15.1

Proteomic and Post Translational Modifications Study of Mouse Brain Endothelial Cells Impaired by Oxidized Low Density Lipoprotein

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Oxidized-low density lipoprotein (ox-LDL) is considered as a key inducing factor in the pathogenesis of atherogenesis. Ox-LDL activates endothelial cells, causes endothelial dysfunction and generates disturbances in coagulatory and anticoagulatory factors. The roles of LDL and ox-LDL have been well studied and high concentrations of either substance can produce impairment of endothelial cell function(s). Proteins post translational modifications (PTMs) changes, especially phosphorylations, have been suggested to be one of the mechanism(s) of these impairments. In this study, a comparative analysis of the proteome between normal and ox-LDL-exposed mouse brain endothelial cell line, bEND3, was carried out. 36 up-regulated and down-regulated proteins were found. Spot shift differences between normal and ox-LDL-impaired bEND3 cells were also noticed in the two dimensional gel electrophoresis, indicating the involvement of PTMs in the mechanism(s) of the impairment. These findings would increase our understanding of the molecular mechanism(s) of the development of atherogenesis, and might result in the development of biomarkers for diagnosis and new medication for the disease.

15.2

Differential Protein and Neuropeptide Expression in Experimental Parkinson's Disease

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Treatment of mice with MPTP is one of several models used to mimic Parkinson's disease (PD) in humans. First, we have developed a peptidomic approach to study a large number of neuropeptides and employed it to an investigation of the endogenous neuropeptide content of PD. Secondly, two-dimensional fluorescence difference gel electrophoresis (DIGE) was used to pinpoint significant differences in protein abundance. Finally, direct molecular imaging of the biological samples using MALDI mass spectrometry (MS) was shown to be a powerful tool for investigating the spatial distribution of peptides and proteins directly on tissue samples. The 2D DIGE analysis detected >3000 proteins and demonstrated significant changes in >50 striatal proteins following dopamine depletion using MPTP in mice. The peptidomics approach was developed using only mg quantities of brain tissue and approx. 600 endogenous neuropeptides were detected in a single analysis. The comparisons of neuropeptides revealed several differences in relative abundance both between the different animal groups and the different brain regions. Several of these neuropeptides were identified as novel. Further, our results show that MALDI MS imaging directly on tissue slices provides unique information regarding the peptide and protein expression in experimental models of PD. For example, dopamine depleted mice showed an increase in the expression of cytochrome C and cytochrome C oxidase, and that increased expression is most intense in the cortex. The present study demonstrates that protein profiles obtained from specific brain regions using DIGE and MALDI MS imaging combined with data from a peptidomic approach allow for the study of complex biochemical processes such as those occurring in experimental PD.

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15.3

Atomic Force Microscopy in Proteomics

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The major problem of the present-day proteomics lies in the absence of the PCR-like reaction; hence the impossibility of copying various molecules, which in turn makes it impossible for the investigator to enhance the concentrations of the assayed biological material. Thus, there arises a methodological barrier in proteomic analyses: the protein molecules occurring in the biological material with concentrations below 10^{-15} cannot be identified easily. It is very actually for tissue proteins, whose concentrations upon their leakage into plasma are lowered manifold and the required limit of diagnostic sensitivity lies in concentrations underfemtomolar. Latest nanotechnological achievements allow such tasks to be effectively solved. These approaches involve construction, instead of concentration detectors whose resolution is not higher than 10^{-15}M, of molecular detectors, counting individual molecules and their complexes. One of these is atomic force microscopy (AFM). Usage of AFM biochips with various types of immobilized macromolecules (proteins, nucleic acids and others) allows the investigator to simultaneously diagnose a variety of diseases through registering the probe/target complexes. The examples of identification of single molecules and their complexes in hepatitis B and C systems, glycodeline system, 3-component cytochrome P450-containing systems by use of atomic force microscopy are given. Such an approach will enable to enhance the concentration sensitivity of the AFM in the sample volume up to a single-molecule level.

This work was supported by the programme "Medical Diagnostic Systems of the Future," by the NSch 325.2003.4, by INTAS Grant # 01-470.

15.4

Genomic and Proteomic Alterations Indicate a Role for PTEN and Akt in Epilepsy Associated Focal Cortical Dysplasias

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Focal cortical dysplasia with Taylor type balloon cells (FCD_{IIb}) constitutes a frequent and histopathologically distinct finding in patients with pharmacoresistant focal epilepsies. Recent data indicate a pathogenetic role of *TSC1*, known to be mutated in Tuberous Sclerosis (TSC), for FCD_{IIb}. TSC1 represents a key factor in the phosphatidylinositol 3-kinase (PI3K) pathway. In order to further elucidate the molecular pathology of FCD_{IIb}, we have analyzed expression profiling patterns and subsequently two additional major components of the PI3K-cascade in FCD_{IIb}, i.e. *PTEN* and *Akt* which operate upstream of TSC1.

Mutational screening of *PTEN* was performed by single-strand conformation polymorphism analysis (SSCP) in 37 FCD_{IIb} compared to 100 controls. Immunohistochemistry with antibodies against *phospho-Akt* (Ser473) was carried out in FCD_{IIb} ($n = 37$).

We found a silent mutation of *PTEN* in exon 2 ($n = 2/37$ vs. 1/100 controls); a polymorphism in exon 8 ($n = 1/37$ vs. 0/100 controls); as well as another mutation, i.e. amino-acid exchange at position 278 (exon 8) with replacement of phenylalanine by leucine (F278L) in one patient, but not in controls. Using laser assisted microdissected cell samples, this alteration was only found in FCD_{IIb} components but not in adjacent CNS tissue.

We demonstrated an increased immunoreactivity for *phospho-Akt* in balloon cells and dysplastic neurons, but not in adjacent normal CNS tissue. These data demonstrate alterations of the PI3K pathway components *PTEN* and *Akt* in FCD_{IIb}. This is in line with the hypothetical role of the PI3K cascade in focal cortical dysplasias with Taylor type balloon cells. Current proteome analyses of will provide further insights into the pathogenesis of FCD.

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15.5

Changes in Expression Profiles of Sub-Proteome in Rat Hippocampal CA1 Region After Forebrain Ischemia

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To explored the proteomic change in CA1 subregions of rat hippocampus between sham-operation rats and rats subjected to 15 min forebrain ischemia, we established an improved microdissection method and sub-proteomic profiling analysis for CA1, by using protein solubility-based sequential sample extraction and modified two-dimensional electrophoresis, which displays cytosolic or membrane proteins/peptides respectively. CA1 membrane proteins had been 2.5 fold enriched, which contribute to the more detailed integrated map containing more than 2500 spots. After analyzing using professional software, we found 3 membrane protein spots disappeared, while 3 cytosolic protein spots produced after ischemia-reperfusion injury, which represent proteins maybe involved in the mechanism of ischemic injury, These spots were not found in existed database of mouse brain proteome and that of neural stem cell from adult rat hippocampus, and remaining further confirmed with identification of mass spectrometry and some other manners. At the same time, sequential extraction was proved to be a more effective way to show enriched membrane protein/peptides, which play important roles in cell events, such as signal transduction.

