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# Genetic profiling and surface proteome analysis of human atrial stromal cells and rat ventricular epicardium-derived cells reveals novel insights into their cardiogenic potential

Sebastian Temme [a,1], Daniela Friebe [a,1], Timo Schmidt [a,1], Gereon Poschmann [b], Julia Hesse [a], Bodo Steckel [a], Kai Stühler [b], Meik Kunz [c], Thomas Dandekar [c], Zhaoping Ding [a], Payam Akhyari [d], Artur Lichtenberg [d], Jürgen Schrader [a,*]

[a] Department of Molecular Cardiology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[b] Molecular Proteomics Laboratory, Biomedical Research Center (BMFZ), Heinrich-Heine-University, Düsseldorf, Germany
[c] Functional Genomics and Systems Biology Group, Department of Bioinformatics, Biocenter, Am Hubland, Würzburg, Germany
[d] Department of Cardiovascular Surgery, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

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ABSTRACT

Epicardium-derived cells (EPDC) and atrial stromal cells (ASC) display cardio-regenerative potential, but the molecular details are still unexplored. Signals which induce activation, migration and differentiation of these cells are largely unknown. Here we have isolated rat ventricular EPDC and rat/human ASC and performed genetic and proteomic profiling. EPDC and ASC expressed epicardial/mesenchymal markers (WT-1, Tbx18, CD73, CD90, CD44, CD105), cardiac markers (Gata4, Tbx5, troponin T) and also contained phosphocreatine. We used cell surface biotinylation to isolate plasma membrane proteins of rEPDC and hASC, Nano-liquid chromatography with subsequent mass spectrometry and bioinformatics analysis identified 396 rat and 239 human plasma membrane proteins with 149 overlapping proteins. Functional GO-term analysis revealed several significantly enriched categories related to extracellular matrix (ECM), cell migration/differentiation, immunology or angiogenesis. We identified receptors for ephrin and growth factors (IGF, PDGF, EGF, anthrax toxin) known to be involved in cardiac repair and regeneration. Functional category enrichment identified clusters around integrins, PI3K/Akt-signaling and various cardiomyopathies. Our study indicates that EPDC and ASC have a similar molecular phenotype related to cardiac healing/regeneration. The cell surface proteome repository will help to further unravel the molecular details of their cardio-regenerative potential and their role in cardiac diseases.



## 1. Introduction

Epicardium-derived cells (EPDC) play a fundamental role in embryonic heart development and cardiac disease (Ruiz-Villalba and Pérez-Pomares, 2012). After birth, epicardial cells are in a relatively dormant state as a single cell-layer covering the myocardium. However, following myocardial infarction (MI), the ventricular epicardium becomes reactivated and is characterized by EMT, a thickening of the epicardial layer and the re-expression of embryonic epicardial genes such as WT1 (Wilms tumor protein 1) and Tbx18 (T-box 18 transcription factor) (Zhou et al., 2011). These adult EPDC can migrate into the injured myocardium to differentiate into distinct cardiovascular cells (Smart et al., 2011; Zhou et al., 2011). Adult EPDC are also involved in the *de novo* formation of cardiomyocytes, however, the number of EPDC which are transformed into cardiomyocytes is quite low and therefore, their contribution appears to be insufficient for effective myocardial regeneration (Masters and Riley, 2014).

While EPDC have been intensively studied in rodents *in vivo* and *in vitro*, studies on human EPDC (hEPDC) are less numerous. Primary hEPDC from patient-derived atrial appendage biopsies can be isolated by stripping the epicardial layer (Clunie-O'Connor et al., 2015). Alternatively, cells types which strongly resemble hEPDC can be isolated from the human atrium by enzymatic techniques and were termed either "cardiac stromal cells" (Rossini et al., 2011) or "cardiac atrial appendage stem cells (CASC)" (Fanton et al., 2016; Koninckx et al., 2013; Windmolders et al., 2015). They show a similar mesenchymal morphology, express mesenchymal markers and have been shown to display strong regenerative potential upon intramyocardial implantation after MI: Implantation of these cells improved cardiac performance (Fanton et al., 2015; Rossini et al., 2011; Winter et al., 2007), attenuated adverse remodeling (Rossini et al., 2011), promoted angiogenesis (Fanton et al.,

* Corresponding author at: Department of Molecular Cardiology, Heinrich-Heine-University Düsseldorf, Universitätsstraße 1, Düsseldorf 40225, Germany.
*E-mail address:* schrader@uni-duesseldorf.de (J. Schrader).
[1] These authors contributed equally to this study.

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2016) and a fraction of the cells differentiated into cardiomyocytes (Fanton et al., 2015; Koninckx et al., 2013; Rossini et al., 2011).

Given the remarkable similarity of EPDC and stromal cells, the aim of the present study was to characterize the molecular phenotype of human ASC (hASC) and rat ventricular EPDC (rEPDC) formed after MI by genetic profiling and systematic analysis of cell surface proteins.

## 2. Materials and methods

### 2.1. Isolation and cultivation of hASC, rASC and rEPDC

Right or left atrial biopsy samples were obtained from 40 patients (66.4 ± 10.2 years of age; 31 men, 9 women) who underwent different cardiovascular surgical interventions (valve replacement/reconstruction, cardiac transplantation, aortic-coronary bypass). The study was approved by the institutional ethics committee (reference number 4125, 4412R und 4646) according to the principles outlined in the Declaration of Helsinki. Human atrial biopsies were immediately transferred into ice-cold PBS. After removal of fat tissue, epicardial and myocardial tissue was separated by a scalpel and cut into 1 $mm^3$ pieces which were transferred to PBS containing 1200 IU/ml collagenase II (Biochrom AG) for 3 h at 37 °C. Cell suspension was filtered (70 μm cell-strainer), centrifuged at 350g and resuspended in culture medium (DMEM supplemented with 20% FCS, 10 ng/ml bFGF (Sigma Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM Glutamax). The filtrate was transferred into a culture flask and incubated for 3 h at 37 °C to eliminate fibroblasts. The supernatant was transferred into a new culture flask and experiments were performed after 2–5 passages.

