanded cells from derma, lung, liver, and heart required more than 3 weeks.

Table 1 Phenotype analysis and genes expression of various fetal tissues-derived culture-expanded clonal cells

Tissues	Immunophenotype analysis by flow cytometry							RT-PCR for gene expression	
	Flk1	CD44	GlyA	CD34	CD45	Class I-HLA	HLA-DR	Nanog	Oct4
Heart	+	+	−	−	−	−	−	−	+
Liver	+	+	−	−	−	−	−	−	+
Lung	+	+	−	−	−	−	−	−	+
Derma	+	+	−	−	−	−	−	−	+
Muscle	+	+	−	−	−	−	−	−	+
Kidney	+	+	−	−	−	−	−	−	+
Adipose	+	+	−	−	−	−	−	−	+

RT-PCR Reverse transcription-polymerase chain reaction

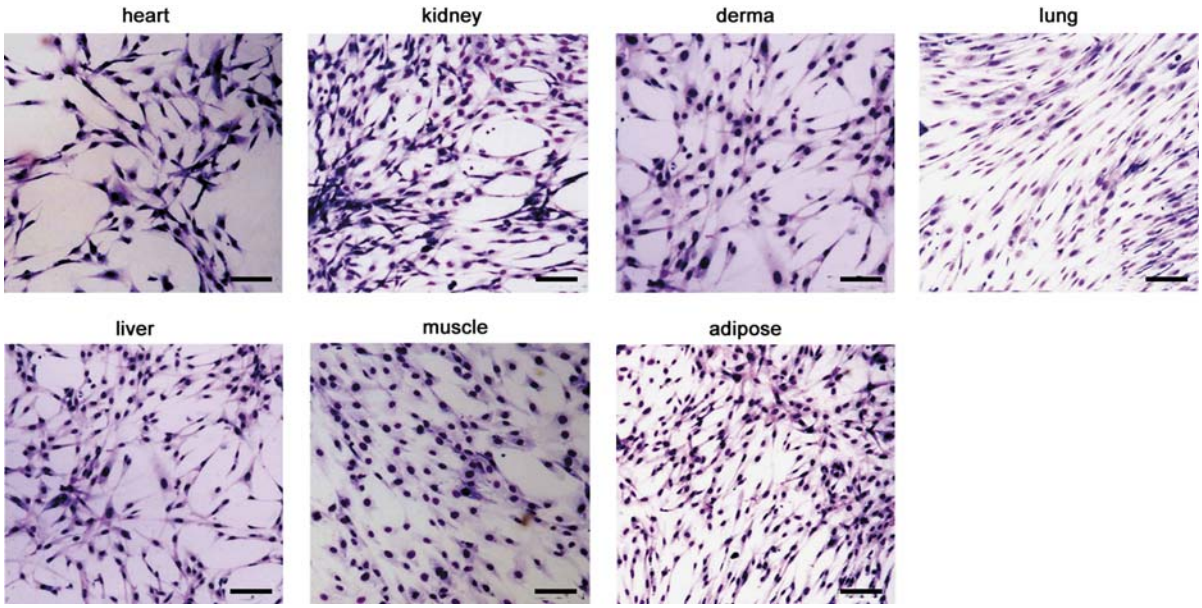

Fig. 1 Morphology of various fetal tissues-derived culture-expanded multipotent stem cells. All culture-expanded cells showed fibroblast-like morphology. Scale bar = 100 μm. Results are a representative example of four experiments. All cell lines had a doubling time of about 30 h

