Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 69 (2005) 1133–1139
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Target identification and validation in drug discovery: the role of proteomics

Karla K. Kopec *, Donna Bozyczko-Coyne, Michael Williams

Worldwide Discovery Research, Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380, USA

Received 1 December 2004; accepted 12 January 2005

Abstract

Proteomics, the study of cellular protein expression, is an evolving technology platform that has the potential to identify novel proteins involved in key biological processes in the cell that may serve as potential drug targets. While proteomics has considerable theoretical promise, individual cells/tissues have the potential to generate many millions of proteins while the current analytical technologies that involve the use of time-consuming two dimensional gel electrophoresis (2DIGE) and various mass spectrometry (MS) techniques are unable to handle complex biological samples without multiple high-resolution purification steps to reduce their complexity. This can significantly limit the speed of data generation and replication and requires the use of bioinformatic algorithms to reconstitute the parent proteome, a process that does not always result in a reproducible outcome. In addition, membrane bound proteins, e.g., receptors and ion channels, that are the targets of many existing drugs, are not amenable to study due, in part, to limitations in current proteomic techniques and also to these being present in low abundance and thus disproportionally represented in proteome profiles. Subproteomes with reduced complexity have been used to generate data related to specific, hypothesis-driven questions regarding target identification, protein-interaction networks and signaling pathways. However progress to date, with the exception of diagnostic proteomics in the field of cancer, has been exceedingly slow with an inability to put such studies in the context of a larger proteome, limiting the value of the information. Additionally the pathway for target validation (which can be more accurately described at the preclinical level as *target confidence building*) remains unclear. It is important that the ability to measure and interrogate proteomes matches expectations, avoiding a repetition of the disappointment and subsequent skepticism that accompanied what proved to be unrealistic expectations for the rapid contribution of data based on the genome maps, to biomedical research.

Keywords: Proteomics; Drug discovery; Target validation; Target confidence building

1. Introduction

Proteomics, defined as the ‘‘global analysis of changes in the quantities, and post-translational modifications, of all proteins in cells’’ [1] involves the full complement of proteins expressed by a genome [2]. These proteins are cell and tissue specific and are affected by age, disease and trauma. Thus, unlike the genome, proteomes (many of which can be generated from the same source) are temporally and spatially dynamic, with each analytical profile representing a unique moment in time. Proteomics can also be used to assess differences in protein expression in cells or tissues in response to drug exposure and to differentiate between proteomes from diseased and normal tissues.

Proteomes are extremely complex with component proteins undergoing extensive post-translational modification. A cell expressing a third of the human genome (~10,000 genes, although there is still ongoing debate as to whether the actual number is smaller [3] or much larger) is thus capable of producing more than 10 MM different proteins [1]. Traditional two dimensional gel electrophoresis (2DIGE) gels are capable of separating only 2000 proteins with larger gels being required to detect upwards of 8500 proteins [4]. Both are still far short of being able to separate the hundreds of thousands of proteins that can potentially exist in a given proteome. Thus proteome complexity far outweighs the technologies currently available for characterization requiring the subfractionation of proteomes to accommodate technological limitations. Similarly, the membrane proteins present on the cell surface that represent the major targets through which many known drugs

* Corresponding author. Tel.: +1 610 738 6282; fax: +1 610 344 0065.
E-mail address: kkopec@cephalon.com (K.K. Kopec).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.01.004

act [5], are not readily amenable to proteomic analysis due, again, to limits in the current technology [1].

Only a fraction of the putative drug targets thought to be present in the genome have been identified to date [6,7]. This, together with a paucity of new drug approvals over the past decade [8,9] has placed a high premium on target identification with proteomics-based approaches having the potential to both identify and validate disease-related target proteins [7,10]. While many academic and industrial institutions have invested significantly in proteomics, the consensus to date, with the exception of clinical proteomics in the cancer area [11], is that the output from proteomics initiatives has fallen short of initial expectations [1]. This has led to considerable skepticism due to concerns of a repetition of the hype associated with the as yet unrealized promise of genome-based medicine [12]. More importantly, it has led to a realization that the logistical complexity in planning, performing and analyzing proteome studies has been underestimated.