15.6

Differential Gene Expression Profiles in the Hippocampus of Senescence-accelerated Mouse

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The senescence-accelerated mouse (SAM) is a useful animal model in the studies of senescence and age-associated disorders due to its inherited aging phenotype. The typical feature of SAM/prone8 (SAMP8), a substrain of SAM, is the early onset of learning and memory deficit during its aging process, while SAM/resistant1 (SAMR1) shows normal aging process. To screen and identify genes that render the impairment of learning and memory with aging, suppression subtractive hybridization (SSH) was used to isolate the differentially expressed genes in the hippocampus of 12-month-old male SAMP8 and age-matched SAMR1. The forward library, cDNA from SAMP8 as tester and cDNA from SAMR1 as driver, includes 864 colonies, and the reverse library includes 960 colonies. The positive ratio of libraries evaluated by PCR was 96.18% and the length of cDNA fragments was ranged from 250 to 2000 bp. Some SSH inserts from two subtractive cDNA libraries were arrayed onto glass by use of robotic printing. The prepared microarray contains 3136 cDNAs, including 1536 cDNAs of different clones in the two SSH libraries, and cDNA segments of β-actin and G3PDH genes as reference. SSH and cDNA microarray hybridization were combined to identify genes that differentially expressed in the hippocampus of SAMP8 and SAMR1. Of all 91 differentially expressed genes, 50 were up- and 41 were down-regulated in SAMP8, and these differentially expressed genes involved in the structures or functions of mitochondrion, purine metabolism, cell skeleton, transcription factors, the Ser/Thr family of protein kinases, the Tyr family of protein kinases, the protein-tyrosine phosphatase family, the Rab family and other signal transduction. Some of the identified genes were tested and confirmed by real time Quantitative fluorescence RT-PCR. Besides the functional known genes, 38 functional unknown genes were also identified. These result indicated that the profiles of gene expression in the hippocampus of SAMP8 and SAMR1 were significant different, which may play important roles in the age-related deficit of the learning and memory in SAMP8, suggesting those genes related to the learning and memory deficient or pathology change in brain of SAMP8 may be potential gene targets for AD therapy.

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15.7
The Human Pituitary Proteome

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We hypothesize that the proteome differs between human pituitary controls and macroadenomas. Comparative proteomics tools have been used to clarify those differences, and to characterize the differentially expressed proteins that hold clues to the molecular mechanisms of macroadenoma formation. The specific focus of this research grant program is human pituitary macroadenomas (>10 mm in diameter) because little is known about their formation, and no hormones are secreted in excess to readily signal their presence. A broader goal is adenomas [microadenomas (<10 mm) and macroadenomas]. We have combined the use of genomics, transcriptomics, and proteomics to study the human pituitary and adenomas. A stable, robust, and reproducible 2D gel-based comparative proteomics system was used for the proteomics study, and a liquid chromatography (LC)-tandem mass spectrometry (MS/MS)-immobilized metal affinity column (IMAC) analytical system for the phosphoproteome. The gel also archives precious human tumor tissue samples. Each differentially expressed protein (DEP) was characterized in an effort to elucidate the basic molecular mechanisms, to develop a biomarker, and to develop effective pharmacological agents. We combine, on the same pituitary tissue sample, comparative proteomics data with the comparative transcriptomics/genomics data from our collaborative research team at Emory University.

15.8
Brain Proteomics

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About 30–50% of the genes in mammals are expressed in the nervous system. A differential expression of genes in distinct patterns is necessary for the generation of the large variety of neuronal phenotypes. Proteomic analysis of brain compartments is useful to understand the complexity, to investigate disorders of the central nervous system, and to search for corresponding early markers. We applied proteomics technologies and studied the identity and levels of human and rat brain proteins as well as changes of their levels and modifications that resulted from various disorders, like Alzheimer's disease and Down syndrome in humans and in animal models of neurological diseases. Up to now, in our laboratory, approximately 500 human brain samples and 150 rat brain samples have been analyzed by two-dimensional electrophoresis and more than 1000 different human and 1000 rat brain gene products have been identified. The proteins, for which altered levels in these disorders have been observed, exert mainly neurotransmission, guidance, and signal-transduction functions, or are involved in detoxification, metabolism, and conformational changes. Some of those proteins may be potential drug targets. We also analyzed fetal brain from controls and subjects with Down syndrome to investigate protein changes in neurodegenerative diseases early in life. Further improvement of proteomics technologies to increase sensitivity and efficiency of detection of certain protein classes is necessary for a more detailed analysis of the brain proteome. The major findings that resulted from the application of proteomics in the investigation of the brain and the potential and limitations of the current technologies will be discussed.

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15.9

Goals and Aims of the HUPO Brain Proteome Project

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The Human Proteome Organisation HUPO was established three years ago with the overall aim of analyzing the human proteome. To handle this huge challenge, different projects were launched focusing on distinct human organs, among others the Human Brain Proteome Project HBPP by German scientists. Within the following months the HBPP members identified interested key persons, techniques and strategies, condensing to a critical workable mass of more than 200 interested scientists. The established committees stated that this project will track the following goals:

Vision: Towards an understanding of the pathological processes of the brain proteome in neurodegenerative diseases and ageing.

Mission: The HUPO Human Brain Proteome Project is an open, international scientific initiative under the umbrella of HUPO. The consortium welcomes all adequate groups and laboratories that are willing to contribute to the goals of HUPO BPP. These goals are:

- Defining and deciphering the normal brain proteome including polymorphisms, modifications, histological localization as well as identification of brain derived proteins in bodily fluids.
- Correlation of the expression patterns of brain proteins and mRNA.
- Identification of disease-related proteins involved in neurodegenerative diseases and ageing by differential protein expression profiling and by techniques and methods available within the participating groups.
- Validation and functional characterization of these proteins by techniques and methods available within the participating groups leading to biomarkers for diagnosis and therapeutic targets.
- The focus will be on Alzheimer's Disease (AD including Down-Syndrome), Parkinson's Disease (PD) and Ageing including corresponding mouse models. However, the elucidation of other neurodegenerative diseases is not less important and can be part of the HUPO HBPP scientific program.
- Besides scientific research the establishing of Training and Education programs to inform the public as well as to propagate techniques and methods used in proteomic research is another important part of the HBPP work.
- Set up of a comprehensive neuroproteomic data base accessible to all participating laboratories and the scientific community.

In addition, two pilot studies have been launched with a differential proteome analysis of normal mouse brain (3 different age brackets) as well as a differential proteome analysis of human brain from biopsies and autopsies involving more than 20 scientific groups.

