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REVIEW PAPER

PROTEOMIC STRATEGIES IN RESEARCH ON THE CARDIOVASCULAR SYSTEM

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Abstract

The evolution of proteomics facilitates protein separation, identification and characterization, which give better insight into molecular mechanisms underlying cardiovascular physiology and pathophysiology. Investigations on the expression of proteins, their interactions and post-translational modifications contributes to our better understanding of disease processes and provides new cardiovascular biomarkers. Up to now, laboratory animals have been employed in proteomic research on human disorders, although rodent models do not genuinely reflect human conditions. Because of the similarities in anatomy, physiology and metabolism, farm animals such as pigs, cows or sheep are increasingly being used as model organisms in human cardiovascular research. It should be noted that application of proteomics has a huge potential for gaining some new insight into physiology and pathophysiology of the cardiovascular system, which cannot be provided with conventional methods. Up to date, complete heart and aorta proteomes of human and several animal species have been established. Several proteomic studies on human diseases, including atherosclerosis, myocardial infarction and dilated cardiomyopathy, have been conducted and proved to be very valuable in bringing key information on their aetiology and progression, as well as new challenges for biomedical investigation. The aim of this review is to summarize achievements in proteomics of cardiovascular physiology and pathophysiology with the use of domestic and laboratory animal models.

Keywords: proteomics, animal models, cardiovascular system.

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INTRODUCTION

Recent advances in proteomic techniques have laid the foundation for sensitive and rapid protein analyses, unambiguous protein identification and verification of changes in protein expression (Doherty et al. 2008, Molacek et al. 2014). The proteomic approach has contributed to better understanding of human and animal physiology, as well as pathophysiological mechanisms of several diseases. Proteomics has proven to be a powerful tool for the characterization of cardiovascular physiology and disease, as it enables to identify and specify the function of a wide range of proteins that directly affect the heart and blood vessels (Heinke et al. 1998, Donners et al. 2004, Yan et al. 2005, Verma et al. 2011, Barallobre-Barriero et al. 2012). In this manner, proteomic analysis provides a unique opportunity for comprehensive and more complete understanding of basic molecular mechanisms that govern cardiovascular physiology. Moreover, disease processes will be reflected in an altered protein pattern of the analyzed tissue. Identification of differentially expressed proteins in healthy and unhealthy conditions may serve as the basis for selecting biomarkers useful in the diagnosis and prevention of a specific disease (Doherty et al. 2008, Sharma et al. 2013).

On account of ethical, legal and practical considerations, numerous animal models have been employed in human science and research. Laboratory animals have been applied in proteomic studies on human disorders, although rodent models do not genuinely reflect human conditions. Because of similarities in anatomy, physiology and metabolic aspects, farm animals such as pigs, cows or sheep are increasingly being used as model organisms in human medicine, including cardiovascular research (Doherty et al. 2008, Verma et al. 2011). The aim of this review is to summarize achievements in cardiovascular proteomics with the use of domestic and laboratory animal models.

Applications of proteomics in heart muscle research

The last decade has brought rapid advances of proteomic technology and its application in research, allowing researchers to specify and identify cardiac muscle proteins (Corbett et al. 1994). This has resulted in generating complete heart proteomes of human and several animal species (Corbett et al. 1994, Muller et al. 1996, Toraason et al. 1997, Weekes et al. 1999, Li et al. 1999, Herosimczyk et al. 2015).

Due to limitations related to sample collection for proteomic analysis from patients suffering from cardiovascular disorders, the search for animal models reflecting analogous pathological processes is crucial. The most important animal models for human cardiovascular diseases are pigs and dogs (Heinke et al. 1998, Heinke et al. 1999, Yan et al. 2005, Barallobre-Barriero et al. 2012). Their suitability for the study of developing cardiovascular disease results *inter alia* from several anatomical and physiological similarities.

Analyzing the data available from sequence database Uniprot (www.uniprot.org), Kooij et al. (2014) found that 6575 from 44 272 proteins identified in human myocardium shared the minimum 90% of the amino acid sequence both with the pig and with the dog. A similar comparison of the amino acid sequence of human and canine cardiac proteins indicates 8861 proteins with analogous homology. Another important animal model includes laboratory rodents, in particular the rat and the mouse. Kooij et al. (2014) demonstrated that 6663 proteins of human, rat and mouse myocardium are characterized by amino acid sequence homology equal to or higher than 90%. Comparison of the mouse proteome with the human proteome reveals that the number of these proteins is equal to 8404, and for the rat and human there are 6948 proteins (Figure 1).

