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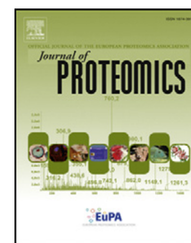

Review

Recent advances in cardiovascular proteomics☆

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ABSTRACT

Cardiovascular diseases (CVDs) are the major source of global morbidity and death and more people die annually from CVDs than from any other cause. These diseases can occur quickly, as seen in acute myocardial infarction (AMI), or progress slowly over years as with chronic heart failure. Advances in mass spectrometry detection and analysis, together with improved isolation and enrichment techniques allowing for the separation of organelles and membrane proteins, now allow for the indepth analysis of the cardiac proteome. Here we outline current insights that have been provided through cardiovascular proteomics, and discuss studies that have developed innovative technologies which permit the examination of the protein complement in specific organelles including exosomes and secreted proteins. We highlight these foundational studies and illustrate how they are providing the technologies and tools which are now being applied to further study cardiovascular disease; provide new diagnostic markers and potentially new methods of cardiac patient management with identification of novel drug targets.

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1. Introduction

Cardiovascular diseases (CVDs) are the major source of global morbidity and death and more people die annually from CVDs than from any other cause. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths [1]. Functionally, heart disease is the inability of the heart to pump sufficient blood to meet the metabolic needs of the body. This disease can occur quickly, as seen in acute myocardial infarction (AMI), or progress slowly over years as with chronic heart failure (HF) [2]. Considering the global health burden of cardiac disease, a greater understanding of the molecular basis of cardiac function will help guide the development of novel diagnostic and therapeutic strategies. In order to improve patient care clinicians require innovations in medical diagnostics that can identify early disease as well as identify novel drug targets that can be used for therapeutics. Techniques such as cDNA and oligonucleotide microarrays make it possible to undertake rapid, global transcriptomic profiling of mRNA expression. However, we and others have found that the presence of RNA does not always correlate with the presence of the protein [3–5] and in particular studies have suggested that RNA expression maybe an unreliable predictor of cell-surface protein [5,6] thereby impeding the identification and discovery of potential membrane embedded drug targets. These caveats can now be overcome by proteomic based studies which provide essential insight into changes in total protein complement during disease as well as insight into post-translation modifications (PTMs) of proteins which are responsible for some of the key biological changes in the function and regulation of proteins. Furthermore, recent technical advances in proteomics and methodologies developed to enrich for membrane proteins will now allow us to investigate cardiac muscle to an unprecedented depth. These technologies provide not only greater scientific insight into cardiac muscle and related diseases, but will also help to develop additional markers of disease progression and even identify novel therapeutic targets to increase our ability to manage cardiac patients. In this review, we outline progress made in these fields and highlight innovative technologies of cardiac research which could potentially improve patient diagnosis and therapies.

2. Proteome and subproteome insight into the heart

The majority of cardiac proteomic research has traditionally been carried out using 2 dimensional gel-based approaches (2-DE) in which proteins are separated in two dimension according to their charge properties (isoelectric point) [7] under denaturing conditions and then their relative molecular mass (M_r) by SDS-PAGE [3,8,9]. This methodology previously played a central role in providing insights into not only the biology of the normal heart [10,11], but also elucidating markers of disease [12,13]. Inherent with the application of 2-DE is its ability to detect PTMs which can cause changes in the isoelectric point and/or the molecular weight of the modified protein, which are readily detected. However, the major limitation of 2-DE to display complete proteomes is the limited dynamic range of 2-DE, with an estimated maximum dynamic range of 10^4 magnitude [3,14], compared with the very high dynamic range of protein abundance, estimated at 10^6 for cells and tissues [3] and 10^{12} for plasma [3,15].

To overcome these restrictions, subcellular fractionation methods have been developed to reduce sample complexity. These methods include differential centrifugation, flow cytometry, immune-based isolation, membrane protein enrichment strategies and/or density gradient isolation of organelles such as the nucleus or mitochondria. These isolation methods are now used either in tandem with 2DE or 1D gel SDS based studies, or more recently, with gel-free LC–MS. Removing the 2DE gel also removes one further major caveat with 2DE which is that membrane proteins are usually underrepresented because of their poor solubility in the isoelectric-focusing sample buffer. With this in mind, Franklin et al. used subcellular gradient fractionation to isolate murine cardiac nuclei followed by further fractionation into acid soluble proteins, chromatin bound molecules, and nucleoplasmic proteins and identified a nuclear proteome of 1048 proteins many of which isolated uniquely to one sub-fraction in the nucleus [16]. They identified 142 integral membrane proteins, the majority of which were exclusive to the nucleoplasmic fraction. The authors further used high mass accuracy techniques to identify peptides that mapped to a total of 54 histone variants, 17 of which were identified by at least 1 unique peptide. This study provided subcellular localisation information of proteins to cardiac nuclei under normal conditions, and laid the foundation for analysis of potential protein trafficking and re-distribution under diseases conditions.

As another example of fractionation applied in cardiac muscle, several studies have assessed mitochondrial proteomes. Mitochondria are essential for cell survival both not only because of their role as metabolic energy providers, but also as regulators of programmed cell death. Mitochondria are double-membrane containing organelles with many membrane embedded proteins, two features that provide a unique challenge concerning solubility. For instance, early 2-DE analysis coupled to MALDI using human placenta identified only 46 proteins [17]. However, more recent work has characterised mitochondria to a greater depth. Studies carried out by Taylor et al. used sucrose density centrifugation to enrich for human mitochondrial proteins in the presence of detergent to aid solubility, applied 1D SDS-PAGE coupled to LC/MS/MS which resulted in the identification of 615 human mitochondrial proteins [18] and more recently Zhang et al. using a similar methodology using isolated mouse mitochondria and identified 940 distinct mitochondrial proteins; 480 proteins of which had not been identified previously [19].

The characterisation of changes within the mitochondrial proteome represents a focused area of research in cardiovascular disease [21–24]. For example, in atrial fibrillation (AF) which is the most common sustained cardiac arrhythmia [25,26] clear changes are seen in the function and morphology of mitochondria [26–28]. Gel free LC/MS/MS carried out by Goudarzi et al. on isolated mitochondrial fractions from 20 right atrial appendages from 10 patients diagnosed with AF and 10 patients without AF identified a total of 700 proteins with 108 proteins differentially expressed in AF patients [26].

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