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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 LCïMS 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 ue