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Quantitative proteomics of changes in succinylated proteins expression profiling in left appendages tissue from valvular heart disease patients with atrial fibrillation

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ABSTRACT

Background: Previous studies have suggested that proteomic modifications are closely associated with cardiovascular diseases. The aim of this study was to identify potential mechanisms by profiling the changes in succinylated protein expression in left appendage tissues from patients with valvular heart disease and atrial fibrillation (AF).

Methods: Using dimethyl labeling for relative and absolute quantification-coupled high-performance liquid chromatography-tandem mass spectrometry, we analyzed the proteomics profiles and succinylation events in 18 left atrial appendage tissue samples from patients who underwent cardiac valvular surgery, including nine patients with permanent AF and nine patients with sinus rhythm (SR).

Results: In total, after setting the quantification ratio > 1.3 and < 1:1.3 representing the up- and down-regulated cutoff values, respectively, 132 proteins were classified as targets of upregulation and 117 proteins as targets of downregulation. Within these proteins, 246 sites exhibited upregulated succinylation and 45 sites exhibited downregulated succinylation. Protein–protein interaction networks showed that the proteins exhibiting lysine succinylation and AF status were highly enriched in energy metabolism, extracellular matrix-related, and cellular structure-related proteins. These results were confirmed by western blot.

Conclusions: The differences in succinylation level of energy metabolism-related proteins indicates the possible involvement of these proteins in AF of valvular heart disease patients, and provide insight for further analysis of their biological functions.

1. Introduction

Atrial fibrillation (AF) is the most common clinical arrhythmia. AF is associated with increased rates of death, stroke and other thromboembolic events, heart failure, degraded quality of life, and reduced exercise capacity [1,2,3]. AF occurs in 1–2% of the general population, and its prevalence is estimated to increase as the population ages. The estimated US prevalence of AF in the year 2050 ranges from 5.6 million to 12.1 million individuals [4]. > 17.9 million adults ≥55 years will develop AF in the European Union in the next 50 years [5]. An epidemiological study suggested that one in five people aged > 40 years old have AF [6]. The incidence of AF in the Chinese population has increased 20 times, and the incidence of AF-related stroke has increased 13 times in past decades [7,8]. With aging of the population speeding up, the incidence rate will further increase. AF has become an important public health issue. Efficacy and safety are still limited, though there are a variety of treatment options, such as antiarrhythmic drugs and radiofrequency ablation. Furthermore, the risk of drug-induced arrhythmias and bleeding and the high recurrence rate of AF are also bothersome to clinicians [9,10]. This severe situation has promoted researchers to explore new mechanisms for the occurrence of AF.

Post-translational modification (PTM) refers to the process of protein covalent activity during translation, usually with specific amino acid residues plus modified groups or proteolytically cut groups. Protein functional diversity is promoted by regulating the activity of a protein, its stability, subcellular localization, and interactions. PTMs are an effective mechanism to expand gene encoding and regulate the physiological functions of cells, and are thought to be involved in

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almost all known cellular disease pathways. An increasing number of PTM types have been identified with the development of mass spectrometry. Studies have shown that PTMs play a crucial role in different biological processes, such as cell regulation and differentiation, organism development, and multiple substance metabolism. Abnormal PTMs of proteins may be closely related to multiple diseases, including cardiovascular diseases [11,12,13]. The lysine succinylation modification is a newly discovered PTM that is thought to be the covalent binding of a succinyl group to a lysine residue with or without enzymes. Succinylation is a process in which a negatively charged four-carbon succinyl group is covalently linked to a primary amine of a lysine residue with or without enzymes. Lysine succinylation transfers larger structural groups and induces a greater change in the charge of a protein than methylation and acetylation [14]. Therefore, lysine succinylation may promote more substantial changes in the structure and function of a protein. Mass spectrometry analyses have indicated that enzymes in many metabolic pathways, including glycolysis, tricarboxylic acid cycle, and key fatty acid metabolic enzymes, have succinylated residues [15]. This suggests that lysine succinylation is widely involved in the biological processes associated with energy metabolism and plays an important role in the processes of inflammation, tuberculosis, ischemia reperfusion injury, and other diseases [16,17]. In this study, we used dimethyl label coupled high-performance liquid chromatography-tandem mass spectrometry to analyze the succinylated protein expression profile in left appendage tissues from patients with valvular heart disease and AF. Our goal was to explore the potential molecular mechanism for the occurrence of AF and provide evidence for the diagnosis and treatment of AF.

2. Material and methods

2.1. Ethical approval

This study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University. The research had been carried out in accordance with the World Medical Association Declaration of Helsinki, and that all subjects provided written informed consent.

2.2. Tissue samples

We collected 18 left atrial appendage (LAA) tissue samples from patients who underwent cardiac valvular surgery, including nine patients with permanent AF and nine patients with sinus rhythm (SR) (without a history of AF) at the Second Xiangya Hospital of Central South University. All patients were divided into the SR group and the chronic AF group according to the clinical symptoms of AF and the results of electrocardiogram or dynamic electrocardiogram. Chronic AF was defined as persistent AF with an onset time longer than 1 year and permanent AF. All patients received routine transthoracic ultrasonography and blood glucose and lipid analyses before surgery, and their body mass index was calculated. Patients aged > 50 years were routinely excluded from coronary artery disease by coronary angiography. In addition, to ensure the safety of patients, a small amount of left auricular tissue was obtained from patients when they underwent a mitral valve replacement, and the tissue samples were rapidly stored in liquid nitrogen for later use. The overall experimental process is presented in Fig. 1A.

2.3. Protein extraction

When analyzed the succinylation profile, we mixed three tissues as one group. Then we have three repeating mixed samples. The frozen powdered LAA tissues were homogenized and lysed using lysis buffer (8 M urea, 1% protease inhibitor, 3 μM trichostatin A, 50 mM nicotinamide and 2 mM ethylene diamine tetraacetic acid) The lysate was centrifuged at 12,000 ×*g* for 10 min at 4 °C to remove the cell debris. The protein concentration of the supernatant was measured using the BCA kit.

2.4. Trypsin digestion

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration < 2 M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion.

2.5. Dimethyl labeling

Following trypsin digestion, the peptides from AF and SR patient samples were separately labeled with heavy and light dimethyl groups, respectively, using an on-column labeling procedure [18]. The isotopically labeled peptide mixtures were pooled, desalted, and dried to a powder by vacuum centrifugation.

