ORIGINAL RESEARCH ARTICLE

Transcriptomes of human mesenchymal cells isolated from the right ventricle and epicardial fat differ strikingly both directly after isolation and long-term culture

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Abstract

Aims   Mesenchymal stromal cells isolated from different tissues are claimed to demonstrate similar therapeutic potential and are often incorrectly named mesenchymal stem cells. However, through comparison of such cells is lacking. This study aimed to compare the transcriptome of mesenchymal cells of the same phenotype isolated from the heart muscle and epicardial fat of the same patient, before and after culture.

Methods and results   Cells were isolated from biopsies of the right ventricle and epicardial fat collected from five patients (three men and two women, mean age 59.4 ± 2.6) who underwent heart transplantation due to ischaemic cardiomyopathy. In both tissues, immunophenotyping revealed three distinct populations: (i)CD31$^-$CD45$^-$CD90$^+$CD34$^+$CD146$^-$, (ii) CD31$^-$CD45$^-$CD90$^+$CD34$^-$CD146$^+$, and (iii) CD31$^-$CD45$^-$CD90$^-$CD34$^-$CD146$^+$, of which only the first one could be grown after sorting. Material for RNA-seq was collected from these cells before culture (250 cells) and at passage 6 (5000 cells). Transcriptomic analysis revealed that cells of the same phenotype (CD31$^-$CD45$^-$CD90$^+$CD34$^+$CD146$^-$) upon isolation preferentially clustered according to the tissue of origin, not to the patient from whom they were isolated. Genes up-regulated in the right ventricle-derived cells were related to muscle physiology while down-regulated genes included those encoding proteins with transmembrane signalling receptor activity. After six passages, heart-derived and fat-derived cells did not acquire similar transcriptome. Cells isolated from the right ventricle in comparison with their epicardial fat-derived counterparts demonstrated higher level of transcripts related, among others, to RNA processing and muscle development. The down-regulated genes were involved in the nucleosome assembly, DNA packaging and replication, and interleukin-7-mediated signalling pathway. Cells from epicardial fat demonstrated higher heterogeneity both before and after culture. Cell culture significantly changed gene expression profile within both tissues.

Conclusions   This study is an essential indication that mesenchymal cells isolated from different tissues do not demonstrate similar properties. Phenotypic identification and ease of isolation cannot be considered as a criterion in any therapeutic utilization of such cells.

Introduction

Mesenchymal stromal cells are described as plastic adherent cells capable of differentiating *in vitro* into osteocytes, chondrocytes, and adipocytes.[1] Cells with such properties can be isolated from various tissues and organs including bone marrow, fat, the umbilical cord, and the heart, and they have been claimed to demonstrate similar pro-angiogenic, immunomodulatory, and anti-apoptotic paracrine activity.[2,3] Thus, mesenchymal stromal cells were extensively investigated in recent years as a novel therapeutic approach for the regeneration of damaged tissues as well as in autoimmune diseases.[2,4] Accordingly, the effect of bone marrow-derived, fat-derived, and umbilical cord-derived cells administration into damaged myocardium has already been assessed in preclinical and clinical studies with the assumption that ease of isolation of putative therapeutic cell population may facilitate the development of successful treatment. This assumption, however, was often made without taking into account that mesenchymal cells isolated from various tissues may differ in terms of biological properties.[1] Indeed, Sacchetti *et al*. reported that cells derived from the bone marrow, skeletal muscle, periosteum, and perinatal cord blood demonstrating a set of cell surface markers considered as characteristic for stromal cells in various tissues differed in their gene expression profile and *in vivo* differentiation capacity.[5]

Similarly, whole transcriptome analysis and surface marker screening revealed that tissue of origin affects properties of human bone marrow-derived, adipose-derived, and tonsil-derived mesenchymal cells.[6] These comparisons, however, focused on cells isolated from anatomically distant sites that serve substantially different functions and were performed after *in vitro* cell expansion. Additionally, genetic variability of patients from which distinct tissues were collected may influence the results. Thus, for a better understanding of mesenchymal stromal cell properties, a direct comparison of cells isolated from distinct but anatomically close tissues derived from the same individual, before and after cell culture, is needed. This would also enhance our knowledge of the components of connective tissue localized in different organs. Accordingly, we aimed to compare the transcriptome of mesenchymal cells with the same immunophenotype isolated from the right ventricle of myocardium and epicardial fat of the same patient, upon isolation and after expansion in culture.

Methods

Patients' characteristics

The investigation conforms with the principles outlined in the Declaration of Helsinki, and all procedures were approved by the Institutional Review Board and Bioethical Committee (KB/430-62/13). Biopsies of the right ventricle and epicardial fat were collected from the hearts of patients suffering from ischaemic cardiomyopathy and undergoing heart transplantation surgery upon obtaining their informed consent. The characteristics of patients from whom the material was collected and used in this study are provided in *Table 1*.

Isolation of cells

Tissue biopsies were collected within 1 h after extraction of heart from transplant recipients. Four pieces of myocardium (approximately 2 cm × 2 cm × 2 cm each) lacking any signs of myocardial lesions (scarring or fibrosis) were excised from the right ventricle (free wall, mid-level, 30 mm from the intraventricular septum) and placed into cold Dulbecco's Modified Eagle's medium (DMEM) with 4.5 g/L glucose (ThermoFisher Scientific) supplemented with 10% fetal bovine serum (FBS, ThermoFisher Scientific). Approximately 10 mL of epicardial fat localized around ventricles and atria was excised and transferred into cold DMEM with 4.5 g/L glucose supplemented with 10% FBS. Both tissues were subsequently minced into small pieces. Fragments of myocardium were washed with phosphate-buffered saline (PBS) supplemented with 1% FBS (300 g, 1 min) and suspended in NB4 collagenase (Serva) solution (0.39 U/mL, dissolved in water containing 5 mM $CaCl_2$ and 25 μg/mL DNase I (Roche), 5 mL of collagenase per 2 mL of tissue). After 1 h of digestion in 37°C, the released cells were transferred into twice the volume of DMEM F12 medium (Lonza) supplemented with 10% FBS and centrifuged [300 g, 10 min, room temperature (RT)]. Subsequently, cells were suspended in 15 mL of DMEM F12 with 10% FBS and filtered through 70 μm strainer. Remaining tissue fragments were further digested in the new collagenase solution, and the released cells were collected as described three additional times every 1 h (4 h of digestion in total). Cell suspensions after each collection were pooled, centrifuged (300 g, 10 min, RT), suspended in 15 mL of DMEM F12 with 10% FBS and filtered through 40 μm strainer.

