

Proteomics in Computer-Aided Drug Design

Ying Wang^{1,2}, Jen-Fu Chiu^{2,3} and Qing-Yu He^{*1,2}

¹Department of Chemistry, ²Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, ³Institute of Molecular Biology, The University of Hong Kong, Pokfulam, Hong Kong SAR, China

Abstract: Proteins are functional molecules in cells and are the major targets for drug action. To design a rational drug, we must firstly find out which proteins can be the drug targets in pathogenesis. Proteomics has great promise in identification of protein targets and biochemical pathways involved in disease processes. Proteomics as a whole increasingly plays an important role in the multi-step drug-development process. The process includes target identification and validation, lead selection, small-molecular screening and optimization, and toxicity testing. Furthermore, sub-disciplines such as computational proteomics, chemical proteomics, structural proteomics and topological proteomics offer significant contributions especially in computer-aided drug design. This review will summarize the recent progress in pharmaco-proteomics and the discipline's potential application in computer-assisted drug design.

Keywords: Proteomics, drug design, drug target, protein target, target identification, lead validation, computer-aided drug design, protein profiling.

1. COMPUTER-AIDED DRUG DESIGN

In principle, the drug discovery process involves three pre-clinical stages before clinical trials, namely target selection, lead identification, and clinical candidate selection (Fig. (1)). Due to rapid advances in structural biology and computer technology, structure-based computer-aided drug design (CADD) using docking techniques, virtual screening and library design, along with target/structure focusing combinatorial chemistry, has become a powerful tool in the multi-step process of drug discovery. As an emerging technology, CADD accelerates drug development by making use of the accumulated information of existing drugs and diseases, combined with inter-disciplinary inputs from other fields. This process extensively uses mathematical models and simulation tools based on the evaluation of potential risks from drug safety and the experimental design of new trials [1-3]. During the early 1980s, structural biologists began to design rational drugs based on protein structures. The first projects were underway in the mid-1980s, and the first successful stories, computer-aided rational design of peptide-based HIV-proteinase inhibitors, were published by the early 1990s [4, 5]. From then on, CADD has become a vital technique in drug candidate screening. The most recent example includes the structure based design of anti-SARS drug, a proteinase inhibitor of viral main proteinase Mpro (or 3CLpro) [6].

As the first step in structure-based CADD, the three dimensional (3D) structure of a target protein or nucleic acid is determined by X-ray crystallography [7] or NMR [8]. Using recently constructed protein and nucleic acid databases (Table 1), new computational methods use the 3D structural information of the unliganded target to design entirely new lead compounds de novo. In this way, large virtual combinatorial libraries of compounds can then be screened computationally before going to the effort and expense of

actual synthesis and biological studies. The ability to rapidly and accurately dock large numbers of candidate molecules into the binding site of a target macromolecule is a key component of lead generation in structure-based drug design [9, 10]. The most widely used computational docking method is the program DOCK [11] which has been and continues to be developed by Kuntz and his colleagues at the University of California and other scientists worldwide [11-14]. The success application of DOCK includes the in silico virtual high throughput screen for high affinity cytochrome p450cam substrates [15] and the computer-assisted design of selective imidazole inhibitors for cytochrome p450 enzymes [16]. Besides DOCK, numerous other programs have been created for virtual screening. Programs such as ADAM [17, 18], AutoDOCK [19-21], FlexX [15, 22-24], and SLIDE [25-27], and other dock databases of compounds can score candidate molecules according to their interactions with the selected site of target protein. De novo generation of ligands can be performed with computer programs including 3D-QSAR [28-30], DISCO [31], GRID [32-35], LUDI [36-38], MCSS [32, 39], and PASSA [40].

With the rapid accumulation of biological and chemical information, CADD has been dramatically reshaping research and development pathways in drug candidate identification. On the other hand, the escalating number of therapeutic candidates are increasing demand on new technologies and strategies to streamline the process of screening for safe and effective therapies. This has inspired the application of molecular approaches including proteomics in an attempt to identify and validate drug candidates effectively in recent years.

2. PROTEOMICS IN COMPUTER-AIDED DRUG DESIGN

2.1 Proteomics

The proteome is the entire protein complement expressed by a genome, and proteomics is the study of the proteome

*Address correspondence to this author at the Department of Chemistry, The University of Hong Kong, Pokfulam, Hong Kong, China; Tel.: (852)2299-0787; Fax: (852)2817-1006; E-mail: qyhe@hku.hk

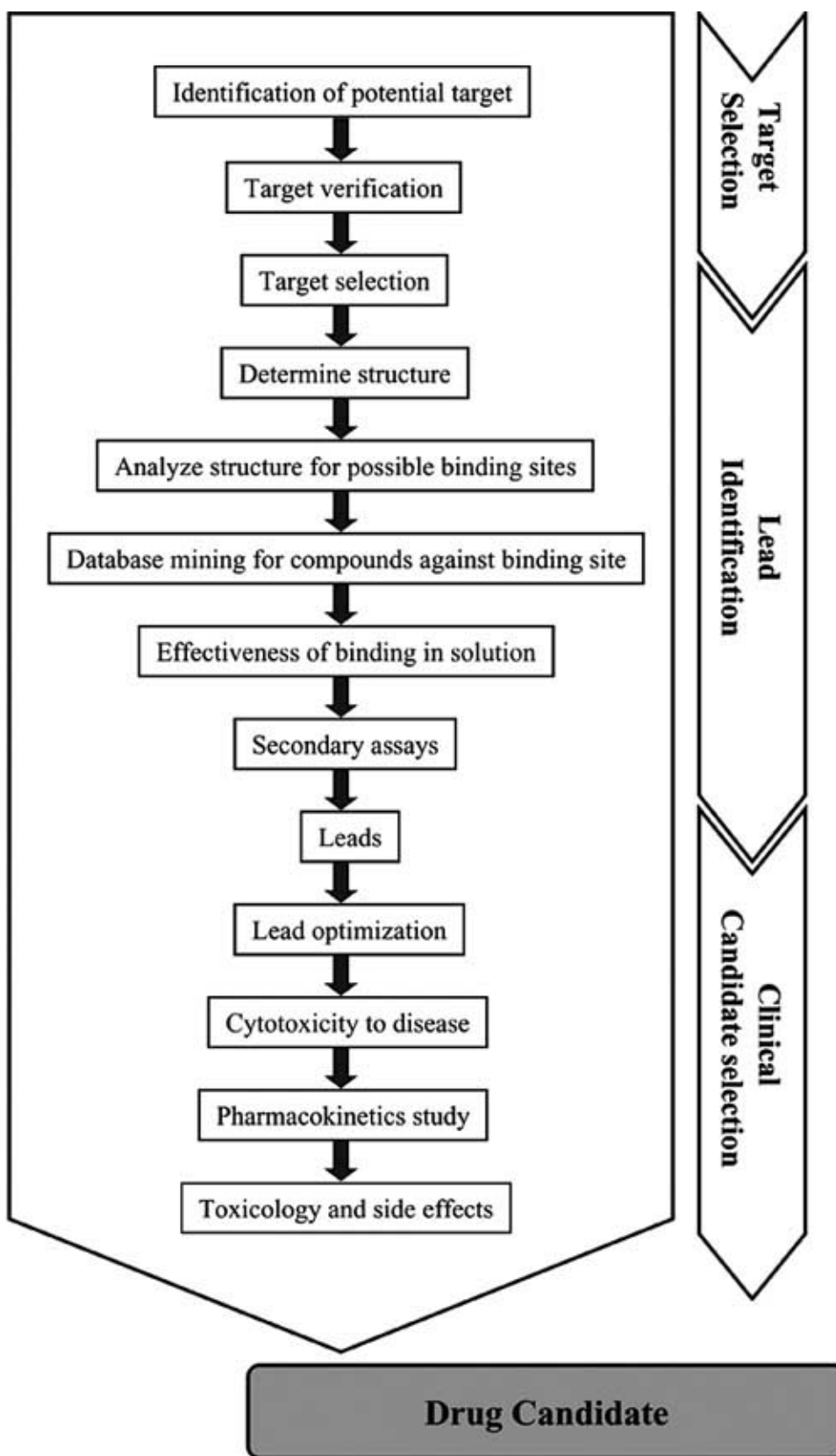


Fig. (1). Schematic flow diagram of drug design and screening process.

