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Label-free quantitative proteomics analysis reveals distinct molecular characteristics in endocardial endothelium

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Abstract

Endocardial endothelium, which lines the chambers of the heart, is distinct in its origin, structure, and function. Characterization studies using genomics and proteomics have reported molecular signatures supporting the structural and functional heterogeneity of various endothelial cells. However, though functionally very important, no studies at protein level have been conducted so far characterizing endocardial endothelium. In this study, we used endothelial cells from pig heart to investigate if endocardial endothelial cells are distinct at the proteome level. Using a high-throughput liquid chromatography-tandem mass spectrometry for proteome profiling and expression, we identified sets of proteins that belong to specific biological processes and metabolic pathways in endocardial endothelial cells supporting its specific structural and functional roles. The study also identified several transcription factors and cell surface markers, which may have roles in the specificity of endocardial endothelium. The detection of sets proteins preferentially expressed in endocardial endothelium offers new insights into its role in the regulation of cardiac function. Data are made available through ProteomeXchange with identifier PXD009194.

Keywords Bioinformatics · Endocardial endothelium · Label-free quantitative proteomics · Transcription Factors · Aortic endothelial cells

Introduction

The endothelium, an inner tissue lining of the vasculature, plays a central role in the regulation of hemostasis, vascular tone, tissue growth, capillary exchange, and angiogenesis. Endothelial dysfunction is the hallmark of cardiovascular and other diseases such as atherosclerosis as the integrity of endothelium is essential for cardiovascular homeostasis. Despite having numerous common features, the endothelium displays structural and functional differences related to biochemical and biomechanical signals, according to its position in the cardiovascular system. Structural and functional heterogeneity of various endothelial cells evolved as an early core feature of this cell lineage [1]. The most notable biomechanical diversity is the spatial differences of shear stress along the cardiovascular tree. Likewise, biochemical diversity also can be explained by local intercellular talk of endothelium with neighboring cells through tissue-specific diffusible mediators. Endothelial heterogeneity is an important characteristic of the cardiovascular system, but the molecular mechanisms responsible for this heterogeneity is poorly characterized.

The heart possesses several endothelial compartments, such as endothelial cells of endocardium, valves, arteries, and capillaries. The endocardium which forms the inner cavity of the heart chamber arises from the heart field mesoderm [2]. Endocardial endothelium modulates cardiac muscle performance and growth, has a barrier function, and shows extensive intercellular overlap and a large number of gap junctions. Endocardial endothelial cells represent a unique and molecularly different population of cells rather than a spatially separated population of endothelium. The de novo

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formation of blood vessels (vasculogenesis) in an embryo is contributed by endothelial precursors called hemangioblasts. Several reports suggested that endocardial endothelial cells originated from a specific type of progenitor cells which are different from the hemangioblasts which contribute to other endothelial cell lineages [3]. The abundance of gap junction for sensory function and secretion of a cardioactive substance such as endothelin-1 and nitric oxide are the two major functions reported in endocardial endothelial cells [4]. Identification of specific molecular features of the endocardial endothelium will not only enhance our understanding of cardiac development, and various disease processes but may also provide the potential for site-specific delivery of therapeutic agents.

Proteomics investigations can provide a better understanding of gene expression by the characterization of proteins since they are the major functional molecules in a cell. Proteomics investigations can serve as a platform for focused investigations designed to confirm novel molecular interactions and pathways. Though not in the heart, characterization studies using proteomics on endothelial cells by various groups reported that the endothelium from different vascular beds expressed different sets of proteins [5–7]. Only a few of these publications focused solely on the proteomic characterization of endothelial cells. Significant proteomics experiments have not yet been done on mature endothelial cells derived from the heart. In the present study, we used high-throughput label-free quantification proteomics analysis to study whether endocardial endothelial functional differentiation was reflected at the level of protein expression. We performed a liquid chromatography-tandem mass spectrometry analysis to characterize the endocardial endothelium from the left ventricle of pig heart and compared that with the pig aortic endothelium. This study identified similar as well as differentially expressed proteins between these two endothelial populations, revealing that the endocardial endothelial and aortic endothelial cells express different sets of proteins even when grown under similar culture conditions. Our analysis shows that endocardial endothelial cells in the cardiovascular system are indeed distinctly differentiated cell types with corresponding characteristic protein expression profiles and can offer novel insights into their molecular mechanisms.