15.10

Proteomic Analysis of Human Fetal and Adult Cerebel

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The proteomic analysis has a strong ability to reveal the mechanism of human brain development, aging and diseases. The comparison of the human fetal and adult cerebel was seldom investigated. Proteomic strategy was employed to study the protein expression differences in the fetal and adult cerebel. In this work, 134 proteins were identified after two dimensional separation and MALDI-TOF-TOF MS/MS identification in which 12 were hypothetical proteins. Both alpha-tubulin 1, the expression of which was gradually lowered to a rather low level until 34 weeks, and beta tubulin, which was almost not expressed at the middle stage of fetal brain development (about 26 weeks), were found highly expressed at the early development stage of fetal cerebel (<16 weeks). This indicated that tubulins played important roles in cell division in fetal cerebel especially at the early development stage. Some proteins, including glial fibrillary acidic proteins, aconitase, alphaenolase, phosphatidylethanolamine-binding protein (PEBP), uracil DNA glycosylase, Mn-superoxiddismutase, creatine kinase precursor, L-lactate dehydrogenase, triosephosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase were expressed much higher in the adult cerebel compared with the fetal one (<16 weeks). Glial fibrillary acidic proteins hardly expressed at the early and middle development stage in fetal cerebel but drastically increased at the late development stage (about 34 weeks) at which the expression level was still far lower than in the adult one. Some house-keeping proteins, such as actin, hemoglobin alpha chain, hemoglobin beta chain, antioxidant protein 2, pyruvate kinase M1, and the metabolic enzymes in TCA were highly expressed at any development stage in the fetal and adult cerebel. In summary, at the early development stage (<16 weeks) of fetal cerebel, the structural proteins was dominant while the enzymes were low; whereas in the adult cerebel and fetal cerebel at the late development stage (about 34 weeks), the amount of enzymes was much higher than structural proteins. These findings may be useful in understanding the molecular mechanism of brain development, aging and diseases.

15.11

The Dynamic Characteristics of Signalling Pathways in Neuron Stimulated by EGF

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Aim: To analyze the dynamic characteristics of the EGF signalling pathways stimulated by EGF in neuron. Methods: Cultured neuron were labeled with [^{32}P]orthophosphate (2.78×10^6 Bq/ml) for 1.5 h and stimulated with EGF (20 nm/L) for 0, 5, 20, 60, 120 min. Reactions were terminated by freezing neuron in liquid nitrogen prior to solubilize in a lysis buffer containing 8M urea, 4% CHAPS, 2% Bio-lyte, pH 3–10). Protein concentrations were determined with Bio-Rad DC Protein Assay kit. The ^{32}P-labled lysates isoelectrically focused on IPG Drystrip pH 4–7 linear gels and subsequently separated by second-dimensional SDS-PAGE. The dried gel was autoradiographed for 5days at –70°C with an intensifying screen. The autoradiography phosphoprotein images were analyzed by PDQuest 2D software, the phosphoprotein spots were evaluated qualitatively with matching to SWISS SPORT protein database. The time courses of MEK1, ERK2, PKC-α and PI3-kinase p85λ-subunit were plotted according to the quantitatively analysis. Result: Autoradiography of the 2-DE-separated ^{32}P-labled neuron lysates revealed more than 100 phosphoproteins. Responses to EGF are marked by more increased labeling of the constitutive phosphoproteins than the appearance of new phosphoproteins. The qualitative analysis of 40 protein spots reveals that various protein phosphorylation modifications are involved in EGF signalling transduction in neuron, including protein kinase such as MEK, ERK, JNK3, PI3K, PKC, CDK, *et al*., protein-tyrosine phosphatase, transcription factor, translation initial factor, intracellular signal molecular, receptor for neurotransmitter, nuclear protein. From these spots, 17 proteins are relevant with cellular signaling transduction, and the levels of phosphorylation are changed with EGF stimulation. The time couses of MEK1, PKCα, ERK2 and PI3-kinase p85λ-subunit phosphorylation modification demonstrated that the maximal peak appears at 5 min for MEK1 and PKCα, but at 20 min for ERK2, 60 min for PI3K p85–2P. Conclusion: The EGF signalling transduction in neuron has a signal integration and a complex self-adaptive control character.

15.12

Fractionation and Characterization of the Mouse Brain Mitochondrial Proteome

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Mitochondrial dysfunction is associated with many neurodegenerative diseases including Alzheimer's and Parkinson's disease. Proteomic studies of genetically-modified mice have revealed protein complexes within the mitochondria that are particularly susceptible to mitochondrial-generated free radicals. An understanding of the role of mitochondria in normal cell function and in disease progression can be enhanced through the identification of all mitochondrial proteins as well as their post-translational modifications. Liquid phase fractionation of mouse brain mitochondrial proteins based on isoelectric point and subsequent 2D gel analysis using both phosphospecific and general protein stains combined with MS analysis has revealed numerous mitochondrial phosphoproteins as well as non-phosphorylated proteins.

15.13

Combined Proteome and Transcriptome Analysis of Alterations in the Mouse Brain Caused by Huntington's Disease

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Within the German Human Brain Proteome Project a multitude of different technologies for analysing the proteome and transcriptome of brain tissue in a parallel high through-put fashion have been developed. In this contribution we present the development of a novel, brain specific, proteome derived DNA microarray based on long oligonucleotides. The oligonucleotides arranged on this microarray were designed to detect transcripts encoding for proteins identified in brain extract.

This novel DNA microarray is dedicated to analyse the transcriptome of brain tissue characterised and quantified in parallel within our consortium by two dimensional gel electrophoresis and mass spectroscopy.

We will present first results of our transcriptome analysis of brain tissue derived from a well characterised Chorea Huntington model mice[1] in relation to control mice. The same brains, extracted from model mice and controls, were analysed with expression profiling and two dimensional gel electrophoresis. Our approach allows a direct comparison of the transcriptome data of the HD brain tissue and the data obtained from the proteome analysis of the same brain tissue and taking the unique origin of each sample and control into consideration, and therefore will lead to a better functional understanding of the mechanisms underlying the effect of Huntington's disease.

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15.14

Proteome Analysis of Molecules Enriched in Growing Axons of the Rat Developmental Brain

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Neurons in the brain form elaborate networks by extending axons. The purpose of the present study is to identify molecules enriched in growing axons of developmental brain. Such molecules may be involved in the formation and maintenance of neuronal networks. We recently developed a high resolution two-dimensional gel electrophoresis (2-DE) system, which utilizes a 93 cm × 103 cm large size gel and detects about 11,000 protein spots expressed in a dynamic range of $1–10^5$ (1). Using this system, we performed two types of proteome analysis in cultured rat hippocampal neurons. One is the screening of proteins enriched in axons, and 200 positive protein spots were detected by the screening of 5,164 proteins. The other is the screening of proteins up-regulated during the initial step of axon formation (from stage 2 to stage 3); 277 positive protein spots were detected out of 6,197 proteins. Mass spectrometric analysis identified molecules detected by these screenings. As an example, we will report a novel protein, the expression level of which increased remarkably during the period of axonal formation and decreased during the period of synapse formation. In addition, this protein was highly concentrated at the tips of axonal growth cones of stage 3 neurons and the overexpression led to formation of multiple axons. These results suggest that the novel protein plays important roles in neuronal network formation.

