TRENDS IN CLINICAL BIOCHEMISTRY: DIAGNOSTICS

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There is an ever-increasing demand for improved diagnostics to support the clinicians in patient evaluation and treatment. In view of this there is an urgent need to fill up the knowledge gap in understanding the cellular mechanisms of the host and also the infectious microbes with integrated strategies particularly in tropical countries. Scientific developments of 21st century facilitate in filling up this gap. The research in the field of clinical biochemistry has expanded, over a period of time, beyond traditional narrow perceptions of the discipline and evolved as a multidisciplinary activity. This is well reflected in diagnostic science and diagnostic technologies. With the advent of genomics and proteomics science, several biochemical, immunological and molecular mechanisms in human diseases are better understood opening up the scope for development of innovative and specific target based diagnostics.

Proteomics and Clinical Biochemistry

Biological and Clinical research today is driven by molecular biology, genomics and proteomics for the functional analysis of cells and tissues in healthy and disease state. Proteomic science finds immense potential in clinical research for a broad spectrum of diseases. In order to identify the causative factors and develop specific diagnostics for diseases, it is necessary to identify the biochemical, immunological, molecular and genetic factors in the host. Further, the interactive mechanisms of the host and the pathogens also need to be understood. The genes and their ultimate products, the proteins of the cells, need to be examined under various physiological conditions, including the diseased state. In such studies, the technology of clinical proteomics has an immense role to play.

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An insight into the nature of enormous number of proteins, their chemical heterogeneity and the dynamics of their synthesis and degradation can be achieved through application of proteomics. Discovery of new diagnostic systems are today possible on comparison of protein expression profiles of various tissues that include cells, organelles, body tissues, body fluids and pathogenic microbes in different physiological states. Widely used techniques for analysis of clinical samples include surface-enhanced laser desorption/ionization mass spectrometry and two-dimensional gel electrophoresis coupled to matrix assisted laser desorption/ionization.

Although there is a better insight of molecular pathogenesis of common types of cancer, the scenario has not changed to the advantage of the patients due to lack of effective strategies for diagnosis. Discovery of cancer biomarkers through use of proteomics facilitate development of new diagnostic science and technology. Factors like Cost effectiveness, feasibility and reproducibility, applicability to large-scale screening and to all body fluids play key role in the application of these biomarkers to clinical diagnosis. A comprehensive, antibody based protein atlas for expression and localization profiles in 48 normal human tissues and 20 different cancers are available today in public domain (<http://www.proteinatlas.org/>). This is a valuable tool for chemical research and development of clinical diagnostics.

Genomics for clinical diagnostics

Availability of human genome sequence and recent advances in biotechnology enabled the molecular profiling of human cells and tissues. DNA micro arrays are useful for obtaining gene expression profiles under different conditions in tissues. These developments have taken clinical diagnostics during the last decade to greater heights. Further, this has widened the scope of diagnosis of a broad spectrum of diseases based on DNA or RNA as biomarker.

Application of Polymerase Chain Reaction (PCR) for amplification of the β -globin gene sequence and diagnosis of sickle cell anemia by restriction fragment length polymorphism

was first reported two decades ago. Since then, the gene based methods, the molecular diagnostics with their versatility of amplification technologies and the highly sensitive detection systems expended the scope of clinical diagnostics. They are useful in identifying pathogenic microbes or disease related gene sequences. The most desirable features of gene-based technologies in clinical diagnostics are specificity, rapidity and direct detection in specimens with high sensitivity. Precise quantification of genes of relevance to a wide range of diseases using clinical specimens is possible today. Gene based diagnostics have a unique feature of detection of pathogenic microbes which sometimes is beyond the scope of conventional diagnostic tests. Such tests have lead to detection of HIV infection even in a new born on the day of birth. Real time PCR is now used routinely in many clinical laboratories all over the world. Immuno PCR coupled with signal amplification and antigen-antibody detection system have the promise of several thousand fold sensitivity over ELISAs .

Gene based diagnostics are also being used for predictive diagnosis of individuals for onset of diseases like breast cancer using specific gene mutations. Further, such strategies can also be used for confirming the diagnosis as well as for monitoring of the diseases such as T-cell leukemia.