Materials and methods

Isolation and culture of endothelial cells

Approval was obtained from the institutional animal ethics committee of Rajiv Gandhi Centre for Biotechnology to collect pig hearts from the local slaughterhouse and to process them. Heart along with aorta from three slaughtered male pigs (*Sus scrofa domesticus*) was collected in phosphate-buffered saline containing 100 U/ml heparin (H3149, Sigma-Aldrich, USA) and antibiotic–antimycotic cocktail (15240, Gibco-Life Technologies, USA). Porcine endocardial endothelial cells and porcine aortic endothelial cells were isolated by an enzymatic method using 0.2% collagenase type-2 (C6885, Sigma-Aldrich, USA) in MCDB 131 (M8537, Sigma-Aldrich, USA). Cells were isolated within 2 h of receiving the sample. Endocardial endothelial cells from freshly collected pig hearts were isolated by the method described by Smith et al. with modifications [8]. After excising the right ventricle and atria, the left ventricle portion was freed from valves, chordae tendineae, and other debris. The left ventricle was filled with 0.2% collagenase type-2 in MCDB 131 and incubated at room temperature for 30 min. The inside of the ventricle was rubbed gently with a cell scraper to remove loosely attached cells. The cell suspension was collected and centrifuged at 2000 rpm for 5 min. The pellet was resuspended in the medium MCDB 131 with 20% FBS (10270, Gibco-Life Technologies, USA) and cultured in a 60×15 mm culture dish (353002, Falcon, USA) at 37 °C, 5% CO_2 until they become confluent. Aortic endothelial cells were also isolated by scraping the internal surface of the aorta and then incubating the cells with 0.2% collagenase type-2 in MCDB 131 [9]. After a 20 min incubation time at room temperature, the cell suspension was collected and centrifuged at 2000 rpm for 5 min. The pellet was resuspended and cultured in MCDB 131 with 20% FBS. Once the cells reached confluence, they were harvested and kept at − 80 °C as cell pellets until proteomic analysis.

Protein sample preparations

Protein profiling and relative quantification analysis were done by Liquid Chromatography-tandem mass spectrometry (LC/MS/MS). Proteins were extracted from the cell pellets by cell homogenization, and cell lysis using RapiGest™ SF surfactant (Waters) in 50 mM ammonium bicarbonate. The protein concentration of each sample was estimated by performing bicinchoninic acid assay (BCA assay). Approximately 100 µg of proteins from each sample was subjected to in-solution trypsin digestion to generate peptides. The protein disulfide bonds were reduced by treating the sample with 5 µl of 100 mM DL-Dithiothreitol in 50 mM ammonium bicarbonate for 30 min at 60 °C and alkylated with 200 mM Iodoacetamide in 50 mM ammonium bicarbonate at room temperature for 30 min in the dark. Proteins were then digested using trypsin, sequence grade, modified (Sigma) in 50 mM ammonium bicarbonate by incubating overnight at 37 °C. The trypsin digestion reaction was stopped by adding 1 µl of 100% formic acid. The digested peptide solutions were centrifuged at 14,000×*g* rpm for 12 min, and the

collected supernatant was stored at −20 °C until the LC/MS/MS analysis [10].

Liquid chromatography

The peptide samples were analyzed by nano-LC–MS^E (MS at elevated energy) using a nanoACQUITY UPLC® System (Waters, Manchester, UK) coupled to a Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (SYNAPT-G2, Waters). Both the systems were operated and controlled by MassLynx4.1 SCN781 software.

Briefly, 3 µl of sample was injected in partial loop mode and was loaded into the reverse phase column with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B using the binary solvent manager. The sample is then trapped in the trap column (Symmetry® 180 µm × 20 mm C$_{18}$ 5 µm, Waters) to remove any salts by employing a high flow rate (15 µl/min) with 99.9% mobile phase A and 0.1% mobile phase B for 1 min. The peptide separation was performed on a 75 µm × 100 mm BEH C$_{18}$ Column (Waters), with a particle size of 1.7 µm. A gradient elution of 1–40% mobile phase B, for 55.5 min at 300 nl/min flow rate was employed. After separation, the column was washed with 80% mobile phase B for 7.5 min and re-equilibrated with 1% mobile phase B for 20 min. The column temperature was maintained at 40 °C. Three technical replicate runs were done for each sample.

Mass spectrometry

Mass spectrometric analysis of eluting peptides from the nano-LC was performed on an SYNAPT® G2 High-Definition MS™ System (HDMS^E System, Waters). It is a hybrid, quadrupole, ion mobility, orthogonal acceleration, time-of-flight mass spectrometer controlled by MassLynx4.1 SCN781 (Waters Corporation, Milford, MA, USA) software. The system combines exact-mass, high-resolution mass spectrometry with high-efficiency ion mobility-based measurements and separations (IMS–MS).

The parameters used were the following: nano-ESI capillary voltage −3.3 KV, sample cone −35 V, extraction cone −4 V; transfer CE −4 V, trap gas flow −2 (ml/min), IMS gas (N$_2$) flow −90 (ml/min). To perform the mobility separation, the IMS T-Wave™ pulse height is set to 40 V during transmission, and the IMS T-Wave™ velocity was set to 800 m/s. The traveling wave height was ramped over 100% of the IMS cycle between 8 and 20 V. The time-of-flight analyzer (TOF) was calibrated with a solution of 500 fmole/µl of [Glu^1]-Fibrinopeptide B human (Sigma). The *lock mass* acquisition was performed at every 30 s by the same peptide delivered through the reference sprayer of the nanoLockSpray source at a flow rate of 500 nl/min. This calibration step set the analyzer to detect ions in the range of 50–2000 m/z.

