BIOINFO Journal of Proteomics
Volume 1, Issue 1, 2011, pp-01-05
Available online at: http://www.bioinfo.in/contents.php?id=170

NEUROPROTEOMICS: A NOVEL FIELD OVERCOMING NEUROLOGICAL DISORDERS

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Received: June 14, 2011; Accepted: July 01, 2011

Abstract- Proteomics is the full-fledged study of proteins, particularly their structures and functions. Proteins are vital parts of all living organisms, as they are the main components of the physiological metabolic pathways of cells. One of the important applications of proteomics is 'Neuroproteomics', for studying the central nervous system and its disorders. These technologies can be applied in discovering the targets for drugs to treat neurological disorders.
As proteomics, treated as a highly complex screening technology, turns from a theoretical approach to practical reality, neuroscientists have to determine the most-appropriate applications for this technology. Even though proteomics boost genomics, it is nothing but in contrast to the basically constant genome due to its dynamic nature. This paper also gives an update of biomarkers of neurological disorders like Alzheimer, Parkinson's disease, Schizophrenia, etc.
Medical advances, as well as habitat changes, have tremendously increased the population's life from out of bounds. Unfortunately, longer life spans have resulted in increase in the number of individuals who will be suffering by some neurodegenerative diseases. For example, the widespread of dementia is estimated to range from 1% for those between 65 and 69 years of age, to 39% in the 90–95 years old population. Most of the population prone to Alzheimer's disease (AD) at some point in their life, either directly or through an afflicted family member. Neurological disorders such as AD, Parkinson's disease (PD), front temporal dementia, amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, Huntington's disease (HD), Lewy body dementia, schizophrenia and stroke often have debilitating effects on the patient and the family members who care for them. Unfortunately, there are no absolute cures for many of these neurological disorders. This review will discuss the advances in proteomics tools and techniques to dominate over harmful neurological diseases.
Keywords: proteomics, neuroproteomics, drug abuse, neurotrauma

Introduction

Neuroproteomics [established in 2004] is currently on the move from an emerging to a mature technology platform [1]. Proteomics serves potential for both study of hypothesis-driven proteomic that focus on only a single or limited number of species for specific analysis and comprehensive characterization of these proteins, as well as discovery-driven efforts aimed at globally characterizing proteomes for finding specific differences between healthy and disease-affected patients. Hypothesis-driven studies reported specific information about cellular pathways associated with the disease, the importance of post-translational modifications and the role specific protein interactions play in modulating protein function can be determined [2-4]. A careful weighing up of the most appropriate technology in the experimental design is required. Researchers need to be aware of different proteomic approaches, and complementary genomic technologies have to be considered in order to select the most promising combination for the question being addressed. This review will therefore first portray the tasks waiting for neuroproteomics [5]. During the period of proteomic investigations, various ''-omics'' were developed, like synaptomics (proteome in the synapse), metabolomics or metabonomics (studies of the metabolites in the cells), ribonomics (proteins binding to mRNA), dependomics (proteome of the dependent organism), peptidomics (peptide pool in the tissue), and some others.

Moreover, there is a widely accepted division of proteomics into three main subgroups:

- Clinical proteomics: Analysis of protein biomarkers of disease.
- Structural proteomics: Determination of the three-dimensional structure of a protein to understand mechanisms and properties of its action in the cell,
- Functional proteomics: Investigations of protein–protein(s) and protein-other molecule(s) interactions to understand complex physiological processes [6].

Yet no absolute cures have been recognized, but there are a wide number of treatments being developed that will minimize the progression of a number of neurological disorders. These treatments will majorly impact the patient's lifestyle if it is possible to slow down the onset of disorders such as AD, PD and HD by 20–40 years

until the patient dies due to natural causes. The great unknown in this study is when to begin treatment. For example, people mistakenly attribute to natural aging because the symptoms in early-stage AD may be subtle and resemble signs. The medical community would face difficulty due an epidemic of people on unnecessary medication if every time someone repeated a statement that they were prescribed a drug to delay the onset of AD, PD or HD-related dementia. In the case of HD, doctors often use simple, vague coordination tests (e.g., standing on one foot and touching your finger to your nose from an outstretched arm) to assess early-stage manifestation of this disorder in patient's that are known to carry the genetic defect responsible for this disease. The discovery of a biomarker of early-stage protein that indicates the onset of such conditions would provide a huge benefit to physicians in knowing when to start treating such patients. These type biomarkers would also be extremely beneficial for monitoring the effect of such drugs [2-4].