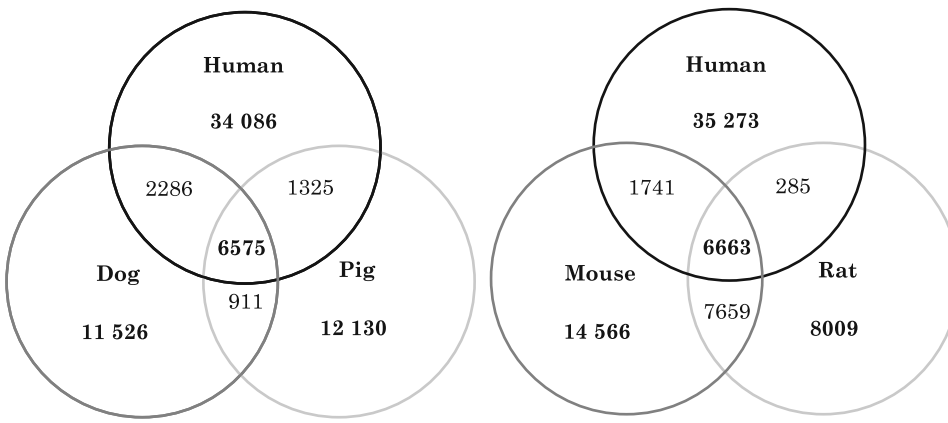

Fig. 1. Diagram showing the number of proteins characterized by amino acid sequence homology higher than 90% between human and animal species (Kooji et al. (2004))

The major analytical strategies of cardiovascular proteomics include two-dimensional gel electrophoresis (2-DE) or two-dimensional differential gel electrophoresis (2D-DIGE) coupled with mass spectrometry (Figure 2). In order to determine protein expression changes and/or to validate results, Western blot analyses followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) should be provided (Figure 2).

A number of studies were performed to identify the protein profile of the heart and its specific regions. Using two-dimensional gel electrophoresis (2-DE), proteins of the human heart tissue were separated, obtaining 3229 protein spots. Of these, 33 were identified by means of microsequencing and 20 by sequence comparison with protein database (Martinsried Institute Protein Sequence Database) (Jungblunt et al. 1994).

A study on the protein profile of the left ventricle and right atrium fragments taken from patients with dilated cardiomyopathy was conducted by Pleissner et al. (1995). The authors demonstrated differences in protein expression levels between these two regions of the heart. Among 197 protein spots separated by *2-DE*, 40 proteins were found to be differentially expres-

