International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article

APPLICATIONS OF PROTEOMICS IN ANIMAL MODEL

Jagatheesh K*[1], Pavankumar P[1], Elangovan N[1], Padmavathi P[1], Swathi D[2] and Mary Tryphena[2]

[1]Molecular Pharmacology Research Lab, Periyar University, Salem, India
[2]Dept of Pharmacology, KLR Pharmacy College, Paloncha, India

***Corresponding author e-mail**: kjagatheesh@gmail.com

ABSTRACT

Proteomics is a relatively new approach for understanding the pathology and pathogenesis of various diseases. It has also been used for characterizing the modifications in protein expression during the development of diseases. Proteomics is defined as a scientific approach used to elucidate all protein species within a cell or tissue, and many researchers are taking advantage of proteomic technology to elucidate protein changes between healthy and diseased states. Animal model plays an important role in the proteomics technology to find out biomarkers, for diagnosis, prognosis and treatment of various diseases. There are several animal models used in proteomic studies they are *Caenorhabditis elegans* (worm), *Drosophila melanogaster* (fly), *Mus musculus* (mouse) and Canine. This review shows several applications of animal models in proteomics.

Keywords: Proteomics, Animal Models and Canine

INTRODUCTION

Proteomics is the study of the large scale of proteins, particularly their structure and functions. The word proteome is a blend of "protein and genome" and was coined by Marc Wilkins in 1994[1]. Proteins are the vital part of living organisms, as they are the main component of the physiological metabolic pathways of cells. Proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. Proteomics encompasses the analysis of protein expression, protein structure and protein interaction. Protein analysis shows how changes in the abundance of particular proteins coordinate the biochemical activities of the cell. Proteomics is much more complicated than genomics mostly because, an organism's whole genome is more (or) less constant, and the proteome differs from cell to cells from time to time. This is because distinct genes are expressed in distinct cell types. Not only does the translation from mRNA cause differences, many proteins are also subjected to a wide variety of chemical modifications after translation. Many of these post translational modifications are critical to the proteins function. The post translational modifications include phosphorylation, ubiquitination, methylation, acetylation, glycosylation, oxidation, nitrosylation.

The analysis of protein structure and protein interactions can provide important functional information. Proteins carry out their activities in the cell by interacting with other molecules. Establishing specific interactions between proteins can therefore help to assign individual functions and link proteins into pathways and networks. Knowledge of a protein's three-dimensional structure can help to predict interactions with other proteins and with smaller molecules, which can be very useful in the development of effective drugs. The comparison of protein structures also provides a further way to determine evolutionary links between genes and investigate their functions. Post-translational modifications of proteins can be determined by different ways. One way which a particular protein can be studied to develop an antibody, this is specific to that of modification. For example, there are antibodies which only recognize certain proteins as

phosphor-specific antibodies. The most common way is two-dimensional gel electrophoresis[2]. Several methods are available to identify probe protein interactions. The traditional method is yeast two hybrid analysis. New methods include protein microarrays, Immunoaffinity chromatography, mass spectrometry, dual polarization, interferometry, microscale thermophoresis and experimental methods such as phage display and computational methods. This review is mainly focused on the use of animal models in proteomic analysis.

Animal models

Animal models are widely used to study the underlying mechanisms and consequences of a disorder. The use of these model organisms to mimic human diseases has become very popular as it enables either the identification of a human gene product (or pathway) that is directly involved in a disease state or the development of biological screens for molecules or gene products that suppress the disease or stop its progression. There are several animal models used in proteomic studies they are *Caenorhabditis elegans* (worm), *Drosophila melanogaster* (fly), *Mus musculus* (mouse) and *Canine*[3].

***Caenorhabditis elegans* (worm)**

C. elegans, the simplest multi cellular organism with a well-understood developmental biology, was introduced as a model by Sydney Brenner in the 1960s. Worms replicate clonally, are easy to grow, and possess a simple nervous system and other mechanisms that make them readily available for genetic studies.

***Drosophila melanogaster* (fly)**

More complex than the nematode, *D. melanogaster* replicates easily and is a multicelluar organism with a complex nervous system. A variety of genetic tools are available with this organism and it possesses the same cell types and biology as vertebrates.

***Mus musculus* (mouse)**

Rodents (rats and mice) are widely used as organisms to study the basic biology, development, genetics, signaling pathways, drug responses and metabolism, and are especially useful as a model for humans because of the repertoire of complex behaviors that are available. Mice are easy to breed, manipulate and handle, and can be genetically engineered. Transgenesis (the introduction of new DNA sequences into the germ line, resulting in the production of transgenic animals) and gene targeting (the integration by homologous recombination of new DNA sequences into the genome of an organism at the sites where its expression can be controlled) technologies are available for the mouse owing to pluripotent embryonic stem cells[36] and germ line manipulations.

Canine

Example for *canine* animal is dog. Some dog breeding is more sensitive to certain drugs than other breeds. Collies and related breeds, for instance, can have adverse reactions to drugs such as ivermectin and loperamide. Drug sensitivities result from a mutation in the multi-drug resistance gene (MDR1). This gene encodes a protein, P-glycoprotein that is responsible for pumping many drugs and other toxins out of the brain. Dogs with the mutant gene can not pump some drugs out of the brain as a normal dog would, which may result in abnormal neurologic signs. The results may be an illness, requiring an extended hospital stay or even death.

There are many advantages to use animal models.

1. They are genetically homogenous within a particular animal model.
2. Environmental factors, like dietary intake, can be tightly controlled.
3. It is also possible to induce diabetes in a well defined manner and to perform intervention and prevention studies in a short time span compared to the time needed for studies in humans.
4. Animal models allow researchers to study tissues that are difficult to obtain from the human subject.

APPLICATIONS OF PROTEOMICS IN ANIMAL MODELS

Proteomics in Biomarker

Proteomics offers potential for tracing mechanism of complex diseases, differential diagnosis, classification of diseases and therapeutic monitoring. The varying abundance of specific proteins in healthy and diseased samples (or) samples representing the progression of a disease can help to reveal useful markers and novel drugs. A small set of proteins fully differentiate tumor samples from controls. This comprises osteopontin, interleukin-18, cystatin C and CD40 antigen. Comparison of protein expression in breast-cancer mouse model, the humanized p53.R270H mice, showed common discriminatory expression of osteopontin. However, other biomarkers showed distinct expression in the two different breast-cancer models, indicating that different mammary tumor sub-types with respect to molecular and estrogen receptor status reveal divergent serum biomarker sets[4].

Proteomic analyses of carcinogen exposed liver showed significant up-regulation of cellular stress responsive proteins that include, superoxide dismutase, heat shock protein 60 and peroxiredoxin. Caspase-8 precursor, vimentin, Rho GDP dissociation inhibitor was identified in rat liver bearing malignant, transformed cells following 18 weeks after carcinogen withdrawal. Annexin A5 and fructose-1, 6-bisphosphatase was regulated after three weeks of exposure indicating their potential usefulness as early predictive biomarkers for liver carcinogenesis[5]. Increasing incidence of cardiovascular diseases, specifically atherosclerosis and heart failure, prioritized the search for novel biomarkers. Biomarkers are needed for the diagnosis, prognosis, therapeutic monitoring and risk stratification of acute myocardial infarction (AMI) and heart failure, leading causes of mortality and morbidity[6]. A correlation was established between an increase in H-FABP expression and decreases in PCNA expression that regulates cardiomyocyte growth and differentiation in the mouse neonatal hearts [7].

