SYMPOSIUM

Physiological Proteomics: Cells, Organs, Biological Fluids, and Biomarkers

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Proteomic research is accelerating rapidly because of marked advances in protein labeling techniques, mass spectrometry (MS), and bioinformatics. Two-dimensional difference gel electrophoresis (2D-DIGE) is being used effectively in conjunction with liquid chromatography tandem MS (LC-MS/MS) and/or matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-ToF MS) and database search software to quantify relative changes in the levels of proteins in two samples. It is now possible in a single study to identify and quantify large numbers of proteins and their posttranslational modifications in different biological samples. Comparisons can be made between groups of animals in different physiological states or in response to experimental treatment. Differences between normal individuals and those in disease states can form the foundation for elucidation of causative factors of disease and the identification of biomarkers for the diseased state. This symposium includes original research that compares the erythrocyte plasma membrane proteome in the normal and the sickle cell state, evaluates the anterior pituitary gland proteome in the ovariectomized rat in response to estrogen, and assesses proteomic methodology employed to identify potentially useful biomarkers in human cells and fluids for clinical medicine. It is directed not only to investigators working in these fields but also to a diverse group of scientists working in the biological and biomedical fields to stimulate cross-disciplinary awareness, interest, and collaboration. Exp Biol Med 230:785–786, 2005

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Proteomics is the study of the proteome, the expressed protein complement of cells, tissues, organs, or biological fluids. It includes the identification of proteins and changes in those proteins that occur in different biological and disease states. Posttranslational modifications of proteins are an important part of the proteome that may play an essential role in determining function.

The field of proteomic analysis has made tremendous progress during the past several years. This is due to advances in protein labeling techniques, mass spectrometry (MS) for proteomic analysis, and bioinformatics for data analysis. It is now possible within a single study to identify numerous proteins and perform relative quantifications on them in two different samples. The techniques employed offer extensive applications to both animal and clinical studies. In addition, they can be used to identify and quantify biomarkers not only to indicate changes in physiological states but also to characterize disease states.

This symposium, titled "Physiological Proteomics," addresses many aspects of proteomics, including applying proteomics, to answer physiological questions in health and disease. The symposium includes studies of cells, organs, and biological fluids. It also includes some of the latest developments in state-of-the-art approaches to conducting proteomics. Physiological proteomics is a new and exciting field with broad applications.

The symposium was presented at the annual Experimental Biology meeting on Monday, April 4, 2005, in San Diego, California. The Society for Experimental Biology and Medicine (SEBM) is grateful to Charles A. Blake, Ph.D., and Steven R. Goodman, Ph.D., for organizing and cochairing the symposium. Manuscripts submitted by three speakers described herein have been peer-reviewed and accepted for publication. To follow is a brief synopsis of their findings and their implications for the application of proteomics to animal models and human health and disease.

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Steven R. Goodman, Ph.D., presented new research on the quantification of changes in the human erythrocyte membrane proteome in association with sickle cell disease. The studies were conducted with David G. Kakhniashvili, Natalya B. Griko, and Lee A. Bulla, Jr. He described how erythrocyte plasma membranes were isolated, membrane proteins were solubilized for two-dimensional difference gel electrophoresis (2D-DIGE) technology, and proteins were identified by liquid chromatography tandem MS (LC-MS/MS) and database software. Dr. Goodman focused on a number of proteins that were identified as being upregulated or downregulated in the sickle cell state. The majority of the regulated proteins were classified in five categories: actin accessory proteins, components of lipid rafts, scavengers of oxygen radicals, protein repair participants, and protein turnover components. Some of these changes may contribute to the malfunctioning of the erythrocyte membrane in this devastating disease. These data are presented in the original research article titled "The Proteomics of Sickle Cell Disease: Profiling of Erythrocyte Membrane Proteins by 2D-DIGE and Tandem Mass Spectrometry" by Kakhniashvili, Griko, Bulla, and Goodman that follows.

Charles A. Blake, Ph.D., detailed design considerations for conducting proteomic analyses of the anterior pituitary gland (AP) with its mixed epithelial cell types. He discussed sample preparation procedures that were employed successfully in conjunction with LC-MS/MS to analyze subcellular fractions of the AP of Golden Syrian hamsters and mice. He also addressed methodology to match proteomic results to specific cell types within the gland. These studies conducted on the AP could serve as a model for proteomic analysis of other heterogeneous organs. An overview of these studies titled "Proteomics of the Anterior Pituitary Gland as a Model for Studying Physiology of a Heterogeneous Organ" by Blake and Helmke follows.

Dr. Blake then presented new research that quantified estrogen-induced changes in the AP proteome of the ovariectomized rat. These studies were conducted with Steve M. Helmke, Lewis M. Brown, Stephen W. Hunsucker, and Mark W. Duncan. He described how the AP-soluble protein fraction was prepared for 2D-DIGE technology and proteins were identified by MALDI-ToF MS and database software. Using this technology, he reports that estrogen upregulates acidic isoforms of growth hormone and prolactin. In addition, a significant number of nonhormonal proteins and their isoforms, many of which are involved in AP hormone secretion, were upregulated or downregulated in response to estrogen. These data are reported in the original research article titled "Estrogen Regulation of the Rat Anterior Pituitary Gland Proteome" by Blake, Brown, Duncan, Hunsucker, and Helmke that follows.

Mark W. Duncan, Ph.D., focused on proteomic methodology employed to identify potentially useful biomarkers and characterize these candidates in the discovery phase. He discussed the importance of employing multiple analytical strategies to identify proteins and their posttranslational modifications and provided examples from studies conducted in his laboratory employing human seminal fluid, urine, tear fluid, and non–small-cell lung cancer cell lines. He addressed 2D-DIGE methodology to quantify gel spots and the subsequent identification of proteins in the spots by MALDI-ToF MS and/or LC-MS/MS. Dr. Duncan pointed out the importance of using immunoassays in addition to MS to quantify proteins in biomarker studies. He also addressed the importance of rigorous design in proteomics, especially in clinical proteomics during the validation phase in the development and testing of disease biomarkers. An overview of these studies titled "Proteomics as a Tool for Clinically Relevant Biomarker Discovery and Validation" by Duncan and Hunsucker follows this introduction.

As a consequence of the symposium, we now have a better understanding of the application of MS and additional proteomic techniques to identify and quantify large numbers of proteins in cells, organs, and fluids. The efficacy of identifying and quantifying large numbers of proteins in two different groups in a single study has enormous potential for determining the effects of experimental treatments on proteomes as well as differences between human proteomes in normal and disease states, changes that could be used as biomarkers to diagnose disease. It is hoped that this symposium and its publication will introduce and provide examples of the power of proteomics, thereby stimulating cross-disciplinary awareness, interest, and collaboration between biomedical scientists and clinicians.

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