Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 3, No. 2, March : 2024

COMPARING PROTEIN ANALYSIS TECHNOLOGIES

Tripti Shrivastava, Scholar, Dr. C. V. Raman University Bilaspur
Dr. Laxmikant Tiwari, Assistant Professor, Deptt of Computer Science, Dr. C. V. Raman University Bilaspur

Abstract
Proteomics technologies have produced an abundance of drug targets, which is creating a bottleneck in drug development process. There is an increasing need for better target validation for new drug development and proteomic technologies are contributing to it. Identifying a potential protein drug target within a cell is a major challenge in modern drug discovery; techniques for screening the proteome are, therefore, an important tool. Major difficulties for target identification include the separation of proteins and their detection. These technologies are compared to enable the selection of the one by matching the needs of a particular project. There are prospects for further improvement, and proteomics technologies will form an important addition to the existing genomic and chemical technologies for new target validation. Proteomics is applicable for protein analysis and bioinformatics based analysis gives the comprehensive molecular description of the actual protein component. Bioinformatics is being increasingly used to support target validation by providing functionally predictive information mined from databases and experimental datasets using a variety of computational tools. This review is focused on key technologies for proteomics strategy and their application in protein analysis.
Keywords: Genomics, Proteomics, Bioinformatics, Microarrays, Mass spectrometry, MALDI

INTRODUCTION

Proteomic technologies are making a significant contribution to target validation. Genomic, transcriptomic and proteomic technologies are currently driving the pharmaceutical industry's search for novel targets that will result in innovative therapies [1]. Drug modulation of a target is likely to have a beneficial effect in a number of diseases i.e.-target validation, is a key step in this process and combines data from molecular biology, cell biology, bioinformatics and in vitro and in vivo experiments. The amount of work needed for validation increasing dramatically for ‘novel targets’ with no known biological function or link to disease. Although experimental work is the key driver in target validation, bioinformatics is playing an increasingly important role in supporting this process as biological knowledge is mined from the numerous databases containing data on DNA, RNA sequences, protein structures, pathways, organisms and disease that exist to uncover disease-links and provide clues to biological function. The hypotheses developed as a result of these efforts can then be tested experimentally in laboratory. This review is focused on key technologies for proteomics strategy and their application in protein analysis. Functional genomics and proteomics have provided several new drug targets. Highthroughput screening and compound libraries produced by combinatorial chemistry have increased the number of new lead compounds (which having some biological activity), creating a bottleneck in the drug discovery pipeline. Validation of diseasemodifying targets is an essential first step in the new drug discovery pipeline. Important technologies for target validation include genomics, proteomics, bioinformatics, microarrays, chemical, and RNAi. Proteomics can be used to study the mode of action of drugs by comparing the proteome of the cells in which the drug target has been eliminated by molecular knockout techniques or with small molecule inhibitors believed to have acted specifically on the same target. Proteomic techniques enable study of protein expression levels, modifications, location, and function in high-throughput automated systems [2]. This has enabled the prediction of all possible protein-coding regions and to choose the best candidates among ‘novel drug targets’. These technologies are used for detection of diseases biomarkers.

ROLE OF BIOINFORMATICS

Bioinformatics is being increasingly used to support target validation by providing functionally predictive information mined from databases and experimental datasets using a variety of computational tools [3]. The most commonly used approach to assign function to proteins is by sequence similarity, but this approach has its limitations, so attention has focused on complementing and extending this approach by the development of complementary methods to function prediction using sequence and structural information. The predictive power of these bioinformatics approaches is strongest when information from several techniques is combined, including experimental confirmation of protein predictions [4]. With the advent of genomics, transcriptomics, proteomics, microarrays etc. bioinformatics has achieved prominence because of its central role in data storage, management and sequence analysis. The importance of bioinformatics in target validation is justified because a rational and efficient mining of the information that integrates knowledge about genes and proteins is necessary for linking targets to biological function. In addition, new developments in bioinformatics will be helpful to infer the protein structural information from raw sequence data, guiding the identification or design of target-specific ligands [5].

