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CD133$^+$ hepatic stellate cells are progenitor cells

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Abstract

Hepatic stellate cells (HSC) play an important role in the development of liver fibrosis. Here, we report that HSC express the stem/progenitor cell marker CD133 and exhibit properties of progenitor cells. CD133$^+$ HSC of rats were selected by specific antibodies and magnetic cell sorting. Selected cells displayed typical markers of HSC, endothelial progenitor cells (EPC), and monocytes. In cell culture, CD133$^+$ HSC transformed into α-smooth muscle actin positive myofibroblast-like cells, whereas application of cytokines known to facilitate EPC differentiation into endothelial cells led to the formation of branched tube-like structures and induced expression of the endothelial cell markers endothelial nitric oxide synthase and vascular-endothelial cadherin. Moreover, cytokines that guide stem cells to develop hepatocytes led to the appearance of rotund cells and expression of the hepatocyte markers α-fetoprotein and albumin. It is concluded that CD133$^+$ HSC are a not yet recognized progenitor cell compartment with characteristics of early EPC. Their potential to differentiate into endothelial or hepatocyte lineages suggests important functions of CD133$^+$ HSC during liver regeneration.

Keywords: AC133; CD133; Endothelial progenitor cells; Hepatic stellate cells; Prominin 1

Liver fibrosis is characterized by excess deposition of collagens, resulting in an impairment of liver function and hemodynamics and finally organ failure. A major player in the process of fibrogenesis is the hepatic stellate cell (HSC). In the quiescent state, HSC are vitamin A (mainly retinyl palmitate) storing cells [1] located between sinusoidal endothelial cells and hepatocytes in the space of Dissé. HSC are further characterized by their stellate-shaped morphology, perinuclear lipid droplets, and expression of the proteins desmin [2] and glial fibrillary acidic protein (GFAP) [3]. Following liver injury HSC become activated, loose their vitamin A stores and transform into a myofibroblast-like phenotype. These myofibroblasts are contractile and synthesize α-smooth muscle actin (α-SMA) and extra-cellular matrix proteins such as collagen (type 1).

Despite intensive research on the biology and pathophysiological role of HSC, their embryonic origin is still a matter of debate, because stellate cells synthesize protein markers of all three embryonic germ layers. Stem cells of the bone marrow can generate cells of all germ layers and HSC were suggested to derive from the bone marrow [4,5]. Moreover, the stem cell markers nestin, CD105, p75 neurotrophin receptor (p75NTR), and c-kit ligand were detected in HSC [6–9]. These findings prompted us to test the hypothesis of whether HSC are undifferentiated stem or progenitor cells. In the course of our studies it turned out that HSC express CD133 (AC133/prominin 1). The cell surface protein CD133 is characteristic for stem/progenitor cells derived from the hematopoietic system and was used here to allow separation and description of a defined HSC population. The selected CD133$^+$ HSC expressed a variety of stem/progenitor cell markers and displayed the capacity to develop into myofibroblast-like, endothelial-like, and hepatocyte-like cells *in vitro*.

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Materials and methods

Cell isolation and selection. Stellate cells were obtained from the liver of male Wistar rats (500–600 g body weight). HSCs were isolated according to a standard protocol using enzymatic digestion of liver tissue and enriched by a single density gradient (8% Nycodenz, Axis-Shield, Oslo, Norway) essentially as described earlier [1]. Obtained HSC were suspended in 10 ml Hanks' balanced salt solution containing 0.25% bovine serum albumin and 50 μg/ml DNase I. Unspecific binding of primary antibody was blocked with immunoglobulins against CD16 and CD32 (1 μg per million cells). Selection of cells was achieved using 3 μg/ml of a biotinylated antibody against CD133 and the EasySep cell separation kit (StemCell Technologies, Vancouver, Canada) according to the manufacturer's recommendations. All antibodies used for cell selection are listed in Supplemental Table S1.

Cultivation and treatment of cells. Selected cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 20 ng/ml platelet-derived growth factor (PDGF-BB) and 10% fetal calf serum (FCS) on plastic in order to facilitate transformation of cells into myofibroblast-like cells. Iscove's modified Dulbecco's medium (IMDM) supplemented with 50 ng/ml vascular endothelial growth factor ($VEGF_{164}$; R&D Systems, Minneapolis, MN, USA), 20 ng/ml basic fibroblast growth factor (bFGF), 10 ng/ml erythropoietin (EPO), 10 ng/ml interleukin-6 (IL-6; Sigma, Taufkirchen, Germany), and 15% FCS (FCS for endothelial cell differentiation; StemCell Technologies) was used to promote differentiation of selected cells into endothelial-like cells. For induction of cell differentiation into hepatocyte-like cells freshly isolated $CD133^+$ HSC were exposed for an initial period of 3 days to IMDM containing 50 ng/ml FGF_4 (R&D Systems), 50 ng/ml bFGF, 40 ng/ml hepatocyte growth factor (HGF; Sigma), 10 ng/ml IL-6, and 10% FCS. The medium was thereafter changed to IMDM containing 50 ng/ml FGF_4 and 5% FCS. All media were exchanged daily. For cell differentiation into endothelial or hepatocyte lineages the $CD133^+$ HSC were cultured on a thick layer of self made collagen (type 1) from rat tail. To investigate the cellular uptake of fluorescent acetylated low density lipoprotein (DIL-AC-LDL; Biogenesis, Poole, UK) the cultured cells were treated for 4 h with 2.5 μg/ml DIL-AC-LDL at 37 °C.

