

Toxicoproteomics: Current Trends in Analytical Techniques

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Abstract

Toxicoproteomics is one most recent field which is unambiguously positioned to elaborate the better understanding of protein expression during environmental disease and toxicity, genetic factor, toxic mechanism and mode of action in response to acute long-run advancement of disease caused or influenced by these types of exposure for public health. This is one of the challenging fields due to the sheer size of the proteome and the massive data that are generated by it. Therefore, an immense improvement of methodologies needs to be applied in toxicoproteomics studies to mount the way for coming a new phase in the area of toxicology research.

Keywords: Toxicoproteomics; Pathogen; Proteome; Antigen; Antibody; Epitopes

Introduction

Toxicoproteomics is the use of proteomic technologies to better understand environmental and genetic factors, toxic mechanisms and modes of action in response to acute exposure to toxicants and in the long-term development of diseases caused or influenced by these exposures. Use of toxicoproteomic technologies to identify key biochemical pathways, mechanisms and biomarkers of exposure and toxicity will decrease the uncertainties that are associated with human health risk assessments. This review provides a comprehensive overview of toxicoproteomics from human health risk assessment perspectives. Key toxicoproteomic technologies such as 2D gel-based proteomic technologies and toxicoproteomic approaches are described, and present examples of applications of these technologies and methodologies in the risk assessment context are presented. The discussion includes a focus on challenges and future directions. It pointed out directions and provided solutions for risk assessment-oriented toxicoproteomic research. It could also serve as a guideline for EPA's proteomic research. So, this review article will be an excellent addition to the literatures in both proteomics and human health risk assessment. The uncovering of the cellular and biochemical mechanism with respect to the response to xenobiotic or toxin exposure in the toxicology is possible via employing the full-strength of genomics and proteomics advanced technologies. Due to a parallel approach, it should be possible to screen for toxic effects more rapidly than with conventional methods, such as histopathology and clinical chemistry. Due to molecular changes the pathological consequences arises and at earlier time-points pathological process the detection of the toxicity could be possible. The potential long-run toxic effects can be identified from lower doses by enforcing the highly sensitive toxicoproteomics technologies. In today's world, the counting of the disease outbreak and its associated risk factor is developing day by day. The major cause is that humans are continuously exposed to the unavoidable diverse range of environmental toxicants and combinations. Toxicoproteomics, the emerging developing field and its application in toxicology research is encouraged and promoted by quantitative and qualitative proteomic technology. This subject area is majorly focalized on the proteomics studies of toxicity. Toxicity is general caused in response to toxic agent (chemical) and environmental exposures. Toxicoproteomics is one of the challenging fields, broadly due to the sheer size of the proteome and the massive data that are generated by it. In the future in the course of the expansion of the identified predictive biomarkers repertoire of the toxicants exposure via toxicoproteomics analysis will provide the critical tools in the evaluation of their safety and also help in the designing of the accurate and apprehensive measures to minimize the adverse effects [1]. The centralized

idea of this new technology are to discover the controlling lead proteins in critical biological pathways which generate the adverse drug effects, biomarkers development, and finally the prediction of toxicity based upon the knowledge of the pharmacogenomic [2-5].

Currently, the increasing popularity of proteomics application in different scientific disciplines has attracted the intense pursuit and the exuberance in the core biology, medicine and now in toxicology area. Primarily, toxicoproteomics made grow under the auspices discipline of toxicogenomics [6] and the proteomics [7], but eventually in the course of the development it got established as an individual discipline. The major defined role of toxicoproteomics are to understanding the process of expression of the alter protein to specific exposure, behavior, expression and responses of the protein in course of the disease and injury. The application of proteomics in the toxicology is a great motivating element for the identification of the biomarker and toxicity signature profiles discovery to identify and understand the environmentally induces disease [8-9]. The drawing attention towards the toxicoproteomics is majorly due to the commercial requirement of discovering the drug exposed associated markers, efficaciousness of toxicity in pharmaceutical sphere, evaluation and validation of the environmental hazards for the better protection of public health which is the major concern of the all developing country. The emerging potential field of the system biology represents its key integration of the functional genomics (transcriptomics, proteomics, interactomics and metabolomics) among the organisms. Major drivers in toxicoproteomics are the commercial need to discover markers associated with drug exposure, efficacy or toxicity in the pharmaceutical arena, and also the urgencies of environmental hazard evaluation for the protection of public health. Finally, an overarching principle among all discovery technologies is that eventual placement of protein changes within biochemical pathways and processes will result from a mechanistic understanding of larger biochemical systems and signaling networks. Systems biology which is a multidisciplinary research, has come into the picture to represent this wider integration of functional genomics disciplines such as transcriptomics, proteomics, interactomics and metabolomics among organisms [10-11].