[41, 42]. Proteomics is a research field that involves large-scale identification, characterization, and quantitation of proteins expressed in a cell, tissue, or organism under given

conditions such as drug treatment [43-45]. The ultimate goal of proteomic analysis is a comprehensive and quantitative description of protein expression and alterations associated

Table 1. Useful Websites in Computer-Aided Drug Design and Proteomics

Database Description	UTL
Ontario Center for Structural Proteomics	http://www.uhnres.utoronto.ca/proteomics/
Network service for comparing protein structures in 3D	http://www.ebi.ac.uk/dali/
Databases and Tools for 3-D Protein Structure Comparison and Alignment	http://cl.sdsc.edu/ce.html
Integrated Sequence—Structure Database	http://www.protein.bio.msu.su/issd/
PROCAT 3D enzyme active site templates	http://www.biochem.ucl.ac.uk/bsm/PROCAT/PROCAT.html
Structural Classification of Proteins	http://scop.mrc-lmb.cam.ac.uk/scop/
Topology of Protein Structure	http://www.tops.leeds.ac.uk/
TopNet for Topological Proteomics	http://networks.gersteinlab.org/genome
Biolmolecular interaction network database	http://www.blueprint.org/bind/bind.php
Protein Data Bank	http://www.rcsb.org/pdb/
Protein sequence analysis and structure prediction	http://www.embl-heidelberg.de/predictprotein/predictprotein.html

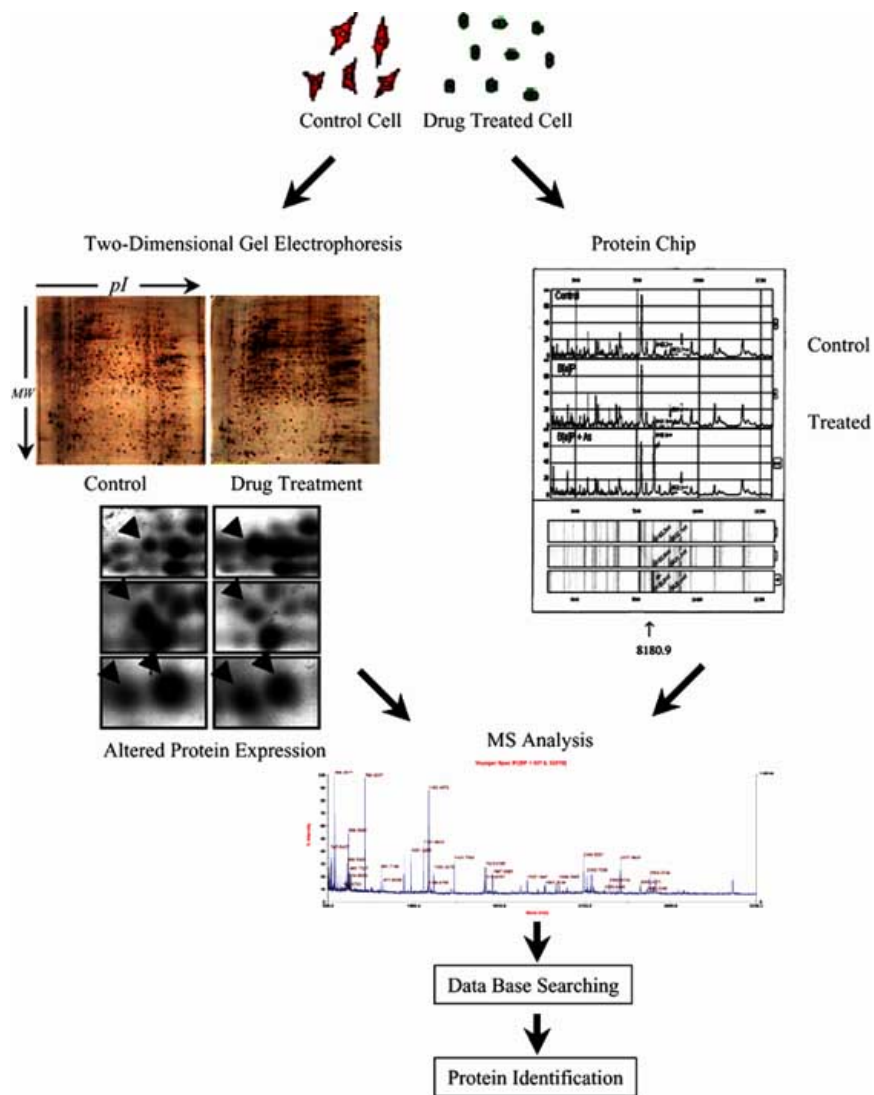


Fig. (2). A example for work flow of proteomic approach. We take drug-treated cells compared to untreated cells by 2DE, and protein marker discovery by Protein Chip array as models.

with biological perturbations under a given condition. This post-genomic technology provides a direct measurement of the presence and relative abundance of proteins, and reveals the consequence of protein functioning in establishing the biological phenotype of organisms in healthy and disease states. Proteins constitute the vast majority of drug targets for pharmaceutical drug design processes. By studying interrelationships of protein expression and modification in health and disease or drug treatment, proteomics contributes important insights into determining the pathophysiological basis of disease [46], validating drug targets [47], and illustrating drug action [48], toxicity and side effects [49]. Table 2 summarizes the major technological platforms in proteomics and their applications in drug discovery. Besides these vital experimental methods for proteomics, sensitive fluorescent dyes have also been developed at Oxford Glyco Sciences (OGS, UK) for detection of low abundant proteins [50]. Fig. (2) shows general workflows of the proteomic approaches of two-dimensional gel electrophoresis (2DE) and protein chip. In this review article, we aim to briefly summarize the recent progresses in pharmaco-proteomics and their potential application in CADD.

2.2 Proteomics in the Multi-step Process of Drug Discovery

Drugs exert their actions mainly by targeting functional proteins. Therefore, it appears straightforward to focus on proteins in order to investigate drug effects. Unfortunately, it is not easy to screen for protein alterations because of their high complexity. Traditional methods such as NMR analysis [51, 52] or yeast two hybrid systems [53, 54] for mapping protein-protein interactions are laborious and cannot meet the need for large scale analysis. Recently developed proteomic approaches have dramatically increased the efficiency and applicability of mapping drug-protein and protein-protein interactions. Proteomics can provide valuable information for drug discovery including target identification and validation [55, 56], lead selection [57], small-molecular screening and optimization [58, 59], and toxicity testing [60, 61].

The opportunities offered by proteomics are not limited to a list of proteins. Instead, the scope of proteomics covers the analysis of protein cellular activities and functions, including the characterization of the flow of information within the cell. For example, protein networks provide information on signal transduction pathways that control cell apoptosis. We performed comparative proteomic analysis of cellular proteins altered by gold (III) porphyrin and cisplatin (as a positive control) treatments, and found that altered protein expression provided information on the mechanism of gold (III) porphyrin as a potential anticancer lead (unpublished data). Another proteomic analysis demonstrated that high levels of arsenite target oxidative-stress pathways leading to apoptosis in rat lung epithelial cells [62].

Besides drug target identification, validation and lead selection, proteomics can also be used in toxicity testing. The Early Detection Research Network in the National Cancer Institute is employing proteomics in the discovery and evaluation of biomarkers for cancer detection and for the identification of high-risk subjects (<http://dtp.nci.nih.gov/>

branches/gcob/gcob%5Fweb27.html) [63]. Combined with traditional biochemical methods, proteomics has been used to elucidate mechanisms of toxic damage in several model systems [64-66]. Bandara *et al.* have found several markers correlated to kidney toxicity of 4-Aminophenol, D-serine, and cisplatin by proteomic evaluation [67]. Using proteomic analysis combined with Western blot, Zhang *et al.* identified that the increased levels of heat shock protein 70 was involved in estrogen and androgen protection of human neurons against intracellular amyloid β_{1-42} toxicity [68].

