International Journal of Cardiology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Atrial remodeling and metabolic dysfunction in idiopathic isolated fibrotic atrial cardiomyopathy

Chang Cui [a,1], Xiaohong Jiang [a,1], Weizhu Ju [a], Jiaxian Wang [a,b], Daowu Wang [c], Zheng Sun [d,e], Minglong Chen [a,*]

[a] Division of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[b] National Center for Human Genetics, National Research Institute for Family Planning, Beijing 100081, China
[c] State Key Laboratory in Reproductive Medicine, Centre for Clinical Reproductive Medicine, Nanjing Medical University, Nanjing 210029, China
[d] Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Baylor College of Medicine, Houston, TX 77054, USA
[e] Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77054, USA

ARTICLE INFO

Article history:
Received 28 December 2017
Received in revised form 18 March 2018
Accepted 18 April 2018
Available online xxxx

Keywords:
IIF-ACM
Histology
Proteomics
Metabolism
Fibrosis
Oxidative stress

ABSTRACT

Background: Idiopathic isolated fibrotic atrial cardiomyopathy (IIF-ACM) is a novel subtype of cardiomyopathy characterized by atrial fibrosis that does not involve the ventricular myocardium and is associated with significant atrial tachyarrhythmia. The mechanisms underlying its pathogenesis are unknown.
Methods: Atrium samples were obtained from 3 patients with IIF-ACM via surgical intervention. Control samples were consisted of 3 atrium biopsies from patients with congenital heart disease and normal sinus rhythm, matched for gender, age and basic clinical characteristics. Comparative histology, immunofluorescence staining, electron microscopy and proteomics analyses were carried out to explore the unique pathogenesis of IIF-ACM.
Results: IIF-ACM atria displayed disordered myofibrils, profound fibrosis and mitochondrial damages compared to the control atria. Proteomics profiling identified metabolic pathways as the most profound changes in IIF-ACM.
Conclusions: Our study suggested that metabolic changes in the atrial myocardium caused mitochondrial oxidative stress and potential cell damage, which further led to atrial fibrosis and myofibril disorganization, the characteristic phenotype of IIF-ACM.

1. Introduction

Atrial myocardium is always affected by multiple cardiac and non-cardiac conditions, such as hypertension [1], obesity [2] and diabetes mellitus [3]. The resulting atrial pathologies, which have a substantial impact on cardiac function, the occurrence of arrhythmias, and stroke risk [4], have been categorized as a new class of cardiomyopathy called atrial cardiomyopathy (ACM) [5]. Isolated atrial lesions can also occur due to age-related amyloid degeneration [6] or mutations in genes such as NPPA [7] and MYL4 [8].

Lately, we reported a group of young patients with unexplained scar-related atrial tachycardia (AT) [9]. Multiple imaging modalities, ventricular voltage mapping, and intracardiac pressure recordings performed during a median 4 years of follow-up ruled out ventricular involvement. In addition, genetic and immunological investigations excluded the involvement of genetic variation or systemic diseases. As the mechanisms of this disease remain unknown, we proposed to name this type of cardiomyopathy as Idiopathic Isolated Fibrotic Atrial Cardiomyopathy (IIF-ACM).

Proteomics is a powerful technology that provides an objective, large-scale analysis of proteins under normal or pathological conditions. It has been applied to the study of complex changes in cardiovascular diseases, such as atrial fibrillation (AF) [10], hypertrophic cardiomyopathy [11] and ischemic cardiomyopathy [3], owing to its ability to examine proteins simultaneously with high throughput [12]. During the last eight years, 3 patients with multiple AT recurrences underwent resections of atrial lesion via minimally invasive surgical interventions in our center to terminate the arrhythmias. These precious atrium samples provided excellent materials for pathological and proteomic studies.

In the present study, we exploited these three atrium specimens from IIF-ACM patients to compare histological, ultrastructural and proteomic characteristics with samples from normal sinus rhythm (NSR) controls. Our results not only provided solid pathological evidence of IIF-ACM, but also revealed proteomic evidence of relationships between atrial remodeling and metabolic dysfunction, shedding light on the inherent mechanism and potential therapeutic strategies for this novel cardiomyopathy.