Animal experiments were performed in accordance with the European Union guidelines described in the directive 2010/63/EU and were approved by the local authorities (reference number 84-02.04.2014.A174). Male Wistar rats (220–280 g body weight, 12–16 weeks of age) used in this study were obtained from Janvier (Le Genest-Saint-Isle, France), and were fed with a standard chow diet receiving tap water *ad libitum*. MI in the rat (60 min ischemia/reperfusion) and isolation of EPDC 5 days after MI was conducted as recently described (Ding et al., 2016). For isolation of rASC, left and right atrial tissue issue (from MI and non-MI rats) was carefully prepared, minced and processed as described above for the human material.

### 2.2. Cell-based assays

*Cell proliferation*: Approximately ~20.000 hASC, or rEPDC of $n = 3$ individual isolates were plated in six-well plates as triplets. After 2–4 days, viable cells were counted using trypan blue (4%).

*Quantitative real time PCR*: To analyze mRNA expression, total RNA was isolated using the RNeasy Micro Kit and cDNA was synthesized applying the QuantiTect Reverse Transcription Kit (Qiagen GmbH) according to the manufacturer's instructions. We used predesigned TaqMan Gene Expression Assays for human and rat samples (Supp. materials and methods) and the StepOnePlus™ System (Life Technologies) following the manufacturer's protocol. Gene expression was normalized to β-actin.

*UPLC*: For ultra-performance liquid chromatography (UPLC), extraction and separation of various purine compounds was carried out as described (Hesse et al., 2017). Creatine derivatives were measured as described (Timohhina et al., 2009) using a Waters Acquity UPLC system.

*Flow cytometry and immunofluorescence*: For flow cytometry, cells were detached from the culture dish using PBS/5 mM EDTA (PBS/EDTA) and washed with FACS buffer (PBS, 0.5% BSA, 2 mM EDTA). After blocking with PBS/5% BSA, and Fc-block, cells were stained with antibodies against CD73, CD90, c-Kit, CD105, CD44 and Pecam-1/CD31and appropriate secondary antibodies (Suppl. Materials and Methods).

For immunofluorescence, cells were seeded on coverslips, fixed with Zamboni's fixative (4% PFA, 0.2 M picric acid (saturated aqueous), 0.1 M $NaH_2PO_4/Na_2HPO_4$, pH = 7.3) and permeabilized with PBS/0.1% Triton-X100 (Sigma) or left non-permeabilized. After blocking with PBS/5% normal serum, cells were stained with antibodies for WT-1, Tbx18, Gata4, Tbx5, troponin T, α-SMA, Pecam-1/CD31 and appropriate secondary antibodies. Nuclei were counterstained with DAPI. Samples were analyzed using a fluorescence microscope (BX61, Olympus) and recorded using high resolution digital cameras [F-ViewII (fluorescence) and UC30 (bright field), Olympus] or a confocal laser scanning microscope (LSM710 meta, Zeiss).

Identification of WT-1 positive cells within the human atrial tissue was performed in air-dried and fixed (10 min Zamboni's fixative) cyrosections (8 μm). Samples were incubated with antibodies for WT-1 and troponin T and with Cy3- or FITC-labeled secondary antibodies.

### 2.3. Isolation and analysis of cell surface proteins

A detailed description of protein isolation and analysis can be found in Supplementary Materials and Methods. In brief, human atrial or rat ventricular cells of three individual isolates (three rat ischemia/reperfusion experiments; three different patient biopsies) were washed with ice-cold PBS and incubated with EZ-Link Sulfo-NHS-SS-Biotin (Thermo Fisher). After biotinylation was stopped, cells were lysed, incubated with Neutravidin-agarose and resuspended in elution buffer to detach biotinylated proteins. For mass spectrometry, three individual samples were processed as described recently (Poschmann et al., 2014) and the resulting peptides were separated by liquid chromatography coupled to the mass spectrometer (Orbitrap Elite (Thermo Fisher) ion trap mass spectrometer or a Q Exactive (Thermo Fisher) quadrupole-orbitrap mass spectrometer). Spectra were searched against proteome datasets downloaded from UniProtKB with a precursor mass tolerance of 20 ppm for the first and 4.5 ppm for the second search and a fragment mass tolerance of 20 ppm (Q Exactive) and 0.5 Da (Orbitrap Elite), respectively. Protein identification was carried out within the MaxQuant framework using standard parameters. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium *via* the PRIDE partner repository with the dataset identifier PXD005013. Bioinformatics functional enrichment analysis was performed using Cytoscape plugin ClueGO. Proteins overlapping between rat and human were analyzed for biological GO processes, pathways from KEGG and REACTOME.

## 3. Results

### 3.1. Human atrial stromal cells and rat EPDC display a similar morphology and express WT-1 and Tbx18

Isolation of stromal cells from human atrial appendage biopsies (hASC) was performed in 40 patients undergoing various surgical interventions using collagenase digestion. Cell preparations, regularly displayed adherent cells with a spindle shaped-morphology (Fig. 1A, left). Cell yield after six days of culture was 1156 ± 1105 cells ($n = 4$) per mg tissue sample. The *in vitro* doubling time was 54 ± 2 h (n = 3) and cells could be cultivated for up to 10 passages. To obtain stromal cells from the atrium of healthy rats (rASC), we used the same protocol and again obtained a homogenous cell population with spindle-shaped appearance (Fig. 1A, middle). Rat ventricular EPDC (rEPDC) were retrieved by collagenase treatment of the heart surface after five days following myocardial infarction (Ding et al., 2016). This procedure permitted the selective removal of the epicardial layer (thickness: ~150 μm 3-4d post MI) and resulted in the cultivation of cells with spindle-shaped structure (Fig. 1A, right). Doubling time was 35 ± 8 h (n = 3) and cells could be kept in culture for up to 20 passages. Cell yield was 307,500 ± 60,140 cells (n = 4) per processed heart after 4d of expansion.

