This Review is part of a new thematic series on **Proteomics: New Methods, Biological Insights, and Clinical Perspectives**, which includes the following articles:

Jennifer E. Van Eyk, Guest Editor

Overview
The Maturing of Proteomics in Cardiovascular Research

Jennifer E. Van Eyk

Abstract: **Proteomic technologies are used to study the complexity of proteins, their roles, and biological functions. It is based on the premise that the diversity of proteins, comprising their isoforms, and posttranslational modifications (PTMs) underlies biology. Based on an annotated human cardiac protein database, 62% have at least one PTM (phosphorylation currently dominating), whereas ≈25% have more than one type of modification. The field of proteomics strives to observe and quantify this protein diversity. It represents a broad group of technologies and methods arising from analytic protein biochemistry, analytic separation, mass spectrometry, and bioinformatics. Since the 1990s, the application of proteomic analysis has been increasingly used in cardiovascular research. Technology development and adaptation have been at the heart of this progress. Technology undergoes a maturation, becoming routine and ultimately obsolete, being replaced by newer methods. Because of extensive methodological improvements, many proteomic studies today observe 1000 to 5000 proteins. Only 5 years ago, this was not feasible. Even so, there are still road blocks. Nowadays, there is a focus on obtaining better characterization of protein isoforms and specific PTMs. Consequently, new techniques for identification and quantification of modified amino acid residues are required, as is the assessment of single-nucleotide polymorphisms in addition to determination of the structural and functional consequences. In this series, 4 articles provide concrete examples of how proteomics can be incorporated into cardiovascular research and address specific biological questions. They also illustrate how novel discoveries can be made and how proteomic technology has continued to evolve. (*Circ Res*. 2011;108:490-498.)**

Key Words: proteomics ■ technology ■ protein isoform ■ posttranslational modification ■ polymorphism

In this accompanying series, we explore how proteomics has matured and its application in cardiovascular research. Cardiovascular science investigates and analyzes the biological systems involved in prevention, treatment, and development of disease. It is often aimed at discovering the fundamental principles that govern or underlie the molecular disease pathways. To achieve this aim, there has been and is a constant need for technological development. In this context, technology refers to techniques, instrumentation, and approaches/strategies that are used to solve problems that are involved in research. Each technology must, therefore, have a particular utility and use. Proteomics is a field that has been driven by the development and strategic application of technology and it continues to rapidly evolve. Proteomic

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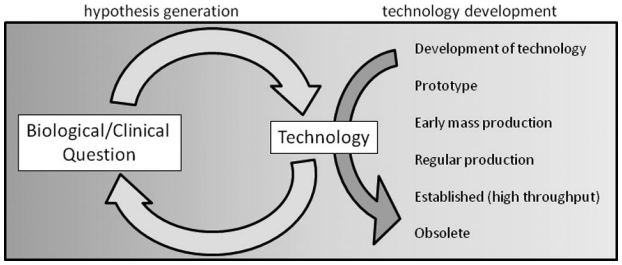

Figure 1. Schema representing the relations that are involved in discovery. Biological or clinical questions drive the development of new technologies that, when applied, will generate new hypotheses. All technology undergoes an evolution from development to finally becoming obsolete and being replaced by new or refined methods.

technologies are used to study the complexity of proteins and their biological roles, including biophysical properties, structure, and function. It is based on the premise that the diversity of proteins, comprising their isoforms, SNPs, and posttranslational modifications (PTMs), underlies biology. Protein alterations occurring because of physiological and pathological processes are reflective of changes at the gene, mRNA, miRNA and metabolite levels. Thus, the proteome consists of information from protein expression, PTMs, processing and turnover, localization and time. PTMs alter the chemical nature of an amino acid residue; which can induce changes in protein structure, activity, binding partners or subcellular localization. PTMs can add or remove a functional group (including a peptide) to a specific amino acid residue within a protein. PTMs include acetylation, amidation, methylation, sumoylation, ubiquitination, prenylation, myristoylatoin, *S*-glutathiolation, sulfation, *N*- and *O*-linked glycosylation, glycation, *S*-nitrosylation (SNO) (*S*-nitrosation) proteolysis and phosphorylation among others. The field of proteomics strives to observe and quantify this protein diversity. It represents a broad group of technologies and methods arising from analytic protein biochemistry, analytic separation, mass spectrometry and bioinformatics. Many technologies are synergistic and complement each other providing different data for each proteome. Proteomic technology development can be linked to specific biological and clinical questions (Figure 1). This is a reiterative situation as the application of different technologies can provide additional insights and generate new hypotheses, which is often assisted by additional methodological refinement and optimization or even creation of new technologies.

