NOTES

Two-dimensional gel electrophoresis analysis of the proteomes expressed in the human hepatoma cell line BEL-7404 and normal liver cell line L-02

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Abstract  Proteome analysis technology has been used extensively in conducting discovery research of biology and has become one of the most essential technologies in functional genomics. The proteomes of the human hepatoma cell line BEL-7404 and the normal human liver cell line L-02 have been separated by high resolution two-dimensional gel electrophoresis (2-DE) with immobilized pH gradient isoelectric focusing (IPG-IEF) in the first dimension and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension (IPG-DALT). The resulting images have been analyzed using 2-D analysis software. Quantitative analysis reveals that 7 protein spots are detected only in hepatoma BEL-7404 cells, 14 only in L-02 cells, and 78 protein spots show significant fluctuation in quantity in both cell lines (P<0.01). These protein spots have been displayed on a proteome differential expression map. Analysis for the reproducibility of 2-DE indicates that the positional variability in the IEF dimension is 0.73 mm, while the variability in the SDS-PAGE dimension is 0.44 mm, and the quantitative variability is 17.6%–19.2%. These results suggest that the reproducibility of 2-DE has been suitable for the study of differential expression of proteomes. Proteome differential expression maps can be useful tools for disease diagnosis, drug-target validation analysis and biological process elucidation.
The word “proteome”, which was coined by Wilkins and Williams of Macquarie University in Australia in 1994 and was first published in *Electrophoresis* in July of 1995, refers to the total protein complement expressed by a genome. Thereafter a newly emerging and challenging discipline, proteomics, was born. Proteomics is the study of various protein properties (expression level, post-translational modifications, interactions etc.) at the whole cell level to obtain a global, integrated view of disease processes, physiological and biochemical processes of cells and regulatory networks at the protein level. The generation and development of proteomics is closely related to the advances in two key technologies, two-dimensional gel electrophoresis (2-DE) of proteins and mass spectrometry, and also promoted by the progresses in bioinformatics. Besides creating a number of 2-D PAGE databases and proteome databases, proteomics technology has also been applied broadly in studying cell growth and regulation, stimulus response, phosphorylation and glycosylation of proteins, protein functions, molecular markers of disease diagnosis, drug development etc. Proteome analysis has gradually become an essential element of functional genomics. To study the total proteins of a proteome is unimaginably difficult, since proteome research per se needs to resolve hundreds and thousands of cellular proteins, and also meets the requirement of obtaining highly reproducible 2-D gels and highly sensitive micro-analysis techniques for protein identification. The application of immobilized pH gradients (IPGs) in the first dimension has greatly improved the reproducibility of 2-DE, e.g. better spot positional reproducibility was obtained among different gels run in internationally separate laboratories. Nonetheless, spot matching rate and quantitative reproducibility were not satisfied. In addition, the submicro-analysis techniques for proteins are being improved and developed. In China, liver cancer is one of the most important diseases that threaten human’s health. Its formation and development is a very complex and multistep process controlled by a lot of factors. Previous studies on such diseases usually focused on single or several genes and/or proteins. It is of great practical and theoretical importance to probe such a pathological and biochemical process, which is controlled by multi-genes and multi-proteins, at the whole cell level using proteomics technology. The hepatoma cells and tissues, which were subject to 2-DE in the past, derived mainly from the model animals such as rats. However, the SWISS-2DPAGE database constructed by Geneva University Hospital, Switzerland, contains the 2-DE reference maps of human liver tissue, hepatoblastoma derived cell line (HEPG2) and its secreted proteins (HEPG2SP). Wirth et al. compared the two-dimensional electrophoretograms of human hepatoma cell lines (e.g. HepG2, etc.) to that of normal liver cells, but the gel maps were not yet digitized and extensively analyzed in quantity at the whole-proteome level. Proteomes are temporally and spatially dynamic and regulable, it is necessary to examine the proteomes of human hepatoma cells at multiple levels and in many ways. In this report, the human hepatoma cell line BEL-7404 and normal human liver cell line L-02 were selected to undergo investigation, the total cellular proteins were separated by 2-DE, and the resulting maps were quantitatively analyzed using 2-D analysis software packages. The reproducibility of 2-DE was also discussed in a view of technology. By analyzing the differential expression of proteomes between the two cell lines, a proteome differential expression map was created.

1 Materials and methods

(i) Chemicals. Dithiothreitol (DTT), urea, sodium dodecyl sulfate, glycine, agarose, glycerol, bromophenol blue, 3-((3-cholamidopropyl)dimethylammonio)-1-propane sulphonate (CHAPS), IPG buffer and Immobiline DryStrips (3-10L) were purchased from Amersham Pharmacia. Acrylamide, Tris and iodoacetamide were from Sigma. Ammonium persulphate, TEMED and 2-D SDS-PAGE standards were from Bio-Rad. N, N’-Methylenebisacrylamide was from Fluka. Other chemicals were analytical grad. All buffers were prepared with Milli-Q water.

(ii) Cell culture and sample preparation. Human hepatoma cell line BEL-7404 and liver cell line L-02 were obtained from the cell bank of the Chinese Academy of Sciences. The cell lines were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum in a 37°C,