EDITORIAL COMMENT

Heart Disease Leaves its Mark

Proteomics-Based Biosignatures in Acute Coronary Syndromes*

W. Robb MacLellan, MD, FACC, Peipei Ping, PhD, Thomas Vondriska, PhD
Los Angeles, California

In 1990, the Human Genome Project officially began with a goal to sequence the entire human genome in 15 years. With progressive technologic advancements and the contributions made by international groups and private industry, a draft of the human genome was published in 2001, well ahead of expectations (1,2). This accomplishment has had a profound impact on the scientific, medical, and pharmaceutical communities and ushered in a new era of diagnostic and therapeutic discovery. The Human Genome Project identified ~32,000 distinct genes in humans; however, there are many more proteins than genes due to the post-translational modifications of proteins, making the human proteome much larger and more complex. Proteomics—the comprehensive and systematic study of global alterations in protein expression in normal or diseased biologic samples—has emerged as a novel field of study to address the technical challenges posed by this complexity.

The sheer number of proteins and post-translational modifications, as well as the dynamic nature of protein expression, in contrast to the relatively stable genome, makes proteomics very challenging. It has only been with technologic advances in the last decade that the tools have become available to begin to apply proteomic methodology to clinical medicine. These advances include enhanced protein separation and staining techniques, allowing identification of less abundant proteins and improvements in mass spectrometry (MS), which allow unequivocal identification of proteins of interest, rather than simply following changes in expression patterns. It is exactly these complexities in proteomics that underlie the high expectations of this technology to deliver improved human health care, as nearly all disease states, even those with genetic origin, ultimately involve malfunctioning, modified, and/or absent proteins. Accordingly, proteins are the final targets of most drugs. Thus, a greater understanding of protein dynamics (e.g., turnover, structure, and activity) will facilitate the design of more accurate and efficacious drugs. In clinical medicine, integration of proteomics-based approaches into standard care has at least four potential advances over current approaches (3–5): 1) protein biomarkers of cardiovascular disease identified by proteomics could lead to earlier and more accurate detection of cardiovascular syndromes; 2) proteomics-derived information may complement existing diagnostic tools in the risk stratification of patients; 3) proteomic information gathered on a patient-by-patient basis will likely facilitate individualized treatment; and 4) proteomic tools and information applied at various stages of treatment will allow for real-time assessment of treatment efficacy and will enhance the ability to modify future therapy options.

The methodologic advancements in the field of proteomics, as described already, now allow the analysis of complex fluid or tissue samples under clinically relevant conditions and protein profiling to study specific diseases or biologic processes. The study by Matteos-Cáceres et al. (6) in this issue of Journal utilizes a proteomics approach to describe novel biosignatures associated with acute myocardial infarction (AMI) and unstable angina (UA). Each year, eight million patients with chest pain are evaluated in emergency departments in the U.S., and nearly one-half of these individuals are admitted to inpatient units for further evaluation and treatment. Despite advances in the diagnosis of acute coronary syndrome (ACS), only a minority of these patients will ultimately be found to truly be suffering from a cardiac condition (7). Cardiac biomarkers, such as cardiac troponins, brain natriuretic peptide (BNP), and highsensitivity C-reactive protein (hs-CRP), have played an increasingly important role in triaging these patients, as well as guiding therapy and determining the subsequent risk of adverse events (8). However, further progress is needed to characterize markers that better identify specific subsets of affected patients, detect underlying pathology, and predict the response to subsequent therapy.

The majority of proteomic investigations in recent years have taken one of two broad approaches: expression proteomics, in which qualitative and quantitative alterations in proteins are catalogued for a given clinical condition; and functional proteomics, in which additional attributes of multiple proteins are studied and a mechanistic link between proteome change and disease phenotype is sought. Investigations like that by Matteos-Cáceres et al. (6) combine these two approaches and illustrate the power of a focused proteomic investigation to target a subset, or sub-proteome, of proteins potentially containing diagnostic and etiologic information related to a disease phenotype. In their study, Matteos-Cáceres et al. (6) employed two-dimensional electrophoresis and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) MS—now standard techniques in proteomics—to display and identify proteins in plasma from patients with either UA or

*Editorials published in the Journal of the American College of Cardiology reflect the views of the authors and do not necessarily represent the views of JACC or the American College of Cardiology.

From the Cardiovascular Research Laboratory, Departments of Physiology and Medicine, David Geffen School of Medicine at University of California at Los Angeles, Los Angeles, California.
AMI. The authors initially identified proteins by peptide mass fingerprinting in the single MS mode. Although this method is adequate to identify proteins under some conditions (such as after two-dimensional electrophoresis), peptide mass fingerprinting is not a robust technique for analysis of complex mixtures (i.e., more than a few proteins) and does not provide optimal accuracy for protein identification. In some cases, however, the investigators employed MALDI-TOF-TOF analyses to identify proteins when single MS analyses proved equivocal. This tandem MS approach offers among the highest sensitivity for protein identification that is currently available and increased the confidence in some of the identifications made by the authors. The importance of this technique should not be overlooked, as it highlights an aspect of proteomics that facilitates the understanding of disease pathogenesis in addition to yielding biomarkers. As mentioned earlier, even the gel pattern (or MS patterns, as used by other investigators [4]) can serve as a biosignature of disease phenotype. However, the ability to reap peptide sequence information (which can only be done with tandem MS experiments) allows for the confident identification of proteins and for the mapping of post-translational modifications and degradation products. These two changes, not obvious from genomic information, can be clearly characterized only with MS/MS approaches, using electrospray or MALDI (as in the Matteos-Cáceres et al. [6] study) ionization sources.