KEY TECHNOLOGIES FOR PROTEOMICS

The current situation in proteomics appears to be characterized by differing opinions and strategic uncertainty in a climate requiring imperative large investment for the new drug developments. Whereas the mainstream strategic are thinking of investors is still completely dominated because of daunting experiences from genomics era. Identifying a potential protein drug target within a cell is a major challenge in modern drug discovery; techniques for screening the proteome are, therefore, an important tool for target validation. Major difficulties for target identification include the separation of proteins and their detection. Now days the fast developing field of proteomics in terms of key technologies are separation of complex protein solutions, analysis of isolated protein, spots recognition by mass spectrometry, data management, and interpretation [6]. Therefore, researchers are focusing on differential and quantitative pattern control in proteomics. This implies coverage of three labeling techniques (fluorescent cyanine dyes, radioactive, and stable isotopes), the detection of labeled proteins in 2D gels and liquid chromatography (LC) and, most importantly, the quantification of identified differential proteins. The corresponding pattern and profile analysis and interpretation will be treated as well.

Protein Identification with Mass Spectrometry Data

Prior to mass spectrometry based protein identification Edman degradation was the method of choice. Edman degradation is still a very powerful technique. With Edman sequencing amino acids are cleaved from the N-terminius of a peptide or protein and each amino acid is then chromatographed using a 20 to 50 min HPLC gradient. Identification is based on correlating the retention time of the eluting amino acid to a standard chromatogram. Mass spectrometry based protein identification is too easy. So far we have used MS sequence database searching techniques to identify a single protein and Progress in identification of proteins by mass spectrometry in the context of differential protein quantification by stable isotope techniques. Identification of proteins by mass spectrometry uses peptide masses or the MS/MS fragmentation of a peptide to identify proteins. In stark contrast mass spectrometry can easily ID 10-20 proteins in about 30 min! Here are a few of the most popular MS ID techniques.

1. Peptide Mass Fingerprinting A protein is first digested with an enzyme and the peptide masses are then used to search a sequence database [7, 8].
2. Sequence Tag A peptide is fragmented in a mass spectrometer and then a short stretch of amino acids is determined. This "tag", (peptide mass, sequence of the tag, starting and ending mass of the tag), is used to search a sequence database. Proteins can be correlated with the fragmentation of a single peptide using this technique [9].

3. MS/MS Peptide Identification A peptide is fragmented in a mass spectrometer and the fragment ion masses are then used to search a sequence database. Proteins can be correlated with the fragmentation of a single peptide using this technique [10].

Mass Spectrometry Suite

As is widely recognised, there has been an explosion of interest in the use of soft ionisation methods for the analysis of biomacromolecules, as well as of small molecules. Such mass spectrometric methods are required for work at the forefront of research into proteomics [11], metabolomics [12] and functional genomics [13] and play an important role in the discovery of novel pharmacophores [14, 15]. Mass Spectrometry have all the latest mass spectrometers including a Micromass LCT™ liquid chromatography electrospray ionisation mass spectrometer (LC-ESI-MS), a Micromass Q-Tof™ LC-ESI-MS-MS that effects tandem mass spectrometry, a Micromass TofSpec-2E™ matrix assisted laser desorption ionisation time-of-flight mass spectrometer (MALDI-TOF-MS), and a gas chromatography mass spectrometer (GC-MS) fitted with an autosampler and pyrolyser unit.

RECENT DEVELOPMENTS

Briefly, the most recent developments involve tandem mass spectrometers with advanced MS/MS capabilities, like MALDI– TOF/TOF–MS (matrix-assisted laser desorption ionization/time of flight; Bruker, Applied Biosciences http://www.bdal.com; http://www.appliedbiosystems.com) and LCQ–FT–MS (http:// www.thermofinniganmat.de). The ‘TOF/TOF’ technique provides the possibility to sequence selected peptides, by analyzing fragment ions generated by the dissociation of precursor ions. MALDI– TOF/TOF allows highly automated and reliable identification of thousands of proteins per week with sensitivities around 1–20 fmol.

The LCQ–FT instrument is a hybrid MS system with high mass accuracy and resolution (approximately 1 ppm). However, regardless of the ever increasing sophistication of mass spectrometry, the first step of a successful proteomics study is still the effective separation and detection of proteins [16-19]. Peptide mass fingerprinting and post-source decay (PSD) analysis, these two MALDI-MSbased identification methods are useful for the high-throughput proteomics [20]. Gel-free techniques are also used for monitoring protein concentration changes and protein modifications, in particular protein phosphorylation, glycosylation, and protein processing. [21] Proteomics approaches coupled with the bacterial ghost (BG) vaccine delivery strategy is useful for an ideal approach for developing safer, cost-effective and efficacious vaccines for human health, which can use in a relatively rapid time frame [22]. Proteomics and metabolomics offer nonbiased applications to address pathophysiologic mechanisms from various levels by integrating signal transduction, cellular metabolism, and phenotype analysis [23].