Proteomics in Toxicology

Toxicology is likely to prove one of the most important applications of proteomics. 2DE is a highly sensitive means of screening for toxicity and probing toxic mechanisms. In one ongoing study, a group at Imperial College London/SmithKline Beecham recently reported an ongoing 2DE- and NMR-based study of glomerular nephrotoxicity in the rat following exposure to puramycin aminonucleoside[8]. By monitoring the proteins in the urine, the study has permitted a more detailed understanding of the nature and progression of the proteinurea associated with glomerular nephrotoxicity than has been previously possible. In a second recent example, a study of lead toxicity in a rabbit model identified a number of proteins that change expression following increased lead exposure, of which several molecules, provisionally identified as glutathione-S-transferase variants, may be developed into valuable markers of lead toxicity in humans[9]. Other notable reports to emerge recently in proteomic toxicology include an analysis of cyclosporine. A toxicity, which revealed a novel toxic mechanism involving the calcium binding protein calbindin-D[10], and the ongoing studies of liver protein changes following exposure of rodents to peroxisome proliferators being conducted by Argonne National Laboratories and Large Scale Proteomics Corporation[11,12]. However, although numerous other toxicological studies have used proteomic tools such as 2DE and MS, the potential of proteomics in routine toxicology have yet to be realized.

Proteomics in Diabetes

Diabetes is a disease caused by deficiency in insulin production (type I), or a deficiency in insulin receptor signaling (type II) that implies glycemic control in affected individuals and results in increased blood sugar levels. This disease has numerous co-morbidities including heart diseases, nephropathy and retinopathy[13].

Type I diabetes (TID): In TID, autoimmune responses observed towards the pancreatic β-cells may affect the protein expression profiles in several tissues. Proteomic analysis will be useful in order to elucidate the changes imposed by various immunological mediators and lack of insulin. As β-cell destruction is central in the pathogenesis of TID, a natural focus of several proteomic studies has been used to examine the effects of cytokines and ion in the islets of Langerhans and β-cells[14-17]. Nerup and coworkers investigated the effect of IL-Iβ on the protein expression profiles of rat islets of langerhans from Wistar Furth (WF) rats[18, 19] and bio-breeding diabetes prone (BB-DP) rats[20, 21]. Islets from WF rats were isolated from neonatal rats and stimulated with IL-Iβ in-vitro. The protein expression profile was analyzed using 2-DPAGE and identified with PMF. 1400 protein spots were detected and 105 of those showed a change in the level of expression after stimulation[18]. When using islets from BB-DP, 1815 protein spots were detected and 82 of those changed their level of expression[20].

Type II diabetes (T2D):

Diet-induced animal model used for insulin resistance, the fructose-fed Syrian golden hamster, to investigate changes in the proteome of the hepatocyte ER. The animals were fed fructose for two weeks to induce insulin resistance. The insulin resistant animals and healthy controls were sacrificed, hepatocytes were isolated and ER fraction was prepared using differential centrifugation. The ER proteome was analyzed using the 2-D PAGE and spots were identified using MS/MS. After analysis, 34 spots were identified as differentially expressed between insulin resistance and control animals. The change in expression of one of the differentially expressed protein ER 60, is involved in degradation of APO, this contribute to the dyslipedimia associated with insulin resistance. Fetal under nutrition can result in increased incidence of T2D. The effects of an isocholic, low-protein diet on the islets of rat fetuses were studied[23]. The serum protein has been the subject of many studies, frequently with

the goal of identifying biomarkers. Serum protein profiling can also be used to achieve a better understanding of the pathophysiology of disease. Peptides and small proteins make up an important subset of the proteome, which is different to study with conventional gel-based methods. Boddle et al.[24] demonstrated the usefulness of MS-based proteomics and quantification of proteins based on the differential analysis of the MS signals for this type of experiment. The authors compared the peptide content of the experiment from different mice[25].

Redox proteomics studies in animal models

These studies were conducted on transgenic *Caenorhabdatis elegans.* It is an invertebrate model to study the role of Aβ aggregations, oxidized stress and cell death. Transgenic *C. elegance* was created by expressing human Aβ using a body wall muscle specific promoter and an Aβ Michigan consisting of the signal peptide fused to the Aβ sequence[26]. This shows an increased ROS formation and protein oxidation which lead to February deposition of peptide supporting soluble aggregates of peptide are toxic[27]. These symptoms are similar to those observed in AD patients supporting Aβ-induced oxidative stress and subsequent neurodegeneration in AD[27, 28]. Increased protein carbonyls and progressive paralysis in *C.elegans* supports amyloid hypothesis states that Aβ induced oxidative stress leads to neuronal cell death in AD[29]. DNA microarray of *C.elegans* shows that stress related genes are upregulated. Those genes are αβ-crystalline and tumor necrosis factor related proteins which are homologous to human genes[30]. To explain the link between oxidative stress, oxidatively modified proteins and Aβ expression redox proteomics were applied in two different forms of *C.elegans*[31], CL 4176, CL 2337; both showed the oxidation of different proteins as that in AD brain. Antioxidant compounds-ginkgobiloba extracts Egb 7lal, ginkgolides, lipoic acid, polyphenol green tea that prevents Aβ induced pathological behavior was tested on *C.elegens*[32, 33].

ANIMAL MODELS RELATED TO CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY

Proteomics of diabetic retinal disease

Investigation of the etiology of this disease is limited due to the absence of animal models that faithfully replicate the disease. The animal model displays only pathological changes related to early stages of diabetic retinopathy. A comprehensive 2-D MALDI-TOF and LC-ESI-MS/MS study has identified a large number of differentially expressed proteins in rat retinas from animals with ten weeks of diabetes[34]. Many investigations use vitreal samples from patients undergoing vitreoretinal surgery as an alteration to investigate the molecular mechanism involved in this disease[35-37]. Proteins in endothelial cells of retina cathepsin, a lysosomal cysteine protease involved in the degradation of extravascular matrix and calretirculin, a calcium binding protein known to have anti-angiogenic activity[38].

Proteomics of glaucoma

The trabecular meshwork is located in the anterior portion of the eye, where it facilitates the outflow of the aqueous portion of the eye into Schlemm's canal. This out flow mechanism for aqueous humor may become impaired by a more resistant trabecular meshwork leading to increased intraocular pressure. If pharmacological intraocular pressure lowering therapy is not responding then surgical removal of the trabecular meshwork, or a trabeculectomy, may be advised. Analysis of surgical removed material using 1-DE, MS/MS has resulted in the identification of a previously unconnected protein Cochlin associated with the trabecular meshwork of glaucoma but not normal cadaver trabecular meshwork[39]. Separation of trabecular meshwork cell proteins by 2-D coupled with MS identification has indicated that the expression of ECM proteins and proteins involved in ECM secretion is increased following transforming growth factor β2 treatment[40]. As aqueous humor TGFβ2 levels are elevated in glaucomatous eyes[41], increased production and secretion of ECM protein by trabecular meshwork cells in response to this cytokine represents a potential mechanism for reduced aqueous out flow in glaucoma. Calpain activation may represent an important mechanism of apoptotic ganglion cell death in glaucoma. Activation of calpains from monkey retinal tissue with extracellular calcium leads to proteolysis of a number of major retinal proteins including vimentin, beta-tubulin, HSP70 and alpha-enolase[42].

