Proteomic analysis of mitochondria reveals a metabolic switch from fatty acid oxidation to glycolysis in the failing heart

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This work characterizes the mitochondrial proteomic profile in the failing heart and elucidates the molecular basis of mitochondria in heart failure. Heart failure was induced in rats by myocardial infarction, and mitochondria were isolated from hearts by differential centrifugation. Using two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry, a system biology approach was employed to investigate differences in mitochondrial proteins between normal and failing hearts. Mass spectrometry identified 27 proteins differentially expressed that involved in energy metabolism. Among those, the up-regulated proteins included tricarboxylic acid cycle enzymes and pyruvate dehydrogenase complex subunits while the down-regulated proteins were involved in fatty acid oxidation and the OXPHOS complex. These results suggest a substantial metabolic switch from free fatty acid oxidation to glycolysis in heart failure and provide molecular evidence for alterations in the structural and functional parameters of mitochondria that may contribute to cardiac dysfunction during ischemic injury.

Heart failure is a major cause of morbidity and mortality throughout the world, as evidenced by increases in the numbers of hospitalizations for heart failure and deaths attributed to heart failure and the costs associated with its care1,2. The prevalence and mortality of heart failure continues to increase, in part, because improved therapy has increased life expectancy and survival after acute myocardial infarction3. These unacceptably high residual mortality and morbidity rates have mandated a reevaluation of cardiac biology with the aim of understanding the cellular and molecular mechanisms of heart failure and identifying novel therapeutic strategies.

Recent investigations have shown that mitochondria contribute to cardiac dysfunction during injury irrespective of the underlying causes of heart failure4-6. Mitochondria are among the most complex organelles, involve in numerous cellular functions, including energy production, fatty acid metabolism, pyrimidine biosyn thesis, and calcium homeostasis. Significantly, mitochondria are the major site of energy production within cells. Previous studies showed that the myocardial PCr content

Received March 31, 2009; accepted June 11, 2009
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Supported by the National Natural Science Foundation of China (Grant No. 3030036)
is reduced in the failing heart, resulting in a decrease in the energy reserves index (PCr/ATP ratio)\textsuperscript{[7,8]}. Those knockout animals for genes encoding adenine nucleotide translocator, mitochondrial Mn\textsuperscript{2+}-SOD and Mitochondrial transcription factor A exhibit phenotypic dilated cardiomyopathy and heart failure\textsuperscript{[9−11]}, suggesting that defective energy supply by mitochondria is implicated in the development and progression of cardiac remodeling and heart failure. However, the molecular mechanisms resulting in mitochondrial damage in heart failure remain unclear. Recent developments in proteomic techniques have provided the opportunity for deeper exploration and better understanding of variations in mitochondrial structure and function\textsuperscript{[12−14]}.

The aim of this study was to characterize the proteomic profile of rat cardiac mitochondria, focusing on changes in protein expression in the failing heart using two-dimensional electrophoresis, and to elucidate the molecular basis of mitochondria in heart failure at the level of the proteome using a systems biology approach. Our study identified a metabolic form switch from free fatty acid oxidation to glycolysis in failing hearts, suggesting that protein dysregulation contributes to the pathogenesis of heart failure.

1 Materials and methods

1.1 Reagent

IPG gels (ReadyStrip, pH 3—10 NL, 17 cm, Catalog163-2009), IPG buffer (Bio-Lyte, pH 3—10), urea, thiourea, mineral oil, CHAPS, iodoacetamide and ammonium persulfate were purchased from Bio-Rad (Hercules, CA). DTT was purchased from Merck (San Diego, CA). Proteinase inhibitor cocktail (Complete mini) and phosphate inhibitor cocktail (PhoSTOP) were purchased from Roche (Basel, Switzerland). Sequencing grade trypsin was obtained from Promega (Madison, WI). Mannitol, sucrose, Tris, glycine, acrylamide, bisacrylamide, SDS, bromophenol blue, HEPES, EDTA, EGTA, PMSF, TEMED, sodium acetate, acetic acid, sodium thiosulfate, silver nitrate, sodium carbonate, ethanol, and formaldehyde were electrophoresis grade or analytical grade chemicals.

1.2 Animal protocols

All procedures were performed in accordance with the Animal Research Committee guidelines of Xi’an Jiaotong University and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (revised in 1996).

The myocardial infarction model was used to induce heart failure in rats as previously described\textsuperscript{[15,16]}. Briefly, male Sprague-Dawley rats (weighing 200—250 g) were anesthetized with 2% sodium pentobarbital (50 mg/kg body weight, i.p.). The left anterior descending coronary artery was ligated with a 5-0 polypropylene suture. ST-segment elevations on electrocardiography were noted in all rats. Control animals underwent a sham protocol, i.e., open chest surgery without coronary occlusion. At 8 weeks after the operation, echocardiography was performed to confirm the presence of heart failure and a subgroup was randomly selected for histological examination\textsuperscript{[17,18]}. Data are reported as mean±SE. Differences among the experimental groups were analyzed using a Student’s two-tailed t-test. P-values of less than 0.05 were considered significant.

1.3 Isolation of mitochondria

Mitochondria were isolated from hearts by differential centrifugation using a previously described method with some modifications\textsuperscript{[14,19]}. Briefly, hearts were collected and immediately washed with 0.01 mol/L PBS (pH 7.4) containing 1 mmol/L EDTA and 1 mmol/L PMSF. After being weighed, each heart was minced and homogenized in an isolation buffer (225 mmol/L mannitol, 75 mmol/L sucrose, 5 mmol/L EGTA, 10 mmol/L HEPES, 10 mmol/L DTT, pH 7.4) in the presence of a protease inhibitor cocktail and a phosphate inhibitor cocktail. The homogenate was centrifuged at 1000×g for 10 min to remove the nuclear fraction and unbroken cells. The supernatant was centrifuged at 7000×g for 10 min and the pellet was retained. The remaining supernatant was centrifuged at 10000×g for another 10 min, and the pellet was retained. The two parts pellets were combined as the mitochondrial fraction, suspended in an isolation buffer and centrifuged at 10000×g for 10 min. The final pellet was either immediately analyzed or stored in liquid nitrogen awaiting analysis. All procedures were performed at 4℃.

Isolated mitochondria were examined for their morphology, integrity and purity by electron microscopy and Western blotting for cytochrome c oxidase.

1.4 Preparation of mitochondrial proteins

The mitochondrial preparations were solubilized in a lysis buffer (7 mol/L urea, 2 mol/L thiourea, 4% (W/V) CHAPS, 40 mmol/L Tris, 5 mmol/L EGTA, 50 mmol/L DTT, pH 7.4) containing a protease inhibitor cocktail and a phosphate inhibitor cocktail and incubated on ice.