Analysis of cells. Freshly isolated and cultured $CD133^+$ HSC were analysed by immunofluorescence staining and Western blot according to standard protocols. The primary and secondary antibodies used are listed in Supplemental Table S1. Actin filaments of HSC were stained by phalloidin-TRITC (Sigma) and the cell nuclei were marked with DAPI (4′,6-diamino-2-phenylindole) by covering of cells with ProLong Gold (Molecular Probes, Invitrogen, Karlsruhe, Germany). Fluorescence images were taken by a confocal laser scanning microscope. The retinol and retinyl palmitate content of selected cells was measured by high-performance liquid chromatography (HPLC) essentially as described elsewhere [10]. The gene expression pattern of $CD133^+$ HSC was investigated by reverse transcriptase-polymerase chain reaction (RT-PCR). Five nanograms of purified mRNA per 20 μl reaction volume was used for synthesis of the first strand cDNA. The PCR was performed according to standard procedures. All PCR products obtained were sequenced to prove specificity of primer sets (Supplemental Table S2). A full view on agarose gels of PCR-products and comparisons of gene expressions is given in Supplemental Figs. S1–S3.

Results

HSC express CD133: selection and characterization of $CD133^+$ HSC

The stem/progenitor cell marker CD133 was detected in HSC, which were isolated from rat liver and enriched by a density gradient. Specific antibodies against CD133 were used to select a defined population of enriched HSC by magnetic cell sorting. About 20–40% of the HSC was separated by this method. Separated cells displayed stellate-like morphology with lipid droplets and synthesized CD133 protein as well as the typical HSC marker proteins desmin and GFAP as shown by immunofluorescence, Western blot, and RT-PCR (Fig. 1A–G). The lipid inclusions of selected cells were analysed by HPLC and low values of retinol, but high amounts of retinyl palmitate were measured (Fig. 1H). The purity of separated cells was tested by RT-PCR. No typical marker proteins of other liver cell types were found in $CD133^+$ stellate cells. The cells were negative for α-SMA, elastin, endothelial nitric oxide synthase (eNOS), vaso-endothelial cadherin (ve-CAD), stabilin 2, scavenger receptor F1 (SCARF1), C-type lectin 13, α-fetoprotein (α-FP), and albumin (Supplemental Fig. S1).

Stem/progenitor cell markers in $CD133^+$ HSC

Selected $CD133^+$ HSC displayed marker proteins of pluripotent stem cells like OCT4 and nanog as demonstrated by immunofluorescence. OCT4 was stained predominantly in the nucleus of all selected cells (Fig. 2A). Nanog was also detected in each $CD133^+$ HSC, but this transcription factor displayed no or only weak nuclear staining (Fig. 2B). The occurrence of both proteins was verified by Western blot analysis (Fig. 3A and B). Other proteins known to be synthesized by stem/progenitor cells such as c-kit, paired-like homeodomain transcription factor 2 isoform c (PITX2c), musashi 1 (MSI1), and CXCR4 were also investigated by immunofluorescence in $CD133^+$ HSC (Fig. 2C–F). PITX2c and MSI1 were stained predominantly in the cell nuclei (Fig. 2D and E). The presence of PITX2c and THY1 was confirmed by Western blot (Fig. 3C and D).

These and additional stem/progenitor cell markers were also investigated by RT-PCR in freshly isolated $CD133^+$ HSC. The mRNAs of OCT3/4, c-kit, c-kit ligand, PITX2c, THY1, FMS-like tyrosine kinase 3 (FLT3), FLT3 ligand, stem cell growth factor (SCGF), breast cancer resistance protein 1 (BCRP1), Slain1, nucleostemin, nestin, p75NTR, wingless type 9b (WNT9b), and MSI1 were detected by this method, whereas nanog mRNA was not found (Fig. 3E).

$CD133^+$ HSC express markers of EPC and monocytes

We detected the mRNA of proteins characteristic for EPC such as fetal like kinase 1 (FLK1), FLT1, angiopoietin receptor 1 precursor (TIE2), runt-related transcription factor 1 (RUNX1), CD105, bone morphogenetic protein-binding endothelial regulator (BMPER), CD31, and von Willebrand factor (vWF; Fig. 3F). In addition to EPC markers, $CD133^+$ HSC were found to synthesize mRNA characteristic of cells derived from the hematopoietic system. CXCR4 and the monocyte marker CD14 were detected at the mRNA and protein level (Figs. 2F and 3G and H).