Proteomics of age-related macular degeneration (AMD)

AMD is characterized by central visual loss resulting from damage to the photoreceptor cells in the macular region of the retina and is initiated by deterioration of the retinal pigment epithelium (RPE), which lies behind this light-sensing cells providing metabolic support[43, 44]. An in-solution digests LC-MS/MS approach of lipid-free preparations of drusen obtained from post-mortem normal and AMD eyes resulted in the identification of 129 proteins[45]. Analysis of the monkey drusen proteome revealed similar proteins as the human study including annexin, complement components, crystallins,

vitrinectin as well as a high prevalence of immunogenic proteins including antibodies against mucrysatllin and annexin II[46]. Lipofuscin also accumulates in the RPE where it may constitute up to 20% of these cells in elderly individuals[47]. The accumulation of lipofuscin in the RPE has also been associated with a number of sights-threatening diseases including AMD[48]. It may contribute to retinal degeneration by augmentation the generation of ROS and mitochondrial and nuclear DNA damage in the aging RPE[49]. Initial studies using 2-DE resulted in the identification of a limited number of proteins from retinal lipofuscin, which were largely abundant cellular proteins[50].

Proteomics of ocular surface disease

Ocular surface disease encompasses surface injuries, dry eyes and other surface problems such as allergies. Tears have been the starting material for a number of proteomic studies of ocular surface disease and indeed other ocular pathologies[51-53]. Tear fluid is reported to have a high protein content[54], and also contains important electrolytes[55]. The protein profile of tear fluid may be altered in both ocular surface diseases and injuries, and also in certain systematic diseases such as diabetes mellitus and cystic fibrosis[56, 57]. Total protein concentration is composed of just four proteins, cystatin, lipocalin, lysozyme and lactoferrin[58]. A direct MALDI-TOF MS approach along with a complementary method involving size exclusion HPLC by Fung et al., comprehensively identified the presence of another abundant class of protein in tear fluid, lachrymal-specific proline rich proteins[59] which are present in various PTM forms. Altered cytokine and chemokine profiles in tears have been reported in ocular inflammatory diseases, with the cytokines secreted differing between different inflammatory diseases[60, 61].

Animal models related to lung diseases

Proteomic studies can also be applied to animal models. This provides a specific opportunity to identify proteins involved in particular metabolic and pathophysiologic pathways. Mouse models are used to study disease processes in homogenous genomic backgrounds. The BALF of O3-sensitive C57Bl/6 and the O3-resistant C3H/HeJ mice used for the identification of proteins involved in the regulation of susceptibility to oxidative stress. They found a significant strain-specific differences in the expression of two isoforms of the antioxidant protein 2 (AOP2). C3H/ HeJ mice expressed only AOP2a, whereas AOP2b found only in C57Bl/6 mice. In addition, the levels of the anti-inflammatory Clara cell protein 16 (CC16) were higher in the resistant C3H/HeJ mice. Thus, AOP2 and CC16 might participate in pathways protecting the respiratory tract from oxidative injury[62]. The study of the mechanism of oxidative lung injury in mice was analyzed. Proteins present in BALF of mice exposed to room air were compared with those exposed to hyperoxia (100% oxygen). Both the amounts of thioether S-methyltransferase and the amounts of 1-cysteine peroxiredoxin were decreased after hyperoxia, indicating their involvement in the pathogenesis of oxidative stress. Thioether S-methyltransferase is abundantly expressed in murine lungs and is important for methylation of thioethers to more water-soluble ions suitable for urinary excretion. 1-cysteine peroxiredoxin, also abundantly expressed in lung tissue, is an antioxidant. Genetically modified mice are of particular interest for studying the impact of the over expression or lack of single proteins on the proteome of cells and biofluids. In the future, interesting results can be expected from proteomics of lungs obtained from transgenic and knockout mice[63].

Proteomics in heart failure

Analysis of ventricular proteomic changes during the initial inception, development, and progression to heart failure revealed large-scale pattern differences[64]. Major alterations observed in heart failure relate to inflammation, calcium signaling, growth and deaths, and cytoskeletal/matrix remodeling targets. In more specific genetic models of cardiomyopathy, such as Rac1 transgenic mice, proteins regulated in heart failure include creatine kinase M-chain, tubulin beta-chain, manganese superoxide dismutase, and malate dehydrogenase[65]. Similarly, in canine models of acute heart failure due to ischemia, certain proteins are up-regulated, including nicotinamide adenine dinucleotide (NAD) isocitrate dehydrogenase and mitochondrial adenosine triphosphate (ATP) synthase D chain, whereas creatine kinase M chain and myosin light chain-1 were decreased[66]. In terms of plasma, the aforementioned protein candidates are also consistent with the efforts of the Human Proteomic Organisation (HUPO) to analyze the human plasma sub proteome systematically. The initial analysis identified families of proteins involved in inflammation, signaling, growth and differentiation, cytoskeletal, channel/receptors, and remodeling processes[67].

Proteomic identification of rat brainstem cytosolic proteins

Nerve injury often results in chronic neuropathic pain (NP) characterized by allodynia, a painful response to a normally nonpainful stimulus, and hyperalgesia, an increased response to a painful stimulus. Neuropathic

pain involves co-regulation of many genes and their translational products in both peripheral and central nervous system. The proteomic analysis showed expressional changes in cytosolic protein levels in rat brainstem tissues following ligation of lumbar 5 and 6 (L5, L6) spinal nerves, which generates a model of peripheral neuropathic pain. Proteins from brainstem tissue homogenates were fractionated by two-dimensional (2-DE) gel electrophoresis to produce a high-resolution map of the brainstem soluble proteins. Several of the identified proteins, including fatty acid binding protein-brain (FABP-B), major histocompatibility complex (MHC) class 1, T-cell receptor (TCR) alpha chain, and interleukin-1 (IL-1), showed significantly higher levels in the NP rat brainstem. Proteomic analysis has identified several proteins with differential expression levels in neuropathic pain. The differential expression of these proteins may reflect their role in primary and/or secondary events leading to the progression of disease and ultimately are contributing to the pathogenesis of neuropathic pain[68].

Animal models related to age-related cerebral degeneration.

Senescence-accelerated mouse prone 10 (SAMP10) strain is a model of age-related neurodegeneration in the limbic forebrain. Two-dimensional fluorescence difference gel electrophoresis was performed and to compare protein expression in the limbic, non-limbic forebrains of SAMP10 and control mice at various ages. Among the protein spots in which patterns of aging in expression in the limbic forebrain differed between SAMP10 and control, three proteins were identified by mass spectrometry: pyridoxal phosphate phosphatase (PLPP), collapsing response mediator protein 2 (CRMP-2) and a-internexin. Expression of PLPP was increased in the limbic forebrain of 3-month-old SAMP10 mice. Levels of CRMP-2 and phosphorylated ainternexin were increased in the limbic forebrain of SAMP10 mice at age 8 months and remained high until 14 months. Aging in SAMP10 mice was associated with an abnormality of PLPP, CRMP-2 and ainternexin, all of which are known to be involved in the brain cytoskeleton formation and associated with acute and chronic neurodegenerative conditions. These proteins are promising targets for further investigation of the mechanisms underlying brain aging[69].

Animal models related to hepatitis B virus proteins

Hepatitis B virus transgenic mice (HBV-Tg mice) have been widely used as animal models in the study of pathogenesis and control of hepatitis B. It is important for the evaluation of such animal models to define the physiological differences between HBV-Tg and wild-type mice. The protein expression and metabolite profiling of HBV-Tg mice were investigated using integrated proteomic and metabonomic techniques. This study focused on 6–8-week-old HBV-Tg mouse liver which represents the typical state of early pathological lesions. These proteins, which changed three fold or more compared with those of the reference gel, were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS). Additionally, dozens of metabolites were discovered as biomarkers in the liver of HBV-Tg mice using HPLC/MS and multivariate data analysis. The integrated results clearly showed that the HBV antigens could alter lipid metabolism in vivo and induce organism oxidative stress[70].

