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Challenges to Acclimatize Proteomic Technologies for Regular Use

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Abstract

Two-dimensional gel electrophoresis is one of the oldest approaches and one of the most powerful protein separation methods available today. The first-dimensional separation of samples is achieved by isoelectric focusing, which separates proteins on the basis of their charge.

Keywords: Polyacrylamide; Electrophoresis; Protein identification; Proteomic analysis; Anticancer therapy; Protein fragments

Introduction

Two types of IEF techniques are currently used: the immobilized pH gradient technique; and the non-equilibrium pH gradient gel electrophoresis. The second-dimensional separation is performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The Two-dimensional gel electrophoresis provides the capability to qualitatively and quantitatively resolve complex protein mixtures to unique spots. The measured protein patterns can be analysed using sophisticated, bio-informatical software to reveal those proteins that are differentially expressed between samples.2D-DIGE is an important proteomic tool, especially for translational research involved in biomarker discovery [1]. When absolute biological variation between samples is the main objective, as it is in biomarker discovery, 2D-DIGE is still one of the methods of choice. Several studies were published, identifying novel prognostic or predictive biomarkers, e.g. biomarkers of drug-resistance. First experiments, to study resistance to anticancer therapy using Two-dimensional gel electrophoresis techniques were performed back in 1986, when Shen investigated the mechanisms of multidrug resistance in human cancer cells. Since then, experimental techniques have continuously been improved and modified for various study designs. For example, Tanaka et al. adapted the Two-dimensional gel electrophoresis technique for a comparative proteomic analysis of basic proteins [2]. In this study, cancer cell lines were analysed with regard to their chemo-sensitivity, using a radical-free and highly reducing method of two-dimensional polyacrylamide gel electrophoresis. This technique is reported to have a superior ability in the separation of basic proteins and the quantification of post-translational modifications, compared to traditional Two-dimensional gel electrophoresis [3]. Different pre-fractionation methods, prior to Two-dimensional gel electrophoresis analysis, as well as various combinations of analysis technique have also been developed to gain detailed knowledge of cellular mechanisms involved in response to anticancer therapy [4].

Methodology

Based upon these developments, detailed studies of different cellular components and protein signalling networks have also been conducted, e.g. the subcellular proteome, the phosphor-proteome, mitochondrial proteome .Using comparative proteomic approaches, long lists of differentially expressed proteins, potentially involved in chemo-resistance mechanisms were published, and reviewed by Zhang. Besides studies based on secondary cell lines, these techniques also found application in the clinical setting. In many studies, biomarker candidates were validated by alternative, more specific techniques such as RT-PCR and Northern blot at the mRNA level or Western blot and immunohistochemistry at the protein level [5]. The identified proteins belonged to a variety of different classes of proteins. However, the limitations of this method include limited reproducibility and inability to detect low abundant proteins. These low levels may result in undetectable proteins which significantly limit the application of this method to clinical samples as shown in (Figure 1).

The combination of Two-dimensional gel electrophoresis based with liquid chromatographic protein separation techniques and complete gel-free LC–MS approaches are more and more recognized [6]. An alternative, non-gel-based, protein separation approach to Two-dimensional gel electrophoresis is Liquid Chromatography. Basically, the components are separated using two phases, a stationary phase and a mobile phase. The procedure is mainly described by the elution of the different components at different rates, due to a varying affinity to interact with the used matrix, which results in a physicochemical separation. This technology is basically used for protein or peptide separations, prior to MS analysis and has been improved to handle proteomic analyses of complex samples. Various chromatography techniques have been developed as methods for protein separation, e.g. reversed-phase, cation exchange, anion exchange, biphasic ion-exchange or size-exclusion. Single- and multidimensional LC can directly be interfaced with the mass spectrometry, enabling automated analysis of large amounts of data for subsequent protein identification [7]. Another 2D chromatographic strategy termed multidimensional protein identification technology has been extensively applied to proteomic analysis. Mud-PIT is in principle a technique in which two liquid chromatographic steps are interfaced back-to-back in a fused silica capillary to permit two-dimensional high-performance liquid chromatography, combined with mass spectrometry for protein identification. However, the application of tryptic digestion of proteins in these technologies introduces some limitations. Unfortunately, the tryptic digestion of protein samples results in a loss of basic information about the intact proteins, e.g. post-translational modifications. Furthermore, low abundance proteins from a complex mixture may not be detectable in the presence of various peptides originating from other proteins [8]. Therefore, the separation of intact proteins by