Process of Technology Development

Technology development is a process involving multiple steps (Figure 1). These steps are (1) the development phase, where the method or technology is born from trial and error experimentation; (2) prototype production, where the consistency and variability is determined; (3) early mass production, where the method requires minimal changes but is tested on a number of different situations or conditions allowing for fine-tuning; (4) regular production with standardization, where problems only rarely are experienced; (5) established method, where the wider scientific community can use the robust technology; and (6) obsolete, where the technology is no longer required or has been replaced with new methods that provides "better" data (eg, qualitative versus quantitative). Often the application of technology requires the establishment of a technical pipeline representing a number of distinct processes that are linked to solve a problem. Of equal value is the adaptation of new technology to more complex samples or experimental conditions. More often methods are initially developed using simple standards such as purified peptides or proteins. The technology then must be vetted in samples of increasing complexity. Moving the technology to a cell subproteome, intact cell or tissue can be extremely time-consuming and require considerable additional experimentation at the prototype or early mass production stages. This can also be true when changing among different subproteomes, cells and tissue types (eg, HeLa cells versus cardiac myocytes; cardiac muscle versus liver; mouse versus human). Proteomic analysis of cardiac myocytes and tissue is particularly challenging compared to many other tissues, and the reasons for this are multifactorial. Chief among them is the fact that the cardiac muscle has a large dynamic range attributable to dominance of the myofilament and mitochondrial subproteomes, and this generally requires extensive fractionation or targeted enrichment to observe low abundant proteins (eg, see elsewhere[1–10]). This unique aspect of cardiac muscle is linked to its intrinsic physiology: the sustained, intense energetic requirements for continuous contractile activity, and the need for a minute to minute adaptation of heart rate, blood pressure, and cardiac output. Another unique aspect of the myocyte proteome is the large number of cardiac-specific isoforms and their regulation by different PTMs. An example illustrating the biological importance of protein isoforms is that of cardiac troponin (cTn)I, which regulates muscle contraction/relaxation, but unlike the skeletal muscle isoforms is phosphorylated by protein kinase A and other kinases (eg, see elsewhere[11–14]). As well, it has been shown to undergo other types of PTMs (*O*-GlcNAcylation and proteolysis) with different pathological conditions (eg, see elsewhere[15–18]). In many cases, a small degree of change to one of these PTMs can result in large physiological

Non-standard Abbreviations and Acronyms

2DE	2D gel electrophoresis
cTnI	cardiac troponin I
GWAS	genome-wide association study
HPRD	Human Protein Reference Database
ITRAQ	isobaric tag for relative and absolute quantification
LC	liquid chromatography
MRM	multiple reaction monitoring
MS	mass spectrometry
***O*-GlcNAc**	*O*-GlcNAcylation
PTM	posttranslational modification
SILAC	stable isotope labeling by amino acids in cell culture
SNP	single-nucleotide polymorphism
SNO	*S*-nitrosylation or *S*-nitrosation
TMT	tandem mass tag