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15.15

Protein Translation in Axons During Neural Network Formation

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Axonal proteins of neuronal cells have been thought to be synthesized in the soma and then transported to the axon. However, recent identification of axonal mRNA indicated that some proteins may be locally translated in the axon. Cytoskeltal proteins like neurofilament, actin, and tubulin, were detected as axonally translated proteins. In the *Drosophila* egg, *nanos* mRNA localizes to the posterior pole and the local protein synthesis plays a crucial role in the anterior-posterior body axis. *ASH1* mRNA localizes in the bud tip of budding yeast during asymmetric cell division. *Actin* mRNA localization in the leading edge is important for cell motility and polarity in chicken embryo fibroblast. Although the local protein translation may play an important role in the formation and the maintenance of cell polarity in many organisms, little is known about protein translation in the neuronal axons during the development of the central nervous system. In order to detect the proteins translated in axons, we performed 1) [^{35}S] metabolic labeling of isolated axons from immature neurons and somatodendrites, and 2) differential display between axon-derived and somatodendrite-derived samples by using two-dimensional gel electrophoresis. Surprisingly our result suggested that various proteins were commonly translated in both the axons and the somatodendrites. Furthermore, our data suggested that several proteins are specifically translated in the axons.

15.16

Protein Expression of Rat Brain, Serum, and Spleen Exposed to Chronic Cold Stress by Proteomic Analysis

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Stress response is composed of alterations in behavior, autonomic function and the secretion of multiple hormones. Many psychiatric disorders, including depression, post-traumatic stress disorder and other anxiety disorders with increasing hormones and unknown factors, result from an interaction between genetic factors and exposure to a sufficiently sensitizing environmental stressor. To study changes of the protein expression levels in rat brain, serum, and spleen exposed to chronic cold stress (CCS), male Sprague-Dawley rats were housed in a cold room maintained at ambient temperature of 4 °C during 1 week. In this literature, we measured that the level of adrenocorticotropic hormone (ACTH) and corticosterone concentrations were determined by radioimmunoassay (RIA) in the serum, and the mRNA expression levels of corticotropin-releasing factor (CRF) and ACTH were examined by RT-PCR in the brain to validate the CCS. Proteome profiles of rat brain, serum, and spleen whether exposed to the stress or not were compared by 2-dimensional electrophoresis (2-DE), MALDI/MS/MS, nano-LC/MS/MS. Several proteins showed significantly different expression level, and some of them might be correlated with neuro-immune system due to exposure of CCS.

15.17

Genetic Polymorphisms of Brain Proteins

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Polymorphisms in DNA and proteins are used in genetic research as a fundamental tool to map genes on the chromosomes, to study the genetic variability in populations and to compare organisms under the aspect of evolution. More recently it became obvious that polymorphisms in many cases may have an influence on the penetrance of certain diseases. Large-scale analysis are in progress to detect single nucleotide polymorphisms (SNPs) associated with distinct diseases. However, it might be of even greater interest to study under this aspect protein polymorphisms which allow the detection not only of sequence variations, but also of genetic variations in expression levels, pre- and post-translational modifications and formations of isoforms. At present two-dimensional electrophoresis (2-DE) of proteins is the only method useful in detecting protein polymorphisms and able to reflect all the types of variations just mentioned. We studied the polymorphisms occurring in brain proteins between the two mouse species *mus musculus* and *mus spretus*. In our previous work we have shown that protein polymorphisms can be used to map modifier loci on the chromosomes. We now investigate the polymorphisms under three other aspects: Frequency in different organs, occurrence like disease-related protein changes in mouse models for neurodegenerative diseases, and effects of polymorphisms and newly occurring mutations on the regulatory network of the cellular proteome. The results will be presented.

15.18

Comparative Proteomic Analysis of Hippocampus in Morphine-dependent Rats

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Chronic exposure to opioids induces dependence, which greatly limits their clinical use. Many studies suggest that the neuroplasticity of hippocampus play an important role in opioid dependence. Comparative proteomic analysis was performed in hippocampus of morphine-dependent Wistar rats. Morphine was injected in rats (10 - 50 mg/kg, sc, tid) for 7 d. And naloxone (5 mg/kg, ip) induced significant withdrawal signs in morphine-dependent rats, which indicated the animal model of morphine dependence was successful . The proteins from the hippocampus were analyzed by two-dimensional electrophoresis and identified by matrix-assisted laser desorption/ionization-mass spectrometry on the basis of peptide mass fingerprinting, following in-gel digestion with trypsin. A total of 33 different proteins were identified, including 14 proteins not being reported in opioid dependence. They are involved in various cellular functions and are mainly localized in the cytosol and in mitochondria. They are related to energy metabolism, signaling transduction, gene transcription, protein fold and degradation, ion transport, cytoskeleton assembly, and so on. Some of them contribute to neuron protection, learning and memory. These results avail to demonstrate the neurobiological basis of opioid dependence.

15.19

Dynamics of Rat Brain Synaptic Membrane Proteome

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Glutamatergic synapses form the major excitatory neurotransmission pathways in the brain, and drive the activity of vast number of neurons. Many of these synapses undergo neuronal activity dependent changes in synaptic efficacy, which are considered as the molecular events that underlie memory and learning. These changes are known to involve post-translational modifications of the synaptic proteins, and the alteration of protein composition.

The molecular description of the synapses and their activity dependent dynamics are demanding because this organelle contains high number of membrane proteins and high degree of protein structural and functional interactions. In the first instance we have defined the proteome of the synapses, using a combination of 2-D gel electrophoresis and LC-MSMS approaches. We then examined the drug of abuse-induced changes of synaptic proteins in rat brain, using iTRAQ reagents from Applied Biosystems for relative quantitation of the peptides. Synaptic membranes from different experimental groups were separately digested by trypsin, and then tagged with iTRAQ reagents. The samples were pooled together, and the peptides fractionated by 2-D liquid chromatography. In the second LC step fractions were collected off-line on a metal target every 15 sec, and up to 25 peptides per LC fraction were submitted to tandem mass spectrometry. Our data reveals that iTRAQ labeling of proteins is simple, yields quantitative results, and increases the sensitivity of the MSMS measurement substantially. We are now studying the functional implications of the protein regulation.

15.20

Identification of Sensory Specific Proteins of Morphine-dependent Rats

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The central feature of drug addiction is compulsive drug use—loss of control over apparently voluntary acts of drug seeking and drug taking. Direct biological effects of morphine action and compensatory reactions of neurons jointly compose the molecular basis for morphine abuse and underlie the phenotype of reinforcement effect, tolerance, dependence and abstinence of morphine treatment.

Neurons in dorsal root ganglia (DRG) are termed "primary afferent neurons" and are known to transduce a variety of sensory modalities. Since opioid receptors are located on the terminals of small-diameter primary afferents and on projection neurons and interneurons of the superficial dorsal horn, the primary afferent and/or the postsynaptic neuron may be the primary locus of the compensatory response to chronic opioid administration.

In the present study, we conduct the proteome analysis of DRG protein expression following chronic morphine treatment in rats. The DRG extracts were separated by high resolution two-dimensional electrophoresis (2-DE) and over 100 protein spots were able to be visualized by sliver staining. As compared with the saline control rats, the significant changes in protein expression were observed in rats following the chronic morphine treatment. Protein spots excised from 2-D gels were further subjected to in-gel digestion with trypsin, and the resulting peptides were characterized by MALDI-TOF-MS and database searching. The two specific spots were aldolase C and proteasome component C8. Our data from proteome analysis clearly indicate that the morphine treatment would induce significant alteration of protein expression in DRG and the expression of some of those proteins might adapt to the chronic morphine treatment.