2.3 Sub-disciplines of Proteomics in Computer-Aided Drug Design

Recognized for their special contribution in drug discovery, a series of sub-proteomic technologies, including computational proteomics, chemical proteomics, structural proteomics, and topological proteomics, are increasingly integrated into emerging drug design research fields.

2.3.1 Computational Proteomics

Computational proteomics refers to the large-scale generation and analysis of 3D protein structural information [69]. Accurate prediction of protein contact maps is the beginning and essential step for computational proteomics. The major resources for computational proteomics are currently available protein and nucleic acid structures. The 3D-GENOMICS (<http://www.sbg.bio.ic.au.uk/3dgenomics/>) [70] and PDB (<http://www.rcsb.org/pdb/>) [71], and other databases (Table 1) provide a broad range of structural and functional annotations for proteins from sequenced genomes and protein 3D structures, which make a solid foundation for computational proteomics.

2.3.2 Chemical Proteomics

Chemical proteomics makes use of synthetic organic chemistry, cell biology, biochemistry, and mass spectrometry to design specific protein-modifying reagents that can be used for functional studies of distinct proteins within a certain proteome [72]. The most important tool of this field is carefully designed chemical probes that can specifically target diverse sets of enzyme families. A chemical probe contains three parts, a reactive ligand that can covalently bind to the target protein/enzyme, a linker region modulating the reactivity and specificity of the reactive ligand, and a tag for identification and purification of the target protein/enzyme [72, 73]. Several kinds of chemical probes have been used in proteomics studies, for example, serine hydrolase probes [74], S-transferase probes [75], and phenyl sulfonate probes [76].

2.3.3 Structural Proteomics

Structural proteomics is the determination of the relationship of all the proteins or protein complexes in a specific cellular organelle and the establishment of the relationship of these proteins in a proteome-wide scale [43, 77]. Combining structural biology with computational and medicinal chemistry, structural proteomics can help design drugs effectively. The major goal of structural proteomics is to determine the 3D structures of as many as possible proteins, so that other proteins in an organelle can be computationally modeled on the basis of similarity of their amino acid sequences [78, 79].

2.3.4 Topological Proteomics

Topological proteomics aims at localizing and characterizing entire protein networks within a single cell, providing quantitative insights into their basic organization, which are valuable information in identifying new drug targets and selecting potential lead compounds [80, 81]. The proprietary technology, Multi-Epitope-Ligan Kartographie (MELK), is an ultra-sensitive topological proteomics technology for analyzing proteins on a single cell level. MELK can trace out large scale subcellular protein patterns simultaneously within a cell, hence unravelling hierarchies of proteins related to a particular cell function or dysfunction [82]. Another topological proteomic program, TopNet, is an automated web tool designed to facilitate the analysis of interaction networks, which is available from TopNet [83] (Table 1).

3. CURRENT ACHIEVEMENTS AND POTENTIAL APPLICATION OF PROTEOMICS IN COMPUTER-AIDED DRUG DISCOVERY

3.1 Identification of Drug Targets

Drug target discovery, which involves the identification and early validation of disease-associated targets, is the first step in the drug discovery pipeline. Disease involves alterations in protein expression and modification and thus offers a basis for detection of drug targets through examining the protein expression profiles. Altered proteins in disease are candidates of drug targets that can be validated by modulating the proteins' activities in a model system to determine the outcome on disease phenotype (target validation). Proteomics is an effective means to globally view and detect protein expression alterations in disease and drug treatment and thus serves in both processes of target identification and validation.

Examples using proteomic approaches for target protein discovery include the identification of transforming growth factor-beta in lung epithelial cells [84] and pancreatic carcinoma cells [85, 86], plasma membrane proteins in breast cancer [76, 77], BCNP1 and MIG2B in chronic lymphocytic leukemia [88], and heat shock protein 90 alpha for tumor cell invasiveness [89]. Others have applied pre-precipitation steps prior to the proteomic approach to identify targets in signaling pathways or certain cellular reactions. By using tandem affinity purification and nanospray microcapillary tandem mass spectrometry together with two-dimensional electrophoresis, Kumar *et al.* has reported their comprehensive analysis of thioredoxin-targeted proteins in *Escherichia coli*. They found a number of proteins associated with thioredoxin that either participate directly (SodA, HPI, and AhpC) or have key regulatory functions (Fur and AcnB) in the detoxification of the cell [90]. The thioredoxin targets in the unicellular photosynthetic eukaryote *Chlamydomonas reinhardtii* have also been identified by proteomics approaches [91]. With an efficient proteomics method, Godl *et al.* have identified the cellular targets of protein kinase inhibitors, and this target may have significant implications on the development of p38 inhibitors as inflammatory drugs [92].

Proteomics can also be used to validate potential protein targets for those highly effective, widely used or newly available drugs with unknown action mechanisms. For

instant, heat shock protein 70 has been reported as a target of farnesyl transferase inhibitor in ovarian cancer 2774 cell line [93] and heat shock protein 90 as a direct target of the anti-allergic drugs disodium cromoglycate and amlexanox [94]. Cellular response of yeast cells to lithium [95] and the enzyme active sites through labeling enzyme targets by sulfonate ester probes [96] have also been investigated by proteomic approaches.

Functional proteomics is particularly useful for mapping protein-protein interactions and for identifying potential targets. Protein-protein interactions, or receptor-ligand interactions, play a critical role in cellular processes such as signal transduction, and thus are essential for the understanding of basic biological processes [97]. In this regard, proteomics is often combined with other methods to study protein-protein interactions in developing suitable drug targets. Serebriiskii *et al.* used an enhanced Dual Bait two-hybrid system together with proteomic approaches to detect peptides, proteins and drugs that selectively interact with protein targets [98]. Other researchers applied luciferase complementation imaging together with proteomics to study kinetic regulation of protein-protein interactions [99]. Rodriguez and his colleagues reported an oriented peptide array library (OPAL) approach to facilitate high throughput proteomic analysis of protein-protein interactions [100]. The interaction of the SCF-like ubiquitin ligase was demonstrated as a potential target of drugs to control differentiation [101].

3.2 Drug Mechanism of Action

Once a target protein is validated, the task of identifying chemical compounds that can appropriately modulate the target can be performed by proteomic techniques, and the cellular mechanisms of drug candidates can also be examined through proteomic analysis. Action mechanisms are the biochemical basis of drug activity. Understanding drug action modes provides valuable insights for drug modification and new drug design. Investigation of altered protein expression in response to drug treatment in established model systems is a commonly used strategy to examine drug action mechanism.

There are numerous examples of research aimed at mapping signaling pathways that are involved in disease processes. A group of researchers investigated apoptotic pathways involved in the selection of inhibitors of fatty acid synthase [59]. LAF389, a synthetic analogue of bengamides, has been found to directly or indirectly inhibit methionine aminopeptidases (MetAps) by a proteomics-based approach. Additionally, a structural study revealed that three key hydroxyl groups on the inhibitor coordinate the di-cobalt center in the enzyme active site [102]. MacKeigan *et al.* found that taxol activated the pathways of mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase by implementing a proteomic approach. By combining with MEK inhibition, taxol synergistically enhances apoptosis by altering the expression of the proteins RS/DJ-1 (RNA-binding regulatory subunit/DJ-1 PARK7) and RhoGDIalpha (Rho GDP-dissociation inhibitor alpha) [103]. RhoGDI alpha.