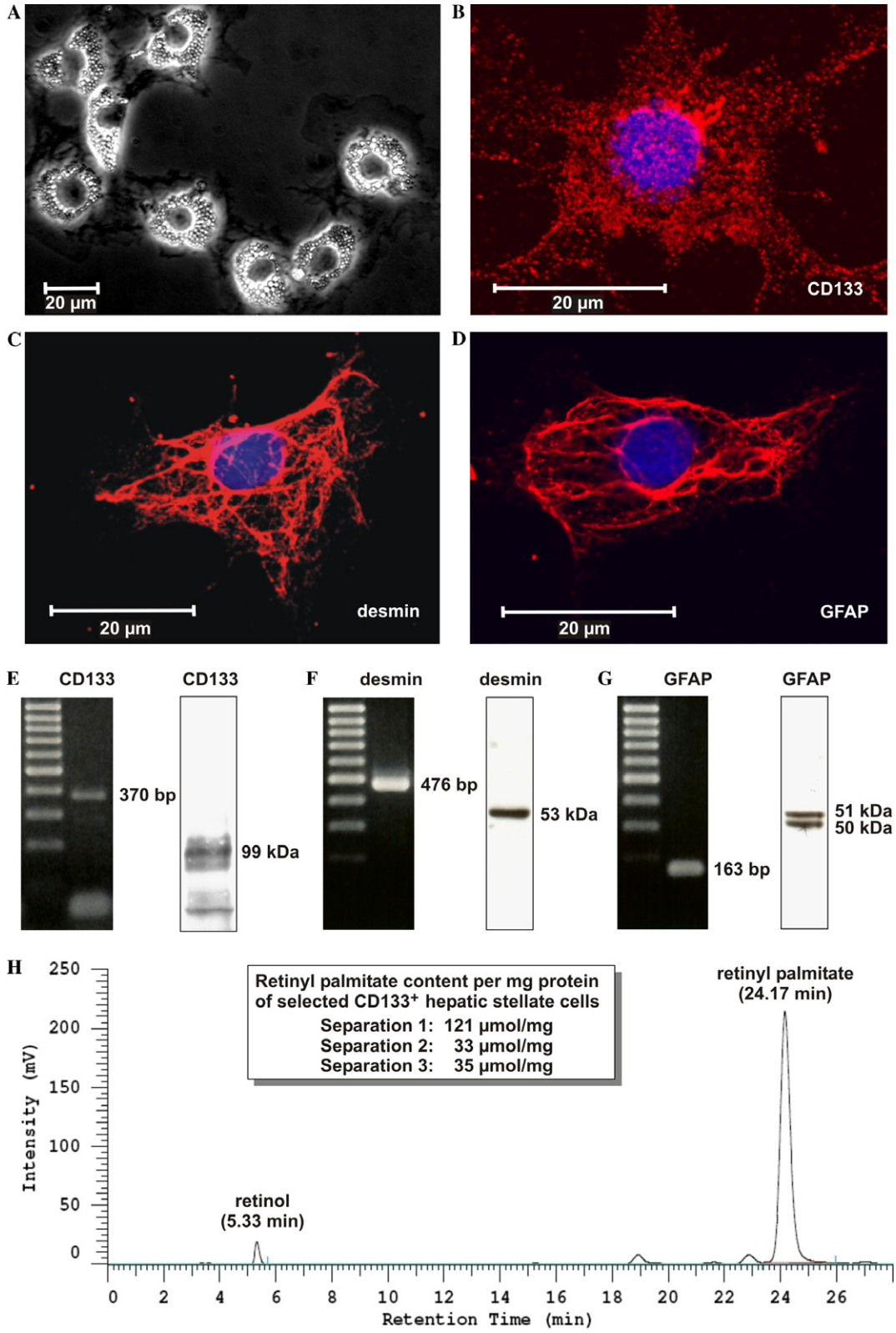

Fig. 1. HSC express CD133 and display typical properties of stellate cells. $CD133^+$ HSC were analysed after magnetic cell sorting (A–H). The cells were cultured on glass coverslips for 24 h (A–D). Phase contrast microscopic image of $CD133^+$ HSC (A). Immunofluorescence staining (red) of CD133 (B), desmin (C), and GFAP (D). Cell nuclei were marked by DAPI (blue, B–D). $CD133^+$ HSC were lysed for RT-PCR and Western blot directly after selection. mRNA (dark lanes) and protein samples (bright lanes) were analysed for CD133 (E), desmin (F), and GFAP (G). The retinol and retinyl palmitate content of freshly selected $CD133^+$ HSC were measured by HPLC (H). Retinyl palmitate values per mg protein of cell lysates of independent cell separations are displayed within the box.