2.6. HPLC-MS/MS analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 μm i.d.). The gradient was comprised of an increase from 9% to 25% solvent B (0.1% formic acid in 90% acetonitrile) over 24 min, 25% to 40% in 8 min and climbing to 10% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 700 nL/min on an EASY-nLC 1000 UPLC system.

The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q ExactiveTM Plus (Thermo) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The *m/z* scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0 s dynamic exclusion. Automatic gain control (AGC) was set at 5E4. Fixed first mass was set as 100 *m/z*.

2.7. Database search

The resulting MS/MS data were processed using Maxquant search engine (v.1.5.2.8). Tandem mass spectra were searched against concatenated with reverse decoy database. Trypsin/P was specified as cleavage enzyme allowing up to 4 missing cleavages. The mass tolerance for precursor ions was set as 20 ppm in First search and 5 ppm in Main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification and oxidation on Met were specified as variable modifications. FDR was adjusted to < 1% and minimum score for modified peptides was set > 40.

2.8. Immune coprecipitation and western blot analyses

We used immune coprecipitation technology combined with western blotting to validate the expression of succinylated proteins in the LAA samples from SR and AF patients by following the Pierce Classic IP kit protocol. We selected two proteins (UQCRB and TPM2) from the total protein profile of expressed succinylated proteins. The Anti-succinyllysine rabbit pAb were custom prepared (PTM BIO, China). Western blotting was performed as described previously [19]. Briefly, 50 μg of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to Hybond-P

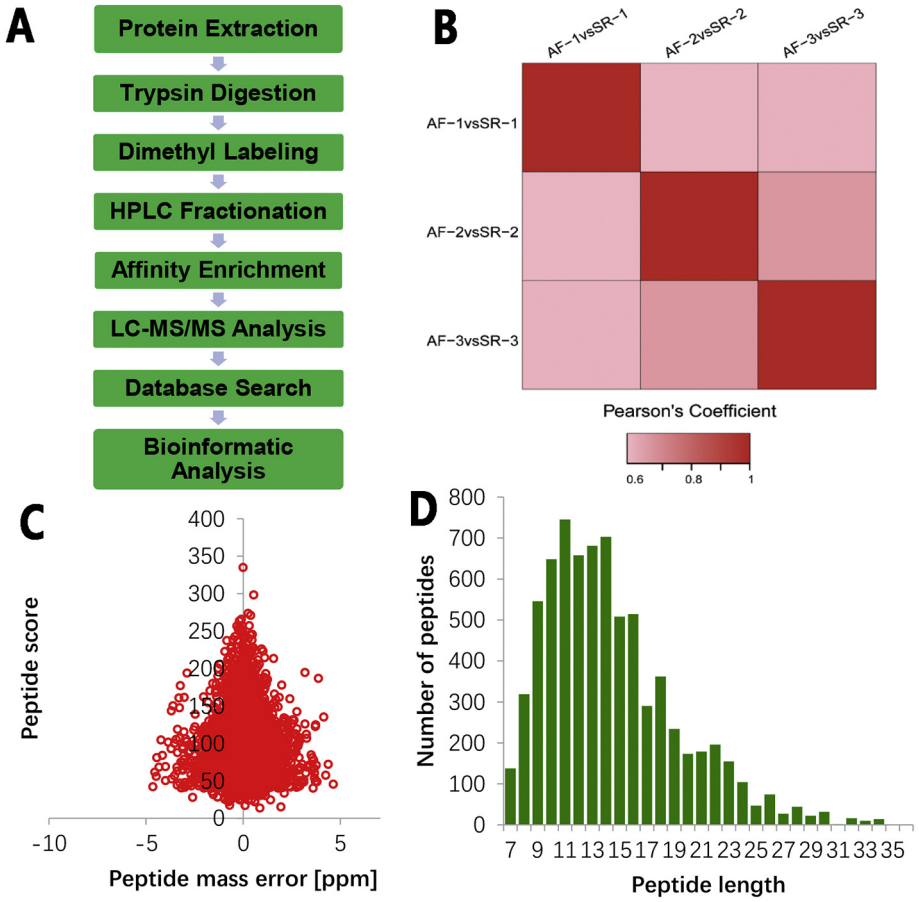

Fig. 1. Quantitative proteomic analysis on AF and SR tissue samples. A. Experimental scheme for the quantitative lysine succinylation analysis on AF and SR. B. Reproducibility of the quantitative proteomic analysis on. C. QC validation of MS data. Mass error indicates distribution of all identified peptides. D. Peptide length distribution identified by quantitative proteomic analysis.

Table 1
general characteristics of AF and SR.

	SR(n = 9)	AF(n = 9)	P
Age (year)	50.5 ± 6.5	55.5 ± 9.0	0.195
BMI (Kg/m^2)	22.2 ± 2.0	22.7 ± 1.8	0.489
Fasting blood glucose (mmol/L)	5.0 ± 0.4	5.2 ± 0.4	0.489
Triglycerides (mmol/L)	2.2 ± 0.5	1.4 ± 0.7	0.010
Total cholesterol(mmol/L)	4.6 ± 0.5	4.3 ± 0.5	0.269
LA size (mm)	37.9 ± 3.1	49.4 ± 8.0	0.001
EF (%)	62.9 ± 8.6	61.3 ± 8.8	0.711
Mitral valve area (cm^2)	1.8 ± 0.3	1.9 ± 0.3	0.746
Mitral regurgitation			0.999
Grade III	6	6	
Grade IV	3	3	
NYHA class (I/II)	9/0	6/3	

polyvinylidene difluoride membranes (Amersham Biosciences, Uppsala, Sweden). After blocking with 5% non-fat dry milk in Tris-buffered saline buffer for 1 h at room temperature, the blots were incubated at room temperature with diluted primary antibodies (1:800) followed by appropriate secondary antibodies (goat-anti-mouse antibody) (Abcam, Cambridge, MA, USA) for 1 h. The signals were visualized with an enhanced chemiluminescence detection reagent (Abcam). Beta-actin (1:3000) served as the loading control.

2.9. Bioinformatics analysis

Gene Ontology (GO) annotation proteome was derived from the UniProt-GOA database (http://www.ebi.ac.uk/GOA/). Proteins were classified by Gene Ontology annotation based on three categories: biological process, cellular component and molecular function. Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) was used to annotate protein pathway. Firstly, using KEGG online service tools KAAS to annotated protein's KEGG database description. Then mapping the annotation result on the KEGG pathway database using KEGG online service tools KEGG mapper. We used Wolfpsort (https://wolfpsort.hgc.jp/) a subcellular localization predication soft to predict subcellular localization. Soft MoMo was used to analysis the model of sequences constituted with amino acids in specific positions of modify-21-mers in all protein sequences. We performed the protein-protein interaction enrichment analysis using the gene annotation and analysis resource Metascape (http://metascape.org/gp/index.html#/main/step1). When performing the bioinformatics analysis, a corrected $P = 0.05$ was considered significant.