The mass spectrometer was operated in resolution mode with a resolving power of 18,000 FWHM, and the data acquisition was acquired in Continuum format. The data were acquired by rapidly alternating between two functions—Function-1 (low energy) and Function-2 (high energy). In Function-1, we acquire only low energy mass spectra (MS), and in Function-2, we acquire mass spectra at elevated collision energy (MS^E) with ion mobility. In Function-1, collision energy was set to 4 V in the Trap region and 2 V in the Transfer region. In Function-2, collision energy was set to 4 V in the Trap region and is ramped from 20 to 45 V in the Transfer region. Each spectrum was acquired for 0.9 s with an interscan delay of 0.024 s. The mass spectrometry-based proteomics data have been uploaded in the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009194.

Data analysis

Endothelial cells were collected from three pigs and cultured independently: each of the cells of the endocardium and aorta (three biological replicates). The mass spectrometry analyses were repeated three times for each isolate (three technical replicates), giving rise to a total of 18 proteomics experiments. The LC-MS^E data were analyzed by using Progenesis QI for Proteomics (Non-linear Dynamics, Newcastle upon Tyne, UK) for protein identification as well as for the relative protein quantification. Data processing includes lock mass correction at post-acquisition. The database for Sus scrofa (pig) was downloaded from NCBI. During database search, the protein false positive rate was set to 1%. The parameters for protein identification was made in such a way that a peptide was required to have at least one fragment ion match, the protein was required to have at least three fragment ion matches, and protein was required to have at least one peptide match for identification. Oxidation of methionine was selected as variable modification, and cysteine carbamidomethylation was selected as a fixed modification. Trypsin was chosen as the enzyme used with a specificity of 1 missed cleavage. Datasets were normalized using the automatic sensitivity method implemented in Progenesis. The protein dataset was filtered by considering only those identified proteins which have at least 2 matched peptides in any one of the technical replicates. The normalized intensity values obtained for commonly identified proteins from 3 biological replicates were used for differential expression analysis by t-test with a false discovery rate (FDR) < 0.05 and the fold change higher than 2. Box and whiskers plot was done with MetaboAnalyst 3.0 platform which is a widely used online platform for metabolomics studies [11].

Bioinformatics analysis

The bioinformatics analysis was done in a stepwise manner, which is briefly described as follows. The Reference Sequence (RefSeq) IDs obtained after the Progenesis QI for proteomics search were converted into gene symbol. The ID conversion was done by online ID conversion tool called Biological Database Network (bioDBnet) [12]. To identify the pathways associated with protein list, online tool called Reactome pathway knowledge base was used [13]. Enriched pathways with the p value ≤ 0.05 are considered significant. The enriched signaling pathways and the corresponding proportion of proteins were used for representation purpose. Cell differentiation markers and transcription factors present in the gene list were identified by Gene set enrichment analysis (GSEA) method. GSEA is a freely available online software tool used for the interpretation of gene expression data. GSEA platform includes a collection of annotated gene sets called Molecular Signatures Database (MSigDB). To categorize the gene set into different gene families including cell

differentiation markers and transcription factors, MSigDB was used [14].