System biology has the potential to affect several areas of biomedical science, healthcare, and engineering. The wide range of datasets which is generated from the Omics technology has upper hand important possibility of greater molecular topology comparatively than to the singular biomarker. Toxicoproteomics along with other Omics analysis has one of a major dogmas, that the specific pattern of protein alteration represent a coherent 'toxicity signature' [12-13], it could be considered with knowing the fact about the variations in the biology, experimental design or technological platforms. According to the researchers finding the idea of emerging technical standards are the major millstones for the continuity of this field and also there is no single platform is best accommodated for the toxicoproteomics study and analysis, the combinational platform will be requiring for desirable coverage of proteome. The primary goal of this technology is to translate identified protein changes into the ameliorated biomarkers and signatures of chemical toxicity [14], in the process of the denominating any protein changes the care must be practiced and observed carefully during toxicoproteomic studies as "new biomarker". 'Biomarkers' at the biochemical and molecular level nailed down to 'singular biological measures with clear reproducible evident of association with disease, health and toxicity adverse effects" but whereas, in scientific and regularity communities the challenges arise due to its wide range of its accountability in terms of use (and misuse) [15-16].

The bailiwicks and platforms of the toxicoproteomics research

In the process of the global protein analyses (proteomics) usually consist of the separation and the identification of the techniques to create a profile of the protein or differentially expressed protein. The toxicoproteomics discipline is majorly to investigate various effects such as drug, chemical, disease or environmental stressor exposure. Proteomic analysis attempts to describe various protein attributes in a global manner. The major factors which generally taken into the consideration by the researcher in order to study the activity of the toxicoproteomics are the proteins complex nature, proteome particular portion target for investigation, consolidative relationship of toxicoproteomics with other Omics technologies) the driving forces behind specific toxicoproteomics projects.

In toxicoproteomics analysis, the two major proteomics tiers are analyzed are: (1) The Tier I of proteomic analysis is basically concentrate to determine individual protein identities like fingerprint, amino acid sequence, their relative or absolute quantities and their spatial location within cell(s), tissues and biofluids of interest whereas, (2) Tier II of proteomics analysis consist of global screening of protein, its functions, protein interactions, 3D structure and specific pattern of post-translational modifications. Both proteomics tier analysis encompass the seven intrinsic attributes of proteins which play a significant role in toxicoproteomic analysis [17]. The utility of the Proteomics platform varies according their respective abilities to deliver data on all protein attributes simultaneously during one analysis. The major goal of proteomic analysis [14] are to achieve maximal proteome coverage (i.e., Tier I analysis) in each sample, high throughput complete analysis, generating an accurate quantitative protein measurement, in a timely period delivering the data and interpretable results and utilization of discovery-oriented, open platforms.

Gel-based proteomics: Two-dimensional or difference gel electrophoresis with mass spectrometry

Since 1975, the 2D gel electrophoresis system with the combination of mass spectrometry (MS) proteomics platform is widely in practice by the researcher, and this platform is commonly used to separate and relatively quantitate samples of protein [18]. In current to separate the protein by its charge is performed by the 2D gels where immobilized pH gradient (IPG) gels is used and then subsequently by mass using SDS-polyacrylamide gel electrophoresis (PAGE) of complex protein samples for effective separation. The approximately 2000 - 3000 proteins can be separated via typical IPG gel of 18 - 24 cm fitted with similar sized SDS-PAGE gel. The resultant every spot doesn't represent unique protein or gene product but often occur as post-translationally modified forms of the same protein. The most sensitive means of protein detection (nano- to microgram range) is fluorescent staining. The intensity and sensitivity of identical protein spots are than likened among treatment groups and a fold changes for each protein is calculated via particularized image analysis software once soon after the electronic alignment (registration) of stained proteins in 2D gels. In other 2D-difference gel electrophoresis (DIGE)-MS technique, the compared protein samples are labeled with either Cy2-, Cy3- and Cy5-based linkers. These labeled samples are mixed together and electrophoresed on the same gel. In the technique, the image analysis errors can be minimize and provide the advantage to accommodate to run the 3-4 samples together on the same 2d gel [19]. The combinational versatile platform standardized discovery-oriented approach is used in the toxicoproteomics study which provides the ready means of protein identification after excision of protein, enzymatic digestion and MS analysis. This platform has its own limitation about the coverage of the proteome that can be realized on 2D gels by even the most sensitive fluorescent stains [20]. The other proteomics platforms like one-dimensional gel-based, 1D-gel liquid chromatography-tandem MS (LC-MS/MS), are also effectively applied for protein separation and identification using SDS-PAGE only (i.e., mass separation) with specifically pre-processed samples alike immuno-depleted plasma [21] or cell secretomes [21]. Such pre-processing sufficiently reduces the original protein complexity to allow small amounts of sample protein (micrograms) or serum (microliters) to be resolved to near protein homogeneity in stained protein bands. Bands are enzymatically digested to obtain diagnostic peptides for protein identification after amino acid sequencing by LC-MS/MS.