TECHNOLOGIES

Proteomics analysis can produce comprehensive molecular description of the differences between normal and diseased states. It can be used to compare the effect of candidate drugs on the disease process. Proteomics can thus be integrated into the drug discovery process along with the genomic and chemical drug discovery and can emerge as a powerful approach for directly identifying highly predictive pharmacogenomic markers in blood or other body tissues. Now days there are number of proteomic technologies are available and are described in more detail in a special report on this topic [24]. These technologies considered being significant for target validation in new drug developments. These technologies are applicable to target validation in oncology [25]. Proteomic techniques are applied for clinical samples analysis to identify new targets for a therapy tailored for an individual patient. The common genomic and transcriptomic profiling technologies and their relevance for clinical use give the special emphasis on two-dimensional gel-technologies (2D-PAGE), particularly as they apply to the study of breast cancer [26]. Two-dimensional gel electrophoresis and MALDI-TOF peptide mass fingerprinting is used for the study of mechanisms of chemoresistance as well as

representing an attractive starting point for the identification of potential protein biomarkers to predict response to chemotherapy in breast cancer [27].

Chromophore-Assisted Laser Inactivation (CALI)

Chromophore-assisted laser inactivation (CALI) is capable of knocking out one subunit/domain at a time or all together and creates an acute loss of protein function at a given point of time. This is a powerful method has been developed by Xerion Pharmaceuticals to inactivate protein function by targeted induction of photochemical modifications. Protein inactivation is transient in living cells because new protein synthesis can replace the damaged protein fraction. This transient knockdown mimics the dose-dependent effects of a drug, which makes CALI especially suitable for identification and validation of targets. After a protein target is functionally validated by CALI, antibodies can be generated using phage display and screened for antibodies that are neutralizing, thereby circumventing structural proteomic approaches. This approach of rapidly developing therapeutic antibody leading from the simultaneous identification and validation of targets, and can be extended to small molecule drugs. CALI is a highly versatile tool for validating disease-relevant targets at the protein level [28]. This approach also takes into account the post-translational modifications like phosphorylation, glycosylation or acylation, thereby enlarging its applicability for many different types of targets.

Pattern Analysis and Interpretation

Proteomic analysis reaches the most interesting stage once mass spectrometry has delivered a final protein list. The quantification of differential proteins is absolutely essential, because is can give clues about the sequence of events (e.g. protein represents an early event in the stem cell differentiation, because it is predominantly expressed in the S1 stage, as compared to a late one, like protein, which is nearly absent in the S1 stage and about twice as abundant in the latest S3 stage as in the intermediate S2 stage). Bioinformatics and data management should be able to automatically generate text files with relevant PubMed entries to each of the accession numbers (PMID). Based on quantification, iterative algorithms are available to correlate al these different data sets biological from sample, biophysical from separation, mass spectra, and literature, with the aim to generate hypotheses. Transcriptomic and genomic data should be integrated the fast and high-throughput identification of proteins by MALDI only uses a fraction of the information available in the spectra (unmodified peptides), and hypotheses can be falsified or verified using the whole detected peptide pool of a given data set, by educated sequencing of selected peptides [29].

Pattern analysis and pattern control is prerequisite for pooling strategies. Extensive fractionation requires ever increasing sample amounts; for example, phosphoproteomes constitute about 10–20% of corresponding total proteomes, whereas some membrane fractions comprise less than 1%. Clinical samples often are not available in endless quantities, laser capture micro dissection results in a few 100 micro-gm of sample. With radioactive methods being the only option for analysis, even if pooling is possible. In proteomics there is actually only one problem with pooling: how to sort out highly abundant single case contaminations from the pool i.e.low abundant contaminations are diluted. This can be achieved by differential displays from the total pools (e.g. each 30 patients) and a set of subpools (each group 5 to 6 patients). In this case a minimum of six differential displays can unambiguously identify the general serum biomarkers and discard single fates only apparent in one of the subpools [30-32].

