LECTURE-38

Proteomics for Translational Research

Handout

PREAMBLE

Proteomics is growing rapidly as a promising platform to translate the knowledge obtained from basic research into diverse clinical applications. Proteomics in clinical research has successfully accelerated identification of potential diagnostic and prognostic biomarkers, and novel drug and vaccine targets for improvement of diagnostics and therapeutics (Hanash 2003; Petricoin and Liotta 2003). Although major focus of proteomics research has focused on oncology; different potential biomarkers for infectious diseases, autoimmune diseases and cardiovascular diseases have also been identified and potential drug/ vaccine candidates have been evaluated (Azad et al., 2006). Apart from the clinical fields, proteomics research has also been implemented in industrial applications; including crop improvement, sanitation assessment, allergens, toxins & food-borne pathogen detection (Agrawal et al., 2012) (Table 1). In this lecture, we will discuss proteomics for translational research in clinical fields; and prospects and challenges associated with translation of proteomics knowledge in clinical application.

OUTLINE OF LECTURE

I. Translation of proteomics in clinical research; prospects and challenges

II. Proteomics in diagnostics: biomarker discovery

III. Proteomics in therapeutics

(a) Identification of drug and vaccine targets

(b) Screening of potential drug molecules

IV. Personalized proteomics to precision medicine/ therapy

V. Conclusions

I. TRANSLATION OF PROTEOMICS IN CLINICAL RESEARCH: PROSPECTS AND CHALLENGES

Different emerging proteomics techniques including gel-based profiling, quantitative mass-spectrometry, and array-based high-throughput proteomics have shown considerable potential in differential proteomic expression analysis for identification of potential biomarkers with promising diagnostic and prognostic implications (Ray et al., 2011a; Paulo et al., 2012). Different nanoproteomics technologies are found to be very efficient in targeted approach for selective detection of very low-abundance target protein markers present in complex biological fluids (Ray et al., 2011b). Additionally, recent studies have suggested the potential of label-free proteomics for screening of probable drug molecules. Hence, translation of proteomics research in clinical fields encompasses (Fig 1):

1. Identification of novel diagnostic and prognostic marker proteins for cancer and other human diseases
2. Ultra-sensitive detection of marker proteins in biological fluids in targeted approach
3. Identification of novel therapeutic targets (drugs and vaccines)
4. Identification of new drug molecules and elucidation of mechanism of action of known drugs (Fig 2)
5. Individualized health care and personalized proteomics for medicine/therapy

In spite of extreme potential of different proteomics techniques, translation of knowledge obtained from basic proteomics research into direct clinical applications is challenging,

indicating huge gap between the “bench-side’ research and “bed-side’ implications. Due to dynamic nature of proteins and complexity of proteome as well as extreme variations among individuals, often it become difficult to translate the findings obtained under lab environments effectively in actual applications.