3. Results

3.1. Patient characterizes

The general characteristics were presented in Table 1. Significant difference was only observed in LA size. And there were no significant differences in other parameters.

3.2. Changes in succinylated proteins between the AF and SR groups

Quantitative lysine succinylation was performed in triplicate (including nine pairs of tissue samples) LAA tissues from valvular heart disease patients with AF using dimethyl label-coupled HPLC-MS/MS. A Pearson's correlation analysis indicated that the three tissue samples had good repeatability (Fig. 1B). We checked the mass error of all identified peptides. The distribution of the mass error was near zero, and most were < 5 ppm, suggesting that the mass accuracy of the MS data fit the requirement (Fig. 1C). The length of most peptides was distributed between 8 and 20, which agreed with the properties of

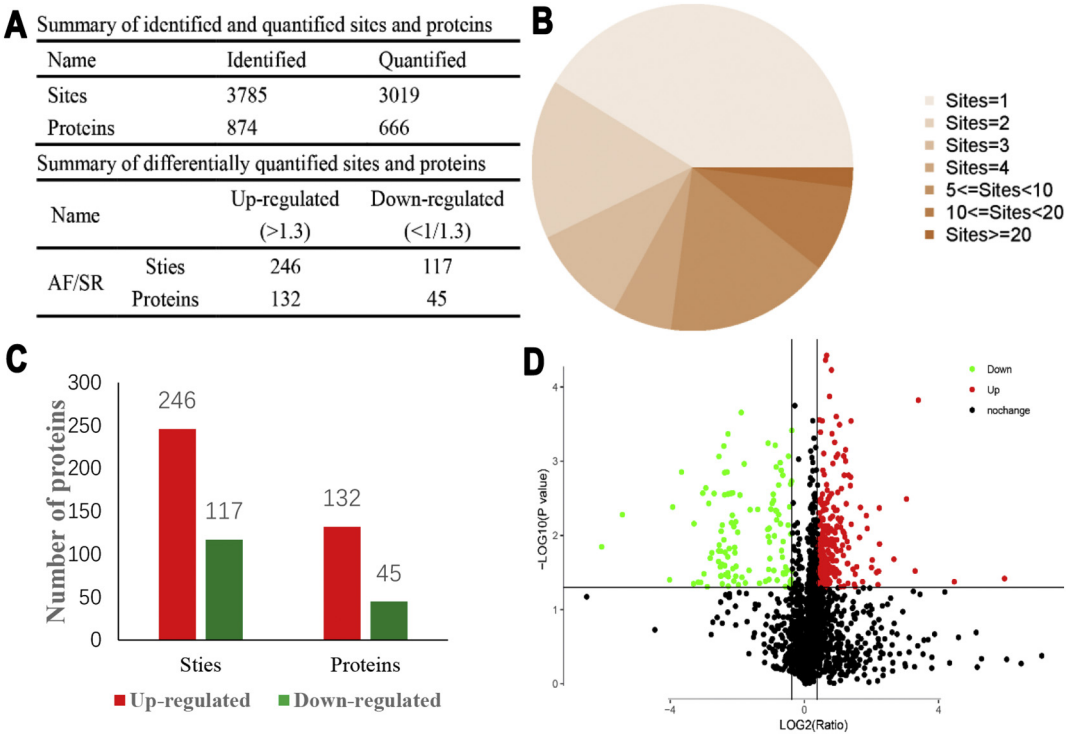

Fig. 2. Succinylation proteins analysis on AF and SR tissue sample. A. identified and quantified sties and proteins. B. Identified protein sties number distribution. C. Differentially expressed sites and proteins numb. D. Volcano plot of differentiated expressed succinylated sites and protein.

tryptic peptides (Fig. 1D). In total, we identified 874 proteins and 3785 sites, including 666 quantifiable proteins and 3019 quantifiable sites (Fig. 2A). Most of the proteins had fewer than four modified sites (Fig. 2B). After setting the quantification ratios of > 1.3 and < 1:1.3 to represent the up- and downregulated cutoff values, respectively, 132 proteins were classified as upregulated and 45 as downregulated. Within these proteins, 246 sites exhibited upregulated succinylation and 117 sites exhibited downregulated succinylation (Fig. 2C and D). These data are presented in Supplementary Material 1.

3.3. Subcellular location classification

We used Wolfpsort software to make predictions and classify the differentially expressed proteins. Approximately 43% (n = 57) of the upregulated succinylated proteins were located in mitochondria, 24% (n = 31) were located in the cytoplasm, and 20% (n = 27) were located in the extracellular matrix (ECM). The remainder were located in the nucleus, plasma membrane, and endoplasmic reticulum. Most of the downregulated succinylated proteins were located in the cytoplasm (31%) and mitochondria (47%). The endoplasmic reticulum harbored 7% of the downregulated succinylated proteins, and the plasma membrane had 5%. The nucleus and extracellular matrix held 4%. These data are presented in Supplementary Material 2.

3.4. Gene ontology (GO) analysis and protein domains involved in succinylation

The differentially expressed proteins were divided into four categories according to the AF/SR ratio (Q1 < 0.667, Q2: 0.667–0.769, Q3: 1.300–1.500; and Q4 > 1.5). The proteins and quantified succinylated sites are presented in Fig. 3A–C using GO enrichment-based clustering of biological processes, cellular components, and molecular functions. Fig. 3A presents the results of biological processes associated with the succinylated sites. The results indicate that the proteins exhibiting downregulated lysine succinylation in the Q1 quantile were highly enriched for actin-mediated cell contraction, actin-myosin filament sliding, and cellular lipid catabolism. Proteins with functions involving nucleobases, nucleosides, ribose phosphate, metabolic monosaccharides, lipid oxidation, hexose, oxidoreduction, carboxylic acid and oxoacid metabolic processes, organic acid catabolism, aerobic respiration, and inner mitochondrial membrane organization were classified as the Q3 quantile. The proteins with upregulated succinylation were also involved in several biological processes, including glycosaminoglycan biosynthesis and catabolism, collagen catabolism and metabolism, aminoglycan catabolism and biosynthesis, proteoglycan metabolism, cation transmembrane and hydrogen transport, blood pressure and circulation regulation, heart contraction, and regulation of cell communication.