Subcellular Proteomics

The subcellular localization of a protein might provide a hint as to the function of the protein. The combination of classic biochemical fractionation techniques for the enrichment of particular subcellular structures with the large-scale identification of proteins by mass spectrometry (MS) and bioinformatics provides a powerful strategy that interfaces cell biology and proteomics. Thus is termed ‘subcellular proteomics’. The proteome analysis at the level of subcellular structures, which can be enriched by subcellular fractionation, represents an analytical strategy that combines classic biochemical fractionation methods and tools for the comprehensive identification of proteins. Among the key potentials of this strategy is the capability to screen not only for previously unknown gene

products but also to assign them, along with other known, but poorly characterized gene products, to particular subcellular structures [33].

CHEMICAL PROBES

Chemical probes to interrogate key protein families for drug discovery. ActivX Biosciences Inc. is developing chemical probes to enable the quantitation of changes in protein activities in any cell type and tissue over a range of normal and pathological conditions. ActivX probes consist of three elements, reactive group that binds the common structural element, a tag, and third is a linker that attaches the tag to the reactive group. The technology can be used to monitor the activity of both secreted and membrane-bound proteins. It has been developed into a gel-free, fluorescence-based highthroughput system that enables precise, quantitative measures of protein activity, on a global scale [34].

Aptamers and High-Throughput Screening

The increases in throughput brought about by automation should potentiate the application of aptamer technology to proteomics. Automated workstations have been developed to select anti-protein aptamers. Aptamers can be used as versatile reagents in competition to binding high-throughput screening (HTS) assays to identify and optimize small-molecule ligands to protein targets. Aptamers link target validation directly with high-throughput screening [35]. Nucleic Acid Biotools has been developed by NasaCell to use these sophisticated ligands for the validation of potential drug targets in disease models. Moreover, aptamers that are specific antagonists of protein function can act as substitute interaction partners in HTS assays. SomaLogic's proprietary photoaptamer technology provides the basis for a new approach to multiplexed protein measurements. Photoaptamers, highly sensitive and specific capture agents, are developed using the PhotoSELEX (systematic evolution of ligands by exponential enrichment) process [36].

METHODS

Two-Dimensional Electrophoresis (2-DE)

Proteins are separated in 2-DE according to their pI (Isoelectric point) and molecular weight. In Two-dimensional electrophoresis (2-DE) analysis the first step is sample preparation; proteins in cells or tissues to be studied have to be solubilized and DNA and other contaminants must be removed. The proteins are then separated by their charge using isoelectric focusing. These steps are usually carried out by using immobilize pH-gradient (IPG) strips, which are commercially available. The second dimension is a normal SDSPAGE, where the focused IPG strip is used as the sample. After 2- DE separation, proteins can be visualized with normal dyes, like Coomassie or silver staining.

Protein Identification by Mass Spectrometry

Mass spectrometers consist of the ion source, mass analyzer, ion detector, and data acquisition unit. First, molecules are ionized in the ion source. Then they are separated according to their massto- charge ratio in the mass analyzer and the separate ions are detected. Mass spectrometry has become a widely used method in protein analysis since the invention of matrix-assisted laserdesorption ionisation/time-of-flight (MALDI-TOF) and electrospray ionisation (ESI) methods. There are several options for the mass analyzer, the most common combinations being time-of-flight (TOF) connected to MALDI and triple quadrupole, quadrupole- TOF, or ion trap mass analyzer coupled to ESI. In proteome analysis electrophoretically separated proteins can be identified by mass spectrometry with two different approaches. The simplest way is a technique called peptide mass fingerprinting (PMF). In this approach the protein spot of interest is in-gel digested with a specific enzyme, the resulting peptides are extracted from the gel and the molecular weights of these peptides are measured. Database search programs can create theoretical PMFs for all the proteins in the database, and compare them to the obtained one. In the second approach peptides after in-gel digestion are fragmented in the mass spectrometer, yielding partial amino acid sequences from the peptides (sequence tags). Database searches are then performed using both molecular weight and sequence information. PMF is usually

carried out with MALDI-TOF, and sequence tags by nano-ESI tandem mass spectrometry (MS/MS). The sensitivity of protein identification by MS is in the femtomole range.