Given the morphological similarity of rEPDC, rASC and hASC, we used flow cytometry to analyze cell surface expression of marker

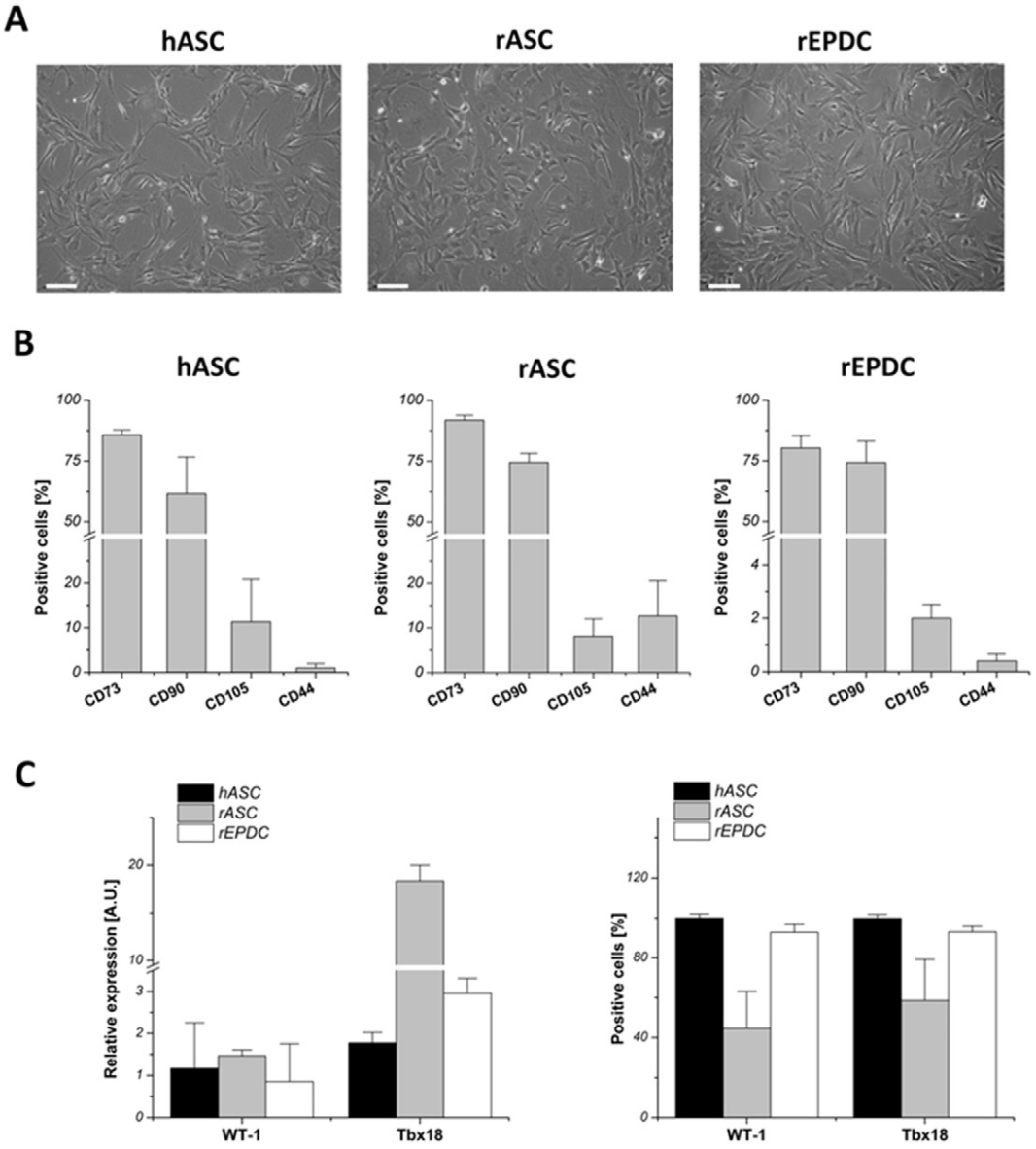


**Fig. 1.** Epicardial and cardiac marker expression in ASC and ventricular rEPDC. (A) Representative bright-field images of cells isolated from human atrium (hASC), rat atrium (rASC) and from the surface of the infarcted rat heart (rEPDC). Scale bar = 50 μm. (B) Quantitative evaluation (% positive cells) of the cell surface expression of CD73, CD90, CD105, CD44. Data are mean values ± SD, n = 3. (C) Quantitative PCR (normalized to *β-actin* mRNA levels) of epicardial marker genes (*WT-1*, *Tbx18*; left panel). Quantification of immunofluorescence staining to determine the number of cells expressing WT-1 and Tbx18 (right panel). Data are mean ± SD of n = 3. (D) Transcript levels of mRNA (upper panel) and protein expression (immunofluorescence; lower panel) of the stem cell marker c-Kit, the cardiac markers Tbx5, Gata4 and cardiac troponin T, as well as α-SMA and Pecam-1/CD31. Data are mean values ± SD of n = 3 independent experiments. n.d. = not detectable.

proteins that were reported for rEPDC (Ding et al., 2016) as well as for mesenchymal stem cells (MSC) (Dominici et al., 2006). CD73 and CD90 were expressed by most of the rEPDC, rASC, hASC (Fig. 1B) but we identified only a low number of CD105 and CD44 expressing cells. While CD73 was homogenously expressed in all samples, the levels of CD90, CD105 and CD44 were heterogeneous (Supp. Fig. S1). Next, we analyzed the expression of WT-1 and Tbx18 - two of the major markers for EPDC (Masters and Riley, 2014). Quantitative PCR showed expression of *WT-1* and *Tbx18* with high *Tbx18* levels in rASC (Fig. 1C). Immunofluorescence revealed that >90% of the cultured hASC and rEPDC express WT-1 and Tbx18, while only 50% of the rASC were positive for WT-1 or Tbx18 (Fig. 1C; Supp. Fig. S2).

