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Endomyocardial Biopsy Derived Adherent Proliferating Cells—A Potential Cell Source for Cardiac Tissue Engineering

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ABSTRACT

Heart diseases are a leading cause of morbidity and mortality. Cardiac stem cells (CSC) are considered as candidates for cardiac-directed cell therapies. However, clinical translation is hampered since their isolation and expansion is complex. We describe a population of human cardiac derived adherent proliferating (CAP) cells that can be reliably and efficiently isolated and expanded from endomyocardial biopsies (0.1 cm^3). Growth kinetics revealed a mean cell doubling time of 49.9 h and a high number of 2.54×10^7 cells in passage 3. Microarray analysis directed at investigating the gene expression profile of human CAP cells demonstrated the absence of the hematopoietic cell markers CD34 and CD45, and of CD90, which is expressed on mesenchymal stem cells (MSC) and fibroblasts. These data were confirmed by flow cytometry analysis. CAP cells could not be differentiated into adipocytes, osteoblasts, chondrocytes, or myoblasts, demonstrating the absence of multilineage potential. Moreover, despite the expression of heart muscle markers like α-sarcomeric actin and cardiac myosin, CAP cells cannot be differentiated into cardiomyocytes. Regarding functionality, CAP cells were especially positive for many genes involved in angiogenesis like *angiopoietin-1*, *VEGF*, *KDR*, and *neuropilins*. Globally, principal component and hierarchical clustering analysis and comparison with microarray data from many undifferentiated and differentiated reference cell types, revealed a unique identity of CAP cells. In conclusion, we have identified a unique cardiac tissue derived cell type that can be isolated and expanded from endomyocardial biopsies and which presents a potential cell source for cardiac repair. Results indicate that these cells rather support angiogenesis than cardiomyocyte differentiation. J. Cell. Biochem. 109: 564–575, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: ENDOMYOCARDIAL BIOPSIES; CARDIAC DERIVED CELLS; GENOME-WIDE MICROARRAY; CELL-BASED THERAPY; TISSUE ENGINEERING

Cardiac diseases are the leading cause of morbidity and mortality in the western world. The research field for heart cell therapies has greatly expanded in the past few years [Beltrami et al., 2001; Quaini et al., 2002] and the concept has been changed. The new exiting paradigm for treating cardiovascular diseases is not only preventing the progression, but also reversing the disease process by enhancing repair and regeneration of damaged tissue [Jolicoeur et al., 2007]. Healing heart failure with cell-based therapies is promising to realize this concept. In this regard cell transplantation into the damaged myocardium for heart regeneration has received extensive attention.

A wide range of different cell types have been tested for cardiac cell therapies, including cells from cardiac tissue (cardiac stem cells, CSC) and from other tissues like bone marrow derived hematopoietic stem cells (HSC), mesenchymal stem cells (MSC), or skeletal myoblasts. First cell therapy trials were initiated using skeletal myoblasts, isolated from muscle biopsies and injected into the myocardium during open-heart surgery or by injection catheter. In

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animal models and in a pilot clinical trial skeletal myoblasts improved heart function [Menasche et al., 2001]. Unfortunately, myoblast transplantation often causes life-threatening arrhythmias [Leobon et al., 2003; Menasche et al., 2003]. Therefore, adult stem and progenitor cells may be more suitable for regenerative medicine [Hirschi and Goodell, 2002]. Bone marrow derived cells (HSC, endothelial progenitor cells (EPC), MSC, side populations (SP)) have been shown to express endothelial and/or cardiomyogenic markers [Jackson et al., 2001] and can profoundly increase functional recovery/improvement after myocardial infarction (MI) [Orlic et al., 2001; Kawamoto et al., 2003; Yeh et al., 2003]. As a further step to find the ''optimal'' cell for cardiac regeneration, different CSC have been identified and isolated by several groups in adult hearts of humans and rodents. These types of cells have the advantage to be cardiac-specific. Among these, cells expressing stem cell factor receptor c-Kit (CD117) [Beltrami et al., 2003], stem cell antigen-1 (Sca1) [Oh et al., 2003], and homeodomain transcription factor islet-1 (Isl1) [Laugwitz et al., 2005], SP cells [Pfister et al., 2005], and cells able to grow in cardiospheres [Messina et al., 2004] have been suggested to be capable of differentiation into cardiomyocytes, either in vivo or in vitro. Several studies are ongoing, but a clinically feasible method for isolating and expanding human CSC for application during an injury event is still lacking [Smith et al., 2008].

In view of clinical translation, cells for cardiac therapy should be relatively easy to isolate, to expand and should foster cardiac regeneration by either differentiating into cardiomyocytes/endothelial cells or by providing an appropriate environment for other cells to do so [Shabbir et al., 2009]. In addition, we strongly believe that cells directly isolated from cardiac biopsies present the best cell source since these cells are already primed by their environment.

In this study we describe for the first time the isolation and extensive expansion of a unique human cardiac derived adherent proliferating cell type (CAP cells) and characterize these cells in order to determine their cardiac regeneration potential and to classify these cells in the context of other stem and differentiated cells. Therefore, we analyzed their growth kinetics, their expression profile using genome-wide microarrays, FACS analysis, immunohistochemistry, and their multilineage differentiation potential. These data were compared with own and literature data for other cell types. According to this initial report, human endomyocardial biopsy (EMB) derived CAP cells present a promising cell source for cardiac repair.

MATERIALS AND METHODS

CARDIAC BIOPSIES

Cardiac biopsies (0.1 cm^3) were obtained from 10 patients (37–67 years old, average age 48.6 years, 3 females, 7 males) undergoing EMB procedure via the femoral vein approach under biplane fluoroscopic control to evaluate unexplained left ventricular dysfunction. The biopsies were taken from the right ventricle side of the interventricular septum [Tschope et al., 2005]. The donation of cardiac tissue was approved by the ethical committee of the Charité-Universitätsmedizin Berlin (No. 225–07).

CAP CELL ISOLATION

Cardiac biopsies were washed with phosphate buffered saline (PBS; Biochrom, Berlin, Germany) and outgrowth cultures were performed in Iscovés medium (Biochrom) supplemented with 10% human allogeneic serum (German Red Cross, Berlin, Germany) and 1% penicillin/streptomycin (Biochrom). The biopsies were cultured under standard cell culture conditions and the medium was replaced every 2–3 days.

Outgrowing cells were subcultured by treatment with 0.05% trypsin/0.02% EDTA (Biochrom). For each individual cardiac biopsy, this harvesting procedure was repeated up to 4 times for newly outgrowing cells. Subsequently, the harvested CAP cells were replated in 9 cm^2 cell culture wells in culture medium consisting of equal amounts of Iscove's/DMEM/Ham's F12 medium (Biochrom) containing 5% human serum, 1% penicillin/streptomycin, 20 ng/ml basic fibroblast growth factor (bFGF; Peprotech, Hamburg, Germany), and 10 ng/ml epithelial growth factor (EGF; Peprotech). When reaching 80–90% confluence, the CAP cells were trypsinized and replated at a density of 6,000 cells/cm^2.

EVALUATION OF GROWTH CURVES

To determine their growth kinetics, CAP cells isolated from tissue samples derived from 3 different donors were cultured up to passage 5 or 7, respectively. When reaching 90% confluence, cell numbers were counted. Since not all harvested cells were used for the determination of growth kinetics, at different time points (t) the theoretical cell numbers (N) were calculated applying the equation $N = N_0 \times e^{\mu t}$ where N_0 represents N at $t = 0$. The cell doubling time (t_d) was calculated applying the equation $t_d = \ln 2/\mu$ where μ represents the growth rate.