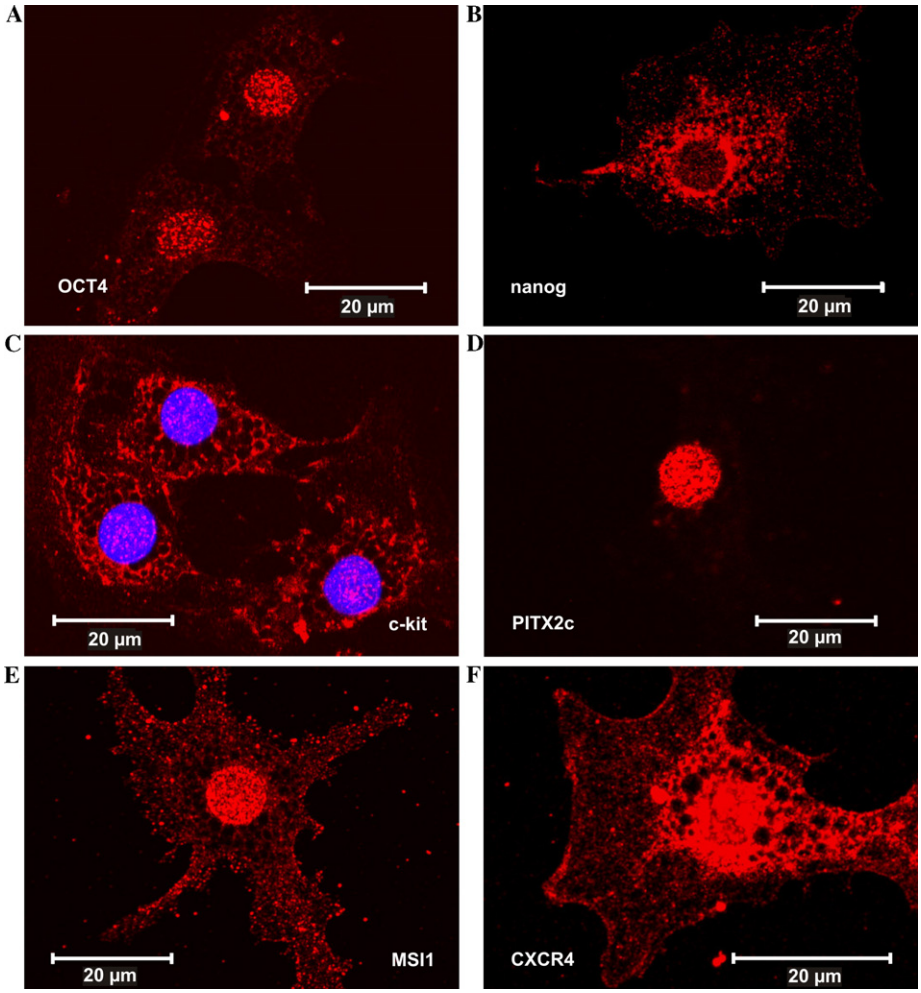

Fig. 2. Stem/progenitor cell marker proteins in CD133$^+$ HSC. Immunofluorescence stainings of CD133$^+$ HSC cultured for 24 h using antibodies against OCT4 (A), nanog (B), c-kit (C, cell nuclei were stained by DAPI), PITX2c (D), MSI1 (E), and CXCR4 (F).

Differentiation capacity of CD133$^+$ HSC in vitro

When CD133$^+$ HSC were cultured with medium containing PDGF-BB on plastic for 7 days, they transformed into myofibroblast-like cells. This development was accompanied by a gradual loss of lipid stores, an increase of cell size, and the onset of α-SMA synthesis (Fig. 4A–D). The expression of CD133 disappeared and FLK1 mRNA levels decreased under this culture condition. Marker proteins of mature endothelial cells (eNOS, ve-CAD) or heptocytes (α-fetoprotein/α-FP, albumin) were neither detected in freshly isolated CD133$^+$ HSC nor in myofibroblast-like cells (Fig. 4E).

Treatment of CD133$^+$ HSC grown on collagen with medium containing $VEGF_{164}$, bFGF, EPO, and IL-6, i.e. conditions known to promote differentiation of EPC to endothelial cells, induced the formation of tube-like structures within 7 days of culture. This process was initiated by a reduction of lipid inclusions, followed by proliferation and migration of cells and finally resulted in the development of branched tubular structures (Fig. 4F–I). The tubular structures displayed discontinuous cavities as shown by confocal laser scanning microscopy (Supplemental Fig. S4). These structures were not formed by other cell types such as isolated sinusoidal endothelial cells or fibroblasts, when treated with the same medium on collagen (Supplemental Fig. S5G and K). After tube formation by CD133$^+$ HSC we observed reduced, but still detectable CD133 expression, indicating that CD133$^+$ HSC maintained characteristics of undifferentiated cells during proliferation. The FLK1 mRNA synthesis declined only slightly under these culture conditions. Furthermore, this cytokine treatment induced the mRNA synthesis of eNOS and ve-CAD (Fig. 4J). The continuous FLK1 expression as well as the synthesis of eNOS and ve-CAD mRNA indicates that endothelial-like cells developed in this culture system. This view was supported by the induction of the stabilin 2 and SCARF1 mRNA (Supplemental Fig. S3Q1 and R1) and uptake of DIL-AC-LDL by cells surrounding tubular structures (Supplemental Fig. S5D). DIL-AC-LDL was not incorporated by freshly isolated CD133$^+$ HSC or myofibroblast-like cells