Abbreviations: IIF-ACM, idiopathic isolated fibrotic atrial cardiomyopathy; AF, atrial fibrillation; AT, atrial tachycardia; TEM, transmission electron microscopy; iTRAQ, isobaric tags for relative and absolute quantitation; SCX, strong cation exchange; NSR, normal sinus rhythm; PCA, principal component analysis; KEGG, Kyoto Encyclopedia of Genes and Genomics; RAO, right anterior oblique.

* Corresponding author.
E-mail address: chenminglong@njmu.edu.cn. (M. Chen).

[1] C.C. and X.J. contributed equally to this work.

https://doi.org/10.1016/j.ijcard.2018.04.080
0167-5273/

2. Methods

2.1. Clinical characteristics

A total of three patients (2 males and 1 female) with IIF-ACM underwent resection of atrial lesions to terminate their multiple AT recurrences. The diseased areas of the right atrium were resected via minimally invasive surgical interventions as described previously by our group [9], which was 3 cm * 1 cm as usual. Controls were three right atrium tissues from patients with congenital heart disease, matched for age and gender. The size of atrial biopsy was routinely 7–8 mm * 4–5 mm. Study procedures were approved by the local Ethics Committee. Before enrollment, patients were screened via questionnaires and pre-operative 12-lead electrocardiography to ensure that they had never experienced AF. Routine 2-D transthoracic echocardiography was performed for all patients. Samples collected during the surgical procedures were snap-frozen in liquid nitrogen and followed by protein analysis and staining.

2.2. Electrophysiological studies

The electro-anatomic mapping was performed as described previously [9]. Briefly, areas of electrical silence or dense scar were defined as those with bipolar voltage ≤0.1 mV for the atria [13] and ≤0.5 mV for the ventricles [14], based on previous studies.

2.3. Myocardial biopsies and histology

For the histological analyses, the atrium samples were fixed in 10% buffered formalin and embedded in paraffin. Five micron thick sections were stained with Hematoxylin & Eosin, Masson trichrome and Congo red. Whole fields of samples were scanned using Pannoramic MIDI (3D HISTECH). Pictures were taken by CaseViewer software.

2.4. Immunofluorescence staining

Paraffin sections were deparaffined, thoroughly dehydrated and incubated with goat serum for 60 min. These sections were then incubated with the following primary antibodies overnight at 4 °C: mouse anti-cardiac troponin T (1:200, Abcam, ab8295), rabbit anti-Cx43 (1:200, Abcam, ab11370), mouse anti-N-Cadherin (1:200, Abcam, ab98952) and rabbit anti-ANP (1:200, Abcam, ab76743). The secondary antibodies were goat anti-mouse IgG (1:500, Abcam, ab150113) and donkey anti-rabbit IgG (1:500, Abcam, ab150074). Nuclei were visualized with DAPI (Life Technologies, P36931). Images were captured using a Zeiss fluorescence microscope (Axio Imager A2, Zeiss). The immunofluorescence was quantified with ImageJ software (National Institutes of Health, 1.8.0_77) as previously described [15].

2.5. Transmission electron microscopy (TEM)

TEM was carried out as described previously by our group [16]. Briefly, atrium tissues were fixed with 4% glutaraldehyde overnight at 4 °C. Samples were then rinsed with PBS and stained in 2% uranyl acetate. Images were captured using a transmission electron microscope (JEM-1010, Jeol Ltd., Tokyo, Japan).

2.6. Proteomic analyses

Isobaric tags for relative and absolute quantitation (iTRAQ) labeling and quantitative proteomics by mass spectrometry were performed as previously described [17]. Briefly, the tissue samples were preconditioned with Collagenase I and Protease [18]. The extracted atrial proteins were then digested with trypsin. Equal volumes of tryptic peptides were labeled with iTRAQ tags according to the manufacturer's instructions (AB Sciex, Foster City, USA). The samples were then fractionated by off-line strong cation exchange (SCX) using a Thermo Biobasic SCX column. Each collected fraction was analyzed by liquid chromatography-mass spectrometry (LC-MS, QStar Elite, AB Sciex). The data were analyzed using Protein Pilot software 4.0 (AB SCIEX, Foster City, USA). In the present study, ratios with p-values <0.05, and fold-changes >1.2 were considered as significant. To further explore the significance of differentially expressed proteins, Ingenuity Pathway Analysis (www.ingenuity.com) was used to search for the relevant molecular functions, cellular processes and pathways of these identified proteins during the pathological changes in the study group. The top canonical pathways identified in this analysis were presented, along with p-values calculated using right-tailed Fisher's exact tests.