Minced adipose tissue was washed twice with 20 mL of PBS supplemented with 1% of FBS (300 g, 1 min, RT), suspended in NB4 collagenase solution (0.39 U/mL, 5 mL of collagenase per 2 mL of tissue), and digested for 1 h in 37°C

Table 1 Characteristics of patients enrolled in the study

Patient	Age	Sex	Disease
1	60	M	Ischaemic cardiomyopathy
2	56	F	Ischaemic cardiomyopathy
3	63	M	Ischaemic cardiomyopathy
4	58	M	Ischaemic cardiomyopathy
5	60	F	Ischaemic cardiomyopathy

with shaking (200 rpm). Tissue suspension was additionally vortexed every 15 min. Subsequently, the released cells were transferred into twice the volume of DMEM F12 medium supplemented with 10% FBS (non-heat-inactivated) and centrifuged (300 g, 5 min, RT). Obtained pellet was vigorously suspended in the supernatant and centrifuged again (300 g, 5 min, RT). The cell pellet was then washed with 20 mL PBS with 1% FBS (300 g, 5 min, RT), suspended in 5 mL of PBS with 1%), and filtered through 40 μm strainer.

Cell sorting and culture

Suspensions of cells isolated from the right ventricle and epicardial fat were centrifuged (300 g, 10 min, RT) and incubated in 10 mL of red blood cells lysis buffer (1.5 M NH_4Cl, 100 mM $NaHCO_3$, 10 mM EDTA, pH 7.4[7]) (7 min, RT). Subsequently, PBS was added to 50 mL, and cells were centrifuged (300 g, 10 min, RT), resuspended in 100 μL PBS with 1% FBS, and incubated with the following set of mouse anti-human monoclonal antibodies (20 min, 4°C): anti-CD90-PE (20 μL, Miltenyi Biotec), anti-CD34-APC (20 μL, Miltenyi Biotec), anti-CD31-APC Cy7 (5 μL, BD Biosciences), anti-CD45-PE Cy7 (5 μL, BD Biosciences), and anti-CD146-FITC (5 μL, BD Biosciences). Additionally, DAPI was used to discriminate live and dead cells. Upon staining, cells were washed with PBS with 1% FBS, filtered through 40 μm strainer and subjected to cell sorting on MoFlo XDP cell sorter (Beckman Coulter). From each tissue, 250 cells of CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ phenotype were directly sorted to 20 μL of 0.1% Triton X-100 (Sigma-Aldrich) in nuclease-free water (Sigma-Aldrich) containing 1 μL of recombinant ribonuclease inhibitor (Sigma-Aldrich) and frozen in −80°C. All further sorted cells of this phenotype were collected in IMDM medium (Lonza) supplemented with 20% FBS, 10 ng/mL bFGF (PeproTech), penicillin (100 U/mL, Sigma-Aldrich), and streptomycin (100 μg/mL, Sigma-Aldrich) and seeded on a 48-well plate. Cells were cultured in this medium until passage 6 when they reached the number allowing for further steps. Cells were then collected using Accutase (GE Healthcare), washed with PBS, suspended in PBS with 1% FBS, and stained with DAPI. Then, 5000 of live cells (DAPI negative) were sorted into RL buffer of Single Cell Purification Kit (Norgen Biotek), and RNA was isolated according to the protocol provided by the manufacturer. RNA was eluted with 14 μL of water and frozen in −80°C.

RNA-Seq analysis

Ion AmpliSeq Transcriptome Human Gene Expression Panel (Thermo Fisher Scientific) was used for library preparation according to the manufacturer's protocol. Briefly, 50 ng of RNA from culture samples or all RNA sample from primary cells was reverse-transcribed, and the cDNA was subjected to multiplex PCR reactions to amplify fragments of the targeted transcripts. The amplicons were then partially digested at primer sequences followed by the adapters' ligation to amplicons and purification on AMPure XP beads (Beckman). The created library was quantified on Bioanalyzer 2100 and diluted to 80 pM before template preparation. Up to eight barcoded libraries were subjected to automated template preparation with Ion PI Hi-Q Chef Kit (Thermo) on the Ion Chef Instrument (Thermo). Barcoded libraries samples were sequenced using Ion PI Chip Kit v3 in Ion Proton (Thermo) according to the manufacturer's instructions. Raw RNA-Seq reads were mapped to hg19 genome using with Ion Torrent RNASeq Analysis plugin (version 5.0.3.0), which utilizes STAR[8] as primary aligner and bowtie2 for remaining unaligned reads.[9] Reads corresponding to *Homo sapiens* genes were counted with HTSeq count.[10] Differential gene expression was assessed with the DESeq2 R package.[11] For the sake of functional analysis, a gene was counted as differentially expressed if its fold change absolute value was higher than 2 and adjusted *P*-value reported by DESeq2 was smaller than 0.05. Overrepresentation of genes associated with biological processes (BP), described in gene ontology (GO) terms and KEGG, was conducted with clusterProfiler[12] package separately for up-regulated and down-regulated genes. *P*-values were corrected for multiple hypothesis testing using the Benjamini–Hochberg procedure to control the FDR.[13]

Results

Upon isolation and staining with antibodies recognizing CD31, CD34, CD45, CD90, and CD146 surface markers, three distinct populations of cells were detected in the right ventricle of myocardium and epicardial fat: (i) CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻, (ii) CD31⁻CD45⁻CD90⁺CD34⁻CD146⁺, and (iii) CD31⁻CD45⁻CD90⁻CD34⁻CD146⁺ (*Figure 1*), from which only the first could be cultured in used conditions and thus was further subjected to RNA-seq analysis. Limited coverage of transcriptome (851 transcripts) was obtained from 250 CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells collected from the right ventricle and epicardial fat upon isolation (*Figure 2A*). Nevertheless, principal component analysis of detected transcripts revealed that samples clustered according to the tissue of origin rather than a patient from whom they were derived (*Figure 2B*) what indicates that mesenchymal cells localized in the heart do not demonstrate a similar gene expression profile to their adipose tissue-derived counterparts.

