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Original article

Proteomic remodeling of proteasome in right heart failure

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ABSTRACT

The development of right heart failure (RHF) is characterized by alterations of right ventricle (RV) structure and function, but the mechanisms of RHF remain still unknown. Thus, understanding the RHF is essential for improved therapies. Therefore, identification by quantitative proteomics of targets specific to RHF may have therapeutic benefits to identify novel potential therapeutic targets. The objective of this study was to analyze the molecular mechanisms changing RV function in the diseased RHF and thus, to identify novel potential therapeutic targets. For this, we have performed differential proteomic analysis of whole RV proteins using two experimental rat models of RHF. Differential protein expression was observed for hundred twenty six RV proteins including proteins involved in structural constituent of cytoskeleton, motor activity, structural molecule activity, cytoskeleton protein binding and microtubule binding. Interestingly, further analysis of down-regulated proteins, reveals that both protein and gene expressions of proteasome subunits were drastically decreased in RHF, which was accompanied by an increase of ubiquitinated proteins. Interestingly, the proteasomal activities chymotrypsin and caspase-like were decreased whereas trypsin-like activity was maintained. In conclusion, this study revealed the involvement of ubiquitin–proteasome system (UPS) in RHF. Three deregulated mechanisms were discovered: (1) decreased gene and protein expressions of proteasome subunits, (2) decreased specific activity of proteasome; and (3) a specific accumulation of ubiquitinated proteins. This modulation of UPS of RV may provide a novel therapeutic avenue for restoration of cardiac function in the diseased RHF.

1. Introduction

Left heart disease, including left heart failure (LHF) associated with ischemia and endotoxemia, has been widely studied. Many advances have been made in the understanding of left heart disease, including characterization of left ventricular anatomy and its response to acute cardiac events. Furthermore, functional studies of the left heart have provided a vast array of data for further understanding left heart disease. In spite of its importance, right heart disease, which is distinctly different from LHF, has not been studied with the same degree as other aspects of left heart disease and remains relatively poorly understood. The primary function of the right ventricle (RV) is to deliver deoxygenated blood to the lungs for gas exchange. The RV effectively serves as a reservoir for blood returning to the heart via the right atrium, thereby optimizing venous return and providing sustained low-pressure perfusion through the lungs. To this end, the RV ejects blood somewhat continuously from the right atria to the lungs. On the contrary, the left ventricle (LV) generates high pressure pulsatile flow through arterial vessels with low compliance. In LHF it is well accepted that the process of cardiac remodeling itself, regardless of the initial cardiac event, is, although compensatory at first, detrimental in the long run [1]. There is now convincing evidence that intervening in the process of remodeling importantly reduces morbidity and mortality in patients with LHF [2,3]. Thus, initially, LV dilatation may be considered as a protective mechanism to maintain cardiac "pump" function. However, this phenomenon ultimately leads to alterations of global function of the LV and ultimately aggravates LHF. RHF is now being increasingly recognized as distinctly different from LHF [4], and an important mediator of overall cardiovascular collapse. However, very little is known about i) the structural and functional evolution of RV dysfunction in RHF, ii) the determining molecular and cellular mechanisms, and iii) the direct (not afterload reducing) interventions that could preserve RV function.

We postulate that the study of RV is very interesting and important to understand the cardiac remodeling mechanisms observed in RHF. Since there is still unexploited potential for therapies that directly target the RV, a better understanding of the complex molecular events that

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initiate and perpetuate the process of RV dilation would facilitate the development of more selective and efficient therapies. Studying RV dilation leading to RHF is therefore fundamental, and requires the evaluation of RV proteome as well as the integration of these findings into the overall disease context.

Proteomic technology allows the examination of global alteration pattern in protein expression in the diseased heart, and can provide new insights into the cellular mechanisms involved in cardiac dysfunction [5]. It is believed that the use of proteomic analysis to investigate heart disease should result in the generation of new diagnostic and therapeutic markers [6]. To search for cardiac specific biomarkers, the analysis of cardiac tissue remains necessary [7] and experimental animal models allow us to study serial changes in cardiac tissue proteins over time, and relate these changes to RHF and cardiac dysfunction. Our objective was thus to find changes in protein expression, which are specifically linked to right ventricular remodeling in RHF. For this purpose, we performed differential proteomic analysis of RV in rats in which RHF was induced either by monocrotaline (MCT) [8] or chronic hypoxia [9] as compared to RV from normal rats. The chronically hypoxic animal model, one of the most commonly used animal models, is obtained by exposure of rats to chronic–hypoxic conditions (four weeks) under hypobaric conditions (keeping animals in a chamber with barometric pressure adjusted to 0.5 atm) [10,11]. These conditions induce a progressive increase in both the mean pulmonary artery pressure (pap) and the Fulton index (the ratio of right ventricle to left ventricle plus septum weight) from 12–15 to 30–35 mm Hg and 0.25% to 0.5%, respectively. An increase in the pap and right ventricle hypertrophy are maximal at three to four weeks of hypoxia [10,11]. The other model is the MCT-treated rat. A single administration of MCT (generally a subcutaneous injection at the concentration of 60 mg/kg) is sufficient to induce PAH [12–14]. The initial injuries result in endothelial degeneration or hyperplasia, hypertrophy of medial smooth muscle, and adventitial edema [15]. These changes result in the augmentation of vascular resistance and the pressure overload of the right ventricle. MCT induced a higher increase in the pap (50–80 mm Hg) than hypoxia [16]. MCT-induced pulmonary hypertension (MCT-PAH) and cardiac dystrophy are irreversible [16]. MCT-PAH is also accompanied by a massive mononuclear infiltration into the perivascular regions of arterioles and muscular arteries. From this point of view (i.e., the inflammatory process), the MCT model is considered as a standard model for idiopathic PAH (IPAH) [17]. All the experiments through the paper were un-pooled experiments to make sure that we avoid any bias effect due to the use of two models. We observed that the RHF is associated with the modulation of numerous gene and proteins in the RV. Importantly, our data provide strong evidence of a ubiquitin–proteasome system (UPS) dysfunction in RHF. Our study offers new insight into the complex integration of UPS expression and activities within metabolic network that are linked to changes in pathophysiological state of RHF.