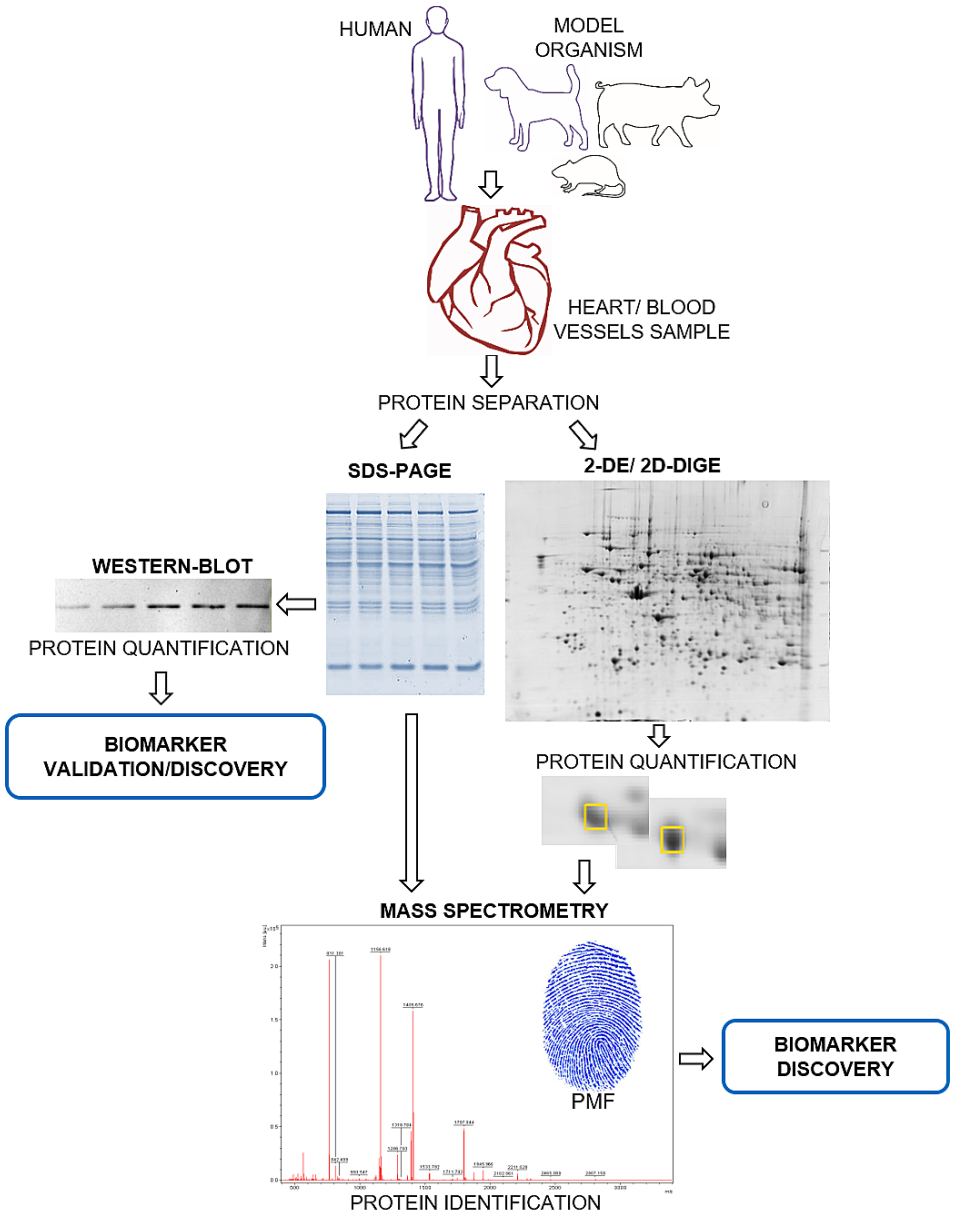

Fig. 2. Examples of analytical strategies used in cardiovascular proteomics

sed between the ventricle and the atrium. Moreover, 3 spots representing myosin light chain 1 (MYL1) and 2 (MYL2) were typical only for the atrium and were not present in the left ventricle. However, 2 spots were characteristic of the left ventricle (phosphoglycerate mutase, ATP synthase alpha chain) (Pleissner et al. 1995).

Proteomic tools were used to determine the proteins that are differentially expressed in the left ventricle of dogs in connection to the development of cardiac disorders. Heinke et al. (1998) conducted a study on 5 dogs which underwent rapid ventricular pacing that produced a cardiomyopathic state resembling dilated cardiomyopathy. Tissue fragments of the left ventricle were separated using 2-DE. In the group of paced dogs, the expression of 69 protein spots differed significantly. Of these, 27 were up-regulated, whereas 42 showed down-regulation. The most numerous groups were represented by proteins involved in energy metabolism (creatine kinase M, pyruvate dehydrogenase, isocitrate dehydrogenase subunit alpha) and cytoskeletal proteins (actin, RAS related protein). Further research using the same experimental design was aimed at the comparison of the protein maps of both groups (Heinke et al. 1999). The authors found 269 spots in the control group and 377 in the experimental group. In the group of paced dogs, 31 protein spots were significantly altered, of which 21 were down-regulated, whereas 10 were up-regulated. Differentially expressed protein spots were identified using peptide mass fingerprinting (PMF). In line with their previous study, the authors identified proteins associated with cell energy production (creatine kinase M, hydroxymethyl glutaryl CoA synthase, cytochrome c oxidase, cytochrome b5). It is suggested that expression changes of these proteins confirm that energy deficit in cardiomyocytes may contribute to cardiac diseases, such as dilated cardiomyopathy. This may indicate that the fatty acid metabolism was reduced by the decreased activity of 3-2trans-enoyl-CoA isomerase, as well as the decreased activity of the electron transport chain due to a lower cytochrome c oxidase and cytochrome b5 expression. Modifications of these energy pathways are manifested by the reduced ATP level of the cardiac muscle in the experimental group. Similar changes were observed in human research on this type of disease. Heinke et al. (1999) observed changes in the cytoskeletal protein expression. Actin was up-regulated, which could be associated with an increased contractility of the cardiac muscle as a result of the stimulation of the heart. Moreover, the expression of triphosphate isomerase and creatine kinase M was elevated. These results are in accordance with the study conducted on patients with dilated cardiomyopathy, by Corbett et al. (1998). Additionally, the authors indicated altered expression of other cytoskeletal proteins (myosin light chain 1, vimentin) and proteins involved in energy metabolism (lactate dehydrogenase, phosphofructokinase, dihydrolipoamide dehydrogenase), which could have important implications for myocyte function (Corbett et al. 1998). Collaborative changes in protein expression may suggest a similar molecular mechanism of this cardiac disorder, thereby the canine model can be of great importance for obtaining sound knowledge of this pathological process (Heinke et al. 1998, 1999).