### 3.2. Expression profiling of rEPDC and ASC

As summarized in Fig. 1D, both hASC and rASC/rEPDC cells displayed a remarkable similar expression profile of various stem cell, cardiac and vascular markers particularly at the protein level: c-Kit (stem cells), Gata4 (early cardiac progenitors), Tbx5 (early myocardial marker), cardiac troponin T (cTnT; late cardiac marker), α-SMA (smooth muscle cells/myofibroblasts) and Pecam-1 (CD31; endothelial cells). While *c-Kit* and *Tbx5* generally show low mRNA expression levels, Tbx5 and Gata4 proteins are highly expressed in all three cell isolates. Interestingly, *cTnT* was expressed in all three isolates but was not detectable at the protein level. A minor fraction of cells was positive for α-SMA or Pecam-1/CD31, suggesting negligible endothelial contamination.

Since paracrine factors released after myocardial infarction are most likely responsible for epicardial EMT including WT-1 upregulation (Zhou et al., 2011), we wondered whether ventricular ischemia also impacts the phenotype of ASC in the rat. As summarized in Supp. Fig. S3, ASC isolated from the atria of non-infarcted and infarcted rat hearts exhibit similar mRNA- and protein levels for WT-1, Tbx18, c-Kit, Gata4, Tbx5, cTnT, α-SMA and Pecam-1/CD31.

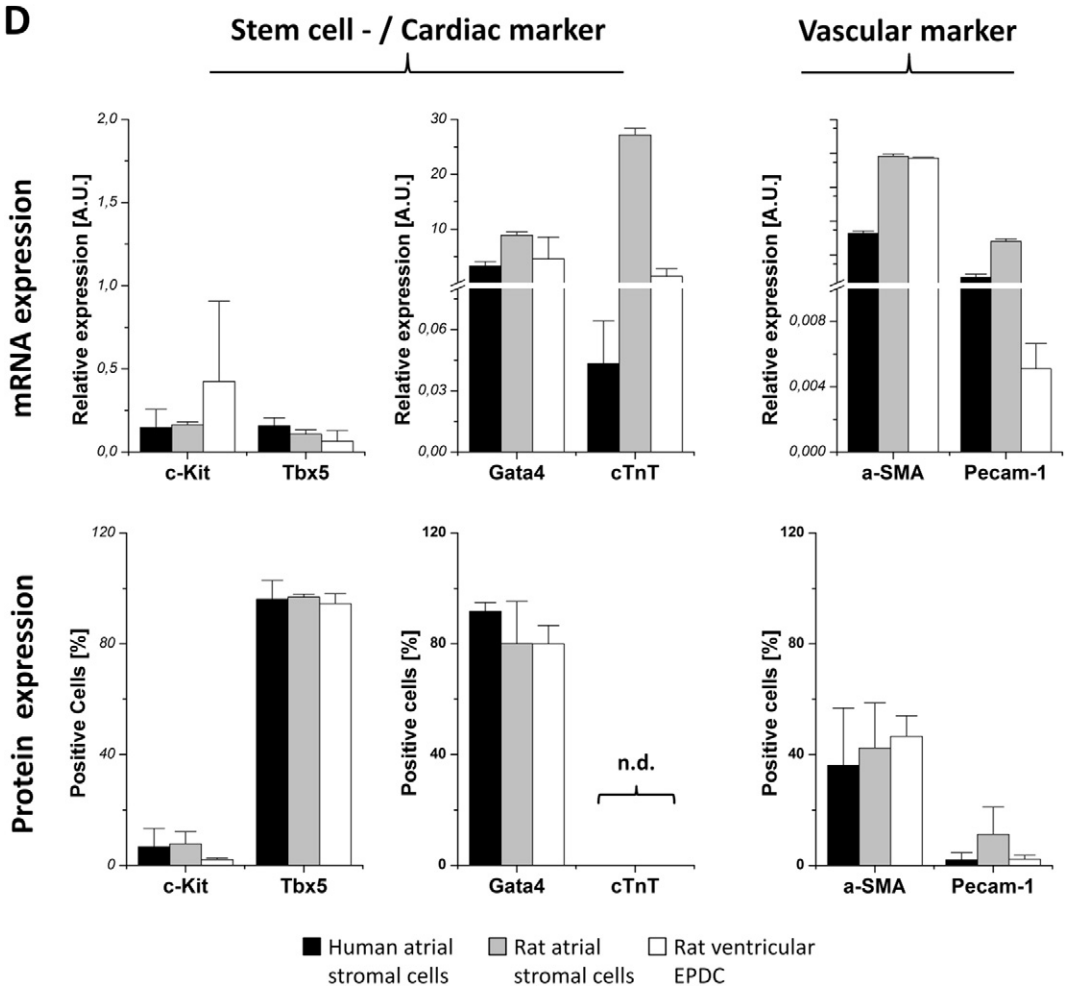


**Fig. 1** (*continued*).

### 3.3. Human ASC isolated from epicardial and myocardial tissue display an identical molecular phenotype

EPDC can be isolated from human atria either by explant cultures (Clunie-O'Connor et al., 2015) or by enzymatic digestion (Rossini et al., 2011). In both cases it is assumed that isolated cells are primarily derived from the epicardial surface. To test this assumption, atrial tissue samples were divided into epicardial and myocardial specimens. Isolation of ASC by enzymatic digestion yielded in both cases morphologically identical spindle shaped cells (Fig. 2A). Expression analysis revealed that mRNA levels (*WT-1*, *Tbx18*, *c-Kit*, *Gata4*, *Tbx5*, *cTnT*, *α-SMA and Pecam-1/CD31*) and the protein expression (CD73, CD90, CD105, CD44, cKit, WT-1, Tbx18, Gata4, Tbx5, cTnT, α-SMA and Pecam-1/CD31) were quite similar (Fig. 2B–D). To verify that WT-1 expressing cells are located within the human atrial myocardium, we analyzed atrial cryosections and found WT-1 positive cells interspersed between troponin T positive cardiomyocytes (Fig. 2E). The number of WT-1 expressing cells was determined to be ~3500 per $cm^2$.