2. Materials and methods

2.1. Heart models

All animal experiments, euthanasia and tissue collection were performed according to protocols approved by the Institutional Animal Care and Use Committee of Bordeaux University. To induce right heart failure (RHF) in rats, six week-old male Wistar rats received a single subcutaneous injection of 60 mg/kg (body weight) monocrotaline (MCT), while control animals were treated with an equal volume of vehicle. In the other group, for induction of RHF by chronic hypoxia, six week-old male Wistar rats were placed in a ventilated chamber system with an inspired O_2 fraction (FiO_2) of 0.10, while control animals were maintained under normoxia. All animals were euthanized on day 28, when they showed accelerated breathing, lethargy, and ruffled fur.

2.2. Tissue sample

Rats were euthanized after intraperitoneal injection with 50 mg/kg of ketamine and 10 mg/kg of xylazine. Hearts were rapidly excised and removed of any blood cells. The right ventricle (RV) and left ventricle (LV) plus septum (LV + S) were dissected and snap-frozen in liquid nitrogen. Samples were stored at −80 °C until further use. Right ventricular hypertrophy was confirmed by the ratio of the right ventricular weight to body weight and compared to the left ventricular weight to body weight ratio.

2.3. Measurement of Fulton index

The Fulton index was measured as the ratio of the RV divided by LV + S weights.

2.4. Hemodynamic studies

Right ventricular pressure (RVP) was measured using the technique as previously described by van Suylen RJ et al. [18]. Mean RVP was calculated by digital integration.

2.5. Right ventricle protein identification by LC–MS/MS

RV proteins were extracted from each tissue sample, six animals per condition, by homogenization and solubilization in 30 mmol/L Tris–HCl pH 8.0, 150 mmol/L NaCl, 1.5% CHAPS and protease inhibitor cocktail (Sigma). The lysates were clarified by centrifugation at 435,000 $\times g_{max}$ for 30 min at 4 °C. Protein concentrations were determined according to the Bradford assay. Isolated RV proteins were solubilized in Laemmli buffer, and subjected to SDS-PAGE gel to evaluate concentration and for cleaning purpose. After entering the resolving gel, separation was stopped. Following a colloidal blue staining, bands were cut out from the SDS-PAGE gel and subsequently cut in 1 mm × 1 mm gel pieces. Gel pieces were destained in 25 mmol/L ammonium bicarbonate 50%, rinsed twice in ultrapure water and shrunk in acetonitrile (ACN) for 10 min. After ACN removal, gel pieces were dried at room temperature, covered with the trypsin solution (10 ng/μL in 40 mmol/L NH_4HCO_3 and 10% ACN), rehydrated at 4 °C for 10 min, and finally incubated overnight at 37 °C. Gel pieces were then incubated for 15 min into 40 mmol/L NH_4HCO_3 and 10% ACN at room temperature on a rotary shaker. The supernatant was collected, and an extraction solution (H_2O/ACN/HCOOH (47.5:47.5:5)) was added onto gel slices for 15 min. The extraction step was repeated twice. Supernatants were pooled and concentrated with a vacuum centrifuge to a final volume of 25 μL. Digests were finally acidified by addition of 1.5 μL of formic acid (5%, v/v) and stored at −20 °C. Peptide mixture was analyzed on a Ultimate 3000 nanoLC system (Dionex) coupled to a nanospray LTQ-Orbitrap XL mass spectrometer (ThermoFinnigan, San Jose, CA). Ten microliters of peptide digests were loaded onto a 300-μm-inner diameter × 5-mm C18 PepMapTM trap column (LC Packings) at a flow rate of 30 μL/min. The peptides were eluted from the trap column onto an analytical 75-mm id × 15-cm C18 Pep-Map column (LC Packings) with a 5–40% linear gradient of solvent B in 105 min (solvent A was 0.1% formic acid in 5% ACN, and solvent B was 0.1% formic acid in 80% ACN). The separation flow rate was set at 200 nL/min. The mass spectrometer operated in positive ion mode at a 2-kV needle voltage and a 24-V capillary voltage. Data were acquired in a data-dependent mode alternating an FTMS scan survey over the range m/z 300–1700 and six ion trap MS/MS scans with Collision Induced Dissociation (CID) as activation mode. MS/MS spectra were acquired using a 3-m/z unit ion isolation window and normalized collision energy of 35. Mono-charged ions and unassigned charge-state ions were rejected from fragmentation. Dynamic exclusion duration was set to 30 s.