Animal models related to liver fibrosis

Liver fibrosis is the sequel of chronic liver diseases and the main reason for increased mortality in affected patients. The pathogenesis of liver fibrosis is complex and modulated by numerous exogenous and genetic factors. While exogenous factors (like alcohol consumption, co-infections etc.) have already been characterized, the identification of gene variants which significantly contribute to liver fibrosis is challenging. The detection of these genes can theoretically contribute to clinical advances in the identification of patients at risk for severe fibrosis and the development of anti-fibrotic therapies based on the pathophysiology behind the gene variants. Animal models bear the advantage of unlimited supply and the possibility to control for environmental factors. Thus, the cross-breeding of inbred mouse strains and their genetic manipulation by transgene and knockout technologies has been used to successfully identify yet unknown gene loci which contribute to liver fibrosis. Thus, animal models seem to be a valuable tool to identify conserved molecular pathways of fibrogenesis[71].

Proteomic analysis in mammary glands of rat offspring

BisphenolA (BPA) is a ubiquitous environmental contaminant with established endocrine disruptor properties. The effect of prenatal exposure to BPA on the rat mammary gland proteome in postnatal rats was investigated. Since the BPA exposure was only during pregnancy and the investigators measured the changes in protein expression at days 21 and 50 postpartum, this appears to occur as a consequence of early developmental alterations. Because there was no change in vaginal opening and circulating estrogen and progesterone concentrations, that the

protein changes in the mammary gland are permanent and may occur as a consequence of epigenetic alterations in this target tissue. Furthermore, the changes in protein expression of vimentin, SPARC, 14–3–3, phospho-AKT, c-Raf, phospho-ERKs, and TGF-β are consistent with increased susceptibility for cancer development[72].

Proteomics in animal models of Alzheimer's and Parkinson's diseases

The risk of developing neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) increases with age. AD and PD are the two most common neurodegenerative diseases that currently affect millions of persons. Proteomics studies have identified a number of common proteins and/or functional categories that change in AD and/or PD model systems and compared protein expression overlap in AD, PD, Huntington's disease, and amyotrophic lateral sclerosis[73]. Alzheimer's disease is an age related neurodegenerative disease. It is characterized by the presence of senile plaques, amyloid β-peptide (Aβ), neurofibrillary tangles and synaptic loss[74]. In addition, gliosis, chronic inflammatory reactions, excitotoxic damage and oxidative stress contribute to the progression of AD[75-81]. Oxidative stress is mainly due to imbalance in the pro-oxidant/antioxidant mechanism. The proteins undergo oxidative damage by the formation of protein carbonyls and 3-nitrotyrosine (3-NT). Protein carbonyls are formed by free radical[82-92] mediated oxidation of amino acid residue side chains. 3-NT is formed by nitration of tyrosine residues that play an important role in regulating the function of the protein[93]. PTM of proteins leads to unfold and degradation of the damaged proteins[94, 95]. In AD increased levels of oxidized proteins are converted with the loss of the activity of the 20s proteosome, a major enzyme for the degradation of oxidized proteins[96-100]. Amyloid beta-peptide (1-42) has been implicated as a mediator of oxidative stress in AD. Additionally, Abeta (1-42) has been shown to induce cholinergic dysfunction when injected into rat brain, a finding consistent with cholinergic deficits documented in AD[101-103]. The overall findings are consistent with the hypotheses that the mechanisms for energy production, protection from oxidative damage and improper protein clearance, and synapse integrity are disrupted and contribute to AD and PD pathogenesis. Animal models that take advantage of known genetic mutations in both familial and sporadic forms of AD and PD have shed light on important physiological processes that may be implicated in disease pathogenesis. Additional studies of this nature, that utilizes proteomics as a tool to understand the disease etiology, will help in the development of treatments to slow or prevent these devastating neurodegenerative disorders[104].

Proteomic analysis of Amylotorphic lateral sclerosis

It is a fatal neurodegenerative disease that affects motor neurons and atrophy. 10% is due to familial or inherited form while 20% is due to mutation in cu^{+2}/Zn^{+2} superoxide dismutase (SODI).

Mouse models

The Wobbler mouse is used[105] which has a spontaneous mutation in gene VPS 54 which is involved in vesicular trafficking[106]. A mutation in the SOD1 gene, particularly glycine at position 93 is replaced by alanine ($SOD1^{G934}$) leads to FALS[107]. This $SOD1^{G934}$is created in transgenic animals[108]. The pathological features observed in mSOD1 transgenic mice include specific & progressive loss of motor neurons, the formation of phosphorylated neurofilament inclusions[109], astrocytosis[110] and muscle atrophy. These features are different in different model of animals. Ex: accumulation of vacuoles was observed in enlarged axons of $SOD1^{G934}$, $SOD1^{G374}$ mice[111, 112] and in $SOD1^{G934}$ rats[113, 114] but not in several other mSOD1 mouse models[115].

In-vitro models

Cultures of motor neurons are used for molecular mechanisms of neurodegenaration studies and identify triggers that induce motor death. Immortalized mouse motor neuron cell lines like NSC34[116] are used as in-vitro models to study ALS. When P62 was co-expressed with SOD1 in NSC34, it greatly increases the aggregates with mSOD1[115]. But the expression of mSOD1, solely in neurons does not lead to ALS[117, 118]. It may be necessary to have mSOD1 in astrocytes or microglia cells to trigger ALS[119]. When a healthy neuron is surrounded by m SOD1 expressing non neuron cells then it undergoes degeneration and when mSOD1 expressing neurons are surrounded by healthy non neuron cells they survived[120].

Proteomic changes in models of ALS

In ALS animal models, alteration of protein chaperones is observed which leads to unfolding of protein formation of aggregate and non-degradation of miss-folded proteins. It may be due to dysregulation of sub groups of chaperones by m SOD1 in such animals. What may be significant, however, is the selectivity of a decrease in specific classes of chaperones including chaperonin sub-units 5, 6A, HSP70,HSP40 and the increase in HSP25, HSP27, protein disulfide isomerase etc.,

It is not only due to altered HSP expressions but also due to protein – protein interaction. Ex: interaction between HSP70 and to its co-chaperone and HSP27, HSP25 and α, β-crystalline which $SOD1^{G934}$. This leads to complex formation. This represents a second mechanism for mediating mSOD1 cytotoxicity. The third mechanism for ALS is oxidation of HSP70, HSP4, and disulfide associated proteins 4–hydroxy–2–non T enol modification in $SOD1^{G934}$ ·Oxidative PTM of specific amino acid in key proteins serves as spinal transduction mechanism[121]. Normally HSP 70 delivers the misfolded proteins to proteosome for degradation but its dysregulation alters its normal action. With m SOD1 over expression in ALS models, the demand for HSP 70 (as they are ATP mediated) and related protein chaperone function increases. So the accumulation of m SOD1, protein aggregation influences energy production. In ALS models proteins involved in glycolysis, tricorboxilic acid cycle and related energy metabolism show a pattern of down-regulation, suggesting a reduction in energy production available for the maintenance of normal motor neuron function.