Multi-dimensional, quantitative LC-MS/MS: MuDPIT, ICAT, iTRAQ and SILAC, and label-free quantitation

Multidimensional liquid chromatography (LC-LC) technique is generally used for protein identification to separate protein digests into nano to micrograms peptides by strong anion exchange charge and hydrophobicity (C18) immediately prior entry into a tandem mass spectrometer [22]. "MuDPIT" platform, "shotgun proteomics" or the multidimensional protein identification technology is a foremost illustrative of LC-MS/MS proteomics and it is only semi-quantitative technique. The advantages of this platform is that it has the vast potential to identify and detect the lower abundance protein that might not get detected in gel-based protein separations approach. This effective platform has got the great utility in the toxicoproteomics finding and discovery studies. In the LC-MS/MS approach the other different types of the variance are incorporated like isotopic labeling strategies for quantification and in-depth proteomics profiling of the protein samples. The isotope coded affinity tags (ICAT), isobaric tag for relative and absolute quantitation (iTRAQ) and stable isotope labeling with amino acids in cell culture (SILAC) are the examples of the such variance platforms of LC-MS/MS approach. In these methods

usually the isotopes used in linkers are of light or heavy forms which can perfectly binds to the functional group of the proteins such as cysteines or amino groups in lysates. The best utility of the SILAC and iTRAQ are particularly seen effective for metabolic incorporation of "light" and "heavy" forms of amino acids (i.e., 1H:2H/12C:13C/14N:15N) into cellular proteins during cell culture incubations. Although this analysis time which is taken is lengthy and slow, the coverage of the protein is greatly expanded with development of this latest multidimensional proteomic platforms. The parameters taken into consideration to achieve the goal of this approach are deliberate sample selection, dose and time selection. Shotgun proteomics approaches (e.g., 1D-LC-MS/MS) derived mass spectral data can also be used for relative or absolute protein quantitation and sample comparison without any tagging or use of stable-isotope labeling in any of several modes with including the integration of ion chromatogram intensities [23,24], spectral counting [25-28], or selected reaction monitoring (SRM) [29,30]. The comprehensive, fully automated label free technique which has its own strengths and weaknesses [31] is introduced by the Higgs, *et al.* (2005) [32] by using the data derived from LC-MS/MS analysis of proteolytic protein digests for relative protein quantification. The major advantage of this approach is its capability to identify the large mass of proteins and providing their abundance information in a statistical robust manner. The platform includes de-noising, mass and charge state estimation, chromatographic alignment, and peptide quantification via integration of extracted ion chromatograms. The development of the protein biomarkers of cisplatin resistance in human ovarian cancer is the best example where this approach has been successfully used This approach has been applied to the development of [33], and more recently, this approach is also utilized to evaluate the effect in the rat nucleus accumbens of ethanol self-administration in the posterior ventral tegmental area of the brain [34], where 1120 proteins were identified and quantified comparatively. The same technique was used to assess the toxic effect of JP-8 jet fuel exposure on rat alveolar type II epithelial cells, at sublethal levels that are occupationally relevant [35]. In this investigation, the approximately 1135 unique proteins were identified with high confidence and quantified. Post hoc bioinformatics analysis for the differentially expressed proteins indicates that the reduced cell viability of jet fuel-exposed cells represented to substantial down-regulation of proteins by turning down in translational and protein synthetic machinery for the those protein, which are usually tangled in different cell activities in all possible modes. Spectral counting method is based on the observation that the total number of detected peptides identifying a specific protein correlates strongly with the abundance of that protein for relative protein quantitation in MS-based experiments. In spite of its utilitlital approach this technique is yet to be exploited by toxicologists. It could be best fitted and applicable for those complex protein analysis which produce relatively small-scale datasets [36,37] and exploring idea of initiating the cellular immune response generated effect of lipopolysaccharide treatment [38]. The emerging application of the SRM in toxicoproteomics to detect and quantify the acrolein metabolite (3-hydroxypropyl) mercapturic acid in urine as a biomarker of cigarette-smoke-induced disease [39] and hypothesis-driven toxicoproteomics approach based on its potential utility is remarkable achievement.

SELDI: Retentate chromatography MS(RC-MS)

The other high-throughput proteomic platform method is Retentate chromatography-MS (RC-MS), which is based on the principle of the adsorptive retention usually from pico- to nanograms protein of a protein subset sample on a thin chromatographic support (i.e., hydrophobic, normal phase, weak cation exchange, strong anion exchange or immobilized metal affinity supports) and creates a laser-based mass spectrum from a chemically absorptive surface. On a thin metal chips the absorptive surfaces are placed and inserted into a (MALDI)-type mass spectrometer which is a specifically modified matrix-assisted laser desorption/ionization. The mass spectrum profile is than created by the laser rapidly from each sample on a metal chip. RC-MS is best suitable platform for discovery of disease biomarker by analysis of serum and plasma samples [40,41]. The surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-MS instrument is nowadays is the lead commercial platform where very minute quantity (i.e in microliters) of biofluids samples are required to perform the analysis relatively in rapid mode. This approach is and resolve several issue area of the proteomics discovery when rapid screening is required for hundreds or thousands of pre-clinical or clinical samples for defining drug or chemical exposure [42,43].

