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Side Population Cells Derived from Adult Human Liver Generate Hepatocyte-like Cells In Vitro

SUNNY ZAHEED HUSSAIN, MD[*,†], **STEPHEN C. STROM, PhD**[‡], **MARTHA R. KIRBY**[§], **SEAN BURNS, MD**[†], **SASKIA LANGEMEIJER**[†], **TAKAHIRO UEDA**[†], **MATTHEW HSIEH, MD**[†], and **JOHN F. TISDALE, MD**[†]

* *Division of Gastroenterology, Children's National Medical Center, Washington, DC*

† *Molecular and Clinical Hematology Branch, NIDDK, NIH*

‡ *Department of Pathology, University of Pittsburgh*

§ *National Human Genome Research Institute, NIH, Bethesda, MD, USA*

Abstract

We sought to determine whether hepatic side population (SP) cells derived from adult human liver possess the potential of a novel candidate hepatic stem cell. Human cadaveric donor liver was subjected to collagenase perfusion and hepatocytes were separated from nonparenchymal cells by differential centrifugation. SP cells were isolated from the nonparenchymal portion after Hoechst 33342 staining. Since CD45 is a panleukocyte antigen, CD45-negative SP cells were separated from the vast majority of CD45-positive SP cells (90%), and hepatic growth medium was used to culture both groups. Both CD45-negative and CD45-positive hepatic SP cells generated colonies in the hepatic growth medium in 2–3 weeks. The colonies yielded large cells morphologically consistent with human hepatocytes, demonstrating granule-rich cytoplasm, dense, often double nuclei, and intracellular lipofuscin pigment. The cultured cells from both sources were positive for markers of human hepatocytes: HepPar, cytokeratin 8 (CK8), and human albumin. Reverse transcriptase–polymerase chain reaction (RT-PCR) performed on both groups demonstrated positivity for additional liver markers including human albumin, CK18, α-1 anti-trypsin, and the human cytochrome P450 enzyme CYP2B6. Double immunostaining (CD45 and HepPar) and RT-PCR confirmed that the hepatocyte-like cells derived from the CD45-negative SP cells acquired HepPar positivity but had no detectable CD45 antigen expression. In contrast, the cultured cells derived from the CD45-positive SP cells also acquired HepPar positivity, but only a minimal fraction expressed the CD45 antigen. We conclude that hepatic SP cells derived from the nonparenchymal portion of human liver are a potential source of human hepatocytes irrespective of their CD45 status, and further animal studies will be required to assess their regenerative potential.

Keywords

human; side population; stem cells; hepatic; CD45

SP cells are identified by flow cytometry because of their unique ability to efflux Hoechst 33342 dye and have been shown in animal studies to be highly enriched stem cells (1,2). While the true adult liver stem cell has not been characterized, human hepatic side population (SP) cells might contain certain cells that might have hepatic progenitor-like properties.

Address for reprint requests: John F. Tisdale, MD, Molecular and Clinical Hematology Branch, NIDDK, National Institutes of Health, Bethesda, MD, USA; E-mail: Johntis@intra.niddk.nih.gov.

The liver in a normal healthy adult maintains a balance between cell gain and cell loss. It is composed mainly of two epithelial cell types, hepatocytes and bile ductular cells, both of which have high regenerative capacity. After hepatic injury, a regenerative process repairs the liver and brings it back to its original mass within 10 days (3). This restoration of mild to moderate cell loss and "wear and tear" renewal is largely achieved by hepatocyte self-replication. A more severe liver injury activates the endogenous stem cell population; these are often termed "oval" cells and are much smaller than mature hepatocytes (4). These oval cells display a distinct phenotype and have been shown to be a bipotential progenitor of two types of cells found in the liver, parenchymal hepatocytes and bile ductular epithelium (5,6). It appears that cells with regenerative potential constitute a small but significant population in both the adult and the developing liver (7), and further work is needed to render them more easily available. The main focus of the current studies is to explore the possibility of a dependable source of hepatic stem cells from within the de novo liver.

SP cells are highly enriched stem cells with robust regenerating potential and can be identified by flow cytometry because of their unique ability to efflux a fluorescent dye, Hoechst 33342. First described by Goodell in murine bone marrow cells (1) while displaying Hoechst fluorescence simultaneously at two emission wavelengths, SP cells represent a small subset of cells that are distinct from the rest of the cell population. These cells contain most of the hematopoietic stem cell (HSC) activity, display cell surface markers of HSCs, and are enriched at least 1000-fold in HSC activity. Similar cells have also been isolated from human fetal liver but have predominantly demonstrated the phenotype of hematopoietic stem cells (8), as the developing fetal liver represents a prominent source of HSCs during development, and a number of studies suggest that the SP phenotype may be useful in identifying candidate stem cells within other organs (9–11). A more recent work has shown the successful isolation of SP cells from adult murine liver and their ability to repopulate the liver in a liver injury model (12). Our study aimed at isolating SP cells from human cadaveric donor liver and studying their phenotypic characteristics and in vitro growth potential.

