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Proteomic approach to autoimmune disorders: A review

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Proteomics is the study of structural and functional endowment of cells, tissues or organs. This science brings together powerful tools—physical separation techniques like 2-D electrophoresis and mass spectroscopy. It also includes various monoclonal antibodies and other probes coupled with which analysis is done by systems biology approach using modern software. Various statistical, probabilistic, humanistic and artificial neural network algorithms and at the same time incorporating elements of chaos and fractal theories are used to study the interactions of multitude of proteins in the cell. This allows separation of large background noise of high concentration proteins inside the cell from pathobiologically and aetiologically relevant protein molecules present in nano, femto or even atto molar concentrations. Pattern recognition algorithms in modern proteomic techniques will help in understanding aetiopathogenesis of disease, discovering diagnostically and prognostically important biomarkers and molecular targets for future drug discovery. These techniques will have important applications in autoimmune disorders and other disorders which are in general difficult to manage. The present review shows how proteomic approach can illuminate various facets of these groups of elusive disorders in near future.

Keywords: Proteomics, autoimmune disorders, autoantibodies, biomarkers, bioinformatics

Introduction

The autoimmune diseases have posed a challenge to clinicians and research scientists ever since the 19th Century. Despite recent acquisition of extensive information, understanding of the mechanisms leading to pathogenic autoimmunity is fragmentary and incomplete. Recent advances in immuno-diagnostics have begun to assemble the missing pieces of the puzzle. Currently, a large number of autoimmune diseases have been diagnosed clinically with support from laboratory investigations. Increasingly different immunological tests have been evolved with higher specificities and sensitivity to diagnose these diseases. These tests are helpful in defining particular subsets of patients with better or poorer prognosis, different patterns of organ involvement, variety of responses to a given treatment or progression of the disease. The evolution of immunoassays has brought considerable benefits in terms of analytical, operational and clinical outcomes. In this respect, immunodiagnostics has proven to be a critical step in the development of many aspects of modern clinical practice.

Proteomics enables correlations to be established between the range of proteins produced by a cell or tissue and the initiation or progression of a disease state. Proteome research helps in the discovery of new protein markers for diagnostic purposes and ~~of~~ novel molecular targets for drug discovery. The abundant information provided by proteome research is complementary to the genetic information generated by genomic research, which at a broad level attempts to catalogue and characterize these proteins, compare variations and their expression levels in health and disease, study their interactions, and identify their functional role. Field of proteomics and bioinformatics will explore an area of autoantibody profiling of various autoimmune diseases in the coming decades. This will prove to be a beneficial tool for classifying patients with varying degrees of severity, subsets of patients based on autoantibody fingerprint, to characterize autoreactive B cell epitope spreading, isotype usage, identification and characterization of biomarker for candidate autoantigen as well as in designing antigen specific immuno-modulating therapies. In near future,

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proteomics will broaden our understanding of immunopathogenic mechanisms of autoimmune disorders which will help in early diagnosis, prognosis and management of patients suffering form various autoimmune disorders[1].

Recent studies to identify antigen specificities of autoantigens, and to identify newer target autoantigens to anti-neutrophil cytoplasmic antibodies (ANCA), myositis specific antibodies, anti-endothelial cell antibodies (AECA), anti-red cell antibodies and anti-endomysial antibodies are based on proteomics. Protein microarrays to identify peptides representing candidate autoantigens is now a powerful technique for immunodiagnosis of autoimmune diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), polymyositis and systemic sclerosis. Advances in the field of proteomics will open a new wing of autoantibody research and will lead to identification of new autoantigens and corresponding autoantibodies. Studies on new autoantigens will be based on combining traditional techniques like 2-D electrophoresis and western blotting with latest mass spectrometric (MS) analysis and protein database retrieval using bioinformatics tool.

Protein-Profiling

Recently, proteomic-based technologies have been successfully used for the identification of antigens targeted by AECA that recognize a restricted set of proteins from a whole protein extract of umbilical vein endothelial cells. These proteins correspond to ubiquitous proteins belonging to highly conserved protein families and exerting pivotal roles in cell physiology, including cytoskeletal proteins (β actin, vimentin, α tubulin) and glycolytic enzymes (glyceraldehydes-3-phosphate dehydrogenase and α enolase)[2]. Increased blood levels of proinflammatory cytokines have been reported to be associated with autoantibody targeting of citrullinated antigens and surrogate markers of disease activity in patients with early RA. Proteomic analysis of serum autoantibodies, cytokines and chemokines enables stratification of patients with early RA into molecular subgroups[3]. A multiplex analysis of serum cytokines using an optimized cytokine bead assay, and integration of these datasets with previously determined antigen array-derived autoantibody signatures have also been reported for the study of cytokines and chemokines derived from subsets of immunoregulatory cells that are selectively upregulated in early rheumatoid arthritis and to study classes of cytokines that are associated with distinct patterns of autoantibody reactivity which provide new insights into associations of anti-citrulline autoantibody responses with production of proinflammatory cytokines[4].