Oxidative stress is a major area of drug treatment. Our proteomics analysis revealed that arsenite induced apoptosis

by stimulating the oxidative stress pathway [62]. Young and his collaborators showed that Ras-mediated oncogenic transformation of ovarian epithelial cells was through activation of antioxidant pathways [104]. The effect of a novel drug lead, IBTP (4-iodobutyl triphenylphosphonium), for ovarian cancer treatment was found to mainly act on mitochondria through oxidative stress [105]. Further proteomics analysis reported that a major cellular response to oxidative stress was the modification of several peroxiredoxins [106].

Apoptosis, or programmed cell death, is a tightly controlled multi-step cellular event. Proteomic approaches have been increasingly employed in mapping out the apoptosis pathways involved in chemotherapy. Trichostatin-A (TSA) was found, through proteomic profile analysis, to cause apoptosis in a pancreatic adenocarcinoma cell lines (Paca44) at G2 arrest [107]. Combining proteomic approaches together with other biological tests, McJilton *et al.* demonstrated that protein kinase cepsilon interacted with Bax and promoted survival of human prostate cancer cells [108]. Ueda's results of two-dimensional immunoblots suggested that the p38 MAPK- MAPKAP kinase 2- BAG2 phosphorylation cascade may be a novel signaling pathway for response to extracellular stresses [109]. Research data from a proteomic-based screening suggested that protein turnover inhibition of caspase-dependent proteolysis by the degrading proteasome was a general event in programmed cell death from *Drosophila* to mammals [110].

Considering the molecular complexity of entire proteomes in tissues and cells, more researchers have applied proteomic analysis to sub-cellular fractions. A group of scientists in the Sidney Kimmel Cancer Center (San Diego, California) used subtractive proteomics and bioinformatics to analyze endothelial cell surface proteins, and found two of these proteins, aminopeptidase-P and annexin A1, as selective *in vivo* targets for antibodies in lungs and solid tumours respectively [111]. Such sub-cellular analysis has also been performed on mitochondria, and S-nitrosylation of cysteine thiol was shown to be a significant part of nitric oxidation mediation [112]. Other scientists have reported that mitochondrial translocation of the actin-binding protein, cofilin, was an early step in apoptosis induction [113]. The signaling pathways of membrane proteins [114, 115], endoplasmic reticulum [116, 117], and golgi apparatus [118] have also been investigated by proteomics.

3.3 Drug Toxicity and Side Effects

Given the low success rate in drug development, detection of potential toxicity and side effects in early stages of drug candidate identification can save money and time by focusing resources on those safe drug leads and candidates. By establishing a database that defines the response of a tissue proteome to specific drugs, comparative proteomics can be used to determine the propensity for a new compound. Proteomic signatures can also be constructed based on the toxicity responses previously observed with known agents. This can provide information to screen similar compounds for modification and improvement in drug design.

Currently, many studies focus on the mechanism of toxic damage by existing drugs, especially in the liver, kidney and

cardiovascular system. Venkatraman *et al.* have identified the modification of mitochondrial proteins in response to ethanol-dependent hepatotoxicity, and demonstrated that chronic ethanol consumption extended to a modification of the mitochondrial proteome much broader than realized previously [49]. Meneses-Lorente *et al.* reported a proteomic signature associated with hepatocellular steatosis in rats after dosing with a compound in preclinical development [118]. Evaluation of drug toxicity to kidney was also performed by proteomic approaches [67, 120].

Studies of drug toxicity related biomarkers are also of great importance for drug screening. Proteomic and immunological techniques were used to identify *in vitro* protein biomarkers of idiosyncratic liver toxicity by Gao and his colleagues, and revealed that BMS-PTX-265 and BMS-PTX-837 were potential toxic biomarkers for up to twenty drugs [121]. Human aldose reductase-like protein-1 (hARLP-1) was the most prominent tumor-associated AKR member detected by proteomic approaches as a strong candidate for immunohistochemical diagnostic marker of human HCC [122].

Besides toxicity, drug resistance is another important reason for the failure of chemotherapies. A systematic proteomic approach for the study of chemoresistance mechanisms of vindesine, etoposide and cisplatin was first undertaken by Sinha *et al.* [123]. Differential proteomic analysis of vinca alkaloid-treated drug-sensitive human leukemia cells (CCRF-CEM) showed that numerous proteins were involved in drug resistance, and some of them could be novel targets for elucidation of resistance mechanisms [124]. Comparative proteomic analysis revealed that methionine adenosyltransferase and S-adenosylmethionine played unique roles in methotrexate resistance in leishmania [125]. Bernstein *et al.* found that resistance to deoxycholate-induced apoptosis was modulated by over-expression of multiple anti-apoptotic proteins and under-expression of multiple pro-apoptotic proteins [126]. By proteomic analysis, landmark proteins for insulin resistance [127] and protozoan parasite leishmania resistance [125] have also been reported. In mapping out mechanisms of drug resistance, one important issue is to differentiate adaptive and acquired resistance. By using proteomic analysis associated with cadmium adaptation in U937 cells, Jeon and his collaborators found that a newly identified protein, calbindin-D28k, is the secondary cadmium-responsive protein that conferred resistance to cadmium-induced apoptosis [128]. The rich information obtained from proteomic study will accelerate lead identification and drug modification and improve drug efficacy and safety in pre-clinical and clinical studies.

3.4 Proteomic Signatures (biomarkers) for Determining Clinical Effects

Biomarkers are usually proteins that have their expression altered in response to a disease condition. Biomarkers can be used as signatures to determine drug efficacy and clinical effects. Biomarkers can also be drug targets for further development [129]. In proteomics, the challenge is to identify unique molecular signatures in complex biological mixtures that can be unambiguously correlated to biological events in order to validate novel drug targets and predict drug responses.

Table 2. Major Technological Platforms for Proteomics

Technique	2DE (Two-Dimensional Electrophoresis)	Protein Chip Array	ICAT (Isotope-Coded Affinity Tags)
Separation based on:	Molecular weight and isoelectric point	Surface affinity and molecular weight	Chemical labeling and relative abundance
Protein ID identified by:	MALDI-TOF MS MS/MS	SELDI-TOF MS MS/MS	LC-ESI MS/MS
Applications in drug discovery	Biomarker discovery Drug screening Action mechanisms Toxicity	Biomarker discovery Drug screening Diagnosis	Biomarker discovery Diagnosis
Remarks	Suitable for cell line, tissue, serum etc.; Identification of over 1000 proteins at the same time; Wide detection range (6-200kDa); Not suitable for low abundant proteins, and very alkaline or very hydrophobic proteins; Affected by post-translational modifications, including phosphorylation, glycosylation, etc.	Capture, detect and analyze proteins directly from crude biological samples; Low limit of detection (< fmole of protein); Small sample size (< 5 µl); Rapid results; Significant result (accurate molecular weight); Wide detection range (0-300 kDa); More automated.	Labeled with biotinylated tags before analysis; Detection and quantitation of low-abundance regulatory proteins; Suitable for abundances of light and heavy proteins; Not suitable for post-translational modified proteins; More automated.

Proteomics has emerged to be a powerful approach for directly identifying highly predictive pharmacogenomic markers in blood or tissues. Previously, we have reported the proteomic analysis of oral tongue carcinoma to globally search for tumor related proteins [130]. A number of tumor-associated proteins were consistently found to be significantly altered in their expression levels in tongue carcinoma tissues, compared with their paired normal mucosae. These proteins are potential biomarkers for tongue carcinoma diagnosis and therapeutic monitoring. A similar analysis for buccal squamous cell carcinoma also produced biomarker candidates that can be the proteomic signature for diagnostic and treatment [131]. Hepatocarcinoma is another cancer that received much attention recently. Several biomarkers have been identified by proteomic approaches, such as aldose reductase-like protein (ARLP) [122], cytokeratin 19 [132], and ferritin light chain [133]. Proteomic approaches have also been applied to biomarker or antigen identification for other cancers, including prostate cancer [73, 75, 134], bladder cancer [17, 135, 136], and ovarian cancer [137-139].