We separated SP cells expressing CD45, a panleukocyte antigen (13), from those not expressing CD45, reasoning that CD45-negative SP cells would most likely represent the cells of a hepatic lineage. Culture of both fractions resulted in the outgrowth of hepatocyte-like cells, and further in vivo work will be required to assess the potential of adult hepatic SP cells.

MATERIALS AND METHODS

Collagenase Perfusion of Cadaveric Liver

Cadaveric human liver cells were obtained from the Liver Tissue Procurement and Distribution System (LTPADS) based at the University of Pittsburgh (NIH Contract N01-DK-9-2310). The use of cadaveric liver for in vitro studies was designated EXEMPT from IRB oversight by the Office of Human Subjects Research (OHSR 2607), National Institutes of Health. The unused cadaveric donor liver was negative for HIV and hepatitis B and C viruses and was subjected to a three-stage collagenase (Sigma–Aldrich) perfusion using methods already described (14). Buffer 1 consists of HBSS without calcium (Bio Fluids), supplemented with 0.5 mM EGTA (Aldrich) and additionally buffered with 10 mM HEPES (Cellgro). Buffer 2 consists of HBSS without EGTA, which ensured that the EGTA was flushed from the liver with buffer 3, containing collagenase (200–500 mg/L in Eagle minimum essential medium with EBSS [Gibco]) and 25 mM HEPES, without glutamine. The primary suspension was centrifuged in three low-speed spins. Mature parenchymal hepatocytes are larger and heavier so they are selectively pelleted. The smaller cells are retained in the supernatant of these centrifugations. This smaller cell fraction was felt to contain the small SP cells and was thus filtered (70 μm) and separated from red blood cells by ACK lysis (Quality Biological). The remaining cells

were then counted and cryopreserved in small aliquots in UW solution with 10% DMSO and 10% FBS.

Hoechst Staining

The liver cells were thawed and resuspended in CMRL (Cellgro) at a concentration of 1×10^6 cells/ml. The cells were then filtered successively with 70- and 40-μm filters. The cell suspension was then stained, first with Bisbenzimide Hoechst 33342 (Sigma), followed by a FITC-conjugated ant-CD45 antibody (Becton Dickinson). Hoechst staining was performed at a concentration of 7.5 μg/ml in a 37°C water bath for 90 min. A control was also set up with 1 ml of this solution with the addition of 50 μl of verapamil (2.455 mg/ml; Sigma). Once Hoechst staining was completed, the cell suspension was placed on ice and FITC-conjugated anti-CD45 was applied at a concentration of 5 μl per 10^6 cells/ml. A control for CD45 staining was set up with 1 ml of this cell suspension with the addition of 5 μl of an isotype control. After staining was completed, the cells were washed with CMRL, centrifuged (1200 rpm, 4°C, 5 min) and resuspended in HBSS before being sorted.

SP Cell Sorting by Flow Cytometry

The stained cells were sorted on a FACSVantage SE (BD Biosciences), using CellQuest software. A UV laser and a 350-nm argon laser were employed to excite the Hoechst 33342, and the SP cells were visualized with tight gating based on the verapamil control. The SP cells were further analyzed for FITC-conjugated CD45 staining, and thus CD45-positive and CD45-negative cells were collected separately in phosphate-buffered saline (PBS).

Cell Culture

The SP cells were cultured in fibronectin-coated 96-well culture plates (BD Biosciences) at a concentration of 5000 cells per 100 μl of culture medium. Dulbecco's modified Eagle medium (Cellgro) with 10% fetal calf serum was supplemented with nonessential amino acids (1 μg/ml; Gibco), penicillin–streptomycin (100 units/ml; Sigma), dexamethasone (50 nm; Sigma–Aldrich), insulin (I Lantus; 10^{-7}M), HGF (20 ng/ml; Sigma–Aldrich), and EGF (10 ng/ml; Sigma–Aldrich). The medium was changed (50%) daily for the first 3 days, then every third day. The cultures were maintained for 4 weeks in a humidified incubator in 5% CO_2.

Cell Staining (Wright–Giemsa and Immunofluorescence)

The cultured cells were detached from the fibronectin-coated plates using trypsin–EDTA and washed with PBS. A small aliquot of each cell suspension (20 μl) was placed on a glass slide for standard Wright–Giemsa staining and immunofluorescence. Standard techniques were used for Wright–Giemsa staining. The expression of HepPar, cytokeratin-8, and human albumin was assayed by immunofluorescence analysis: HepPar (Dako), a liver-specific mouse monoclonal anti-human antibody was used at a 1:25 dilution. Cytokeratin 8 (Dako), a mouse monoclonal anti-human antibody, was used at a 1:25 dilution. Both HepPar and cytokeratin 8 are intracytoplasmic primary antibodies and require a secondary FITC-conjugated rabbit anti-mouse antibody to be detected by immunofluorescent microscopy. Human albumin staining was performed using a FITC-conjugated rabbit anti-human antibody (Dako). All slides were blocked by 5% rabbit serum to counter the background staining and then washed for 15 min in PBS before the FITC-conjugated antibodies were applied. Separate positive (mature human hepatocytes) and negative (human peripheral blood cells) controls were obtained for each staining. At the end, the slides were mounted with Vectashield (Vector Laboratories, USA) with DAPI (4,6-diamidino-2-phenylindole; Sigma) and examined under a fluorescent microscope.