### 3.4. Comparison of hASC and rEPDC with human BMSC

Since ASC and rEPDC are of mesenchymal origin, we measured the expression profile of human bone marrow derived SC (BMSC). As shown in Fig. 3A, BMSC do not express *WT-1, Gata4* and *cTnT* and the transcripts of *c-Kit*, *Tbx18* and *Pecam-1/CD31* are also low. On the other hand, flow cytometry revealed that BMSC as well as hASC, rASC and rEPDC show high expression of CD73 and CD90 and low expression levels of CD105, CD44, c-Kit and Pecam-1/CD31 (compare data in Fig. 3A/B with Fig. 1D).

BMSC are also different to hASC and rEPDC with respect to their content of high energy phosphates. Using UPLC we measured creatine (Cr), phosphocreatine (CrP), ATP, ADP, AMP and NAD and found that analyzed metabolites were quite comparable between rEPDC, hASC and BMSC with the exception that BMSC did not contain measurable CrP and Cr (Fig. 3C).

### 3.5. Identification and comparison of cell surface proteins in hASC and rEPDC

Due to the similar phenotype of hASC and rEPDC, we carried out a comparative analysis of the surface membrane proteome. Cell surface biotinylation was used to specifically label and enrich membrane proteins of rEPDC and hASC from three preparations each. Successful biotinylation and protein isolation was confirmed by immunofluorescence and SDS-PAGE separation (Supp. Fig. S4A + B). For mass spectrometry, samples were subjected to trypsin-mediated in-gel digestion and resulting peptide mixtures were analyzed by high resolution ion trap based LC-MS. In total, we identified 1248 proteins in hASC and 1211 proteins in rEPDC samples, of which 239 human and 396 rat proteins were confirmed as being surface proteins (displaying signal peptides, transmembrane region or GPI anchor) and were present in at least two out of three samples.

In a first step, we compared the GO annotations of both datasets according to subcellular structure, protein class and biological process which revealed a similar pattern of the GO classification between