2. Proteomic technologies: separating the wheat from the chaff

The use of proteomics in drug target discovery is limited by a number of conceptual and technical challenges (Table 1). These include: the infinite number of proteomes that can be generated from a single source based on the effects of age, disease and tissue manipulation; the vast amount of protein-expression data [13] unique to a proteome that ''relates to only one particular situation, in one particular tissue, in one particular gel system'' [1] that is being used to populate proteomic databases and cannot be cross-referenced to other datasets; the masking of low abundance proteins by more abundant and separable 'housekeeping' proteins, e.g., cytoskeletal and matrix proteins; the inability to amplify these low abundance proteins in the absence of a proteomic technology analogous to the polymerase chain reaction (PCR); subtle to major differences in the initial sample from which the proteome is extracted even when using homogenous cell culture sources; the inherent complexity of the initial proteomic sample that requires extensive purification to accommodate current 2DIGE gel and mass spectrometry (MS) methodologies [14] that can result in extensive protein loss particularly of those proteins in low abundance; the challenges in isolating hydrophobic transmembrane proteins, e.g., receptors and ion channels; the availability and specificity of antibodies to the numerous proteome components in both their native and post-translational forms, e.g., phosphorylated/dephosphorylated; the time required for data generation, analysis and integration; data replication semantics, including statistical validation of the results; and validation of the putative drug target. One consequence of these challenges is that the proteins that may be of the greatest value as potential drug targets are probably lost as a result of the purification process used to find them, an excellent biological example of Heisenberg's Principle.

2.1. Protein array prioritization

The ability to identify novel disease-related proteins, when these represent only a minor part of a proteome or when they are not well characterized, makes it difficult to prioritize proteins for further investigation. Thus, proteins whose biology and function are, to some degree under-

Table 1
Proteomeics challenges and possible solutions

Challenge	Technical issue	Solution?
Proteome heterogeneity	No single proteome—proteomes are temporally and spatially dynamic—affected by age, disease, trauma and drug treatment	Improved experimental design. Proteome consortia at initiation co-coordinating experimentation to provide multiple data sources for same experimental paradigm [40]. Replication and integration of similar studies —support of results using non-proteomic approaches [41]
Database populating	Overloaded with data that cannot easily be compared or integrated (GIGO)	Additional biological insight, co-coordinating experimentation, use of systems-based approaches [38,40–42]. More rigorous statistical analysis.
Low abundance protein/loss of protein during analysis	Important proteins, e.g., GPCRs and ion channels not easily amenable to isolation and separation. Presence masked by ''housekeeping'' proteins (cytoskeleton etc). No protein amplification step similar to PCR	Improved subproteome separation techniques. Larger scale, real time separation, purification and analysis techniques with supporting bioinformatics capabilities [37]
Data replication	Not all proteins seen in apparently similar proteomes [25]	Replication of full experiment not just proteomic analysis. Co-coordinating experimental approaches
Target validation	Target validation only occurs when a drug-like compound, selective for the novel disease target shows efficacy in its target patient population [9]	Recognition that preclinical ''target validation'' is in reality *target confidence building*. Develop reliable paradigms via consortia approaches [1,40]

stood, e.g., recognizable motifs, tend to be prioritized for analysis while those lacking in detail end up at the bottom of the list until additional information becomes available. This can result in a subjective prioritization of data akin to the proverbial search for lost keys under a street lamp and emphasizes the need for a null hypothesis-based approach in analyzing proteomic data sets. Without this, the value of such data and its timely utilization can be limited leading to erroneous conclusions based on information lacking physiological context. An increase in the isoforms of protein 14-3-3 in Alzheimer's disease and Down syndrome led to the suggestion that this protein may play a role in neurodegenerative disease pathology [15]. However, this same protein has been implicated in tumorigenesis, multiple sclerosis and ALS [16], highlighting the need to assess such findings in a context broader than a single experimental series ideally involving pathway analysis in different cells and tissues.

2.2. Hydrophobic transmembrane receptors

While receptors, both G-protein coupled (GPCRs) and ion channels, are the targets through which 70% of currently available drugs act [5,17], issues with sample preparation and purification results in these proteins being underrepresented in many proteome arrays. Other proteins integral to the membrane account for 20–30% of the genome and may represent a wealth of as yet unexploited drug targets [18,19]. However, membrane proteins are positively charged, enhancing their interactions with negative charges on the membrane surface [20] and typically contain hydrophobic transmembrane domains which adds to the difficulty of isolation.