Simultaneous Immunostaining for HepPar and CD45

Double immunostaining for CD45 and HepPar was performed to ascertain the CD45 status of the cultured cells derived from both CD45-positive and CD45-negative SP cells. Small cell suspensions (50 μl) were placed on glass slides and allowed to dry at room temperature (RT) for 20 min. The slides were then submerged in 100% acetone for 15 min at −20°C. Thereafter, they were kept at RT for 10 min and then washed in PBS for 15 min (5 min × 3). Blocking was performed for 20 min with 2% bovine serum. After being washed for 15 min with PBS, the HepPar (mouse anti-human) antibody was applied (1:25) for 30 min in the dark, followed by the FITC-conjugated secondary (rabbit anti-mouse) antibody (1:20). Repeated washing with PBS was done for 15 min. PE-conjugated anti-CD45 antibody was then applied for 30 min in the dark. The slides were then washed again in PBS before mounting and observation under an immunofluorescence microscope. Positive and negative controls were concomitantly stained and analyzed and included normal human peripheral blood cells and mature human hepatocytes.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) for CD45 and Human Liver Markers

RT-PCR was performed on mRNA isolated from the cultured cells derived from both the CD45-positive and CD45-negative SP cells. Total cellular RNA was obtained using RNA STAT-60 (Tel-Test, Friendswood, TX) and was treated with DNAse (Invitrogen). cDNA was prepared by reverse transcription using an RNA PCR Core (GeneAmp Applied Biosystems). To determine the expression of CD45, amplification of cDNA (Advantage PCR; BD Biosciences) was performed with Taq polymerase using CD45 primers (5′ AACAGTGGAGAAAGGACGCA and 5′TGTGTCCAGAAAGGCAAAGC) for 20 cycles: denaturation at 94°C (1 min), primer annealing at 55°C (1 min), and primer extension at 72° C (1 min). cDNA from negative (HepG2 cell line; ATCC, USA) and positive (white blood cells) controls were concurrently amplified with each sample and §-actin was used as a universal control.

To identify liver markers (15–17), the cDNA was amplified by use of a GeneAmp PCR System 9700 Cycler (PE Applied Biosystems) under the following conditions: human albumin (forward, 5′-TTGGAAAAATCCCACTGCAT; reverse, 5′-CTCC AAGCTGCTCAAAAAGC), at 95°C for 120 sec, followed by 35 cycles at 94°C for 0 sec and 72°C for 20 sec; human cytokeratin 18 (CK-18: forward, 5′-GAGATCGAGGCTCTCAAGGA; reverse, 5′-CAAGCTGGCCTTCAGATTTC), at 95°C for 120 sec, followed by 40 cycles at 94°C for 0 sec, 58°C for 5 sec, and 72°C for 20 sec; α-1 antitrypsin (AAT: forward, 5′-AGACCCTTTGAAGTCAAGGACACCG; reverse, 5′-CCATTGCTGAAGACCTTAGTGATGC), at 95°C for 15 min, 94°C for 30 sec, 68°C for 30 sec, and 72°C for 1 min, followed by 40 cycles at 72°C for 10 min ; and human cytochrome P450 enzyme (CYP2B6: forward, 5′-GATCACACCATATCCCCGGA; reverse, 5′-CACCCTACCACCCATGACCG), for 40 cycles at 95°C for 15 sec, 60°C for 30 sec, and 72° C for 30 sec. For universal control, human glyceraldehyde 3-phosphate dehydrogenase (GAPDH: forward, 5′-GTC TTCTCCACCATGGAGAAGGCT; reverse, 5′-CATGCCAGTGAG CTTCCCGTTCA) at 95°C for 120 sec, followed by 30 cycles at 94°C for 0 sec, 58°C for 5 sec, and 72°C for 16 sec. Human white blood cells and mature human hepatocytes were used as negative and positive controls, respectively.

RESULTS

Isolation of SP Cells from Nonparenchymal Cells

SP cells comprised up to 1% of the nonparenchymal fraction of the liver and were further sorted into CD45-negative and CD45-positive SP cells (Figure 1). Approximately 90% of the SP cells

were CD45-positive, ranging from 45,000 to 500,000 per sort. Approximately 10% of SP cells derived from the liver were CD45-negative, ranging from 5000 to 50,000 cells per sort.

Culture of Human SP Cells

Both CD45-negative and CD45-positive SP cells differentiated into cells resembling human hepatocytes (Figure 2) 3 weeks after in vitro culture in hepatic growth medium. They began to form colonies (Figures 2B and E) at about 2–3 weeks and were allowed to grow for up to 4 weeks. The cells were then removed from the 96-well plate using trypsin–EDTA and, upon staining with Wright–Giemsa, demonstrated typical hepatocyte-like morphology, with granule-rich cytoplasm and dense, often double, nuclei, along with displaying the characteristic lipofuscin pigment (Figures 2C and F).