The chicken embryo is used as a model to study the heart's development and pathogenesis. Bon et al. (2010) carried out a comprehensive analysis of the proteome of the ventricle (V), great arteries (GA) and the outflow tract

(O) in chicken throughout three stages of the embryonic development (at day 5, 7 and 10). The authors used one-dimensional electrophoresis (1-DE) to separate proteins and nanoliquid chromatography mass spectrometry (nano LC-MS/MS) for protein identification. In total, approximately 600 protein spots from 3 different tissue samples (V, GA, O) were obtained and 267 proteins were identified. Differences in the expression of several proteins: myosin-heavy chain 6, myosin-heavy chain 7, titin, connectin, collagen alpha-1 and xin, was observed, depending on the development stage and structure. The pathway analysis indicated a particularly important contribution of myosin 6 and myosin 7 in the cardiovascular system's development and function. Myosin-heavy chain 6 was expressed in all heart tissues tested at the initial stage. In contrast, myosin-heavy chain 6 was not detected in GA tissue at the later stages. Myosin-heavy chain 7 was expressed in O and V on the 7th day of the embryonic development and in GA on the 5th day of the embryonic development, but was unobserved at other stages. Moreover, other important proteins were identified, such as supervillin and xin, which are involved in actin organization in cells, ankyrin 3, which participates in the maintenance of normal electrical activity in cardiomyocytes and in the development of excitable membrane domains in heart tissue, desmoplakin, which is of key importance for cellular organization (Bon et al. 2010)

Proteomic techniques are being widely used in studies on the cardiovascular system in humans and in laboratory animals. However, proteomic data of the heart and arteries (including aorta) of farm animals are not available in the literature. Proteomic strategies enable us to evaluate each element of the cardiovascular system, thus they may provide relevant information about ongoing physiological processes and contribute to the broadening of our current knowledge on the system's function (Arrell et al. 2001, Oda, Matsumoto 2016).

Applications of proteomics in blood vessel function research

Vascular smooth muscle cells (SMCs) play a key role in the development of cardiovascular disorders. Protein expression changes or their products (secretome) may serve as a biomarkers for the developing or ongoing pathological changes. Aortic smooth muscle cells (ASMCs) provide a good model for proteomic studies that focus on the search of mechanisms controlling cell alterations during pathogenesis. It is based on the evaluation of variable biological activity of these cells in relation to the development of blood vessel damage (Gallego-Delgado et al. 2005, Rzucidlo et al. 2007). Dupont et al. (2005) established proteomic reference maps of SMCs obtained from the thoracic artery of patients undergoing a coronary artery bypass surgery. Using 2-DE, the authors obtained protein maps specific to the ASMC proteome and secretome. By means of matrix-assisted laser desorption/ionization mass spectrometry, they identified 83 and 18 different intracellular and secreted proteins, respectively. The biggest group was composed of cytoskeletal prote-

ins involved in the regulation of actin polymerization, cell shape control and contraction processes. Different expression of these proteins can be associated with atheromatosis. Other important protein classes included proteins engaged in protein biosynthesis and proteolysis and proteins involved in metabolic pathways or defense mechanisms.

Proteomic analysis of carotid plaque samples taken from patients with atherosclerotic plaque progression was carried out by Donners et al. (2004). Proteins were separated using 2-DE, identified by mass spectrometry and protein expression was validated by means of Western blotting and immunohistochemistry. The authors detected about 800 spots, 29 of them were unique for thrombus-containing plaques and 21 were specific for advanced stable plaques. This was the first study indicating that vinexin-β, a vinculin-binding protein, is expressed in advanced atherosclerotic plaques, albeit at the same level as in stable and thrombus-containing plaques. Moreover, α_1-antitrypsin, an acute-phase protein, was shown to be differentially expressed during progression of atherosclerotic plaque (Donners et al. 2004).

For atherosclerosis research, Mayr et al. (2005) analyzed the proteome of aortic SMCs of mice using 2-DE, MALDI-TOF MS and MS/MS. The authors found approximately 2400 protein spots and identified 235 of them as representing the products of 154 unique genes. As a result of extensive analyses, the first map of aortic SMCs was presented. The largest group consisted of enzymes and structural proteins, which constituted more than of half of the identified proteins. Moreover, proteins involved in protein synthesis, chaperons, antioxidants and signaling molecules were also found (Mayr et al. 2005). Husi et al. (2014) conducted analysis of proteins of aortic vessels from diabetic mice and controls, in order to investigate diabetes-induced molecular changes in the vascular system. Using liquid chromatography tandem mass spectrometry, 996 proteins were detected in both diabetic and control samples. Of this number, 72 proteins were differentially expressed, indicating their contribution to the pathogenesis of diabetic arteriopathy. Several proteins and pathways were identified to be implicated, such as oxidative stress-response proteins (aldolase reductase, mirinoglobulin-1, fatty acid binding protein 4), apoptosis modulators (alpha-1-antitripsin, T-complex protein 1 subunit zeta, gasdermin, leucine rich PPR motif-containing protein, collagens type 6), proteins associated with hypertrophy and hypertension, decreased glycolysis and fatty acid metabolism (ATP--citrate synthase, transketolase, pyruvate dehydrogenase), evident increase in vascularization (Husi et al. 2014).