Protein capture arrays: Antibody arrays

Protein capture arrays is the current state-of-the- art in the arena of the proteomics tool. It is applicable for parallel array of proteins

(any mass), peptides, capture ligands or adsorptive protein surfaces [44]. The captured molecules which can be arrayed through this approach are recombinant proteins, aptamers, peptides, drug libraries but the most prevailing array is antibody arrays, which straightly separate proteins from each other by affinity binding to specific protein targets. Although, the commercially available antibody array platform have varying sensitivity based on the target proteins like cytokine/chemokine arrays, cellular function protein arrays, and cell signaling arrays but however, the antibody arrays are not currently available for any given cell type, biofluid or species. Due to limitation to sets of protein but high screening quality of this platform may contribute some application in toxicoproteomics. Toxicoproteomics Concept in Identifying Biomarkers of Toxicant Action. Toxicoproteomics is considered to be a valuable approach for identification of molecular signatures and methodical understandings of several environmental toxicants response in biological systems. There are major advancement is required in the new toxicological research for effective screening of environmental risks on complex living systems. The data obtained from the Laboratory investigation (through *in vitro*, *in vivo* and some clinical studies) affirmed that the environmental toxicant produces the extensive adverse effect on the health and causes several neurological disorders and cancer. The in-depth investigation is required to understand the network and the event takes place at the cellular level for cell to determine these toxicants and how cell put effort to maintain genomic stability and prevent carcinogenesis because the response to carcinogens/toxicants is a complex matter to be revealed. The biomarkers may play a fundamental role and links at the relevant level of exposure and enable us to elaborate our understanding of phenomenon behind their carcinogenic potential. Toxicoproteomics approach successfully demonstrated the identification of the pesticides-inducing neoplastic changes in mammalian skin system. Investigation suggest that SOD 1, calcyclin (S100A6) and calgranulin-B (S100A9) are associated with glyphosate (organophosphate herbicide) inducing tumor promoting potential and may be useful as biomarkers for tumor promotion. In mancozeb (carbamate fungicide)-induced carcinogenesis molecular mechanism study via 2-DE and MS suggest that the level of S100A6 and S100A9 was significantly up regulated in the mancozeb exposed mouse skin and found higher in mancozeb-exposed human keratinocytes, HaCaT cells. The reported protein like carbonic anhydrase 3, Hsp-27, S100A6, galectin-7, S100A9, S100A11, SOD 1 by using quantitative proteomics approach in mouse skin exposed to cypermethrin (a synthetic pyrethroid insecticide) suggest that it play a substantial roles in several cellular functions along with the oxidative stress response, proliferation, binding of calcium ions and apoptosis. The disruption in any of this cellular function leads to carcinogenesis. This confers that these proteins were confederated with initiation of cell proliferation and might be responsible for the neoplastic transformation of mouse skin preneoplastic lesions by cypermethrin [45].

The role of toxicoproteomics in assessing organ specific toxicity

The role of toxicoproteomics in assessing organ-specific toxicity can be very well defined in the toxicoproteomics field. Toxicoproteomics is basically the application of the proteomic technologies in the toxicobiology to have the better understanding of environmental and genetic factors, toxic mechanisms, modes of action in response to acute exposure to toxicants and in the long-term development of diseases caused or influenced by these exposures [46].

Toxicoproteomics studies in liver injury

The liver is considered to the main organ for biotransformation and elimination of pharmaceuticals from the body [47]. Since 1950 after the pioneering investigations about the link between toxic metabolites and chemical carcinogenesis reveals the importance of the reactive metabolites in the pathogenesis of drug-induced toxicity and has been a major focus of research area. Initially the toxicoproteomics studies attempted for drug-induced liver injury using rodent models of toxicity. For liver toxicity investigation animal models are often selected for prevalence of one phenotype such as necrosis, hepatitis, cholestasis, steatosis, fibrosis, cirrhosis or malignancy [48]. Drug-induced hepatotoxicity is the major cause of the liver injury. The drug-induced liver disease pathogenesis is usually involves due to participation of the parent drug or metabolites which directly affect the cell biochemistry or elicit an immune response. The adverse hepatic events caused by drugs are deliberated as either predictable with high incidence or unpredictable with low incidence. The predictable drug induced liver injury, such as paracetamol, usually shows the adverse effect within a few span of time and it's a resultant of direct liver toxicity of the parent drug or its metabolites [49], whereas, the unpredictable events manifest as overt or symptomatic disease and can

occur with intermediate (1 - 8 weeks) or long (1 year) periods of latency such as phenytoin [50], and isoniazid is an example of the latter [51]. It has been seen that the majority drug-induced hepatic adverse events are unpredictable due to immune-mediated hypersensitivity reactions or idiosyncratic which result in the cell death and lead to clinical manifestation of hepatitis [52,53]. hepatotoxicity has attracted considerable attention that highlight underlying susceptibility factors to drug-induced injury including age, sex, drug-drug interactions, and genetic polymorphism in metabolic pathways involved in activation or disposition of therapeutic drugs and leads the removal of several widely-prescribed drugs from the marketplace [54]. Recently, toxicogenomics study were conducted to classify evaluate the hepatotoxicants and it was found that 25 well-known model compounds or substances showing hepatotoxicity during testing [55]. Such model agents are acetaminophen, bromobenzene, carbon tetrachloride, hydrazine and others which are responsible for causing acute hepatonecrotic injury. Hepatic gene expression profiles were analyzed by support vector machines; SVMs (a supervised learning method) to generate classification rules. The SVM method was combined with recursive feature elimination to improve classification performance.