Immunofluorescence Staining of Cultured Cells

These hepatocyte-like cells were analyzed by immunofluorescence for various known human hepatocyte markers including HepPar, CK8, and human albumin. These hepatocyte-like cells demonstrated HepPar positivity (Figure 3A), with a control which demonstrated no immunofluorescence (Figure 3C). CK8 (Figure 3B) was also detected among these cells. Human albumin, although nonspecific, is a sensitive liver marker and was also detected among the cultured cells (Figure 3D). Both positive (mature human hepatocytes) and negative (mature human white blood cells) controls (Figures 3E and F) were checked for each immunofluorescent antibodies. These results were reproduced on three occasions.

CD45 Antigen Status of Cultured Cells

Cultured cells from both the CD45-positive and the CD45-negative fractions produced cells which phenotypically resembled mature hepatocytes. Immunufluorescence using double immunostaining (Figure 4) with FITC-conjugated antibody to HepPar and PE-conjugated CD45 showed that the cultured cells derived from the CD45-negative SP cells acquired HepPar positivity but had no detectable CD45 antigen expression. In contrast, the cultured cells derived from the CD45-positive SP cells also acquired HepPar positivity, but a very small fraction also expressed the CD45 antigen.

RT-PCR for CD45 Antigen and Liver Markers

The cultured cells derived from CD45-negative SP cells were devoid of CD45 antigen mRNA. In contrast, cultured cells derived from CD45-positive SP cells were indeed positive for CD45 antigen mRNA (Figure 5).

RT-PCR for hepatocyte markers demonstrated that the hepatocyte-like cells derived from both CD45-positive and CD45-negative fractions were positive for human albumin, CK18, AAT, and the human cytochrome P450 enzyme CYP2B6, generating 350-, 357-, 360-, and 275-bp PCR amplicons, respectively (Figure 6).

DISCUSSION

The derivation of mature hepatocytes from both hepatic and extrahepatic progenitor cells remains a focus of investigators involved with liver stem cell research. There have been a number of studies suggesting that bone marrow–marrow progenitor cells may differentiate into hepatocyte-like cells in rodents (18–22). Studies utilizing human bone marrow-derived cells have produced similar results both in vitro and in animal studies (17,23–25); however, the phenotype of the hepatocyte-like cells has not been adequately defined in most cases, and the demonstration of cellular fusion (26,27) posed a major limitation to the interpretation of animal transplantation studies. Human umbilical cord blood has also been suggested to be a reliable

source of transplantable hepatic progenitor cells (15), and some limited attempts have been made to demonstrate that these cells can differentiate without cellular fusion (28). Both adult and fetal liver have long been considered to harbor hepatic progenitor cells because of anatomical homology, and several studies have now substantiated this hypothesis. Adult human liver-derived progenitor cells have exhibited both hepatocytic and biliary markers upon differentiation (16,29). Hepatoblasts isolated from human fetal liver in the second trimester that expressed biliary (CK-19) and hepatocyte (glycogen) markers at initial isolation eventually expressed markers for both hepatocytes (albumin) and biliary epithelium (GGT) (30).

We sought to explore the regenerative potential of SP cells derived from adult human liver. As mammalian liver is known to harbor hematopoietic cells, we attempted to empirically separate the hepatic and hematopoietic SP stem cells of the basis of their CD45 antigen status, given the fact that CD45 is a universal hematopoietic surface antigen (13). Wulf *et al.* have recently demonstrated in a murine model that both CD45-positive and CD45-negative hepatic SP cells can give rise to colonies of both hematopoietic and hepatic lineages in vitro and, upon transplantation, can contribute to liver regeneration (12). Our findings with in vitro culture of human hepatic SP cells are consistent with those of the murine model. Both CD45-positive and CD45-negative SP cells derived from cadaveric human liver differentiated into large cells that resembled hepatocytes, demonstrating characteristic granular cytoplasm with dense and double nuclei and lipofuscin pigment (Figures 2C and F).

We further evaluated hepatocyte differentiation by immunofluorescence (HepPar, CK8, and human albumin) markers of hepatocyte differentiation (Figure 3). Both CD45-positive and CD45-negative SP cells differentiated into cells that expressed HepPar, CK8, and human albumin. HepPar is monoclonal mouse antibody directed against the mitochondrial fraction of human hepatocytes and does not label hematopoietic or nonparenchymal cells (31). CK8 labels only hepatocytes among the other cells derived from human liver, as it is directed against the epithelial cells (32). Human albumin is not a specific marker for human hepatocyte but has a high sensitivity. We made efforts to define the exact CD45 antigen status of these cultured hepatocyte-like cells by double staining for both HepPar and CD45 (Figure 4). The cultured cells derived from the CD45-negative SP cells acquired HepPar positivity but were negative for CD45 antigen expression. The hepatocyte-like cells derived from CD45-positive SP cells, on the other hand, also expressed HepPar, but only minimal CD45 staining was detectable. This observation was subsequently confirmed by RT-PCR for CD45 antigen (Figure 5). These findings suggest that the CD45 surface antigen expression status is not important in terms of the hepatic differentiation potential of the SP cells derived from human liver, at least in vitro.

We confirmed hepatocyte differentiation by reverse transcriptase PCR for both sensitive (albumin) and specific (AAT, CK18, and cytochrome P450) markers of hepatocyte differentiation (Figure 6). Although albumin is a nonspecific marker, AAT (33) and CK18 (32) are relatively specific markers for human hepatocytes. CYP2B6 expression is considered hepatocyte specific, as cytochrome P450 enzymes are expressed only in mature hepatocytes (17).