The cellular component analysis indicated that proteins in Q1 exhibiting downregulated succinylation events were involved in myosin and muscle myosin complexes and filaments. By contrast, proteins exhibiting upregulated succinylation involved many cellular components, including ECM components, the respiratory chain and mitochondrial respiratory chain complexes, sarcolemma, transmembrane transporter complex, collagen beaded filament membrane rafts, protein complexes, and intrinsic components of the membrane. The Q3 proteins were associated with the mitochondrial envelope, membrane, inner membrane, organelle inner membranes, mitochondrial matrix, and myelin sheath (Fig. 3B).

We also analyzed the molecular functions related to succinylation events (Fig. 3C). The proteins exhibiting downregulated succinylation were mainly enriched in nucleotide and nucleoside phosphate binding, small molecule binding, calmodulin binding, and microfilament motor activity. The upregulated succinyl-modified proteins were involved in macromolecular complex binding, integrin, protein complexes, the ECM, glycosaminoglycan and collagen binding, hormone activities, and ECM structural constituents. Some proteins were involved in oxidoreductase activity, NAD and coenzyme binding, hydrolyase activity, and magnesium ion binding.

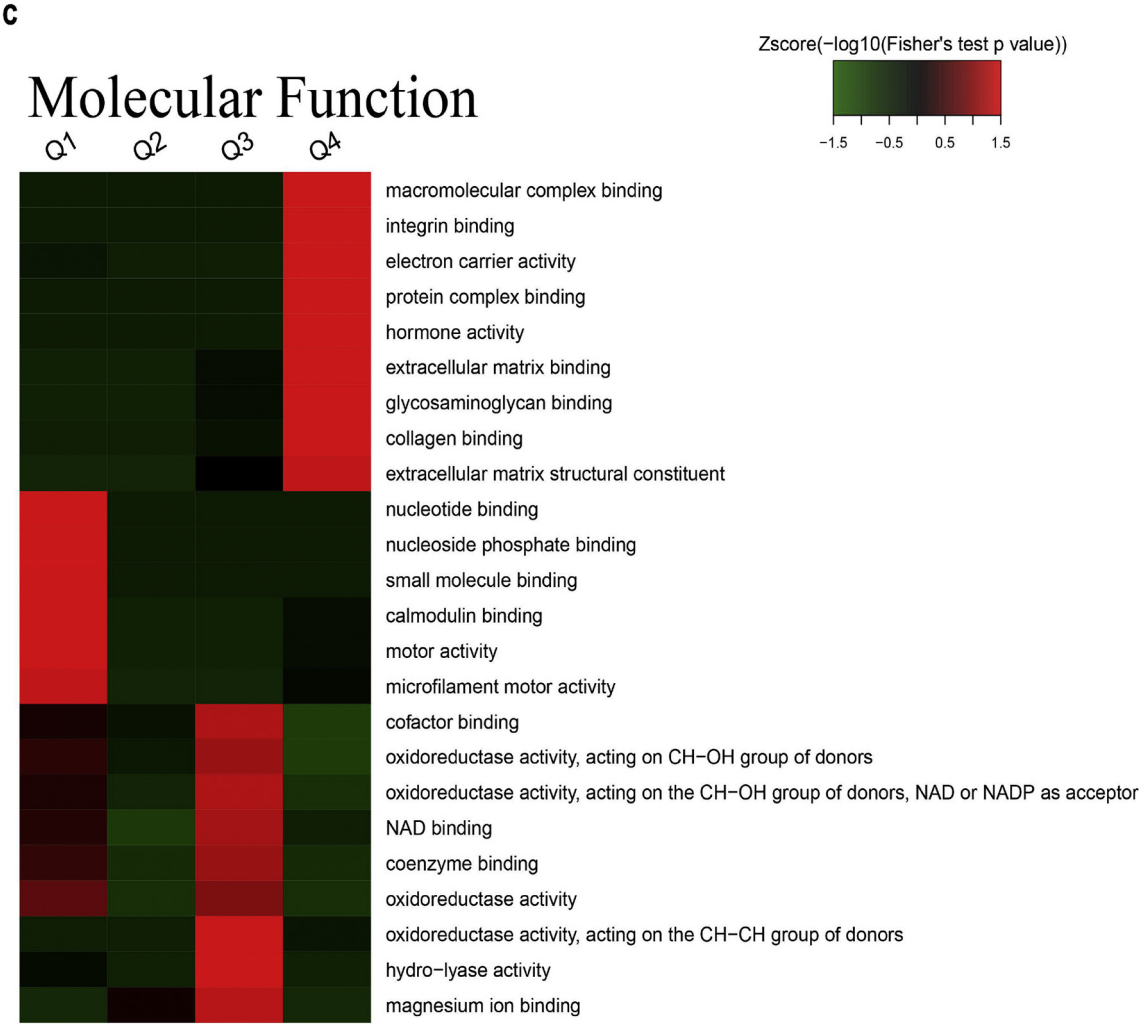

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Fig. 3. Functional enrichment-based clustering analysis for quantified succinylated proteins. According to the functional differences observed between increased and decreased proteins, GO-term association and enrichment analysis using the DAVID program (https://david.ncifcrf.gov/) were performed. A. Biological process analysis. B. Cellular component analysis. C. Molecular function analysis.