RNA ISOLATION AND GENOME-WIDE CAP CELL MICROARRAYS

To obtain RNA for gene expression profiling, passage 3 CAP cells derived from 3 donors were trypsinized and 6,000 cells/cm^2 were plated in a 25 cm^2 cell culture flask. CAP cells were cultured until they reached 90% confluence. Total RNA was isolated as described before [Chomczynski, 1993], using TRI Reagent LS (Sigma–Aldrich, Taufkirchen, Germany). The RNA was controlled for integrity and purity with the Agilent Bioanalyzer and NanoDrop spectrophotometer.

RNA of the 3 individual donors was used for microarray analysis applying genome-wide HG-U133 plus 2.0 microarrays (Affymetrix, Santa Clara) according to Affymetrix recommendations. In brief, 2 μg of total RNA was used to synthesize biotin-labeled cRNA. Fragmented cRNA (10 μg) was hybridized to GeneChips for 16 h at 45°C. Washing, staining, and scanning of the GeneChips was performed applying the GeneArray scanner controlled by Affymetrix GeneChip operating software 1.4 (GCOS 1.4). Raw expression data were processed, normalized, and subsequently analyzed with the GCOS 1.4 software.

MICROARRAY DATA MINING AND STATISTICAL ANALYSIS

The expression profiles of human CAP cells were analyzed to obtain insights into their characteristics. The first aim was to investigate whether human CAP cells ($n = 3$ donors) express marker genes, which are described for distinct human stem cells and fibroblasts.

Thus, CAP cell genes whose expression was detected as 100% present or 100% absent were selected and compared with literature data reviewing marker genes for different cell types.

The second aim was to determine in which biological processes CAP cell genes are involved. Here we focused on processes that are of special interest for cell-based cardiac therapy. To further characterize differences between CAP cells, stem cells, and normal dermal human fibroblasts (GSE11418; see third aim), we concentrated on genes whose expression were detected as 100% present in CAP cells and/or in MSC and/or in fibroblasts. Selected processes were "angiogenesis" (gene ontology, GO 0001525), "heart-development" (GO 0007507), and "muscle development" (GO 0007517).

The third aim was to test for differences and similarities of CAP cells with human bone marrow derived MSC and to identify putative priming toward differentiated cell types present in cardiac tissue. Therefore, expression profiles of CAP cells and other reference cell types/tissues were compared to MSC. With exception of CAP cells and MSC, all profiles were available from the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database [Barrett et al., 2009]. These included profiles from periosteal progenitor cells, neonatal dermal fibroblasts, and adult dermal fibroblasts of passages 4 and 13 (GSE11418; GEO serious accession numbers), clones of human embryonic stem (ES) cells and human induced progenitor stem cells (GSE9865), neurogenic progenitor cells (GSE13307), human umbilical vein endothelial cells (HUVEC; GSE9677), human pulmonary artery endothelial cells (HPAEC; GSE4567), myocardial biopsies from heart transplant donors (GSE1145), skeletal muscle (GSE6798), and three different groups of bone marrow progenitor cells positive for CD235a, CD45, or CD11b (GSE9894). Profiles were analyzed using Affymetrix GCOS1.4 signal and pairwise comparison analysis. All data were integrated into the BioRetis database (http://www.bioretis-analysis.de) that evolved from the SiPaGene database [Menssen et al., 2009]. For each array, signals were normalized by quantiles according to a pre-defined standard. Group comparisons were performed and, for increased as well as decreased expression of each cell type compared to MSC, the top 50 candidate genes as determined by consistent regulation and magnitude of fold change were selected. This resulted in 806 different genes. For these genes, signals of all different cell types were log-transformed, z-normalized by gene, and used to perform principal component analysis (PCA) and hierarchical cluster analysis (HCA) using the software Genesis 1.7.2 [Sturn et al., 2002].

FLOW CYTOMETRY ANALYSIS

Following trypsin–EDTA treatment, single cell suspensions ($n = 3$ donors) of passage 3 CAP cells were washed with PBS/0.5% BSA. Fluorescein isothiocyanate (FITC) labeled mouse anti-human CD44, CD45, CD90, and R-phycoerythrin (PE) labeled mouse anti-human CD14, CD34, CD73, and CD166 were purchased from Pharmingen (Heidelberg, Germany), FITC-labeled mouse anti-human CD105 was purchased from Acris (Herford, Germany). For staining with FITC and PE coupled antibodies, 2.5×10^5 CAP cells were incubated in a single-step reaction for 15 min on ice. Prior to FACS analysis on a FACS Calibur cytometer (Becton Dickinson, Heidelberg, Germany), cell samples were washed. Apoptotic cells and cell debris were stained with propidium iodide (Sigma–Aldrich) and excluded. Unstained cells serve as negative control. Data were evaluated using CellQuest software (Becton Dickinson).

IMMUNOFLUORESCENCE STAINING OF BIOPSIES AND CELLS

Cardiac biopsies were embedded in TissueTec (Sakura Finetek, Torrance) and cryopreserved. Passage 2 CAP cells were seeded on Lab-Tek chamber slides (Nalgen, Roskilde, Denmark), fixed with 2% formaldehyde (Sigma–Aldrich) for 30 min and briefly rinsed with TBS (0.1 M Tris buffered saline, pH 7.6; Dako, Hamburg, Germany). Both, sliced cryosections and CAP cells on slides were permeabilized with 0.5% Triton X-100 (Sigma–Aldrich) in PBS for 15 min and subsequently washed 3 times with PBS. Following 60 min incubation with blocking buffer, samples were incubated with primary antibodies or IgG control antibodies (Dako) for 30 min at 37°C. The slides were extensively washed with TBS and fluorophore conjugated secondary antibodies were added for 30 min at 37°C. As primary antibodies mouse anti-human cardiac troponin T and myosin (Acris), rabbit anti-human connexin 43 (Novus Biologicals, Littleton), and mouse anti-human α-sarcomeric actin (Sigma–Aldrich) were used. As secondary antibodies goat anti-mouse cyanine 3 (Cy3; Dianova, Hamburg, Germany) or swine anti-rabbit FITC (Dako) were applied. For CD90 staining, directly conjugated rabbit anti-human CD90 FITC (Dianova) was used. After a further washing step, nuclei were stained with 0.2% bisbenzimide solution (Hoechst, Frankfurt a. M., Germany). Samples were covered with cytomation fluorescent mounting medium (Dako). Images were obtained on an Olympus CKX41 laser microscope (Olympus, Hamburg, Germany).