Detection of biomarkers in blood after liver injury

Blood is one of the most accessible and informative biofluids for specific organ pathology in preclinical studies. Biomarkers that can be assayed in biological fluids from preclinical species may hold relevance to human subjects [56]. The Human Proteome Organization (HUPO) is currently undertaking a comprehensive mapping of soluble human blood elements of the plasma proteome for an improved understanding of disease and toxicity [57]. Results from an international survey of soluble human blood proteins by chromatographic and electrophoretic separation have revealed several thousand resolvable proteins for which MS has provided evidence for over 1000 unique protein identifications [58,59]. Researchers are also mapping the mouse [60] and rat [61] serum and plasma proteomes for use in preclinical and experimental studies. An excellent review has been published for 2D gel mapping of rat serum and rat tissue proteomic studies [61]. The sensitivity of 2D gel proteomic approaches to detect and measure alterations in the mouse or rat plasma proteomes has only recently been tested by various labs and examined changes in the mouse plasma proteome focusing upon inflammation after cutaneous burn injury with superimposed *Pseudomonas aeruginosa* infection [62] and in another study 2D-MS proteomics approach is utilize to note the changes and the great changes were noted for haptoglobin and hemopexin [63]. 1D-Gel LC-MS/MS analysis is used to study the comparative plasma proteome upon a few microliters of plasma from lymphoma-bearing SJL mice experiencing systemic inflammation [64].

In toxicoproteomics study of liver injury the application of serum or plasma protein profiling is still at the initial.

Toxicoproteomic studies in kidney injury

Kidney is a primary organ for preclinical assessment in pharmaceutical development since its metabolic and excretory functions often render it susceptible to drug-induced toxicity [65]. The major organ for filtration, reabsorption and secretion to maintain homeostasis of water-soluble salts and small molecules is kidney and it has got a considerable capability of biotransformation of drugs and xenobiotics. Renal damage can be due to several different mechanisms affecting different segments of the nephron, renal microvasculature or interstitium. The nature of renal injury may be acute and recoverable. The ability to perform kidney transplants and other organ replacements have saved many lives but relies on immunosuppressive drug treatment to prevent organ rejection. However, over time even the immunosuppressive drugs can also cause the risk of renal toxicity. The major goal for toxicoproteomic and toxicogenomic technologies is to develop a new nephrotoxic marker amenable for multiple preclinical models and high-throughput screening [65-67].

Computational Toxicoproteomics

The protein posttranslational modifications (PTMs) are found in rich quantity in the toxicoproteomic samples the significant performance penalties obtained for in the course of PTMs identification through standard database searching. There are number of the algorithms which have been used in the toxicoproteomics analysis for PTMs. The small number of the known modification with the provided

list of the known masses and sequence specification can be search via application of the 'Sequest algorithm' [68], The 'Mascot error-tolerant approach is used to searches for a global known PTMs list automatically [69]. In spite of having the effective algorithms the database searches become unsuccessful to identify the massive quantity of tandem mass spectra (MS/MS) because of the unexpected chemical and posttranslational modifications in toxicoproteomics samples. In toxicoproteomics data sets, the unanticipated (blind) mass shifts search will expose vast range of the unidentified missed modification via standard database search in near future. The several advanced approaches of informatics has been formulated to detect the blind modification from clinical samples such as MS/MS de novo sequencing and the OpenSea alignment algorithm [70], unrestrictive PTM search algorithm and MS-Alignment approach [71], an empirically derived fragmentation model [72], Popitam (a new algorithm dedicated to automated protein identification from tandem mass spectrometry (MS/MS) data by searching a peptide sequence database [73], ModifiComb method for mapping hundreds types of PTMs at a time, including novel and unexpected PTMs [74], an Error-tolerant Algorithm Based on an Extended Sequence Tag Approach [75], a powerful and convenient web server 'MODi' for identifying multiple peptide PTMS from tandem mass spectra [76], Sequential Interval Motif Search approach [77], a Spectral Clustering Approach to MS/MS Identification of Post-Translational Modifications [78], Lookup Peaks (A Hybrid of de Novo Sequencing and Database Search for Protein Identification by Tandem Mass Spectrometry) [79]. The contemporary de novo peptide sequencers 'PepNovo' [80] miss to interpret large portions of identifiable spectra. The MS-alignment [71] method, applied by the InsPecT [81] software, acquaints an arbitrary mass shifts in peptide database while matching its predicted spectrum to an MS/MS. Recently, the emerging sensitive method is partial sequence tagging which is employed for mutations and PTMs detections. The detection of unanticipated modifications is enabled by the 'The GutenTag' software which is an automated inference of sequence tags from MS/MS [72]. For highly accurate tag inference, the Tabb laboratory introduced 'DirecTag' software (An accurate sequence tags from peptide MS/MS through statistical scoring) [82] whereas, TagRecon software was introduced for high-throughput mutation identification through sequence tagging [83]. The spectral clustering method, exemplified by the Bonanza software to detect the unanticipated PTMs by examining the mass shift differences between unmodified peptide identifications and unidentified spectra [78] whereas the "fraglet" method, exemplified by the ByOnic software [79], which matches peptides database to the based on fragment peaks matching MS/MS without matching precursor masses and the differences of masses between the candidate matches is interpreted as a modification. Despite of advancement in the potential detective computational toxicoproteomics techniques, the recognition of protein unforeseen modifications and blind- PTM search persists as an exotic conception for biologists. TagRecon is capable of uncover the large number of known PTMs and unexpected PTMs, and is one of the sections of an integrated bioinformatics pipeline holding a flexible protein assembler, a superior performance search engine database and a user-friendliness PTM results reviewer [84].