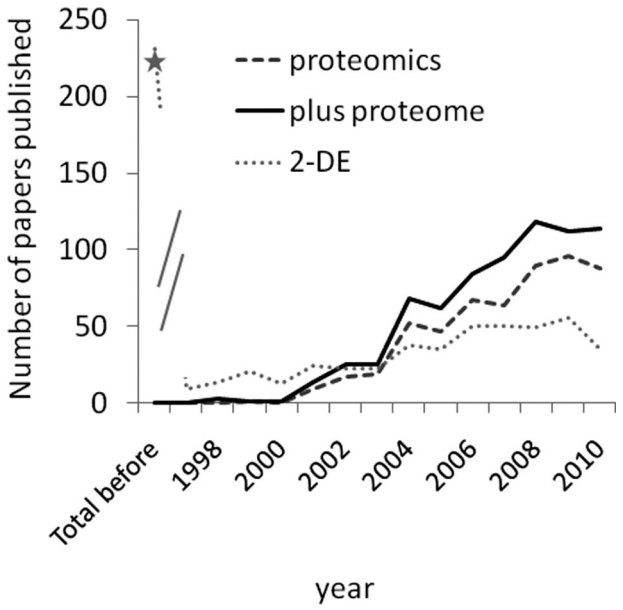

Figure 2. The number of articles published per year using proteomics in the area of cardiac and heart research has been steadily increasing. PubMed was searched using (1) "proteomics" alone or with "proteome" and (2) either "heart or cardiac" as subjects. Articles that were not in English or were cited as reviews were excluded from the total count. There were no articles before 1998. Note, however, that when a search was carried out where "proteomics/proteome" was replaced with 2DE, articles earlier than 1998 were identified.

alterations (eg, 15% substitution of the truncated cTnI form results in ≈50% reduced maximal force[19]). Because subtle PTM changes can have large functional consequences, it is necessary to develop and use techniques that can assess multiple PTMs, identify and quantify each modified residue, and be very accurate (a necessity when quantifying low abundant PTMs). In addition to the problems outlined above, the final issue for cardiac muscle proteomic studies is that there are no dividing cell culture systems for ventricular cardiomyocytes. Approaches dependent on complete saturation metabolic labeling (such as stable isotope labeling by amino acids in cell culture also known as SILAC[20]) are therefore not possible. To detail cardiac protein changes, unique metabolic labeling strategies or alternative technologies will need to be extended or adapted.

Technology Progression in the Field of Proteomics

Since the mid- to late 1990s, the application of proteomic analysis has been increasingly used in cardiovascular research. As shown in Figure 2, there has been a continuous increase in the publication of studies using proteomic (or proteome) in PubMed. Two-dimensional gel electrophoresis (2DE), a traditional protein separation method in proteomics that combines separation of intact proteins by pI and molecular weight that was developed in the mid-1970s,[21,22] continues to increase, but in the last few years has contributed less toward the total number of proteomic based studies. This is because of the increasing number of other protein and peptide separation methods which have been developed or refined over the last decade along with improvements in the sensitivity and accuracy of mass spectrometers and more comprehensive bioinformatics. 2DE is among the few methods that allow the determination of quantity and potential PTM or isoform status of proteins (those that differ in the pI of the protein). It continues to be improved with methods for solubilization and loading of difficult proteins (eg, hydrophobic or basic) onto the gel, more sensitive protein stains and labeling that allow simultaneous analysis of multiple samples, and improved image alignment and quantification (reviewed elsewhere[23–26]). Other intact protein separation methods involving liquid phases (free-flow electrophoresis), capillary electrophoresis, and liquid chromatography (LC) have similar benefits as 2DE and, interestingly, often observe a different set of proteins (reviewed elsewhere[27–30]). This is attributable to the solubilization or separation parameters. By using more than one method in a multidimensional arrangement (off-line or in-line with mass spectrometry [MS]), an investigator can increase proteome coverage (number of proteins observed) and often protein coverage (number of peptides observed for each protein). As examples from our group, we have compared a number of different protein and peptide separation methods,[1,9,31,32] and, in some, cases there is only <30% overlap in the proteins observed between methods. Today, many studies observe and quantify 1000 to 5000 proteins, whereas 5 years ago, this was not feasible even with extensive separation methods. To date, the type of data that proteomics can help achieve can be broken into (1) protein identification (which allows one to create a database to determine the protein composition of a specific protein complex, organelle, or cell); (2) protein quantification (either determining the relative ratio or absolute concentration); (3) identification of protein isoforms; (4) determining whether a protein has a PTM, determining the type of PTM, and identifying the specific modified amino acid residues; and finally (5) quantification of each modified residue. Certainly, the level of technical difficulty increases as one moves up to more specific and quantifiable data.