16 male Wistar rats were randomly divided into control group and morphine group, 8 in each group. To establish animal model of morphine dependence, the rats were subcutaneously injected with morphine. The aldolase C level in DRG was significantly higher in morphine-dependent rats than in normals rats. The relative amount of aldolase C in locus Ceruleus was lower in morphine-dependent rats than in normals rats. There was no significant difference in other nucleus between the two groups. The level of aldolase C are altered in some brain region of morphine-dependent rats, suggesting that the regulating of aldolase C expression in some brain regions may be involved in the development of opioid dependence.

15.21

Tid1 Acts as a Signaling Protein and as a Chaperone Protein for the Trk Family of Receptor Tyrosine Kinases

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Through Trk receptor tyrosine kinases, neurotrophins stimulate a variety of cellular responses by multiple pathways. Five intracellular tyrosine residues (Y^{499}, Y^{679}, Y^{683}, Y^{684} and Y^{794}) are phosphorylated in response to neurotrophin stimulation and as such serve as docking sites for the recruitment of signalling proteins to form an active receptor complex. Thus in the active TrkA receptor complex the signalling adapter proteins Shc and FRS2 bind at pY^{499}, while PLCγ-1 and the Csk Homology Kinase (CHK) bind to pY^{794}. In a yeast two-hybrid screen, we identified Tid1, *t*umorous *i*maginal *d*isc 1, as a novel Trk receptor binding protein. In this study, we report that Tid1 associates with Trk receptor tyrosine kinases and facilitates NGF-induced neurite outgrowth. Protein-protein binding assays and transfection studies have shown that Tid1 binds to Trk at the activation loop ($Y^{683}Y^{684}$ in rat TrkA) and that Tid1 is tyrosine phosphorylated by Trk. Moreover, Tid1-Trk co-immunoprecipitation and Tid1 tyrosine phosphorylation are detected in neurotrophin-stimulated primary mouse cortical neurons. Further characterization revealed that the C-terminal portion of Tid1 mediates binding to Trk. Over-expression studies have shown that both $Tid1_L$ and $Tid1_S$ significantly facilitate NGF-induced neurite outgrowth in nnr5 cells expressing TrkA through increased activation of mitogen-activated protein kinase. Tid1 constitutively binds the endogenous adapters, APS and Grb2, and the chaperone proteins, HSP70/HSP90, indicating that Tid1 is involved in multiple signaling pathways.

Further studies have shown that the siRNA mediated knockdown of endogenous Tid1 significantly reduces the cellular response to NGF in nnr5-TrkA cells. Unlike ShcB, an adaptor protein that binds to the mature form of Trk receptor, Tid1 preferentially binds to the immature form of TrkA. Furthermore, fractionation studies show that Tid1 colocalizes with both mature and immature forms of TrkA in the endoplasmic reticulum and other organelles of nnr5 cells. Tid1 knockdown reduces TrkA expression on the cell surface and significantly delays formation of immature TrkA in nnr5-TrkA cells. Taken together, we show that Tid1 has dual functions, i.e., as a signaling protein for TrkA signal transduction and as a chaperone protein involved in the processing of the Trk family of tyrosine kinases.

15.22

Alteration in Protein Expression in the Hippocampus of Morphine-treated Mice

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Hippocampus plays an important role in the rewarding responses and drug-seeking behavior. Chronic opiate treatment results in an alteration of synaptic plasticity in hippocampus, which contributes to develop opiate dependence. The maladaptive synaptic plasticity in hippocampus following chronic opiate treatment might involve changes in protein expression. To identify potential proteins that might be implicated in this effect, Proteomic analysis were performed on the hippocampus derived from morphine-dependent mice. Image analysis of silver-stained 2-D gels revealed that 4 protein spots were significantly down-regulated ($p < 0.05$) in morphine-dependent mice compared to naïve mice. These protein spots were further identified by mass spectrometry as 4 different proteins: Lactate dehydrogenase 2, dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase (E2), NADH dehydrogenase, and Heat shock protein cognate 74. The 2-D gels and silver staining results were further verified by functional analysis. The proteins identified are important for energy metabolism and modulation of ATP levels, suggesting that modulation of ATP levels via alteration in expression of identified proteins in response to chronic exposure to morphine may play a role in change in hippocampal plasticity, leading to opiates dependence.

15.23

Preliminary Study of Proteomics on Nervous System

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People are suffering from nervous system diseases many of whose mechanisms have not been known. A lot of relevant proteins have been studied using traditional technologies, which contributed to realizing the mechanisms and helping with their therapy partly, there is no cure efficient enough to relieve patients thoroughly yet. So more special proteins need to be discovered to supply sufficient information on therapy. Undoubtedly, proteomics plays a pivotal role in the research field of nervous system for it can offer a comprehensive protein expression profile. In this experiment, cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS), brain tissue of patients with intracerebral hemorrhage (ICH), sciatic nerve of hens were used to establish a technical system of two-dimensional electrophoresis. In sample preparation, ultracentrifugation and sedimentation were used respectively to the three kinds of samples. In CSF sample preparation, different precipitators were added to CSF, which resulted in different products. And also in CSF sedimentation, ultrafiltrating membranes with cutting molecular weight of 10KD were used to compare with the other methods, and led to the best result. During IEF, different lengths of immobilized pH strips and different compositions of rehydrating buffer were applied. And during SDS-PAGE, the same 12% of gels were used. Silver nitrate was applied to stain proteins. Finally, different 2-D maps were received because of the above differences. There were 359 spots detected in CSF of control, 397 in CSF of MS, and 524 spots in brain tissue of control, 568 spots in brain tissue of ICH, and 697 spots in sciatic nerve by ImageMaster 2D-Elite software. The technology of proteomics was applied to the study of CSF, brain tissue and sciatic nerve, which helped revealing the pathogenesis of nervous system through displaying some special proteins, and helped offering some drug targets for the therapy of diseases.

15.24

Separation and Identification of Up-regulated Proteins Involved in Injured Rat Spinal Cords

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The inability of the central nervous system (CNS) to regenerate in adult mammals makes exploring associated proteins looking-forward-to work. Here we reported a list of up-regulated proteins involved in injured spinal cords of young adult rats. Two month male Wistar rats were operated either only thoracic laminectomy (as sham control) or plus complete spinal cord transection by razor at thoracic 8–9 segments. The comparative proteomic analysis between sham and treated whole spinal cords was conducted 5 days after operation. As a result, twenty eight up-regulated (2-fold or more) proteins in injured spinal cords were separated by two-dimensional gel electrophoresis and identified by mass spectrometry. Among this list, seven proteins were identified with 2–4 peptide tags sequenced by tandem mass spectrometry. Proteins in this sublist include glial fibrillary acidic protein (GFAP), valosin-containing protein, transferrin, disulfide isomerase ER-60, glutamate dehydrogenase, annexin III and brain lipid binding protein. Voltage-dependent anion channel 2 and endoplasmic reticulum protein 29 were identified only by one sequenced tag; another seven proteins were first identified by peptide mass fingerprint and further confirmed by immunological blot. Proteins in this sublist include arrestin, olfactory cyclic nucleotide-gated channel 2 (CNG2), glypican, dynactin, dynein, cyclin D1 and vimentin; the other twelve proteins were identified unambiguously (criteria of minimum of five matched peptide masses in NCBInr database) only by peptide mass fingerprint data, these proteins include tubulin, fibrinogen, ATP synthase, mitochondrial aconitase, Ca^{2+}-independent phospholipase A2, amidophosphoribosyl transferase, heat shock protein 27, zinc finger protein, zinc finger homeodomain enhancer-binding protein 1, farnesoid activated receptor, peroxisome proliferator-activated receptor and glucose-regulated protein (GRP78).