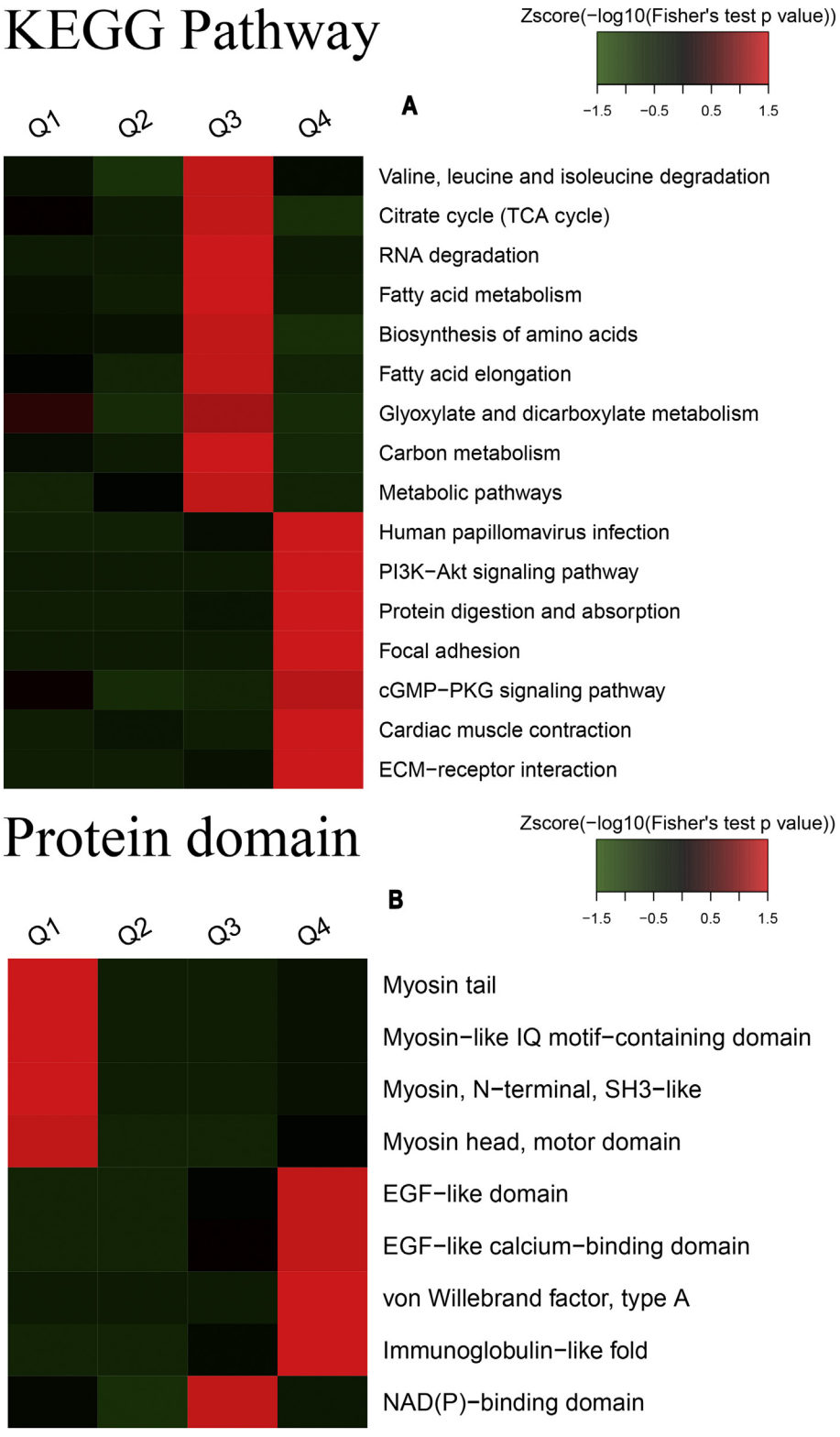

Fig. 4. Protein-complex and KEGG-pathway analysis of quantitatively altered succinylated proteins. The CORUM protein complex database for humans (http://mips.helmholtz-muenchen.de/genre/proj/corum/) was used for protein-complex analysis. The KEGG (http://www.genome.jp/kegg/) database was used to investigate enriched pathways using the DAVID Functional Annotation Tool against the background of *Homo sapiens*. A. KEGG pathway analysis. B. Protein complex analysis.

3.5. KEGG pathway and protein domain analyses of quantitatively altered succinylated proteins

We conducted a KEGG pathway analysis of quantitatively altered proteins (Q4). The results showed that the pathways involved were human papillomavirus infection, the PI3K-Akt signaling pathway, protein digestion and absorption, focal adhesion, the cGMP-PKG signaling pathway, cardiac muscle contraction, and ECM–receptor interactions. The Q3 enriched proteins were involved in metabolic signaling pathway processes (citrate cycle, fatty acid metabolism, biosynthesis of amino acids, fatty acid elongation, glyoxylate, and dicarboxylate metabolism) and RNA, valine, leucine, and isoleucine degradation (Fig. 4A).

The protein domain analysis indicated that the enriched proteins in the Q1 quantile were mainly involved in the myosin tail, myosin-like IQ motif-containing domain, myosin head, and the motor domain. Q3 was enriched in proteins involved in the NAD(P)-binding domain, and Q4 was involved in the epidermal growth factor-like calcium-binding domain, immunoglobulin-like fold, and von Willebrand factor (Fig. 4B).

3.6. Motif analysis of quantitatively altered succinylated proteins

To determine whether there was a common sequence motif required for succinylation of mitochondrial proteins, we compared the amino acid sequences of all succinylated and non-succinylated sites using iceLogo (Fig. 5A–5E). A preference was observed for glutamic acid at the −3 position and for aspartic acid at the −5 position (Fig. 5A), and positively charged residues were included (Fig. 5B). The SIRT3 substrates had no partiality to a negative charge preceding the acK site and preferred a positive charge at the −3 position (Fig. 5B). Interestingly, the sequence motif of the highly conserved sequences from Fig. 5C showed a stronger preference for K at the −1 and −2 positions.

3.7. Protein–protein interaction networks associated with differentially succinylated proteins

We used the protein–protein interaction networks to further analyze physical and functional interactions. The results show that proteins exhibiting lysine succinylation in AF were highly enriched in energy metabolism-related, ECM-related, and cellular structural-related proteins (Figs. 6A–6C and 7). Seventeen sites in the oxidative phosphorylation-related succinylation protein interaction network exhibited upregulated modifications in AF, four sites exhibited downregulated modifications, and one site involved both upregulated and downregulated modifications (Fig. 6A). For energy metabolism-related modifications related to the tricarboxylic acid cycle, the glycolipid and amino acid metabolism-related succinylated-protein network consisted of 26 upregulated modifications, nine sites exhibiting downregulated modifications, and seven sites exhibiting both up- and downregulated modifications. The protein interaction network associated with ECM-related protein modifications included 15 sites exhibiting upregulated modifications and one site exhibiting both up- and downregulated modifications. The succinylated protein interaction network associated with cardiac muscle contraction included six sites exhibiting upregulated modifications, four sites exhibiting downregulated modifications, and three sites involving both up- and downregulated modifications.