Detergents are frequently used to solubilize membrane proteins prior to 2DIGE or gel-free analysis using MS. However, proteins produced in this manner are prone to precipitation in the first 2DIGE dimension and are lost to subsequent analysis [20,21]. Detergents can also affect gel-free approaches interfering with column purification and suppressing ionization during the MS analysis.

However, newer zwitterionic detergents compatible with 2DIGE improved the solubilization and analysis of two membrane proteins, the human histamine H_2 receptor (hH_2R) and the rat $P2X_3$ receptor ($rP2X_3$), a GPCR and ATP-gated ion channel, respectively, as assessed by immunoblotting [22]. Neither target could be visualized using commonly used protein stains, even after increasing protein load on the gel. MS identified only a single peptide from the $P2X_3$ channel and failed to identify the GPCR highlighting the need for technological advances to analyze membrane subproteomes.

2.3. Sample complexity

To address the challenges present by the multitude of proteins present in a proteome and of isolating membrane proteins, smaller scale "subproteomes" are obtained by subcellular fractionation, affinity labeling and chromatography of the parent proteome and also by using chemical proteomics [23,24]. Combinations of classical and newer purification procedures can reduce sample complexity, allowing the visualization of lower abundance, yet potentially important, proteins. Analysis of a subproteome from the synaptic plasma membrane using multiple purification steps including SDS-PAGE, cation exchange and reverse phase HPLC to reduce sample complexity prior to MS analysis, identified a number of proteins involved in synapse physiology [25]. Combining information obtained from ESI (Electrospray injection)- and MALDI (Matrix-assisted laser desorption ionization)-MS approaches enhanced protein identification as a result of greater sequence coverage, demonstrating the utility of sample fractionation and purification and different MS systems in elucidating components of the synaptic subproteome.

Organic solvents and novel surfactants can also enhance membrane protein isolation and identification by enhancing solubility during membrane extraction and during subsequent analysis. Chloroform/methanol increased the identification of highly hydrophobic proteins isolated from chloroplast membranes while at the same time excluding hydrophilic proteins [21]. Methanol extraction combined with biotinylation of cysteine-containing peptides and streptavidin affinity chromatography also increased hydrophobic protein isolation and identification [26], increasing by 10% the number of proteins identified, 50% of which were novel and included ATP-binding cassette (ABC) transporter proteins and others typically in low abundance. Proteomic analysis of a mouse forebrain *N*-methyl-D-aspartate receptor complex (NRC), using immunoaffinity, peptide affinity chromatography SDS PAGE and MS, resulted in the identification of 34 unknown proteins [27]. However, other NRC-associated proteins were not found indicating that proteome analysis is not always consistent, emphasizing the need for the replication of well planned and executed studies. Additional complications of data generated from complex brain proteomes come from studies of mouse [28] and human fetal [29] brain proteomes where 8500 (mouse) and 1700 (human) proteins were identified. In the mouse proteome, MS only identified 6% (500) of these. In the human brain proteome, the 1700 proteins were identified as products of 437 genes [29]. In a rat brain proteome, 190 proteins were identified, 30 of which had only been predicted from nucleic acid sequences [30]. The latter study proved the existence of these proteins in brain; however the immediate utility of the 30 proteins in target identification beyond annotation of the genome was unclear. While generating considerable data, the utility of these brain based studies in terms of alternate target discovery has yet to be established.

Protein-protein interaction analysis and signal pathway mapping also fall under the rubric of proteomics with yeast two-hybrid and *gal* pull down techniques adding information on binary protein and receptor-scaffold interactions [27,31,32].