15.25

Rat Spinal Cord Injury and Regeneration-related Genes Cloned by Improved Subtractive Hybridization

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The mechanisms underwent spinal cord injury (SCI) are poorly understood. In the present study, we used PCR-based subtractive hybridization for analysis of genes differentially expressed during restoration after spinal cord injury, identifying of target genes and seeking pathways for drug development and therapeutic intervention.

Subtractive hybridization was used to construct subtraction library to identify up-expressed genes in injured spinal cord. In this study, we adopted several new techniques on the basis of traditional subtraction method, such as SMART reverse transcription method, long-distance PCR, streptavidin magnetic bead-mediated subtraction and spin column chromatography. Using the cDNA derived from injured spinal cord as tester and from normal spinal cord as driver, a cDNA subtractive library was constructed after two rounds of subtractive hybridization. The amplified cDNA fragments from the secondary PCR were ligated into pGEM®-T Easy [(a)] vector. Of the 110 clones randomly picked out using α-complementary screening method, 93 clones were found to have the recombinant plasmids. Sequenced and analyzed, 51 differentially expressed sequences (not including repeat sequences) were gained. Reverse dot blot was conducted to remove the pseudo-positive clones and finally 40 differentially expressed sequences were gained. Then we designed primers for several ESTs and conducted semi-quantative RT-PCR to confirm that they are upregulated in damaged spinal cord.

Compared with Genbank database, we found that 32 ESTs show high homology to known sequences and the other 8 represent novel sequences. Among 32 known ESTs, such genes as synuclein, clusterin, alpha acid glucosidase, vimentin, reticulon, and neurofascin are involved in development of nervous system, survival of neurons, nurite outgrowth or nerve degenerative diseases. 38 ESTs can find their localization on the rat genome and the other two sequences show little similarity with rat genome database. In order to find clues for further research, we analyzed these sequences with SMART and Interproscan software, the results showed that a bZIP domain existed in ORF of 69 sequence. This structure is one of the conservative domains where transcription factors bind their DNA regulatory sequences specifically, indicating that 69 protein might be a crucial transcription factor involved in spinal cord injury and regeneration. Then 3′-and 5′-RACE technique were adopted to clone the full sequence of this gene and we successfully obtained the 3321bp sequence (Genbank accession number AY546001), which located in the 4q22 on the rat chromosome. The complete coding sequence (CDS) of this gene is 1566bp, coding for 521 amino acids. To study the expression and localization of 69 gene, the CDS was ligated to pEGFP-N1 and transfected into COS7. Observed under the fluorescence microscopy, the fluorescence light was observed only in the nucleus in many COS7 cells transfected with pEGFP-N1–69, signifying that the role of 69 protein might be a transcription factor. We have already exp3ressed 69 protein in prokaryotic system, which laid a solid foundation for studying the biologi3cal features of this gene during spinal cord injury and regeneration.

15.26

A Proteomics Analysis by 2D-PAGE/MALDI-TOF-MS/MS on SH-SY5Y Cells After Chronic Lithium and Kenpaullone Treatment

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Glycogen synthase kinase-3 (GSK-3), a serine/threonine specific protein kinase, has been implicated to play a critical role in the pathogenesis of Alzheimer's disease and endocrine disorders such as type II diabetes. It is therefore of great interest to have a better understanding of the signaling events associated with GSK-3 activation. In this study, we provided a preliminary account on profiling of protein expression changes after GSK-3 inhibition in neuronal cells. Human neuroblastoma cells SH-SY5Y were treated with GSK-3 inhibitor kenpaullone (5 μM) and lithum (5 mM) for 24 hours and then the total proteins were separated by two-dimensional polyacrylamide gel-electrophoresis (2D-PAGE). Over two thousands protein spots were visualized by silver staining on the gels. Proteins that were differentially up- or down-regulated after drug treatment were excised from the gels, in-gel trypsin-digested and extracted, and then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS) for protein identification by searching the protein database MSCOT. The protein IDs of approximately 20 proteins out of 50 were successfully identified. This study demonstrated that differences in protein expression and/or post-translational modification (particularly the protein phosphorylation) from drug-treated SY-SY5Y cells could be analyzed by 2D-PAGE/MALDI-TOF-MS/MS approaches. This study can help us to yield insights into the potential molecular mechanisms, and perhaps to identify novel targets for the treatment of neurodegenerative and endocrine diseases mediated by GSK-3β.

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15.27

BDNF Neuroprotection Against Glutamate Toxicity Is Protein Synthesis-dependent: Proteomic Studies in Hippocampal Neurons

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Neuronal death underlies the symptoms of many human neurological disorders, including stroke and Alzheimer's, Parkinson's and Huntington's diseases. Brain injury following transient or permanent focal cerebral ischemia (stroke) develops from a complex series of pathophysiological events that evolve in time and space. Ischemic stroke results from a transient or permanent reduction in cerebral blood flow that is restricted to the territory of a major brain artery. Although most of the neurons die by necrosis in the impact region, in the penumbra region neurons generally die by apoptosis. Cell death by apoptosis is due to massive activation of glutamate receptors, which increases the intracellular calcium concentration, oxygen radical production and mitochondrial and DNA damage.

BDNF (brain-derived neurotrophic factor) mRNA and protein levels are higher in ischemia-resistant brain regions and lower in more vulnerable areas. Furthermore, mice deficient in a single allele for BDNF have increased susceptibility to cerebral ischemia. The objective of this work is to determine the mechanisms underlying neuroprotection by BDNF in excitotoxic conditions. We found that incubation of cultured hippocampal neurons with BDNF leads to activation of TrkB receptors inducing a transient increase in the activity of the PI-3K (phosphatidylinositol 3-kinase) and Ras/MAPK pathways. After 12–24h of stimulation with BDNF the signalling activity was similar to the control, but BDNF still protected neurons from glutamate toxicity. Neuroprotection by BDNF against glutamate induced toxicity was inhibited by anisomycin, indicating that it occurred through a protein synthesis dependent mechanism.

In order to identify these proteins, hippocampal neurons were incubated with radiolabelled amino acids, in the presence or absence of BDNF, for 12h. A proteomic analysis was performed consisting on identification of soluble and membrane/membrane-associated proteins. Protein solubilization and resolution was improved in 2D-SDS-PAGE using zoom-in IPG gels, with linear pH gradients ranging from 4.5 to 5.5 and 5.5 to 6.7. Several spots were identified as new spots in extracts prepared from BDNF treated cells. Furthermore, a down and upregulation of several spots was also observed under the same conditions.