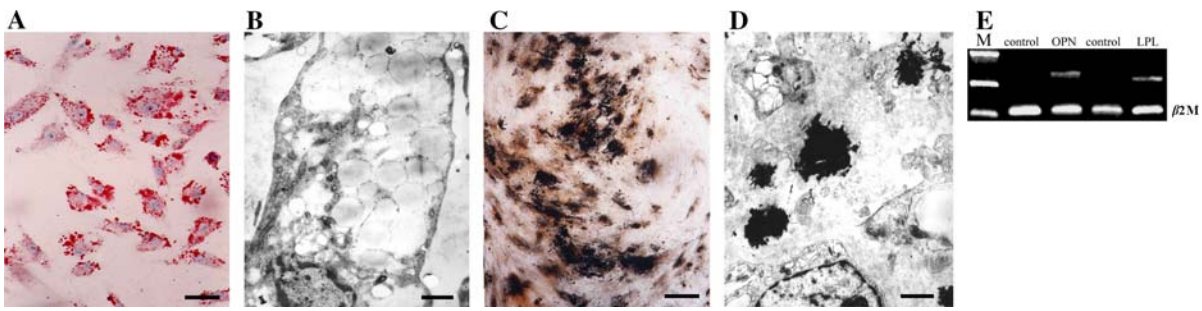

Fig. 2 Various fetal tissues-derived culture-expanded clonal cells could differentiate into adipocytes and osteocytes. Heart-derived clonal cells in the adipogenic induction group were positively stained for Oil Red-O (**a**) and positively stained by the Von Kossa showed calcium accumulation after osteogenic induction (**c**), while the undifferentiated control cells stained negatively (data not shown). Ultrastructure of the induced adipocytes (**b**) and osteocytes (**d**) under transmission electron. **e** RT-PCR for mRNA expression of osteopontin (OPN), lipoprotein lipase (LPL), and β2-microglobulin (β2M). The uninduced cells serve as control. As for the other tissue-derived culture-expanded clonal cells, they all could differentiate into adipocytes and osteocytes in vitro (data not shown). Scale bar = 50 μm (**a**, **c**); 1 μm (**b**, **d**). M = DNA marker. Results are a representative example of four experiments

The differentiation of the culture-expanded clonal cells from different fetal tissues into cells with endothelial characteristics

The culture-expanded clonal cells after 40 population doublings from different fetal tissues could be induced to differentiate into cells with endothelial characteristics. We 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 (Fig. 3a). Parallel experiments with human umbilical vascular ECs served as positive control. Results were further confirmed by FACS analysis (Fig. 3b) and Western blot (Fig. 3c). When the cells were plated in Matrigel containing VEGF and bFGF, they began to migrate after 24 h and formed aggregates after 2–3 days.