2.4. Chemical proteomics

This is a technique that uses small, drug-like molecules either tethered to a resin [23,24] or exposed to protein chips [33]. Proteins binding the ligand are then viewed as potential drug targets. In addition to providing insights into the selectivity and mechanism of action of a compound, chemical proteomics can also identify previously unknown protein targets for a compound that may aid in the identification of potential side effects. Knowledge of the co-crystal structure of the compound and a target protein can significantly enhance the outcome of such studies [34] but can also impose a priori, rather than null, criteria for subsequent proteome analysis. If information on the target protein is lacking or an in vitro bioassay for the tethered or biotinylated compound is unavailable, the optimization of affinity chromatography probes and the downstream validation of identified proteins becomes a significant challenge. Chemical proteomics was used to examine the selectivity of the mitogen-activated protein kinase (MAP) p38 inhibitor, SB 203580 [34] which is widely used as a pharmacological tool to examine the role of p38 in inflammation and other disease states. Using an active analog of SB 203580 as an affinity probe, 12 kinases including p38 were identified in HeLa cells where SB 203580 had comparable IC_{50} values. SB 203580 was actually a more potent inhibitor of the ominously named RICK (Rip-like interacting caspase-like apoptosis-regulatory protein (CLARP) kinase/Rip2/CARDIAK) kinase than p38 kinase (IC_{50} = 16 nM versus 38 nM) and also inhibited two other kinases, GAK (cyclin-G associated kinase; $IC_{50} \sim 135$ nM) and CK1α ($IC_{50} \sim 124$ nM). As a result of this analysis, many studies that used SB 203580 as a selective p38 kinase inhibitor will require re-evaluation. This study involved two high resolution steps with affinity chromatography followed by 2DIGE to reduce sample complexity, in this instance the HeLa cell kinase subproteome. While demonstrating the potential of proteomics when applied to a specific question, this study was somewhat reductionistic in that it involved only a single cell line. It was also in marked contrast to de novo chemical proteomics studies that may involve the analysis of many thousands of cellular proteins from more complex proteomes where knowledge of target structure is totally absent.

Another example of this issue is the identification of maleate dehydrogenase as a primary target for the novel anticancer agent, E7070 involved both proteomics and transcription profiling strategies with subsequent target validation [24]. Binding proteins enriched from HCT cells were interrogated with two affinity matrices containing probes based on analogs of E7070, one active and the other inactive. Many high abundance proteins, e.g., tubulin and chaperones, were present in the 285 proteins identified by LC-MS/MS from both the active and inactive compound resins making it difficult to identify proteins specific for the active analog of E7070. By using fluorescent two-dimensional differential in-gel electrophoresis (2D-DIGE) following affinity purification and labeling of the proteins eluted from the active and inactive resins with Cy5 and Cy3 fluorescent dyes, respectively, four proteins were visualized by 2D-DIGE gel. Three of these were also observed using a cleavable isotope coded affinity tag reagent (ICAT) approach in combination with multidimensional LC-MS/MS. This latter technique, while reducing sample complexity, actually resulted in more peptides being identified by MS than were originally found after affinity chromatography with E7070. These results were also confirmed by DNA microarray studies demonstrating both the value of reducing sample complexity—even a subproteome—and of using alternative approaches to provide context to proteomic findings.

Sample complexity can also be reduced by combining chromatography and MS in tandem with the protein chip technology used in SELDI-MS (surface-enhanced laser desorption ionization MS) [35]. Chips are available that have surfaces with ion exchange, metal affinity, reverse and normal phase chromatographic properties and covalently bound proteins for affinity purification, e.g., antibodies, streptavidin, and protein A. SELDI-MS has been used to generate differential protein maps from CSF proteomes from inflammatory and neuropathic pain models and has been used to assess the effects of the COX-2 inhibitor, nimesulide, on these profiles [36]. Chemical proteomics has also been used to assess drug effects on the cellular proteome using a high throughput microscopy-based technique that may also have the potential to identify novel drug targets [37].

3. Technical challenges and developments

As the technologies applied to proteome analysis evolve, there are tactical aspects of proteome experimentation that need to be addressed to enhance progress. Subfractionation of a proteome requires that the data derived from the subproteome is reassembled into the context of the parent proteome using some form of bioinformatics algorithm. This process addresses several aspects of the proteome experiment: (i) replication; (ii) data integration; and (iii) target validation.