Biomarker is then applied to the scenario that it could be used as a test given at a routine physical when a patient reaches a certain age. While dealing with other neurological disorder like traumatic brain injury or stroke, the physician must make quick decisions on the most effective treatment to decrease short- and long-term disability to the patient. In these cases, biomarkers indicate the level of immediate care that is required in order to save the patient's life. In addition, biomarkers that show the probability and severity of any long-term injury effects would also be beneficial effectively in determining future medical and planning requirement [3].

Neuroproteomics has difficulty in defining on a molecular level the pathways of consciousness, senses, and self. Neurological disorders are distinct in that they do not always exhibit outward signs and symptoms. The need of exclusive technology, sophisticated software and skilled manpower hikes the challenge. Defining the disorders quite becomes difficult and so neuroproteomics is a step in the right direction of recognizing biomarkers that can be used to detect diseases. Biomarkers may exhibits in the form of genes, proteins and other molecules, or morphological characteristics. Depending on the information they provide, biomarkers can be used in diagnostics as prediction tools e.g. sub-clinical markers, vulnerability markers, or as diseases signatures e.g. disease markers, stage or progression markers [8].

Objectives

Neuroproteomics is one of the novel applications of proteomics to the study of the CNS and its disorders. Proteomic technologies can be applied to the discovery of drug targets to treat neurological disorders. Diseases with protein pathological such as Alzheimer's disease can be studied under this technology. The important receptors for CNS drugs include proteins viz., G-protein-coupled receptors, N-methyl- D -aspartate receptors and protein kinases. Molecular diagnostics can be based on proteins detected present in cerebrospinal fluid and these same proteins can serve as drug targets. Proteomics boost pharmacogenomics and will facilitate the improvement of personalized medicines for neurological disorders [7].

Proteomics has made leaps and bounds in the last 10 years especially in the fields of oncology and cardiovascular medicine. Neuroproteomics comparatively playing catch up mainly due to the relative complexity of neurological disorders. Schizophrenia is such a disorder caused due to the results of multiple factors both genetic and environmental. Over 2 million people affected in the US alone, it has become a major concern of clinical and public health worldwide. Several studies described the potential of cerebrospinal fluid as a source of neuro-specific biomarkers. Genetic association studies are having foresight in identifying candidate genes for schizophrenia. Moreover, flourishing fields such as metabonomics, bioinformatics, and neuroimaging techniques are aiming to complete the picture by filling in knowledge gaps. International cooperation, using the innovative tools and databases of emerging field known as Bioinformatics, in the form of genomics and protein databases and brain banks is facilitating research efforts. No recent developments described here in qualifies as a discovery of biomarker, many are likely to stepping-up towards that goal [8].

Quantitative neuroproteomics is one of the classical and novel tools for studying neural differentiation and function. Various mechanisms such as neural stem cell proliferation, differentiation and maturation perform the most critical role in the development and wiring of neuronal connections. This process involves the activation of multiple serial events, which helps in guiding the undifferentiated cells to different lineages through distinctive developmental programs, constructing neuronal circuits and thus shaping the adult or matured nervous system. Furthermore, any alterations within these strictly regulated pathways can lead to severe diseases like neurological and psychiatric diseases. The analysis and characterization of the high dynamic protein expression changes and other factors affecting protein functions (for example post-translational modifications, the alterations of protein interaction networks) is of pivotal importance for the understanding of the molecular mechanisms responsible for cell differentiation [9].

Methods

The neuroproteomics research involves two important methods:

a. Protein separation technique:
   Proteins must be separated for the proper working of neuroproteomics. Commonly, two dimensional polyacrylamide gel electrophoresis (2D-PAGE) protein separation technique is used. In neuroproteomics, proteins are individually analyzed and correlated between different proteins for recognizing actual cause of neurological disorder.
b. Protein identification technique:

Advanced methods were developed to overcome the limitations of protein separation technique viz., 2D-PAGE. The method used for protein identification is liquid chromatography mass spectrometry (LC-MS) along with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This method can also process with large protein species size but in small amount of protein sample [16].

These are mostly used as the basis of neuroproteomics studies. But there is a need to develop these techniques for obtaining greater accuracy.