One contentious issue is the possible contribution of CD45-negative SP cells in the generation of hepatocyte-like cells from CD45-positive SP cells. Careful assessment of verapamil and isofluorescence controls indicates the potential for 0.01% CD45-negative cell contamination at the most, estimating 50 to 60 cells among 5000 to 6000 CD45-negative SP cells employed for in vitro culture. Conversely, it is possible that CD45-positive blood stem cells were present among the CD45-negative cells, however, it is unlikely that they were the most significant contributors to the hepatocyte-like cells grown from the CD45-negative SP cells. However, neither our recent work nor the work of Wu *et al.* definitively establishes the cell responsible for such observations, and further animal transplantation studies will be required to assess their

in vivo regenerative potential and, should regenerative potential be demonstrated, the mechanism for such regeneration, i.e., direct contribution, fusion, or “transdifferentiation.”

We demonstrate for the first time that both CD45-positive and CD45-negative human hepatic SP cells are capable of differentiating into cells which phenotypically resemble hepatocytes, express markers of hepatocytes including expression of HepPar, CK8, CK18, human albumin, and AAT, as well as human cytochrome P450 (CYP2B6), and may be representative of endogenous hepatic stem cells. Further studies in immunodeficient mice will be required to assess the in vivo potential of human hepatic SP cells. The percentage yields of hepatocyte-like cells from both CD45-positive and CD45-negative SP cells were similar (75–80%). We speculate that using hepatic SP cells without additional sorting for CD45 status would be more effective in the transplantation setting, avoiding the additional step in processing; however, a comparison of the in vivo potential of both CD45-positive and CD45-negative SP cells will be required to address this question, and such studies are currently under way.

CONCLUSION

Human hepatic SP cells sorted on the basis of their expression of the CD45 surface antigen are capable of differentiating into hepatocyte-like cells in vitro, similarly to their CD45-negative counterparts. Further in vivo studies will be necessary to determine the regenerating ability of hepatic SP cells.

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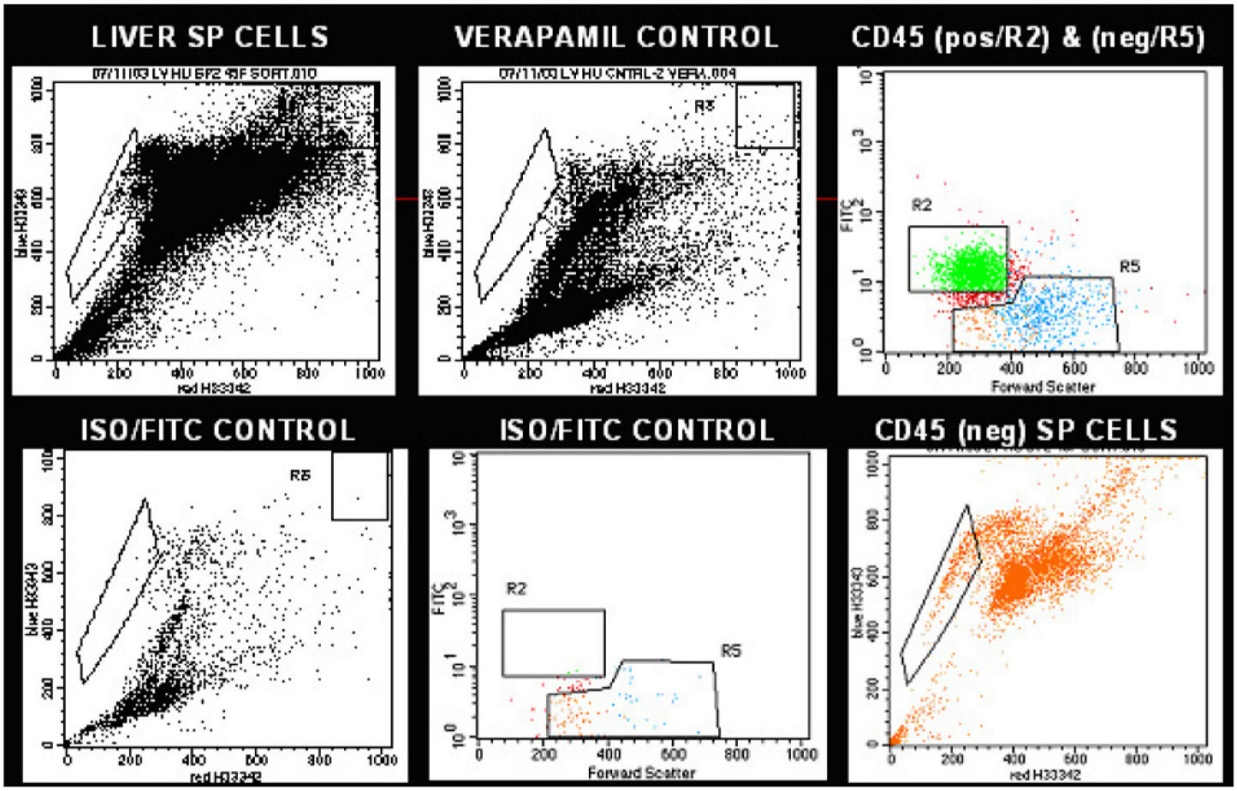

Fig 1.