Additionally, hierarchical clustering of differentially expressed genes revealed a higher heterogeneity of cells isolated from epicardial fat (*Figure 2C*). In a pairwise samples comparison, three genes were up-regulated and 57 down-regulated (adj. *P*-value <0.05) in the right ventricle-derived
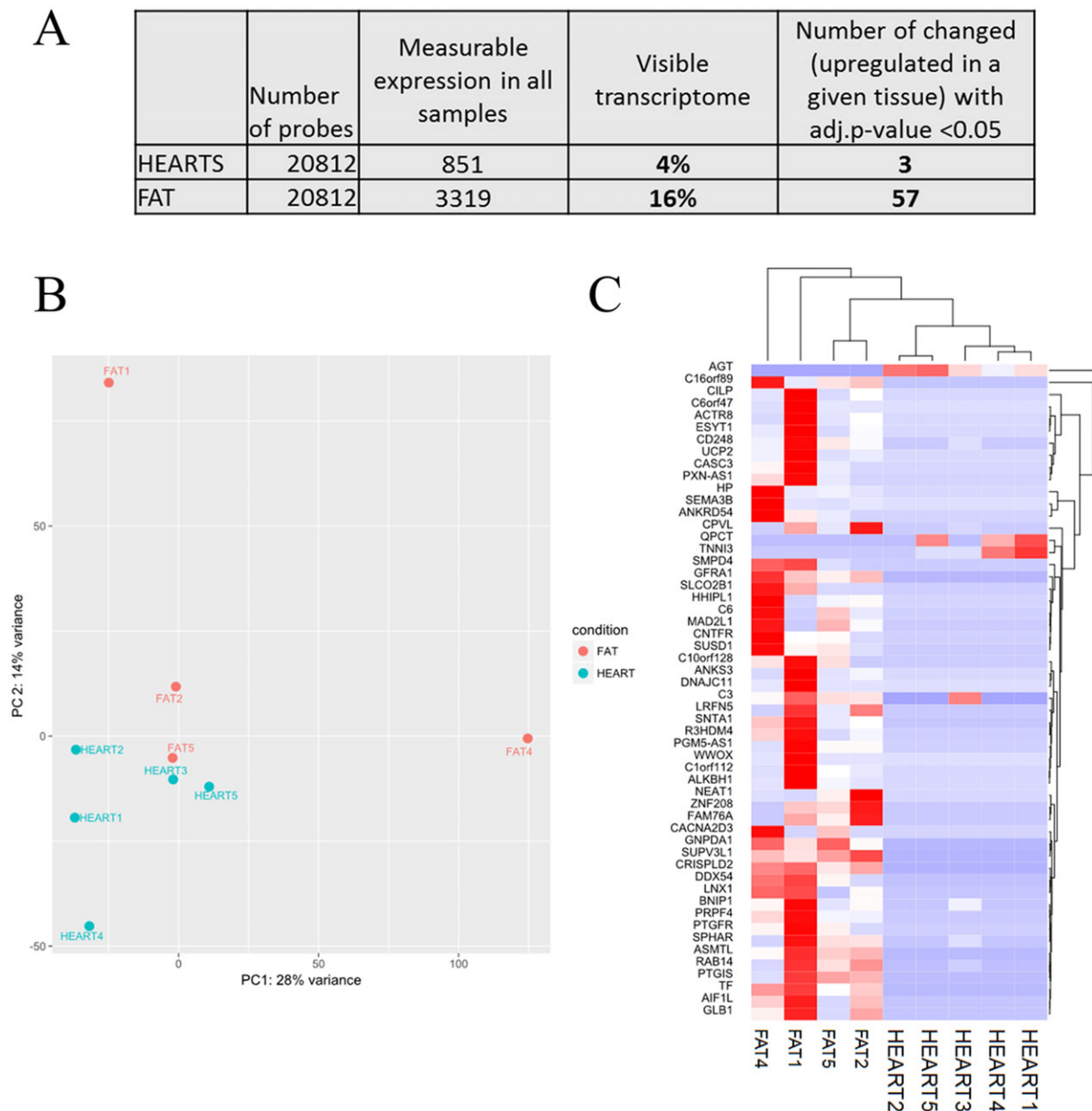
Figure 1 Immunophenotyping of freshly isolated cells from the right ventricle and epicardial fat. Representative gating strategy utilized to sort CD9031⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells from the right ventricle and epicardial fat.
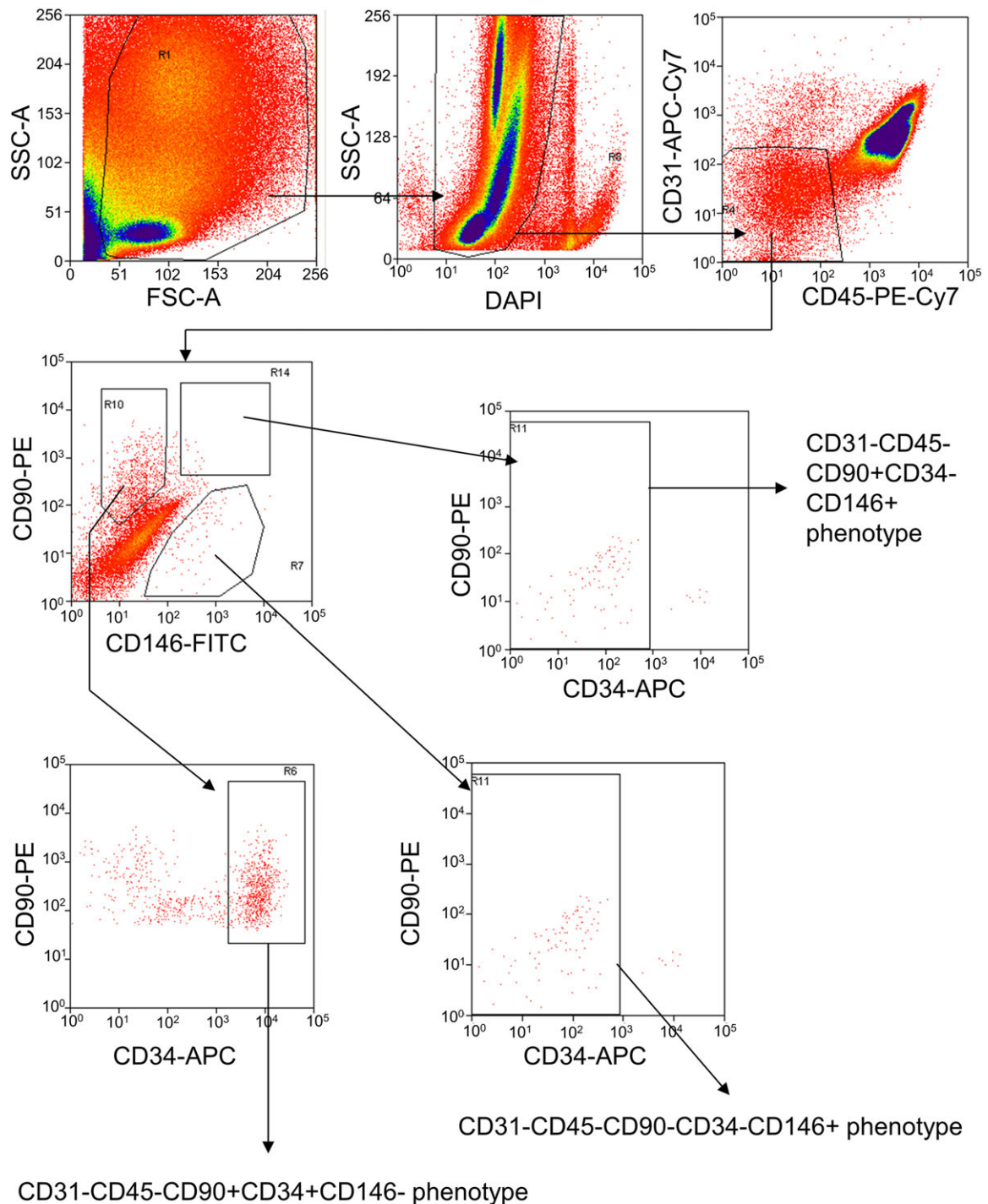
cells in comparison with their adipose tissue-derived counterparts. Because there were few significant differentiating genes after correction for multiple hypotheses, we then used the top 10% transcripts sorted according to a *P*-value as an input for functional analyses with GO. For transcripts up-regulated in the heart, this analysis yielded significant over-representation of genes among GO terms belonging to 20 BP, one molecular function (MF), and seven cellular component (CC) categories (adj. *P*-value <0.05; *Table S1*). The down-regulated genes were enriched in none BP, two MF,