Protein Identification and Quantification

Protein identification is at the heart of most proteomics. This can be accomplished using MS or protein-specific capture/detecting regents such as antibodies. Antibody detection and quantification of intact proteins has also been improving with the large-scale production of antibodies against human proteins by both industry and academia. Of note is the human protein atlas (http://www.proteinatlas.org), which currently has more than 13 150 antibodies that recognize gene products from 10 100 human genes.[33,34] The use of antibody arrays (reversed or direct) provide the ability to screen for ever-increasing numbers of proteins, whereas the opposite, antigen arrays, can allow detection of autoantibodies (reviewed elsewhere[35,36]). This work has stimulated the production of a new generation of affinity (capture and sensor [detecting]) reagents, including peptoids and aptamers (eg, see elsewhere[37,38]).

MS is also a commonly used method that directly analyzes proteins or the respective peptides obtained via either enzymatic or chemical digestion. It is, however, the resulting peptides that provide amino acid sequence data. Although it is

often possible to deduce sequence information directly from the spectra, currently the most common data analysis pipelines include comparing the experimental spectra to those derived from a theoretical digestion (ie, in silico) of a selected protein database by way of a particular "search engine" algorithm (eg, Mascott, X!Tandem, Sequest, OSSMA). To ensure rigorous standards of data analysis and reporting, a number of important issues should be considered. First, the mass accuracy of the data affects the reliability of the identifications; therefore, the type of MS instrumentation used needs to be taken into consideration. Second, there are many different data search engine algorithms available, and each algorithm is likely to produce a different result from the same data. Third, many search engines will make some attempt to assign sequence information to poor quality spectra, which can result in incorrect identifications. Fourth, it is critical to select a protein database appropriate for the sample. Databases can vary with respect to accuracy and completeness, as well as contain protein name redundancy, whereby the same amino acid sequence may be represented by multiple accession numbers. Fifth, data clarity can be compromised if a spectrum can be assigned to multiple amino acid sequences, because of ambiguity, or if a peptide sequence can be assigned to multiple proteins, because of homology. Consequently, analysis of MS data and assignment of peptide/protein identification can be difficult.

Protein quantification is an important aspect of a proteomic analysis. There are essentially 2 types of quantification that are currently used in proteomics: relative ratio and absolute quantities. For the former, 2DE is most often used for proteins, whereas quantitation at the peptide level is achieved using metabolic (SILAC, on proliferating cells) or chemical labeling (most often labeling of the digested peptides include isobaric tag for relative and absolute quantification [also called iTRAQ[39]]) and tandem mass tags (also called TMT[40]) or label-free (based on spectral counts[41]). Comparison is made between 2 or more samples, allowing the relative quantitative relation between them to be determined. On the other hand, absolute quantification means that the precise value of each peptide or protein in a sample is determined independent of other samples. Previously, to obtain absolute quantitation of a protein, we were forced to rely on ELISA or quantitative Western blots, both of which require high-quality antibodies. When high-quality antibodies are available, these methods are very valuable. However, this is rare (although becoming less rare because of the large-scale efforts in reagent production as discussed previously) in research where candidate proteins arise from proteomic studies, or when specific protein isoforms (or specific modified amino acids) need to be investigated. With the recent adoption of multiple reaction monitoring (MRM), it is possible to obtain absolute quantification (relative ratios can also be determined) of target proteins without the need for antibodies. This MS method is a long-established technology in the field of metabolite quantification but has only in the last few years been optimized for the measurement of larger molecules, including peptides (eg, see elsewhere[42–45]). In essence, a proteotypic peptide (an ionizable peptide with a unique amino acid sequence that is consistently observed by MS) that results from an enzymatic digestion of the sample (eg, trypsin) is specifically selected within the MS instrument. MRM enhances: (1) protein studies across different species; (2) analysis when sample size is limited; (3) the study of proteins that have no (or poor quality) antibodies; (4) simultaneous quantification of multiple proteins (note upwards of 40 proteins in a single analysis[46]); (5) quantification of protein isoforms; and (6) quantification of site-specific PTMs within one or more proteins (for review comparing ELISA and MRM, see elsewhere[47]).

