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Original Article

Impaired mitochondrial biogenesis is a common feature to myocardial hypertrophy and end-stage ischemic heart failure

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ABSTRACT

Mitochondrial (mt) DNA depletion and oxidative mtDNA damage have been implicated in the process of pathological cardiac remodeling. Whether these features are present in the early phase of maladaptive cardiac remodeling, that is, during compensated cardiac hypertrophy, is still unknown.
We compared the morphologic and molecular features of mt biogenesis and markers of oxidative stress in human heart from adult subjects with compensated hypertrophic cardiomyopathy and heart failure. We have shown that mtDNA depletion is a constant feature of both conditions. A quantitative loss of mtDNA content was associated with significant down-regulation of selected modulators of mt biogenesis and decreased expression of proteins involved in mtDNA maintenance. Interestingly, mtDNA depletion characterized also the end-stage phase of cardiomyopathies due to a primary mtDNA defect. Oxidative stress damage was detected only in failing myocardium.

1. Introduction

Heart failure (HF) is a complex chronic clinical syndrome and a leading cause of morbidity and mortality in industrialized countries worldwide [1]. Central to the pathogenesis of HF is left ventricular (LV) contractile dysfunction, due either to an ischemic insult (i.e., myocardial infarction), or to nonischemic causes (e.g., hypertension, valvular heart diseases, genetic cardiomyopathies, etc.). These insults induce an inexorable series of maladaptive phenomena that manifest clinically as changes in the size, shape, and function of the heart, collectively referred to as *pathological cardiac remodeling*. The initial event in pathologic remodeling is LV hypertrophy, which eventually evolves to LV dilation and decreased contractility [2].

Clinical and experimental studies have shown that both impaired oxidative phosphorylation (OXPHOS) and increased mitochondria-derived oxidative stress are implicated in the pathophysiology of HF [see for a Review 3,4]. Thus, the causes of mitochondrial dysfunction in HF are the object of intense investigations, in view of possible therapeutic applications [5]. Previous studies have pointed to altered mitochondrial biogenesis as one of the causal mechanisms of OXPHOS

Abbreviations: HF, heart failure; LV, left ventricular; OXPHOS, oxidative phosphorylation; mtDNA, mitochondrial DNA; MIC, mitochondrial cardiomyopathies; LCM, laser capture microdissection; COX, cytochrome *c* oxidase; SDH, succinate dehydrogenase; TEM, transmission electron microscopy; *PPARα*, peroxisome proliferator-activated receptor alpha *PPARA*; *PGC-1α*, peroxisome proliferator-activated receptor gamma coactivator 1 alpha, *PPARGC1A*; *NPPA*, natriuretic peptide A; *NRF1*, nuclear respiratory factor 1; *ERRα*, estrogen-related receptor alpha *ESRRA*; *TFAM*, mitochondrial transcription factor A; *POLG*, polymerase (DNA directed) gamma; *HPRT1*, hypoxanthine phosphoribosyltransferase 1; SDS-PAGE, sodiumdodecylsulphate–polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; DNPH, 2,4-dinitrophenylhydrazine; DNP, antidinitrophenyl; CAT, catalase; GPx, glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; SOD2, superoxide dismutase 2; MDA, malondialdehyde.

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dysfunction in cardiac remodeling. Down-regulation of specific genes (i.e., peroxisome proliferator-activated receptor alpha, *PPARA* aliases *PPARα*; peroxisome proliferator-activated receptor gamma, coactivator 1 alpha, *PPARGC1A* aliases *PGC-1α*) involved in energy metabolism modulation and mitochondrial biogenesis has been shown in several experimental models of HF [6–11]. However, recent works suggest that the mechanisms at play are more complex in human than in animal models. For example, Karamanlidis et al. showed that mitochondrial dysfunction in the failing human heart is attributable to mitochondrial DNA (mtDNA) depletion rather than decreased transcriptional activity of the mitochondrial genome [12] and pointed to altered mtDNA replication and oxidative-dependent mtDNA damage as possible mechanisms. In addition, an unresolved issue with potential therapeutic implications is whether and to what extent the derangement in mitochondrial biogenesis observed in HF is present in the early phase of cardiac remodeling, that is, during compensated hypertrophy.

In the present work, we aimed to evaluate mitochondrial biogenesis and oxidative damage in compensated myocardial hypertrophy and HF in the adult human heart. To this purpose, we compared ultrastructural, biochemical, and molecular features of myocardial samples from patients with sarcomeric hypertrophic cardiomyopathy (HCM) (as a model of compensated myocardial hypertrophy) and end-stage ischemic cardiomyopathy. For further comparison, we looked for changes in mitochondrial biogenesis and oxidative stress in hypertrophic nonfailing and failing hearts associated with a primary mtDNA defects (all patients with mitochondrial tRNA mutations).