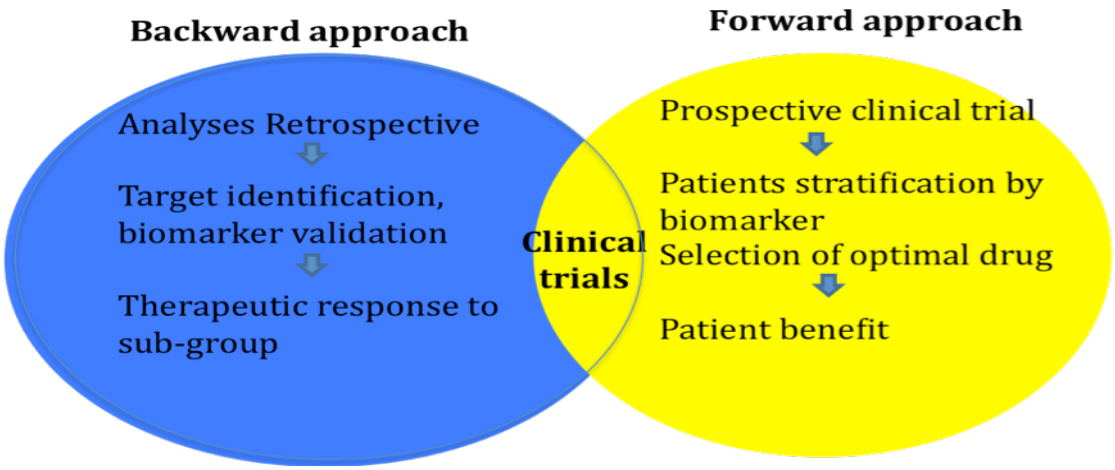

Fig 1. Identification of potential biomarkers by two different approaches - prospective and retrospective.

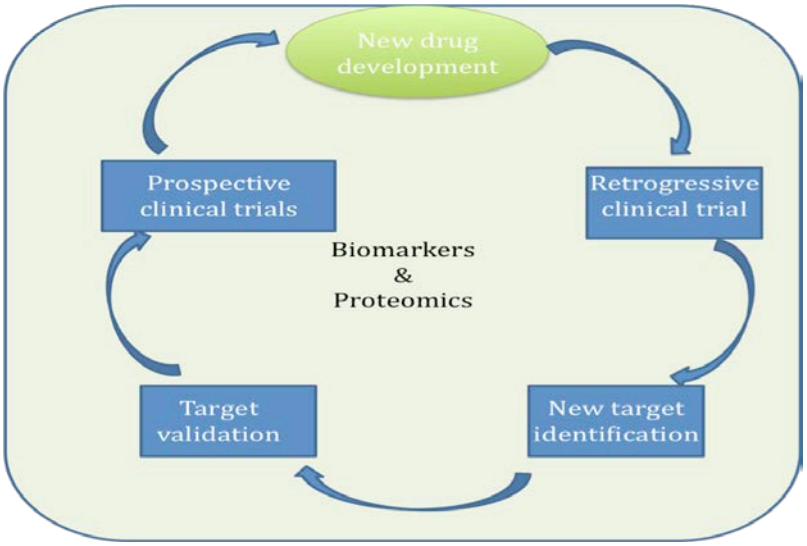

Fig 2. Depiction of process of drug development and decision making by utilizing proteomics.

These are following basic limitations associated with translational proteomic research:

1. Field is developing and quite a few approaches are still in proof-of-principle level; lack of standard protocols
2. There is no protein amplification method (like PCR used for gene amplification)
3. Fragile character of proteins and difficulties in extraction and isolation of proteins from specific organelles
4. Presence of various isoforms of a single protein
5. Huge variation in proteome with time within a same individual
6. Variations among the individuals of same or different populations; difficult to establish gold-standard biomarkers applicable for all populations
7. Broad dynamic range of protein concentrations and complexity of biological fluids

II. PROTEOMICS IN DIAGNOSTICS: BIOMARKER DISCOVERY

Biomarkers are indicator biomolecules that assist to detect diseased conditions at an early stage, make discrimination between different diseases, and useful for monitoring progression/severity of disease. Proteomics is a useful platform for comprehensive analysis of protein expression levels in control and diseased conditions and can indicate the alterations in host proteome due to the external infections or unhealthy conditions. Therefore, over the last decade different researchers have adopted diverse proteome profiling techniques for identification of marker proteins in biological samples; including serum/plasma, urine, saliva, tissue, CSF etc. and reported a plethora of potential proteins that can serve as classifier molecules for accurate discrimination of disease states from healthy normal; as well as can differentiate among multiple diseases (Anderson 2010; Drake et al., 2005; Pisitkun et al., 2006; Hu et al., 2006). Gel-based proteomics technologies, particularly, classical two-dimensional electrophoresis and 2D-DIGE have been massively applied for differential proteome profiling of diseased and normal samples. In recent years, quantitative MS techniques including iTRAQ, ICAT, MRM and label-free MS approaches, and microarray-based approaches, particularly reverse phase protein microarrays gained popularity for identification of protein markers for human diseases.