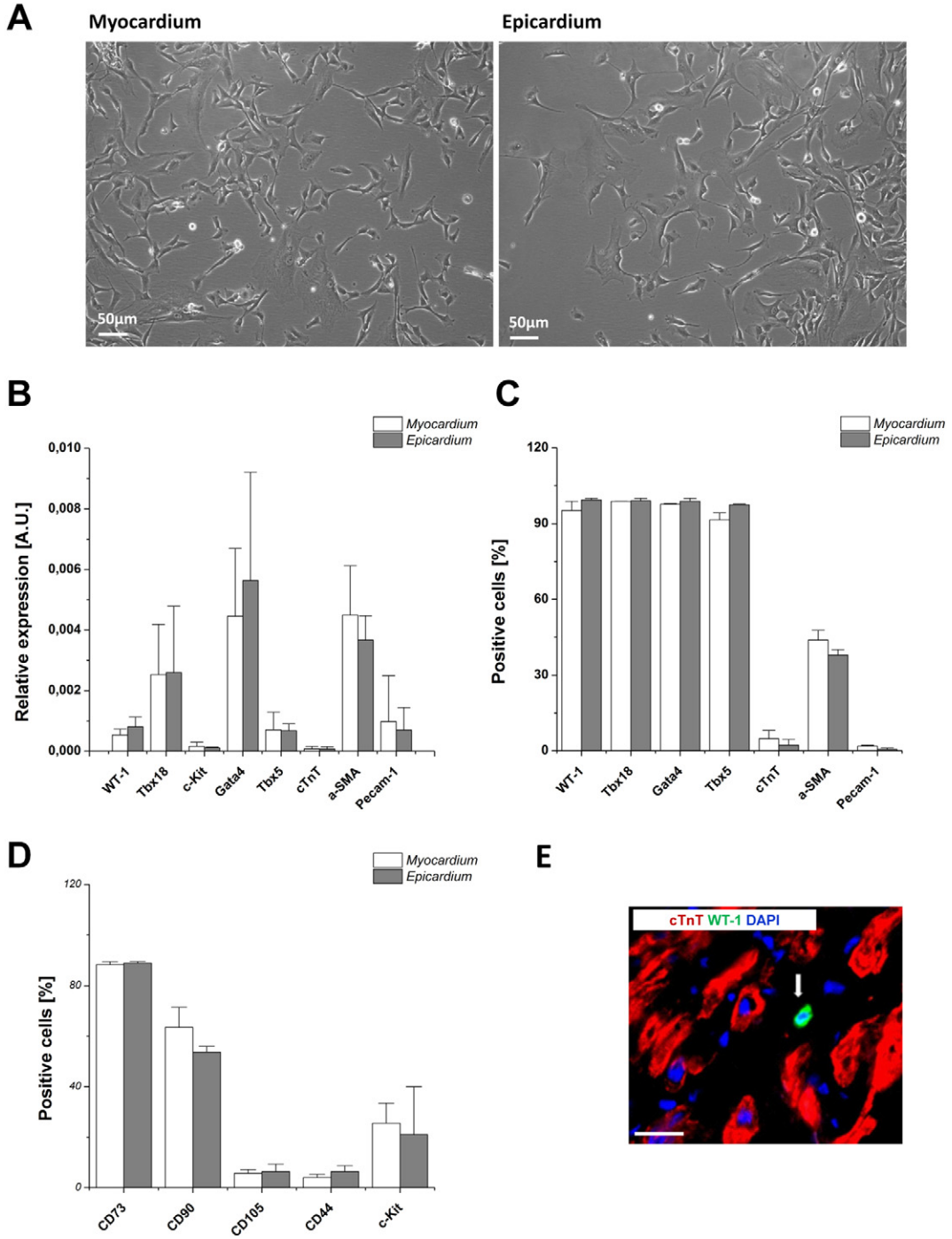


**Fig. 2.** Comparison of hASC isolated from epicardium and myocardium. (A) Bright-field micrographs of ASC isolated from myocardium (left) or epicardium (right). (B–D) Characterization by qPCR (mRNA, B) or immunofluorescence (C) and flow cytometry (D). Data are mean values ± SD, n = 3. (E) Identification of WT-1 positive cells (green) within cryosections of human atrial tissue biopsies. Cardiomyocytes were stained for cardiac troponin T (red). Scale bar = 50 µm.

hASC and rEPDC (Supp. Fig. S4C). We also found that the variation of the hASC data is higher than the rEPDC dataset, most likely due to the patient inter-individual variability (Supp. Fig. S5). Next, we aligned both datasets which revealed 149 common proteins present in both hASC and rEPDC whereas 90 proteins were exclusive for hASC and 247 proteins were only found in the rEPDC dataset (Supplementary table 1). Bioinformatics functional enrichment analysis of the 149 overlapping proteins identified several significantly enriched GO and pathway terms (Supplementary table 2). As shown in the pie chart of Fig. 4, a large fraction of IDs was annotated to the ECM, cell-cell communication and receptors, enzymes and transporters, angiogenesis/arteriogenesis, immunological processes, cell motility & migration as well as development & differentiation, and finally stress response. A similar analysis was carried out solely for the ECM proteins (Supplementary table 3). Analysis of ECM-subcategories revealed that most of the proteins identified are involved in cell adhesion followed by ECM-receptor interactions, integrins, ECM organization, cell junction organization, collagens or basigin interactions. The 149 overlapping proteins were also subjected to an unbiased functional category enrichment and network analysis which revealed fourteen ontology terms from the KEGG database to be enriched (Supplementary table 4). Nine of these categories could be grouped into a cluster connected by several central integrin family