Aorta proteins of typical subtotal nephrectomy (SNX) and sham-operated (SO) rat models were analyzed using 2-DE for protein separation and Q-TOF MS for protein identification (Lin et al. 2010). 97 differentially expressed spots were identified, of these 29 were up-regulated and 53 were down-regulated. These proteins were categorized according to their function into chaperons, cytoskeleton proteins, extracellular proteins (osteoglicin, dermatopontin), proteins involved in oxidative stress, ion transporters and lipid

transporters (apolipoprotein A1, apolipoprotein E) and many others. Interestingly, several of differentially altered proteins were associated with the ageing process, e.g. increased MFG-E8 accumulation in the aorta of SNX rats was observed. An increase in the abundance of MFG-E8 leads to an increase in the tensile strength, affecting and adversely aortic functions. Chaperons, such as α-B-crystalline, HSP27, chaperonin containing TCP1, HSP70, were down-regulated in SNX aorta, which could contribute to altered mechanical properties in the SNX rat aorta. Another protein associated with mechanical characteristics is collagen, whicht is involved in cell migration and arterial remodeling. The SNX aorta differently expressed several proteins related to collagen synthesis, e.g. osteoglycin and dermatopontin, which were up-regulated, and HSP47 that was down-regulated. Moreover, many of cytoskeletal proteins exhibited expression changes, such as transgelin, Rho GDP dissociation inhibitor α, myosin regulatory light chain, tropomyosins, lamin A and vimentin. Altered accumulation of proteins associated with cytoskeleton and contractile function indicates changes in vascular tonic contraction and arterial wall characteristics. Authors observed decreased expression of several crucial anti-oxidant enzymes, e.g. glutathione peroxidase, GST m3 and m5, aldehyde dehydrogenase 2, biliverdin reductase and DJ-1 in the SNX aorta tissue (Lin et al. 2010).

Another study on the aorta from spontaneously hypertensive (SHR) and normotensive (WKY) rats resulted in the identification of several differently expressed proteins associated with vascular remodelling and other pathological changes. Of these, six proteins were up-regulated in hypertensive rats: non-muscle myosin alkali light chain, Mob4B protein, glutathione S-transferase Y-b subunit, similar to 6-phosphogluconolactonase, RhoGDIα (S15 and S17), and four proteins were down-regulated: ubiquitin-conjugating enzyme E2N (UBE2N), ribosomal protein S12, Blvrb protein, osteoglycin precursor (OGN). Mob4B and UBE2N are implicated in the imbalance between proliferation and apoptosis of cells. Blvrb protein and 6-phosphogluconolactonase play a cell protective role from oxidative damage and toxic substances. OGN down-regulation in the hypertensive rats may be related to higher formation of collagen (Bian et al. 2007).

Protein profiles of calcified abdominal aortic aneurysms (CAAs) and calcified thoracic aortic aneurysms (CTAs) in patients were established by Matsumoto et al. (2012). The authors analyzed protein expression changes compared to normal aorta tissues. Using very strict criteria for protein identification, the authors detected 138 proteins with altered expression in the CAA aorta and 134 differentially expressed proteins in the CTA aorta. Well-known proteins that participate in the development and progression of aortic aneurysmal formation were found in both CAAs and CTAs. Of the proteins with significant expression changes in CAAs, type I and type III collagens were increased, whereas in CTAs type I collagen, fibrinogen α, β, γ chains and prothrombin exhibited expression, and fibulin-5 was shown to be decreased. Several proteins associated with vascular calcification were de-

tected, such as matrix Gla protein, α-2-HS-glycoprotein (fetuin-A) and biglycan, which were up-regulated, and mimecan (osteoglycin), which was down-regulated in CAA aorta, as well as α-2-HS-glycoprotein, decorin and biglycan, being increased in CTA samples (Matsumoto et al. 2012). Other authors have separated proteins of the abdominal aortic wall from patients with rupturing abdominal aortic aneurysm (AAA) and healthy controls. This analysis enabled the researchers to detect 85 proteins expressed only in aneurysm tissue and 18 proteins specific to healthy controls. The authors confirmed several previously reported proteins, implicated in the development of aneurysm, such as alpha-1-antitrypsin, alcohol dehydrogenase, annexins and glyceraldehyde-3-phosphate dehydrogenase, fibrinogen gamma, albumin, transferin, alpha enolase. Additionally, new proteins were found to be associated with aneurysm formation, mainly extracellular proteins and those related to the cytoskeleton, e.g. alpha actin, collagen VI, type II keratin, vimentin, destrin, transgelin and others (Molacek et al. 2014).