Targeted Analysis of PTMs

The area of PTM proteomics is challenging. Many PTMs are transient or labile with respect to MS analysis and are often present in low abundance, resulting in incomplete amino acid sequence coverage and a failure to fully characterize the PTMs. Biological complexity further impedes analysis; a single protein can have a number of amino acid residues that are modified, comprising one or different types of PTMs. Furthermore, each modified residue may have a different effect on function. Quantitative data on each modified amino acid residue are needed if we are to understand the dynamic changes of the proteome, the crosstalk and selectivity of different PTMs, and their impacts on signaling cascades. Many useful methods for PTM analysis have the following characteristics: (1) use enrichment of low-abundance modified proteins (or peptides); (2) simultaneously identify and quantify a protein; (3) identify the actual modified amino acid residue; (4) quantify the extent of a PTM under different conditions; and (5) quantify the relative differences of a particular modified residue in a protein with multiple modified residues. Most PTMs do not have such methods developed yet. Even so, progress has been made, either through the use of multiple methods, each allowing one to observe more of the PTM-proteome, or via a continuous refinement, often in incremental steps. Phosphorylation is an example where multiple synergistic approaches have been developed (reviewed elsewhere[48–50]). It is (by far) the most common PTM described today (Figure 3). This PTM is of particular interest to the broader scientific community. The analysis of phosphoproteins can be done by multiple approaches, each with their own advantages and disadvantages. Often, 2DE is used because a change in pI of a protein in the first dimension can be indicative of phosphorylation (or dephosphorylation) attributable to the addition (or loss) of a charged phosphate group. However, a shift in pI can be attributable to a number of PTMs, and, hence, a secondary set of experiments is required to prove that the protein is phosphorylated. The treatment of the sample before 2DE with a phosphatase and monitoring the reverse shift in pI can be one unambiguous approach (eg, see elsewhere[51,52]). However, a particular phosphatase (even those with broad specificity like alkaline phosphatases) does not dephosphorylate all proteins. Thus, if a protein does not show a shift after treatment this does not mean that the protein is not phosphorylated. ProQ diamond is a protein stain that displays enhanced binding to negatively charged proteins, like those which are phosphorylated.[53] However, false positives can occur thus secondary proof is required. The new phos-tag 1D gels can retard phosphorylated proteins and can provide insight in to extent of modifica-