Summary and Future Prospects

Toxicoproteomics using proteomic pattern technology can have important direct applications within the pipeline of the drug development and as well as potentially powerful bedside applications. The incorporation of high throughput screening of conditioned media, body fluids from animals into hit-to-lead screening, lead screening and preclinical validation may be possible. In future, we can envision in which specific plasma mass, serum and urine spectral proteomics of variety of major organ toxicities such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and reprotoxicity, are used to rapidly screen against experimental compounds either for toxic liability or for protective intervention efficacy. In future this proteomics approach analysis is extensively used by the researchers and the pathologist at every major stages of the disease management. This approach can be utilized in different types of the tissue and organ for protein profiling studies of adverse effects of therapeutics. Proteomic approaches are now revealing a new blood serum and tissue biomarkers in animal models of human neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis [85,86]. Proteomic in depth investigation has also been conducted and observed in cardiotoxicity models with doxorubicin [87] and renin-angiotensin models of hypertension [88]. In the arena of pharmaceutical development, the expectations envision of Omics technologies are very extensive. The toxicology application and its settings are still being explored to match platform sensitivity for differential protein expression with preclinical biological samples. Future trends in toxicoproteomics studies will see developments in several areas where special attributes of proteins can be exploited by proteomics in preclinical assessment. This approach can be continued to be applied in the preclinical test-

ing of the drugs and their high throughput investigation of their adverse toxicity effect. This review covers recent studies that have used simultaneous measurement of multiple protein markers for prediction of tissue toxicities. In addition, conventional and newer proteomic techniques have been evaluated for applicability to drug toxicology studies. The potential of toxicoproteomics to predict adverse drug reactions in clinical use have also been explored. The major concern area of this toxicoproteomics studies are required great attention. Firstly, the major refinements in MS/MS approach with intimately integrated multi-dimensional separation schemes will continue to dominate proteomic analysis for identification and quantification. MS instruments and software will become more user-friendly and accessible, such as the recently introduced orbitrap MS/MS instruments. Second, "reduction of sample complexity" or any pre-purification strategy prior to toxicoproteomics analysis will be very useful upon innovative application to appropriate biological samples and problem areas (i.e., immunodepletion of albumin, immunoglobulins in plasma) or research problem areas (i.e., phosphoprotein enrichment in protein signaling). Third, Tier II proteomics will begin to be applied to toxicoproteomics problem areas such as global and targeted protein phosphorylation [89-91] and chemoproteomics [92] using pharmaceuticals or enzyme substrates like ATP [93] as mass capture-ligands for proteins. Fourth, toxicoproteomics is readily positioned to exploit accessible biofluids (i.e., serum/plasma, urine and cerebral spinal fluid) for biomarker development [94] and could be combined with transcriptomic analysis of blood leukocytes for a parallel approach in biomarker discovery [95]. Fifth, the astute use of genetically altered animals and cell models will enhance discovery of protein targets and mechanistic insights into adverse drug reactions. Finally, continued efforts for integration of proteomics, transcriptomics and toxicology data to derive mechanistic insight and biomarkers will be a continuing goal to maximize return on the investment in Omics technologies [96,97]. In 2007 by EPA, the ToxCast computational toxicology research program was launched to develop a cost-effective approach for efficiently prioritizing the toxicity testing of thousands of chemicals and the application of this information to assessing human toxicology. This was a part of the federal Tox21 consortium [98]. Predictive toxicology plays a critical role in reducing the failure rate of new drugs in pharmaceutical research and development. The modern computational toxicologist is more productively engaged in understanding the gaps and driving investigative toxicology towards addressing them [99].

Challenges for toxicoproteomics in preclinical risk assessment are: use as a discovery tool for specific proteins affected by drug and toxicant action; better understanding of biochemistry and cell biology; and biomarker development. The discipline of proteome mapping will be a different and more complex enterprise from the high-throughput, linear-sequencing activities that have been so useful in mapping of the human genome. While the immensity of mapping and measuring the attributes in any one proteome is a large undertaking, biofluid proteomes such as serum/plasma, urine and cerebrospinal fluid hold the most immediate promise for preclinical assessment in terms of better biomarkers. Although there are many challenges for toxicoproteomics in preclinical assessment, the opportunities are also close at hand for a greater understanding of toxicant action, the linkage to accompanying dysfunction and pathology, and the development of predictive biomarkers and signatures of toxicity.

