# Proteomic analysis of cardiac ventricles: baso-apical differences

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#### Abstract

The heart is characterized by a remarkable degree of heterogeneity. Since different cardiac pathologies affect different cardiac regions, it is important to understand molecular mechanisms by which these parts respond to pathological stimuli. In addition to already described left ventricular (LV)/right ventricular (RV) and transmural differences, possible baso-apical heterogeneity has to be taken into consideration. The aim of our study has been, therefore, to compare proteomes in the apical and basal parts of the rat RV and LV. Two-dimensional electrophoresis was used for the proteomic analysis. The major result of this study has revealed for the first time significant baso-apical differences in concentration of several proteins, both in the LV and RV. As far as the LV is concerned, five proteins had higher concentration in the apical compared to basal part of the ventricle. Three of them are mitochondrial and belong to the "metabolism and energy pathways" (myofibrillar creatine kinase M-type, L-lactate dehydrogenase, dihydrolipoamide dehydrogenase). Myosin light chain 3 is a contractile protein and HSP60 belongs to heat shock proteins. In the RV, higher concentration in the apical part was observed in two mitochondrial proteins (creatine kinase S-type and proton pumping NADH:ubiquinone oxidoreductase). The described changes were more pronounced in the LV, which is subjected to higher workload. However, in both chambers was the concentration of proteins markedly higher in the apical than that in basal part, which corresponds to the higher energetic demand and contractile activity of these segments of both ventricles.

Keywords  $Proteomics \cdot Heart \cdot Ventricle \cdot Myocardial heterogeneity \cdot Two-dimensional electrophoresis \cdot Ventricular myocardium$ 

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# Introduction

The heart is characterized by a remarkable degree of heterogeneity [1], the basis of which is a subject to active investigation. Since different cardiac pathologies affect different cardiac regions, it is important to understand the molecular mechanisms by which these regions respond to pathological stimuli. Important dissimilarities between the two ventricles can already be found in the early phases of ontogenetic development. The right ventricle (RV) and the RV outflow tract are derived from the anterior heart field, whereas the left ventricle (LV) and atria are derived from the primary heart field [2]. Transcription factors such as HAND1 and HAND2 appear to play an important role in chamber-specific heart formation [3]. RV and LV free-wall thickness and force development are equal throughout fetal life. After lung expansion and clamping of the umbilical cord at birth, the peak systolic pressure in the LV and the systemic vascular resistance rise, while RV pressure, pulmonary artery



pressure and pulmonary vascular resistance decrease. The increased workload resulting from these circulatory changes is considered to be the stimulus for a more rapid growth of the LV [4, 5]. The RV and LV of the adult mammalian heart work in series to deliver oxygenated blood to the body. Although both have identical cardiac outputs, their afterload pressures differ significantly (25 vs 120 mmHg); the workload ratio of the left to right heart approaches 5 [6]. The reported ratios of LV to RV mass range between 3.4 [7] and 2.6 [8] and are not proportional to the fivefold difference in work. This discrepancy suggests that in addition to workload there are other underlying differences between the ventricles that determine LV/RV mass, including architecture, contractile properties, and metabolic activities [6]. In contrast to the ellipsoidal shape of the LV, the RV appears triangular when viewed in cross section. The RV wall is mainly composed of superficial circumferential and longitudinally oriented deep muscle layers; the LV contains obliquely oriented myofibers superficially, longitudinally oriented myofibers in the subendocardium, and predominantly circular fibers in between. This arrangement contributes to the more complex movement of the LV, which includes torsion, rotation, translation, and thickening [9]. Oxygen consumption in the resting RV is less than that in LV [10, 11], implying that the metabolic stress (ATP generation rate/maximum ATP generation rate) of the RV is lower, based on a wet weight comparison. Additionally, oxidative capacity in the LV is apparently more sensitive to hypoxia than RV [12]. Furthermore, the RV has a higher concentration of collagenous proteins [13] and higher activity of aerobic glycolytic metabolism than LV [14]. Moreover, several reports have demonstrated significant right/left differences in myocardialsusceptibility to various insults: RV is more resistant to ischemia-induced injury [15] as well as to anthracycline toxicity as compared to LV [16].