Diagnostic markers help to detect disease at an early stage of development; while prognostic biomarkers assist to discriminate among diseased states and help to differentiate severe and non-severe forms of diseases (Lee et al., 2011). In proteomics analysis work-flow for biomarker discovery, generally in initial discovery phase small/moderate sized populations are screened for identification of potential candidates,

while in validation phase, identified markers are tested using bigger clinical cohorts (Fig 3).

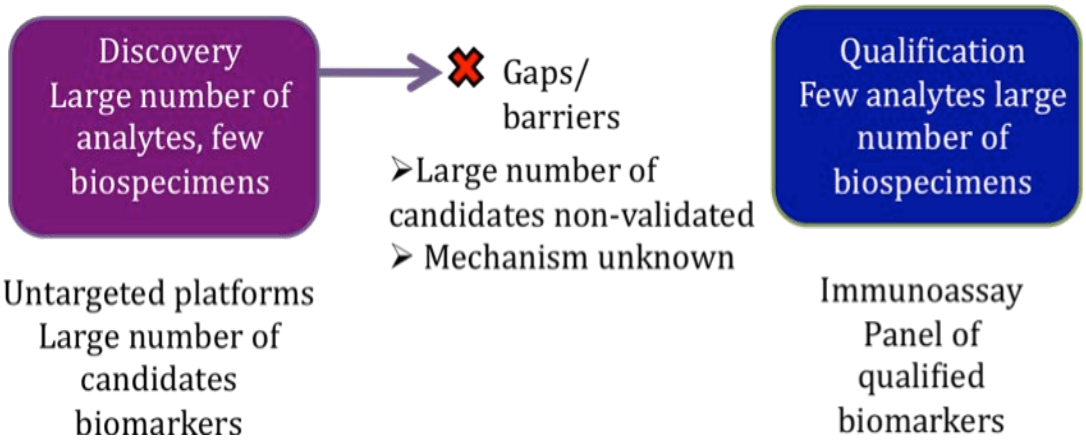

Fig 3. Representation of typical proteomics research pipeline from discovery to qualification.

Once the possible candidates with ability of discrimination between controls and diseased states are identified; specificity, sensitivity and accuracy of the marker proteins are evaluated in multiple levels of trials. Often combination of a panel of marker proteins is required rather than a single marker for accurate detection of any complex disease/disorder, which has similar/overlapping manifestations with other diseases (Fig 4). According to a 2008 report of US Food and Drug Administration (FDA), protein-based assays for 109 unique protein targets in plasma or serum have been approved for further analysis (Anderson 2010). Among the different approved markers vascular endothelial growth factor (VEGF), haptoglobin, β-2 microglobulin, α-fetoprotein, carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125) are the most promising candidates for cancer diagnosis (Anderson 2010). Although, different promising

proteomics techniques have reported potential biomarkers for early detection of cancers and other different types of infectious and non-infectious diseases, but unfortunately, majority of them has not translated in practical clinical application. The foremost impediments for establishment of gold-standard biomarkers are associated with extreme biological variations and non-specificity of the identified marker for any definite diseases.