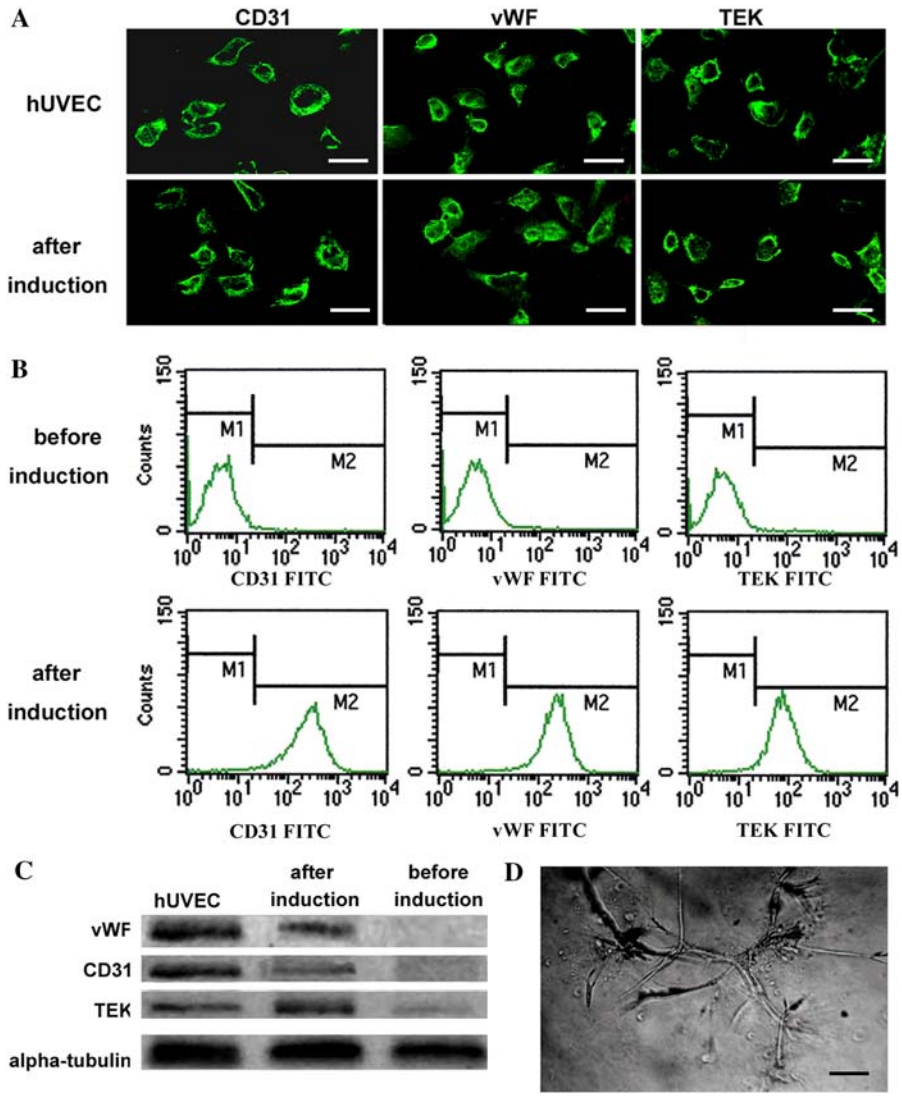

Fig. 3 Endothelial differentiation of various fetal tissues-derived culture-expanded in vitro. **a** Heart-derived clonal cells could differentiate into endothelial cells evaluated by immunofluorescence microscopy for CD31, Tek, and vWF. Human umbilical vascular endothelial cells (hUVEC) served as positive control. Results were further confirmed by FACS (**b**) and Western blot for vWF, Tek, and CD31 (**c**). Cells seeded in the Matrigel began to aggregate within 2–3 days and migrated gradually to form vascular plexus on day 7 (**d**). As for the other tissue-derived culture-expanded clonal cells, they all could differentiate into cells with endothelial characteristics in vitro (data not shown). Scale bar = 25 μm (**a**); 40 μm (**d**). Results are a representative example of four experiments

Capillary-like tube structures shot out from the aggregates and gradually formed a capillary net at about day 7 (Fig. 3d).

Neuroectoderm and hepatocyte-like cells differentiation

The culture-expanded clonal cells after 40 population doublings from different fetal tissues were plated on matrigel with FGF-4 and HGF for 20 days, then more than 45% culture-expanded cells acquired morphologic and phenotypic characteristics of hepatocyte-like cells. Immunoflurescent staining analysis demonstrated that these hepatocyte-like cells stained positive for CK18, ALB, and HNF-1α (Fig. 4a), while the undifferentiated cells did not stain negative with any of the antibodies used (data not shown). In addition, RT-PCR showed these differentiated cells expressed CK18 and ALB (Fig. 4d).

As for neuroectoderm differentiation, the differentiation potential is similar to all culture-expanded cells after 40 population doublings from different fetal tissues. After these culture-expanded cells incubated in FN-coated wells with 100 ng/ml bFGF for 14 days, about 47% of these cells acquired morphologic and phenotypic characteristic of neurons (NF^+; Fig. 4b), about 16% of oligodendrocytes ($GalC^+$; Fig. 4b) and about 12% of astrocytes ($GFAP^+$; Fig. 4b), while the undifferentiated cells were not stained with any of the antibodies used (data