Protein profiles of stenotic and nonstenotic human aortic valves were compared by Martin-Rojas et al. (2012). By means of 2D-DIGE and mass spectrometry, the authors identified 43 differently expressed proteins. Of these, 35 proteins exhibited higher expression, whereas 8 proteins were decreased. The detected proteins are involved in cardiovascular processes as antioxidant enzymes (e.g. superoxide dismutase (Cu–Zn), glutathione S-transferase P), structural and contractile proteins (e.g. lumican, vimentin), proteins associated with inflammation (e.g. serum amyloid P-component, alpha-1-antitrypsin), transport proteins (e.g. transthyretin, apolipoprotein A1). These proteins play roles in homeostasis, fibrosis, coagulation and other processes, and may be involved in pathological processes of the degenerative stenosis (Martin-Rojas et al. 2012).

Cardiovascular proteomics of farm animals – human disease models

Farm animals such as pigs, cows, sheep or horses are important alternatives to laboratory animals, widely used as model organisms in cardiovascular physiology and pathophysiology research. Livestock animals are becoming increasingly important for proteomics in human disease. In terms of omnivorousness, metabolism, body size and lifespan, the pig is of particular significance, since it is much more similar to humans than rodents are. It should be noted that proteomics is broadly applied in physiology and biomedical research, much more often carried out on pigs than on other farm animals. Better understanding of physiological processes requires good knowledge of the expression level, structure and function of proteins. Therefore, application of proteomics in animal model research is justified (Bendixen et al. 2011, de Almeida, Bendixen 2012, Ceciliani et al. 2014).

Due to the high production cost and large size, cattle are not well suited as a model for practical research. However, studies on naturally occurring bovine diseases may be useful for investigating human disorders by analogy.

An important example is dilated cardiomyopathy (Doherty et al. 2008). Weekes et. al. (1999) conducted a proteomic study on the ventricular tissue from Simmental Red Holstein crossbred cattle with hereditary dilated cardiomyopathy (DCM) and from healthy controls. The study revealed that 35 proteins were altered in DCM cattle, and of these 12 were successfully identified. Several proteins were mitochondrial enzymes (e.g. isocitrate dehydrogenase, isovaleryl CoA dehydrogenase, mitochondrial thioredoxin-dependent, cytochrome c oxidase, polypeptide VA) and proteins involved in oxidative process (mitochondrial stress-70 protein, peroxidase reductase). Moreover, ubiquitin C-terminal hydrolase exhibited significantly higher expression, suggesting that inappropriate ubiquitination may contribute to DCM pathogenesis.

Recently, more attention has been paid to the pig as a human disease model. Regarding the cardiovascular system, pigs have been used to investigate atherosclerosis and myocardial infarction (Turk et al. 2004, 2005). The heart function and its characteristics, such as the size, heart rate and myocardial blood flow, in a miniature pig are very similar to those in the human (Verma et al. 2011). Barallobre-Barriero et al. (2012) carried out an experiment on a pig model of ischaemia/reperfusion (I/R) injury, in order to monitor extracellular matrix (ECM) remodelling processes after myocardial infarction. In total, the authors identified 139 extracellular proteins belonging to different protein families, such as proteoglycans, collagen subunits, glycoproteins and others. Several proteins were proven to contribute to cardiac remodelling, including cartilage intermediate layer protein 1, extracellular adipocyte enhancer binding protein 1, matrilin-4, collagen α-1, dermatopontin, beclin, asporin and prolargin (Barallobre-Barriero et al. 2012). Yan et al. (2005) used a swine model of chronic ischaemia to investigate if chronic ischaemia triggered autophagy. The authors demonstrated an increase in the expression of several proteins that participate in the process of autophagy, including cathepsin B and D, heat shock cognate protein Hsc73, beclin 1 and the processed form of microtubule-associated protein 1 light chain 3 (Yan et al. 2005). Sheikh et al. (2009) conducted a study on the right ventricular hypertrophy using a pig model. By means of proteomic tools, the authors compared the right ventricular myocardial tissue of experimental animals and healthy controls. In total, 18 differentially expressed proteins were identified, including structural proteins (vinculin, tropomyosin β-chain, tropomyosin 1 α-chain, calsarcin-1), metabolic enzymes (alpha enolase, F1-ATPase β-chain, malate dehydrogenase cytoplasmic, medium chain acyl-CoA dehydrogenase chain A), proteins involved in stress response (e.g. superoxidase dismutase [precursor]) and other functional groups. Decreased abundance of the F1-ATPase β-chain was observed, which may be associated with early dysfunction in energy metabolism and impaired contractile processes. Moreover, the up-regulation of calcineurin confirmed its contribution to cardiac hypertrophy.