Double-staining (Hoechst 33342 and FITC-conjugated CD45) and sorting by flow cytometry separating CD45-negative and CD45-positive human hepatic SP cells. The *x* and *y* axes represent the red and blue emissions of Hoechst 33342, respectively, upon excitation by ultraviolet light (scale 0, 200, 400, …, 1000 signifies relative linear fluorescence index). Verapamil blocks the efflux pump and thus abolishes the SP population. Tight gating (R2 for CD45-positive and R5 for CD45-negative SP cells) was used to specifically obtain both the CD45-positive and the CD45-negative SP cells.

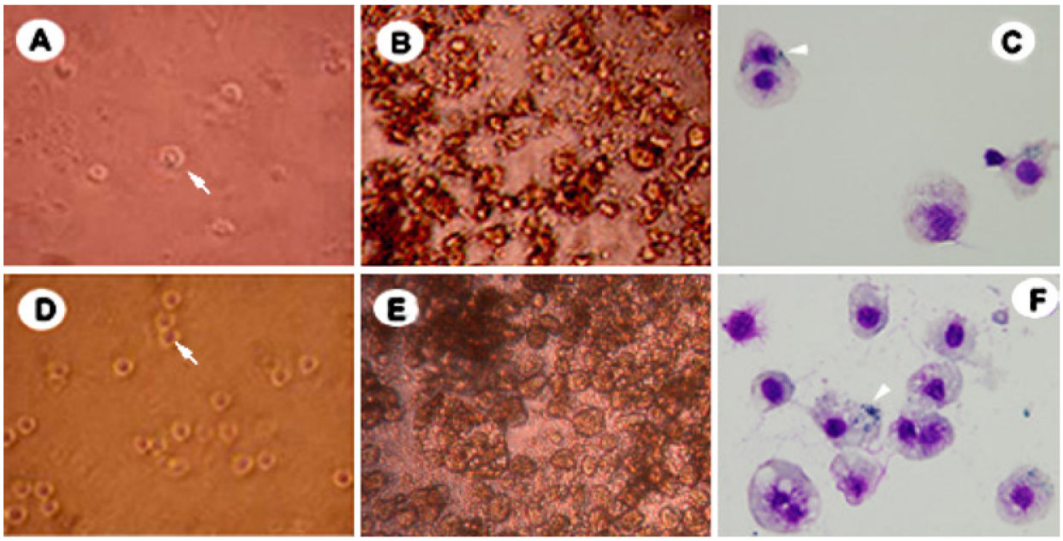

Fig 2.

Culture of human hepatic SP cells. (A) CD45-negative SP cells in the 96-well culture plate, on day 1. (B) CD45-negative SP cells forming colonies on day 14. (C) Wright–Giemsa stain showing hepatocyte-like cells on day 28, with the characteristic granular cytoplasm, double nuclei, and lipofuscin pigment (white arrowhead). (D) CD45-positive SP cells on day 1. (E) CD45-positive SP cells forming colonies on day 14. (F) Wright–Giemsa stain showing hepatocyte-like cells on day 28 (derived from CD45-positive SP cells). White arrowhead shows characteristic lipofuscin pigment. (Original magnifications: A and D, ×60; C, ×100; B and E, ×40.)

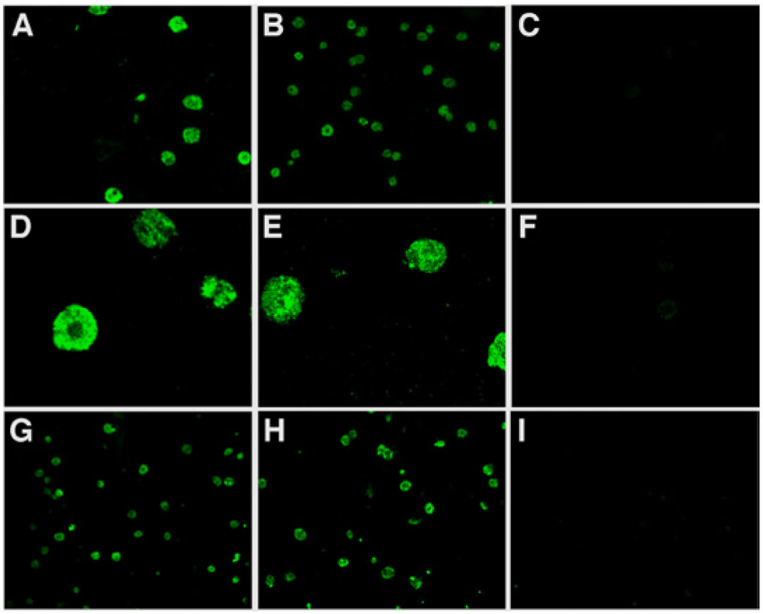

Fig 3.