MULTILINEAGE DIFFERENTIATION ASSAYS

The multilineage differentiation potential of human CAP cells ($n = 3$, passage 4) was analyzed by applying slightly modified standard protocols often described for the successful differentiation of human MSC [Gimble et al., 1992; Jaiswal et al., 1997; Johnstone et al., 1998]. Briefly, for adipogenesis, 6,000 CAP cells/cm^2 were seeded. Five days after reaching confluence, CAP cells were treated for 3 days with induction medium consisting of DMEM (4.5 g/L glucose) supplemented with 10% human serum, 1 μM dexamethasone (Sigma–Aldrich), 0.2 mM indomethacin (Sigma–Aldrich), 10 μg/ml insulin (Actraphane, Novo Nordisk, Bagsvaerd, Denmark), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma–Aldrich), and then for 2 days with maintenance medium consisting of DMEM (4.5 g/L glucose), human serum, and 10 μg/ml insulin. This cycle of 3 days of induction and 2 days of maintenance was repeated for 3 times. Control cells received only maintenance medium. Osteogenesis was induced for up to 28 days in Iscoves's/DMEM/Ham's F12 medium supplemented with 1% human serum, 100 nM dexamethasone, 10 mM β-glycerolphosphate (Sigma–Aldrich), and 0.05 mM L-ascorbic acid 2-phosphate (AsAP; Sigma–Aldrich). Control cells were cultured in medium without the last three substances. To form high-density micromass cultures for chondrogenic induction, 2×10^5 CAP cells were centrifuged and subsequently cultured for up to 28 days in a defined medium consisting of DMEM (4.5 g/L glucose), ITS+1 (Sigma–Aldrich), 100 nM dexamethasone, 0.17 mM AsAP, 1 mM sodium pyruvate (Sigma–Aldrich), 0.35 mM L-proline

(Sigma–Aldrich), and 10 ng/ml transforming growth factor-β3 (TGFβ3; R&D Systems, Wiesbaden, Germany). Controls were cultured without TGFβ3. For myogenic differentiation 6,000 CAP cells/cm^2 were seeded and cultured in complete Iscove's medium. After reaching 90% confluence, cells were treated for 48 h with 10 μmol/L 5-azacytidine (Sigma–Aldrich) and then cultured for 4 weeks in complete Iscove's medium.

HISTOLOGICAL METHODS AND IMMUNOHISTOCHEMISTRY

To analyze adipogenic differentiation, Oil Red O (Sigma–Aldrich) staining of lipid droplets was performed. To study osteogenesis, visualization of alkaline phosphatase activity with Sigma fast BCIP/NBT (Sigma–Aldrich) and von Kossa staining (Sigma–Aldrich) of a bone-specific mineralized matrix, were carried out. Chondrogenesis was analyzed by staining of cartilage proteoglycans with Alcian blue 8GX (Roth, Karlsruhe, Germany) counterstained with nuclear fast red (Sigma–Aldrich), and by immunohistochemistry using the EnVision + System, Peroxidase rabbit kit AEC (Dako). Cryosections (6 μm) were incubated for 1 h with primary rabbit anti-human type I and type II collagen antibodies (Acris). Subsequently, samples were treated according to the manufacturer's protocol and counterstained with hematoxylin (Merck, Darmstadt, Germany). Myogenesis was analyzed by the formation of elongated and polynucleated myocytes and immunohistochemistry using the EnVision + System, System, peroxidase mouse kit AEC (Dako), and a primary mouse-anti human myosin heavy chain antibody.

RESULTS

ISOLATION AND GROWTH CURVES OF HUMAN CAP CELLS

Human CAP cells were isolated from cardiac biopsies (0.1 cm^3) of the right ventricle side of the interventricular septum and expanded in standard cell culture medium containing allogeneic human serum, bFGF, and EGF. In our outgrowth culture system, first outgrowing adherent cells were detected on days 3–5 showing a fibroblast-like cell morphology (Fig. 1A). After about 13 days the first cells were harvested (harvest 1; H1) and subcultured (Fig. 1B–D). During cell culture up to passage 3 CAP cells stretched and presented a stable fibroblast-like phenotype (Fig. 1D).

After 17 days, a second harvest (H2) of outgrowing cells from the same cardiac biopsy was performed. Overall, cells were harvested 4 times (H3: about day 25, H4: about day 34). Independent of the harvest, cells showed a similar morphology and growth kinetics (Fig. 2A; H1–H4, n = 3 donors, 5 passages). From passages 1–4, cells showed an average doubling time (t_d) of 49.9 h (H1: 49.5 h, H2: 40.9 h, H3: 57.5 h, H4: 51.6 h). A mean cell number of 4 harvests (H1–H4) was calculated for 3 donors. Single primary cultures showed a low number of cells in P1 and were expanded up to a maximum of 2.93×10^7 cells in P3 (mean of n = 3: 2.54×10^7), and 5.86×10^8 cells in P5 (mean of n = 3: 4.8×10^8, Fig. 2B). Even when examining only the first cell harvest (H1), high cell numbers could be achieved (Fig. 2C). In passage 3, roughly 39 days after EMB and also a time point until CAP cells from all 3 donors approximately showed exponential growth behavior, up to 4.93×10^7 cells

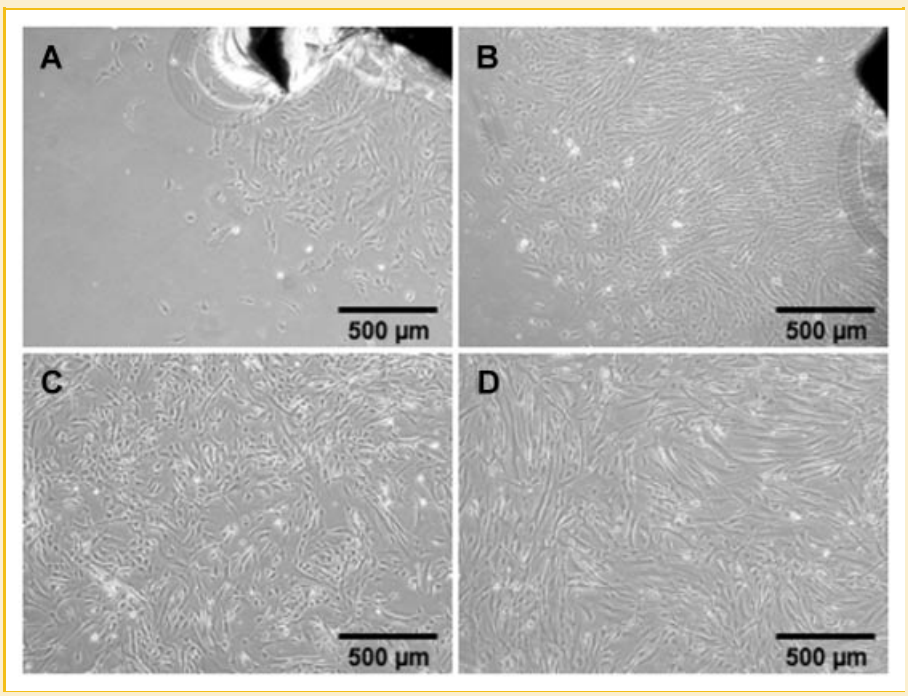

Fig. 1. Morphology of human CAP cells. Human cardiac derived adherent proliferating (CAP) cells were isolated from cardiac biopsies of the right ventricle side of the interventricular septum obtained by endomyocardial biopsy (EMB) procedure. A: On day 8, adherent cells with fibroblast-like morphology grew out from biopsies. B: After about 13 days CAP cells grew confluent and were harvested (H1). During subculture up to passage 1 (C) and passage 3 (D), CAP slightly cells stretched and presented a stable fibroblast-like phenotype.