Vascular smooth muscle cells (VSMCs) play an important role in blood vessel plasticity, and are able to migrate and proliferate if blood vessel da-

mage occurs (Owens et al. 2004, Boccardi et al. 2007). The first protein map of VSMC was published in 2001 by McGregor et al. (2001). These authors analyzed human saphenous vein medial VSMC, mapping 149 unique proteins. Of these, 129 were successfully identified by MALDI- MS and nanospray -MS. The most comprehensive VSMC map was obtained by Mayr et al. (2005). Proteins from aortic SMCs of mice were separated using 2-DE and identified by MALDI-TOF MS and MS/MS. Of 2400 spots detected, 235 protein were identified, representing 154 distinct proteins. A 2D reference map of human VSMC protein expression was presented by Dupont et al. (2005).

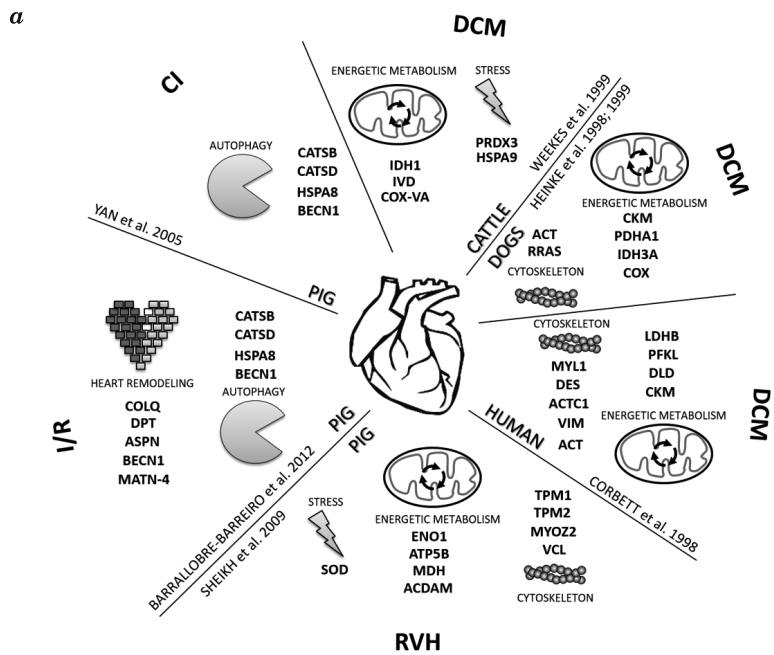

Fig. 3*a*. Graphical representation of results of the recent cardiac proteomic studies performed on humans and animal models. The scheme shows abbreviated names of proteins that were differentially expressed in response to dilated cardiomyopathy (DCM), right ventricular hypertrophy (RVH), chronic ischemia (CI) and ischemia/reperfusion injury (I/R). Protein name abbreviations: **IDH1** – Isocitrate dehydrogenase; **IVD** – Isovaleryl CoA dehydrogenase; **COX-VA** – Cytochrome c oxidase polypeptide VA; **PRDX3** – Preoxidase reductase; **HSPA9** – Mitochondrial stress-70 protein; **ACT** – Actin; **RRAS** – Ras related protein; **CKM** – Creatine kinase M; **PDHA1** – Pyruvate dehydrogenase; **IDH3A** – Isocitrate dehydroghenase subunit alpha; **COX** – Cytochrome c oxidase; **MYL 1** – Myosin light chain 1, ventricular; **DES** – Desmin; **TPM1** – Myosin 1 α-chain; **TPM2** – Tropomyosin β-chain; **MYOZ2** – Calsarcin-1; **VCL** – Vinculin; **ENO1** – Enolase α; **ATP5B** – F1-ATPase β-chain; **MDH** – Malate dehydrogenase, cytoplasmic; **ACDAM** – Medium chain acyl-CoA dehydrogenase chain A; **SOD** – Superoxidase dismutase; **COLQ** – Collagen subunit; **DPT** – dermatopontin; **ASPN** – Asporin; **BECN1** – Beclin; **MATN-4** – Matrilin-4; **CATSB** – Cathepsin B; **CATSD** – Catheopsin D; **HSPA8** – Heat shock cognate protein HSC 73