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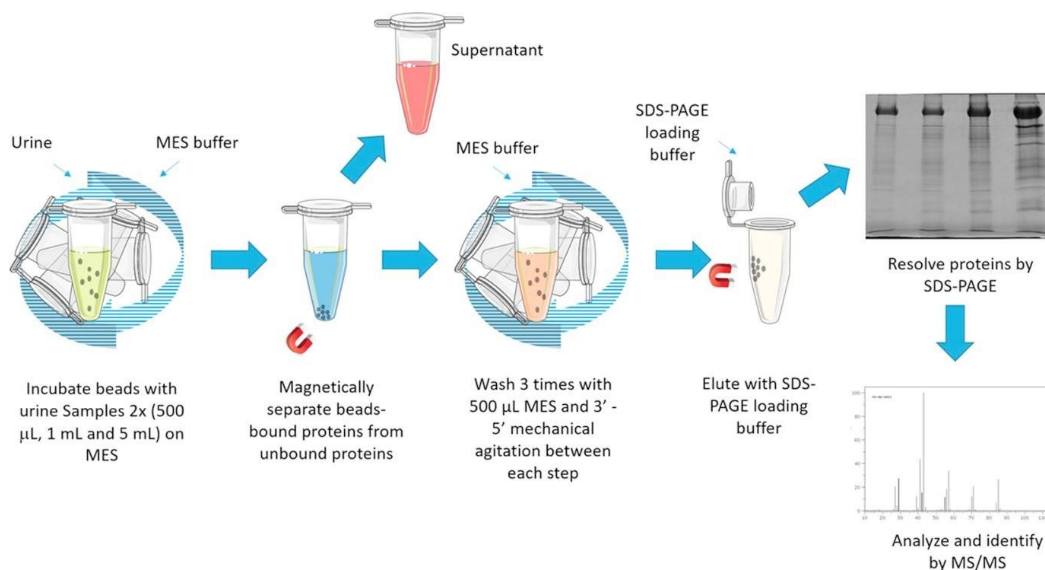

Figure 1: Low abundance proteins.

liquid chromatography may offer advantages over tryptic approaches and the use of gel-based methods. In general, these technologies show advantages over gel-based techniques with regard to speed, sensitivity, scope of analysis and dynamic range. In the field of onco-proteomics these methods have been integrated in the mass spectrometry-based discovery and characterization of novel biomarker candidates for guiding individualized anticancer therapy [9].

Results

Mass spectrometry is a method of choice for analytical characterization of potential drug molecules and protein identification. This technology is widely used to detect and identify the chemical composition of samples, after ionization, on the basis of their mass-to-charge ratio. As described earlier, mass spectrometry is often combined with different protein separation techniques to discovery of protein biomarker [10]. Many variants of mass spectrometry-based approaches have been developed for gel-free proteomic analysis. These methodologies apply different pre-fractionation techniques, such as selective surface binding, magnetic bead pre-fractionation or liquid chromatography. The basic principle of the surface-enhanced laser desorption/ionization- time of flight and the matrix-assisted laser desorption/ionization techniques is the fact that the sample is pulsed with laser energy causing proteins or protein fragments to ionize, and fly through a vacuum tube to the detector plate. Their time of flight is affected by the mass of the particle and its charge [11]. The detector plate records the intensity of the signal at a given m/z value, and a spectrum is generated. The different peaks in the spectrum correspond to different m/z protein species.

Discussion

Seldi-Tof is a proteomic technology used for the quantitative analysis of protein mixtures after selectively capturing proteins on pre-treated surfaces. In contrast to the Maldi technology, the seldi technology uses selective surfaces for binding a subset of proteins based on absorption, partition, electrostatic interaction or affinity chromatography on a solid-phase protein chip surface [12]. Therefore, stainless steel or aluminum-based chips are coated with chemicals or