COMPARISON OF TECHNOLOGIES

2D-PAGE is absolutely superior for pattern analysis of complex samples; a comprehensive control of 2D patterns requires real differential display strategies, which are not available with standard staining procedures. Generally, 2D gels are said to be difficult to use with certain proteins, like membrane proteins and basic proteins, but for these subproteomes there are always excellent alternatives. 2D-PAGE is slower than LC-based methods and more laborious, despite considerable progress in automation, but it does provide superior information. In principle, 2D-PAGE can analyze 70– 80% of the proteome of a given sample and, moreover, the residual proteome can be salvaged and treated with alternative approaches if appropriate. Multidimensional LC–MS methods have highthroughput and clear advantages for small proteins and peptides (1– 20 kDa), but lose a lot of relevant information because of their confinement to cysteine-containing peptides (in a total tryptic digest of human peptides only 3% of the peptides contain cysteine). The inherent complexity of samples might remain invisible because of limited pattern resolution. SELDI, with chromatographic arrays, has similar pros and cons. Resolution is inferior; in typical examples only a few dozen peaks were detected [52, 53].

Chromophore-assisted laser inactivation (CALI) can provide rapid information about protein function in cellular and diseaserelevant pathways. Specific inactivation has been achieved for greater than 90% of the proteins tested by assaying loss of activity and/or in vivo phenotypes. Advantages of MIPS include automation, comprehensive protein coverage and amenability to "gel-free" analyses.

Advantages of Caprion's organelle or subproteomic approach are that it provides functional insight into signaling pathways and protein orientation. It can deliver drug targets and functional insights unattainable by other proteomics approaches. Whereas the conventional approaches provide only incomplete protein identification showing abundant proteins only with no information on protein location, the CellCarta Cell Maps provide comprehensive protein identification including low abundance proteins with their location and orientation.

ActivX activity-based proteomics platform enables precise, quantitative measures of protein activity, on a global scale. It can be used to solve crucial challenges across the entire spectrum of the drug development process, from target discovery and validation to toxicity profiling and patient stratification in clinical testing at low cost. The major advantages of using aptamers in HTS assays are speed of aptamer identification, high affinity of aptamers for protein targets, relatively large aptamer–protein interaction surfaces, and compatibility with various labeling/detection strategies. Aptamers might be particularly useful in HTS assays with protein targets that have no known binding partners such as orphan receptors.

Ciphergen's Protein Biomarker System enables researchers to ask and rapidly answer important clinical questions by investigating the proteome from crude clinical samples ranging from laser capture micro-dissected cells, biopsies, tissue, urine and serum using Ciphergen's patented ProteinChip array-based Expression Difference Mapping and integrated SELDI–TOF–MS detection processes. The system utilizes Biomarker Patterns software, which automates pattern recognition-based statistical analysis methods to correlate protein expression patterns from clinical samples with disease phenotypes.

ZeptoMARK protein profiling system provides the precision necessary to quantify changes of 10–20% in protein expression or activation. It enables considerable savings in sample and reagent consumption as well as in labor and assay time.

TMAs are available from several vendors such as Invitrogen, Ambion (http://www.ambion.com), BD Biosciences. Cytomyx has developed superior Tissue Microarray technology, which enables the systematic imprinting of tissue specimens on microscope, slides. Tissue Microarray slides provides the capability to perform rapid analysis of comprehensive panels of normal and disease specimens.

CONCLUSION

Functional genomics and proteomics approaches allow highthroughput expression profiling in human disease tissue. Current validation strategies represent a "bottleneck" to translate differential gene expression patterns into novel diagnostic and therapeutic strategies for numerous disorders. The integration of data mining in human disease samples and corresponding animal models might provide an avenue to precisely identify target genes with a high pathogenetic relevance. Functional assays have to be adapted to high-throughput to cope with the elementary step to functionally evaluate differentially expressed proteins or proteins patterns for diagnostic or therapeutic purposes. Proteomics analysis can produce comprehensive molecular description of the differences between normal and diseased states; it can be used to compare the effect of candidate drugs on the disease process. Proteomics can thus be integrated into the drug discovery process along with the genomic and chemical drug discovery and can emerge as a powerful approach for directly identifying highly predictive pharmacogenomic markers in blood or other body tissues. By providing predictions of the biological function and potential disease-related roles of putative targets in advance of experimental work, proteomics and bioinformatics is increasingly contributing to the target validation process as hypotheses that can be tested in vitro and in vivo are generated in silico. Although intensive biochemical, genetic and animal studies will still be required to validate potential drug targets, the information provided by proteomics will facilitate and help direct this process.