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15.28

The HUPO Human Brain Proteome Project Pilot Phase—Differential Analysis of Mouse and Human Brains

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Since 2001 several initiatives have been established under the roof of HUPO—a non-profit organization promoting proteomic research and proteome analysis of human tissues, initiating several worldwide projects—that analyze the proteome of a distinct human organ, e.g. the Human Brain Proteome Project (HBPP). Within this project a pilot phase has been started that addresses i) a quantitative proteome analysis of mouse brain of three different ages and ii) a differential quantitative proteome analysis of biopsy and autopsy human brain samples. Taken together, more than 20 different groups from Austria, Belgium, China, Germany, Greece, Korea, Ireland, Switzerland, the UK and the US are analyzing samples and are the initiation point of the HBPP standardization efforts. Data from the pilot studies as well as from subsequent work will be collected at a new developed Data Collection Center that will be built up to the World Neuroproteome Database (WNPD), offering a close interface with actual scientific research.

Our group participates in the pilot studies using different 2D-PAGE systems (the large gel system according to Klose, 1975 and the IPG-system according to Görg *et al*. 1985), comparing and evaluating the results of both methods. After differential quantitative analysis the protein spots of interest are excised from the gel, tryptically digested and analyzed using an iontrap (LCQ Deca XP, Thermo Electron) or MALDI-TOF/TOF (Ultraflex, Bruker Daltonics) mass spectrometer. So far several differences were detected in the mouse brain proteomes of different age stages. The mass spectrometric analyses are still under work and will be presented at the meeting in October.

15.29

The HUPO Brain Proteome Project—Talk at the Neuroproteomics Session in Beijing

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The brain is the most complex tissue of higher organisms, differing from other organs due to its many different cell types, its structure at the cellular and tissue level, and by the fact that one of the most important cell type in the brain, the neuron, stops dividing in adult life. Elucidating the protein complement of the brain is therefore a significant challenge for current technologies in proteome analysis.

At the same time, the brain is of paramount interest to medical research and pharmaceutical industry because of the social impact of the more common neurological diseases such as Alzheimer, Parkinson, Multiple Sclerosis, Prion Diseases and Stroke. The prevalence of some of these diseases is increasingly high, e.g. every 5th person over 80 years in industrial countries is suffering from Alzheimer.

There is no strict relationship between the genome and the protein complement, as one gene can code for several proteins, protein expression levels are not predictable from mRNA expression level and proteins are often modified and processed after translation. In addition, it is to be shown if brain function is mediated largely by brain specific proteins or proteins common to different organs. Thus, the scientific problems of brain proteomics can not be solved with the resources of single laboratories or groups.

Therefore, the HUPO Brain Proteome Project (BPP, www.hbpp.org) was established under the patronage of the Human Proteome Organisation (HUPO) in order to coordinate worldwide neuroproteomic efforts, as well as to reduce duplicate efforts. One aim of HBPP is the characterization of the human and mouse brain proteomes and the utilization of these data (identified proteins, mRNA profiles, protein/protein interactions, protein modifications and localization, validated targets) to elucidate human neurodegenerative diseases with the focus on Alzheimer's and Parkinson's Disease. Gained data, SOPs and new technologies obtained through these studies will be accessible to all active members of the HBPP. The 1st HUPO HBPP Workshop already took place at Castle Mickeln, Duesseldorf, Germany, in early September 2003. A pilot phase addressing the available methods of the participating groups has just started (Feb/March 2004). Quantitative proteome analysis will be done to study aging processes (mice) and protein post mortem stability (human), leading to a reliable neuroproteome database.

15.30

Proteome Analysis of Proteins Enriched in Axons of Cultured Hippocampus Neurons

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Neurons have the polarized shape consisted of the single-long axon and the somato-dendritic region. Polarity is not only the morphological feature of neurons but also necessary for fundamental functions of them. However, the mechanism for the establishment of neuronal polarity is poorly understood. Recent findings suggest that some proteins and some post-translational modification procedures are distributed to axon or somato-dendrite selectively, and such specific localizations of these proteins are considered important for the formation and maintenance of neuronal polarity.

To search for molecules exhibiting specialized localization in cultured hippocampus neurons, we prepared [^{35}S]labeled proteins from axons and somato-dendrites separately, and compared the spot intensity of each protein spot detected by autoradiography in the two-dimensional electrophoresis (2-DE) gels. In the differential display and MALDI-TOF MS analysis, we identified a tyrosine phosphatase, SHP-2, as one of the proteins enriched in axons rather than somato-dendrites. We carried out western blot analysis on 2D-gel with anti-SHP-2 antibody to confirm that the protein spot is surely SHP-2. Moreover, we found other protein spots are also reactive to anti-SHP-2 antibody, and, in differential display analysis, one of them were detected with higher intensity on the gel including somato-dendritic proteins than that of axonal proteins. These data suggests the possibility that SHP-2 is post-translationally modified in cultured hippocampus neurons and exhibits different distribution in axon or somato-dendrite depending on the modification states.

15.31

Investigations of Primary Astrocyte Injury and Regeneration-related Proteins by Comparative Proteomics

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Astrocyte, the predominant glia cell in CNS, is one of the earliest cells respond to CNS lesion. To investigate the response and the dynamic changes in protein expression of astrocytes after injury, the 2D electrophoresis was employed to visualize differentially expressed proteins via a new designed mechanical injury model in vitro. We first isolated and purified astrocytes from rat spinal cord of the rat. The easy-controlled template was put under the culture dishes to reveal the cut matching to the cross-sectional lines on it by a sharp razor blade. The cultured dishes were divided into four groups: 24 hour, 72 hour and 120 hours after injury and normal cultured dishes as control. All cultured cells were harvested for the protein sample preparation in 2DE electrophoresis study.

The proteins, extracted from cultured astrocytes with lysis solution via ultrasonic and ultracentrifugation, was used for 2DE electrophoresis after quantitated by Bradford measurement method. 12 gels, with 3 gels every group were used in this investigation. Those spots ranged from isoelectric point (pI) 3–10 and molecular weight (MW) 10–100 kDa were showed clearly in the protein expression 2DE maps. And the dynamic changes of proteins at the injury model after 24, 72 and 120 h were also analyzed and confirmed. 1785, 1806, 1809 and 1841 protein spots were detected respectively in control, 24 h, 72 h and 120 h via ImageMaster software. Three categories of dynamic changes were identified and total different 50 spots were detected in this study from the 2DE gels. One sort, with total 15 spots, can only be found at a special stage after injury; another sort, with only 6 spots, vanish only at a special stage after injury; the last sort, of total 29 spots, is up-regulated or down-regulated at different period after injury.

The differently displayed spots identified via MALDI-TOF MS can sort as: (i) metabolism, e.g. Dimethylarginine dimethylaminohydrolase 1 (DDAH1) and NAD-independent Isocitrate Dehydrogenase (NAD-IDH), (ii) cyto-skeletal and intracellular protein transport, e.g. beta-actin and Vimentin, (iii) molecular chaperoning, e.g., prohibition and Valosin-containing protein (VCP); (iv) others. The results of this study indicate that those proteins may involve in the process of SCI.

As a conclusion, an astrocyte culture method was successfully established in this study and the purity of cultured astrocytes was over 97%, verified by immunofluorescence. And the injury model of astrocytes has been improved in this study for a better reproducibility and an enough quantity of reaction astrocytes. Some differentially displayed proteins that show dynamic changes were obtained by using the improved astrocyte lesion model and the 2D electrophoresis techniques and ten of them were identified via PMF analysis.