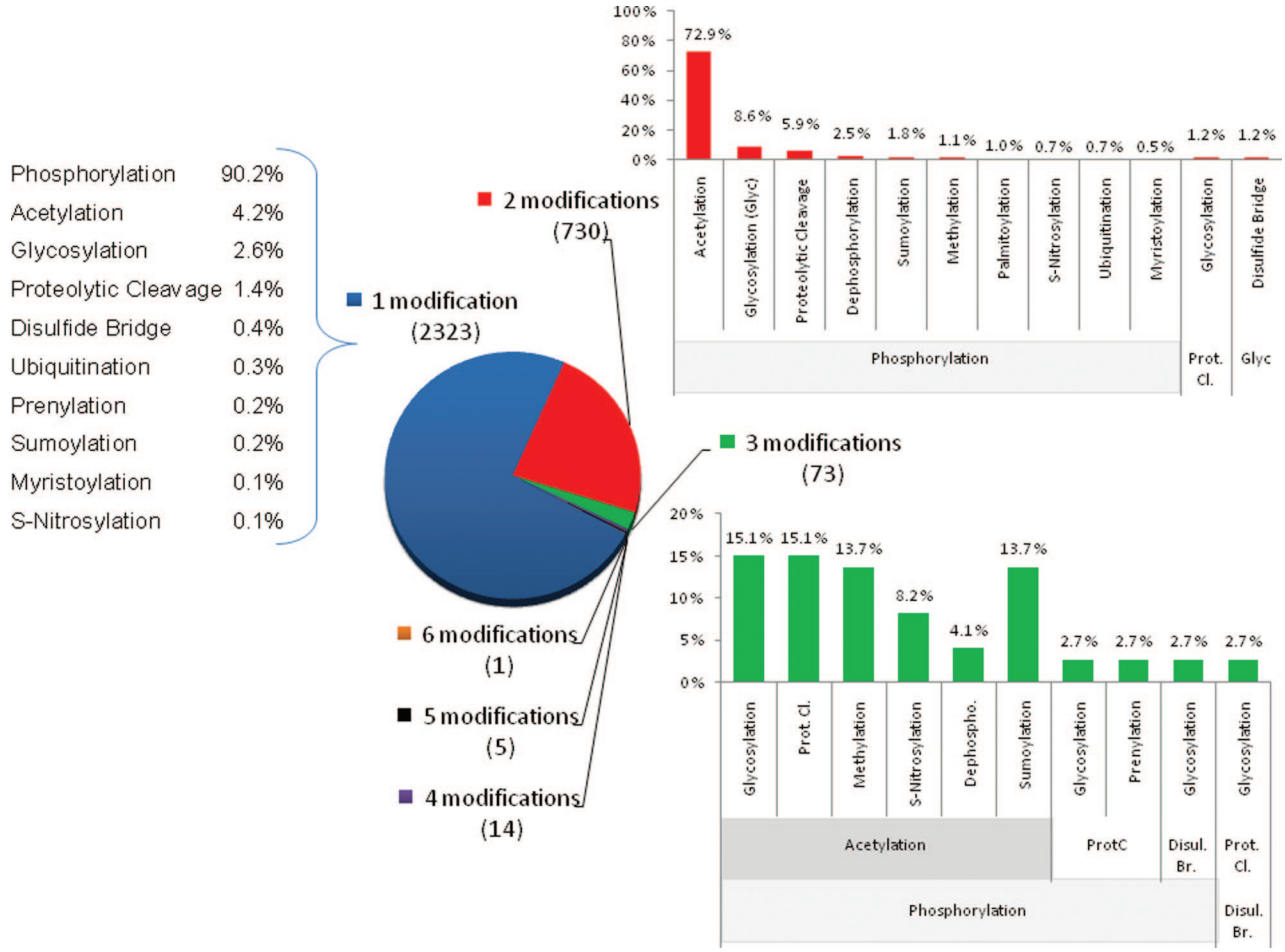

Figure 3. The number of annotated human cardiac proteins with one or more different types of PTMs. The human protein reference database (currently contains 30 047 protein entries and 93 710 PTM sites) was searched for proteins that are known to be expressed in the heart. For this subgroup of proteins, all of the different PTMs annotated were determined (phosphorylation [combined with dephosphorylation]; acetylation, glycosylation [*N*- and *O*-linked, Glyc], proteolytic cleavage [Prot.Cl.], disulfide bridge [Disul. Br], ubiquitination, prenylation, sumoylation, myristoylation, and SNO). The number of different PTMs were determined and represented as a pie chart. The percentage of each PTM for those proteins that have only 1, 2, or 3 types of modifications are shown.

tion (most often based on western blots).[54] None of these protein based methods provides information about the actual phosphorylated amino acid residues. That requires additional MS analysis. To determine phospho-sites, there are also multiple techniques: direct analysis based on neutral loss and the phospho-peptide enrichment methods (immobilized metal affinity chromatography and TiO_2 affinity chromatography, among others). Importantly, each method sees only a subset of phospho-peptides. This means that it is difficult to unambiguously detect all phosphorylation sites in either an isolated protein or a complex protein mixture. Over the last few years, there has been considerable work defining the phosphoproteome within some of the subproteomes of cardiac muscle. This includes extensive analysis of the myofilament,[55] 20S proteasome,[56] and the mitochondria using both direct targeting of phospho-peptides[57] or proteins (eg, see elsewhere[58–60]). However, none of these methods provides quantitative data without additional steps.