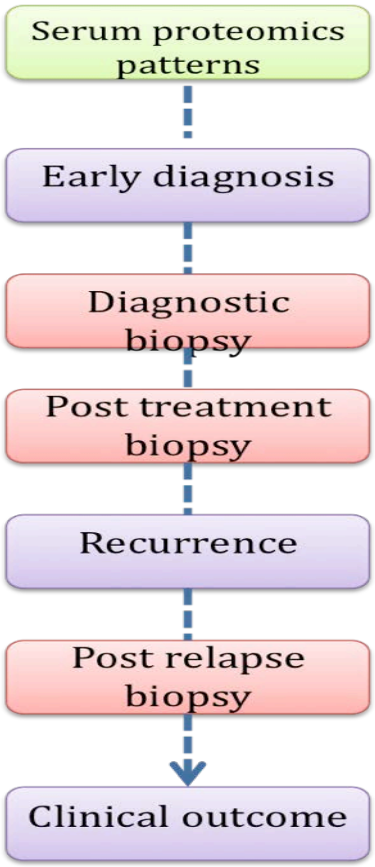

Fig 4. Flow chart shows utility of clinical proteomics

III. PROTEOMICS IN THERAPEUTICS

Proteomics deals with the study of entire proteome present in the specific cells/tissues at a given time period. Proteins play major role in various metabolic processes. Alterations in the protein levels/activity may disrupt the normal physiological conditions in the organisms and results in disease (Cooper et al., 2004). By using proteomics approach we can identify changes taking place in various proteins and metabolic pathways in the organism under the pathological conditions. By targeting the responsible agent for the disease, we can cure the disease. With latest developments in the field of proteomics, investments have been increased in this field for the identification of new drug targets and the development of vaccines prior to onset of the disease.

(a) Identification of drug and vaccine targets

Nowadays proteomics is widely used in the identification of drug targets in various cancers and infectious diseases (Cooper et al., 2004, Zwick et al., 2002). In case of cancers various kinases gets activated which are involved in increased proliferation of cells. These kinases are proteins of interest for the cancer drug development. Some of the drugs, which are targeted against the kinases are under the evaluation stage prior to their use in the clinical application (Traxler et al., 2001). In case of malaria (*falciparum* malaria) cysteine proteases are good targets for the drug development as they are involved in various metabolic pathways in the malarial parasite (Lee et al., 2003).

In conventional drug development methods, the drugs are developed that act at the up-stream levels, which results in the inhibition of the up-stream product resulting in the non-formation of down-stream products. However, this kind of approach often leads

to the side effects (toxic effects) as the required inhibitor/ drug concentration is high. If we are able to develop drugs against a few target proteins involved in a metabolic process responsible for particular disease, which will be better than targeting a single protein. In multi-protein targeting, the required amount of a drug will be minimum, which reduces the toxic side effects by preventing the accumulation of a particular drug (Elnaggar et al., 2012). Proteomics helps in the identification and characterization of various proteins involved in the metabolism based on which drugs for various proteins in the metabolic pathway could be developed.

Expression of proteins (up-regulation or down-regulation) in various pathological conditions can be studied by using various proteomic techniques. Up-regulation of the proteins associated with a metabolic pathway may be associated with the disease condition. In such conditions the up-regulated proteins could be targeted, which may be potential targets for curing the disease. Such kinds of strategies are under study for the discovery of drugs, for example bevacizumab (monoclonal antibody to VEGF) along with sorafenib, a RAF kinase inhibitor are used for the treatment of cancers. Bevacizumab (monoclonal antibody to VEGF) sequesters the VEGF molecule and decreases its availability for their binding on to the VEGFR. On the other hand sorafenib inhibits the activity of raf kinase, which is involved in the angiogenesis and activated in response to VEGF. Thus inhibiting the VEGF signaling at two different points results in the reduction of angiogenesis. This treatment showed good results on the ovarian cancer patients (Azad et al., 2006). The same strategy is being used in various cancer diseases for the drug development. Quantitative proteomic techniques (iTRAQ, SELDI-

TOF, ICAT etc.) made it possible to identify the proteomic alterations in large number of patients.

The cell surface proteins of the parasites (in case of infectious diseases) and the cancer cells (undergone alteration because of mutations) will be better targets for the development of antibody based drugs as they are unique, the drugs developed against them acts effectively and specifically on the parasite/ cancer cells. However, such kinds of drugs are not effective in case of intracellular parasites.