Conclusions

The expected advanced development in the field of the toxicoproteomics will facilitate the major outbreaks in the pharmaceutical and industrial lead compound by recognizing, much earlier than is recent possible, which have the propensity to cause human toxicity and simultaneously, to predict the target population for either the pharmacological or toxicological effects. In the current genetic medicine and personalized medicine era the therapeutic strategies will be tailored to the requirement of the individuals with known genome sequence variations is likely to emerge in the upcoming future. The development of the biomarker have the potential for monitoring subtle gene expression changes and this in near future may facilitate the opportunity to screen ongoing changes in accessible human tissues (blood, urine, plasma etc). This will not only going to be beneficial for the environmental, occupational health practitioner and biologist to able to identify the toxic compound and its mechanisms at the cellular level but it is also expected that the advancement in the proteomics ,genomic and technological level may propel medical advancement and facilitate the opportunities to intervene during disease progression and development of new era of the drug designing and development. The past decade was the witnessed in the major significant development in the study of mass spectrometry based proteomics with bioinformatics synergy meet the biological discovery programs

expectation. For mining the proteome in the context of discovery program aimed to identify the minute-level protein identification and expression detection from human tissues from the rare available sample normally in microgram quantity. Currently, in order to deal with the multi-target drug designing and polypharmacology study, the virtual screening approaches are broadening. In human disease sample and corresponding animal models may lead an avenue to identify precisely the target gene, protein with a high pathogenetic relevance by introducing data mining techniques. Proteomics analysis to evaluate the differentially expressed protein and proteins pattern can provide the comprehensive understanding of target and its mechanism at molecular level.

Conflict of Interest

None.

Glossary

AMT: Accurate Mass Tags obtained from highly accurate mass spectrometry platforms.

AMU: Atomic Mass Units.

Antibody array: An orderly spotting (microarray) of antibodies on a glass slide (chip) or membrane (macroarray) for selective affinity binding to proteins in a sample. Each antibody should bind to a unique sequence on each protein. Detection of proteins bound to antibodies may involve fluorescence or chemiluminescence.

Biofluids: fluids or secretions accessible for proteomic analysis (i.e., serum, urine, cerebral spinal fluid).

Biomarker: usually a singular measure of a protein, enzyme activity, or a small molecule in a biological sample that is associated with health, toxicity or disease.

cDNA microarray: an orderly spotting (microarray) of cDNA fragments (i.e., 500 to 1,000 bp) on a glass slide (chip) to which an extracted mRNA sample (i.e., fluorescent tagged for detection) is hybridized to detect the presence of a corresponding transcript in the sample. Each cDNA should correspond to a unique sequence on each gene.

CE: capillary electrophoresis.

DNA microarray platform: the combination of technologies for to measure the transcriptome from a biological sample. The 2 primary platforms are cDNA microarrays and oligonucleotide microarrays.

DIGE: Differential Gel Electrophoresis; 2D-DIGE-MS is a variation on traditional 2D-MS in using a dual fluorescent labeling method for control and test sample proteins prior to 2D gel electrophoresis. DIGE eliminates the need for registration and comparison of different 2D gels since scanning is performed for each dye at different wavelengths on the same gel.

Dynamic range: the difference in high and low abundance proteins in a biological sample. For example, serum has a high dynamic range for proteomic analysis.

ESI: Electrospray Ionization; An ionization source in a mass spectrometer that creates ionized peptides (or proteins) in the gas phase from a fine liquid spray of microdroplets in a high voltage field just prior to their introduction into a tandem mass spectrometer.

EST: Expressed Sequence Tag.

FT-ICR: Fourier Transform-Ion Cyclotron Resonance mass spectrometry; a type of mass spectrometer capable of extremely high mass accuracy and ion resolution.

Genome: the entire set of genes encoded by the DNA of an organism.

Homolog: a gene related to a second gene by descent from a common ancestral DNA sequence.

ICAT: Isotope Coded Affinity Tags; a type of proteomic platform that uses different radiolabeled linker reagents to tag proteins and quantitatively measure and identify them from biological samples in a comparative manner.

Immunosubtraction: a fractionation method usually performed upon serum to remove noninformative, abundant proteins (i.e., removal of albumin from serum) by immunoaffinity

column chromatography to assist proteomics platforms in finding more desirable, low abundance proteins from sample.

Ion trap mass spectrometry: peptide ions with a desired range of m/z are sorted and then separated and presented to a detector. This

mass spectrometer is capable of generating single mass spectra or tandem mass spectra, but it is a low resolution instrument compared to other MS/MS instruments.

Ionization source: a device that creates gaseous ions from a dried or liquid sample. Examples are ESI or MALDI.

IPG: immobilized pH gradient; refers to new type of isoelectric focusing gel that is used to separate proteins by charge with isoelectric focusing that is the first step in 2D gel electrophoresis.

Knowledgebase: a computer-automated system that can derive new knowledge from -Omic data content.

LC: liquid chromatography (i.e., HPLC or nanoflow LC)

MALDI-Tof: matrix-assisted laser desorption ionization time of flight; a mass spectrometer platform that creates gas-phase ions from dried sample peptides (or proteins) by mixing of a sample with a crystalline matrix. The matrix has an absorption wavelength that closely matches that of the MALDI-Tof instrument's laser. A laser pulse acts as the ionization source to excite the sample/matrix to ionize peptides which enter the time-of-flight mass analyzer as singly charged ions. MALDI-Tof creates a peptide mass fingerprint to identify proteins.