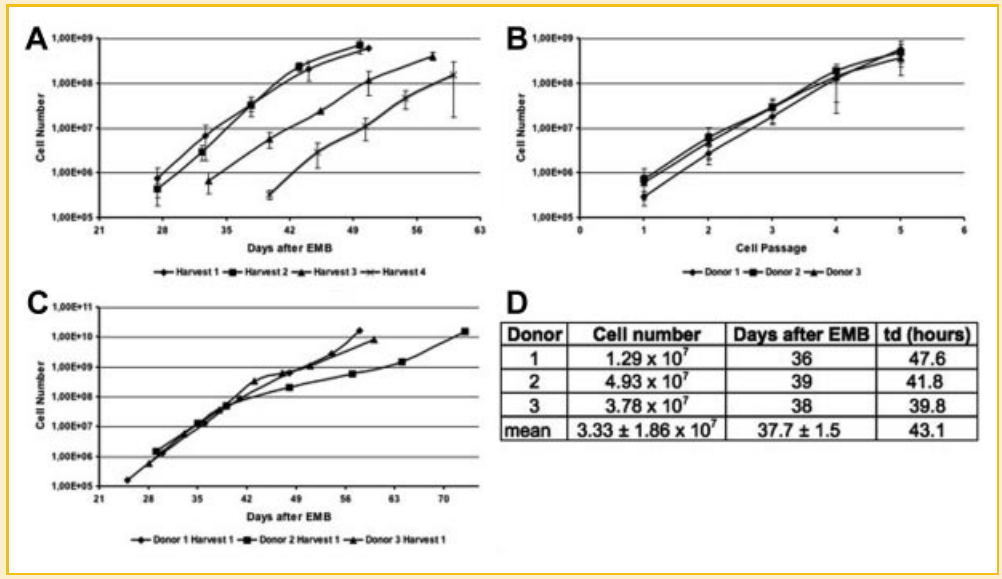

Fig. 2. Growth kinetics of human CAP cells. A: CAP cells derived from biopsies of three donors, four harvests per biopsy (H1–H4) and cultured for up to passage 5 showed comparable growth kinetics. H1 was performed on day 13, H2 on day 17, H3 on day 25, and H4 on day 34. Symbols reflect the end of each passage. The average doubling time (t_d) in passages 1–4 was 49.9 h. B: A mean value of four harvests was calculated on the basis of three donors. Single primary cultures were expanded resulting in up to 2.93×10^7 cells in P3 and 5.86×10^8 cells in P5. C: CAP cells from H1 of three donors were cultured up to passage 7 and showed a high expansion potential. D: At passage 3, a time point until CAP cells from all three donors approximately showed exponential growth behavior, the mean cell number was $3.33 \pm 1.86 \times 10^7$ and the mean doubling time 43.1 h.

(donor 2) were obtained. At that time the average cell number was $3.33 \pm 1.86 \times 10^7$ and the mean cell doubling time was 41.8 h (Fig. 2D).

Summarized, human CAP cells display a fibroblast-like morphology, and have a high proliferation capacity. Both features were often described for mesenchymal stem and progenitor cells but also for fibroblasts. Thus, we performed different further studies to characterize and classify this new cell type in more detail.

HUMAN CAP CELLS EXPRESS DISTINCT STEM CELL AND FIBROBLAST MARKERS BUT ARE PREDOMINANTLY CD90 NEGATIVE

Genome-wide Affymetrix HG-U133 plus 2.0 array analysis was performed (n = 3 donors) to investigate the gene expression profile of human CAP cells. On the basis of this profile, we investigated whether CAP cells express markers which according to published data are somehow or other related to different stem and progenitor cells like MSC, HSC, EPC or CSC, or to differentiated fibroblasts. Clearly, this part of the study was performed to get an overview, it was not intended to perform complex bioinformatics or statistics.

In summary, human CAP cells expressed several marker genes also known from human MSC (Table I). They expressed a broad pattern of genes coding for cell surface antigens such as the hyaluronan receptor CD44, the transferrin receptor CD71, the ecto-5′-nucleotidase CD73 (SH2), the TGFβ1 and TGFβ3 receptor endoglin (CD105, SH2), and the activated leukocyte cell adhesion molecule (ALCAM; CD166). However, we could not detect genes

TABLE I. Marker Gene Expression of Human CAP Cells (Microarray Data, n = 3 Donors)

Marker genes expressed by:	
Mesenchymal stem cells	
Present:	CD13, CD29, CD44, CD49a, CD71, CD105, CD166, gp130, CD73, ICAM1, ICAM3, IL1-R, ALCAM-1, LFA-3 (CD58), INFGR, TGFB1R, TGFB2R, bFGFR, EGFR, αSMA Collagen types I, III, IV, V, and VI, Fibronectin, Laminin
Absent:	CD90, VCAM1
Hematopoietic stem cells	
Present:	CDCP1
Absent:	CD45, CD133, CD14, CD34, CD11a, CD11b,
Endothelial progenitor cells	
Present:	CD31, VE-cadherin, VEGFR-2,
Absent:	vWF, CD133, CD14, CD45, HSPB2, CD34, CD146
Cardiac stem cells	
Present:	SSEA1, Sca1
Absent:	Isl1, Oct 3/4, MDR-1, Abcg2
Fibroblasts	
Present:	Vimentin, αSMA, FSP-1, DDR2, FAP, P4HA1, Col1A2
Absent:	CD90, Desmin, VCAM1

Further details of MSC, HSC, EPC, CSC, and fibroblast marker expression can be found for instance in the following references: *MSC*: [Pittenger et al., 1999; Barry and Murphy, 2004], *HSC*: [Wognum et al., 2003], *EPC*: [Middleton et al., 2004], *CSC*: [Goodell et al., 1996; Cai et al., 2003; Oh et al., 2003; Laugwitz et al., 2005], and *fibroblasts*: [Kalluri and Zeisberg, 2006; Flavell et al., 2008; Dudas et al., 2009; Porter and Turner, 2009].

encoding CD90 (Thy1) and the vascular cell adhesion molecule 1 such as the lipopolysaccharide receptor CD14, the leukocyte common antigen CD45, the early HSC marker CD34, CD11a (ITGAL; integrin αL), and CD11b (ITGAM; integrin αM). Human CAP cells expressed some but not all EPC markers. They expressed genes encoding the platelet/endothelial cell adhesion molecule CD31 (PECAM1), vascular endothelial cadherin (VE-cadherin), and the kinase insert domain receptor (KDR; also known as VEGFR2). In contrast, we could not detect genes encoding the von Willebrand factor (vWF), CD133, the melanoma cell adhesion molecule MCAM (CD146), the heat shock protein HSPB2, and as already mentioned, for CD14, CD34, and CD45. Regarding CSC markers, genes coding for stage-specific embryonic antigen 1 (SSEA1) and Sca1 were detected, whereas genes coding for transcription factors ISL LIM homeobox 1 (Isl1) and Oct3/4, and for Abcg2 (ATP-binding cassette, subfamily G (WHITE), member 2) and MDR1 were not detected.

Fibroblast marker genes encoding for Vimentin, α-smooth muscle actin (α-SMA), the fibroblast-specific protein (FSP1, also called S100A4), the discoidin domain receptor 2 (DDR2), the fibroblast activation protein (FAP), poly-4-hydroxylase (P4HA1), and pro-collagen1 α2 (Col1A2) were detected in CAP cell cultures. However, these markers are also expressed by MSC. Other genes coding for markers of fibroblasts like VCAM1, desmin, and CD90, expressed by MSC, could not be detected.