Recombinant antibodies

Availability of high performance antibodies against the target analytes is essential for development of high quality immunodiagnostics. The use of good quality polyclonal and monoclonal antibodies are now being replaced by recombinant antibodies. Recombinant antibodies are produced by “phage display” technology. Targeted single chain antibodies can be produced using gene libraries for selection of recombinant antibody fragments. Recent advances in Immunodiagnostics for cardiac markers and antibiotics etc. are based on such innovations. Production of high quality recombinant antibodies also expedited the development of sensitive micro titer plate arrays, latex agglutination, lateral flow membrane based assays for a broad range of analytes used for field and point of care tests.

Phage display technique for production of antibodies involves fusion of gene segments encoding the antigen binding variable V domains of antibodies to gene of the coat protein of bacteriophage. Bacteriophage containing such gene fusions are used to infect bacteria. The resulting phage particles have coats that express the antibody like fusion protein displayed on the surface of bacteriophage. Phage display library is a collection of recombinant phages displaying different antigen

binding domains on its surface. This technology also facilitates identification of peptide candidates that mimic the epitopes of receptor monoclonal antibodies from a combinatorial peptide phage-display library. The high quality of antibodies both for immunodiagnostics and therapeutics bring great promise to healthcare system.

Recombinant antibodies paved the way for the application of proteomics to clinical diagnostics. The high sensitivity of antibodies in the femtomolar range, linear and reproducible labelling and signal amplification protocols lead to study of proteome activity in clinical specimens with ease.

Protein Microarrays – applications

Highly sensitive protein microarrays are now in use for a variety of research problems. Protein microarrays can be used for identification of protein-protein interactions, small molecular targets, protein phospholipids interactions for understanding the biochemical mechanisms in pathogenesis and cellular mechanisms for basic research. Protein arrays find direct application for clinical diagnostics as well as for monitoring the progress of disease sites. DNA microarrays in genomics research have been used to study gene expression patterns, transcription factor binding sets, SNPs, sequence mutations and detections on a grand scale. However, they give no information regarding the gene products, the proteins and their function. Protein microarray technology offers a novel way of discovering previously unknown multifunctional proteins and also to discover new functionality for well studied proteins.

The biochemical properties of proteins can be studied using analytical, functional and reverse phase microarrays. Antibody microarrays are analytical arrays. Small molecular proteins labeled with fluorescence, affinity, fluorochemical or radioisotope tags are used for detection of reactive proteins. Label free detection methods are also being reported to overcome the problems of labeling such as interference and steric hindrance etc.

Surface plasmon resonance, carbon nanowires, carbon nanotube, micro electro mechanical systems and cantilevers offer great promise for new detection systems for clinical diagnostics.

Clinical diagnostics for Allergic and invasive Aspergillosis

During past decade there is a great advancement in the scientific knowledge of allergic and invasive Aspergillosis, Invasive Aspergillosis is often fatal in immuno compromised

host and the allergic form is highly debilitating in an immuno competent host. Although the work was initiated in 1952, only during past 10 years significant knowledge on the genes, allergens, antigens of *Aspergillus fumigatus*, their molecular characterization, identification of epitopes and their use in immunodiagnosics etc., has been acquired. Further, the genome sequences of *A. fumigatus*, *A. nidulans* and *A. flavus* are available now. Some important genes and ESTs of *Aspergilli* are identified and characterised. This has facilitated better understanding of biochemical and immunochemical mechanisms of the pathogenic fungus in invasion and during allergic reactions. Diagnosis of allergic and invasive Aspergillosis has evolved from microscopy and cultures to Peptide based, monoclonal antibody based and gene based diagnostic tests. It is anticipated that reproducible formats will soon be available in the international markets with high quality

reagents. There is good scope for development of predictive diagnostic of allergic and invasive Aspergillosis based on single nucleotide polymorphisms in the genes of lung surfactant proteins and mannose binding lectins and few others. Immuno proteomic analysis of *A. fumigatus* resulted in the identification of novel allergens and allergens with new functionalities offering scope for development of new diagnostic and therapeutic reagents.

Confluence of numerous recent innovations in genomics, proteomics and micro fluidics is already influencing the developments in clinical diagnostics in a noticeable fashion. These advances are anticipated to result in more meaningful health care to patients in coming years.

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