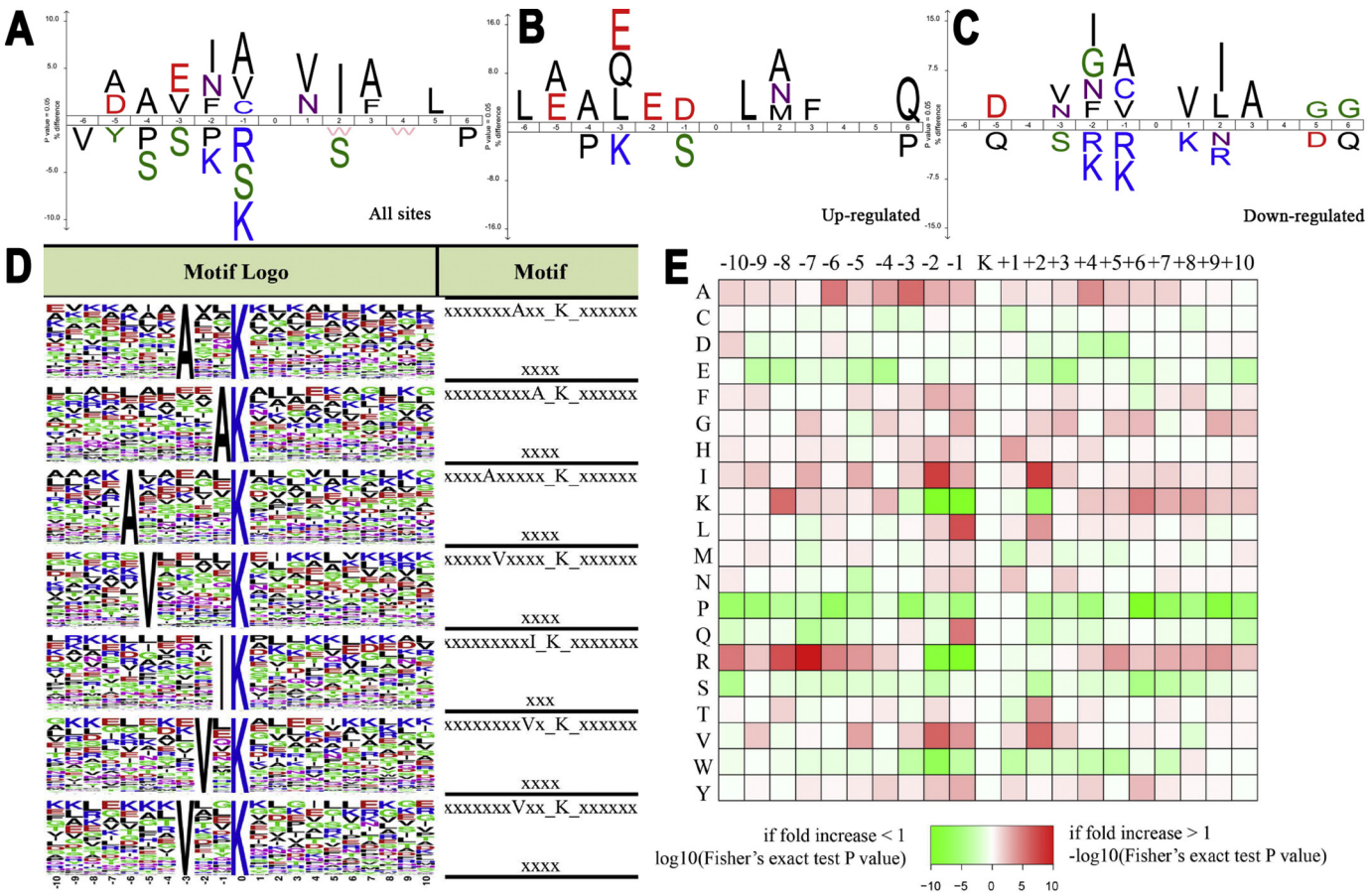

Fig. 5. Motif analysis of the identified succinylation peptides. A. Consensus sequence logos plot for acetylation sites ± six amino acids from the lysine of all acK sites identified. B. from the identified lysine residues significantly increased in AF/SR tissue C. for highly conserved acetylation sites significantly decreased in AF/SR tissue. D. sequence logo of acetylation motifs, E. heat map of amino acid frequencies of the sequences flanking Kac sites.