Despite advances in understanding the targets of autoimmune destruction, the causes of autoimmunity remain unclear. Mounting evidence suggests that complementary proteins might have a role in the induction of autoimmunity through a complex network of idiotypic–anti-idiotypic antibodies. This idea was initially promulgated nearly two decades ago and further substantiated by the discovery that a protein, termed cPR-3 (c, complementary), encoded by the anti-sense strand of the human proteinase-3 gene, is able to initiate an autoimmune response by first eliciting the formation of antibodies against itself (Fig. 1)[5]. These antibodies, in turn, provoke the formation of antibodies against themselves and these are able to recognize and bind proteinase-3, which results in an autoimmune response. This process is termed 'autoantigen complimentarily' and results in the formation of an idiotype—anti-idiotype network[6]. Cloning and expressing genetically engineered peptides with potential epitopic region of chosen immunodiagnostic antigens are also under study. Some of the bioinformatics strategies are used to predict putative epitopic regions of specific antigens (PR3, MPO, PSA) by designing primers to amplify regions that express putative epitopic regions. A single peptide designed is then expressed in bacterial and/or mammalian cells, which is further tested for their ability to raise antibodies which would also identify the antigen in its native form.

OnChip-Autoantibody Analysis

It has been shown that different viral infections can trigger B cell responses leading to polyclonal activation and production of autoantibodies. Comparison of serum reactivity against a specific panel of autoantigens based on proteomics approach suggests that viral infections may leave specific 'autoantibody fingerprints' in virally infected host. Mass spectrometric identification of proteins is suitable for comparing overall profile of autoantibody proteins in different populations and for subsequent identification of relevant autoantigens where an efficient proteomic approach can be successfully

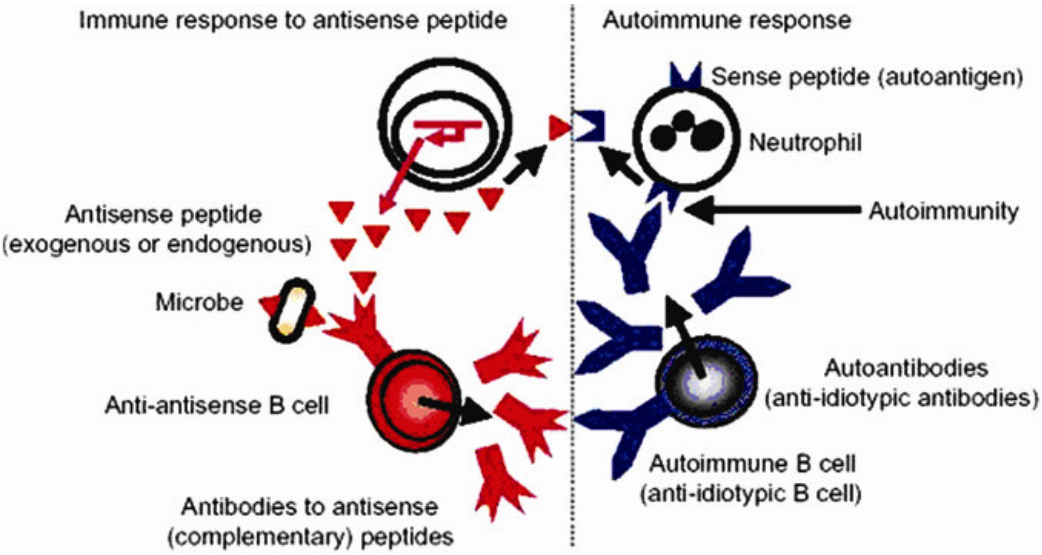

Fig. 1—Autoimmunity trigged by cPR-3 (105-201), a peptide complementary to human autoantigen proteinase-3.

utilized. Immunoassays on microarrays hold appeal for studies having the potential to quantify a number of selected proteins. Recently, considerable progress has been made in this area in terms of increased assay sensitivity and complexity, i.e., degree of multiplexing. Rolling-circle amplification facilitates the use of such arrays utilizing its powerful degree of signal enhancement and simultaneous detection of a large number of defined analytes. Such robust systems will enable routine use of antibody arrays in both biomedical research and diagnostic modalities.

The ability to determine molecular fingerprinting of autoantibodies (antibody signatures) may provide useful clinical diagnostic and prognostic information. Several proteomics approaches are useful for the identification of antigens recognized by these autoantibodies. Serological proteome analysis combines separation of tumour cell proteins on 2-D electrophoresis gels, western blotting with sera of patients and healthy subjects, followed by identification of the detected antigens by MS. The use and limitations of reverse phase protein microarrays for testing patient's serum containing autoantibodies are also considered. Lastly, the most important difficulty of any proteomically identified autoantibody signature is validation in patient cohorts or clinical samples[7-8].

The identification of autoantibodies to tumour cell proteins by proteomics approaches has tremendous impact on cancer biomarker discovery. The humoral immune response represents a form of biological amplification of signals that are otherwise weak due to very low concentrations of antigen, especially in the early stages of cancers. In addition, proteomics can detect immunoreactivity directed against a particular protein after post-translational modifications. 2-D gel based western blots, protein antigen microarrays, and multiplex ELISA reactions have been applied to antigen based biomarker detection and validation. Recent advances in the field of proteomics are the identification and characterization of autoantibodies against novel serum markers in cancer detection at early stages. Proteomic based identification of autoantibodies against peroxiredoxin VI and CDC25B as novel markers in esophageal squamous cell carcinoma had also been reported[9].