To add insight into the underlying mechanisms of differences between the LV and RV, great effort has been put into gene expression analyses of LV and RV [17, 18]. However, such analysis has yet to be performed at the proteomic level: proteomic technology allows us to examine global differences in protein expression and asses the posttranslational modification status of LV and RV; McGregor reviewed over 5000 proteins in both ventricles [19]. So far there are only few reports dealing with the comparison of protein profiling in the RV and LV. Comunian et al. proposed the first comparative characterization of LV and RV using multidimensional protein identification in mice; they have observed significant quantitative differences in the representation of individual proteins [20]. Special attention was paid to the transmural differences in the RV and LV (epi-endocardial). It has been observed that some histochemical changes as well as ultrastructural picture revealed significant transmural differences [21, 22]. The first attempt to assess chamber-specific transmural heterogeneity in myofilament protein phosphorylation by top-down mass spectrophotometry was done by Gregorich et al. [23]. They have observed that phosphorylation of cardiac troponin I and T vary in the two chambers; however, no significant transmural differences were observed in the phosphorylation of the myofilament proteins analyzed.

During development, the differences in gene expression in the ventricles were studied by Krejci et al. in the chick embryo. While the number of differentially expressed genes increased during the septation, the general pattern seemed to be that the right ventricle is lagging about 2 days behind the left ventricle [24].

In addition to the transmural heterogeneity, possible basoapical differences, observed already during embryonic life, have to be taken into consideration. Differential growth of regions within the ventricular free walls is important for molding of the entire organ [25]. Two proliferative centers were described in the apical parts of RV and LV [26, 27] and later confirmed by de Boer et al. [28]. Furthermore, there is a recognized difference in left ventricular wall thickness between the apex and the base [29], present already at the early developmental stage [30]. It was hypothesized that this difference is due to differential loading stemming from the ventricular activation sequence from apex to base, due to arrangement of the cardiac conduction system; the early-activated thinner apical myocardium is contracting against less resistance than the thicker late-activated basal myocardium [31]. The data about the possible baso-apical differences in the protein composition of individual ventricles are, however, still lacking. The aim of our study was, therefore, to compare proteomes in the apical and basal parts of the RV and LV.

### **Materials and methods**

#### Sample preparation

Adult male Wistar rats (n=5) 3 months old  $(484 \pm 26 \text{ g body})$  wt) were used in this experiment. Rats were killed by cervical dislocation, and their hearts were dissected and washed in ice-cold saline. Free LV and RV walls were separated and divided transversally into three parts: basal, middle, and apical. Samples taken from basal and apical parts were frozen and stored in liquid nitrogen until use (Fig. 1; Supplement Table 1).

#### **Extraction of proteins**

Lyophilized transmural samples (0.5 mg dry weight (ca  $2 \text{ mm}^3$ )) were homogenized, and subjected to sonication (water basin) in 0.5-ml tube (15 min, 20 °C) in 135 mL of



**Fig. 1** Four-chamber view of a 3-month-old male rat heart arrested in diastole. The squares show the apical sampling sites while the circles indicated the sampling sites for the basal specimens. LA left atrium, LV left ventricle, RA right atrium, RV right ventricle. Scale bar 5 mm

lysis buffer (7 mol/L urea, 2 mol/L thiourea, 2% 3-((3-cholamidopropyl)dimethylammonium)-1-propanesulfonate (CHAPS, w/v), 0.6% Bio-Lyte® ampholytes (3–10 buffer, w/v), 1% dithiothreitol (w/v)), Bio-Rad, Hercules, USA), and centrifugation (1000 g, 20 °C, 3 min). The supernatant was taken for subsequent 2-DE analysis (analyses were performed separately (n=5) with replication in case of inconsistent spots, samples were not pooled).