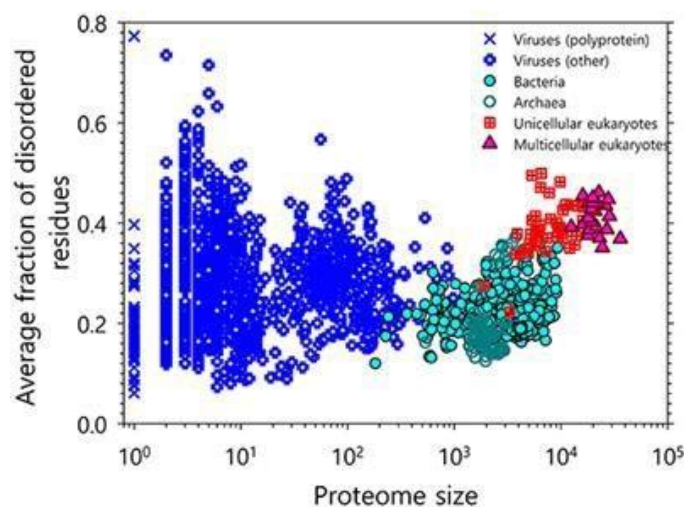

Figure 2: Intrinsically disordered protein properties.

biological substances to capture protein samples based on their intrinsic properties as shown in (Figure 2).

These pre-fractionation steps enable the detection of low abundant proteins. Until now, SELDI has mainly been used to characterize patients at risk of the development of cancer based on the direct analysis of body fluids like serum, plasma, and urine. Nonetheless, there are approaches to use Seldi-Tof as a clinical proteomics tool for the identification of protein biomarker candidates, being predictive for response to anticancer therapy. In general, Maldi techniques immobilize protein samples in an energy absorbing matrix. The entire repertoire of proteins in the sample interacts with the matrix from which a selected subset of proteins is bound to, a function of the composition of the selected matrix. The matrix chemicals absorb energy, which is subsequently passed to the sample proteins. Protein structural information, such as peptide molecular weight, amino-acid sequence composition, type and location of post-translational modification, could be obtained by MS analysis [13]. Two MS technologies are common and widely used, the matrix-assisted laser desorption ionization time of- flight mass spectrometry and the electrospray ionization mass spectrometry.

Maldi–Tof–Ms generates ions from solid-phase samples and measures their mass in a flight tube, whereas Esi–Ms generates ions from liquid samples and measures their mass using either quadru-pole or time of flight detector. Maldi–Ms is the most commonly used technique for peptide mass fingerprinting. Maldi–Ms is a fast, robust, easy to perform, sensitive, and accurate technology, which can be adapted to high-throughput. Lc-Maldi approaches have also been used to identify protein biomarker for the prediction of response to anticancer therapy. These studies were performed using cell lines, as well as patient's tumour and serum samples. Mass spectrometry technologies in combination with protein separation techniques have the ability to investigate complex patterns of protein expression and modification. Despite the complexity of the human proteom, the constantly improved proteomic technologies will ultimately enable the measurement of individual molecular profiles of patients on the protein level, with the potential to guide personalized medicine. Similar to the western blot technology, immunohistochemistry is a well-known method which has developed over the years with respect to reproducibility and sensitivity [14]. In 1941 already, Coons published a paper describing an immunofluorescence technique for detecting cellular antigens in tissue sections, which marked the beginning of immune-histo-chemistry. The fundamental concept behind Immunohistochemistry is the detection of antigens within tissue sections using specific antibodies. Once antigen–antibody binding occurs, a coloured histo-chemical reaction becomes visible by light microscopy or in the case of fluoro-chromes using ultraviolet light. Immunohistochemistry has long been used as an adjunctive diagnostic tool in a variety of cancers. It has provided clinicians with correlative insight into potential prognosis and differential diagnosis. The initially simple method of Immunohistochemistry has become more complex over the years. Currently, extremely sensitive methods are available to detect one or multiple antigens simultaneously or even to examine hundreds of tissues in the same section for the presence of a particular Antigen. Automation using automated slide stainer increased throughput and reproducibility [15]. Automated staining according to very stringent and standardized conditions has become more and more important since the introduction of targeted anticancer therapy, wherein target expression is one of the essential preconditions. For example, Her2 testing has become an important part of the clinical evaluation of all breast cancer patients throughout different countries, and accurate Her2 results are necessary for identifying patients who benefit from Her2-targeted therapy. IHC analysis is deeply integrated in breast cancer treatment by being able to determine the Her-2 status, the testing of progesterone receptor, estrogen receptor and the proliferation marker Ki-67.

Conclusion

Hence IHC is routinely used to predict response to both her-2 and hormonal targeted therapies, but is not yet suitable for the prediction of either efficiency or toxicity of anticancer drugs.

Acknowledgement

None

Conflict of Interest

None

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