3.1. Replication

As in all branches of science, replication of a finding, initially in the laboratory making the finding and, sub-

sequently, in independent laboratories, is a key part of the process of building confidence in new scientific knowledge [38]. In proteomics, because of: (a) technology limitations (despite some of the advances outlined above); (b) the dynamic nature of a proteome and; (c) the complexity of the initial sample, it is often very difficult to replicate data from a single proteome sample, let alone from a repeat experiment in the same laboratory. Thus replication between laboratories is a major concern with the need for 'routine, reliable and efficient technologies'' for data acquisition and analysis in proteomics being a high priority [1]. Added to these considerations is the actual time required for analysis. This can take from months to, in one instance where triplicate measures were required, nearly 3 years—certainly not a process that has immediate impact in biomedical research.

3.2. Data integration

Because of the ''infinite number of proteomes'' [1], databases can become swamped with information that, because of the issues discussed above, makes the comparison of data within and between experiments and between researchers nearly impossible—if indeed such comparisons are being routinely attempted. The ability of genomics and proteomics to generate lists of genes and proteins has given cause for concern [1,39,40] with the suggestion that the process would benefit significantly from the biological insight and context of more integrated, systems-based approaches [41,42], including pharmacology [43] and physiology, especially when the latter are triaged from the clinical setting [11,44,45]. While proteomics has provided interesting findings in regard to initial target identification [23,33,34], the ability to meaningfully consolidate this information in the broader context of the total proteome has yet to be achieved. Genomic and proteomic data can indeed be valuable in hypothesis generation [39], despite the latter being described as an ''ignorance-based approach'' [46], as it can lead to additional testing and validation in appropriate disease models [41]. However, it may also contribute to the larger failure of proteomics in that it creates bias and a drive towards preconceived conclusions – the absence of a null hypothesis [47].

3.3. Target confidence building versus validation

Transitioning from the identification of a putative drug target using genomic and proteomic approaches [38] to its validation as a *bona fide* drug target is a daunting challenge, representing the next rate-limiting step in the utilization of ''omics'' technologies [Table 1]. At the preclinical level, target validation can be more accurately described as *target confidence building* being distinct from target validation, as the latter only occurs when a drug-like compound, selective for the novel disease target, is shown to have efficacy in its target patient population [10]. Once a target has achieved some status—as yet difficult to generically define (there are for instance currently in excess of 30 candidate genes for schizophrenia)—from a list of disease-related genes and is confirmed by proteomics analysis, strategies are required that can build confidence in the selection of the protein from a subset of potential targets to more effectively bridge the gap from target identification to clinical trial initiation [48]. This should potentially lead to fewer clinical failures allowing the anticipated potential of genomic and proteomic strategies to be realized in a predictive rather than retrospective manner.

The challenge however, remains in identifying those methodologies that can most effectively translate genomic and proteomic information into high confidence targets. One key element is to have sufficiently high throughput in the technologies to make the testing of lists of potential targets rapid, reliable, reproducible and comparable. Many of the techniques required reflect the classical tools of biochemistry and pharmacology and include affinity chromatography, immunoblotting, immunoprecipitation, enzymology, radioligand binding and autoradiography to which are being added newer technologies like surface plasmon resonance and MALDI-MS. These technologies can provide evidence of the interaction between compounds and the protein target with additional confidence being provided by various cell-based functional assays, systems-based approaches [41,42,49] and targeted gene knockouts for the target of interest [50].

Prioritization of potential new drug targets is essential in drug discovery research where only a small number of well validated, high confidence targets can be advanced to the clinic due to the high cost of downstream studies and high attrition rates [51]. Ignoring for one moment the polygenomic nature of the majority of human diseases, to advance compounds active at the 30 candidate genes for schizophrenia mentioned above, none of which currently appears better than any other, to clinical validation would cost between $750 and $900 MM, representing a large investment to find perhaps the one new drug target with an acceptable therapeutic index that will improve treatment of this disease.

4. Future considerations

For proteomics to deliver on its ability to provide viable disease-associated targets for drug discovery, solving the technological and experimental challenges outlined above is a high priority as is the establishment of a viable scientific pathway for target validation. The latter should not continue to be confused with target confidence building as expectations for the two are very different. This will

require, to paraphrase Horton [52], a broader acknowledgement of the inherent difficulties of proteomics in order to appropriately advance the science to meet its anticipated promise rather than continuing the population of databases (and journals) with data that are lacking in context and, as a result, value.

References

[1] Huber LA. Is proteomics heading in the wrong direction? Nat Rev Mol Cell Biol 2003;4:74–80.