FLOW CYTOMETRY ANALYSIS OF CELL SURFACE MARKER PRESENTATION

One important microarray result was that human CAP cells express several MSC and fibroblast markers but at the gene expression level were CD90 negative. To verify these microarray data and to further characterize CAP cells with respect to their MSC/fibroblast resemblance, cells were flow cytometry analyzed (n = 3 donors) for the presentation of typical human MSC marker epitopes. Clearly, human CAP cells showed a homogeneous cell population (Fig. 3). They were uniformly positive for CD44 (Fig. 3A), CD73 (Fig. 3B), CD105 (Fig. 3C), and CD166 (Fig. 3D). In contrast, CAP cells were negative for CD14 (Fig. 3E), CD34 (Fig. 3F; 2% positive cells), and CD45 (Fig. 3G). Unstained cells serve as negative control (black line). Again, unlike MSC and fibroblasts, CAP cells were predominantly negative for CD90 (Fig. 3E; 2.1% positive cells).

IMMUNOHISTOCHEMICAL STAINING OF CARDIAC BIOPSIES AND CAP CELLS

To further analyze CD90 presentation, cryosections of cardiac biopsies and CAP cells (n = 3 donors) were stained with an FITC-labeled monoclonal CD90 antibody (green). Nuclei were stained with bisbenzimide solution (blue). As exemplary shown in Figure 4, all tested cardiac biopsies predominantly were CD90 positive (Fig. 4A) whereas all CAP cells were CD90 negative (Fig. 4B).

In addition, cardiac biopsies and cells were stained for distinct heart muscle markers. Biopsies were positive for all tested heart muscle markers, namely α-sarcomeric actin, cardiac troponin T, the gap junction protein connexin 43, and cardiac myosin (Suppl. Fig. 1). CAP cells were positive for α-sarcomeric actin and cardiac myosin (Suppl. Fig. 2). They were negative for the other markers (data not shown). No staining was detectable using an isotype control (data not shown).

CAP CELLS DO NOT SHOW A MULTILINEAGE DIFFERENTIATION POTENTIAL

The next consistent step to analyze CAP cells with respect to their MSC/fibroblast resemblance was to study the potential multilineage

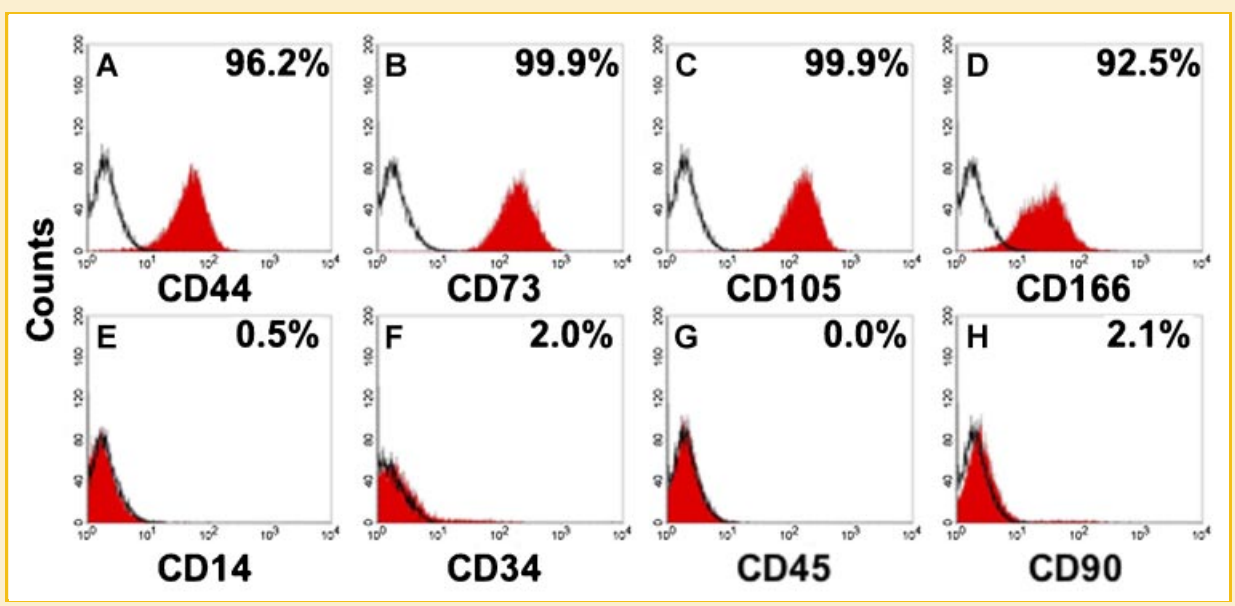

Fig. 3. Flow cytometric analysis of human CAP cells. FACS analysis of surface markers routinely used for the characterization of human bone marrow derived mesenchymal stem cells (MSC). The analysis demonstrated that human CAP cells were positive for reactivity to antigens (A) CD44, (B) CD73, (C) CD105, and (D) CD166 but negative for reactivity to antigens (E) CD14, (F) CD34, and (G) CD45. These results are in accordance with reactivity of MSC. H: Unlike MSC or fibroblasts, CAP cells were negative for CD90. Unstained cells serve as negative control (black line). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

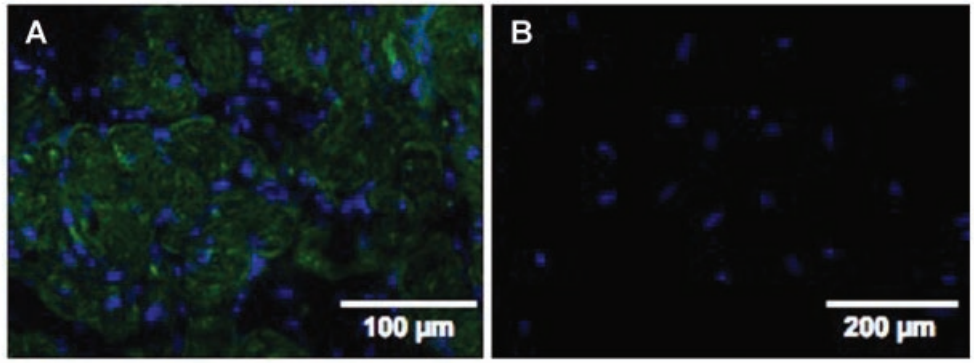

Fig. 4. Immunohistochemical CD90 staining of cardiac biopsies and CAP cells. To visualize CD90 presentation in human cardiac biopsies and CAP cells a FITC-labeled CD90 antibody (green staining) was used. (A) Human heart biopsies were CD90 positive (green) whereas (B) CAP cells were CD90 negative. Nuclei were stained with bisbenzimide solution (blue) and detected in both cardiac biopsies and CAP cells.

capacity of CAP cells. During adipogenesis, in insulin treated control cultures (Suppl. Fig. 3A) and also in adipogenic stimulated cultures (Suppl. Fig. 3D) the formation of a very small amount of Oil Red O stained lipid droplets were detected. A pronounced adipogenic development potential comparable to MSC was not observed. In osteogenic induced cultures, the activity of alkaline phosphatase was lower than in control cultures (Suppl. Fig. 3B,E). On day 28, a negative von Kossa staining of bone-specific mineralized extracellular matrix (Suppl. Fig. 3C,D) indicated that no mineralization/osteogenesis occurred. Following chondrogenic induction with TGFβ3, on day 28 Alcian blue staining demonstrated a very weak secretion of cartilage typical proteoglycans in control cultures (Suppl. Fig. 4A) and a more pronounced secretion in induced cultures (Suppl. Fig. 4B). Both controls and induced high-density mass cultures secreted type I collagen (a marker of undifferentiated cells) (Suppl. Fig. 4B,E). Even on day 28, cartilage-specific type II collagen was not detected indicating that no chondrogenic development occurred (Suppl. Fig. 4C,F). Regarding myogenic differentiation, no typical elongated or polynucleated cells, could be observed. Moreover, muscle myosin staining was negative (data not shown).