Results

Proteome profile and expression differences between the endothelial cells

A total of 1234 proteins were identified altogether, showing highest number of proteins (1017) for the Aortic endothelium, and 882 proteins for the endocardial endothelium. The distribution of proteins among the two endothelial cell types was shown in a Venn diagram (Fig. 1a). 665 proteins were common for both aortic and endocardial endothelium. Two hundred and seventeen and 352 proteins were unique for endocardial and aortic endothelium, respectively. The proteins found unique in endocardial endothelium are listed in Supplementary Table 1. The principal component analysis
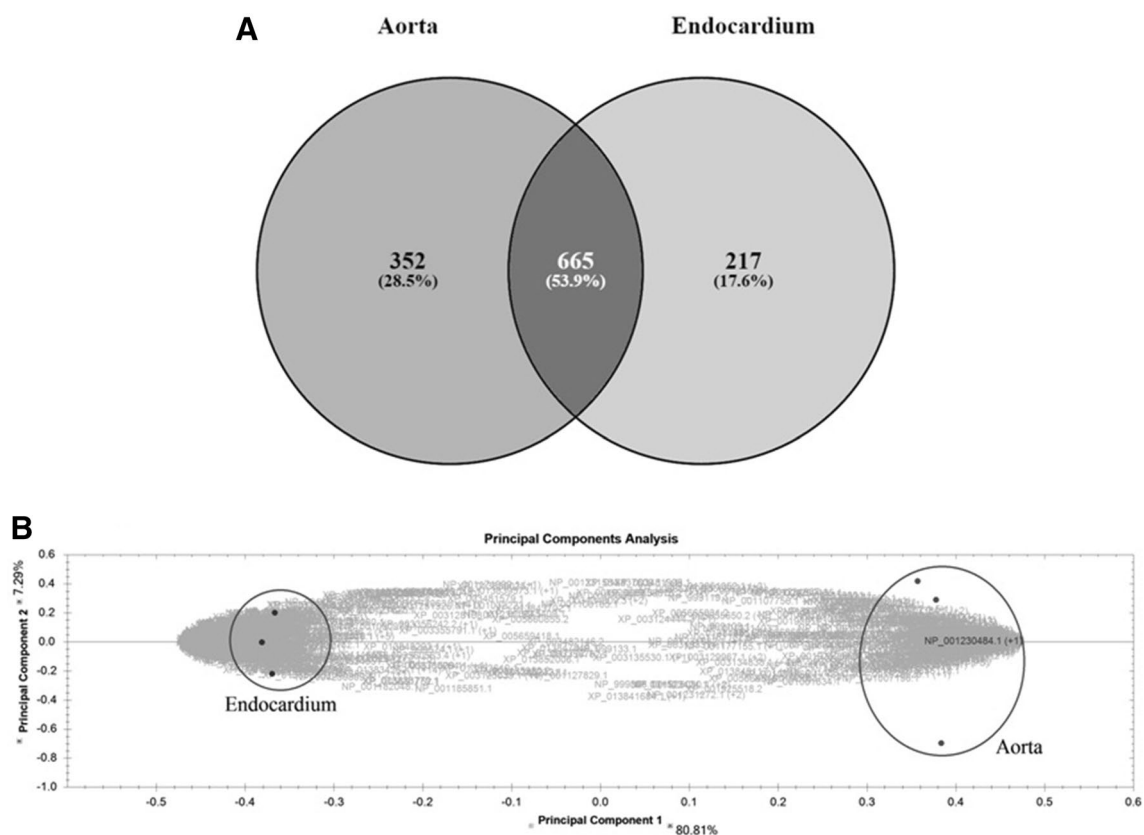

Fig. 1 Venn diagram **a** showing the distribution of all the proteins identified from endothelial cells of the endocardium, and aorta of a pig heart. A total of 1234 proteins were identified altogether, having 1017 proteins for aortic endothelium, and 882 for the endocardial endothelium. The distribution shows 665 proteins common for both aortic and endocardial endothelium and 217 and 352 proteins

unique for endocardial and aortic endothelium, respectively. Principal component analysis (PCA) score plot. **b** Showing the separation of endothelial and aortic samples by Progenesis QI for proteomics analysis. Triplicate data acquired from a single biological replicate were used for the representation purpose

of mass spectrometry data shows a significant difference in aortic and endocardial endothelial samples (Fig. 1b).

The commonly found endothelial proteins were compared between vascular beds for their protein expression (relative protein quantification). We found that the level of expression of a considerable number of the proteins was different in endocardial endothelial cells when compared to aortic endothelium. Proteins which show a twofold or greater (FDR < 0.5) difference in endocardial endothelium when compared to aortic endothelium are listed in Supplementary Table 2. Noticeably, proteins such as PROCR, CAV1, VDAC2, and PTGIS (Fig. 2) were significantly altered in endocardial endothelium.

Analysis of biological classifications

Reactome pathway analysis of the identified proteins in both endocardial and aortic endothelial cells was performed to search for the associated signaling pathways.

This analysis predicted 43 common signaling pathways in both the endothelial cells and from this 18 and 40 pathways were specific to endocardial and aortic endothelial cells, respectively (data not shown). The top 10 signaling pathways (Fig. 3) such as cellular responses to stress, hemostasis, signaling by Rho GTPases, signaling by Wnt, signaling by VEGF, MAPK family signaling cascades, programmed cell death, insulin receptor signaling cascade, IRS-mediated signaling, and prolonged ERK activation events were enriched in both types of endothelial cells (Fig. 3). Noticeably, pathways such as cellular responses to stress, hemostasis, signaling by VEGF, MAPK family signaling cascades, cellular response to hypoxia, and response to elevated platelet cytosolic Ca^{2+} were highly enriched in endocardial endothelial cells. Similarly, platelet activation, signaling, and aggregation; the formation of the cornified envelope; regulation of HSF1-mediated heat shock response; detoxification of reactive oxygen species; and ion homeostasis were uniquely enriched in endocardial endothelium as well (Fig. 3).