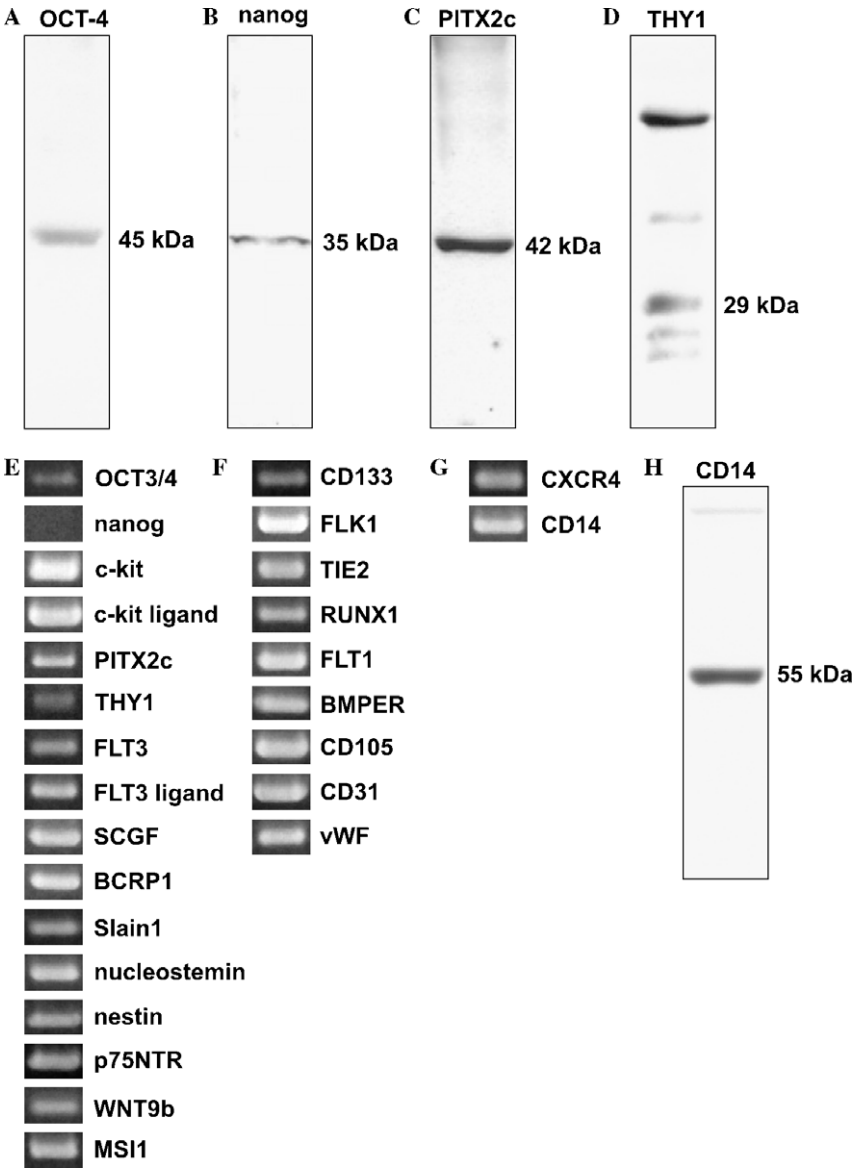

Fig. 3. Detection of marker proteins of stem/progenitor cells, EPC, and monocytes immediately after selection of $CD133^+$ HSC. The stem/progenitor cell markers OCT4 (A), nanog (B), PITX2c (C), and THY1 (D) were investigated by Western blot analysis. $CD133^+$ HSC were also analysed by RT-PCR for molecular markers of stem/progenitor cells (E), EPC (F), and monocytes (G). The monocyte marker CD14 was detected by Western blot (H).

(not shown). Very little or no expression of the myofibroblast marker α-SMA was observed after tube formation. Also markers of hepatocytes such as α-fetoprotein and albumin were not detectable (Fig. 4J).

$CD133^+$ HSC cultured on collagen were treated with FGF_4, HGF, bFGF, and IL-6 to investigate whether these cells can also differentiate into the hepatocyte lineage. Under these conditions, selected cells lost their lipid stores, started to proliferate, and between days 4 and 7 of cytokine treatment rotund cells appeared in the culture system (Fig. 4K–O). The cytokine cocktail induced the mRNA synthesis of hepatocyte markers like α-fetoprotein, albumin, and multidrug resistance protein 2 (MRP2; Fig. 4P; Supplemental Fig. S3). Rotund cells, approximately 25–50 μm in diameter, were removed from the cell culture to investigate their protein synthesis by immunofluorescence staining. The cells synthesized the hepatocyte marker α-fetoprotein (Fig. 4O), indicating that non-polarized hepatocyte-like cells developed from $CD133^+$ HSC. Under these culture conditions, the expression of CD133 and FLK1 decreased. The mRNA of molecular markers of endothelial cells or myofibroblasts was not induced. Interestingly, an expression of CD34 was not detected in freshly isolated $CD133^+$ HSC as investigated by RT-PCR (Fig. 4E, J, and P) and immunofluorescence staining (not shown), but increased under culture conditions favoring the development of myofibroblast-like, endothelial-like, and hepatocyte-like cells (Fig. 4E, J, and P).