CAP CELLS EXPRESS IMPORTANT GENES INVOLVED IN ANGIOGENESIS, HEART AND MUSCLE DEVELOPMENT

As CAP cells showed no multilineage differentiation potential, we were interested in which biological processes relevant for cell-based heart therapy CAP cell genes are involved. To be consistent, we characterized differences between CAP cells, stem cells, and normal human dermal fibroblasts, and therefore concentrated on genes that were detected as 100% present in CAP cells and/or in MSC and/or in fibroblasts. This part of the study was performed to get an overview; complex bioinformatics or statistical analysis were not planned.

Here, Affymetrix microarray analysis showed that CAP cells expressed most of the genes involved in "angiogenesis" (GO 0001525). The expression of 36 angiogenesis-related genes was detected as 100% present either in CAP cells, MSC, or fibroblasts (Table II). From 36 genes, 31 of them were reproducible expressed in CAP cells. *CSPG4 (chondroitin sulfate proteoglycan 4)*, *FGF1 (fibroblast growth factor 1)*, and *EREG (epiregulin)* were expressed in two of three donors (67% detection) and *EPAS1* (endothelial *PAS domain protein 1*) in one of three donors (33% detection). Again, CAP cell expression of CD90 could not be detected. In MSC the expression of 27 genes related to angiogenesis revealed 100% detection, one gene was detected in two out of three donors (67%) and two genes in only one donor (33%). Expression of six genes was not detected. Normal dermal human fibroblasts expressed 24 angiogenesis genes in all three donors, eight genes were positive in only one donor, and expression of four genes was not detected.

Human CAP cells expressed *vascular endothelial growth factor (VEGF)*, *VEGFB*, *VEGFC*, *PIGF* (*phosphatidylinositol glycan anchor biosynthesis, class F*), and the *KDR* (*VEGFR2*) receptor. Moreover, the cell surface glycoprotein genes *NRP1* and *NRP2*, the AvB3 receptor gene (*ITGB3*, *integrin β3*), and *VE-cadherin* were also expressed: all genes are involved in angiogenesis [Veikkola et al., 2000]. *PIGF* expression was also 100% detected in MSC and fibroblasts, *VEGFB* was only 67% expressed in MSC and fibroblasts, and *ITGB3* expression 0% in MSC and 67% in fibroblasts, respectively.

Concerning "heart development" (GO 0007507), 18 genes were expressed in all three donors either in CAP cells, MSC, or fibroblasts (Table III). A reproducible (100%) expression was shown for 31 genes in human CAP cells, 12 genes were detected as present in all three MSC donors, and 16 in all fibroblast donors. The expression of 46 genes related to "muscle development" (GO 0007517) was 100% detected as present in CAP cells and/or MSC and/or fibroblasts (Table III). As also seen for "heart development" most of these genes (41) were 100% present in the CAP cell group. Twenty-four genes were reproducible expressed in the MSC group and 27 in the fibroblast group.

"INTEGRATED UNIQUENESS" OF CAP CELL GENOMIC PROFILES

To further test for similarities and differences of human CAP cells with human bone marrow MSC as well as to identify a putative priming toward differentiated cell types present in cardiac tissue, genes were selected for differential expression between MSC and CAP cells or MSC and any of the tested cell types. Selecting the 50 most significant genes from each comparison, a total of

TABLE II. Microarray Analysis of Genes Involved in Angiogenesis (GO 0001525)

Gene symbol	Gene Title	% CAP detection	% MSC detection	% NHDF detection
Angiogenesis (GO 0001525)				
AGGF1	Angiogenic factor with G patch and FHA domain 1	100	100	100
ANG	Angiogenin, ribonuclease, RNase A family	100	67	100
ANGPT1	Angiopoietin 1	100	100	100
ANGPTL4	Angiopoietin-like 4	100	100	33
ANPEP	Alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	100	100	100
APOLD1	Apolipoprotein L domain containing 1 /// apolipoprotein L domain containing 1	100	100	33
ARTS-1	Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator	100	100	100
CANX	Calnexin /// calnexin	100	100	100
COL15A1	Collagen, type XV, alpha 1	100	0	100
CSPG4	Chondroitin sulfate proteoglycan 4 (melanoma-associated)	67	0	100
EMCN	Endomucin	100	0	0
EPAS1	Endothelial PAS domain protein 1	33	100	100
EREG	Epiregulin	67	100	33
FGF1	Fibroblast growth factor 1 (acidic)	67	100	33
FGF2	Fibroblast growth factor 2 (basic)	100	100	100
HTATIP2	HIV-1 Tat interactive protein 2, 30 kDa	100	100	33
IL8	Interleukin 8	100	100	100
JAG1	Jagged 1 (Alagille syndrome)	100	100	100
KDR	Kinase insert domain receptor (a type III receptor tyrosine kinase)	100	0	0
MMP19	Matrix metallopeptidase 19	100	100	100
NARG1	NMDA receptor regulated 1	100	100	100
NRP1	Neuropilin 1	100	100	100
NRP2	Neuropilin 2	100	100	100
PGF	Placental growth factor, vascular endothelial growth factor related protein	100	100	100
RHOB	Ras homolog gene family, member B	100	100	100
RNASE4	Ribonuclease, RNase A family, 4	100	100	100
ROBO4	Roundabout homolog 4, magic roundabout (*Drosophila*)	100	0	0
SCG2	Secretogranin II (chromogranin C)	100	100	0
SHB	Src homology 2 domain containing adaptor protein B	100	0	33
TGFB2	Transforming growth factor, beta 2	100	33	33
THY1	Thy-1 cell surface antigen	0	100	100
TNFAIP2	Tumor necrosis factor, alpha-induced protein 2	100	33	100
TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	100	100	100
TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13 /// tumor necrosis factor (ligand) superfamily, member 12–member 13	100	100	33
VEGF	Vascular endothelial growth factor	100	100	100
VEGFC	Vascular endothelial growth factor C	100	100	100

Genes whose expression was detected as 100% present in CAP cells and/or MSC and/or normal donor human dermal fibroblasts. Human CAP cell and MSC profiles are own data; fibroblast profiles were available from the Gene Expression Omnibus (GEO) database. n = 3 donors.

806 different genes were identified and applied for PCA (Fig. 5) and HCA (Suppl. Fig. 5).

Both PCA and HCA demonstrated that CAP cells derived from three different donors form a separate cluster. Although with many overlapping profile components, CAP cells were more different from MSC than periosteal progenitor cells (PC) and dermal fibroblasts (Fb; adult (aFb) and neonatal (nFb)). PCA projected the clusters for embryonic progenitor cells, muscle tissue, and hematopoietic cells in different directions away from the MSC cluster. Reprogrammed induced progenitor cells were located between MSC and ES cells. Endothelial cells including HPAEC and HUVEC were also forming separate clusters that were close to the partially reprogrammed induced progenitors but shifting away toward the hematopoietic cluster. Neurogenic progenitors (VM, CTX) were close to the embryonic cluster, but clearly different from CAP cells as shown by the heat map of the HCA.