Figure 2 RNA-seq analysis of freshly isolated cells. (A) A number of transcripts detected in samples isolated from the right ventricle (HEARTS) and epicardial fat (FAT). (B) Principal component analysis (PCA) of transcripts detected in cells isolated from both tissues. HEART: CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from the right ventricle (1, 2, 3, 4, 5—patient ID). FAT: CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from epicardial fat (1, 2, 4, 5—patient ID). (C) Hierarchical clustering based on differentially expressed transcripts detected in cells from both tissues.

A	Number of probes	Measurable expression in all samples	Visible transcriptome	Number of changed (upregulated in a given tissue) with adj.p-value <0.05
HEARTS	20812	851	4%	3
FAT	20812	3319	16%	57

and two CC categories. In general, the functional categories with up-regulated genes were related to muscle physiology while for down-regulated genes, these included genes encoding proteins with transmembrane signalling receptor activity (*Table S1*).

After expansion in culture, cells from both tissues demonstrated mesenchymal-like morphology (data not shown). However, immunophenotyping on passage 6 revealed that they lost expression of CD34 marker and a lower percentage of cells expressed CD90 marker (*Figure S1A,B*). For further evaluation of the impact of *in vitro* expansion on cells characteristics, 5000 of live cells from the right ventricle and epicardial fat were sorted and subjected to RNA-seq analysis, providing substantial coverage of transcriptome (*Figure 3A*). Principal component analysis of detected transcripts revealed that upon culture samples again clustered