Vaccines are used for the prevention of the diseases. Proteomics plays a major role in the identification of proper target molecules for the development of vaccines. In case of infectious diseases the vaccines development will be based on the identification of the cell surface proteins of the pathogen and the specific protein expression at the time of infection (Walters et al., 2010). Servin et al., studied the cell surface antigens to develop vaccines against *S. pyogenes.* Cell surface antigens play major role in the bacterial interaction with the host system and they are available for the host immune system to interact with and generate memory against the parasite (Servin et al. 2007). Proteomics-based approaches have been followed for the development of vaccines against various pathogens like *Staphylococcus aureus, Schistosomiasis mansoni, Francisella tularensis* etc (Glowalla et al., 2009; Wilson et al., 2004; Eyles et al., 2007).

(b) Screening of potential drug molecules

With the advances in field of proteomics, now it is possible to screen large number of drug molecules in a short time by using various labeled/label free proteomic techniques. Proteomic techniques like affinity chromatography, mass spectrometry, microarrays, surface plasmon resonance (SPR) etc. revolutionized the drug discovery by high throughput screening of the drug targets. In affinity chromatography method of screening, the drug molecules which are assumed to bind with some of the proteins from the cell lystes are immobilized onto a column and the protein sample/cell lyste is allowed to pass over the immobilized ligand. After passing the protein sample the column is washed with wash buffers to remove the unbound proteins. Then the proteins that are bound to ligand (drug) molecule are eluted out by altering the pH of the buffer (elution buffer) followed by their separation on SDS-PAGE. The protein bands observed on the polyacrylamide gel is subjected to mass spectrometry for the identification of the protein molecules, which are interacting with the drug molecule (Sleno et al., 2008).

Reverse-phase microarrays & tissue microarrays are used to screen proteomic alterations specific to a disease in large number of patients. In this method the patient's tumor tissue lysates (in case of cancer) and the normal tissue lysates are spotted on the nitrocellulose slide separately. Antibody against the target protein is applied on to the slides and the expression levels are detected by using a secondary antibody tagged with an enzyme or a fluorescent molecule. The drug molecules can be developed and targeted against these target proteins. The drug molecules, which bind only to the tumor tissue lysates, could be further screened for their utility in the clinical applications. Whole body arrays contain the tissue lysates from the various tissues of the body, which

are useful for the identification of the probable drug cross-reactive sites from various parts of the body (Petricoin et al., 2002).

Label-free technique SPR is used to study various protein-ligand interactions, which are weak. SPR uses the total internal reflection as its principle for the identification of molecular interactions. The proteins of interest or the tissue lysates from the cancer cells/ diseased tissue are immobilized on to a thin glass plate coated with a metallic film (in generally gold coating). Then the target drug is allowed to pass over the tissue lysate or specific proteins on the SPR chip. If the proteins on the chip bind to the drug molecules, it results in the increase in mass at that point on the chip, which can be detected by the changes in the refractive index of the medium. Large numbers of drug molecule screens have been performed using such technology (Jönsson et al., 1991).

Table1a. List of potential drug targets identified in various diseases by using proteomic approaches

Disease	Potential drug target protein	Proteomic techniques	Reference
Prostate cancer	Phosphorylated Akt	Reverse-phase arrays	Paweletz et al. 2001
Falciparum malaria	Plasmepsins, plasmepsins-like enzymes	2DE, MS	Cooper et al. 2004
Sepsis-induced acute renal failure	Merpin-1-α	2D-DIGE, MALDI-MS	Holly et al. 2006
Tuberculosis (Drug resistant)	Rv1446c, Rv3028c, Rv0491, Rv2971, and Rv2145	2DE, MALDI-TOF-MS	Jiang et al., 2007

Table 1b. Application of proteomics for the discovery of potential vaccine targets

Microbe/Disease	Potential target protein (for vaccine development)	Proteomic techniques	Reference
Helicobacter pylori	Le ^{b} -binding adhesin, Other membrane proteins	2D-LPE, MALDI-TOF-MS	Nilsson et al., 2000
Trypanosoma cruzi	TS/gp85, mucins and Other membrane proteins	Multidimensional liquid chromatography, MALDI-TOF-MS	Nakayasu et al., 2011
Streptpcoccus sps.	spy0416	Nano-LC/MS/MS	Rodrı´guez-Ortega et al., 2006