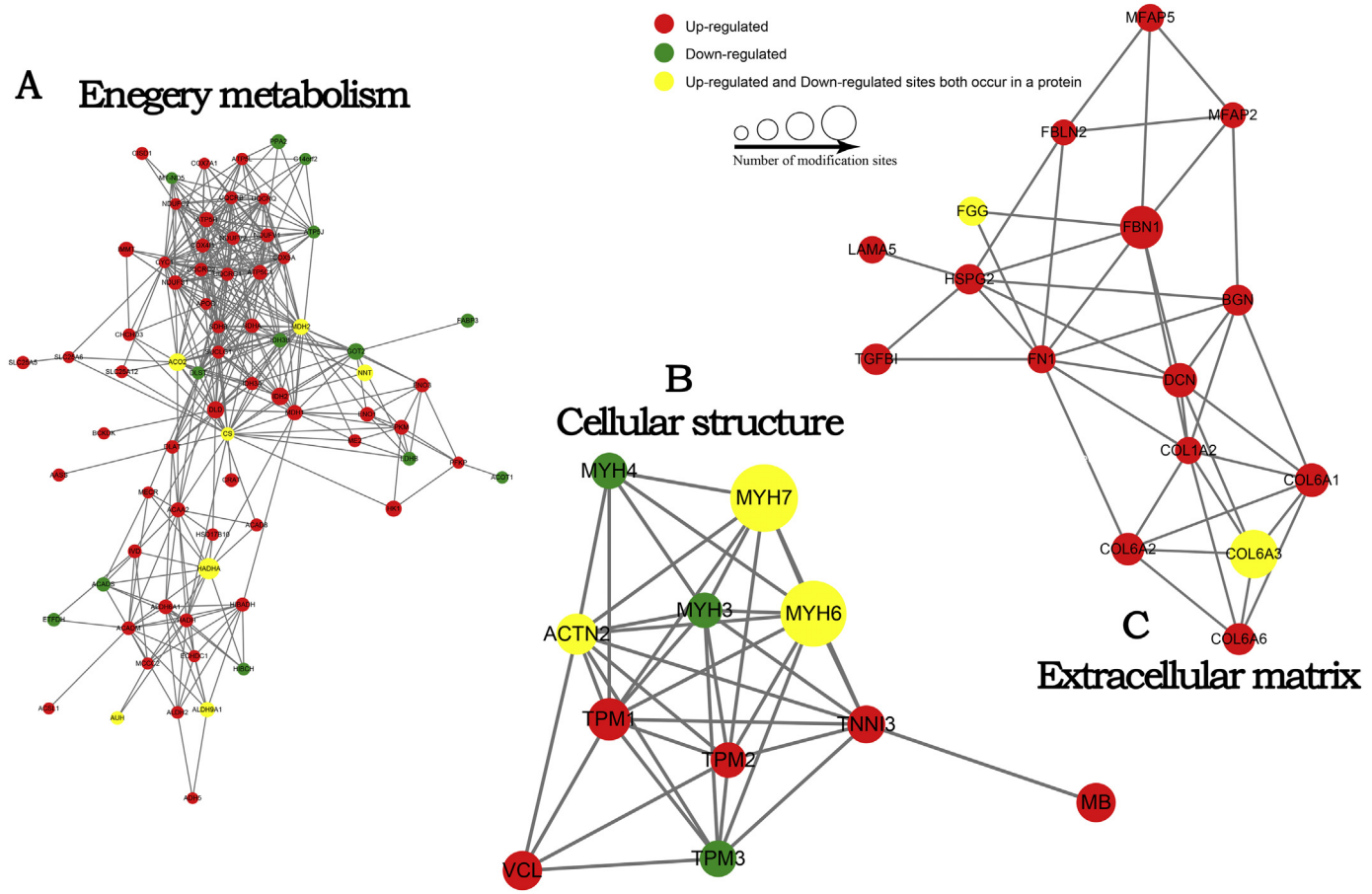

Fig. 6. Succinylated protein-protein interaction network analysis: energy metabolism (A), extracellular matrix related proteins (B) and cellular structure related proteins (C).

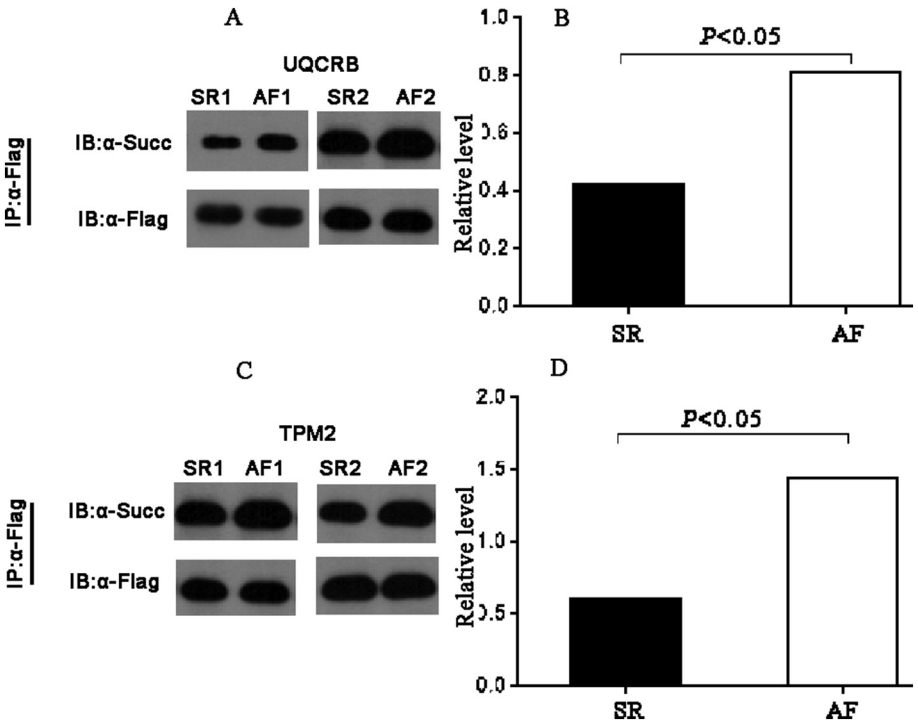

Fig. 7. Up-and down-regulated succinylated proteins in AF/SR.

3.8. Validation results

We identified many differentially expressed succinylated sites according to the quantitative proteomics of changes in succinylated protein expression profiling. We selected two proteins for validation. These two proteins had high succinylated levels and represent energy metabolism (cytochrome *b*-c1 complex subunit 7, UQCRB) and cellular structure (TPM2)-related protein succinylation, respectively. According to the gray-scale value of the straps, the expression levels of succinylated UQCRB and TPM2 in AF patients were significantly higher than those in the SR patients ($P < 0.05$.).

4. Discussion

To the best of our knowledge, this is the first report to analyze protein succinylation in patients with AF. Our results indicate that AF status altered protein succinylation levels, resulting in both up- and downregulated succinylation events. We also analyzed the specific succinylated sites. Modifications to 246 succinylation sites in 132 proteins (AF/SR > 1.3) were quantified as upregulated modifications and modifications to 117 sites in 35 proteins were quantified as downregulated. Furthermore, some proteins contained both up- and downregulated lysine-succinylated sites. These differentially expressed succinylated sites were present in energy metabolism-related, ECM-related, and cellular structural-related proteins. Although we did not prove the significance of a particular succinylated protein via knockout, we identified most of the succinylated proteins in these tissues. These results indicate that succinylation modification events may be involved in the occurrence of AF in patients with valvular heart disease.

PTMs are an effective way to modify proteins. PTMs are ubiquitous in various prokaryotes and eukaryotes and play important roles in various life processes. Among the 20 amino acids, lysine sites are most frequently modified after translation; modifications include acetylation, propionylation, butyrylation, malonylation, and succinylation [20,21,22,23]. Previous studies have reported that PTMs are involved in many cardiovascular diseases, including atherosclerosis, heart failure, hypertension, and arrhythmias [24]. Protein lysine succinylation results from the reaction of the dicarboxylate compound succinyl-CoA with lysine residues. Adding a succinyl moiety to lysine adds a mass of 100 Da and imparts a negative charge at physiological pH. Using the MS/MS method, Zhang et al. identified 14 succinylation-modified sites in *Escherichia coli* [25]. In addition to prokaryotic cells, lysine succinylation has been detected in yeast and SL2 cells [26]. Therefore, modifying lysine residues by succinylation has aroused the interest of researchers. It is well known that persistent balanced energy metabolism is important for maintaining normal cardiac function [27]. Myocardial energy synthesis is composed of fatty acid and glucose production substrates and mitochondrial oxidative phosphorylation produces ATP. The direct source of energy for myocardial activity is ATP [28]. ATP produced by the myocardium originates from the catabolism of glucose, lactic acid, lipids, ketones, and amino acids. Under normal circumstances, the energy source for myocardial activity mainly comes from oxidative catabolism of fatty acids, particularly beta-oxidation of long-chain fatty acids [29]. Myocardial energy metabolism is impaired in many cardiac pathological processes, and impaired energy metabolism function can seriously affect cardiac systolic function [30]. Studies on animal models of early chronic AF suggest that mitochondria, a key structure of energy metabolism in atrial myocytes, may undergo changes, such as deformation and edema [31]. Several studies have shown that changes in energy metabolism occur in both chronic and paroxysmal AF in animal models and humans. Our results show that patients with chronic AF and SR with valvular heart disease appear to have abnormally expressed atrial supply system-related proteins, including sugar metabolism-related, lipid metabolism-related, and biological oxidation-related proteins.