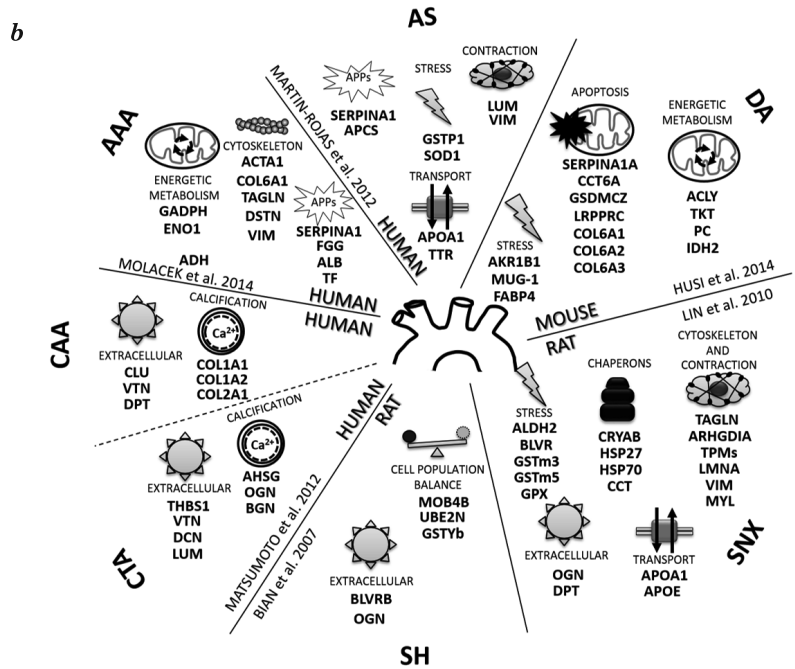

Fig. 3*b*. Schematic diagram of results of the recent cardiovascular proteomic studies performed on humans and rodent models. The graph contains abbreviated names of proteins that were differentially expressed in response to aortic stenosis (AS), diabetes (DA), subtotal nephrectomy (SNX), spontaneous hypertension (SH), calcified thoracic and abdominal aortic aneurysms (CTA, CAA) as well as rupturing abdominal aorta aneurysm (AAA). Protein name abbreviations: **SERPINA1** – alpha-1-antitrypsin; **APCS** – Serum amyloid P-component; **GSTP1** – Glutathione S-transferase; **SOD1** – superoxidase dismutase (Cu-Zn); **APOA1** – Apolipoprotein A1; **TTR** – Thransthyretin; **LUM** – Lumican; **VIM** – Vimentin; **AKR1B1** – Aldolase reductase; **MUG-1** – Murinoglobulin-1; **FABP4** – Fatty acid-binding protein 4; **SERPINA1A** – Alpha-1-antitripsin; **CCT6A** – T-complex protein 1 subunit zeta; **GSDMCZ** – Gasdermin; **LRPPRC** – Leucine rich PPR motif-containing protein; **COL6A** – Collagens type 6; **TAGLN** – Transgelin; **ARHGDIA** – Rho GDP dissociation inhibitor *α*; **TPMs** – Tropomyosins; **LMNA** – Lamin A; **MYL** – Myosin regulatory light chain; **APOE** – Apolipoprotein E; **OGN** – Osteoglicin; **DPT** - Dermatopontin; **CRYAB** – *α*-*β*-crystalline; **HSP27** – Heat shock protein 27; **HSP70** – Heat shock protein 70; **CCT** – Chaperonin containing TCP1; **ALDH2** – Aldehyde dehydrogenase; **BLVR** – Biliverdin reductase; **GSTm3** – Glutathione S transferase mu3; **GSTm5** – Glutathione S transferase mu5; **GPX** – Glutathione peroxidase; **MOB4B** – Mob4b protein; **UBE2N** – ubiquitin-conjugating enzyme E2N; **GSTYb** – Glutathion S-transferase Y-b subunit; **BLVRB** – Biliverdin reductase B; **AHSG** – *α*-2-HS-glucoprotein; **BGN** – Biglycan; **THBS1** – Trombospondin-1; **VTN** – Vitronectin; **DCN** – Decorin; **CLU** – Clusterin; **COL1A** – type I collagen *α*-1; **COL2A** – type II collagen *α*-2; **GAPDH** – glyceraldehydes 3-phosphate dehydrogenase; **ENO1** – alpha enolase; **ADH** – Alcohol dehydrogenase; **ACTA1** – Alpha actin; **COL6A1** – Collagen VI; **TAGLN** – Transgelin; **DSTN** – Destrin

Conclusions and future prospects

Proteomics is a valuable tool for studies on the physiology of the cardiovascular system and diagnosis/prevention of cardiovascular diseases. Application of proteomic techniques has enabled researchers to specify and identify proteins of heart regions and different types of blood vessels, and to specify their function in the cardiovascular system. The comparative proteomic approach has provided biomarkers of several heart and circulatory diseases, including atherosclerosis, myocardial infarction and dilated cardiomyopathy. Using animal models in human research has contributed to better understanding of human biology. Recently, farm animals have been successfully employed in proteomic studies on human cardiovascular disorders. Therefore, proteomics is a valuable tool for cardiovascular research, allowing us to explore mechanisms of disease onset and progression and to increase the efficiency of diagnosis and/or monitoring of treatment (Figure 3).

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