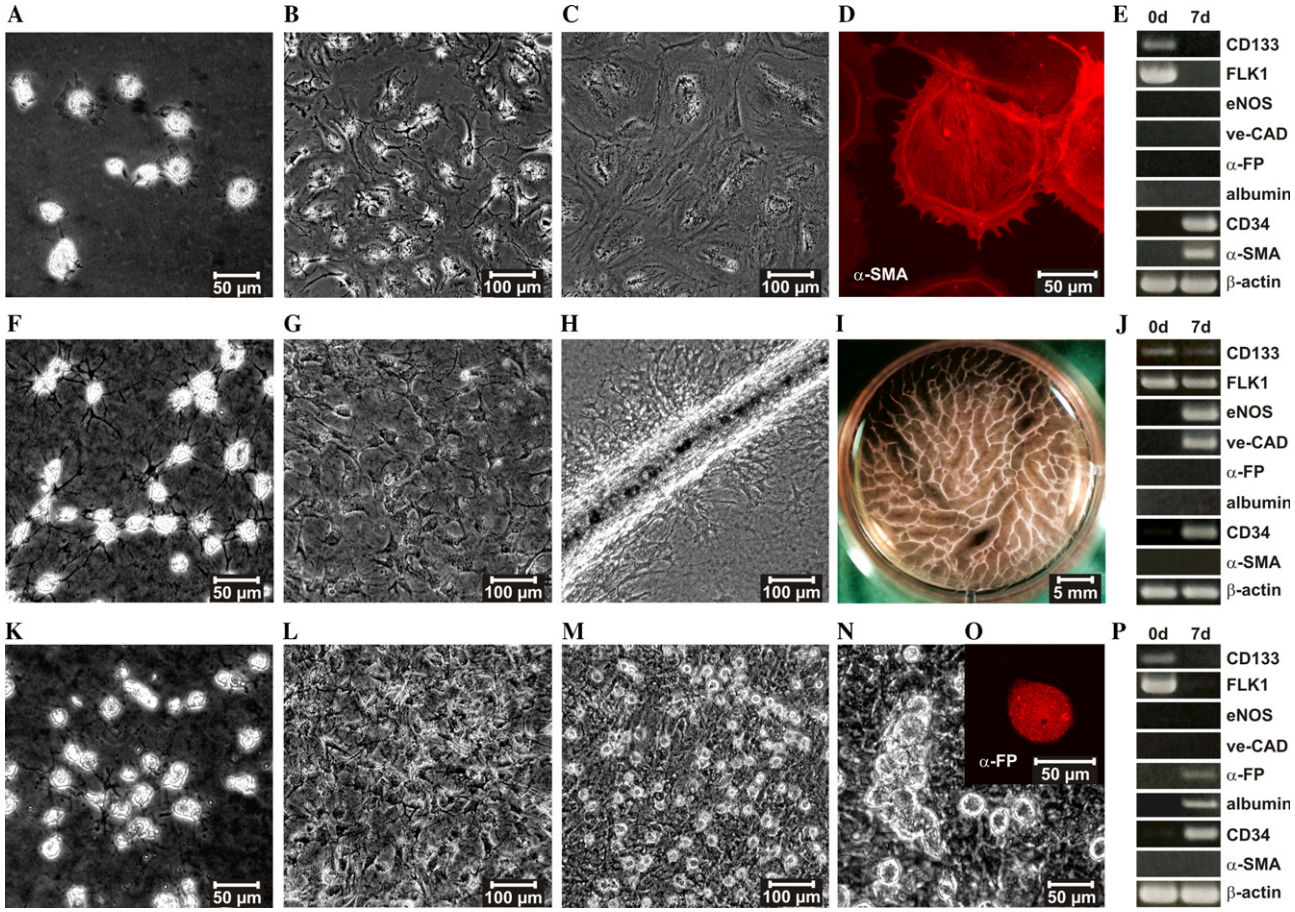

Fig. 4. Culture experiments with $CD133^+$ HSC to reveal their differentiation capacity. Selected HSC were cultured with medium supplemented with PDGF-BB to promote development of myofibroblast-like cells in plastic culture dishes (A–E). Phase contrast microscopic images of $CD133^+$ HSC after 1 (A), 3 (B), and 7 days (C) of culture. Immunofluorescence staining of α-SMA after 7 days of culture (D). $CD133^+$ HSC were lysed for RT-PCR analysis directly without culturing (0d) and after 7 days (7d) of cultivation (E). The selected HSC were also cultured with medium containing $VEGF_{164}$, bFGF, EPO, and IL-6 on collagen to facilitate EPC differentiation (F–J). Phase contrast microscopic images of $CD133^+$ HSC cultured for 1 (F), 3 (G), and 7 days (H). Macroscopic view of tube-like structures after 7 days of culture (I). RT-PCR analysis of freshly selected and cultured HSC after treatment with medium promoting EPC differentiation (J). $CD133^+$ HSC were cultured on collagen and treated with medium containing FGF_4, bFGF, HGF, and IL-6 to facilitate cell differentiation into hepatocytes (K–P). Phase contrast microscopic images of $CD133^+$ HSC were taken after 1 (K), 3 (L), and 7 days (M and N). The hepatocyte marker α-fetoprotein (α-FP) was detected by immunofluorescence staining in rotund cells (O). Changes in gene expression of $CD133^+$ HSC after treatment with FGF_4, bFGF, HGF, and IL-6 were analysed by RT-PCR in freshly isolated and cultured cells (P).

Discussion

$CD133^+$ HSC possess characteristics of typical stellate cells and EPC

The present study shows that stellate cells of the rat liver express the hematopoietic stem/progenitor cell marker CD133. $CD133^+$ HSC exhibit typical properties of stellate cells such as the characteristic cell morphology with perinuclear lipid droplets, high retinyl palmitate content, and synthesis of the HSC marker proteins desmin and GFAP. When $CD133^+$ HSC were cultured on plastic they transformed into myofibroblast-like cells as described in many studies using isolated HSC. However, stellate cells expressing CD133 displayed a variety of features also found in progenitor cells such as EPC. Bone marrow-derived early EPC are negative for molecular markers of more mature endothelial cells like ve-CAD and eNOS, but their expression appears during differentiation of EPC [11]. A similar situation was observed in freshly isolated $CD133^+$ HSC. These cells expressed markers of EPC, and proteins of mature endothelial cells (eNOS, ve-CAD, stabilin 2, SCARF1) were induced after application of cytokines which favor differentiation of EPC into endothelial cells. In line with this, features of mature endothelial cells like endocytosis of DIL-AC-LDL emerged during endothelial differentiation of $CD133^+$ HSC, indicating that functional endothelial-like cells developed from $CD133^+$ HSC. The formation of tube-like structures observed in cultured $CD133^+$ HSC is another feature pointing towards EPC [12]. The view that $CD133^+$ HSC possess characteristics of EPC was further strengthened by their CD14 expression. This monocyte marker was reported to occur in EPC with low abundance of CD34 [13]. Albeit CD34 was already

described for HSC of the human fetal liver [14], this well-established marker of hematopoietic cells and EPC was not expressed by freshly isolated $CD133^+$ HSC, but increased in cultured cells. At present, the relevance of elevated CD34 expression during cultivation of $CD133^+$ HSC is unknown.