Klebsiella pneumoniae	OmpA, OmpK36, OmpK17, FepA, OmpW, Colicin I receptor	2DE, Immunoblotting, MALDI-TOF-MS	Kurupati et al., 2006
Streptococcus suis serotype 2	Muramidase-released protein, surface protein SP1 & glyceraldehyde-3-phosphate dehydrogenase (GapdH)	2DE, Immunoblotting, MALDI-TOF-MS	Zhang et al., 2008

Table 2. Application of proteomics in different translational research (few illustrative examples)

Field	Application	Proteomic techniques	Reference
Food industry	Identification of food allergens	2DE, western blotting	Akagawa et al., 2007
Agriculture	Study of proteomic alterations in wheat grain in response to heat stress	2DE, MALDI-TOF-MS	Majoul et al., 2004
Oil industry	Development of high oil yielding *J. curcas* plants	2DE, LTQ-ESI-MS/MS	Yang et al., 2008
Sports	To detect the doping agents	LC-MS/MS	Kay et al., 2010
Pharmaceutical industry	To detect the purity of the drugs	MS	Monsarrat et al., 2005

IV. PERSONALIZED PROTEOMICS TO PRECISION MEDICINE/THERAPY

Personalized medicine and therapy is one of the major focuses of healthcare in recent years (Jain 2004). Individualized therapy and personalized omics analysis are gaining popularity due to the extreme variations among the individuals and each person's unique response towards drug treatment and diseased states. Proteomics aids in understanding the biological and molecular functions of proteins, their involvements in different physiological pathways and interaction networks, and therefore has tremendous implications for therapeutic interventions and identifying body response during and post-drug treatment. After obtaining success with pharmacogenetic and pharmacogenomic approaches; pharmacoproteomics is also emerging effectively, since proteomics-based characterization of diseases and clinical groups are more appreciable for individual's preventive care or drug therapy (Reddy et al., 2011; Ray et al., 2011). Characterization of personalized omics including genomics, transcriptomics, proteomics and metabolomics is highly effective for establishment of disease-oriented medicine and identification of at-risk personals and families with susceptibility to hereditary cancer and other fetal disorders (Chen and Snyder 2013).

The major purpose of personalized omics and individualized therapy include;

- Identification of gene and protein-level variations among individuals
- Establishment of personal omics profile
- Analysis of individual's response pre/during/post disease state
- Development of patient-tailor therapy
- Understanding disease pathobiology and early detection of diseased conditions

However, the field of pharmacoproteomics and personalized proteomics is presently at a very early stage of development. The fundamental improvement of proteomics

research, data miming and systems biological approaches are required to establish for making this discipline more effective and reliable for clinical applications.

V. CONCLUSIONS

Ultimate aspiration of clinical proteomics is to translate the findings of basic research into direct practical clinical applications through development of processes/products, which can effectively improve diagnostics and therapeutics, and in turn provide beneficial outcome for mankind. Despite the promising outcomes in proof-of-principle level experiments; proteomics is far way from the routine implications for disease diagnosis and treatment. In order to obtain a comprehensive picture of the living system, it is essential to perform organized studies by combining the proteomics findings with other omics level research, which can effectively unravel mechanistic insights of biological networks for identification of novel diagnostic and therapeutic targets. High-throughput proteomic technologies competent for screening large number of analytes rapidly are very promising for identification of new drug molecules and diagnostic indicators. Eventual success of proteomics in translational research will depend on the world wide initiatives for sharing of scientific findings among different research groups through the development of data repositories, correlation of new research findings with existing knowledge and circumvention of the fundamental limitations associated with proteome level research. To this end, establishment of Human Proteome Organization (HUPO), Human Proteome Project (HPP) and other proteome projects are phenomenal achievements for translation of proteome research into direct “bed-side” applications.

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