Fig. 2 Box and Whiskers plot of upregulated proteins identified in endocardial endothelial cells compared to aortic endothelial cells. Peptide intensities from the LC/MS/MS data of each endothelial protein sample ($n = 3$) were used to create the plot by MetaboAnalyst 3.0. The differences shown between the endothelial cells were significant ($p < 0.05$)
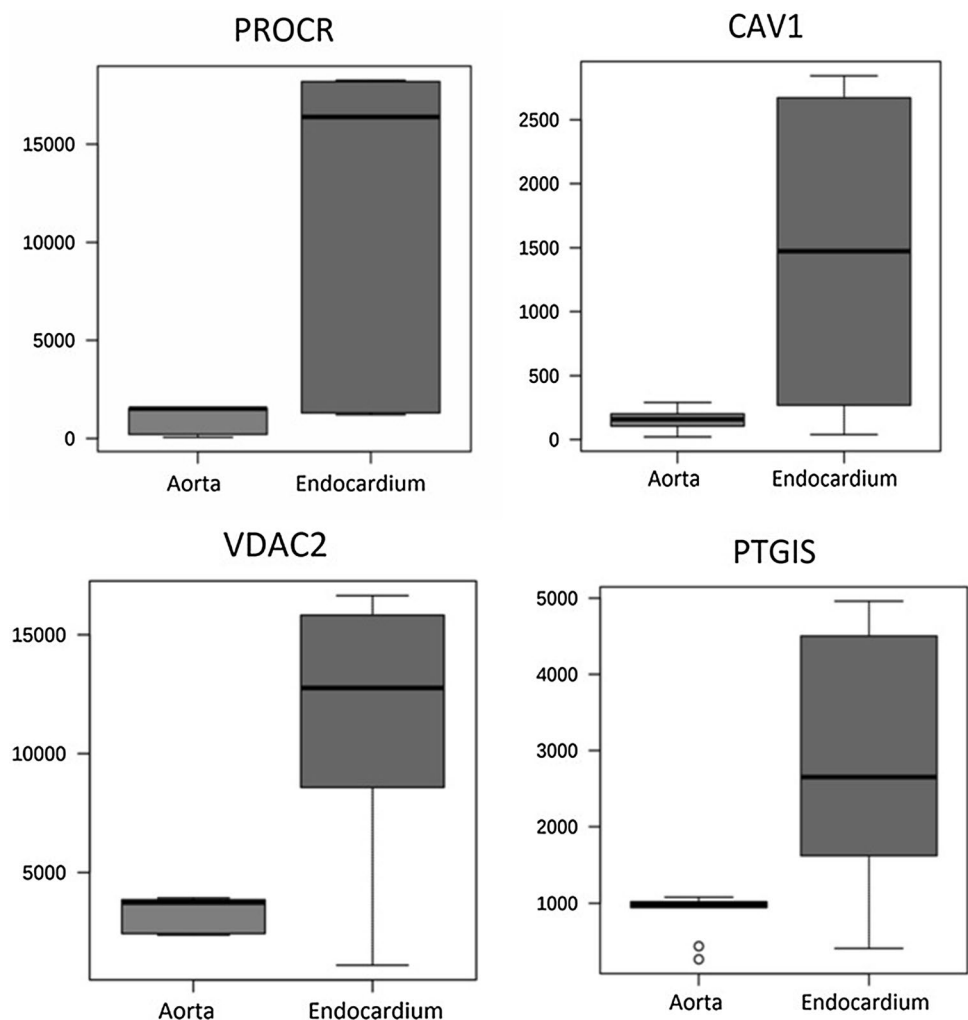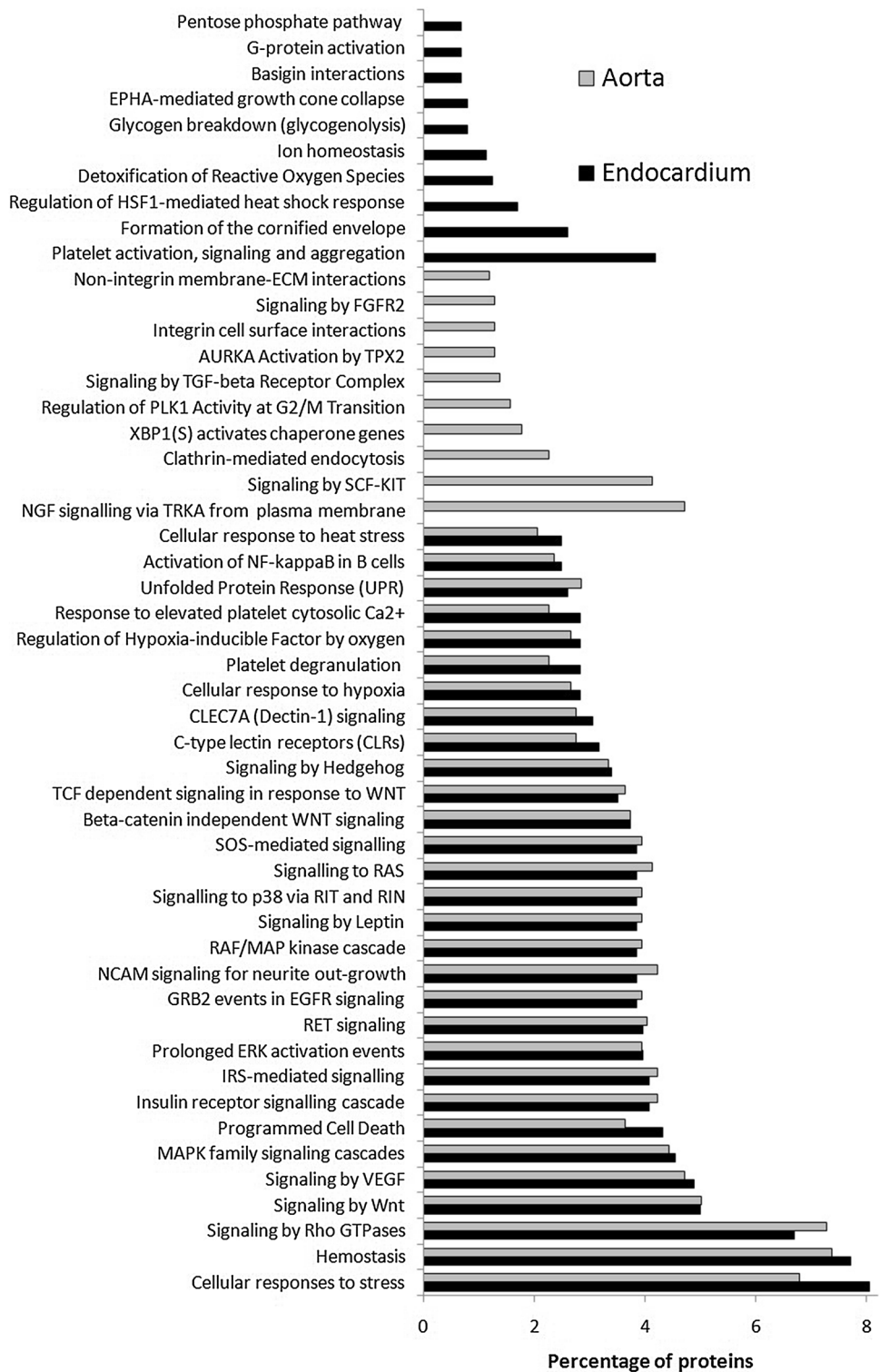
Fig. 3 Signaling pathways enriched in the proteome of aortic and endocardial endothelial cells by reactome analysis. Figure shows top 10 unique and 30 common pathways having significant ($p < 0.05$) enrichment in both endocardial and aortic endothelium