Stem/progenitor cell markers in $CD133^+$ HSC

Apart from EPC characteristics, $CD133^+$ HSC expressed many genes commonly used to identify stem/progenitor cells. Some of them are new or less frequently used markers such as WNT9b, Slain1, nucleostemin, and PITX2c. WNT9b as well as MSI1 are expressed by stem cells in the crypts of the small intestine [15,16]. Slain1 and nucleostemin are newly discovered stem cell markers also found in pluripotent embryonic stem cells [17,18]. The PITX2c isoform appears in primitive hematopoietic stem/progenitor cells and its expression declines during their differentiation [19]. A similar behaviour was observed here in primary cultures of total HSC (not shown). $CD133^+$ HSC also show protein synthesis of OCT4 and nanog, markers formerly known to be expressed in embryonic stem cells. These markers were recently also described in EPC [13]. The mRNA of nanog was not detected in freshly isolated $CD133^+$ HSC, but the occurrence of the protein nanog was verified by Western blot analysis and immunofluorescent cell staining. In contrast to OCT4, the homeobox transcription factor nanog was not clearly observed in the nuclei of $CD133^+$ HSC, suggesting a reduced function of nanog at this stage.

$CD133^+$ HSC and liver regeneration

Despite controversies regarding the quantitative contribution, stem/progenitor cells participate in liver regeneration and the question on the role of HSC in this process arises. The appearance of so-called ductular structures during renewal of liver tissue is thought to represent a stem cell response. It is at present speculative whether the observed formation of tube-like structures by $CD133^+$ HSC *in vitro* corresponds to the ductular structures *in vivo*, but transplanted EPC were reported to form tube-like structures in the injured liver [12]. Moreover, hematopoietic progenitor cells characterized by CD133, CD14, CD45, and CXCR4 appear in the peripheral blood of healthy humans after partial hepatectomy and these cells differentiate into hepatocytes *in vitro* [20]. Also $CD133^+$ HSC display the cell surface proteins CD14 and CXCR4, raising the question of whether $CD133^+$ HSC can differentiate into hepatocytes. In fact, after treatment of $CD133^+$ HSC with the cytokines FGF_4, HGF, bFGF, and IL-6 rotund cells appeared and typical hepatocyte markers such as α-fetoprotein, albumin, and MRP2 were induced, as demonstrated in the present study. This expression pattern and the non-polarized cell morphology indicates that immature hepatocyte-like cells developed from $CD133^+$ HSC. Due to their capacity to generate cells of hepatocyte and endothelial lineage, the possibility exists that $CD133^+$ HSC can directly support renewal of injured liver tissue. Taniguchi and colleagues [12] already demonstrated supportive effects of transplanted EPC in this process. It will be interesting to investigate the contribution of $CD133^+$ HSC to liver regeneration *in vivo*.

The identification of $CD133^+$ HSC as not yet recognized progenitor cells with characteristics of early EPC indicates that stellate cells fulfill functions beyond participation in liver fibrogenesis. However, HSC are rather exceptional progenitor cells due to their high retinyl palmitate loads. Retinoic acid was shown to induce neuronal and astrocyte markers such as GFAP in $CD133^+$ hematopoietic stem cells from human umbilical cord blood [21]. Therefore, retinoids may support the neuronal expression pattern of HSC. In addition, retinoic acid maintains mesenchymal stem cells in an undifferentiated state [22], this might be also the case in HSC. In line with this, application of retinol and retinoic acid can counteract stellate cell activation [23]. Nevertheless, the molecular mechanisms responsible for the maintenance of HSC in an undifferentiated state await further evaluation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.11.029.

References

[1] H.F.J. Hendriks, W.A. Verhoofstad, A. Brouwer, A.M. DeLeeuw, D.L. Knook, Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver, Exp. Cell Res. 160 (1985) 138–149.
[2] Y. Yokoi, T. Namihisa, H. Kuroda, I. Komatsu, A. Miyazaki, S. Watanabe, K. Usui, Immunocytochemical detection of desmin in fat-storing cells (Ito cells), Hepatology 4 (1984) 709–714.
[3] A. Gard, F. White, G. Dutton, Extra-neural glial fibrillary acidic protein (GFAP) immunoreactivity in perisinusoidal stellate cells of rat liver, J. Neuroimmunol. 8 (1985) 359–375.
[4] S. Baba, H. Fujii, T. Hirose, K. Yasuchika, H. Azuma, T. Hoppo, M. Naito, T. Machimoto, I. Ikai, Commitment of bone morrow cells to hepatic stellate cells in mouse, J. Hepatol. 40 (2004) 255–260.
[5] F.P. Russo, M.R. Alison, B.W. Bigger, E. Amofah, A. Florou, F. Amin, G. Bou-Gharios, R. Jeffery, J.P. Iredale, S.J. Forbes, The bone marrow functionally contributes to liver fibrosis, Gastroenterology 130 (2006) 1807–1821.
[6] T. Niki, M. Pekny, K. Hellemans, P. De Bleser, K. Van Den Berg, F. Vaeyens, E. Quartier, F. Schutt, A. Geerts, Class VI intermediate