Figure 3 RNA-seq analysis of *in vitro* expanded cells (passage 6). (A) Number of transcripts detected in samples isolated from the right ventricle (HEARTS) and epicardial fat (FAT). (B) Principal component analysis (PCA) of transcripts detected in cells isolated from both tissues. HEART: CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from right ventricle (1, 2, 3, 4, 5—patient ID). FAT: CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from epicardial fat (2, 3, 4, 5—patient ID). (C) Hierarchical clustering based on differentially expressed transcripts detected in cells from both tissues. (D) Hierarchical clustering based on 40 most differentially expressed transcripts detected in cells from both tissues.
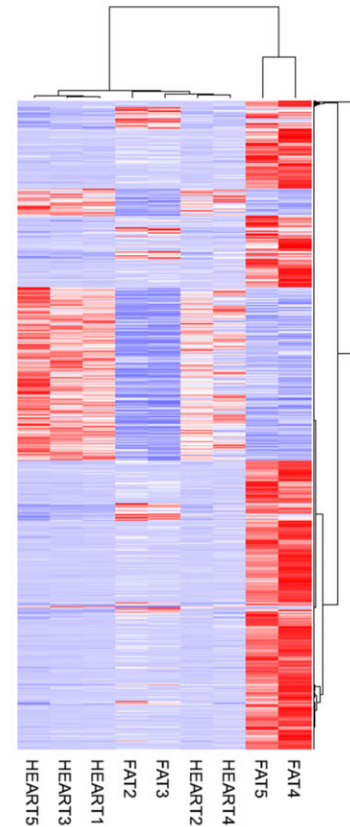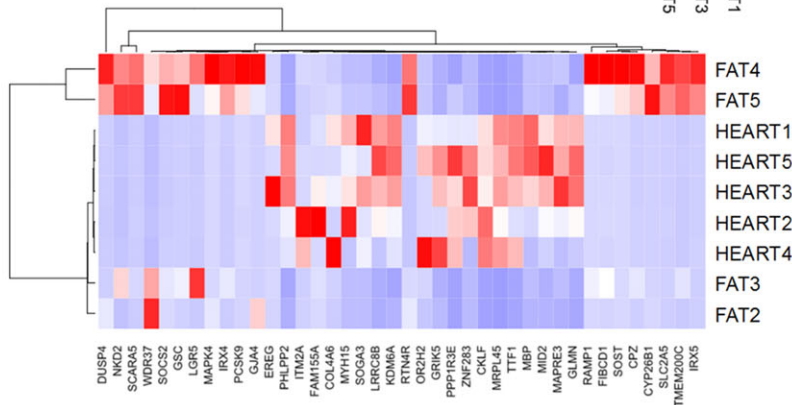
Figure 4 Comparison of freshly isolated and *in vitro* expanded epicardial fat-derived cells. (A) Principal component analysis (PCA) of transcripts detected in freshly isolated and *in vitro* expanded CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from epicardial fat (FAT). Before: freshly isolated cells; after: *in vitro* expanded cells (1, 2, 3, 4, 5—patient ID). (B) Gene ontology terms overrepresented among the genes differentiating (adj. *P*-value <0.05) freshly isolated and *in vitro* expanded cells down-regulated after cell culture. (C) Gene ontology terms overrepresented among the genes differentiating (adj. *P*-value <0.05) freshly isolated and *in vitro* expanded cells up-regulated after cell culture.

ID	Description
GO:0050727	regulation of inflammatory response
GO:0072376	protein activation cascade
GO:0003013	circulatory system process
GO:0006956	complement activation
GO:0006954	inflammatory response
GO:1901681	sulfur compound binding
GO:0061134	peptidase regulatory activity
GO:0008201	heparin binding
GO:0008009	chemokine activity
GO:0005539	glycosaminoglycan binding
GO:0004497	monooxyganse activity
GO:0033293	monocarboxylic acid binding
GO:0060205	cytoplasmic membrane-bounded vesicle lumen
GO:0031983	vesicle lumen
GO:0031012	extracellular matrix
GO:0072562	blood microparticle

ID	Description
GO:0000904	cell morphogenesis involved in differentiation
GO:0034330	cell junction organization
GO:0046777	protein autophosphorylation
GO:0043087	regulation of GTPase activity
GO:0035295	tube development
GO:0043547	positive regulation of GTPase activity
GO:0060560	developmental growth involved in morphogenesis
GO:0007265	Ras protein signal transduction
GO:0031589	cell-substrate adhesion
GO:0004672	protein kinase activity
GO:0050839	cell adhesion molecule binding
GO:0005911	cell-cell junction
GO:0045202	synapse
GO:0044815	DNA packaging complex
GO:0000786	nucleosome
GO:0005913	cell-cell adherens junction