Advances of proteomic technology also hold great promise for improvements in the understanding, diagnosis and therapy of central nervous system disorders. Jin *et al.* revealed a role of stathmin in adult neurogenesis by proteomic and immunochemical characterization [140]. Others have reported that collapsin response mediator protein (CRMP-2) was a marker of changes developed in rat hippocampus [141]. Great efforts have been made in searching for new and accurate biomarkers for cardiovascular system disease [142, 143], HBV and HCV [144, 145], and arthritis [146].

Biomarker discovery could make great contributions to the characterization of the pharmacology of drug candidates and to the understanding of diseases subtypes to which a therapeutic intervention applies. Proteomic approaches have

been proved to be promising techniques in the process of biomarker discovery.

4. FUTURE PROSPECTS

The successful stories of proteomics application in drug discovery in recent years have demonstrated the potential value of proteomics in drug development. Proteomic approaches can provide valuable information for target identification and validation, lead selection, small-molecular screening and optimization. In particular, those sub-disciplines of proteomics have demonstrated promising application for CADD. However, application of proteomic approaches is rather limited at present due to its inefficiency in detecting low abundant or post-translationally modified proteins. With the development of related techniques, proteomics is sure to play a more important role in rational drug design. The key point to drug development in the future is the integration of proteomics with CADD to use the vast amount of information and techniques available for accelerating drug discovery process.

ACKNOWLEDGEMENTS

This work was partially supported by Hong Kong Research Grants Council Grants HKU 7227/02M (to Q.Y.H.), HKU 7218/02M and HKU 7395/03M (to J.F.C.), the Department of Chemistry, and the Areas of Excellence scheme of Hong Kong University Grants Committee.

ABBREVIATIONS

2-DE	=	2-dimensional gel electrophoresis
CADD	=	Computer-aided drug design
pI	=	Isoelectric point

ICAT	=	Isotope-coded affinity tags
LC-ESI MS/MS	=	Liquid chromatography-electrospray ionization tandem MS
MALDI-TOF	=	Matrix-assisted laser desorption ionization time of flight
MELK	=	Multi-Epitope-Ligan Kartographie
MS	=	Mass spectrometry
SELDI	=	Surface enhanced laser desorption ionization