filament protein nestin is induced during activation of rat hepatic stellate cells, Hepatology 29 (1999) 520–527.
[7] N. Trim, S. Morgan, M. Evans, R. Issa, D.R. Fine, S. Afford, B. Wilkins, J.P. Iredale, Hepatic stellate cells express the low affinity nerve growth factor receptor p75 and undergo apoptosis in response to nerve growth factor stimulation, Am. J. Pathol. 156 (2000) 1235–1243.
[8] S.K. Meurer, L. Tihaa, B. Lahme, A.M. Gressner, R. Weiskirchen, Identification of endoglin in rat hepatic stellate cells, J. Biol. Chem. 280 (2005) 3078–3087.
[9] K. Fujio, R.P. Evarts, Z. Hu, E.R. Marsden, S.S. Thorgeirsson, Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat, Lab. Invest. 70 (1994) 511–516.
[10] W. Stahl, A.R. Sundquist, M. Hanusch, W. Schwarz, H. Sies, Separation of beta-carotene and lycopene geometrical isomers in biological samples, Clin. Chem. 39 (1993) 810–814.
[11] M. Hristov, C. Weber, Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance, J. Cell. Mol. Med. 8 (2004) 498–508.
[12] E. Taniguchi, M. Kin, T. Torimura, T. Nakamura, H. Kumemura, S. Hanada, T. Hisamoto, T. Yoshida, T. Kawaguchi, S. Baba, M. Maeyama, H. Koga, M. Harada, R. Kumashiro, T. Ueno, S. Mizuno, H. Ikeda, T. Imaizumi, T. Murohara, M. Sata, Endothelial progenitor cell transplantation improves the survival following liver injury in mice, Gastroenterology 130 (2006) 521–531.
[13] P. Romagnani, F. Annunziato, F. Liotta, E. Lazzeri, B. Mazzinghi, F. Frosali, L. Cosmi, L. Maggi, L. Lasagni, A. Scheffold, M. Kruger, S. Dimmeler, F. Marra, G. Gensini, E. Maggi, S. Romagnani, CD14 + CD34 low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors, Circ. Res. 97 (2005) 314–322.
[14] D.L. Suskind, M.O. Muench, Searching for common stem cells of the hepatic and hematopoietic systems in the human fetal liver: CD34+ cytokeratin 7/8+ cells express markers for stellate cells, J. Hepatol. 40 (2004) 261–268.
[15] A. Gregorieff, D. Pinto, H. Begthel, O. Destrée, M. Kielman, H. Clevers, Expression pattern of Wnt signaling components in the adult intestine, Gastroenterology 129 (2005) 626–638.
[16] T. Kayahara, M. Sawada, S. Takaishi, H. Fukui, H. Seno, H. Fukuzawa, K. Suzuki, H. Hiai, R. Kageyama, H. Okano, T. Chiba, Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine, FEBS Lett. 535 (2003) 131–135.
[17] R.Y.L. Tsai, R.D.G. McKay, A nuclear mechanism controlling cell proliferation in stem cells and cancer cells, Genes Dev. 16 (2002) 2991–3003.
[18] C.E. Hirst, E.S. Ng, L. Azzola, A.K. Voss, T. Thomas, E.G. Stanley, A.G. Elefanty, Transcriptional profiling of mouse and human ES cells identifies SLAIN1, a novel stem cell gene, Dev. Biol. 293 (2006) 90–103.
[19] B.A. Degar, N. Baskaran, R. Hulspas, P.J. Quesenberry, S.M. Weissman, B.G. Forget, The homeodomain gene Pitx2 is expressed in primitive hematopoietic stem/progenitor cells but not in their differentiated progeny, Exp. Hematol. 29 (2001) 894–902.
[20] U.M. Gehling, M. Willems, M. Dandri, J. Petersen, M. Berna, M. Thill, T. Wulf, L. Müller, J.M. Pollock, K. Schlagner, C. Faltz, D.K. Hossfeld, X. Rogiers, Partial hepatectomy induces mobilization of a unique population of haematopoietic progenitor cells in human healthy liver donors, J. Hepatol. 43 (2005) 845–853.
[21] Y.K. Jang, J.J. Park, M.C. Lee, B.H. Yoon, Y.S. Yang, S.E. Yang, S.U. Kim, Retinoic acid-mediated induction of neurons and glial cells from human umbilical cord-derived hematopoietic stem cells, J. Neurosci. Res. 75 (2004) 573–584.
[22] I. de Hemptinne, C. Vermeiren, J.-M. Maloteaux, E. Hermans, Induction of glial glutamate transporters in adult mesenchymal stem cells, J. Neurochem. 91 (2004) 155–166.
[23] T. Sato, R. Kato, C.A. Tyson, Regulation of differentiated phenotype of rat hepatic lipocytes by retinoids in primary culture, Exp. Cell Res. 217 (1995) 72–83.