Immunofluorescence staining of hepatocyte-like cells derived from CD45-positive SP cells, employing various markers for human hepatocytes. Mature human hepatocytes and human peripheral blood cells were used as positive and negative controls for each marker. (A) HepPar staining of hepatocyte-like cells. (B) Positive control for HepPar. (C) Negative control for HepPar. (D) Cytokeratin 8 (CK8) staining of hepatocyte-like cells. (E) Positive control for CK8. (F) Negative control for CK8. (G) Albumin staining of hepatocyte-like cells. (H) Positive control for albumin. (I) Negative control for albumin. Similar results were obtained with the hepatocyte-like cells derived from CD45-negative SP cells. (Original magnifications: A, ×40; B, C, G–I, ×20; D–F, ×60.)

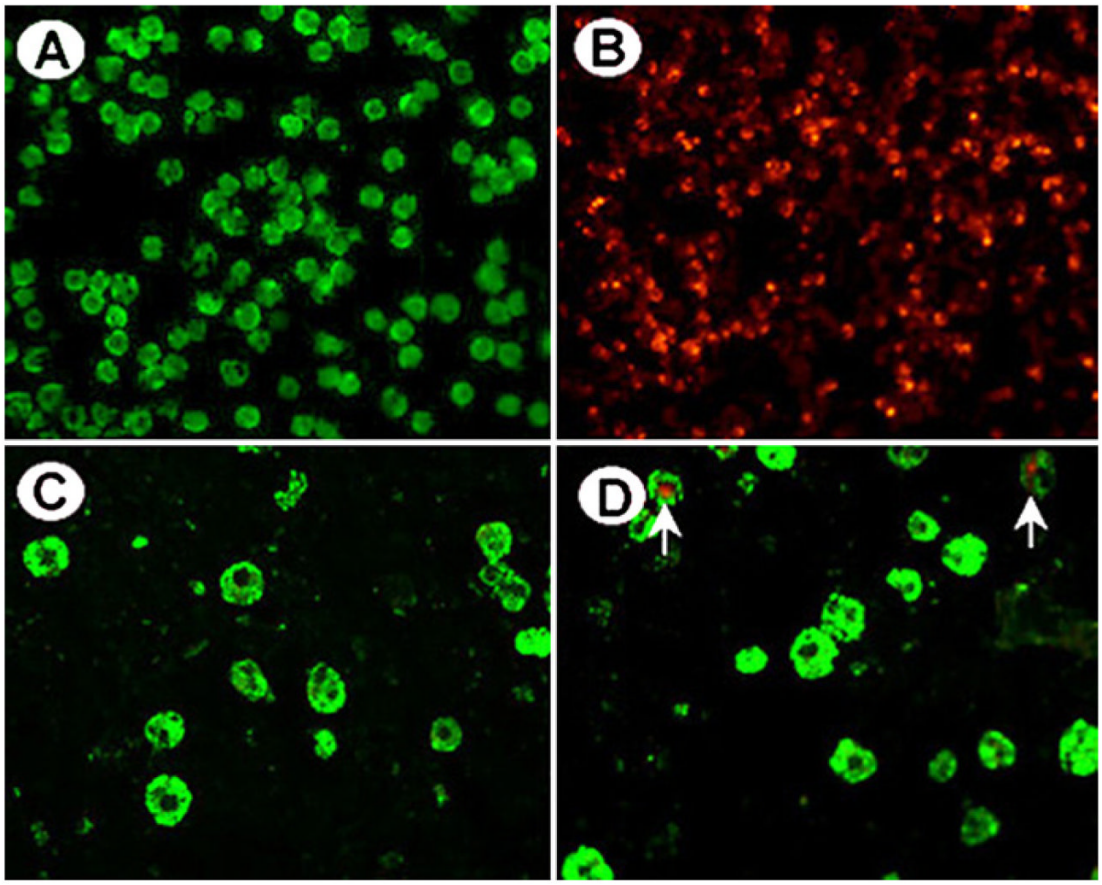

Fig 4.

Double staining (HepPar-FITC and CD45-PE) of cultured hepatic SP cells. (A) Positive control (mature human hepatocytes) showing only HepPar (green immunofluorescence. (B) Negative control (white blood cells) showing only CD45 antigen expression (orange–red). (C) Cells derived from CD45-negative hepatic SP demonstrated only HepPar (green immunofluorescence). (D) Cells derived from CD45-positive hepatic SP cells demonstrated HepPar (green) staining. However, there was minimal but obvious CD45 positivity (red–orange; arrows) detectable in some cells. (Original magnifications: A and B, ×20; C and D, ×40.)