CHALLENGES PROTEOMICS IN ANIMAL MODELS

Proteomics studies in animal models form the basis for the next step—the study of human patients. However, animal models particularly rodent models, are highly biased there are number of important challenges discussed below

1. Handling and storage of clinical samples need to be optimized and standardized. Freeze/thaw protocols need to be validated fully, particularly for the study of blood cells.
2. The enormous volume of data generated by proteomics platforms such as mass spectrometry and protein arrays will require creative advances in bioinformatics.
3. Inter- and intra-assay normalization for autoantibody profiling remains to be resolved, a task that is of the highest priority for the future.
4. Improvements in technologies, particularly more reproducible methods for printing proteins, and better methods for performing and quantitating mass spectrometry, are required.
5. The generation of high-affinity, non–cross-reactive antibodies is perhaps the biggest challenge facing the future development of reverse-phase protein microarrays. Without prior protein separation, the accuracy of the results depends solely on the specificity of the antibodies that are used.
6. Newly developed detection technologies using alternative fluorophores need to be validated further. Fluorophores that emit in the infrared range permit scanning at higher wavelengths.
7. The development of reagents such as MHC-tetramers for studying human antigen-specific immune responses may fundamentally alter our understanding of autoimmune disease pathogenesis[120-121].
8. Finally, Since the Determination of significant differences between mass spectrometry datasets from biological samples is one of the major challenges of proteome informatics.

CONCLUSION

Proteomics in an animal model is used to identify and understand the biochemistry of proteins. Proteomics is the foundation for constructing and extracting useful knowledge to biomedical research. This new direction promises greater understanding of disease pathogenesis, diagnostic tests and identification of disease biomarkers for treatment and prognosis. It is highly probable that some of these techniques will become accepted as diagnostic and prognostic tools in the future of clinical medicine. In this article we have reviewed animal models applied in various diseases and applications. These techniques have a unique strength in studying proteins in their native state.

REFERENCES

1. http://en.wikipedia.org/wiki/Proteomics
2. Gorg A, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. Proteomics. 2004, 4 (12), 3665-85.
3. Patrick Nef. Key animal models for the identification and validation of drug targets. DDT. 2001; 6 (15).

4. Wendy Rodenburg, Jeroen LA Pennings, Conny TM van Oostrom, Marianne Roodbergen, Raoul V Kuiper, Mirjam Luijten and Annemieke de Vries. Identification of breast cancer biomarkers in transgenic mouse models: A proteomics approach. Proteomics Clin. Appl. 2010; 4: 603–612.
5. Fella K, Gluckmann M, Hellmann J, Karas M, Kramer PJ, Kroger M. Use of two-dimensional gel electrophoresis in predictive toxicology: identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. Proteomics. 2005; 5: 1914–1927.
6. Stanley BA, Gundry RL, Cotter RJ, Van Eyk JE. Heart disease, clinical proteomics and mass spectrometry. Disease Markers. 2004; 20: 167–178.
7. Tang MK, Kindler PM, Cai DQ, Chow PH, Li M, Lee KK. Heart-type fatty acid binding proteins are unregulated during terminal differentiation of mouse cardiomyocytes, as revealed by Potomac analysis. Cell and Tissue Research. 2004; 316: 339–347.
8. Cutler P, Bell DJ, Birrell HC, Connelly JC, Connor SC, Holmes E, Mitchell BC, Monte SY, Neville BA, Pickford R. An integrated proteomic approach to studying glomerular nephrotoxicity. Electrophoresis. 1999; 20: 3647-3658.
9. Kanitz MH, Witzmann FA, Zhu H, Fultz CD, Skaggs S, Moorman WJ, Savage RE. Alterations in rabbit kidney protein expression following lead exposure as analyzed by two-dimensional gel electrophoresis. Electrophoresis. 1999; 20: 2977-2985.
10. Aicher L, Wahl D, Arce A, Grenet O, Steiner S: New insights into cyclosporine A nephrotoxicity by proteome analysis. Electrophoresis. 1998; 19: 1998-2003.
11. Anderson NL, Esquer-Blasco R, Richardson F, Richardson F, Foxworthy P, Eacho P. The effects of peroxisome proliferators on protein abundances in mouse liver. Toxicol. Appl. Pharmacol. 1996; 137: 75-89.
12. Giometti CS, Tollaksen SL, Liang X, Cunningham ML: A comparison of liver protein changes in mice and hamsters treated with the peroxisome proliferator Wy-14,643. Electrophoresis. 1998; 19: 2498-2505.
13. Nathan D M. Long-term complications of diabetes mellitus. N. Engl. J. Med. 1993; 328: 1676-1685.
14. Anderson HU, Jorgensen KH, Egeberg J, Mandrup-Poulsen T, Nerup J. Nicotinamide prevents interleukin-1 effects on accumulated insulin release and nitric oxide production in rat islets of langerhans. Diabetes. 1994; 43: 770-777.
15. Mandrup-Poulsen T, Bendtzen K, Nerup J, Dinarello CA, Svenson M, Nielsen JH. Affinity-purified human interleukin 1 is cytotoxic to isolated islets of langerhan. Diabetolgia. 1986; 29: 63-67.
16. Borg LA, Cagliero E, Sandler S, Welsh N, Eizirik D. Interleukin-1 beta increases the activity of superoxide dismutase in rat pancreatic islets. Endocrinology. 1992; 130: 2851-2857.
17. Chen MC, Schuit F, Pipeleers DG, Eizirik DL. IL-1beta induces serine protease inhibitor 3 (SPI-3) gene expressions in rat pancreatic beta-cells. Detection by differentially display of messenger RNA. Cytokine. 1999; 11: 856-862.
18. Anderson HU, Fey SJ, Larsen PM, Nawrocki A, Hejnaes KR, Mandrup-Poulsen T, Nerup J. Interleukin-1beta induced changes in the protein expression of rat islets: a computerized database. Electrophoresis. 1997; 18: 2091-2103.
19. Larsen PM, Fey SJ, Larsen MR, Nawrocki A, Andersen HU, kahlar, H, Hailmann, C, Voss MC, Roepstorff P, Pociot F, Karlsen AE, Nerup J. Proteome analysis of interleukin-1beta-induced changes in protein expression in rat islets of langerhans. Diabetes. 2001; 50: 1056-1063.
20. Christensen UB, Larsen PM, Fey SJ, Andersen HU, Nawrocki A, Sparre T, Mandrup-Poulsen T, Nerup J. Islet protein expression changes during diabetes development in islet syngrafts in BB-DP rats and during rejection of BB-DP islet allograftes. Autoimmunity. 2000; 32: 1-15.
21. Sparre T, Christensen UB, Moses Larsen P, Fey SJ, Wrzesinski K, Roepstorff P, Mandrup-Poulsen T, Pociot F, Karlsen AE, Nerup J. IL-1beta induced protein changes in diabetes prone BB rat islets of Langerhans identified by proteome analysis. Diabetologia. 2002; 45: 1550-1561.
22. Morand JP, Marcri J, Adeli K. Proteomic profiling of hepatic endoplasmic reticulum-associated proteins in an animal model of insulin resistance and metabolic dyslipidemia. J. Biol. Chem. 2005; 280: 17626-17633.
23. Sparre T, Reusens B, Chief H, Larsen MR, Roepstorff P, Fey SJ, Mose Larsen P, Remacle C, Nerup J. Intrauterine programming of fetal islet gene expression in rats-effected of maternal protein restriction during gestation revealed by proteome analysis analysis. Diabetologia. 2003; 46: 1497-1511.