Prominent targets of succinylation are the major metabolic processes, including TCA cycle, the pathways breaking down amino acids and fatty acids and the protein complexes responsible for energy transfer and ATP synthesis [32]. We also found that oxidative phosphorylation-related succinylation modification were significantly upregulated. Previous studies found that altering the availability of succinyl-CoA (by genetically ablating specific enzymes of the TCA cycle) affects the global succinylation pattern in yeast, suggesting that succinyl-CoA used for succinylation comes from mitochondria. In the mitochondria, electron generated by three tricarboxylic acid cycle oxidative phosphorylation to produces ATP by the mitochondrial respiratory chain. High energy phosphate bonds in ATP combine with creatine to from creatine phosphate [33]. Creatine phosphate can be dispersed into the muscle fiber and release ATP under the catalysis of creatine kinase, and used as the energy of myocardial contraction and relaxation [34]. The protein-protein networks indicated that there were 17 sites exhibiting up-regulated in modifications in AF, four sties exhibiting down-regulated modifications, and one site involved both up-regulated and down-regulated modifications. In the valvular heart disease, oxidation of free fatty acid and glucose were inhibited. The process of oxidative phosphorylation is impaired, phosphocreatine levels can be reduced by 30%–70%, and creatine transporter function also decreased. At this point, a small amount of ATP supplied by glycolysis is the only source of myocardial cell survival [35]. The improvement of myocardial energy metabolism may be a new approach in the treatment of AF.

Estimates of succinylation stoichiometry on target proteins in *E. coli*. Are reported but they are only limited to a small number of proteins. The mammalian Sirtuin (Sirt) family of proteins includes seven members, three of which (Sirt3, Sirt4, and Sirt5) are predominantly located in the mitochondria. Sirt5 catalyzes the desuccinylation of lysine residues in a reaction that requires consumption of NAD+ [36]. The study of Sirt5 and its desuccinylase activity has recently been described. In the present study, we identified several metabolic pathway succinylated protein, including glycolysis (PKM) fatty acid oxidation (HADHA ACAA2), PDHc TCA cycle (CS SDHA MDH2), ETC OXPHOS ATP handing (ATP5H), redox balance and sensing (IDH2 ACO2 PRDX1 ME2) and mitochondrial chaperones (HSPD1 HSPE1). Our results are consistent with the proposed role of Sirt5 inrepresding succinylation in previous report. Two studies made the seminal discovery that Sirt5, a mitochondrial sirtuin with only marginal deacetylase activity, had the ability to function as desuccinylase. Park et al. reported that Sirt5 desuccinylates subunits of pyruvate dehydrogenase complex (PDHc) and this reduces PDH's acivity, and a similar regulation mode is suggedted for respiratory complex II [37]. Another study indicated that Sirt5 has activating effects, as it desuccinylates Hmgcs2 to increase hepatic ketogenesis [38]. Sirt5 is also suggested to desuccinylate and activate enzymes regulating fatty acid oxidation in the liver and skeletal muscle [38]. Both of these studies detected Sirt5 in mitochondria and cytoplasm, indicating that Sirt targets both compartments. The former identified 120 extramitochondrial proteins whose succinylation was regulated by Sirt5. The later on did find significant succinylation in the cytoplasm of liver extracts or in MEFSs. Nevertheless, despite their differences with us, these overlap results show that Sirt5-regulated succinylation also exists in cardiac function and suggests that lysines targeted by both PTMs may have important regulatory roles. Very little data are available exploring the magnitude of protein succinylation, its implications in protein function, and its impact on cardiac function. Nevertheless, mitochondria isolated from Sirt5−/− hearts exhibit considerable increases in global protein succinylation [39]. In fact, Succinyl-CoA levels and Sirt5 activity have emerged as the main regulators of protein succinylation and desuccinylation respectively. A recent study found that both succinylation contribute to maturational alterations in energy metabolism in the newborn heart, and the overall succinylation continued to increase even in adult hearts, implicating a distinct role of protein succinylation in cardiac metabolism in matured hearts [40]. Furthermore, in a mouse mode with Sirt5 WT and KO,

Sadhukhan et al. report that protein lysine succinylation predominantly accumulates in the heart when Sirt5 is deleted. Sirt5-deficient mice exhibit defective fatty acid metabolism, decreased ATP production, and hypertrophic cardiomyopathy. This study suggests that regulating heart metabolism and function is a major physiological role of lysine succinylation and SIRT5 [41]. Boylston et al. also found that the unique proteins in Sirt5−/− mouse heart participate in metabolic processes such as fatty acid β-oxidation and branched chain amino acid catabolism, and included respiratory chain proteins. Moreover, they also assessed whether the loss of Sirt5 would impact ischemia-reperfusion injury and they found an increase in infarct size in Sirt5−/− hearts compared to WT type after ischemia and reperfusion. These results further demonstrated that Sirt5−/−not only increased the protein succinylation but also was related to ischemia-reperfusion injury in the heart [42]. These studies suggested the potential roles of Sirt5 in stress-induced cardioprotection. Similar mechanism may occur in valvular heart disease patients with atrial fibrillation. Further studies are required.

In conclusion, proteomic and succinylation levels were significantly altered between AF and SR patients. Most of the differentially expressed proteins were mainly involved in upregulated energy metabolism-related succinylation modification sites. These results indicate that changes in energy metabolism-related protein succinylation affected the occurrence of AF in patients with valvular heart disease. Improving myocardial energy metabolism may be a new approach to treat AF. However, these hypotheses require confirmation through additional experimentation.

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Discourse

The authors declare no conflicts of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.05.002.

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