The investigators reported four areas within the plasma protein map corresponding to isoforms of alpha-1-antitrypsin (AAT), fibrinogen gamma chain (FGG), apolipoprotein A-I and gamma-immunoglobulin heavy chains that changed with AMI and UA. One of these proteins—AAT—is a strong inhibitor of several proteolytic enzymes, and low AAT levels have been suggested to play a role in atherogenesis (9). The authors found that AAT isoform I was absent in the plasma of patients with AMI and UA, with more subtle changes in the expression of isoforms 5, 6, and 7, which differed between AMI and UA, suggesting a unique biosignature existed for each condition. Although it is tempting to speculate on a link between these changes and pathophysiologic mechanisms, it should be noted that these findings merely suggest an association between ACS and AAT, and more definitive proof will await larger prospective studies combined with mechanistic data from animal-based experiments. Likewise, although statistically significant, all the differences, except with AAT isoform I, were very modest. Given the fact that gender, age, race, differences in medication use, and co-morbid conditions can effect the proteome (10), the choice of control group is crucial. A major methodologic problem plaguing similar genetic studies is the selection of controls, which may be an important source of bias. The choice of control groups in future studies will need to be closely scrutinized if the findings generated are to lead to useful pathophysiologic insights or, alternatively, if they are to be useful as clinical screening tools. Nonetheless, the report by Matteos-Cáceres et al. (6) is potentially an important study, as there is a paucity of data in the literature regarding proteomic changes associated with coronary syndromes.

This report likely represents the first of many in this field, as there is certainly additional important information that can be generated on ACS by similar studies. As recognized by the authors, all electrophoretic techniques are limited by stain sensitivity and dynamic range of protein expression and thus are not able to visualize all proteins. In response to this limitation, the authors focused their analysis on regions of overt difference between the control and diseased two-dimensional electrophoresis plasma maps, but this approach does not allow comprehensive coverage. Therefore, this study would not identify potentially important changes in proteins below this threshold, a limitation that can only be overcome by repetitive, unbiased gel sampling. An advantage of this approach, however, is that by focusing on an area of marked alteration between the normal and diseased patients, the authors increased their chances of identifying proteins with altered function that are directly related to the disease phenotype. Future studies that utilize cutting-edge MS techniques and equipment will undoubtedly generate additional information. Tandem MS approaches, aimed at characterizing protein modification, have the potential to yield additional information on post-translational modifications, cleavage events, and other protein alterations that are, in themselves, biomarkers. Likewise, despite the ability of gel staining to give a relative estimation of protein abundance, true MS quantitation requires protein-labeling techniques (e.g., isotope-coded affinity tagging). The implementation of these quantitative techniques to clinical proteomic investigations will increase the applicability of the findings to diagnosis and care.

As these sophisticated proteomic markers/signatures for coronary syndromes and other diseases continue to develop, the challenge will lie in translating the information gained from these screens into clinically useful diagnostic tests or therapeutic interventions. Although MS-based signatures can be easily generated in a reasonably outfitted proteomics laboratory, the time and expertise to carry out these techniques, as well as the cost of the necessary equipment, are likely to prohibit the application of these technologies “as is” in the clinical setting. Therefore, unless significant advances occur in the field of MS in the near future, assays will need to be developed once a relevant biosignature is identified, which are both more sensitive and inexpensive, such as targeted immunoassays. Likewise, if identification of clinically useful biosignatures is to occur, future studies will need to address current limitations with quantitation. It will be essential that investigations deliver truly quantitative information on protein expression or modification changes associated with coronary syndromes or other cardiovascular diseases. Moreover, qualitative and quantitative changes must be analyzed on much larger cohorts of patients with carefully chosen control populations.
These studies will likely require more extensive, perhaps multi-institutional clinical trials to orchestrate collection of plasma (or other body fluid) samples and to coordinate with scientists with expertise in proteomics who can evaluate these end points. Proteomics holds substantial promise to improve the diagnosis and care of patients, but how both basic science and clinical investigators deal with the considerable challenges will determine whether this promise is to be delivered.

Reprint requests and correspondence: Dr. W. Robb MacLellan, Cardiovascular Research Laboratory, David Geffen School of Medicine at UCLA, 675 C.E. Young Drive, MRL 3-645, Los Angeles, California 90095-1760. E-mail: rmaclellan@mednet.ucla.edu.

REFERENCES