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Review

Proteomics quality and standard: From a regulatory perspective

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ABSTRACT

Proteomics has emerged as a rapidly expanding field dealing with large-scale protein analyses. It is anticipated that proteomics data will be increasingly submitted to the U.S. Food and Drug Administration (FDA) for biomarker qualification or in conjunction with applications for the approval of drugs, medical devices, and other FDA-regulated consumer products. To date, however, no established guideline has been available regarding the generation, submission and assessment of the quality of proteomics data that will be reviewed by regulatory agencies for decision making. Therefore, this commentary is aimed at provoking some thoughts and debates towards developing a framework which can guide future proteomics data submission. The ultimate goal is to establish quality control standards for proteomics data generation and evaluation, and to prepare government agencies such as the FDA to meet future obligations utilizing proteomics data to support regulatory decision.

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Contents

Proteomics has emerged as a rapidly expanding field dealing with large-scale qualitative and quantitative protein analyses. The number of published research articles related to proteomics has shown a steady increase according to a search in Thompson Reuters Web of Knowledge Science Citation Index (Fig. 1). The annual increase rate is over 100% before 2002, and about 25% on average during the past decade. Although the recent relative increase rate becomes smaller because of the larger base of published papers, the absolute number of publications is still steadily increased. Proteomics has the promise to gain insights into the overall picture of proteome-wide alterations of protein abundance, interactions, activities, post-translational modifications and so forth under various physiological and pathological conditions. It is anticipated that a growing number of proteomic data will be submitted to regulatory agencies for biomarker qualification and/or in

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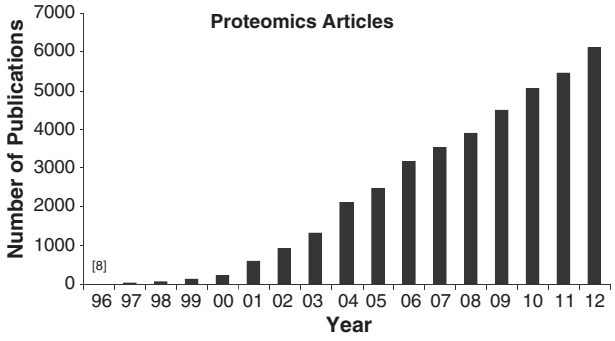

Fig. 1 – Annually published research articles (excluding reviews, abstracts, editorials, or commentaries) containing the keyword "proteomics", "proteomic", or "proteome" based on a search in Thompson Reuters Web of Knowledge Science Citation Index.

conjunction with drug applications to support, at the molecular level, drug efficacy and toxicity levels. The increasing submission of proteomic data and biomarkers will bring regulatory agencies great challenges in evaluating this type of data. It is not practical to validate a large number of proteins individually; therefore, it becomes of paramount importance that objective parameters and reliable procedures can be utilized to reflect the overall quality of a given proteomics dataset. Hence, establishing quality control standards for proteomics data generation and evaluation will help regulatory agencies meet obligations to utilize proteomics data in conjunction with drug review and biomarker qualification processes [1,2].

Compared to genomics, proteomics is a much more complicated proposition because of not only the huge number of protein species generated by variant splicing and post-translational modification, but also additional dynamic characteristics such as protein–protein interactions, interactions with other non-protein-type molecules, proteolysis, subcellular localization, high dynamic range of concentrations (particularly in plasma as a highly accessible sample type for biomarker discovery), etc. Therefore, proteomics could face more complex, challenging, and unique issues. To accurately evaluate proteomics data, simple and relevant questions have to be addressed before more specific questions relevant to a particular case are asked. For instance, how efficient is a protein extraction method? Will different methods for protein concentration measurements generate different results? These questions retain much less attention in the proteomics community than other issues such as the performance of liquid chromatography-mass spectrometry (LC-MS) platforms. Since proteins have to be extracted from membrane, cytoplasm, nucleus, and other organelles for proteomic analyses, diverse methods have been developed and utilized in protein extraction and purification processes. While in theory all proteins should be included in the analysis, it is often not the case in real experiments when diverse extraction methods with different yield efficiencies were utilized even for the same type of tissue or cell samples. It is conceivable that different protein extraction methods may yield variable amounts of protein for subsequent proteomics analyses. Thus, would it be appropriate to determine and state protein yield (*e.g.*, mg protein/g tissue) for a particular protein extraction method and a particular type of sample? In addition, different methods to determine protein concentrations have been employed in practice. While it is not practical to endorse one method and abandon others, would it be necessary to set up a kind of conversion standard among different methods for protein concentration measurement, so that inter-methodological results could be appropriately compared and assessed?

Major standardization efforts for certain proteomic approaches and for setting scientific publication guidelines have been made by proteomics communities. Several attempts have been initialized such as the Proteomics Standards Initiative by the Human Proteome Organization (HUPO-PSI) [3] and the Clinical Proteomic Technology Assessment for the Cancer by National Cancer Institute [4]. A series of proposals have been recommended and published [5–17]. More recently, the ProteomeXchange (www.proteomexchange.org) consortium has been set up to provide a single point of submission of MS proteomics data to the main existing proteomics repositories such as PRIDE and PeptideAtlas. The primary goals of these initiatives were aimed at facilitating data comparisons from different laboratories and for overall data quality evaluation. Although these frameworks recommend the minimal reporting information regarding experimental procedures, unfortunately they do not address all parameters that can have major influences on the accuracy of experimental data. To date, there is no established guideline and standard available concerning the generation of proteomics data for regulatory evaluation. A recent report by the HUPO Test Sample Working Group revealed that out of the 27 laboratories which examined the same sample that consisted of 20 highly purified human proteins, only 7 laboratories reported all 20 proteins correctly [18]. Notably, this was only at the qualitative level, *i.e.*, identification of the correct proteins rather than determination of the relative abundance of each protein in the sample. Many MS-based quantitative proteomic approaches were developed in the past decade for global measurement of proteome changes or targeted analysis of biomarkers of disease or drug response [19–23]. These technologies were further improved more recently to achieve ultra high resolution, selectivity, sensitivity, and speed of peptide measurement [24–27], which resulted in higher throughput and nearly complete analysis of proteomes [28–31]. Although recent efforts were made to initiate the evaluation of the intra- and inter-laboratory performance of targeted proteomics approaches [32,33], the performance of majority of global quantitative proteomic approaches has not been evaluated thoroughly. What should be considered as the "gold-standard" for proteomics data evaluation? In our opinion, similar as already proposed by others [34], a high quality proteomics dataset should fulfill at least two criteria: 1) repeatability of data generated within the same laboratory and 2) consistency of data generated from different laboratories. However, without further quantitative definition of consistence, questions will remain such as what is the minimum level of quality required to accept or reject proteomics data?

In order to begin addressing these questions, we reviewed research articles published in leading proteomics journals including *Molecular & Cellular Proteomics*, *Journal of Proteome Research*, *Journal of Proteomics*, *Proteomics*, *Proteome Science*, and *Proteomics-Clinical Applications*. Our objective was to use the published papers in a calendar year as an example to provide a snapshot of the diversity of subjects and methodologies in