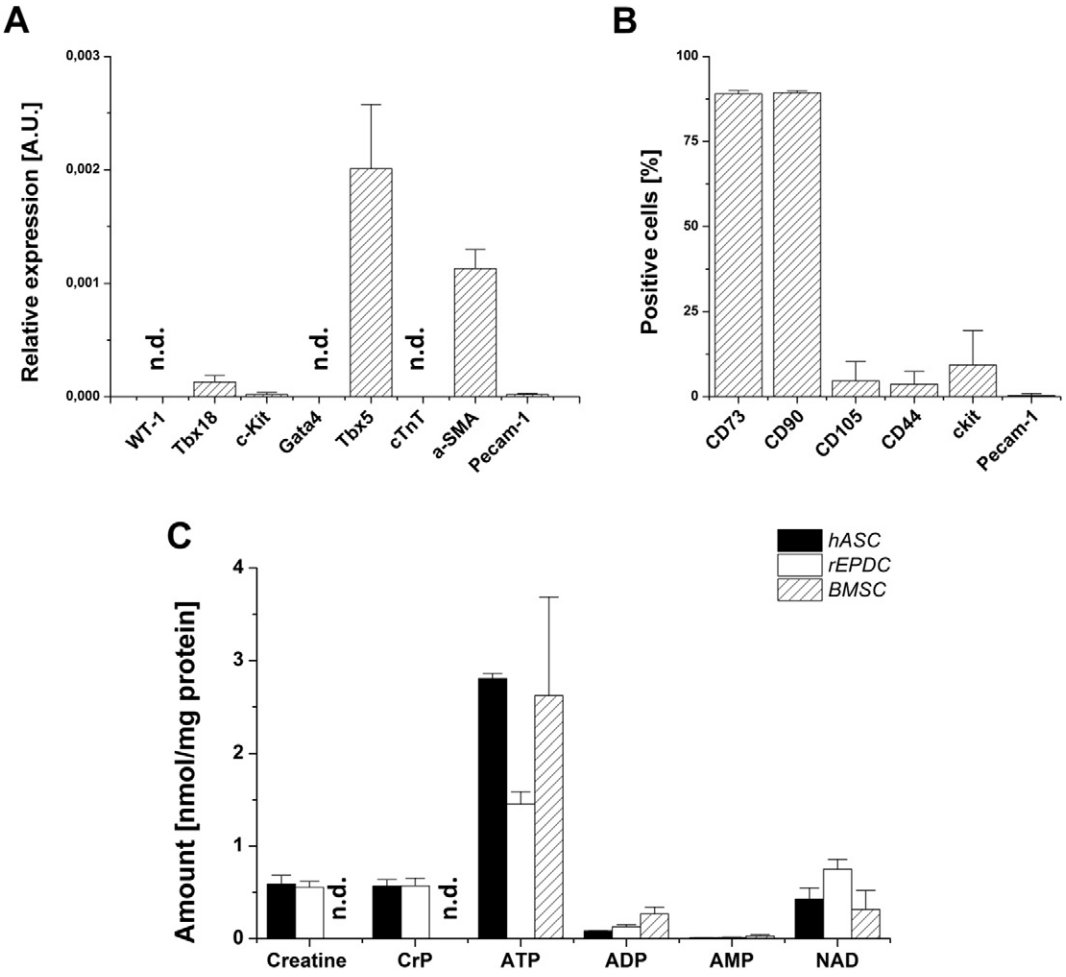


**Fig. 3.** Comparison of ASC with BMSC. (A) Expression profile of cultivated human bone marrow derived stromal cells (BMSC): mRNA transcripts of *Tbx-18*, *c-Kit*, *α-SMA*, *WT-1*, *cTnT* and *Pecam-1* were analyzed. (B) Cell surface expression of the stem/progenitor cell markers CD73, CD90, CD105, CD44, c-Kit and Pecam-1/CD31 as determined by flow cytometry (right). (C) Levels of creatine, phosphocreatine (CrP), ATP, ADP, AMP and NAD were determined by UPLC analysis. Data are mean values ± SD of n = 3–4 individual samples each; n.d. = not detectable.

members that were connected to heart associated ontologies (dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy) (Supp. Fig. S6).

A list of selected functionally relevant proteins is displayed on the bottom of Fig. 4. Among proteins related to migration/differentiation, we found the tyrosine kinase UFO (AXL), the discoidin receptor 2 (DDR2), Plexin-B2, Reticulon-4, CD166, Anthrax toxin receptor, frizzled 2, NELL, basigin or Notch 2. In addition, we identified proteins involved in immune responses like the LPS receptor CD14, attractin, CD276, CD81, OX-2, ICAM-1, Nectin-2 or MHC class I molecules. Several proteins identified are involved in blood vessel development or angiogenesis: EPH receptor A2, EPH receptor B2, Neuropilin-1, aminopeptidase N (CD13), PDGF receptor beta, Integrin α-5, -β-3 or fibronectin.

## 4. Discussion

Here we report the observation that stromal cells isolated from human and rat atria (hASC/rASC) as well as rat epicardium-derived cells (EPDC) isolated from the cardiac surface after myocardial infarction, display a very similar phenotype both morphologically and at the level of key markers of EPDC. Surface proteome analysis revealed a substantial overlap in the expression of various proteins. Together our data suggests that ASC are EPDC-like cells which may have a common developmental origin and a similar potential in cardiac regeneration.

Using enzymatic digestion, isolated rat ventricular EPDC and ASC from rat and human atria uniformly displayed a spindle-shaped appearance and resemble EPDC that have undergone EMT. The majority of the isolated ASC and EPDC expressed EPDC-specific markers (WT-1, Tbx18) and several stem/progenitor cell markers (CD73, CD90). We also found the expression of cardiac genes (Tbx5, Gata4 and cTnT) that all have been previously associated with cardiomyocyte differentiation. The molecular signature of ASC and rEPDC suggests that both cell types retained their embryological origin and, it is therefore likely that both ASC and ventricular EPDC have the potential to contribute to the cardiomyocyte lineage particularly when exposed to a supportive cardiac microenvironment. *In vivo* studies, however, indicate that the cardiomyogenic differentiation potential of rodent ventricular EPDC appears to be rather low (Smart et al., 2011). On the other hand, cardiac atrial appendage stem cells (CASC) have been successfully used in stem cell therapy where differentiation into cardiomyocytes and functional integration of CASC have been observed (Hendrikx et al., 2016). Furthermore, ASC have been shown to improve angiogenesis and may impact on ventricular remodeling possibly by production of ECM and interaction with the local immune system. The cardio-regenerative potential of ASC and EPDC is further supported by our comparative analysis with human bone marrow derived SC (BMSC) which revealed important differences: BMSC did not express WT-1, Gata4 and cTnT and were low in Tbx18. More importantly both hASC and rEPDC contained phosphocreatine (CrP) and creatine (Cr) which was not found in BMSC. CrP is well known to serve as a reserve of high-energy phosphates particularly in cardiac muscle. The presence of Cr/CrP supports the notion that ASC and EPDC are cardiac progenitor cells which are involved in heart regeneration (Masters and Riley, 2014).