# Separation of proteins by two-dimensional electrophoresis (2-DE)

Isoelectric focusing and separation by 2-DE were performed on homogeneous 11% SDS–polyacrylamide gel as described previously [32]. An appropriate amount of the supernatant (125  $\mu$ L for a 7-cm strip) was transferred to Ready Strip<sup>TM</sup> IPG Strips (pH range 3–10 NL, Bio-Rad) overnight by active in-gel rehydration (50 V, 15 °C). Isoelectric focusing

was carried out at 15 °C with a Protean® IEF cell system (Bio-Rad) under mineral oil. Proteins were focused until 28 kVh per strip was reached. Prior to separation in the second dimension, the strips were equilibrated and transferred to a homogeneous 11% SDS-polyacrylamide gel. Finally, 5 µL of Precision Plus Protein<sup>TM</sup> Standards (molecular weight range 10-250 kDa, Bio-Rad) was added at the top end of the gel. Mini gels were run in the Mini-Protean Tetra Cell system (5 min for 50 V and then 45 min for 250 V). Gels were stained with colloidal Coomassie brilliant blue stain (Bio-Safe, Bio-Rad) according to the manufacturer's recommendations to enable the visual detection of the various abundances in the spots. Spot normalized volume was used to select statistically significant differential spots  $(n=5, \dots, n=5)$ samples were not pooled) by PDQuest Advanced (Bio-Rad). Protein levels showing significant quantitative differences (Student's t test,  $p \le 0.05$ ) were selected for mass spectrometric analysis.

# Identification of proteins—nano-liquid chromatography-tandem mass spectrometry (nLC-MS/MS)

Spots with differential expression were excised from the Coomassie-stained gels and then processed as described previously [33]. The separation of peptides was achieved via a linear gradient between mobile phase A (water) and B (acetonitrile), both containing 0.1% (v/v) formic acid. The nano-LC apparatus used for protein analysis was a Proxeon Easy-nLC (Proxeon, Odense, Denmark) coupled to a maXis Q-TOF (quadrupole-time of flight) mass spectrometer with ultra-high resolution (Bruker Daltonics, Bremen, Germany) by nanoelectrosprayer. The nLC-MS/MS instruments were controlled with the software packages HyStar 3.2 and micrOTOF-control 3.0. The data were collected and analyzed using the software packages ProteinScape 3.0 and DataAnalysis 4.0 (Bruker Daltonics). Peptide mixtures were injected into a NS-AC-11-C18 Biosphere C18 column (particle size: 5 µm, pore size: 12 nm, length: 150 mm, inner diameter: 75 µm), with a NS-MP-10 Biosphere C18 pre-column (particle size: 5 µm, pore size: 12 nm, length: 20 mm, inner diameter: 100 µm), both obtained from NanoSeparations (Nieuwkoop, Netherlands). All nLC-MS/MS analyses were performed in duplicates.

# **Database search**

Database searches were performed as described in [34], and the taxonomy was restricted to *Rattus norvegicus* to remove protein identification redundancy. Proteins were identified by correlating tandem mass spectra with the UniProt/SwissProt database (http://www.uniprot.org), using the MAS-COT online search engine for protein identification using mass spectrometry data (http://www.matrixscience.com). An initial peptide mass tolerance of  $\pm 0.05$  Da was used for MS/ MS analysis. Only significant hits (MASCOT score  $\geq 80$  for proteins) were accepted.

# Results

#### **Baso-apical differences in the LV and RV**

A total of about 150 most abundant proteins were identified in the LV and RV. It has been observed that the quality of protein composition was identical in basal and apical parts of both ventricles. However, significant baso-apical differences were observed in the quantitative representation of some proteins.

As far as the LV is concerned, the concentration of five proteins was significantly higher in the apical part as compared with the basal part: myosin light chain 3, L-lactate dehydrogenase B chain, creatine kinase M-type, dihydrolipoyl dehydrogenase, 60 kDa heat shock protein (Table 1; Fig. 2a). From these five proteins with different baso-apical concentrations, three participate in the metabolism and energy pathways (L-lactate dehydrogenase B chain, creatine kinase M-type, dihydrolipoyl dehydrogenase); three of them are mitochondrial (L-lactate dehydrogenase B chain, dihydrolipoyl dehydrogenase, 60 kDa heat shock protein) (Table 1; Fig. 3) (additional data about differently produced proteins in both ventricles are listed in Supplement Table 2).