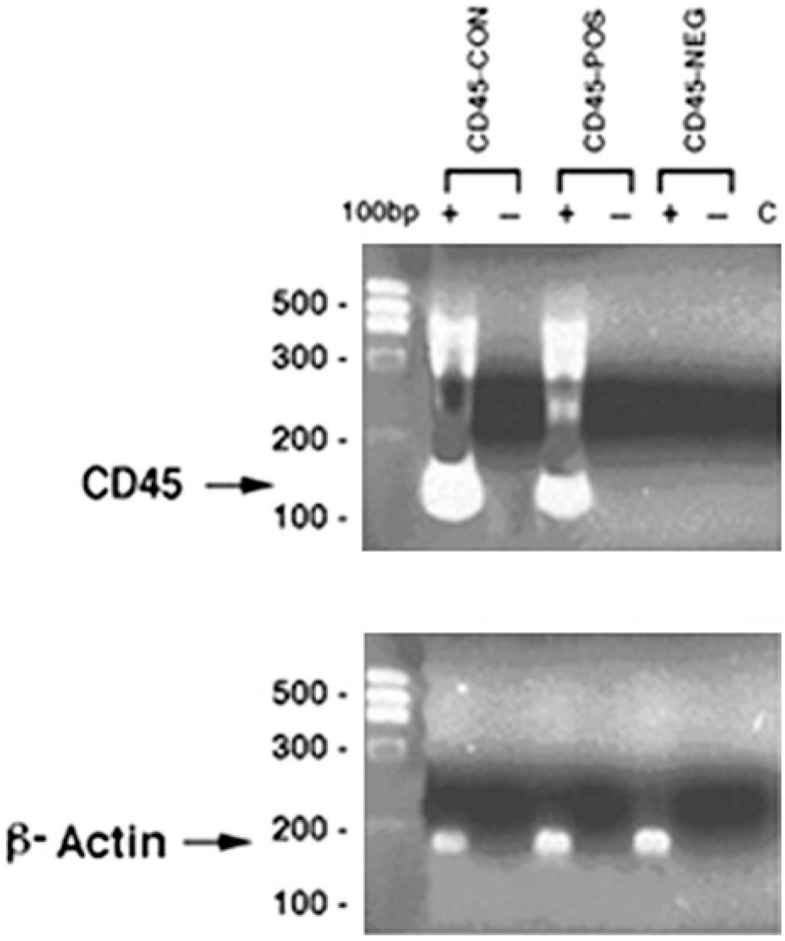

Fig 5.

Results of RT-PCR for CD45 expression. PCR products were run on a 1.5% agarose gel. Lanes labeled with a (+) had reverse transcriptase added, and those with a (−) had no reverse transcriptase added. Lanes labeled with a C represent controls with water where no cDNA was added. The CD45-CON lane represents the positive control (white blood cells). The CD45-POS lane represents cultured cells derived from CD45-positive SP cells. The CD45-NEG lane represents cultured cells derived from CD45-negative cells. *β*-Actin controls were run concurrently and were positive for all samples, confirming the validity of the PCR reactions.

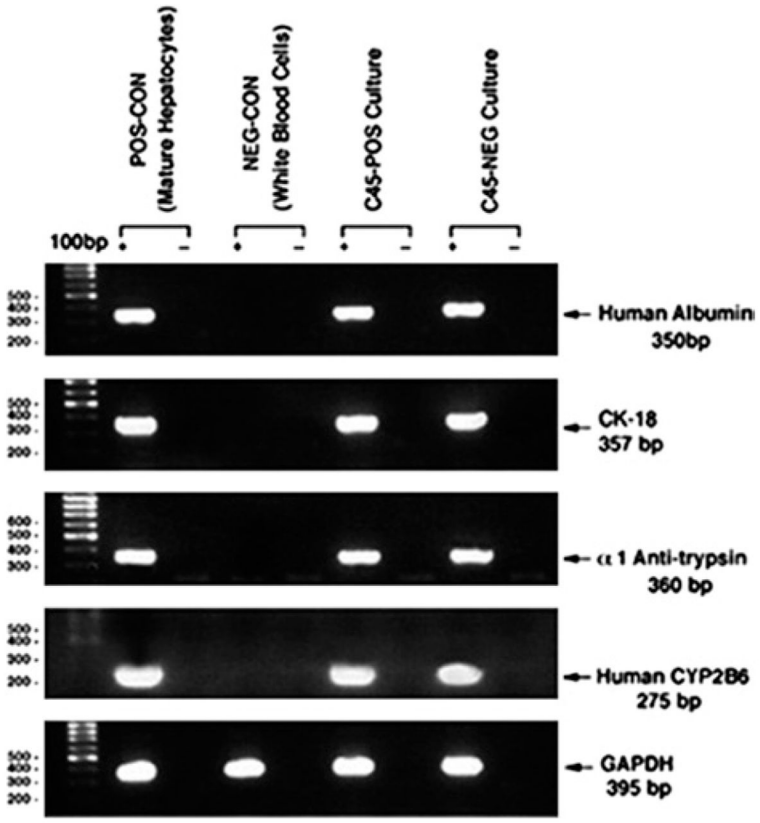

Fig 6.

Results of RT-PCR for liver markers: PCR products were run on a 1.5% agarose gel. Lanes labeled with a (+) had reverse transcriptase added, and those with a (−) had no reverse transcriptase added. The POS-CON lane represents the positive control (mature human hepatocytes). The NEG-CON (white blood cells) represents the negative control. Both the CD45-NEG and the CD45-POS lanes, representing cultured hepatocyte-like cells derived from CD45-negative and CD45-positive SP cells, respectively, show positivity for human albumin, cytokeratin 8 (CK8), α-1 AAT (AAT), and the human cytochrome P450 gene (CYP2B6), respectively. GAPDH controls were positive for all cDNA-positive (+) samples, confirming the validity of the PCR reactions.