Applications of Neuroproteomics

Neurodegeneration is the umbrella term for the progressive loss of structure or function of neurons, including death of neurons. Neurodegenerative diseases result from deterioration of neurons. The methods used for neuroproteomics to function properly are protein separation techniques (2D-PAGE, etc.) and protein identification (Chromatographic techniques).

Neuroproteomics for Alzheimer's disease

Proteomics studies undertakes the identification of unknown proteins along their separation, often using 2D-electrophoresis, digestion of particular proteins of interest by trypsin, determination of the molecular weight of the resulting peptides, and database searching to make the identification of the proteins. Alzheimer's disease (AD) is the leading cause of dementia, a condition that gradually destroys brain cells and leads to progressive decline in mental functions. The disease is characterized by accumulation of misfolded neuronal proteins, amyloid and tau, into insoluble aggregates known as extracellular senile plaques and intracellular neurofibrillary tangles, respectively [10]. Application of proteomics to Alzheimer's disease (AD) has just begun which is the major dementing disorder of the elderly. Differences in protein expression and post-translational modification (mostly oxidative modification) of proteins from AD brain and peripheral tissue, as well as in brain from rodent models of AD have yielded insights into potential molecular mechanisms of neurodegeneration in this dementing disorder. Proteomics studies are supposed to relevant to AD, from which new understandings of the pathology, biochemistry, and physiology of AD are beginning to emerge [11].

Neuroproteomics for Parkinson's disease

Parkinson's disease is the second most common neurodegenerative disorder, affecting nearly 3% of the population over the age of 60 years [12]. The aging population of many countries may prone to PD which is a major challenge to many national health care budgets. The epidemic scale of the disease helps researchers to identify its causes and improve the effectiveness of available treatment options, to ascertain PD research with a prominent place on many national research agendas.

This review discusses the potential offered in for sighting the technological advances made in proteomics. Proteomics can provide a clearer understanding of the pathogenesis and protective mechanisms against Parkinson's disease (PD), by giving insight into the role played by the aggregation and deposition of proteins in human PD and in chemically induced models of the disease [13]. In addition, to provide for more effective treatment options, it can reveal the patterns of PD-specific cellular markers and to allow the therapeutic regime to start earlier when likely to have more beneficial effect.

Despite the widespread, growing interest in the field, insight into the application of proteomics tools and technologies is only slowly offering to the PD research community and relatively few studies have been performed in the PD context. The technology has grown rapidly as a sub-discipline of the life sciences to its current position, where it is having major applications as clinical proteomics, with the view to identify new biomarkers for diagnosis and to better understand the mechanisms, risks, state and progression of PD [14-15].

Sensitive proteomics techniques have been developed to determine the structure, localization, biochemical activity, interactions (i.e., protein–protein, protein–lipid) and the cellular roles played by a multitude of proteins, with the results translated either to a natural physiological or to a pathological state of events. Mostly mass spectroscopic (MS) techniques are used in peptide-sequencing. MS technology and two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) are frequently applied conjunctionally to quantify proteins according to their degree of expression,. This combined experimental approach allows for inter-sample comparisons relating to the disease state as well as between experimental subjects and normal controls [29].

Neuroproteomics in drug abuse research

It is well-known that drug addiction involves permanent synaptic plasticity of various neuronal circuits. Neuroproteomics is being applied to study the drug addiction effect across the synapse. Research is being conducted by isolating distinct regions of the brain in which synaptic transmission takes place and defining the proteome for that particular region. Different stages of drug abuse must be studied in order to map out the progression of protein changes along the course of the drug addiction.

These stages include:

1. Enticement
2. Ingesting
3. Withdrawal
4. Addiction, and
5. Removal.

It begins with the change in the genome through transcription that occurs due to the abuse of drugs [16]. The number of discovery proteomic studies of drug abuse has begun to increase in recent years, facilitated by the adoption of new techniques such as 2D-DIGE and iTRAQ. For these new tools to provide the greatest

insight into the neurobiology of addiction, however, it is important that the addiction field has a clear understanding of the strengths, limitations, and drug abuse-specific research factors of neuroproteomic studies. We would also came to know about improving animal models, protein sample quality and stability, proteome fractionation, data analysis, and data sharing to maximize the insights gained from neuroproteomic studies of drug abuse. For both the behavioral researcher interested in what proteomic study results mean, and for biochemists joining the drug abuse research field, a careful consideration of these factors is needed. As compared to genomic, transcriptomic, and epigenetic methods, appropriate use of new proteomic technologies offers the potential to provide a novel and global view of the neurobiological changes underlying drug addiction. Proteomic tools can also help to identify key proteins involved in drug abuse behaviors, with the ultimate goal of understanding the etiology of drug abuse and identifying targets for the development of therapeutic agents and also neuroproteomics may give clinicians even earlier biomarkers to test for to prevent permanent neurological damage [17].