24. Budde P, Schulte I, Appel A, Netiz S, Kellmann M, Tammen H, Hess R, Rose H. Peptidomics biomarker discovery in mouse models of obesity and type 2 diabetes. Comb. Chem. High Throughput Screen. 2005; 8: 775-781.
25. Barglow KT, Cravatt BF. Discovering disease-associated enzymes by proteome reactivity profiling. Chem. Biol. 2004; 11: 1523- 1531.
26. Link, C. D., Expressed of human beta-amyloid peptide in transgenic C. elegans. Proc. Natl. Acad. Sci. USA. 1995; 92: 9368-9372.
27. Drake J, Link CD, Butterfield DA. Oxidative stress precedes fibrillar deposition of AD amyloid beta-peptide (1-42) in a transgenic C.elegans model, Neurobiol. Aging. 2003; 24: 415-420.
28. Vanfleteren JR. Oxidative stress and ageing in C.elegens, Biochem. J. 1993; 292: 605-608.
29. Markesbery WR. Oxidative stress hypothesis in AD, Free Radic. Biol. Med. 1997; 23: 134-147.
30. Wu Y, Lou Y. Transgenic C. elegans as a model in Alzheimer's research. Curr. Alz. Res. 2005; 2: 37-45.
31. Boyd-Kimball D, Poon HF, Lynn BC, Cai J, Pierce WM Jr, Klein JB, Ferguson J, Link CD, Butterfield DA. Proteomic identification of proteins specifically oxidized in C. elegans expressing human A beta (1-42) implications for AD. Neurobiol. Ageing. 2006; 27: 1239-1249.
32. Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, Link CD, Luo Y. Amtloid-beta-induced pathological behaviors are suppressed by Ginnkgo biloba extract EGb 761 and ginkgolides in transgenic C. elegans, J. Neurosci. 2006; 26: 13102-13113.
33. Brown MK, EvansJL, Luo Y. Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in C. elegans, Pharmacol. Biochem. Behave. 2006; 85: 620-628.
34. Quin GG, Len AC, Billson FA, Gillies MC. Proteome map of normal rat retina comparison with the proteome of diabetic rat retina: new insight into the pathogenesis of diabetic retinopathy. Proteomics. 2007; 7: 2636-2650.
35. Ouchi M, West K, Crabb JW, Kinoshita S, Kamei M, Exp. Eye Res. 2005, 81, 176-182.
36. Garcia-Ramirez M, Canals F, Hernandez C, Colome N, Ferrer C, Carrasco E, Garcia- Arumí J, Simo R. Proteomic analysis of human vitreous fluid by fluorescence-based difference gel electrophoresis (DIGE): A new strategy for identifying potential candidates in the pathogenesis of proliferative diabetic retinopathy. Diabetologia. 2007; 50: 1294-1303.
37. Gao BB, Clermont A, Rook S, Fonda SJ Srinivasan VJ, Wojtkowski M, Fujimoto JG, Avery RL, Arrigg PG, Bursell SE, Aiello LP and Feener EP. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through perkallikrein activation. Nat. Med. 2007; 13: 181-188.
38. Pike SE, Yao L, Setsuda J, Jones KD, Cherney B, Appella E, Sakaguchi K, Nakhasi H, Atreya CD, Teruya-Feldstein J, Wirth P, Gupta G, Tosato G. Calreticulin, calreticulin fragments are endothelial cell inhibitors that suppress tumor growth. Blood. 1999; 94: 2461-2468.
39. Bhattacharya SK, Rockwood EJ, Smith SD, Bonilha VL, Crabb JS, Kuchtey RW, Robertson NG, Peachey NS, Morton CC and Crabb JW. Proteomics reveal Cochlin deposits associated with glaucomatous trabecular meshwork. J. Biol. Chem. 2005; 280: 6080-6084.
40. Zao X, Ramsey KE, Stephan DA, Russell P. Gene and protein expression changes in human trabecullar meshwork cells treated with transforming growth factor-beta. Invest. Opthalmol. Vis. Sci. 2004; 45: 4023-4034.
41. Picht G, Weldge-Luessen U, Grehn F, Lutjen-Drecoll E. Transforming growth factor beta 2 levels in the aqueous humor in different types of glaucoma and the relation to filtering bulb development. Graefes Arch. Clin. Exp. Ophthalmol. 2001; 239: 199-207.
42. Nakajima E, David LL, Bystrom C, Shearer TR, Azuma. Chaplain-specific proteolysis in primate retina: contribution of calpins in cell death. Invest. Ophthalmol. Vis. Sci. 2006; 47: 5469-5475.
43. Suter M, Reme C, Grimm C, Wenzel A, Jäättela M, Esser P, Kociok N, Leist M and Richter C. Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J. Bol. Chem. 2000; 275: 39625-39630.
44. Bressler NM, Bressler SB, Fine SL. Age-related macular degeneration. Surv. Ophyhalmol. 1988; 32: 375-413.

45. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc. Natl. Acad. Sci. USA. 2002; 99: 14682-14687.
46. Umeda S, Suzuki MT, Okamoto H, Ono F, Mizota A, Terao K, Yoshikawa Y, Tanaka Y, Yoshikawa Y, Tanaka Y. Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of muscular degeneration in cynomolgus monkey (Macaca fascicularis). FASEB J. 2005; 19: 1683-1685.
47. Feeney-Burns L, Hilderbrand ES, Eldridge S. Agring human RPE: morphometric analysis of macular, equatorial, and peripheral cells. Invest. Ophthalmol. Vis. Sci. 1984; 25: 195-200.
48. Holz FG, Bellmann C, Margaritidis M, Schutt F, Otto TP, Völcker HE. Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. Graefes Arch. Clin. Exp. Ophthalmol. 1999; 237: 145-152.
49. Godley BF, Sharmsi FA, Liang FQ, Jarrett SG, Davies S, Boulton M. Blue light induces mitochondria DNA damage and free radical production in epithelial cells. J. Biol. Chem. 2005; 280: 21061-21066.
50. Schutt F, Ueberle B, Schnolzer M, Holz FG, Kopitz J. Proteome analysis of lipofusin in human retinal pigment epithelial analysis of lipofuscin in human retinal pigment epithelial cells. FEBS Lett. 2002; 528: 217-221.
51. Souza GA, Godoy LM, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. Genome Biol. 2006; 7: R72.
52. Tsai PS, Evans JE, Green KM, Sullivan RM, Schaumberg DA, Richards SM, Dana MR, Sullivan DA. Proteomic analysis of human meibomian gland secretions. Br. J. Ophthalmol. 2006; 90: 372-377.
53. Li N, Wang N, Zheng J, Liu XM, Lever OW, Erickson PM, Li L. Characterization of human tear proteome using multiple proteomic analysis techniques. J. Proteome Res. 2005; 4: 2052-2061.
54. Fullard RJ, Tucjer DL, Changes in human tear protein levels with progressively increasing stimulus. Invest. Ophthalmol. Vis. Sci. 1991; 32: 2290-2301.
55. Salas-Auvert R, Colmenarez J, Ledo DH, Colina M, Gutierrez E, Bravo A, Soto L, Azuero S. Determination of anions in human and animal tears fluid and blood serum by chromatography. J. Chromatogr. A. 1995; 706: 183-189.
56. Mrugacz M, Kacmarski M, Bakunowicz-Lazarcyz A, Zelazowska B, Wysocka J, Minarowska A. IL-8 and IFN-gamma in tear fluid of patients with cystic fibrosis. J. Interferon Cytokine Res. 2006; 26: 71-75.
57. Grus FH, Sabuncuo P, Dick HB, Augustin AJ, Pfeiffer N. Changes in the tear proteins of diabetic patients of diabetic patients. BMC Ophthalmol. 2002; 2: 4.
58. Gachon AM, Lacazette E. Tear lipocallin and the eye's front line of defense. Br. J. Ophthalmol. 1998; 82: 453-455.
59. Fung KY, Morris C, Sathe S, Sack R, Duncan MW. Characterization of the in vivo forms of lacrimal-specific proline-rich proteins in human tears fluid. Proteomics. 2004; 4: 3953-3959.
60. Shoji J, Inada N, Sawa M. Antibody array-generated cytokine profiles of tear of patient with vernal kerato-conjugativitis or giant papillary conjunctivitis. Jpn. J. Ophthalmol. 2006; 50: 195-204.
61. Leonardi A, Curnow SJ, Zhan H, Calder VL. Multiple cytokinase in human tear specimens in seasonal and chronic allergic eye disease and in conjunctival fibroblast cultures. Clin. Exp. Allergy. 2006; 36: 777-784.
62. Wattiez R, Noel-Georis I, Cruyt C, Broeckaert F, Bernard A, Falmagne P. Susceptibility to oxidative stress: proteomic analysis of bronchoalveolar lavage from ozone-sensitive and ozone-resistant strains of mice, Proteomics.2003; 3 (5): 658–665.
63. Ray P, Chen L, Tyurin VA, Kagan VE, Witzmann FA. Proteomic and inducible transgenic approaches to study disease processes, Am. J. Respir. Cell Mol. Biol. 2003; 29 (3): 42–S46.
64. Gramolini AO, Kislinger T, Liu PP, MacLennan DH, Emili A. Analyzing the cardiac muscle proteome by liquid chromatography mass spectrometry (LC-MS) based expression proteomics. Methods in Molecular Biology. 2006; 357: 15–32.
65. Buscemi N, Murray C, Doherty-Kirby A, Lajoie G, Sussman MA, Van Eyk JE. Myocardial subproteomic analysis of a constitutively active Rac1-is expressing transgenic mouse with lethal myocardial hypertrophy. Am. J. Physiol. Heart Circ. Physiol. 2005; 289: 2325–33.
66. Sawicki G, Jugdutt BI. Detection of regional changes in protein levels in the in vivo canine model of acute heart failure following ischemiareperfusion injury: functional proteomics studies. Proteomics. 2004; 4: 2195–202.