DISCUSSION

This study represents the initial step toward our overall aim to develop a cell-based regenerative medicine approach for cardiac regeneration. For the first time we report a unique population of CAP cells that can be reliably and efficiently isolated and expanded from human cardiac biopsies taken from the right ventricle side of the interventricular septum. In comprehensive studies we analyzed the potential cardiac regeneration capacity of CAP cells and classified these cells in the global context of other differentiated and stem cells. Briefly, genome-wide expression profiling of CAP cells demonstrated that the expression of their typical marker genes is also present in MSC, EPC, CSC, and fibroblasts but not in human hematopoietic (stem) cells. On the protein level, CAP cells presented MSC and also heart muscle markers. Strikingly, they were predominantly CD90 negative, which is a characteristic MSC and fibroblast marker, and showed no multilineage developmental potential. However, microarray data revealed the expression of most of the genes involved in angiogenesis. Finally, PCA and comparison with profiling data from several undifferentiated and differentiated reference cell types and tissues revealed a unique identity of CAP cells stated above.

Most of the routinely clinical applied regenerative medicine approaches like skin or cartilage repair base on the application of tissue inherent differentiated cells. Thus, it does not seem to be a mandatory provision to work with stem cells to induce repair or

TABLE III. Microarray Analysis of Genes Involved in Heart Development (GO 0007507) and Muscle Development (GO 0007517)

Gene symbol	Gene Title	% CAP detection	% MSC detection	% NHDF detection
Heart development (GO 0007507)				
CITED2	Cbp/p300-interacting transactivator	100	100	100
DVL1	Dishevelled, dsh homolog 1 (*Drosophila*)	100	100	100
DVL3	Dishevelled, dsh homolog 3 (*Drosophila*)	100	100	100
ECE2	Endothelin converting enzyme 2	100	67	100
ERBB2	v-erb-b2 Erythroblastic leukemia viral oncogene homolog 2	100	100	100
FBN1	Fibrillin 1	100	100	100
GATA4	GATA-binding protein 4	100	0	0
GJA1	Gap junction protein, alpha 1, 43 kDa (connexin 43)	100	100	100
MKKS	McKusick–Kaufman syndrome	100	67	100
NCOA6	Nuclear receptor co-activator 6	100	100	100
PDLIM5	PDZ and LIM domain 5	100	100	100
PLCE1	Phospholipase C, epsilon 1	33	100	100
PTEN	Phosphatase and tensin homolog	100	100	100
SHOX2	Short stature homeobox 2	0	0	100
SRI	Sorcin	100	100	100
TBX5	T-box 5	67	0	100
TCF25	Transcription factor 25 (basic helix–loop–helix)	100	100	100
TGFB2	Transforming growth factor, beta 2	100	33	33
Muscle development (GO 0007517)				
AEBP1	AE-binding protein 1	100	33	100
BVES	Blood vessel epicardial substance	100	100	100
CAPN3	Calpain 3 (p94)	100	0	100
COL6A3	Collagen, type VI, alpha 3	100	100	100
CSRP2	Cysteine and glycine-rich protein 2	100	100	100
DMD	Dystrophin	100	100	67
EGR3	Early growth response 3	100	100	33
EMD	Emerin (Emery-Dreifuss muscular dystrophy)	100	100	100
EVC	Ellis van Creveld syndrome	100	33	100
FCMD	Fukuyama type congenital muscular dystrophy (fukutin)	100	100	100
FGF2	Fibroblast growth factor 2 (basic)	100	100	100
FHL1	Four and a half LIM domains 1	100	100	100
FHL3	Four and a half LIM domains 3	100	33	67
GATA6	GATA-binding protein 6	100	100	33
HBEGF	Heparin-binding EGF-like growth factor	100	67	33
ITGA11	Integrin, alpha 11	100	100	100
ITGA7	Integrin, alpha 7	0	100	67
LAMA2	Laminin, alpha 2 (merosin, congenital muscular dystrophy)	100	0	100
MAPK12	Mitogen-activated protein kinase 12	100	67	33
MBNL1	Muscleblind-like (*Drosophila*)	100	100	100
MEF2A	MADS box transcription enhancer factor 2, polypeptide A	100	67	100
MEF2B	MADS box transcription enhancer factor 2, polypeptide B	100	67	33
MEF2C	MADS box transcription enhancer factor 2, polypeptide C	100	33	33
MEF2D	MADS box transcription enhancer factor 2, polypeptide D	100	0	33
MKX	Mohawk homeobox	0	0	100
MRAS	Muscle RAS oncogene homolog	100	67	33
MTM1	Myotubularin 1	100	67	33
MTSS1	Metastasis suppressor 1	100	100	100
MUSK	Muscle, skeletal, receptor tyrosine kinase	0	0	100
OAZ1	Ornithine decarboxylase antizyme	100	100	100
SGCB	Sarcoglycan, beta	100	100	100
SGCD	Sarcoglycan, delta	33	67	100
SGCE	Sarcoglycan, epsilon	100	100	100
SIX1	Sine oculis homeobox homolog 1 (*Drosophila*)	33	100	67
SMTN	Smoothelin	100	33	67
SPEG	SPEG complex locus	100	33	67
SRI	Sorcin	100	100	100
TAGLN	Transgelin	100	100	100
TAGLN2	Transgelin 2	100	100	100
TAGLN3	Transgelin 3	100	0	0
TCF12	Transcription factor 12	100	100	100
TEAD4	TEA domain family member 4	100	33	33
TK1	Thymidine kinase 1, soluble	100	67	33
TTN	Titin	100	0	100
UTRN	Utrophin (homologous to dystrophin)	100	100	67
VAMP5	Vesicle-associated membrane protein 5 (myobrevin)	100	100	100

Genes whose expression was detected as 100% present in CAP cells and/or MSC and/or normal donor human dermal fibroblasts. Human CAP cell and MSC profiles are own data; fibroblast profiles were available from the Gene Expression Omnibus (GEO) database. n = 3 donors.

regeneration. In view of clinical translation it is important that cells for cardiac therapy, no matter if CSC or differentiated cells, are easy to isolate and expand and that they foster cardiac repair. Cells isolated from cardiac biopsies seem to have an advantage over other cells since their environment primes them (cardiac-specific niche). Here, human CAP cells were reliably and efficiently isolated from cardiac biopsies. During cell expansion we obtained a mean cell number of $3.33 \pm 1.86 \times 10^7$ at passage 3 from one biopsy (0.1 cm^3) and first harvest. The EMB procedure allows sampling of up to seven biopsies, and therefore the possible cell number is very high. This