REFERENCES

- [1] Carlson, H.A.; McCammon, J.A. *Mol. Pharmacol.*, **2000**, *57*, 213-218.
- [2] Street, A.G.; Mayo, S.L. *Structure Fold Des.*, **1999**, *7*, R105-109.
- [3] Veselovsky, A.V.; Ivanov, A.S. *Curr. Drug Targets Infect. Disord.*, **2003**, *3*, 33-40.
- [4] Erickson, J.; Neidhart, D.J.; VanDrie, J.; Kempf, D.J.; Wang, X.C.; Norbeck, D.W.; Plattner, J.J.; Rittenhouse, J.W.; Turon, M.; Wideburg, N. *Science*, **1990**, *249*, 527-533.
- [5] Adam, B.L.; Qu, Y.; Davis, J.W.; Ward, M.D.; Clements, M.A.; Cazares, L.H.; Semmes, O.J.; Schellhammer, P.F.; Yasui, Y.; Feng, Z.; Wright, G.L.Jr. *Cancer Res.*, **2002**, *62*, 3609-3614.
- [6] Anand, K.; Ziebuhr, J.; Wadhvani, P.; Mesters, J.R.; Hilgenfeld, R. *Science*, **2003**, *300*, 1763-1767.
- [7] Varney, M.D.; Appelt, K.; Kalish, V.; Reddy, M.R.; Tatlock, J.; Palmer, C.L.; Romines, W.H.; Wu, B.W.; Musick, L. *J. Med. Chem.*, **1994**, *37*, 2274-2284.
- [8] Dunbar, P.G.; Durant, G.J.; Rho, T.; Ojo, B.; Huzl, J.J.; Smith, D.A.; el-Assadi, A.A.; Sbeih, S.; Ngur, D.O.; Periyasamy, S. *J. Med. Chem.*, **1994**, *37*, 2774-2782.
- [9] Kuntz, I.D. *Science*, **1992**, *257*, 1078-1082.
- [10] Good, A.C.; Ewing, T.J.; Gschwend, D.A.; Kuntz, I.D. *J. Comput. Aided Mol. Des.*, **1995**, *9*, 1-12.
- [11] Kuntz, I.D.; Blaney, J.M.; Oatley, S.J.; Langridge, R.; Ferrin, T.E. *J. Mol. Biol.*, **1982**, *161*, 269-288.
- [12] Erickson, J.A.; Jalaie, M.; Robertson, D.H.; Lewis, R.A.; Vieth, M. *J. Med. Chem.*, **2004**, *47*, 45-55.
- [13] Schafferhans, A.; Klebe, G. *J. Mol. Biol.*, **2001**, *307*, 407-427.
- [14] Yamamoto, Y.; Ishihara, Y.; Kuntz, I.D. *J. Med. Chem.*, **1994**, *37*, 3141-3153.
- [15] Keseru, G.M. *J. Comput. Aided Mol. Des.*, **2001**, *15*, 649-657.
- [16] Verras, A.; Kuntz, I.D.; Ortiz de Montellano, P.R. *J. Med. Chem.*, **2004**, *47*, 3572-3579.
- [17] Adam, B.L.; Vlahou, A.; Semmes, O.J.; Wright, G.L. Jr. *Proteomics*, **2001**, *1*, 1264-1270.
- [18] Mizutani, M.Y.; Tomioka, N.; Itai, A. *J. Mol. Biol.*, **1994**, *243*, 310-326.
- [19] Buzko, O.V.; Bishop, A.C.; Shokat, K.M. *J. Comput. Aided Mol. Des.*, **2002**, *16*, 113-127.
- [20] Goodsell, D.S.; Morris, G.M.; Olson, A.J. *J. Mol. Recognit.*, **1996**, *9*, 1-5.
- [21] Osterberg, F.; Morris, G.M.; Sanner, M.F.; Olson, A.J.; Goodsell, D.S. *Proteins*, **2002**, *46*, 34-40.
- [22] Hindle, S.A.; Rarey, M.; Buning, C.; Lengau, T. *J. Comput. Aided Mol. Des.*, **2002**, *16*, 129-149.
- [23] Knegtel, R.M.; Bayada, D.M.; Engh, R.A.; von der Saal, W.; van Geerstein, V.J.; Grootenhuis, P.D. *J. Comput. Aided Mol. Des.*, **1999**, *13*, 167-183.
- [24] Vigers, G.P.; Rizzi, J.P. *J. Med. Chem.*, **2004**, *47*, 80-89.
- [25] Hawkins, C.A.; Watson, C.; Yan, Y.; Gong, B.; Wemmer, D.E. *Nucleic Acids Res.*, **2001**, *29*, 936-942.
- [26] Marzilli, L.G.; Saad, J.S.; Kuklenyik, Z.; Keating, K.A.; Xu, Y. *J. Am. Chem. Soc.*, **2001**, *123*, 2764-2770.
- [27] Sinay, P. *Nature*, **1999**, *398*, 377-378.
- [28] Jozwiak, K.; Ravich, S.; Collins, J.R.; Wainer, I.W. *J. Med. Chem.*, **2004**, *47*, 4008-4021.
- [29] Purushottamachar, P.; Kulkarni, V.M. *Bioorg. Med. Chem.*, **2003**, *11*, 3487-3497.
- [30] Santos-Filho, O.A.; Mishra, R.K.; Hopfinger, A.J. *J. Comput. Aided Mol. Des.*, **2001**, *15*, 787-810.
- [31] Myers, A.M.; Charifson, P.S.; Owens, C.E.; Kula, N.S.; McPhail, A.T.; Baldessarini, R.J.; Booth, R.G.; Wyrick, S.D. *J. Med. Chem.*, **1994**, *37*, 4109-4117.
- [32] Bitetti-Putzer, R.; Joseph-McCarthy, D.; Hogle, J.M.; Karplus, M. *J. Comput. Aided Mol. Des.*, **2001**, *15*, 935-960.
- [33] Cruciani, G.; Watson, K.A. *J. Med. Chem.*, **1994**, *37*, 2589-2601.
- [34] Polanski, J.; Gieleciak, R.; Magdziarz, T.; Bak, A. *J. Chem. Inf. Comput. Sci.*, **2004**, *44*, 1423-1435.
- [35] Tomioka, N.; Itai, A.; Itaka, Y. *J. Comput. Aided Mol. Des.*, **1987**, *1*, 197-210.
- [36] Bogacewicz, R.; Trylska, J.; Geller, M. *Acta. Pol. Pharm.*, **2000**, *57*, S25-28.
- [37] Bohm, H.J. *J. Comput. Aided Mol. Des.*, **1992**, *6*, 593-606.
- [38] Wolf, K.; Dormeyer, M. *Parasitol. Res.*, **2003**, *90*, S91-96.
- [39] Joseph-McCarthy, D.; Hogle, J.M.; Karplus, M. *Proteins*, **1997**, *29*, 32-58.
- [40] Tondel, K.; Anderssen, E.; Drablos, F. *J. Comput. Aided Mol. Des.*, **2002**, *16*, 831-840.
- [41] Wasinger, V.C.; Cordwell, S.J.; Cerpa-Poljak, A.; Yan, J.X.; Gooley, A.A.; Wilkins, M.R.; Duncan, M.W.; Harris, R.; Williams, K.L.; Humphery-Smith, I. *Electrophoresis*, **1995**, *16*, 1090-1094.
- [42] Wilkins, M.R.; Sanchez, J.C.; Gooley, A.A.; Appel, R.D.; Humphery-Smith, I.; Hochstrasser, D.F.; Williams, K.L. *Biotechnol. Genet. Eng. Rev.*, **1996**, *13*, 19-50.
- [43] He, Q.Y.; Chiu, J.F. *J. Cell. Biochem.*, **2003**, *89*, 868-886.
- [44] Pandey, A.; Mann, M. *Nature*, **2000**, *405*, 837-846.
- [45] Tyers, M.; Mann, M. *Nature*, **2003**, *422*, 193-197.
- [46] Hanash, S. *Nature*, **2003**, *422*, 226-232.
- [47] Rosamond, J.; Allsop, A. *Science*, **2000**, *287*, 1973-1976.
- [48] Graves, P.R.; Kwiek, J.J.; Fadden, P.; Ray, R.; Hardeman, K.; Coley, A.M.; Foley, M.; Haystead, T.A. *Mol. Pharmacol.*, **2002**, *62*, 1364-1372.
- [49] Venkatraman, A.; Landar, A.; Davis, A.J.; Chamlee, L.; Sanderson, T.; Kim, H.; Page, G.; Pompilius, M.; Ballinger, S.; Darley-Usmar, V.; Bailey, S.M. *J. Biol. Chem.*, **2004**, *279*, 22092-22101.
- [50] Chi, S.W.; Ayed, A.; Arrowsmith, C.H. *EMBO J.*, **1999**, *18*, 4438-4445.
- [51] Fleming, K.G.; Engelman, D.M. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 14340-14344.
- [52] Page, M.J.; Amess, B.; Townsend, R.R.; Parekh, R.; Herath, A.; Brusten, L.; Zvelebil, M.J.; Stein, R.C.; Waterfield, M.D.; Davies, S.C.; O'Hare, M.J. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 12589-12594.
- [53] Huang, J.; Schreiber, S.L. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 13396-13401.
- [54] Kang, J.; Kim, T.; Ko, Y.G.; Rho, S.B.; Park, S.G.; Kim, M.J.; Kwon, H.J.; Kim, S. *J. Biol. Chem.*, **2000**, *275*, 31682-31688.
- [55] Drummelsmith, J.; Brochu, V.; Girard, I.; Messier, N.; Ouellette, M. *Mol. Cell Proteomics*, **2003**, *2*, 146-155.
- [56] Greenbaum, D.C.; Baruch, A.; Grainger, M.; Bozdech, Z.; Medzihradsky, K.F.; Engel, J.; DeRisi, J.; Holder, A.A.; Bogoy, M. *Science*, **2002**, *298*, 2002-2006.
- [57] Bleicher, K.H.; Bohm, H.J.; Muller, K.; Alanine, A.I. *Nat. Rev. Drug Discov.*, **2003**, *2*, 369-378.
- [58] Baker, K.; Blecinski, C.; Lin, H.; Salazar-Jimenez, G.; Sengupta, D.; Krane, S.; Cornish, V.W. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 16537-16542.
- [59] Kridel, S.J.; Axelrod, F.; Rozenkrantz, N.; Smith, J.W. *Cancer Res.*, **2004**, *64*, 2070-2075.
- [60] Imanishi, S.; Harada, K. *Toxicol.*, **2004**, *43*, 651-659.
- [61] Keightley, J.A.; Shang, L.; Kinter, M. *Mol. Cell Proteomics*, **2004**, *3*, 167-175.
- [62] Lau, A.T.; He, Q.Y.; Chiu, J.F. *Biochem. J.*, **2004**, *382*(Pt 2), 641-650.
- [63] Verma, M.; Wright, G.L.Jr.; Hanash, S.M.; Gopal-Srivastava, R.; Srivastava, S. *Ann. NY Acad. Sci.*, **2001**, *945*, 103-115.
- [64] Edvardsson, U.; von Lowenhillem, H.B.; Panfilov, O.; Nystrom, A.C.; Nilsson, F.; Dahllof, B. *Proteomics*, **2003**, *3*, 468-478.
- [65] Fountoulakis, M.; Berndt, P.; Boelsterli, U.A.; Cramer, F.; Winter, M.; Albertini, S.; Suter, L. *Electrophoresis*, **2000**, *21*, 2148-2161.
- [66] Petricoin, E.F.; Rajapaske, V.; Herman, E.H.; Arekani, A.M.; Ross, S.; Johann, D.; Knapton, A.; Zhang, J.; Hitt, B.A.; Conrads, T.P.; Veenstra, T.D.; Liotta, L.A.; Sistiare, F.D. *Toxicol. Pathol.*, **2004**, *32*(S1), 122-130.
- [67] Bandara, L.R.; Kelly, M.D.; Lock, E.A.; Kennedy, S. *Toxicol. Sci.*, **2003**, *73*, 195-206.