67. Berhane BT, Zong C, Liem DA, Huang A, Le S, Edmondson RD, Jones RC, Qiao X, Whitelegge JP, Ping P, Vondriska TM. Cardiovascular-related proteins identified in human plasma by the HUPO Plasma Proteome Project pilot phase. Proteomics 2005; 5: 3520 –30.
68. Oscar Alzate, Syed-Rehan A. Hussain, Virginia M. Goettl, Arun K. Tewari, Francesca Madiai, Robert L. Stephens JR, Kevin V. Hackshaw. Proteomic identification of brainstem cytosolic proteins in a neuropathic pain model. Research report Mol. Brain Res. 2004; 128: 193–200.
69. Ayako Furukawa, Shinji Oikawa, Sanae Hasegawa-Ishii, Yoichi Chiba, Noriko Kawamura, Shiro Takei, Keisuke Yoshikawa, Masanori Hosokawa, Shosuke Kawanishi, Atsuyoshi Shimada. Proteomic analysis of the aging brain in SAMP10 mouse: A model of age-related cerebral degeneration. Mechanisms of Ageing and Development. 2010; 131: 379–388.
70. Fu Yang, Shikai Yan, Ying He, Fang Wang, Shuxia Song, Yingjun Guo, Qi Zhou, Yue Wang, Zhongying Lin, Yun Yang, Weidong Zhang, Shuhan Sun. Expression of hepatitis B virus proteins in transgenic mice alters lipid metabolism and induces oxidative stress in the liver. J. Hepatology. 2008; 48: 12–19.
71. Susanne N, Hermann E. Liver fibrosis: from animal models to mapping of human risk variants. Best Prac. Res. Cli. Gastroenterology. 2010; 24: 635–646.
72. Angela M, James A, Jose Russod, Coral A. Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A. J.Proteomics. 2010; 73: 1241 – 1253.
73. Zabel C, Andreew A, Mao L, Hartl D. Protein expression overlap: more important than which proteins change in expression? Expert Rev. Proteomics. 2008; 5 (2): 187–205.
74. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzhemer's disaese. Neurology. 1991; 41: 179-486.
75. Mattson MP, Gary DS, Chan SL, Duan W. Perturbed endoplasmic reticulum fuction, Synaptic apoptosis and the pathogenesis of Alzheimer's disease. Biochemical Society Symposium. 2001; 151-162.
76. Butterfield DA, Hensley K, Cole P, Subramaniam R, Aksenov M, Aksenova M, Bummer PM, Haley BE, Carney JM. Oxidatively assessed by analysis of spin label incorporation kinetics: relevance to Alzheimer's diseases. J. Neurochem. 1997; 68: 2451-2457.
77. Gahtan E, Overmier JB, Inflammatory pathogenesis in Alzheimer's disease: biological mechanisms and cognitive sequrli. Neurosci. Biobehav. Rev. 1999; 23: 615-633.
78. Poon HF, Hensley K, Thongboonkerd V, Merchant ML, Lynn BC, Pierce WM, Klein JB, Calabrese V, Butterfield DA. Redox proteomics analysis of oxidatively modified proteins in G93A-SOD1 transgenic mice- a model of familial amyotrophic lateral sclerosis.Free Radic. Biol. Med. 2005; 39: 453-462.
79. Mastres CL, Cappai R, Barnham KJ, Villemagne VL. Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics. J. Neurochem. 2006; 97: 1700-1725.
80. Butterfield DA, Drake J, Pocernich C, Casrtegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends Mol. Med. 2001; 7: 548-554.
81. Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, Albert MS, Hyman BT, Irizarry MC. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology. 2004; 62: 925-931.
82. Butterfield DA, Stadtman ER. Protein oxidation processes in aging brain. Adv. Cell Aging Gerontol. 1997; 2: 161-191.
83. Sies H, Cadenas E. Oxidativestress: damage to intact cells and organs, Philos. Trans. R. Soc. Lond. B Biol. Sci. 1985; 311: 617-631.
84. Mikkelsen RB, Wardman P. Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms, Oncogene. 2003; 22: 5734-5754.
85. Thannickal VJ, Day RM, Klinz SG, Bastien MC, Larios JM, Fanburg BL. Ras-dependent and independent regulation of reactive oxygen species by mitogenic growth factors and TGF-beta 1, FASEB J. 2000; 14: 1741-1748.
86. Aksenov MY, Aksenova MV, ButterfieldD A, Geddes JW, Markesbery WR. Protein oxidation in the brain AD, Neuroscience. 2001; 103; 373-383.
87. Baynes JW, Thorpe SR. Glycoxidation and lipoxidation in atherogenesis, Free Radi. Biol. Med. 2000; 28: 1708-1716.
88. Butterfield DA. Amyliod beta-peptide (1-42) induced oxidative stress and neurotoxicity: implications of nerodegeneration in AD brain, A review, Free Radic. Res. 2002; 36: 1307-1313.