2.7. Statistical analyses

All analyses were performed using SPSS Statistics 19.0 (IBM). Unless stated otherwise, all data were expressed as the means ± SD, as estimated by Student's t-test. A p-value of <0.05 was considered significant for all statistical tests.

3. Results

3.1. Patient population

Clinical and echocardiographic results are presented in Supplement Table. 1. There were no differences as to age, gender, ejection fraction or atrial size between the two groups of patients. In particular, the control patients were in NSR and did not exhibit AF during the recruitment process. The samples were derived from a non-scar-related chamber and were therefore considered to be as close as possible to normal atrium tissues.

3.2. Histological analyses revealed extensive fibrosis in the atria of IIF-ACM patients

Among the IIF-ACM patients, three-dimensional voltage maps revealed large areas of electrical silence in the right atrial free wall (Fig. 1A), whereas other chambers were quite healthy (Supplement Fig. 1). Through thoracotomy, large fibrotic areas were observed, with islands of viable cardiac muscles among the fibrotic tissues (Fig. 1B). Under Hematoxylin-Eosin staining (Fig. 1C), irregularly arranged myocardium and collagen in pale pink was observed in IIF-ACM patients, compared with the NSR controls. Masson's trichrome staining (Fig. 1D, E) of the atrial myocardium samples at the corresponding site showed profound and extensive fibrosis in the IIF-ACM group. In addition, histological appearance under Congo red staining did not differ between the two groups, ruling out the possibility of cardiac amyloidosis in IIF-ACM patients (Fig. 1F). The figures of other two patients and two controls were shown in Supplement Fig. 2.

3.3. Immunofluorescence staining revealed atrial remodeling in IIF-ACM patients

Subsequently, the sample sections were immunostained for a panel of cardiac markers. Cardiac troponin T (cTnT) staining revealed low-density and disordered myofibrils in the IIF-ACM group although both groups showed robustly expressed connexin 43 (Cx43) (Fig. 2A, B). It has been reported that loss of cardiac-specific N-Cadherin leads to slow conduction and arrhythmogenesis [19]. We also found that, compared with NSR controls (Fig. 2C), IIF-ACM showed a dramatically decreased expression of N-Cadherin (844.60 ± 280.58 vs 148.81 ± 57.10, mean ± SD, $n = 6$ per group, $^{***}p < 0.001$ estimated by Student's t-test, Fig. 2D). There was no difference in the expression levels of ANP. Taken together, the immunostaining results demonstrated the atrial remodeling of IIF-ACM. The pictures of other two patients and two controls were shown in Supplement Fig. 3.

3.4. Proteomics profiling identified differentially expressed proteins

To provide insight into the pathogenesis of the disease, we used proteomics profiling to compare the protein levels between IIF-ACM atria and the controls. iTRAQ labeling and LC-MS/MS identified a total of 4225 unique proteins. 2D principal component analysis (2D PCA) of all proteins showed an obvious distinction between the IIF-ACM and the control groups (Fig. 3A), indicating a relatively low intragroup variation and significant differences between the groups. 539 proteins (284 decreased, 255 increased) showed differential expression between the IIF-ACM and control groups (Fig. 3B). Ontology analysis revealed significant enrichment for proteins involved in collagen metabolism, action potentials, and skeletal morphogenesis among the differentially expressed proteins (Fig. 3C). These findings were consistent with the observed morphological changes.

3.5. Altered expressions of enzymes in lipid and carbohydrate metabolism in IIF-ACM atria

Identified proteins were further functionally classified using Ingenuity Pathway Analysis to define the main biological processes varied in IIF-ACM and NSR control patients (Supplement Fig. 4). Interestingly, energy metabolism was among the top enriched pathways altered in IIF-ACM atria versus the controls. These metabolic processes included citrate cycle (TCA cycle), 2-oxocarboxylic acid metabolism, glycerophospholipid

Please cite this article as: C. Cui, et al., Atrial remodeling and metabolic dysfunction in idiopathic isolated fibrotic atrial cardiomyopathy, Int J Cardiol (2017), https://doi.org/10.1016/j.ijcard.2018.04.080