- [68] Zhang, Y.; Champagne, N.; Beitel, L.K.; Goodyer, C.G.; Trifiro, M.; LeBlanc, A. *J. Neurosci.*, **2004**, *24*, 5315-5321.
- [69] Maggio, E.T.; Ramnarayan, K. *Drug Discov. Today*, **2001**, *6*, 996-1004.
- [70] Fleming, K.; Muller, A.; MacCallum, R.M.; Sternberg, M.J. *Nucleic Acids Res.*, **2004**, *32*, D245-250.
- [71] Sanishvili, R.; Yakunin, A.F.; Laskowski, R.A.; Skarina, T.; Evdokimova, E.; Doherty-Kirby, A.; Lajoie, G.A.; Thornton, J.M.; Arrowsmith, C.H.; Savchenko, A.; Joachimiak, A.; Edwards, A.M. *J. Biol. Chem.*, **2003**, *278*, 26039-26045.
- [72] Jeffery, D.A.; Bogoy, M. *Curr. Opin. Biotech.*, **2003**, *14*, 87-95.
- [73] Adam, G.C.; Cravatt, B.F.; Sorensen, E.J. *Chem. Biol.*, **2001**, *8*, 81-95.
- [74] Liu, Y.; Patricelli, M.P.; Cravatt, B.F. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 14694-14699.
- [75] Adam, G.C.; Sorensen, E.J.; Cravatt, B.F. *Nat. Biotechnol.*, **2002**, *20*, 805-809.
- [76] Adam, G.C.; Sorensen, E.J.; Cravatt, B.F. *Mol. Cell. Proteomics*, **2002**, *1*, 828-835.
- [77] Adam, P.J.; Boyd, R.; Tyson, K.L.; Fletcher, G.C.; Stamps, A.; Hudson, L.; Poyser, H.R.; Redpath, N.; Griffiths, M.; Steers, G.; Harris, A.L.; Patel, S.; Berry, J.; Loader, J.A.; Townsend, R.R.; Daviet, L.; Legrain, P.; Parekh, R.; Terrett, J.A. *J. Biol. Chem.*, **2003**, *278*, 6482-6489.
- [78] Renfrey, S.; Featherstone, J. *Nat. Rev. Drug Discov.*, **2002**, *1*, 175-176.
- [79] Sali, A.; Glaeser, R.; Earnest, T.; Baumeister, W. *Nature*, **2003**, *422*, 216-225.
- [80] Linding, R.; Jensen, L.J.; Diella, F.; Bork, P.; Gibson, T.J.; Russell, R.B. *Structure (Camb.)*, **2003**, *11*, 1453-1459.
- [81] Amaral, L.A.; Scala, A.; Barthelemy, M.; Stanley, H.E. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 11149-11152.
- [82] Girvan, M.; Newman, M.E. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 7821-7826.
- [83] Schubert, W. *Adv. Biochem. Eng. Biotechnol.*, **2003**, *83*, 189-209.
- [84] Yu, H.; Zhu, X.; Greenbaum, D.; Karro, J.; Gerstein, M. *Nucleic Acids Res.*, **2004**, *32*, 328-337.
- [85] Kanamoto, T.; Hellman, U.; Heldin, C.H.; Souchelnytskyi, S. *EMBO J.*, **2002**, *21*, 1219-1230.
- [86] Imamura, T.; Kanai, F.; Kawakami, T.; Amarsanaa, J.; Ijichi, H.; Hoshida, Y.; Tanaka, Y.; Ikenoue, T.; Tateishi, K.; Kawabe, T.; Arakawa, Y.; Miyagishi, M.; Taira, K.; Yokosuka, O.; Omata, M. *Biochem. Biophys. Res. Commun.*, **2004**, *318*, 289-296.
- [87] Rosty, C.; Christa, L.; Kuzdzal, S.; Baldwin, W.M.; Zahurak, M.L.; Carnot, F.; Chan, D.W.; Canto, M.; Lillemoe, K.D.; Cameron, J.L.; Yeo, C.J.; Hruban, R.H.; Goggins, M. *Cancer Res.*, **2002**, *62*, 1868-1875.
- [88] Boyd, R.S.; Adam, P.J.; Patel, S.; Loader, J.A.; Berry, J.; Redpath, N.T.; Poyser, H.R.; Fletcher, G.C.; Burgess, N.A.; Stamps, A.C.; Hudson, L.; Smith, P.; Griffiths, M.; Willis, T.G.; Karran, E.L.; Oscier, D.G.; Catovsky, D.; Terrett, J.A.; Dyer, M.J. *Leukemia*, **2003**, *17*, 1605-1612.
- [89] Eustace, B.K.; Sakurai, T.; Stewart, J.K.; Yimlamai, D.; Unger, C.; Zehetmeier, C.; Lain, B.; Torella, C.; Henning, S.W.; Beste, G.; Scroggins, B.T.; Neckers, L.; Ilag, L.L.; Jay, D.G. *Nat. Cell Biol.*, **2004**, *6*, 507-514.
- [90] Kumar, J.K.; Tabor, S.; Richardson, C.C. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 3759-3764.
- [91] Lemaire, S.D.; Guillon, B.; Le Marechal, P.; Keryer, E.; Miginiac-Maslow, M.; Decottignies, P. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 7475-7480.
- [92] Godt, K.; Wissing, J.; Kurtenbach, A.; Habenberger, P.; Blencke, S.; Gutbrod, H.; Salassidis, K.; Stein-Gerlach, M.; Missio, A.; Cotten, M.; Daub, H. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 15434-15439.
- [93] Hu, W.; Wu, W.; Verschraegen, C.F.; Chen, L.; Mao, L.; Yeung, S.C.; Kudelka, A.P.; Freedman, R.S.; Kavanagh, J.J. *Proteomics*, **2003**, *3*, 1904-1911.
- [94] Okada, M.; Itoh, H.; Hatakeyama, T.; Tokumitsu, H.; Kobayashi, R. *Biochem. J.*, **2003**, *374*, 433-441.
- [95] Bro, C.; Regenberg, B.; Lagniel, G.; Labarre, J.; Montero-Lomeli, M.; Nielsen, J. *J. Biol. Chem.*, **2003**, *278*, 32141-32149.
- [96] Adam, G.C.; Burbaum, J.; Kozarich, J.W.; Patricelli, M.P.; Cravatt, B.F. *J. Am. Chem. Soc.*, **2004**, *126*, 1363-1368.
- [97] Archakov, A.I.; Govorun, V.M.; Dubanov, A.V.; Ivanov, Y.D.; Veselovsky, A.V.; Lewi, P.; Janssen, P. *Proteomics*, **2003**, *3*, 380-391.
- [98] Serebriiskii, I.G.; Mitina, O.; Pugacheva, E.N.; Benevolenskaya, E.; Kotova, E.; Toby, G.G.; Khazak, V.; Kaelin, W.G.; Chernoff, J.; Golemis, E.A. *Genome Res.*, **2002**, *12*, 1785-1791.
- [99] Luker, K.E.; Smith, M.C.; Luker, G.D.; Gammon, S.T.; Piwnica-Worms, H.; Piwnica-Worms, D. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 12288-12293.
- [100] Rodriguez, M.; Li, S.S.; Harper, J.W.; Songyang, Z. *J. Biol. Chem.*, **2004**, *279*, 8802-8807.
- [101] Liao, E.H.; Hung, W.; Abrams, B.; Zhen, M. *Nature*, **2004**, *430*, 345-350.
- [102] Towbin, H.; Bair, K.W.; DeCaprio, J.A.; Eck, M.J.; Kim, S.; Kinder, F.R.; Morollo, A.; Mueller, D.R.; Schindler, P.; Song, H.K.; van Oostrum, J.; Versace, R.W.; Voshol, H.; Wood, J.; Zabladoff, S.; Phillips, P.E. *J. Biol. Chem.*, **2003**, *278*, 52964-52971.
- [103] MacKeigan, J.P.; Clements, C.M.; Lich, J.D.; Pope, R.M.; Hod, Y.; Ting, J.P. *Cancer Res.*, **2003**, *63*, 6928-6934.
- [104] Young, T.W.; Mei, F.C.; Yang, G.; Thompson-Lanza, J.A.; Liu, J.; Cheng, X. *Cancer Res.*, **2004**, *64*, 4577-4584.
- [105] Lin, T.K.; Hughes, G.; Muratovska, A.; Blaikie, F.H.; Brookes, P.S.; Darley-Usmar, V.; Smith, R.A.; Murphy, M.P. *J. Biol. Chem.*, **2002**, *277*, 17048-17056.
- [106] Rabilloud, T.; Heller, M.; Gasnier, F.; Lucche, S.; Rey, C.; Aebersold, R.; Benahmed, M.; Louisot, P.; Lunardi, J. *J. Biol. Chem.*, **2002**, *277*, 19396-19401.
- [107] Cecconi, D.; Scarpa, A.; Donadelli, M.; Palmieri, M.; Hamdan, M.; Astner, H.; Righetti, P.G. *Electrophoresis*, **2003**, *24*, 1871-1878.
- [108] McJilton, M.A.; Van Sikes, C.; Wescott, G.G.; Wu, D.; Foreman, T.L.; Gregory, C.W.; Weidner, D.A.; Harris Ford, O.; Morgan Lasater, A.; Mohler, J.L.; Terrian, D.M. *Oncogene*, **2003**, *22*, 7958-7968.
- [109] Ueda, K.; Kosako, H.; Fukui, Y.; Hattori, S. *J. Biol. Chem.*, **2004**, *279*, 41815-41821.
- [110] Adrain, C.; Creagh, E.M.; Cullen, S.P.; Martin, S.J. *J. Biol. Chem.*, **2004**, *279*, 36923-36930.
- [111] Oh, P.; Li, Y.; Yu, J.; Durr, E.; Krasinska, K.M.; Carver, L.A.; Testa, J.E.; Schnitzer, J.E. *Nature*, **2004**, *429*, 629-635.
- [112] Foster, M.W.; Stamler, J.S. *J. Biol. Chem.*, **2004**, *279*, 25891-25897.
- [113] Chua, B.T.; Volbracht, C.; Tan, K.O.; Li, R.; Yu, V.C.; Li, P. *Nat. Cell Biol.*, **2003**, *5*, 1083-1089.
- [114] Tam, E.M.; Morrison, C.J.; Wu, Y.I.; Stack, M.S.; Overall, C.M. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 6917-6922.
- [115] Hitchcock, A.L.; Auld, K.; Gygi, S.P.; Silver, P.A. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 12735-12740.
- [116] Desjardins, M. *Nat. Rev. Immunol.*, **2003**, *3*, 280-291.
- [117] Wrzeszczynski, K.O.; Rost, B. *Cell Mol. Life Sci.*, **2004**, *61*, 1341-1353.
- [118] Wu, C.C.; MacCoss, M.J.; Mardones, G.; Finnigan, C.; Mogelsvang, S.; Yates, J.R. 3rd; Howell, K.E. *Mol. Biol. Cell.*, **2004**, *15*, 2907-2919.
- [119] Meneses-Lorente, G.; Guest, P.C.; Lawrence, J.; Muniappa, N.; Knowles, M.R.; Skynner, H.A.; Salim, K.; Cristea, I.; Mortishire-Smith, R.; Gaskell, S.J.; Watt, A. *Chem. Res. Toxicol.*, **2004**, *17*, 605-612.
- [120] Charlwood, J.; Skehel, J.M.; King, N.; Camilleri, P.; Lord, P.; Bugelski, P.; Atif, U. *J. Proteome Res.*, **2002**, *1*, 73-82.
- [121] Gao, J.; Ann Garulacan, L.; Storm, S.M.; Hefta, S.A.; Opitck, G.J.; Lin, J.H.; Moulin, F.; Dambach, D.M. *Toxicol. In Vitro*, **2004**, *18*, 533-541.
- [122] Zeindl-Eberhart, E.; Haraida, S.; Liebmann, S.; Jungblut, P.R.; Lamer, S.; Mayer, D.; Jager, G.; Chung, S.; Rabes, H.M. *Hepatology*, **2004**, *39*, 540-549.
- [123] Sinha, P.; Poland, J.; Kohl, S.; Schnolzer, M.; Helmbach, H.; Hutter, G.; Lage, H.; Schadendorf, D. *Electrophoresis*, **2003**, *24*, 2386-2404.
- [124] Verrills, N.M.; Walsh, B.J.; Cobon, G.S.; Hains, P.G.; Kavallaris, M. *J. Biol. Chem.*, **2003**, *278*, 45082-45093.
- [125] Drummelsmith, J.; Girard, I.; Trudel, N.; Ouellette, M. *J. Biol. Chem.*, **2004**, *279*, 33273-33280.
- [126] Bernstein, H.; Payne, C.M.; Kunke, K.; Crowley-Weber, C.L.; Waltmire, C.N.; Dvorakova, K.; Holubec, H.; Bernstein, C.; Vaillancourt, R.R.; Raynes, D.A.; Guerriero, V.; Garewal, H. *Carcinogenesis*, **2004**, *25*, 681-692.