The same concentration gradient (i.e., a higher concentration in the apical part) was observed also in two proteins in the RV: creatine kinase S-type (S-CK) and NADH:ubiquinone oxidoreductase core subunit V1 (Fig. 3). On the other hand, the opposite gradient (a higher concentration in the basal part) was detected for aldo-keto reductase family member C1 and succinate semialdehyde dehydrogenase (Table 1; Fig. 2b). All four RV proteins with different baso-apical concentrations participate in metabolism and energy pathways, and three of them are mitochondrial (S-CK, NADH:ubiquinone oxidoreductase, and succinate semialdehyde dehydrogenase) (Table 1).

## **RV/LV differences**

In two proteins, there were detected higher concentrations in the LV: myosin light chain 3 in the apical part and two spots of myosin-binding protein C in the basal part (Table 2; Fig. 4) (additional data about differently produced proteins in the left ventricle in comparison with the right ventricle are listed in Supplement Table 2). Both proteins participate in the contractile function.

# Discussion

For the proteomic analysis, 2DE was used, which is the most common method in proteomics despite well-known problems associated with membrane proteins and a rather inaccurate characterization of the molecular mass (membrane and small proteins could be missed out) and isoelectric point of a selected protein. Despite these limitations, 2D gels have a unique ability to separate complete proteins with all

Table 1 List of baso-apical differentially expressed proteins in the left ventricle (part A), the right ventricle (part B)

Part A	UniProt <sup>a</sup>	Ventricle	Protein	Apical/ basal (fold)	$p^{b}$	Mw <sup>c</sup>	pI <sup>d</sup>	n. in Fig. 2a <sup>e</sup>
	P16409	Left	Myosin light chain 3	1.758	0.024	22.1	4.9	1
	P42123	Left	L-lactate dehydrogenase B chain	1.624	0.048	36.6	5.6	2
	P00564	Left	Creatine kinase M-type	1.679	0.029	43	6.6	3
	Q6P6R2	Left	Dihydrolipoyl dehydrogenase	1.582	0.029	54	9	4
	P63039	Left	60 kDa heat shock protein	2	0.025	60.9	5.8	5
Part B	UniProt	Ventricle	Protein	Apical/ basal (fold)	р	Mw	pI	n. in Fig. 2b <sup>e</sup>
	Q6P8J7	Right	Creatine kinase S-type	1.809	0.045	47.4	9.4	6
	Q91YT0	Right	NADH:ubiquinone oxidoreductase core subunit V1	1.81	0.006	50.7	9.4	7
	Q3MHS3	Right	Aldo–keto reductase family 1, member C1	0.425	0.049	37.2	7.8	8
	P51650	Right	Succinate-semialdehyde dehydrogenase	0.495	0.015	66.1	9.9	9

<sup>a</sup>Protein number in UniProt database (http://www.uniprot.org)

<sup>b</sup>Significance

<sup>c</sup>Theoretical molecular weight

<sup>d</sup>Theoretical isoelectric point

<sup>e</sup>Spot number in the Fig. 2



**Fig. 2** Representative two-dimensional gel electrophoresis maps of the LV (**a**), and RV (**b**). **a** Protein differences between basal and apical part of the LV. Arrows express up- or down-regulation to apical part of LV (1- myosin light chain 3; 2- L-lactate dehydrogenase B chain; 3- creatine kinase M-type; 4- dihydrolipoyl dehy-

drogenase; 5–60 kDa heat shock protein). **b** Protein differences between basal and apical part of RV. Arrows indicate up-regulation or down-regulation to apical part of RV (6- creatine kinase S-type; 7- NADH:ubiquinone oxidoreductase; 8- aldo-keto reductase family member C1; 9- succinate semialdehyde dehydrogenase)