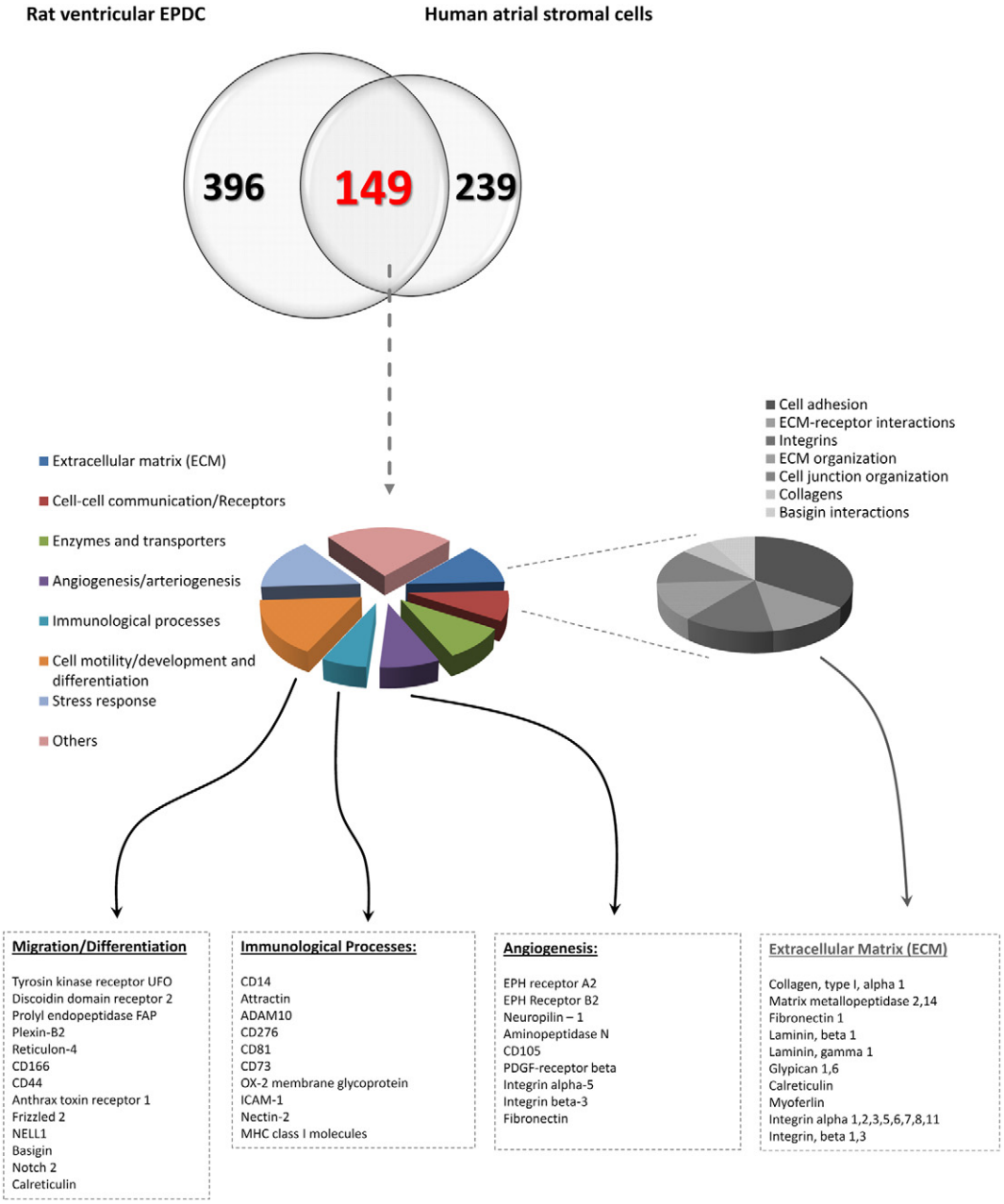


**Fig. 4.** Enriched GO-terms in the overlapping dataset of hASC and rEPDC. Venn diagram showing common cell surface proteins detected in hASC and rEPDC (top). Overlapping proteins were analyzed for biological gene ontology (GO) processes, KEGG pathways and REACTOME. Subsequently proteins associated with ECM (blue sector) were sorted into subcategories (grey pie-chart, right). Distribution of proteins in color-coded or pattern-coded sectors indicates the total number of proteins within each group. A list of manually selected proteins from the categories is shown below. Analysis was based on data obtained from cell surface proteins of three preparations each.

We have recently reported that EPDC release of substantial amounts of IL-6 particularly after adenosine $A_{2B}$ receptor activation (Hesse et al., 2017). Human atrial stromal cells similarly secrete high quantities of IL-6 even in absence of $A_{2B}$ receptor stimulation (unpublished observation). IL-6 has been shown to be critically involved in the generation of fibrosis for example by direct activation of myofibroblasts or *via* CD4 T-cells (Le et al., 2014). Interestingly, IL-6 can directly act on EPDC (Hesse et al., 2017) as well as on hASC (unpublished observation) in an autocrine manner showing that both cell types express the IL6Rα receptor subunit and signaling unit gp130. Mass spectrometry also revealed gp130 but not IL6Rα which might be due to shedding of IL6Rα. IL6 released by hASC is therefore likely to shape the inflammatory response and in addition may be involved in the development of atrial fibrillation. In fact, a recent study identified calreticulin and α5 integrin – both proteins were found to be expressed on hASC and ventricular EPDC – to be increased in atrial tissue from patients with atrial fibrillation (Zhao et al., 2013).

The present study is the first to systematically identify proteins on the surface of rEPDC and hASC showing a large degree of overlap in the expression pattern of biologically important proteins. Knockout studies illustrate the importance of integrins during cardiomyogenesis: *e.g.* mice deficient of α4 integrin – expressed by rEPDC – die by E15.5 and exhibit cardiac defects including absence of epicardium and coronary vessels, and abnormal epicardial progenitor migration (Yang et al., 2010). In addition, embryonic stem cells lacking β1 integrin exhibit a delayed expression of various cardiac markers after the onset of cardiac differentiation, including abnormal specification to atrial, ventricular, and sinusnodal type cells (Fässler et al., 1996). Among the identified receptors, EPH receptors and their ligands, ephrins, represent the largest group of the receptor tyrosine kinase families that impact on many

developmental processes (Pasquale, 2005). The EPH/ephrine system is critical for cardiovascular development and human cardiac stem cells were reported to express the EPH receptor A2 (also found on rEPDC/hASC) which promotes cardiac stem cell migration after infarction (Goichberg et al., 2011). In addition, plexins and neuropilin were identified on rEPDC/hASC which are cell surface receptors for semaphorins that regulate cell-cell interactions and control tissue regeneration (Grieskamp et al., 2011). Our proteomic analysis also revealed a large number of receptors for growth factors (IGF, PDGF, EGF and anthrax toxin) expressed on rEPDC/hASC which already were postulated in a different context to be involved in cardiac and vascular regeneration. A recent report showed that IGFs significantly improved stem cell-mediated cardiac repair (Jackson et al., 2015) and PDGF was also suggested to regulate cardiac repair after infarction (Zhao et al., 2011). EGF was reported to be essential for cardiogenesis and is functionally linked to Gata4 in inducing cardiac differentiation (Ma et al., 2015). Specific stimulation of EPDC/ASC by growth factors may therefore promote vasculogenesis and cardiac repair. Thus, the cell surface proteome repository reported here will help to further unravel the role of EPDC/SC in the cardiac healing/regeneration process.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2017.11.006.

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