[2] Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, et al. Progress with gene-product mapping for the Mollicutes: Mycoplasma genitalium. Electrophoresis 1995;16(7): 1090–4.

[3] Southan C. Has the yo-yo stopped? An assessment of human protein-coding gene number. Proteomics 2004;4(6):1712–26.

[4] Kim SI, Voshol H, van Oostrum J, Hastings TG, Cascio M, Glucksman MJ. Neuroproteomics: expression profiling of the brain's proteomes in health and disease. Neurochem Res 2004;29(6):1317–31.

[5] Drews J. Strategic trends in drug discovery. Drug Disov Today 2003;8(9):411–20.

[6] Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov 2003;1:727–30.

[7] Lindsay MA. Target discovery. Nat Rev Drug Discov 2003;2: 831–8.

[8] Horrobin DF. Realism in drug discovery-could Cassandra be right? Nat Biotechnol 2001;19:1099–100.

[9] Milne GM. Pharmaceutical productivity—the imperative for new paradigms. Ann Rep Med Chem 2003;38:383–96.

[10] Williams M. Target validation. Curr Opin Pharmacol 2003;3: 571–7.

[11] Petricoin EF, Liotta LA. Clinical applications of proteomics. J Nutr 2003;133:2476S–84S.

[12] Collins FS, Green ED, Guttmacher AE, Guyer MS, USNHGRI. A vision for the future of genomics research. Nature 2003;422: 835–47.

[13] Patterson SD. Data analysis—the Achilles heel of proteomics. Nat Biotech 2003;21:221–2.

[14] Ferguson PL, Smith RD. Proteome analysis by mass spectrometry. Ann Rev Biophys Biomol Struct 2003;32:399–424.

[15] Fountoulakis M, Cairns N, Lubec G. Increased levels of 14-3-3 gamma and epsilon proteins in brain of patients with Alzheimer's disease and Down syndrome. J Neural Transm Suppl 1999;57:323–35.

[16] Bridges D, Moorhead GB. 14-3-3 proteins: a number of functions for a numbered protein. Sci. STKE 242: re20, 2004.

[17] Wu CC, Yates III JR. The application of mass spectrometry to membrane proteomics. Nat Biotechnol 2003;21(3):262–7.

[18] Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001;305(3): 567–80.

[19] Wallin E, von Heijne G. Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. Protein Sci 1998;7(4):1029–38.

[20] Schwartz R, Ting CS, King J. Whole proteome pI values correlate with subcellular localization of proteins for organisms within the the three domains of life. Genome Res 2001;11(5):703–9.

[21] Ferro M, Seigneurin-Berny D, Rolland D, Chapel A, Salvi D, Garin J, et al. Organic solvent extraction as a versatile procedure to identify hydrophobic chloroplast membrane proteins. Electrophoresis 2000; 21(16):3517–26.

[22] Henningsen R, Gale BL, Straub KM, DeNagel DC. Application of zwitterionic detergents to the solubilization of integral membrane proteins for two-dimensional gel electrophoresis and mass spectrometry. Proteomics 2002;2:1479–88.

[23] Jeffery DA, Bogyo M. Chemical proteomics and its application to drug discovery. Curr Opin Biotechnol 2003;14:87–95.

[24] Oda Y, Owa T, Sato T, Boucher B, Daniels S, Yamanaka H, et al. Quantitative chemical proteomics for identifying candidate trug targets. Anal Chem 2003;75:2159–65.

[25] Stevens SM, Zharikova AD, Prokai L. Proteomic analysis of the synaptic membrane fraction isolated from rat forebrain. Brain Res Mol Brain Res 2003;117(2):116–28.

[26] Goshe MB, Blonder J, Smith RD. Affinity labeling of highly hydrophobic integral membrane proteins for proteome-wide analysis. J Proteome Res 2003;2:153–61.

[27] Husi H, Ward MA, Choudhary JS, Blackstock WP, Grant SGN. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. Nat Neurosci 2000;3(7):661–9.

[28] Klose J, Nock C, Herrmann M, Stuhler K, Marcus K, Bluggel M, et al. Genetic analysis of the mouse brain proteome. Nat Genet 2002;30(4): 385–93.

