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Short Communication

Quantification of Functional Proteins by Mass Spectrometry

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DESCRIPTION

Accurate quantification of proteins and peptides in complex biological systems is one of the most challenging areas of proteomics. Mass spectrometry-based approaches have made great development enabling accurate and sensitive quantification with functional proteins. Mass Spectrometry (MS) is playing an important role in basic science since the invention of Electro Spray Ionization (ESI), traps and MALDI technique. MS is widely applied in genomics, proteomics, transcriptomics and metabolomics. It is a powerful tool for the identification, quantification, and characterization of biomolecules [1]. Quantitative measurement of proteins by mass spectrometry is widely accepted in research areas nowadays. In particular, the application of quantitative mass spectrometry offers itself new opportunities and great possibilities for innovative development diagnostic and prognostic tests to identify new treatments. It aims to enable the design of personalized patient care, ultimately extending or reducing healthy life expectancy. In most cases, ELISA is highly accurate, sensitive, and relatively less costly and simple assay for protein analysis. Quantitative mass spectrometry of proteins has evolved dramatically over the past decade. In early days proteins are labeled with stable isotope enrichment reagents to introduce mass labels into proteins of interest for relative protein quantification. Additionally, a 'label-free' method was developed for the relative quantification of proteins in complex mixtures. In some cases the mass spectrometry can quantify the most challenging proteins or cannot be measured by immunological methods. Various types of mass spectrometry are available and different types of mass spectrometers are used for different cases. Sometimes different spectrometer can be used to develop an assay for the same analyte. A mass spectrometer selection can depend on several factors, necessary throughput, complexity of the sample, and method of introduction of the sample to the mass spectrometer [2]. Mass spectrometry's capacity to precisely differentiate various protein isoforms or modified forms, even in mixtures, introduces extra advantages to the design of quantitative techniques and simplifies the creation of multiplex assays. The dynamic range of the proteins in a sample is more closely related to limitations on the sensitivity of quantitative assays than the high sensitivity of mass spectrometers. Spectrometry can accurately distinguish between different isoforms or modified forms of proteins in mixtures. The development of High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)-based proteomics technologies relies mainly on liquid chromatography-mass spectrometry, sample separation and preparation techniques, identification, and bioinformatics. Currently, the shotgun method is the most commonly used large-scale protein identification strategy. Label-free analytical approaches are the most widely used strategy for proteome quantification due to their simplicity and minimally invasiveness [3]. No additional biomolecules are involved and it is convenient and economical as well. However, researchers can only obtain the relative abundance of proteins by the LFQ method. LFQ is often used to search for tumor biomarkers in clinical trials. This is because tumor-adjacent tissue or normal tissue is often used as a control. Stable isotope labeling with amino acids in cell culture is a highly accurate and reliable *in vivo* metabolic labeling method for both absolute and relative protein quantification. Proteins are labeled as light and heavy forms by adding isotopes of Lys (13 C or 15 N) and Arg (13 C or 15 N) to cell culture media lacking Lys and Arg. Quantification is initiated by MS1 levels detected by mass spectrometry. Using SILAC, scientists quantified the expression levels of extracellular vesicles in TGFBR2-deficient colon cancer, revealing 48 TGFBR2 regulatory proteins. The SILAC strategy is widely used to identify Post-Translational Modifications (PTMs) of proteins and amino acids. Isobaric Tags for Relative and Absolute Quantification (iTRAQ) technique were developed by the American ABI Company in 2004. Up to 8 samples can be labeled at a time, depending on the iTRAQ kit containing reporter groups, peptide-reactive groups, and balance groups. iTRAQ technology can compare proteins in many different samples with good reproducibility and high sensitivity. Qualitative and quantitative analysis can be performed simultaneously. This technique is increasingly used in proteomics research [4]. It has been successfully used in the field of medical research to analyse and identify various proteins within tumour tissue to find biomarkers. To quantify more protein samples, Tandem Mass Tag (TMT) labeling was developed. TMT can quantify up to 16 samples simultaneously. The TMT kit also contains reporters, normalizers, and amine-reactive groups. TMT is designed tofurther increase the number of multiplexed samples without

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sacrificing protein identification and quantitative quality goals, resulting in improved quantitation accuracy. TMT is widely used in clinical sample studies. Researchers labeled samples from 10 of her HCC patients and found that plasminogen was a prognostic biomarker for her HBV-associated acute to chronic liver failure. Similar strategies have been used for biomarker detection for gastric cancer, glioma and glioblastoma. The secret to discover sample simplification techniques is also to improve the sensitivity of mass spectrometric protein assays. Throughput is the main obstacle in mass spectrometry-based protein quantification [5]. There are two formats available for immunological assays: 96- and 384-well. In batch mode, plate readers could measure readouts of complete plates. Mass spectrometers, on the other hand, need to examine each sample individually. Each sample will take a few seconds to process, even in situations when analysis is carried out very quickly, like in a MALDI-TOF study. This challenge might be addressed by multiplexed mass spectrometric detector advancements in the future.

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