REFERENCES

[1] Whittaker, P.A. (2003) *Trends Pharmacol. Sci.,* 24, 434-439.
[2] Schrattenholz, A. (2004) *Drug Discov. Today: Technol.,* 09, 1(1), 1-8.
[3] Gomase, V. S. (2004) *Bioinform. India J.*, 2(1), 65-70.
[4] Gomase, V.S.; Chikhale, N. J. and Ingale, A.G. (2005) *Bioinformat.India J.*, 3(1), 79-84.
[5] Whittaker, P.A. (2004) *Drug Discov. Today: Technol.,* 10, 1(2), 125-133.
[6] Crameri, R. (2005) *Allergy*, 60(10), 1227-37
[7] Henzel, W.J.; Billeci, T.M.; Stults, J.T.; Wong, S.C.; Grimley, C.and Watanabe, C. (1993) *Proc. Natl. Acad. Sci. USA*, 90(11), 5011-5015.
[8] Henzel, W.J.; Watanabe, C. and Stults, J.T. (2003) *J. Am. Soc.Mass. Spectrom*., 14 (9),931-942.
[9] Mann, M. and Wilm, M. (1994) *Anal. Chem*., 66 (24), 4390-4399.
[10] Yates, J.R. 3rd; Eng, J.K.; McCormack, A.L. and Schieltz, D. (1995) *Anal. Chem*., 67(8), 1426-1436.
[11] Yates, J. R. (2000) *Trends Genet.*, 16, 5-8.
[12] Fiehn, O.; Kopka, J.; Dörmann, P.; Altmann, T.; Trethewey, R. N. and Willmitzer, L. (2000) *Nat. Biotechnol.*, 18, 1157-1161.
[13] Dyer, M. R.; Cohen, D. and Herrling, P. (1999) *Drug Discov. Today*, 4, 109-114.
[14] Allsop, A. E. (1998) *Curr. Opin. Microbiol.*, 1, 530-534.
[15] Page, M. J.; Amess, B.; Rohlff, C.; Stubberfield, C. and Parekh, R. (1999) *Drug Discov. Today*, 4, 55-62.
[16] Tyers, M. and Mann, M. (2003) *Nature*, 422, 193-197.
[17] Fusaro, V.A. and Stone, J.H. (2003) *Clin. Exp. Rheumatol*., 21, S3-14.
[18] Purcell, A.W. and Gorman, J.J. (2004) *J. Prot. Res.*, 3(3), 572-581.
[19] Aebersold, R. and Mann, M. (2003) *Nature*, 422, 198-207.
[20] Gevaert, K. and Vandekerckhove, J. (2000) *Electrophoresis,* 21(6), 1145-54.
[21] Gevaert, K.; Van Damme, P.; Ghesquière, B.; Impens, F.; Martens, L.; Helsens, K.; Vandekerckhove, J. (2007) *Proteomics*, 7(16), 2698-718.
[22] Khan, A.S.; Mujer, C.V.; Alefantis, T.G.; Connolly, J.P.; Mayr, U.B.; Walcher, P.; Lubitz, W.; Delvecchio, V.G. (2006) *J. Chem. Inf. Model.,* 46(1), 111-115.
[23] Mayr, M.; Madhu, B.; Xu, Q. (2007) *Trends Cardiovasc. Med*., 17(2), 43-48.
[24] Jain, K.K. (2002) *Technol. Cancer Res. Treat.,* 1, 231-236.