Figure 5 Comparison of freshly isolated and *in vitro* expanded cells. (A) Principal component analysis (PCA) of transcripts detected in freshly isolated and *in vitro* expanded CD31⁻CD45⁻CD90⁺CD34⁺CD146⁻ cells isolated from the right ventricle (HEART). Before: freshly isolated cells; after: *in vitro* expanded cells (1, 2, 3, 4, 5—patient ID). (B) Gene ontology terms overrepresented among the genes differentiating (adj. *P*-value <0.05) freshly isolated and *in vitro* expanded cells down-regulated after cell culture. (C) Gene ontology terms overrepresented among the genes differentiating (adj. *P*-value <0.05) freshly isolated and *in vitro* expanded cells up-regulated after cell culture.
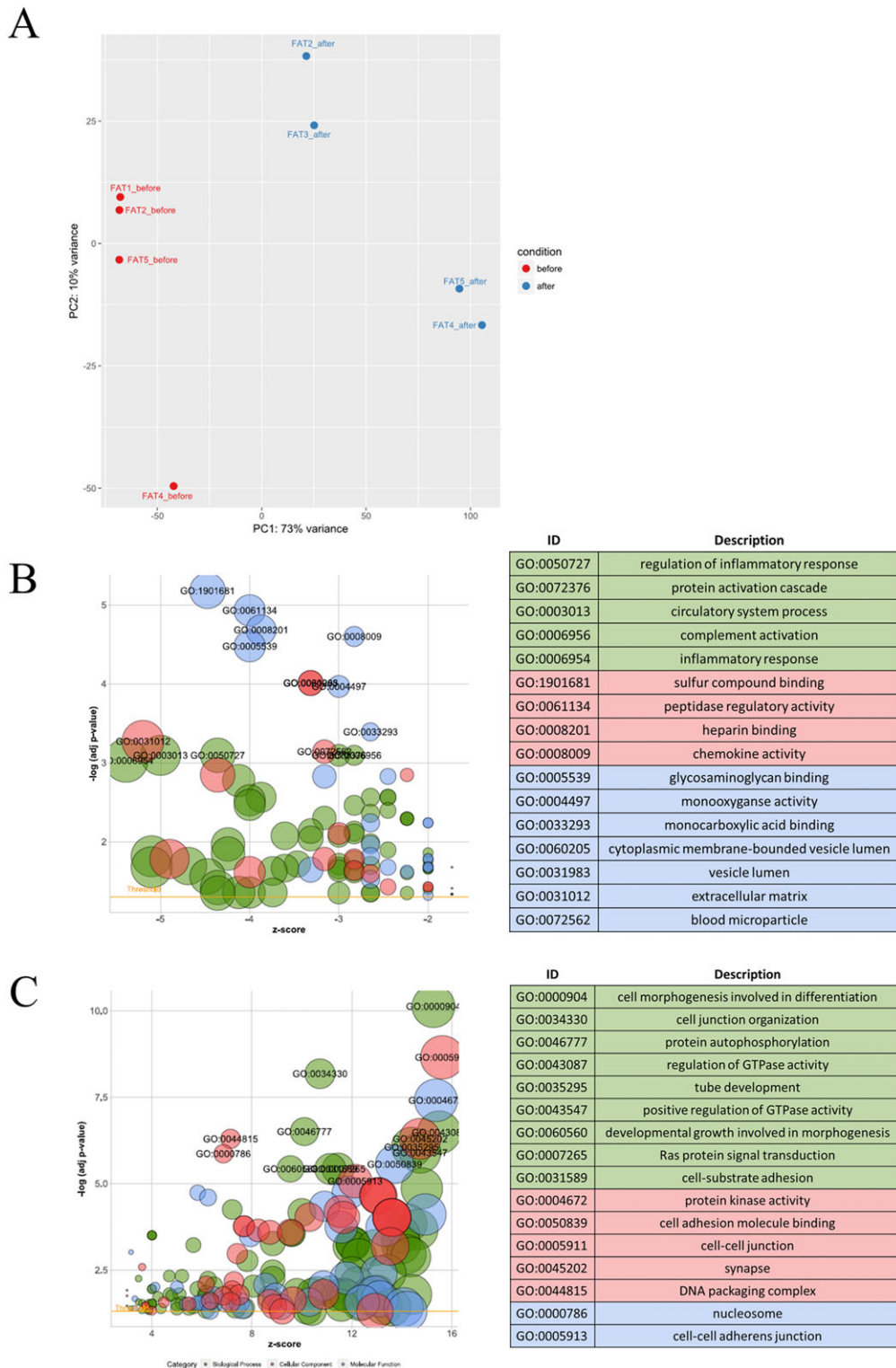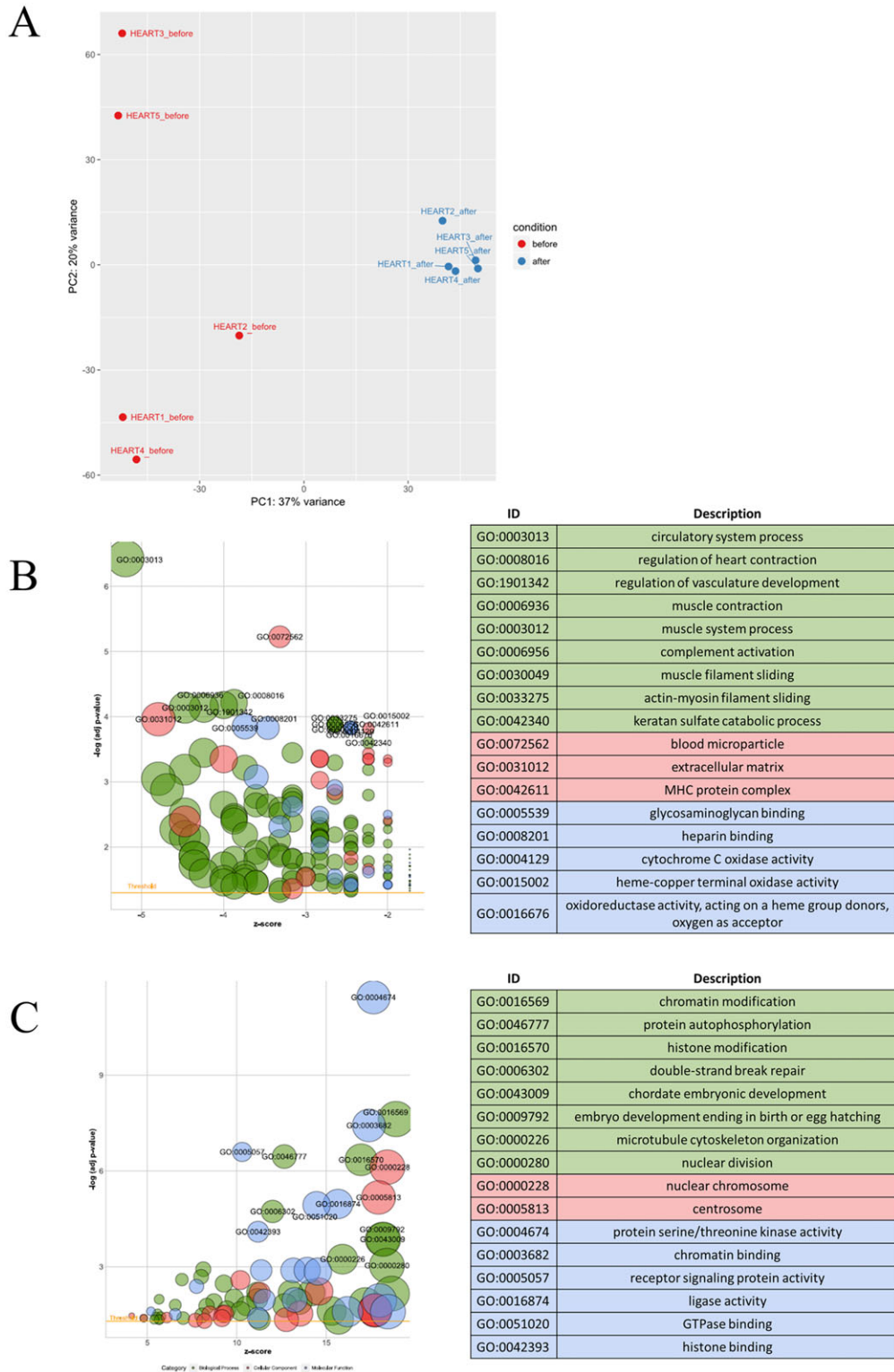