Mass analyzer: a device that separates a mixture of ions by the mass to charge ratios. Examples are Tof, triple quadrupole, ion trap and FT-ICR. Downloaded from tpx.sagepub.com by guest on May 5, 2016 Vol. 32, No. 6, 2004 TOXICOPROTEOMICS 641

Mass spectrometer: an instrument used to identify proteins in proteomics that consists of three integrated devices including an ionization source, a mass analyzer and a detector. Examples are MALDI-Tof; ESI-MS/MS; FT-ICR and Tof/Tof.

Metabolomics: the comprehensive and quantitative study of intermediary metabolites, hormones, and small bioorganic molecules.

MS/MS: 2 mass analyzers coupled together to form a tandem mass spectrometer. These mass spectrometers are capable of deducing a peptide's amino acid sequence (sequence tag) by interpreting the m/z pattern second mass spectrum pattern. An ESI ion source is often coupled with a tandem mass spectrometer (i.e., ESI-MS/MS).

m/z: mass-to-charge ratio; mass spectrometers measure the m/z ratios of peptide (or protein) ions. A peptide ion is typically formed following ionization of the molecule via the addition of 1 or more protons.

MudPIT: Multidimensional protein identification technology; a type of proteomic platform designed to separate proteins by 2-dimensional liquid chromatography just prior to tandem mass spectrometry to identify peptides and proteins.

Noncoding regions: noncoding RNA genes that are not transcribed into proteins but are involved in control of gene expression and protein synthesis.

OligoDNA microarray: an orderly spotting (microarray) of oligonucleotides (i.e., 60 to 70 bp) on a glass slide (chip) to which an mRNA sample (fluorescently tagged for detection) is hybridized to detect a corresponding transcript in the sample. Each oligonucleotide sequence should correspond to a unique sequence on each gene.

On-line separation: the direct coupling of separation techniques like liquid chromatography or capillary electrophoresis with the mass spectrometer.

ORFeome: ORFs or open-reading frames are the protein coding sequences or exons within a genome. The ORFeome is the entire set of ORFs within a given organism.

Ortholog: orthologs are genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

Paralog: paralogs are genes related by duplication within a genome. Orthologs retain the same function in the course of evolution, whereas paralogs evolve new functions, even if these are related to the original one.

Peptide mass fingerprinting: a characteristic set of protein masses from an enzyme digest that match the predicted digest pattern of a unique protein that can be found by searching protein databases. Fingerprinting is often performed by MALDI-Tof.

Peptide sequence tag: an amino acid sequence deduced by tandem mass spectrometry that can be used for protein identification by searching protein or gene databases for matches.

Protein attributes: biophysical features of proteins that influence separation, analysis and identification in proteomics (i.e., primary sequence, charge, posttranslational modification, 3D structure, protein-protein interactions, cellular location).

Proteomic platform: combination of components or technologies for global protein analysis. Examples are 2D-MS, ICAT, MudPIT, antibody arrays and RC-MS.

Proteomics: technologies for global and quantitative measurement of all proteins and their isoforms in a biological sample.

PTM: posttranslational modification. Common PTMs are phosphorylation, glycosylation, and ubiquitination.

RC-MS: retentate chromatography mass spectrometry; a proteomic platform consisting of a chromatographic surface analyzed by a MALDI mass spectrometer. SELDI is the most common commercial platform for RC-MS. The data from RC-MS is usually a mass spectrum of native protein masses that can form a characteristic protein profile data output to distinguish health, toxicity and disease from biological samples.

Reverse-phase protein arrays: Protein samples to be identified are immobilized on glass slides that is the reverse of what is normally done when arraying the capturing reagents (i.e., antibodies) on slides. This permits large scale arrays of many different types of patient samples or treatment samples for parallel sample analysis by subsequent probing with a single antibody or other appropriate probe.

SELDI-MS: surface-enhanced laser desorption ionization mass spectrometry; a commercial RC-MS platform.

Signature profile: a set of up- and down-regulated transcripts (or proteins) that can distinguish between health, disease or toxicity in a biological sample.

SNP: single nucleotide polymorphism.

Splice variant: Some genes have different transcriptional start sites with the result that multiple transcripts (and proteins) are possible from a single gene.

Subproteome: a portion of a specific proteome. Organelles are subproteomes of the cellular proteome.

Systems biology: the study of a biological system (or organism) by the systematic and quantitative analysis of all of the components that constitute the system.

Systems toxicology: the application of systems biology to toxicology to understand and predict how biological systems (or organism) are perturbed by toxicant exposure.

Tandem mass spectrometry: the configuration of two mass analyzers (MS/MS) in a mass spectrometer.

Toxicogenomics: the application of gene expression technologies (transcriptomics, proteomics and metabolomics) to toxicology that is analyzed and interpreted by bioinformatics.

Toxicoproteomics: seeks to identify critical proteins and pathways in biological systems that are affected by and respond to adverse chemical and environmental exposures using global protein expression technologies.

Two dimensional gel electrophoresis-MS (2D-MS): proteomic platform consisting of two dimensional gel electrophoresis that separates proteins by charge and mass and then allows for protein identification by mass spectrometry. Separated proteins on multiple 2D gels are compared for quantitative differences to determine differential protein expression.

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