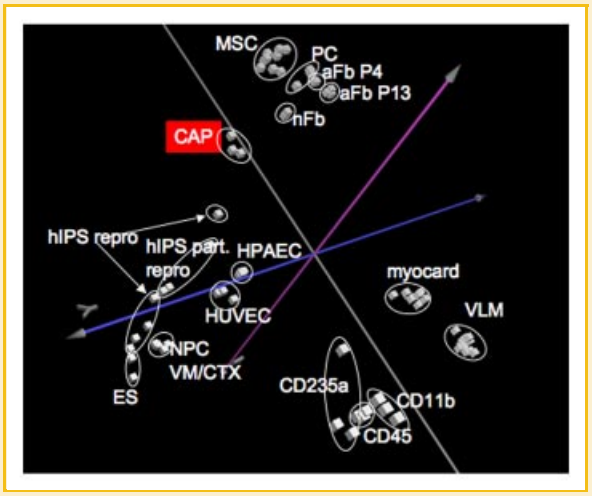

Fig. 5. Principal component analysis. The three-dimensional plot shows that human CAP cells from three different donors form a separate cluster, indicating similar characteristics in gene expression between the three donors. Compared to MSC, CAP cells were shifted toward the progenitor cluster, but not to the muscle or, the hematopoietic cluster. This suggests that CAP cells have their own molecular characteristics and preparation of this type of cells is reproducible. aFb P13 = adult dermal fibroblasts passage 13, aFb P4 = adult dermal fibroblasts passage 4, CD11b = myelocytic precursors from bone marrow, CD235a = erythrocytic precursors from bone marrow, CD45 = lymphocytic precursors from bone marrow, ES = human embryonic stem cells, hIPS part. repro = induced pluripotent stem cells partially reprogrammed, hIPS repro = induced pluripotent stem cells reprogrammed, HPAEC = human pulmonary artery endothelial cells, HUVEC = human umbilical vein endothelial cells, MSC = mesenchymal stem cells, myocard = left ventricular myocard, nFb = neonatal dermal fibroblasts, NPC = neurogenic progenitor cells from ventral midbrain (VM) or cortex (CTX), PC = periosteal cells, VLM = vastus lateralis muscle tissue. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

procedure has shown to be of very low risk for patients because surgery is only minimal invasive [Holzmann et al., 2008].

To classify CAP cells, we accomplished genome-wide gene expression profiling of these cells. Due to their fibroblast-like morphology, adherent growth and good expansion capacity, which are features already reported for human MSC and fibroblasts, we selected genes and compared with literature data. CAP cells showed no expression of typical hematopoietic cell surface antigens, verifying that these cells do not belong to the hematopoietic cell lineage. Furthermore, we analyzed CAP cells for their expression of marker genes that have already been described for different cell types residing in heart: endothelial cells, especially EPC, CSC, and fibroblasts. They were negative for the most prominent EPC marker genes but expressed *CD31*, *VE-cadherin*, and *VEGFR2*. CAP cells also expressed several fibroblast marker genes (derived from cardiac and other tissues). However, these "fibroblast markers" are not specific and some of them are also expressed for instance by MSC. The identification and isolation of most CSC cell types is based on surface markers presented by blood or bone marrow derived stem cells or by ES cells. Some ES marker genes were expressed by CAP cells, such as *SSEA1* and *Sca1*, but most of the genes were not (*Isl1*, *Oct3/4*, *Abcg2*, and *MDR1*). CAP cells expressed many genes already described as marker genes for human bone marrow derived MSC. This observation initially led us to the assumption that CAP cells represent mesenchymal progenitor cells. However, microarray analysis demonstrated that CAP cells were *VCAM1* negative and predominantly CD90 negative, both markers which are present on MSC [Pittenger et al., 1999]. In agreement with these differences, we could not induce CAP cells to differentiate into the adipogenic, osteogenic, or chondrogenic lineage using standard assays, a main characteristic of MSC [Pittenger et al., 1999; Dominici et al., 2006]. The small amount of lipid droplets in control and adipogenic cultures could be explained by a sensitivity of these cells to glucose or insulin, two supplements present in the basal and induction medium [Gimble et al., 1992]. During chondrogenic induction, Alcian blue staining revealed a very low secretion of acidic proteoglycans in control cultures and a low secretion in TGFβ3 induced cultures. Clearly, proteoglycan secretion is cartilage-typical but not specific. Since no formation of cartilage-specific type II collagen was detected, chondrogenesis could not be demonstrated [Johnstone et al., 1998]. Since MSC are defined by their multilineage potential human CAP cells cannot be classified as such cells.

In context of cardiac tissue repair, a differentiation potential of CAP cells to cardiomyocytes is of special interest. Our expression profiling data showed several genes expressed by CAP cells, which are involved in heart and muscle development. Nevertheless, applying standard protocols for the differentiation into muscle cells [Zuk et al., 2001], we could not induce myogenesis of CAP cells. Therefore, we have to assume that CAP cells do not develop into skeletal muscle cells or cardiomyocytes. Clearly, this will be analyzed in additional animal studies. Interestingly, CAP cells presented α-sarcomeric actin and cardiac myosin.

An alternative therapeutic effect of cell therapy is more and more discussed. Stem cells as well as differentiated cells can target diseased organs and secrete bioactive factors providing a regenerative environment, referred to as trophic activity, stimulating for instance mitosis and differentiation of tissue-intrinsic reparative or stem cells [Caplan and Dennis, 2006]. Shabbir et al. [2009] reported a heart failure therapy mediated by trophic activities of MSC. Cardiac fibroblasts are one important cell type in the heart. They play a key role in the normal myocardial function and in myocardial remodeling [Porter and Turner, 2009]. Most likely the fibroblast halfway mediate their effects via trophic effects and they also serve as tissue-intrinsic reparative cells targeted by other cell types.

Furthermore, our gene expression profiling data revealed the expression of a multitude of genes associated with angiogenesis. Angiogenic factors are one research focus targeting the treatment of cardiac diseases. Therapeutic angiogenesis may be a realistic approach in treating ischemic heart disease [Sim et al., 2002; Spillmann et al., 2006]. Among the genes involved in angiogenesis which were reproducibly expressed in CAP cells, there are promising regulators like *angiopoietin-1* (*Ang1*), *VEGF* and its receptor *KDR*, *NRP1*, *2*, and *fibroblast growth factor receptors-1*, *2* (FGF1, 2). Ang1 is essential in embryonic vasculogenesis and in adult angiogenesis [Novotny et al., 2009]. The ability of VEGF to promote angiogenesis and its interaction with the receptor KDR has been studied extensively [Schenone et al., 2007]. Furthermore, neuropilins

NRP1 and 2 are both receptors for various members of the VEGF family. Neuropilins represent multifunctional co-receptors for neuronal and cardiovascular development [Pellet-Many et al., 2008]. FGF1 and FGF2 are also known to play an important role in control of angiogenesis [Slavin, 1995] and are potent angiogenic inducers [Murakami et al., 2008]. Finally, based on an angiogenesis model reported by Veikkola et al. [2000], CAP cells were positive for many genes involved in this biological process. More detailed studies to investigate if these cells differentiate to endothelial cells or if they induce other cells to do this are in progress.

Finally, we tested for differences and similarities of CAP cells with undifferentiated MSC as well as if CAP cells express any functional profile components related to any differentiated cell type of cardiac tissue (muscle, blood, endothelium) and not to an undifferentiated cell type (MSC). Therefore, the expression profile of these cells was compared with many other cardiac relevant undifferentiated and differentiated cell and tissue types. In PCA, CAP cells clustered between a group of fibroblasts, bone marrow (MSC) and periosteum derived (PC) mesenchymal stem cells, and a group of endothelial cells (HPAEC, HUVEC). As expected, based on PCA, the CAP cell cluster was also separated from the ES and iPS cluster, and from different blood cell clusters. It was also separated from myocard and muscle tissue clusters, impressively verifying the uniqueness of this cell population.

In conclusion, for the first time we report a unique human endomyocardial biopsy derived population of CAP cells that have not the multilineage differentiation potential of stem cells, but in contrast to human CSC is reliably and efficiently to isolate and expand. As CSC, they have the advantage over other cells that they are cardiac-specific and can therefore be primed by their cardiac environment. If CAP cells can differentiate into other cardiac cell types or induce other cells to do this has to be investigated in further studies. Reflecting the data of this initial study we expect that these cells rather support angiogenesis than cardiomyocyte differentiation.

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