Neuroproteomics in Neurotrauma

Traumatic Brain Injury (TBI) is characterized by a direct physical impact or trauma to the head followed by a dynamic series of injury and repair events as shown in figure [18]. Neurotrauma in the form of traumatic brain injury afflicts more populations annually than Alzheimer's and Parkinson's disease combined, yet few researchers have used neuroproteomics to investigate the underlying complex molecular events that exacerbate TBI. The application of proteomics in neurotrauma is all but unexplored, seemingly contrary to the fact that 5.3 million Americans live with disabilities that resulted from traumatic brain injury (TBI) according to the National Institute of Neurological Disorders and Stroke—NINDS (www.ninds.nih.gov) [19].

Different separation techniques, protein identification methods are used post-TBI proteome change. Source of sample such as brain tissues, cerebrospinal fluid (CSF), blood, serum and plasma are used in the TBI studies [20-23]. Proteomic analysis can help us understand complex protein–protein association and molecular pathways in the nervous system. Protein-pathway mapping has been successfully attempted with the post-synaptic density-associated NMDA [N-methyl-d-aspartate] receptor complex with over 100 associated proteins identified [24-25].

Bioinformatics also played a vital role in TBI studies. Separations and MS data must be assembled and processed to provide biologically relevant information. Due to the large amount and complexity of data, computer software is required for all but the smallest proteomic experiments. Protein data are collected in non-redundant publicly available archives, and can be subset into tissue- and species-specific databases to reduce analysis time to provide fewer false positives/negatives [26]. The most widely used database-searching algorithms are Sequest and Mascot [27-28]. In both methods, tandem-MS product-ion spectra of protease-specific peptide precursor ions are correlated with theoretical product-ion spectra derived from the database. Neuroproteomics and field such as neurotrauma has to be developed for resolving from the limitations like extremes in protein number and dynamic range in the heterogeneous brain. Neuroproteomics have also been applied in the neurological disorders like schizophrenia, prion disease, Huntington disease, etc.

Future Aspects

Advances in technology have flourished the field of neuroproteomics equipped with refined tools for the study of the expression, interaction and function of proteins in the nervous system. Along with bioinformatics, neuroproteomics can address the organization of dynamic, functional protein networks and macromolecular structures that underlie physiological, anatomical and behavioral processes that will intensify the neuroproteomic research. Furthermore, neuroproteomics is contributing to the disease mechanisms elucidation and is a powerful tool for the identification of biomarkers [29].

The greatest challenge facing the optimal utilization of this technology lies in detecting and quantifying low-abundant and hydrophobic proteins, therefore, there is intense need for the development of such technologies. The accurate detection of post-translational modifications, their origin and the role they play in PD should have a prior importance. However, it is regretted that protein chips and miniature separation systems will play a significant role in overcoming these limitations [30-i]. Rapidly developing techniques that considerably enhanced information gained from different resources proteomes, integrate proteomics with other disciplinary areas such as cell biology, biochemistry, molecular genetics, and chemistry. This consolidation will certainly demonstrate proteomics incredible power and possibilities for further applications by ease of tackle with immense challenges. The goals for the future concern the achieving, integration, and handling of vast amounts of data, establishment of criteria for protein identification by MS, and a wide access to proteomic results would be made possible through different fields like bioinformatics. It is necessary to cross the barriers of limited resolution, mass range, detection level, and other reasons of protein underrepresentation in analyzed proteomes. Once achieved, the door that allows complete identification of specific protein markers and the comprehension of complex networks of protein/peptide interactions involved in particular brain-related disorders will be opened [6].

Conclusion

Neuroproteomics is very essential field for demolishing the harmful neurological disorders. Even though neuroproteomics research area has boosted due to advances in the protein separation, identification and quantification technologies, but still there are some

limitations in the sense of proper implementations of techniques, due to the complexity in whole proteome of an organism, that are going to be applied in the neuroproteomics studies. Bioinformatics is also playing a vital role towards the approaches for neuroproteomics through computerized data analysis and manipulations with greater accuracy and with short time period of research.

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