- [127] Sanchez, J.C.; Converset, V.; Nolan, A.; Schmid, G.; Wang, S.; Heller, M.; Sennitt, M.V.; Hochstrasser, D.F.; Cawthorne, M.A. *Proteomics*, **2003**, *3*, 1500-1520.
- [128] Jeon, H.K.; Jin, H.S.; Lee, D.H.; Choi, W.S.; Moon, C.K.; Oh, Y.J.; Lee, T.H. *J. Biol. Chem.*, **2004**, *279*, 31575-31583.
- [129] Braddock, M.; Quinn, A. *Nat. Rev. Drug Discov.*, **2004**, *3*, 330-339.
- [130] He, Q.Y.; Chen, J.; Kung, H.F.; Yuen, A.P.; Chiu, J.F. *Proteomics*, **2004**, *4*, 271-278.
- [131] Chen, J.; He, Q.Y.; Yuen, A.P.; Chiu, J.F. *Proteomics*, **2004**, *4*, 2465-2475.
- [132] Ding, S.J.; Li, Y.; Tan, Y.X.; Jiang, M.R.; Tian, B.; Liu, Y.K.; Shao, X.X.; Ye, S.L.; Wu, J.R.; Zeng, R.; Wang, H.Y.; Tang, Z.Y.; Xia, Q.C. *Mol. Cell. Proteomics*, **2004**, *3*, 73-81.
- [133] Park, K.S.; Kim, H.; Kim, N.G.; Cho, S.Y.; Choi, K.H.; Seong, J.K.; Paik, Y.K. *Hepatology*, **2002**, *35*, 1459-1466.
- [134] Petricoin, E.F.; Ornstein, D.K.; Liotta, L.A. *Urol. Oncol.*, **2004**, *22*, 322-328.
- [135] Kageyama, S.; Isono, T.; Iwaki, H.; Wakabayashi, Y.; Okada, Y.; Kontani, K.; Yoshimura, K.; Terai, A.; Arai, Y.; Yoshiki, T. *Clin. Chem.*, **2004**, *50*, 857-866.
- [136] Celis, J.E.; Wolf, H.; Ostergaard, M. *Electrophoresis*, **2000**, *21*, 2115-2121.
- [137] Jacobs, I.J.; Menon, U. *Mol. Cell. Proteomics*, **2004**, *3*, 355-366.
- [138] 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.; Liotta, L.A. *Lancet*, **2002**, *359*, 572-577.
- [139] Zhu, W.; Wang, X.; Ma, Y.; Rao, M.; Glimm, J.; Kovach, J.S. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 14666-14671.
- [140] Jin, K.; Mao, X.O.; Cottrell, B.; Schilling, B.; Xie, L.; Row, R.H.; Sun, Y.; Peel, A.; Childs, J.; Gendeh, G.; Gibson, B.W.; Greenberg, D.A. *FASEB J.*, **2004**, *18*, 287-299.
- [141] Khawaja, X.; Xu, J.; Liang, J.J.; Barrett, J.E. *J. Neurosci. Res.*, **2004**, *75*, 451-460.
- [142] Pinet, F.; Poirier, F.; Fuchs, S.; Tharoux, P.L.; Caron, M.; Corvol, P.; Michel, J.B.; Joubert-Caron, R. *FASEB J.*, **2004**, *18*, 585-586.
- [143] Labugger, R.; McDonough, J.L.; Neverova, I.; Van Eyk, J.E. *Proteomics*, **2002**, *2*, 673-678.
- [144] He, Q.Y.; Lau, G.K.; Zhou, Y.; Yuen, S.T.; Lin, M.C.; Kung, H.F.; Chiu, J.F. *Proteomics*, **2003**, *3*, 666-674.
- [145] Takashima, M.; Kuramitsu, Y.; Yokoyama, Y.; Iizuka, N.; Toda, T.; Sakaida, I.; Okita, K.; Oka, M.; Nakamura, K. *Proteomics*, **2003**, *3*, 2487-2493.
- [146] Xiang, Y.; Sekine, T.; Nakamura, H.; Imajoh-Ohmi, S.; Fukuda, H.; Nishioka, K.; Kato, T. *Arthritis Rheum.*, **2004**, *50*, 1511-1521.

Received: August 20, 2004

Accepted: October 16, 2004