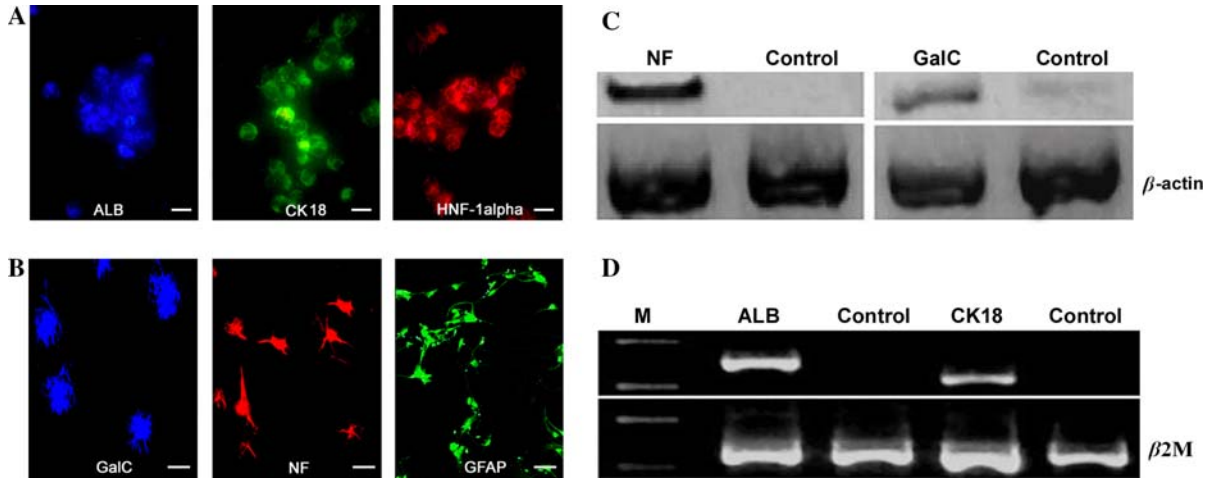

Fig. 4 Various fetal tissues-derived culture-expanded clonal cells could differentiate into neuroectoderm and endoderm in vitro. **a** Heart-derived clonal cells cultured with FGF-4 and HGF for 14 days were stained by ALB (*blue*), CK18 monoclonal antibody (*green*), and HNF-1α (*red*) antibodies. **b** Heart-derived clonal cells grown in the presence of 100 ng bFGF/ml for 14 days show immunoreactivity with NF-200 (*red*), GalC (*blue*), and GFAP monoclonal antibody (*green*) after induction. **c** These results were confirmed by Western blot with monoclonal antibodies against NF-200 and GalC. As for the other tissue-derived culture-expanded clonal cells, they all could differentiate into neuroectoderm and hepatocyte-like cells in vitro (data not shown). **d** RT-PCR products of ALB, CK18, and β2-microglobulin (β2M). Scale bar = 20 μm. *M* = DNA marker. Results are a representative example of four experiments

not shown). These results were confirmed by Western blot analysis (Fig. 4c).

Discussion

Stem cell plasticity is a hot research field although the mechanism underlying plasticity remains a matter of debate. Recently, cell fusion was suggested as an explanation for stem cell plasticity. Some researchers have raise doubts about whether transdifferentiation or de-differentiation actually occurs (Spees et al. 2003; Terada et al. 2002; Vassilopoulos et al. 2003; Wang et al. 2003; Ying et al. 2002). Their findings, using either in vitro or in vivo systems, has suggested that stem cells "transdifferentiation" or "plasticity" is a result of cell fusion events that may lead to the detection of Y chromosome (when the donor is male and the recipient is female) or other donor cell-specific genes in "differentiated cells" which coexpressed recipient-specific genes.

Our in vitro studies demonstrate that single cell-derived human fetal BM $Flk1^{+}CD34^{-}$ cells, never co-cultured with tissue-specific mature cells or embryonic stem cells, could differentiate into hematopoietic and endothelial cells (Guo et al. 2003), indicating that the in vitro behavior of these cells cannot be attributed to cell fusion. In addition, considering our findings and some others' in vivo transplantation studies reporting robust (7–57%) levels of transdifferentiation (Ianus et al. 2003; Galli et al. 2000; Lagasse et al. 2000; Rietze et al. 2001), it is unlikely that the plasticity potential of adult stem cell is due to cell fusion events. Even Grompe and his colleagues reported that cell fusion was responsible for most donor-derived hepatocytes in their setting, cell fusion was still a rare event (Wang et al. 2003). Thus, it is reasonable to assume that, in our experiments, cell fusion, if at all present in vivo, may be a rare event in the paradigm of "transdifferentiation or plasticity" of adult stem cells.

Then the question comes: how to explain the stem cell plasticity. We suppose that there is a presently undefined subfraction of stem cell population that exists in a number of tissues beyond embryonic development. We term them PMSCs. They differ from embryonic stem cell in that they gradually lose some differentiation potentials during development and adopt some special phenotypes once in a certain tissue. They can give rise to different kinds of stem cells; therefore, help to maintain balance during development and metabolism. They provide cells not

only for the damaged tissues they reside in, but also for damaged tissues different from them by migration triggered by proinflammatory cytokines or growth factors. This will be of great importance when stem cells in damaged tissues are destroyed or not enough in number.

In this study, we analyzed the fetal tissue-derived culture-expanded stem cells for expression of markers known to be expressed on culture-expanded MSCs and assessed the multilineage potential for adipogenic, osteogenic, neural, and hepatic differentiation. We found that these cells with similar characteristics of fetal BM-derived $Flk1^{+}CD34^{-}$ PMSCs (Fang et al. 2004; Guo et al. 2003) can be culture-isolated from different fetal tissues. These cells have the similar morphology, phenotype, and in vitro differentiation ability. In addition, as fetal bone marrow-derived $Flk1^{+}CD34^{-}$ PMSCs, these multipotent stem cells from different fetal tissues had the capacity for self-renewal and the multilineage differentiation even after being expanded for more than 40 population doublings in vitro. Whether PMSCs with the similar characteristics of fetal BM-derived $Flk1^{+}CD34^{-}$ PMSCs (Fang et al. 2003, 2004; Guo et al. 2003) can be generated from adult human has not been tested. However, Verfaillie and her coworkers found that the age of the donor seemed not to influence the generation of pluripotent adult progenitor cells (Reyes et al. 2001, 2002). Therefore we have thought that the PMSCs hypothesis may be true, and this will help us to further understand the properties of plasticity and may greatly contribute to the potential use of stem cells for cell transplantation, artificial tissue construction and gene therapy.

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