Preface

Over the past few years, the power and potential of proteomics has become widely recognized. The use of proteomics for the study of complex diseases is increasing and is particularly applicable to cardiovascular disease, the leading cause of death in developed countries. The ability to investigate the complete proteome provides a critical tool toward elucidating the complex and multifactorial basis of cardiovascular biology, especially disease processes. Proteomics involves the integration of a number of technologies with the aim of analyzing all the proteins expressed by a biological system in response to various stimuli under different pathophysiological conditions. The proteomic approach offers the ability to evaluate simultaneously the changes in protein expression and cell signaling pathways in response to such conditions as atherosclerosis, cardiac hypertrophy, stroke, or heart failure.

Cardiovascular Proteomics: Methods and Protocols covers many of the above aspects of the proteomic approach in the cardiovascular field. This volume takes the reader through the complete process of proteomic analysis, from the obtention of specific heart proteins (troponin I) to the new techniques of identifying risk biomarkers of atherome plaque rupture, analyzing the secretome of explanted endarterectomies cultured in vitro, or the application of phage display techniques to decipher the molecular diversity of blood vessels. Determining global changes in the protein expression levels of cardiac myocytes in response to states of ischemia, hypertension, hypertrophy, heart failure, or infarction may disclose new molecules directly involved in these pathologies. Thus, detailed protocols for the isolation and the short- and long-term culture of adult mouse cardiac myocytes have been included.

Cardiovascular Proteomics: Methods and Protocols covers not only the most recent advances of separating proteins by two-dimensional electrophoresis (2-DE; zoom gels, large formats, cup-loading, basic pH ranges) and 2D difference gel electrophoresis, but also the newly developed strategies of liquid chromatography coupled to mass spectrometry (LC-MS) or the SELDI-TOF approach to searching for biomarkers of stroke in human serum or of hypertension in the serum of animal models. A key requirement for analysis of the serum proteome is the depletion of the most abundant proteins (mainly albumin and immunoglobulin G) in order to detect lower abundance proteins that might prove to be potential biomarkers of disease. Thorough descriptions of the most effective methods (immunoaffinity substraction, delipidation combined with specific precipitation) to deplete these proteins have been included.

In recent years the proteome (and secretome) of the most relevant cellular elements of the cardiovascular system has begun to be depicted with the

concomitant construction of 2-DE maps and databases. The proteomic strategies and protocols to study the proteome of endothelial, arterial smooth muscle cells, foam cells, circulating blood monocytes, and platelets constitute an essential part of *Cardiovascular Proteomics: Methods and Protocols*. These databases will provide a consistent basis for comparative studies of protein expression levels in these cells from healthy and patient subjects.

Special mention is given to two research areas of high current interest: the analysis of subproteomes and the characterization of posttranslational modifications. *Cardiovascular Proteomics: Methods and Protocols* includes protocols for subcellular fractionation and the obtention of several organelles, cytosol, membranes, and so on, and particularly of heart mitochondria and the analysis of its proteome (comprised of more than 1000 polypeptides). The methods for the purification and characterization of the proteins in others subproteomes (myofilaments, caveolae from endothelial cells, phosphoproteome of human platelets) are also described in detail. The analysis and characterization of complex associations of proteins are central aims of proteomics. A good example is included in *Cardiovascular Proteomics: Methods and Protocols* with the description of the proteomic analysis of the subunit composition of complex I (ubiquinone oxidoreductase) from bovine heart mitochondria. There are numerous examples of cardiovascular functions whose molecular pathways are mediated through posttranslational processes, such as phosphorylation and glycosylation. Several protocols are included illustrating these topics (identification of targets of phosphorylation in mitochondria, analysis of phosphorylated isoforms of HSP-27 in atherosclerotic plaques, identification of *S*-nitrosylated proteins in endothelial cells).

The application of proteomics to cardiovascular disease holds great promise and offers exciting advances towards predictive, preventive, and personalized medicine. It is conceivable that the analysis of a simple plasma sample will provide unique prognostic information about an individual's risk of atherothrombotic disease or heart failure. Similarly, proteomic analysis of a myocardial biopsy specimen may provide useful prognostic information in patients with unexplained heart failure or in cardiac transplant recipients. It is our intent that our book will contribute to this important task.

We hope that readers will find *Cardiovascular Proteomics: Methods and Protocols* a useful snapshot of the currently available technologies, a starting point for evaluating the applicability of these techniques to their own research. The editor is especially grateful to all the contributing authors for the time and effort they have put into writing their chapters and particularly to the Methods in Molecular Biology series editor, John Walker, for his continuous advice and support through the editorial process.

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