ID	Description
GO:0003013	circulatory system process
GO:0008016	regulation of heart contraction
GO:1901342	regulation of vasculature development
GO:0006936	muscle contraction
GO:0003012	muscle system process
GO:0006956	complement activation
GO:0030049	muscle filament sliding
GO:0033275	actin-myosin filament sliding
GO:0042340	keratan sulfate catabolic process
GO:0072562	blood microparticle
GO:0031012	extracellular matrix
GO:0042611	MHC protein complex
GO:0005539	glycosaminoglycan binding
GO:0008201	heparin binding
GO:0004129	cytochrome C oxidase activity
GO:0015002	heme-copper terminal oxidase activity
GO:0016676	oxidoreductase activity, acting on a heme group donors, oxygen as acceptor

ID	Description
GO:0016569	chromatin modification
GO:0046777	protein autophosphorylation
GO:0016570	histone modification
GO:0006302	double-strand break repair
GO:0043009	chordate embryonic development
GO:0009792	embryo development ending in birth or egg hatching
GO:0000226	microtubule cytoskeleton organization
GO:0000280	nuclear division
GO:0000228	nuclear chromosome
GO:0005813	centrosome
GO:0004674	protein serine/threonine kinase activity
GO:0003682	chromatin binding
GO:0005057	receptor signaling protein activity
GO:0016874	ligase activity
GO:0051020	GTPase binding
GO:0042393	histone binding

more according to the tissue of origin (*Figure 3B*) what indicates that *in vitro* expansion does not unify gene expression profile of mesenchymal cells isolated from distinct tissues. Additionally, hierarchical clustering of differentially expressed transcripts showed higher heterogeneity of epicardial fat-derived cells, as was also observed in samples collected upon isolation, and revealed a set of genes up-regulated explicitly in mesenchymal cells from the hearts (*Figure 3C,D*). The functional analysis with GO using the top 10% transcripts sorted according to a *P*-value assigned transcripts up-regulated in the hearts to five BP, one MF, and one CC category (adj. *P*-value <0.05; *Table S2*). These terms were related to RNA processing and muscle development. On the other hand, the down-regulated genes were enriched in 27 BP, four MF, and four CC categories and were involved in the nucleosome assembly, DNA packaging and replication, and interleukin-7-mediated signalling pathway (*Table S2*).

Importantly, principal component analysis of transcripts detected both upon isolation and after *in vitro* expansion revealed that cell culture substantially affects the transcriptome of cells derived from investigated tissues (*Figures 4 and 5*). Interestingly, samples from epicardial fat (*Figure 4A*) showed a high degree of heterogeneity both before and after expansion, whereas their counterparts isolated from right ventricle clustered close together at passage 6 (*Figure 5A*). Further GO analysis indicated that in case of adipose tissue-derived cells *in vitro* culture significantly down-regulated genes involved in, among others, regulation of inflammatory response, protein activation cascade, chemokine activity, sulfur compound binding, and glycosaminoglycan binding (*Table S3*, *Figure 4B*) whereas it up-regulated transcripts involved in, among others, cell morphogenesis, protein autophosphorylation, cell junction organization, and cell adhesion molecule binding (*Table S3*, *Figure 4C*). Within the heart-derived cells, on the other hand, lower levels of transcripts involved in processes such as regulation of heart contraction, regulation of vascular development, complement activation, and positive regulation of response to external stimulus were detected upon expansion (*Table S4*, *Figure 5B*) concomitantly with a higher expression of genes involved in, among others, chromatin modification, double-strand break repair, and protein serine/threonine kinase activity (*Table S4*, *Figure 5C*).

Discussion

The main finding of our study is the demonstration of significant differences in the transcriptome of mesenchymal cells of the same phenotype isolated from two distinct tissues localized within one organ and the profound effect of *in vitro* expansion on gene expression profile. We investigated the

components of the right ventricle and epicardial fat isolated from the hearts of patients undergoing heart transplantation as this model may further enhance our comprehension to what extent the tissue of isolation shall be taken into account in cell-based therapies. This is an important issue in preclinical and clinical studies utilizing mesenchymal stromal cells.[14] Accordingly, *bona fide* mesenchymal stem cells are localized in the bone marrow within the haematopoietic niche, demonstrate *in vitro* capacity for clonal growth and differentiation into osteocytes, chondrocytes, and adipocytes, and reconstitute bone marrow receptive for haematopoietic stem cells engraftment upon *in vivo* administration.[15]

Nevertheless, cells of similar *in vitro* differentiation potential can be isolated from different tissues including more easily accessible adipose tissue (e.g. collected during liposuction) and therefore are similarly termed mesenchymal stem or stromal cells.[2] Such fat-derived cells are often used in preclinical and clinical studies without thorough characterization. For instance, a stromal vascular fraction obtained from liposuction-collected adipose tissue was used in APOLLO clinical trial targeting acute myocardial infarction; however, stromal vascular fraction represents a crude and heterogeneous mix of cell populations.[16,17] Importantly, Sacchetti *et al*. already reported that CD45$^-$CD34$^-$HLA$^-$II$^-$CD146$^+$CD105$^+$CD90$^+$CD73$^+$ cells capable of *in vitro* differentiation into osteocytes, adipocytes, and chondrocytes isolated from the bone marrow, skeletal muscle, periosteum, and perinatal cord blood do not demonstrate similar gene expression profile and *in vivo* biological properties.[5] Notably, only bone marrow-derived cells formed bones with established haematopoietic environment upon *in vivo* delivery whereas myogenic capacity was attributed to skeletal muscle-derived counterparts but not others.[5] Similarly, distinct biological features were described for mesenchymal cells obtained from human embryonic stem cells, fetal limb, and bone marrow even though they shared a common phenotype.[18] Thus, a comprehensive investigation of any cell type is needed before considering its application in cell-based therapy.