89. Butterfield DA, Perluigi M, Sultana R. Oxidative stress in AD brain: new insights from redox proteomics, Eur. J. Pharmacol. 2006; 545: 39-50.
90. Nakamura K, Hori T, Sato N, Sugie K, Kawakami T, Yodoi J, Brégégère F, Milner Y, Friguet B. Redox regulation of a src family protein tyrosine kinase p56lck in T cells, Oncogene. 1993; 8: 3133-3139.
91. Smith MA, Perry G. AD disease: protein-protein interaction and oxidative stress, Bol. Estud. Med. Biol. 1996; 44: 5-10.
92. Staal FJ, Anderson MT, Staal GE, Herzenberg LA, Gitler C. Redox regulation of signal transduction: tyrosine phophorylation and calcium influx, Proc. Natl. Acad. Sci. USA. 1994; 91: 3619-3622.
93. Davies KJ. Degradation of oxidized proteins by the 20s proteosome, Biochimie. 2001; 83: 301-310.
94. Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease, Chem. Res. Toxicol. 1997; 10: 485-494.
95. Dalle-Donne I, Scaloni A, Butterfield DA. Redox proteomics: from protein modifications to cellular dysfunction and diseases, John Wiley and Sons, Hoboken, NJ 2006.
96. Schater E, Williams JA, Lim M, Levine RL. Differential susceptible of plasma proteins to oxidative modifications: examination by western blot immunoassay, Free Radic. Biol. Med. 1994; 17: 429-437.
97. Keiier JN, Hanni KB, Markesbery WR. Impaired proteosome function in AD, J. Neurochem. 2000; 75: 436-439.
98. Petropolus I, Conconi M, Wang X, Hoenel B, Brégégère F, Milner Y, Friguet B. Increase of oxidatively modified prtein is associated with a decrease of proteosome activity and content in aging epidermal cells, J, Gerontol. A Biol. Sci. Med. Sci. 2000; 55: 220-227.
99. Shringarpure R, Grune T, Sitte N, Davies KJ. 4-Hydeo-xynonenal-modified amyliod-beta peptide inhibits the proteosome: possible importance in AD, cell. Mol. Life Sci. 2000; 57: 1802-1809.
100. Starke-Reed PE, Oliver CN. Protein oxidation and proteolysis during aging and oxidative stress, Arch. Biochem. Biophys. 1989; 275: 559-567.
101. Butterfield DA. Beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications of AD, Chem. Res. Toxicol. 1997; 10: 495-506.
102. Rena AS, Joshua BO, Allan Butterfield D. Proteomics in animal models of Alzheimer's and Parkinson's diseases. Ageing Res. Reviews. 2009; 8: 1–17.
103. Duchen LW, Strich SJ. A hereditary motor neurone disease with progressive denervation of muscle in the mouse the mutant 'wobbler'. J. Neurol. Neurosurg. Psychiatry. 1968; 31: 535-542.
104. Schmitt-john T, Dreeper C, mussmann A, Hahn P, Kuhlmann M, Thiel C, Hafner M, Lengeling A, Heimann P, Jones JM, Meisler MH, Jockusch H. Mutation of Vps54 causes motor neuron disease and defective spermiogenisis in the wobbler mouse. Nat. Genet. 2005; 37: 1213-1215.
105. Daniel RR, Teepu Siddique, David Patterson, Denise AF, Peter Sapp, Afif Hentati, Deirdre Donaldson, Jun Goto, Jeremiah PO, Han-Xiang Deng, Zohra Rahmani, Aldis krizus, Diane Mckenna-Yasek, ANnarueber Cayabyab, Sandra MG, Ralph berger, Rudolph ET, John JH, Brian Herzfeldt, Raymond Van den bergh, Wu-yen hung, Thomas bird, Gang deng, Donald WM, Celestine smyth, NigelGL, Edwin soriano, Margaret AP, Jonathan haines, Guy AR, James S. Gusella, H. Robert Horvitz and Robert H. Brown. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amylotrophic lateral sclerosis. Nature. 1993; 362: 59-62.
106. Gurney ME, Pu H, Dhiu AY, Dal Canto MC. Motor neuron degeneration in ice that express a human Cu, Zn superoxide dismutase mutation. Science. 1994; 264: 1772-1775.\
107. Wrong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA, Sisodia SS, Cleveland DW, Price DL. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron diseases characterized by vascular degeneration of mitochondria. Neuron. 1995; 14: 1105-1116.
108. Bruijin LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW. ALS-linked SODI mutant G85R mediates damage to astrucytes and promotes rapidly progressive with SOD1-containing inclusions. Neuron. 1997; 18: 327-338.
109. Dal Canto MC, Gurney ME. Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. Am. J. Pathol. 1994; 145: 1271-1279.
110. Dal Canto MC, Gurney ME. A low expressor line of transgenic mice carrying a mutant human Cu, Zn superoxide dismutase (SOD1) gene develops pathological changes that most closely resemble those in human amyotrophic lateral sclerosis. Acta Neuropathol. (Berl). 1997; 93: 537-550.

111. Howland DS, Liu J, She Y, Goad B, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutantant-mediated myotrophic lateral sclerosis (ALS). Proc. Natl. Acad. Sci. USA. 2002; 99: 1604-1609.
112. Jonsson PA, Graffmo KS, Anderson PM, Brannstrom T, Lindberg M, Oliveberg M, Marklund SL. Disulphide-reduced superoxide dismutase-1 in CNS of transgenic amyotrophic lateral sclerosis models. Brain. 2006; 129: 451-464.
113. Morrison BM, Gordon JW, Ripps ME, Morrison J H. Quantitative immunocytochemical analysis of the spinal cord in G86R superoxide dismutase transgenic mice: neurochemical correlates of selective vulnerability. J. Comp. Nerol. 1997; 373: 619-613.
114. Cashman NR, Durham HD, Blusztajn JK, Oda K, Tabira T, Shaw IT, Dahrouge S, Antel JP. Neuroblastoma x spinal cord (NSC) hybrid cell lines resemble developing motor neurons. Dev. Dyn. 1992; 194: 209-221.
115. Lino MM, Schneider C, Caroni P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathway or motoneuron diseases. J. Neurosci. 2002; 22: 4825-4832.
116. Pramatarova A, Laganiere J, Roussel J, Brisebios K, Rouleau GA. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. J. Neurosci. 2001; 21: 3369-3374.
117. Wang J, Xu G, Slunt HH, Gonzales V, Coonfield M, Fromholt D, Copeland NG, Jenkins NA, Borchelt DR. Coincident thresholds of mutant protein for paralytic disease and protein aggregation caused by restrictively expressed superoxide dismutase cDNA. Neurobiol. Dis. 2005; 20: 943-952.
118. Clement AM, Nguyen MD, Roberts EA, Gracia ML, Boillée S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, Brown RH Jr, Julien JP, Goldstein LS, Cleveland DW. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science. 2003; 302: 113-117.
119. Hensely K, Mhatre M, Mou S, Pye QN, Stewart C, West M, Williamson KS. On the relation of oxidative stress to neuroinflammation: lesions learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. Antioxid. Redox Signal. 2006; 8; 2075-2087.
120. Steven m. Chan and paul j. Utz The Challenge of Analyzing the Proteome in Humans with Autoimmune Diseases Ann. N.Y. Acad. Sci. 2005;1062: 61–68.
121. Stella Elkabes and Hong Li Proteomic strategies in multiple sclerosis and its animal models Proteomics Clin Appl. 2007 ; 1(11): 1393–1405.