As mentioned above, in some cases, there is a continuous improvement of technology aimed at one particular problem. An example is the PTM, *O*-GlcNAcylation (*O*-GlcNAc), which was discovered more than 25 years ago (see reference 61 and review reference 62). Today it is clear that *O*-GlcNAc rivals protein phosphorylation in terms of abundance, dynamic cycling, and distribution on nuclear, mitochondrial and cytoplasmic proteins.[63–69] Within the heart this PTM has roles in aging, protection, and disease (eg, see references 70–76 and review references 77–79). However, this understanding was slow to be gained as GlcNAcylation is difficult to detect and quantify by standard methods and required continued technology development.[80–83] The existing MS-based method can simultaneously quantify site occupancy for both *O*-GlcNAc and phosphate on hundreds of proteins (recent reviews[84,85]). The interplay between these different types of PTM and the potential to influence the action of each other is becoming a more common theme not only for *O*-GlcNAc versus phosphorylation but also with other PTMs. For example, interplay between phosphorylation and SNO is also possible (eg, see elsewhere[86]). SNO modification has been found for a number of phosphatases in a number of systems, although not yet in the heart. To follow up this and other PTM interplay in the future, technologies targeting less common PTMs will be essential. It is clear that this is an area of future growth.

Proteome Complexity

It is generally accepted that the proteome is complex and that some of this complexity arises from PTMs. To grabble with this, we have asked 2 questions: "What is the extent of PTMs reported in the human heart?" and "How many proteins have more than one type of PTM, a potential indication of signaling system crosstalk?" We turned to the well-annotated Human Protein Reference Database (HPRD) (www.hprd.org)[87] that was established by Dr Akhilesh Pandey (Johns Hopkins University). HPRD involves a large team of biologists, bioinformaticists, and software engineers who oversee and manually curate the information based on the literature with frequent reannotation. Our search was limited to only those human proteins that have been shown to be present in the heart. In total, there were 5079 human cardiac proteins annotated. Sixty-two percent of these have at least one documented PTM. For a breakdown regarding the number of proteins with a specific PTM and also heart proteins with specific cellular component/localization, see the upcoming review in this series. To investigate the potential for crosstalk between different types of signaling pathways that result in PTM, we determined the number of annotated human cardiac proteins with one or more different types of PTMs (Figure 3). Of these, a total of 3146 proteins, 75% have one PTM type. Phosphorylation dominates, accounting for more than 90% of all proteins with a single modification type. Not surprisingly, this is also true if the protein has more than one type of modification. It is somewhat surprising that acetylation is the next most prominent PTM in cardiac proteins followed by glycosylation (combing both *N*- and *O*-linked). There are a number of caveats to this information. The first, this database is only for human proteins (note: PTMs observed only in other species are not included). Second, the database is continuously being updated but only reflects what is present to date. Third, the information in the HPRD reflects only those proteins for which site data (the actual modified amino acid) is known. There has been continuous development of technological tools for the identification of phosphorylated amino acids residues, in part, driven by the wide interest in this particular PTM as a regulator of biological processes (as described above in the section Targeting the Analysis of PTMs). This has resulted in several studies identifying large number of phospho-sites in human cells or tissues. For example, recently 2225 phospho-sites were identified in 1023 proteins isolated from human liver,[88] whereas Mann and colleagues reported more than 20 000 phosphorylation sites in 6027 proteins from HeLa cells.[89] As tools are developed for other PTMs to allow easier determination of the actual modified amino acid residues, it will be very interesting to see how these numbers change.