Biomarker Discovery

Biomarkers are valued tools used across the biological science spectrum, from research to diagnosis. Proteomics has the potential for advanced biomarker discovery. A number of proteomics platforms are available today for diagnostic and prognostic aspects of autoimmune diseases. The classical example of such biomarker of high specificity is anti-Citrullinated α enolase autoantibodies and its diagnostic and prognostic implication in RA and SLE[9]. Both 2-D gel electrophoresis and mass spectrometry can be used to analyze complex mixtures of proteins and/or peptides in such systems. Newer approaches to address the limitations of 2-D gel electrophoresis, by detecting less abundant proteins through the use of protein

chips arrayed with specific capture molecules like antibodies, are being pursued actively. Latest proteomic technologies for autoantibody profiling include multiplex analysis using protein chips/ microarrays of antigen specific immunobeads, addressable tags, nanoparticles and arrays of living cells. Peptide–MHC tetramer arrays, reverse-phase protein microarrays, cDNA and oligonucleotide microarrays, antibody microarrays, bead-based multiplex assays, autoantigen microarrays, whole-proteome microarrays are such types of assays.

The completion of the human genome project and the availability of highly sensitive and accurate mass spectrometers have led to the ongoing proteomics revolution. Future advances in proteomics depend heavily on the quality and possibilities of related software. The required software can be seen as consisting of two segments. The first one is the analysis of the proteome population of proteins, i.e. proteome status. Unlike the genome, the compositions of the proteome change dynamically with the developmental state of the cell. Proteome analysis requires elaborated software to identify individual proteins by mass and mobility in 2-D electrophoresis and matrix assisted laser desorption/ionization (MALDI) experiments. The second software segment is focused on the proteome structural analysis. The primary amino acid sequences of proteins provide important advances in analysis of individual proteins of the proteome and making predictions regarding the functional status of the proteome. Exclusive prognosis can be based on estimating changes in the primary sequences and related 3-D structures of a set of proteins, i.e. protein markers[10]. Considering that on-chip procedure is quite easy to use, much less time consuming than Western blotting, and much more sensitive at least in the low molecular weight range, the SELDI-TOF technology is a very promising approach for the screening of auto-antibodies in autoimmune diseases. Due to its versatility, this on-chip technology could allow the large-scale screening for complex auto-antibody distributions for diagnostic purposes and early detection of autoimmune diseases could be made possible[11].

A wealth of information available in bioinformatics includes a description of protein function, its domain structure, subcellular localization, post-translational modifications, variants, similarities to other proteins, structural classification, etc. Computational methods to define changes in the 3-D structure and stability of proteome proteins versus sequence variations and to make predictions about the proteome functional status are an integral part of proteomics. Newer methods of protein 3-D structure prediction, including homology modeling, molecular dynamics, protein 3-D structure visualization software (spdvb, Rasmol) are gaining impetus. The ongoing structural genomic project with its goal of solving the 3-D structures of all proteins encoded by the human genome serves as the main initiative to annotate basic proteome proteins.

Conclusion

It has been observed that proteomic approach provides us with another strong analytical tool for (1) understanding the underlying mechanisms which lead to the development of various autoimmune disorders; (2) finding out subsets of a disease which may need different approaches to therapy; and (3) knowing a more inclusive theory of pathogenesis of a particular autoimmune phenomena depending on the proteomic evolution of the disease. This synthesis will pave the way for (a) new biomarker discovery as diagnostic or prognostic marker; (b) development of new ways to manage the disease, i.e. targeted intervention to modulate metabolic machinery in the cell or tissue early in the disease; and (c) many autoimmune diseases, which produce antibodies or reactive immuno competent cells that may damage, kill, remove or stimulate cellular proliferation. The range of autoantibodies and spectrum of their action in different autoimmune diseases at different stages of disease will usher a new dawn in understanding and managing these diseases which so far have been proved to be unsatisfactory and in certain cases elusive.

Following are some of the important websites for proteomics and bioinformatics tools:

1 Data retrieval tools-http://www.ncbi.nlm.nih.gov/Entrez
2 Comparison of protein sequences to sequence databases and for statistical significance of matches-http://blast.ncbi.nlm.nih.gov/BLAST.cgi
3 Database of protein domain, families and functional sites-http://au.expasy.org/prosite
4 CATH is a hierarchical classification of protein domain structures, which clusters proteins at four major levels : Class (C), Architecture (A), Topology (T) and Homologous super family (H)-http://www.cathdb.info/
5 For structural classification of protein (SCOP)-http://scop.mrc-lmb.cam.ac.uk/scop/

6 Tools and resources for studying the structure of biological macromolecules and their relationships to sequence, function and disease-http://www.rcsb.org/pdb/home
7 Molecular visualization of proteins-http://spdbv.vital-it.ch/;http://www.umass.edu/microbio/rasmol/
8 Human genome project-http://www.genome.gov

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