2. Materials and methods

2.1. Patients

All studies conformed to Sapienza, University of Rome Ethical Committee protocols. Hypertrophic, nonfailing, myocardial samples were obtained from septal myectomy procedures performed on patients with sarcomeric HCM with obstructive physiology (HCM group, $n=10$). LV myocardial samples from patients with end-stage HF (HF group, $n=15$) were obtained from transplant procedures. We selected only failing hearts with proven ischemic etiology based both on clinical records and gross morphologic findings. We used as controls [nonfailing heart (NHF) group] LV myocardial samples obtained either from donor hearts, which were unsuitable for transplantation ($n=5$), or from autopsies of subjects who died for noncardiac causes (i.e., traffic accident), obtained from the Forensic Pathology Service, Institute of Legal Medicine of Seville, Spain ($n=5$, obtained within 4 h from death).

In addition, we analyzed LV myocardial samples obtained either at autopsy (performed within 4 h from death, $n=3$) or cardiac transplant ($n=3$) from patients with genetically proven mitochondrial cardiomyopathies (MICs).

2.2. Gross analysis, histology, and enzyme histochemistry

All explanted hearts were weighted and photographed. The epicardial coronary arteries were examined, and the presence and degree of luminal narrowing were evaluated. The myocardium was carefully inspected with short axis sections. Myectomy samples from HCM patients were grossly inspected and measured after surgery.

For routine histological analysis, multiple samples from the left ventricle, ventricular septum (VS), and coronary arteries were embedded in paraffin, and 5-micronmeter-thick sections were stained with hematoxylin–eosin, periodic acid–Schiff and Masson trichrome stain.

Myocardial samples from left ventricle and myectomy were collected, avoiding areas of scarring, and immediately snap-frozen in liquid nitrogen-chilled isopentane, both in cryovials for molecular analyses and in Optimal Cutting Temperature Compound embedding compound for enzyme histochemistry and laser capture microdissection (LCM) experiments.

To identify mitochondrial respiratory chain-deficient cardiomyocytes, we performed sequential cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) reactions on frozen sections from the left and right ventricles. Combining the histo-enzymatic mitochondrial reactions for COX (brown) and SDH (blue) normally results in a brown precipitate within the cell in the presence of normal COX activity. As a consequence of mtDNA depletion and/or multiple deletions, mitochondrial protein synthesis is impaired, resulting in a significant reduction in COX activity. In contrast, activity of the entirely nuclear-encoded SDH is retained. Thus, cells with mtDNA defects are highlighted as blue, respiratory-deficient cells following the sequential COX/SDH reaction.

2.3. Ultrastructural and morphometric analysis

Mitochondrial ultrastructure was studied in myocardial samples by transmission electron microscopy (TEM). One-cubic millimeter myocardial blocks were dissected from three hearts from each study group and immediately fixed in 2.5% phosphate buffered glutaraldehyde. Tissue dehydration and resin embedding and staining were performed as described [13]. Grids were observed with a Zeiss EM 10 (ZEISS Obercocken, Germany) (TEM), and 15 random fields at 8000× magnification were acquired and stored as TIFF images (Digital Micrograph 3.4TM, Gatan GMBH, Munchen, Germany). Image analysis was performed using ImageJ 64 1.48a (GNU License, National Institute of Health, Bethesda MD, USA). Mitochondria were counted, the perimeter of each organelle was manually traced, and the mitochondrial area was automatically measured. Both the total and the mean cross-sectional mitochondrial area were then obtained for each acquired field. The sarcomere area was also manually measured for each image, and the ratio between total mitochondrial area and sarcomere area was then derived.

2.4. Molecular analyses

Prior to each experiment, sections stained with hematoxylin and eosin and Masson trichrome stain were obtained from each frozen myocardial sample for morphological examination to exclude the presence of extensive fibrosis.

Total DNA from LV myocardial tissue was extracted by Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Total RNA was isolated from LV tissue using the SV total RNA isolation kit (Promega, Madison, WI, USA). RNA amount was measured with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA), and total RNA was reverse-transcribed to cDNA using System Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Life technologies Italia, MB, Italy) according to manufactures guidelines.

2.5. Quantification of mtDNA and LCM analysis

Total DNA was obtained both from myocardial homogenates and from myocytes microdissected by LCM (Leica LMD 7000, Leica Microsystem, MI, Italy). For the latter, serial 5-μm-thick frozen sections were mounted on a polyethylene foil slide and stained with hematoxylin and eosin. Sections were observed under light microscope with a 40× objective. Small groups of myocytes (5–10 cells) were microdissected by a ultraviolet laser and collected on an adhesive cap of nanotubes as previously described [14]. Samples were digested with proteinase K (25μg/100μl) over night at 37°C, preamplified by TaqMan® PreAmp Master Mix kit (Life Technologies Italia, MB, Italy) according to manufactures guidelines. Absolute quantification of mtDNA was performed by the standard curve method as previously detailed [14]. The technique involves obtaining the ratio of an unknown variable (number of copies of mtDNA) to a known factor (number of copies of a nuclear DNA gene). With each assay, a standard curve for mtDNA and nDNA was generated using serial known dilutions of a vector in which the regions used as template for the two amplifications were cloned tail to tail to have a ratio of 1:1 of the reference molecules [15]. The absolute mtDNA copy number per cell was obtained by the ratio of mtDNA to nDNA values multiplied by two (as two copies of the nuclear gene are present in a cell). Evaluation of mtDNA