Fig. 3 Functional categorization and subcellular localization of significantly highly expressed proteins in the apical part of the LV and RV in comparison to their basal part. Numbers in brackets indicate numbers of significantly differently expressed proteins

pId

4.9

6.5

6.5

141.2

141.2

n. in Fig. 4<sup>e</sup>

1

2

3

	list of significantly higher expressed proteins in left ventricle in comparison with the right ventricle									
UniProt <sup>a</sup>	Ventricle part	Protein	LV/RV (fold)	$p^{b}$	Mw <sup>c</sup>					
P16409	apical	Myosin light chain 3	1.71	0.02934	22.1					

2.05

3.18

Myosin-binding protein C, cardiac-type

Myosin-binding protein C, cardiac-type

<sup>a</sup>Protein number in UniProt database (http://www.uniprot.org)

<sup>b</sup>Significance

P56741

P56741

<sup>c</sup>Theoretical molecular weight

basal

basal

<sup>d</sup>Theoretical isoelectric point

<sup>e</sup>Spot number in the Fig. 4



Fig. 4 Representative two-dimensional gel electrophoresis map differences between LV and RV. The arrows express up-regulation of proteins in the apical part of the LV (1-myosin light chain 3) and in the basal part of the LV (2, 3-both spots are isomers of myosin-binding protein C)

their modifications, and 2D gel-based proteomics presents a powerful tool for studying modification landscapes in the heart muscle [35].

#### **Baso-apical gradients in LV**

The major result of our proteomic study has revealed for the first time significant baso-apical differences in concentration of some proteins, both in the LV and RV. As far as the LV is concerned, five proteins have a higher concentration in the apical as compared to the basal part of the ventricle (Table 1). They include the following:

Myofibrillar creatine kinase M-type (MM-CK) reversibly catalyzes transfer of phosphate between ATP and various phosphagens (e.g., creatine phosphate). It is necessary to mention that the heart muscle expresses four creatine kinase isoforms; MM-CK represents about 88% of the total CK activity in the human heart [36]. The expression of multiple components of cardiac CK system under pathological conditions was summarized by Zervou et al. [37]. The expression of CK in the cardiac muscle is not homogenous: in addition to our results, transmural endoepicardial variations of CK were detected in the canine LV wall [38]. These results suggest the possible functional 3D gradient of CK in the LV.

0.01462

0.03485

L-lactate dehydrogenase is very important enzyme of energy metabolism. This protein reduces pyruvate to L-lactate when oxygen supply is absent or reduced; it performs the reverse reaction during the Cori cycle. Enzymes involved in the L-lactate metabolism have recently been reviewed [39].

Myosin light chain 3 is contractile protein and its higher apical concentration is in agreement with the increased contractile force in this part of the ventricular wall. This finding supports the observation of Taylor et al. that phosphorylation of myosine binding protein-C contributes to the genesis of ventricular wall geometry, linking myofilament biology with multiscale cardiac mechanisms and myoarchitecture [40].

Dihydrolipoamide dehydrogenase serves as E3 subunit of three mitochondrial enzyme complexes: branched chain  $\alpha$ -ketoacid dehydrogenase complex,  $\alpha$ -ketoglutarate dehydrogenase complex, and pyruvate dehydrogenase complex [41].

60 kDa heat shock protein (HSP60) increases in response to different pathological conditions, e.g., inflammation, infection, hyperthermia, and presence of toxic metabolites [42].

Three of these proteins are mitochondrial and belong to the "metabolism and energy pathways" group of proteins (myofibrillar creatine kinase M-type, L-lactate dehydrogenase, dihydrolipoamide dehydrogenase); myosin light chain 3 is a contractile protein and HSP60 is the protein involved in folding of other proteins and possibly helping to stabilize them under pathological conditions. The biological function of these proteins corresponds with the higher contraction energetic demand of the apical part of the LV (Table 1; Fig. 3).

#### **Baso-apical gradients in the RV**

In the RV, higher concentration in the apical part has been observed in two proteins; both of them belong to the "metabolism and energy pathway" group (Table 1; Fig. 3).