[29] Fountoulakis M, Juranville JF, Dierssen M, Lubec G. Proteomic analysis of the fetal brain. Proteomics 2002;2(11): 1547–76.

[30] Shin JH, Yang JW, Juranville JF, Fountoulakis M, Lubec G. Evidence for existence of thirty hypothetical proteins in rat brain. Proteome Sci 2004;2(1):1–8.

[31] Legrain PJ, Wojcik J, Gauthier JM. Protein-protein interactive maps: leads towards cellular function. Trends Genet 2001;17(6): 346–52.

[32] Bockaert J, Marin P, Dumuis A, Fagni L. The 'magic tail' of G protein-coupled receptors: an anchorage for functional protein networks. FEBS Lett 2003;546:65–72.

[33] Huang J, Zhu H, Haggarty SL, Spring DR, Hwang H, Jin F, et al. Finding new components of the target of rapamycin (TOR) signaling network through chemical genetics and proteome chips. Proc Natl Acad Sci USA 2004;101:16594–9.

[34] Godl K, Wissing J, Kurtenbach A, Habenberger P, Blencke S, Gutbrod H, et al. An efficient proteomics method to identify the cellular targets of protein kinase inhibitors. Proc Natl Acad Sci 2003;100(26): 15434–9.

[35] Merchant M, Weinberger SR. Recent advancements in surface-enhanced laser desorption/ionization-timeof flight-mass spectrometry. Electrophoresis 2000;21(6):1164–77.

[36] Gineste C, Ho L, Pompl P, Bianci M, Pasinetti GM. High-throughput proteomics and protein biomarker discovery in an experimental model of inflammatory hyperalgesia: effects of nimesulide. Drugs 2003; 63(Suppl. 1):23–9.

[37] Perlman ZE, Slack MD, Feng Y, Mitchison TJ, Wu LF, Altschuler SJ. Multidimensional drug profiling by automated microscopy. Science 2004;306:1194–8.

[38] Kramer R, Cohen D. Functional genomics to new drug targets. Nat Rev Drug Discov 2004;3:965–72.

[39] Firestein GS, Pisetsky DS. DNA microarrays: boundless technology or bound by technology? Guidelines for studies using microarray technology. Arthritis Rheum 2002;46:859–61.

[40] Choudhary JS, Grant SGN. Proteomics in postgenomic neuroscience: the end of the beginning. Nat Neurosci 2004;7:440–5.

[41] Butcher EC, Berg EL, Kunkel EJ. Systems biology in drug discovery. Nat Biotechnol 2004;22(10):1253–9.

[42] Hood L, Heath JR, Phelps ME, Lin B. Systems biology and new technologies enable predictive and preventative medicine. Science 2004;306:640–3.

[43] Williams M. A return to the fundamentals of drug discovery. Curr Opin Investigational Drugs 2004;5(1):29–33.

[44] Petricoin EF, Zoon KC, Kohn EC, Barrett JC, Liotta LA. Clinical proteomics: translating benchside promise to bedside reality. Nat Rev Drug Discov 2002;1:683–95.
[45] Petricoin EF, Fishman DA, Conrads TP, Veenstra TD, Liotta LA. Lessons from Kitty Hawk: from feasibility to routine clinical use for the field of proteomic pattern diagnostics. Proteomics 2004;4: 2357–60.
[46] Lazo JS, Ducruet AP, Koldamova RP. Sleuthful pharmacology. Mol Pharmacol 2003;64(2):199–201.
[47] Kenakin T. Pharmacologic analysis of drug-receptor interaction, second ed. New York: Raven; 1997. p. 344–346.
[48] Horrobin DF. Modern biomedical research: an internally self consistent universe with little contact with medical reality. Nat Rev Drug Discov 2003;2:151–4.
[49] Hauptschein RS, Eustace BK, Jay DG. Global high-throughput screens for cellular function. Exp Hematol 2002;30:381–7.
[50] Zambrowicz BP, Sands AT. Knockouts model the 100 best-selling drugs—will they model the next 100. Nat Rev Drug Discov 2003; 2:38–51.
[51] Duyk G. Attrition and translation. Science 2003;302:603–5.
[52] Horton R. AIDS: the elusive vaccine. New York Rev. Books, vol. 51, 2004. p. 53–8.