Transcription factors and cell surface markers in endocardial endothelium

Transcription factors and cell differentiation markers were identified from the LC/MS/MS data by performing Gene set enrichment analysis (GSEA) using Molecular Signature Database (MSigDB). Total of 26 and 22 transcription factors were identified from endocardial and aortic endothelium, respectively (Table 1). Transcription factors including CAND1, CCT4, CSRP1, ENO1, FHL1, FUBP1, ILF2, ILF3, MYBBP1A, PDLIM5, PSMC5, SND1, SUB1, and YBX1 were observed in both

Table 1 Transcription factors in Endocardial and Aortic endothelial cells

Gene symbol	Description	Peptide count (maximum)	Unique peptides (maximum)
Endocardial endothelium			
CAND1	Cullin-associated NEDD8-dissociated protein 1	4	4
CCT4	T-complex protein 1 subunit delta	11	9
CNBP	Cellular nucleic acid-binding protein	2	2
CSRP1	Cysteine and glycine-rich protein 1	3	3
ENO1	Alpha-enolase	28	22
FHL1	Four-and-a-half LIM domains protein 1	7	6
FHL2	Four-and-a-half LIM domains protein 2	3	3
FUBP1	Far upstream element-binding protein 1	4	3
GTF2I	General transcription factor II-I	3	2
HMGB1	High mobility group protein B1	7	3
HMGB2	High mobility group protein B2	5	3
HMGN2	Non-histone chromosomal protein HMG-17	3	3
ILF2	Interleukin enhancer-binding factor 2	8	7
ILF3	Interleukin enhancer-binding factor 3	11	10
LARP1	La-related protein 1	6	2
MYBBP1A	Myb-binding protein 1A	15	12
PDLIM5	PDZ and LIM domain protein 5	7	7
PSMC5	26S protease regulatory subunit 8	5	3
PTTG1IP	Pituitary tumor-transforming gene 1 protein-interacting protein	2	2
SND1	Staphylococcal nuclease domain-containing protein 1	13	12
STAT1	Signal transducer and activator of transcription 1	6	6
SUB1	Activated RNA polymerase II transcriptional coactivator p15	3	2
TRIM25	E3 ubiquitin/ISG15 ligase TRIM25	3	3
TRIP11	Thyroid receptor-interacting protein 11	6	5
YBX1	Nuclease-sensitive element-binding protein 1	5	5
ZNF185	Zinc finger protein 185	3	3
Aortic endothelium			
ADNP2	ADNP homeobox protein 2	6	3
ANP32A	Acidic leucine-rich nuclear phosphoprotein 32 family member A	3	2
BCLAF1	Bcl-2-associated transcription factor 1	3	2
BTF3	Transcription factor BTF3	3	3
CAND1	Cullin-associated NEDD8-dissociated protein 1	3	3
CBX3	Chromobox protein homolog 3	3	3
CCT4	T-complex protein 1 subunit delta	12	10
CSRP1	Cysteine and glycine-rich protein 1	5	5
ENO1	Alpha-enolase	26	20
FHL1	Four-and-a-half LIM domains protein 1	7	9
FUBP1	Far upstream element-binding protein 1	7	6
ILF2	Interleukin enhancer-binding factor 2	8	8
ILF3	Interleukin enhancer-binding factor 3	17	14
LIMA1	LIM domain and actin-binding protein 1	4	2
LSR	Lipolysis-stimulated lipoprotein receptor	3	2
MYBBP1A	Myb-binding protein 1A	14	12
PDLIM5	PDZ and LIM domain protein 5	5	3
PSMC5	26S protease regulatory subunit 8	6	5
SF1	Splicing factor 1	2	2
SND1	Staphylococcal nuclease domain-containing protein 1	20	17
SUB1	Activated RNA polymerase II transcriptional coactivator p15	4	4
YBX1	Nuclease-sensitive element-binding protein 1	7	5

Transcription factors were identified by Gene Set Enrichment Analysis using Molecular Signatures Database. The table shows the number of matched peptides, as well as the unique peptide, obtained corresponding to each protein by LC/MS/MS analysis

endothelial cells. Transcription factors such as CNBP, FHL2, GTF2I, HMGB1, HMGB2, HMGN2, LARP1, PTTG1IP, STAT1, TRIM25, TRIP11, and ZNF185 were uniquely observed in endocardial endothelial cells. Similarly, ADNP2, ANP32A, BCLAF1, BTF3, CBX3, LIMA1, LSR, and SF1 were observed only in the aortic endothelial cells. Among the observed cell differentiation marker proteins BSG, ENG, ITGA5, ITGB1, PROCR, SLC3A2, TFRC, and THY1 were observed in both endocardial and aortic endothelial cells (Table 2). Cell differentiation markers such as ANPEP, CD63, IL18RAP, and PECAM1 were uniquely enriched in endocardial endothelial cells, and CD44, ITGAV, LY75, TLR9, and VCAM1 were only observed in aortic endothelial cells as well.

Discussion

In this study, we described the endocardial endothelial cells diversity in comparison with aortic endothelium at the protein expression level in a high-throughput manner, which are in agreement with its established morphological and functional role [15, 16]. The differentially expressed proteins and the enriched pathways suggest the existence of intrinsic cell specificity in endocardial endothelium as compared to aortic endothelium.

The mechanistic and functional specificity of endocardial endothelial cells can be understood by the interpretation of uniquely and differentially expressed proteins. For example, endothelial protein C receptor (PROCR) was highly upregulated in endocardial endothelial cells. PROCR plays a critical role in preventing thrombus formation on the surface of endothelium and also mediates other cytoprotective

Table 2 Cell differentiation markers in endocardial and aortic endothelial cells

Gene symbol	Description	Peptide count (maximum)	Unique peptides (maximum)
Endocardial endothelium			
ANPEP	Aminopeptidase N (CD13)	9	9
BSG	Basigin precursor (CD147)	9	8
CD63	CD63 antigen (CD63)	5	5
ENG	Endoglin (CD105)	13	11
IL18RAP	Interleukin-18 receptor accessory protein (CD281b)	4	2
ITGA5	Integrin alpha-5 (CD49)	12	11
ITGB1	Integrin beta-1 precursor (CD29)	10	13
PECAM1	Platelet endothelial cell adhesion molecule (CD31)	15	13
PROCR	Endothelial protein C receptor (CD201)	10	10
SLC3A2	4F2 cell-surface antigen heavy chain (CD98)	3	3
TFRC	Transferrin receptor protein 1 (CD71)	8	7
THY1	Thy-1 membrane glycoprotein (CD90)	2	2
Aortic endothelium			
BSG	Basigin precursor (CD147)	11	9
CD44	CD44 antigen isoform X7 (CD44)	5	5
ENG	Endoglin precursor (CD105)	9	7
ITGA5	Integrin alpha-5 (CD49)	10	9
ITGAV	Integrin alpha-V precursor (CD51)	4	2
ITGB1	Integrin beta-1 precursor (CD29)	15	13
LY75	Lymphocyte antigen 75 precursor (CD205)	2	2
PROCR	Endothelial protein C receptor precursor (CD201)	6	5
SLC3A2	4F2 cell-surface antigen heavy chain (CD98)	3	3
TFRC	Transferrin receptor protein 1 (CD71)	8	8
THY1	Thy-1 membrane glycoprotein precursor (CD90)	2	2
TLR9	Toll-like receptor 9 isoform X1 (CD289)	4	12
VCAM1	Vascular cell adhesion protein 1 precursor (CD106)	4	4