Creatine kinase S-type (sarcomeric)(S-CK) is an important mitochondrial protein [43]; its concentration in the apical part was higher in comparison with the basal part, similarly as in the case of another CK in the LV. Our results, together with the observation of transmural variation of CK observed by Robitaille et al., suggest a possible functional 3D gradient of CK in the whole heart [38].

Proton pumping NADH:ubiquinone oxidoreductase (member of mitochondrial complex 1) is the largest and most complicated enzyme of the respiratory chain; the role of subunits and functional analysis of mitochondrial complex 1 has been recently summarized by Wirth et al. [44].

On the other hand, in two cases, higher protein concentration was observed in the basal part of the RV.

Aldo-keto reductase family member C1 belongs to the aldo-keto reductase superfamily, which catalyzes the NADPH-dependent reduction of various substrates including steroid hormones [45].

Succinate semialdehyde dehydrogenase is one of the two enzymes necessary for gama-aminobutyric acid (GABA) recycling from the synaptic cleft [46].

Our results thus confirmed the hypothesis that cardiac heterogeneity in protein composition also involves significant differences between the apical and basal part of both chambers; the observed changes were more pronounced in the LV, characterized by a higher workload. In this connection, it is necessary to mention that regional mechanical function in the LV varies longitudinally from basal to apical segments. Similarly, RV contraction is sequential, starting with the contraction of the inlet and trabeculated myocardium and ending with the contraction of the infundibulum, which is of longer duration than contraction of the inflow region [47]. The timing of electrical excitation in the LV wall is also known to be heterogeneous, with excitation generally spreading from apex to base [48]. Different protein compositions of the basal and apical parts of both ventricles are likely closely related to the described functional basoapical gradients. These differences, corresponding to different workloads, are also demonstrated at the morphological level by different thickness of the wall [29].

These differences already appear very early in the embryonic development prior to ventricular septation [30].

#### Comparison of the LV and RV

The comparison of protein composition between the LV and RV in the present study has surprisingly revealed differences in only two proteins; both of them belong to the group of

contractile proteins. Myosin light chain 3 expression was higher in the apical part of the LV; on the other hand two spots of myosin-binding protein C were higher in the basal part of the LV (Table 2).

The data on the comparison of proteomic characteristics of the LV and RV are very rare and controversial. At the first attempt, Comunian et al. in their study on the mouse heart utilized multidimensional protein technology to characterize murine LV and RV proteomes [20]. They identified thousands of distinct proteins: 16 proteins were more abundant in the LV compared to RV, 47 proteins were more abundant in the RV. Differences between proteomes of the LV and RV, both under aerobic conditions and in response to ischemia/reperfusion, were observed by Cadete et al. in rats [49]. They found ten protein spots whose levels were different between the aerobic LV and RV. Manakov et al. investigated proteome differences in the LV and RV in spontaneously hypertensive rats [50]. They identified 26 differently expressed proteins, out of which 18 were upregulated in the RV and 8 in the LV. The first analysis of the human myocardium demonstrated that the LV and RV have distinct proteomes, and that the differences further depend on the type of disease [51]. On the other hand, proteomic analysis of Phillips et al. has surprisingly revealed that expression levels for more than 600 RV and LV proteins detected were similar between the rabbit and pig hearts [6]. They have suggested that the RV-LV differences in overall workload are managed by modifying the amount of cytosol, rather than its composition. It seems, therefore, that the results dealing with the protein profiling of the LV and RV are dependent on the analytic technology, species and the type of pathological conditions. In addition, we are convinced that the protein composition of both ventricles may be influenced by age and sex of investigated experimental model.

# Conclusions

Our results for the first time suggest that heterogeneity of protein composition of the ventricular myocardium involves, beside the already described LV/RV and transmural differences also significant quantitative differences between the apical and basal part of both ventricles. The described changes were more pronounced in the LV. In both chambers, the concentration of proteins, mitochondrial and contractile, was markedly higher in the apical than that in the basal part, which corresponds to the higher energetic demand and contractile activity of these segments of both ventricles.

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#### **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures have been performed in accordance with the ethical standards and with the approvement of the Ethical Committee of the Institute of Physiology CAS in Prague.

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