Future Expanding Proteomic Coverage and Understanding

Although often inferred in protein identification, it is often difficult to distinguish specific protein isoforms, mutant or SNP and polymorphisms. This is most likely because of incomplete detection by the MS (a result of inherent sampling biased, ionizablity of individual peptides, and instrument sensitivity thus reducing the coverage of a protein) and that our bioinformatic tools and protein databases have yet to be sufficiently encompassing to allow the specific peptides to be identified. This is needed. In view of the great amount of data being generated from genomic studies (such as genome wide association studies [GWAS]) about SNPs and their link to human diseases, proteomic methods must move toward linking with this knowledge. Thus, more sensitive MS instruments, more robust methods to increase coverage (eg, enhanced protein and peptide separation and use of multiple enzymes), and expanded databases will help. One can envision means to specifically target disease-linked SNPs or mutations that would allow for quantification of the different forms. One possibility is MRM, because this is a peptide-based method. This level of proteome characterization will occur, but the method still needs to be refined in the "regular production stage" of development. Already, the triple quadruple MS instruments are in clinical laboratories, where they are currently used for absolute quantification of metabolites and drugs. Thus, one can see a program development for protein/peptide based quantification of biomarkers in that setting.[90] However, what is needed is to move MRM into regular science research laboratories as a means to replace quantitative western blots and ELISA technology. The driving force will be the ability to greatly expand the number of analytes to quantify and to provide more accurate quantification with a larger dynamic range. To do this will require robust MS instruments that are user-friendly and a large number of consumables or kits comprising standard labeled peptides. The purchase of a MRM peptide kit for a particular protein, isoform, mutant, SNP, or PTM would be equivalent to the purchase of an antibody.

Equally important to the field of proteomics has been the simultaneous development of methods for large-scale protein characterization. These include initiatives in protein structure (eg, Protein Structure Initiative [PSI]; eg, reviewed[91,92]), which currently it is feasible to determine the 3D structure of >100 proteins per year. There has also been a push to define protein interacting partners also referred to as the "interactome." The interactome can target the identification of binding partners to a single protein but also can include the analysis of "complete" signaling cascades (eg, transforming growth factor β[93]), proteins that bind common small signaling molecules (eg, cAMP[94]) or specific classes of proteins (eg, kinase/phosphatases[95]). These studies on protein complexes have increased our understanding of basic protein biochemical properties, as well as pushed the field toward broad network analysis (eg, see elsewhere[96]). Along these same lines, the biophysical protein characterization (eg, Structural Genomics Consortium [SGC]; reviewed elsewhere[97]) and in vivo cell localization (reviewed elsewhere[98,99]) are now being carried out in higher-throughput mode, although often in model systems.

Conclusions

Proteomics is becoming integrated and incorporated into cardiovascular research and, as such, will impact new discoveries. In this series, 4 articles are presented that provide concrete examples of how proteomics can be incorporated into research and address specific biological questions. Mayr

and colleagues present how proteomics has allowed a better definition of stem cell populations and their phenotype; Costello and colleagues have summarized the importance of oxidative modifications and their growing role and definition in cardiovascular disease; Agnetti et al discuss organelles and their interplay with PTM regulation, and, at the end, Gerzneten and colleagues describe the pipeline required to developed circulating biomarkers with clinical relevance. These articles summarize some novel aspects within cardiovascular research where proteomics has helped to provide solutions and insights into biology that would have been difficult without these approaches. Nonetheless, considerable roadblocks remain to fully integrate proteomics into cardiovascular research and translational medicine As discussed in this review series, these include additional PTM-specific mapping tools, routine quantitative methods, more extensive databases, and more sensitive and cost effective instrumentation, to name a few. Furthermore, to exploit the proteomic data, we must find ways to integrate the data and the development of models to understand the complexity of the cell and organ. In addition, we need better approaches to link between a proteome change and direct function and cell localization in context to health and disease. Excitingly, however, technology does not stand still, and it will continue to undergo transformation, with older technologies improving and newer methods being developed. Proteomics research within the cardiovascular field is evolving rapidly, and if the past predicts the future, even more insights and novel findings will occur as the broader scientific community adopts and uses proteomic methods.

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