Cell differentiation markers were identified by Gene Set Enrichment Analysis using Molecular Signatures Database. The table shows the number of matched peptides, as well as the unique peptide, obtained corresponding to each protein by LC/MS/MS analysis

effects by activating protein C [17]. Similarly, caveolin-1 (CAV1) was found to be upregulated in endocardial endothelium. Caveolin-1 is a plasma membrane caveolae protein, regulates the NO production by interacting with endothelial nitric oxide synthase [18]. Prostacyclin synthase (PTGIS) involved in the conversion of prostaglandin H2 to prostacyclin, a potent inhibitor of platelet aggregation and vasodilator, was also upregulated in the endocardial endothelium. Differential expression PTGIS in a different region of endocardium due to hemodynamic shear stress response was also reported [19]. Transport of ATP, phosphocreatine, and calcium between cytoplasm and mitochondrial compartments mainly mediated through voltage-dependent anion channels (VDACs). Recent evidence shows that alternate splice variant of VDACs are present in the plasma membrane as well. VDAC1 has been identified as a novel binding partner for eNOS in the systemic circulation and modulates its activity [20]. Both VDAC1 and VDAC2 were also upregulated in endocardial endothelial cells.

Interestingly, pathway analysis of our data shows the enrichment of stress response-related proteins in endocardial endothelium as compared to the aorta. Hemodynamic wall shear stress-dependent change in the ventricular endothelial gene expression pattern by transcriptome analysis reported the role of stress response in endocardial endothelial cell gene expression [21]. Endothelial cells mediate the haemostasis function by taking part in different functions such as synthesis, storage, and release of different vasoconstrictors, anticoagulants, vasodilators, fibrinolytic proteins, and procoagulants. Proteins related to haemostasis function have enriched almost equal in both types of the endothelial cell [22]. Signaling by VEGF is another notable pathway enriched in endocardial and aortic endothelial cells. Recently Wu et al. reported that ventricular endocardial cells are the major source of coronary artery endothelium, VEGF signaling is essential for this differentiation [23].

The enrichment of shared transcription factors in both endothelial cells indicates that their combinatorial control is regulated by multiple transcription factors, rather than a specific transcription factor, which might determine endothelial specificity [24, 25]. We have also identified 12 cell surface markers for endocardial endothelium in this study. Surface markers are of great interest in the case of the endocardial endothelium as the potential target for detection. For instance, Endoglin (ENG) is already reported as cell surface marker of vascular and endocardial endothelium [26]. Platelet endothelial cell adhesion molecule isoform X1 (PECAM-1, CD31) is a single chain transmembrane protein, which mediates adhesive interactions between adjacent endothelial cells as well as between leukocytes and endothelial cells. PECAM-1 is one of the earliest adhesion molecules expressed by developing endothelial cells [27]. The common cell surface markers are present on endothelial cells, supporting the idea of a common embryonic precursor, but in rare cases, some markers are rather exclusive for each endothelial cell types [28]. However, more studies are required to establish the specificity of the markers found in this study for the endocardial endothelium.

Endocardial and aortic endothelial cells are derived from different progenitor cells such as cardiogenic mesoderm and hemogenic mesoderm [29]. The uniquely identified transcription factors and cell surface markers in both endothelial cells further confirm this disparity in the developmental origins. Endocardial endothelial cells make the blood–heart barrier and modulate the function of cardiomyocytes by its endocrine and sensory roles. In addition, endocardial endothelial cells modulate the function of cardiomyocytes by secreting signaling molecules such as endothelin, nitric oxide, angiotensin-II, and prostaglandins. Angiopoietin, neuregulin, vascular endothelial growth factor (VEGF), and fibroblast growth factor are the other mediators produced by endocardial endothelial cells [30, 31]. We have identified several proteins in endocardial endothelium, which are relevant to signaling and modulation of cardiomyocytes in our study.

In conclusion, this study, for the first time, characterizes the proteome of endocardial endothelium and further establishes the fact of molecular diversity of endothelial cells of different location and origin. This diversity was preserved even when they are taken out and cultured away from their normal physiological environment. Our results support the notion that there is intrinsic and specific diversity that exists in endocardial and aortic endothelial cells. Data also support the various modulatory roles of endocardial endothelium on cardiac function.

Compliance with ethical standards

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