Interestingly, our immunophenotyping of cells isolated from the right ventricle and epicardial fat revealed the presence of three distinct populations differing in the expression of CD90, CD34, and CD146 markers. As only CD31$^-$CD45$^-$CD90$^+$CD34$^+$CD146$^-$ population could be expanded *in vitro* in the culture conditions utilized in this study; we narrowed our transcriptomic analysis to these cells. Nevertheless, further investigation of other observed cell types would be of importance for better characterization of various components of connective tissue within one organ. Among them, CD31$^-$CD45$^-$CD90$^+$CD34$^-$CD146$^+$ cells would most closely resemble MSCs described by Sacchetti *et al*.; however, further studies are needed to find optimal culture conditions for such population isolated from heart and epicardial fat.[5] Interestingly, CD34 was proposed to characterize MSCs *in vitro*, yet its expression was lost upon expansion of cells investigated in this study.[19,20]

The major strength of our study is the direct comparison of mesenchymal cells isolated from distinct tissues of the same patient. Such an approach limits the genetic variability of different tissue donors (i.e. umbilical cord blood vs. bone marrow or muscle in other studies[5,6]) and thus allows to focus on gene expression profile dependent on anatomical localization of cells. Indeed, the performed RNA-seq analysis indicated that mesenchymal cells clustered according to the tissue of origin rather than the patient from whom they were isolated. Additionally, we describe a substantial impact of cell culture on cellular transcriptome, which has not been addressed in other studies.[5,6,18] We believe that it should be considered as an important factor for designing any cell-based therapy. Finally, this study provides comprehensive information on gene expression profile of mesenchymal cells located in the heart, which may add to the understanding of the biological properties of cell populations present in this organ, which is difficult to access for such analysis.

However, we performed an analysis of cells derived from diseased hearts, taken from patients undergoing transplant surgery, which may not reflect the properties of mesenchymal cells present in healthy cardiac tissues. Further studies are also needed to evaluate whether cells isolated from different tissues within other organs demonstrate such substantial transcriptomic differences. Another limitation of our study is the cell culture protocol (media, number of passages, etc.), which may direct specific gene expression. Last but not the least, limited transcriptome coverage in the RNA-seq analysis of freshly isolated cells may narrow the comparison of their transcriptomes to the highly expressed genes.

Nevertheless, our findings revealed that cells expressing the same mesenchymal markers isolated from tissues localized within one organ have distinct transcriptomes. Thus, ease of isolation shall not be primarily taken into account in designing cell-based therapies. Instead, we believe that thorough characterization, including transcriptomic and functional studies, should stand behind further preclinical evaluation of any cell type. High heterogeneity of adipose-derived cells observed in our analysis additionally raises a question on the efficacy of treatments based on mesenchymal cells. Our data indicate that the mesenchymal cells of various origin have different properties what undermines the rationale for using them as cell therapy source in cardiac diseases.

Conflict of interest

M.O.Z. is a consultant for Abbott Inc., Boston Scientific, and AtriCure.

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Immunophenotyping of cells from the right ventricle and epicardial fat on passage 6. A. Gating strategy for flow cytometry analysis. B. The phenotype of cells isolated from the right ventricle (HEART) and epicardial fat (FAT). Results presented as mean ± SEM of a percentage of cells positive for analysed markers from 5 patients enrolled in the study. Red box – phenotype of initially isolated and sorted population.

Table S1. Gene expression levels and Gene Ontology terms and KEGG pathways overrepresented among the 10% genes sorted according to a p. value in heart vs adipose primary samples comparison. Individual sheets contain a list of GO terms or KEGG pathways for upregulated (up) or downregulated (down) genes;ID – GO or KEGG identifier, GeneRatio – the ratio of number of differentiating genes in a given term/pathway to the number of differentiating genes with GO or KEGG identifier; BgRatio – the ratio of number of not differentiating genes in a given term/pathway to the number of expressed genes with GO or KEGG identifier; *p*-value - p-value in hypergeometric test; q-value - p-value after FDR correction; GeneID - list of genes assigned to a given term/pathway; Count – number of differentiating genes contributing to a given term/pathway; BP - biological processes BP, MF - molecular function; CC - cellular component.

Table S2. Gene expression levels and Gene Ontology terms and KEGG pathways overrepresented among the 10% genes sorted according to a p. value in heart vs adipose cultured samples comparison. Individual sheets contain a list of GO terms or KEGG pathways for upregulated (up) or downregulated (down) genes;ID – GO or KEGG identifier, GeneRatio – the ratio of number of differentiating genes in a given term/pathway to the number of differentiating genes with GO or KEGG identifier; BgRatio – the ratio of number of not differentiating genes in a given term/pathway to the number of expressed genes with GO or KEGG identifier; *p*-value - p-value in hypergeometric test; q-value - p-value after FDR

correction; GeneID - list of genes assigned to a given term/pathway; Count – number of differentiating genes contributing to a given term/pathway; BP - biological processes BP, MF - molecular function; CC - cellular component.

Table S3. Gene expression levels and Gene Ontology terms overrepresented among the genes differentiating (adj. p.value <0.05) primary and cultured adipose cells. Individual sheets contain a list of GO terms for upregulated (up) or downregulated (down) genes;ID – GO identifier, GeneRatio – the ratio of number of differentiating genes in a given term to the number of differentiating genes with GO identifier; BgRatio – the ratio of number of not differentiating genes in a given term to the number of expressed genes with GO identifier; p-value - p-value in hypergeometric test; q-value - p-value after FDR correction; GeneID - list of genes assigned to a given term/pathway; Count – number of differentiating

genes contributing to a given term; BP - biological processes BP, MF - molecular function; CC - cellular component.

Table S4. Gene expression levels and Gene Ontology terms overrepresented among the genes differentiating (adj. p.value <0.05) primary and cultured heart cells. Individual sheets contain a list of GO terms for upregulated (up) or downregulated (down) genes;ID – GO identifier, GeneRatio – the ratio of number of differentiating genes in a given term to the number of differentiating genes with GO identifier; BgRatio – the ratio of number of not differentiating genes in a given term to the number of expressed genes with GO identifier; p-value - p-value in hypergeometric test; q-value - p-value after FDR correction; GeneID - list of genes assigned to a given term/pathway; Count – number of differentiating genes contributing to a given term; BP - biological processes BP, MF - molecular function; CC - cellular component.

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