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Editorial

Current Status of Two-Dimensional Gel Electrophoresis and Multi-Dimensional Liquid Chromatography as Proteomic Separation Techniques

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Editorial

Proteomics is very important component in the era of post-genomics because it can address functions of genes and some important non-gene-determined biological issues such as Post-Translational Modifications (PTMs), splicing, translocation, and spatial structure. Proteome is very complex, including multiple parameters such as kind of proteins, copy number of each protein, PTMs, isoforms, spatial structure of each protein, protein-protein interaction, and protein-other molecule interaction, etc. Moreover, proteome is dynamic, and alters with different conditions such as different physiological processes, different pathological processes, and different disease status, etc [1-3]. Measurement of proteomic alteration would lead to discovery of important protein biomarkers for a given condition. Also, protein abundance alters in a huge range among different proteins within a proteome. Proteomic separation, identification, and bioinformatics are basic techniques in the field of proteomics. The protein species in a proteome must be separated prior to identification. The common proteomic separation techniques include Two-Dimensional Gel Electrophoresis (2DGE) [4,5] and Multi-Dimensional Liquid Chromatography (MDLC) [6,7]. However, 2DGE and MDLC have their own advantages and disadvantages, and cannot be replaced with each other.

2DGE separates proteins based on two basic features of proteins – Isoelectric Point (pI) in the direction of Isoelectric Focusing (IEF) and Molecular Weight (Mw) in the direction of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) [4]. 2DGE is an extensively used separation technique in the field of proteomics since the terms "proteome" and "proteomics" were proposed in 1994 [8]. Four main contributions of 2DGE in the field of proteomics are (1) 2DGE-based reference map of a proteome to expand proteome database [9,10], (2) 2DGE-based comparative proteomics to identify Differentially Expressed Proteins (DEPs) between a given condition and controls [11,12], (3) 2DGE-based Western blot coupled with a specific antibody to visually detect a kind of PTM in a proteome [13-15], and relatively quantify PTM-differential proteins between a given condition and controls [16,17], and (4) 2DGE-based Western blot coupled with a specific protein antibody to visually detect isoforms of that given protein in a proteome [18,19]. World-2DPAGE Constellation (http://world-2dpage.expasy. org) collected many 2DPAGE resources [20], including SWISS-2DPAGE (http://world-2dpage.expasy.org/swiss-2dpage), World-2DPAGE Repository (http://world-2dpage.expasy.org/repository), World-2DPAGE Portal (http://world-2dpage.expasy.org/portal), and World-2DPAGE List (http://world-2dpage.expasy.org/list). Of them, World-2DPAGE list contains references to known 2DPAGE database servers and 2DPAGE related servers and services, currently up to 60 databases and nearly 400 gel images, which is the most complete 2DPAGE list. World-2DPAGE portal is a dynamic and virtual portal, which integrates over 250 maps for 23 species; totally including nearly 40,000 identified spots, and is the biggest gel-based proteomics dataset available from a single interface. Two- dimensional difference in-gel electrophoresis (2D DIGE) is an improved 2DGE method, which uses flouresence dyes to label protein samples, and then equally mixes the labelled protein samples for 2DGE. 2D DIGE significantly lets spot-matching much easier, protein quantification more accurate and uses less amount of protein sample, compared to the classic 2 DGE. However, 2DGE and 2D DIGE are very labor-intensive, time-consuming, and low-throughput. It is difficult to distinguish the co-migration and overlapping of proteins with similar pI and Mr values which results in multiple proteins in

one single spot [15], to well-separate the extremely acid/basic proteins and extremely high/low-mass proteins, and to detect low-abundance proteins [1] although besides wide-range gradient IPG strip such as pH 3-10, a series of medium- and narrow-range of IPG strips, for example, pH 3-7, pH 4-7, pH 6-9, pH 6-11, pH 3.5-4.5, pH 4-5, pH 4.5-5.5, pH 5-6, and pH 5.5-6.7 IPG strips are developed to improve separation capability in a limited range of proteins [5,21]. Therefore, 2DGE and 2D DIGE are limited in maximizing the coverage of a proteome relative to MDLC. However, 2DGE has its own advantages in visualization of protein component of a proteome, detection of protein isoforms or variants that are mainly derived from splicing and PTMs [13-17,22]. Protein isoforms or variants are very important issue because different isoforms or variants of a given protein are associated with a given condition such as a corresponding pathophysiological status, which play important roles in multiple biological processes [23,24].

MDLC coupled with tandem mass spectrometry (MDLC-MS/MS) technique is superior to 2DGE in maximizing the coverage of a proteome and detecting low-abundance proteins [6,7]. The basic procedure of that strategy includes enzymatic peptides from a complex proteome, followed by separation in the first-dimension with any one of several different chromatography's, and in the second-dimension with reversed-phase chromatography; the separated enzymatic peptides are on-line input into mass spectrometer for MS/MS analysis, followed by protein identification with database search. MDLC-MS/MS-based proteomics techniques were developed rapidly, mainly including stable isotope-labeled MDLC-MS/MS [25] such as ICAT (Isotope-Coded Affinity Tags) [26-29], ^{18}O [30,31], ICPL (Isotope-Coded Protein Labeling) [32,33], IPTL (Isobaric Peptide Termini Labeling) [34,35], iTRAQ (Isobaric Tags For Relative And Absolute Quantification) [36,37], TMT (peptide Tandem Mass Tag) [38,39], and SILAC (Stable Isotope Labeling Of Amino Acids In Cell Culture) [40,41], and non-labeled MDLC-MS/MS such as label-free [42,43], SRM/MRM (Selected or Multiple Reaction Monitoring) [44,45], SWATH (Sequential Window Acquisition Of All Theoretical Spectra) [46,47], and AQUA (Absolute Quantification) [25,48], according to whether the sample is isotope-labeled or not. Those MDLC-MS/MS techniques have extensively used in the field of proteomics because of their high-throughput, high-accuracy, and high-sensitivity in analysis of a proteome, and that they easily overcome the disadvantages of 2DGE and 2D DIGE. However, MDLC is extensively used to separate the enzymatic peptide mixture, whereas 2DGE is extensively used to separate and visualize the intact protein mixture. MDLC is limited in identification of isoforms or variants of a given protein.

In summary, 2DGE is limited in maximizing proteome coverage but has its advantage in analysis protein isoforms or variants that are mainly derived from splicing and different PTMs. MDLC has its significant advantage in maximizing proteome coverage but is limited in analysis of intact protein isoforms or variants. Therefore, 2DGE and MDLC are complementary proteomic separation techniques with their own advantages and disadvantages in analysis of a proteome.

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