[25] Jain, K.K. (2002). *Methods Enzymol.*, 356, 157-167.
[26] Neubauer, H.; Fehm,T.; Schütz, C.; Speer, R.; Solomayer, E.; Schrattenholz, A.; Cahill, M.A.; Kurek, R. (2007) *Recent Results, Cancer Res*.,176, 89-120.
[27] Chuthapisith, S.; Layfield, R.; Kerr, I.D.; Hughes, C.; Eremin, O. (2007) *Int. J. Oncol.,* 30(6), 1545-1551.
[28] Rubenwolf, S.; Niewöhner, J.; Meyer, E.; Petit-Frère, C.; Rudert, F.; Hoffmann, P.R. and Ilag, L.L. (2002) *Proteomics*, 2(3), 241- 246.
[29] Boguski, M.S. and McIntosh, M.W. (2003) *Nature*, 42, 233-237.
[30] Vogt, J.A.; Schroer, K.; Hölzer, K.; Hunzinger, C.; Klemm, M.; Biefang-Arndt, K.; Schillo, S.; Cahill, M.A.; Schrattenholz, A.; Matthies, H. and Stegmann, W. (2003) *Rapid Commun. Mass. Spectrom.*, 17(12), 1273-1282.
[31] Vogt, J.A.; Hunzinger, C.; Schroer, K.; Hölzer, K.; Bauer, A.; Schrattenholz, A.; Cahill, M.A.; Schillo, S.; Schwall, G.; Stegmann, W. and Albuszies, G. (2003) *Mol. Cell. Proteomics*, 2(9), 795.
[32] Washburn, M.P.; Wolters, D. and Yates, J.R. III (2001) *Nat. Biotechnol.,* 19, 242-247.
[33] Dreger, M. (2003) *Mass Spectrom Rev.,* 22, 27-56.
[34] Lill, J. (2003) *Mass Spectrom Rev.,* 22, 182-194.
[35] Burgstaller, P.; Girod, A. and Blind, M. (2002) *Drug Discov. Today*, 7, 1221-1228.
[36] Bock, C.; Coleman, M.; Collins, B.; Davis, J.; Foulds, G.; Gold, L.; Greef, C.; Heil, J.; Heilig, J.S.; Hicke, B.; Hurst, M.N.; Husar, G.M.; Miller, D.; Ostroff, R.; Petach, H.; Schneider, D.; Vant-Hull, B.; Waugh, S.; Weiss, A.; Wilcox, S.K. and Zichi D. (2004) p*roteomics,* 4(3), 609-618.
[37] Sickmann, A.; Mreyan, M. and Meyer, H.E. (2003) *Adv. Biochem. Eng. Biotechnol*., 83, 141-176.
[38] Thompson, D.M.; King, K.R.; Wieder, K.J.; Toner, M.; Yarmush, M.L. and Jayaraman, A. (2004) *Anal. Chem.,* 76 (14), 4098-4103.
[39] Blank, K.; Mai, T.; Gilbert, I.; Schiffmann, S.; Rankl, J.; Zivin, R.; Tackney, C.; Nicolaus, T.; Spinnler, K.; Oesterhelt, F.; Benoit, M.; Clausen-Schaumann, H. and Gaub, H.E.; (2003) *Proc. Natl. Acad.Sci. USA,* 100 (20), 11356-11360.
[40] Ng, J.H. and Ilag, L.L. (2003) *Biotechnol. Annu. Rev*., 9, 1-149.
[41] Hecht, M.H.; Das, A.; Go, A.; Bradley, L.H. and Wei, Y. (2004) *Protein Sci.,*13, 1711-1723.
[42] Huang, R.P. (2003) *Comb. Chem. High Throughput Screen.,* 6, 769-775.
[43] Liotta, L.A.; Espina, V.; Mehta, A.I.; Calvert, V.; Rosenblatt, K.; Geho, D.; Munson, P.J.; Young, L.; Wulfkuhle, J. and Petricoin, E.F. (2003) *Cancer Cell*, 3, 317-325.
[44] Torhorst, J.; Bucher, C.; Kononen. J.; Haas, P.; Zuber, M.; Köchli, O.R.; Mross, F.; Dieterich, H.; Moch, H.; Mihatsch, M.; Kallioniemi, O.P. and Sauter, G. (2001) *Am. J. Pathol.,* 159(6), 2249-2256.
[45] Simon, R. and Sauter, G. (2002) *Exp. Hematol*., 30, 1365-1372.
[46] Hewitt, S.M. (2004) *Methods Mol. Biol.,* 264, 61-72.
[47] Nilbert, M. and Engellau, J. (2004) *Acta Orthop. Scand. Suppl.,* 75, 29-34.
[48] Chapman, K. (2002) *Biochem. Soc. Trans.,* 30, 82-87.
[49] Pawlak, M.; Schick, E; Bopp, M.A.; Schneider, M.J.; Oroszlan, P. and Ehrat, M. (2002) *Proteomics*, 02(4), 383-393.
[50] Jain, K.K. (2002) *Methods Enzymol.*, 356, 157-167.
[51] Liotta, L.A. and Petricoin, E.F. (2000) *Breast Cancer Res.,* 2, 13-14.
[52] Petricoin, E.F.; Ardekani, A.M.; Hitt, B.A.; Levine, P.J.; Fusaro, V.A.; Steinberg, S.M.; Mills, G.B.; Simone, C.; Fishman, D.A.; Kohn, E.C. and Liotta, L.A. (2002) *Lancet*, 359(9306), 572-577.
[53] Xu, Q. and Lam, K.S. (2003) *J. Biomed. Biotechnol*., 3, 257-266.