15.32

Proteomic Study of Neuroblastoma Differentiation by Retinoic Acid

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Neuroblastoma is the most frequent solid tumor and the second most common malignancy in childhood. And it is considered the dismal entity due to the difficulty to treat and the consequence of poor prognosis. Neuroblastoma can regress spontaneously, and spontaneous or induced differentiation is also seen with significant frequency. Neuroblastoma cell line is a good model for neuronal development. To elucidate molecular events in differentiation and biological behaviour of tumor, global change of protein expression before and after neuroblastoma differentiation induced by all-trans retinoic acid has been discovered using proteomics strategy. Human neuroblastoma cell line SH-SY5Y was treated with 10 μM all-trans retinoic acid for 8 days to induce neuron-like differentiation with the control of untreated cells. Total protein lysates of treated and untreated cells were resolved using 2-dimensional electrophoresis. After gel image analysis, 73 differentially expressed protein spots were detected, in which expressions of 51 spots were down-regulated after differentiation and 22 spots up-regulated. Differentially expressed protein spots were excised and trypsinated in gel. 40 proteins were identified in which 31 down-regulated and 9 up-regulated. From analysis of identification results, neuronal differentiation relates to energy metabolism, membrane trafficking, mRNA synthesis, protein synthesis, signal transduction and oncogenes. Semi-quantitative RT-PCR and Western blot analysis of some differentially expressed proteins showed that the expression level of mRNAs was relevant to that of proteins.

15.33

A New Proteomic Approach to Study Multicomplex Modifications During Neuron Activity and Development

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Proteomics tools reveal new ways to analyse network of proteins characterized by a high complexity, like the PSD multicomplex. In this study, we show how a new proteomic and pharmacological approach based on a conservative sub-cellular fractionation reveals changes in protein synthesis, degradation and post-transalational modifications in rat hyppocampal neurons.

We developed a differential detergent fractionation (DDF) protocol which permits to obtain distinct and functionally different partition of neuron proteoma, while preserving protein-protein and protein to cytoskeleton interaction.

Hyppocampal neurons were maintained in culture, pharmacologically treated and then fractionated by sequential exposition with detergent-containing buffer; immunoblotting with at least ten antibodies was used to characterize the distribuition profile of citosolic, membrane-, organelle, cytoskeletal and PSD associated proteins. 1-D and 2-D analysis were generated for each fraction and for each conditions. A wide range analysis of protein multicomplex enables the development of new concepts and approaches for neuroscience research.

15.34

Proteomics to Dissect the Cell Regulatory Machinery in Brain

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Recent advances in proteomic technologies enable protein characterization at a proteome-wide scale and in a systematic way, opening new doors in studies of biological systems. In cells, proteins often exist as complexes with other cellular proteins and multi-protein complexes linking a protein to its cellular targets and regulators appear as a molecular principle for conducting its physiological functions. Hence, systematic identification of cellular protein complexes and interaction networks would provide a holistic view of the cell regulatory machinery. Proteomics has provided a powerful technology platform for comprehensive analysis of protein interactions. The proteomic analysis of protein-protein interactions usually comprises the following steps: (1) biochemical isolation of multi-protein complexes from cells, tissues or animals; (2) protein identification and sequence analysis by mass spectrometry; (3) demonstration of how they interact with one another; and eventually (4) assembly of protein complexes and networks within a specific biological context. We have employed this proteomic strategy to analyze protein-protein interaction networks and therefore, to unravel the cell regulatory machinery in the brain. Cyclin-dependent kinase 5 (Cdk5) in association with its neuron-specific activator p35 plays an essential role in a wide variety of cellular activities occurring in neurons of the central nervous system, including neuronal differentiation, cell signaling, and synaptic plasticity. Deregulation of the Cdk5/p35 enzyme activity is neurotoxic and has strong implications in an increasing number of neurological diseases. In the brain, Cdk5/p35 was found to exist in macromolecular complexes with many other proteins. Using proteomic approaches, we have identified a number of protein complexes of Cdk5/p35. Characterization of identified protein complexes has helped us in further understanding what cellular activities it is involved and how it exerts its actions in these events. It allows us move towards understanding complex molecular mechanisms underlying many of the important processes occurring in neurons including pathogenesis of neurodegenerative diseases.

15.35

Some Properties of the Binding of Neuronal Tau and DNA

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Tau proteins belong to the family of microtubule-associated proteins. Research shows tau associate with chromosomes scaffold and localizes in the nuclear and the nucleolar organization regions in neuronal and some nonneuronal cells, but its role in this subcellular compartment is still unknown. In our study, the binding of tau to DNA was investigated by the electrophoretic mobility shift assay. We found that tau bound to double-stranded DNA but not single-stranded DNA. And the binding is in an aggregation-dependent and phosphorylation-independent manner. Formation of tau-polynucleotide complex was interfered at alkaline pH and high concentration of NaCl. Nevertheless, the DNA-bound tau can be replace by alkaline protein such as histone. Electron microscopy revealed that the protein associated with the nucleic acid in a necklace manner. Here, AFM is used to study the interaction of neuronal tau and DNA. The results show the appearance of the tau-DNA complex is dependent upon the molar ratio of tau/DNA. Tau-DNA complex forms a “beads-on-a-string” structure when the molar ratio is low (tau/bp, 1:700) and it shows a “beads-on-a-coil” structure when the ratio becomes higher (tau/bp, 1:350).

In order to detect the length and the conformation of DNA interaction with tau, a series of different length and different sequence of complement strands nuclear tides were constructed. The result shows that one tau molecule just binds to a 13-bp polynucleotide without any discrimination. It means that tau molecular binds to DNA in one helix (in B-DNA, 10 bp make one helix and the length of a helix is 34nm). Furthermore, we found that protein can recognizes and associates with B-DNA, but not A-DNA and Z-DNA .The result suggests that the interaction between tau and DNA depends on a strict conformation both protein and polynucleotides.

15.36

Proteome, Transcriptome and Metabolome of Single Cell: Harvesting with Patch-Clamp Pipettes

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Although all cells of multicellular organisms have an identical genome, individual cells express a different set of genes resulting in a multiplicity of specialised cells with different functions. One of the present goal of biological research is to decipher in a single cell the set of genes transcribed in messenger RNA (Trancriptome), the set of proteins translated (Proteome) and the set of metabolites synthesized (Metabolome). This task is difficult due to rarity of materials in a single cell.

Indeed, the volume of a single cell of 10 μm diameter is 1 picoliter. It weights, mainly because of its water content, 1 nanog. It contains 10 picog of DNA, 50 picog of total RNA, 1 picog of messenger RNA or around 1 million of copies of messenger RNA molecules. The protein content of a single cell is around 100 picog. A protein representing 1% of the total proteins corresponds to 1 picog or 50 attomoles for proteins whose molecular weights are 20000. In a cell the most abundant ion [K^+] is at 100mM and thus a single cell contains 100 femtomoles of K^+. In a neurocrine cell, the high neurotransmitter content is between 1 and 10 femtomoles, second messengers are much less abundant at a maximum concentration of 1μM or 1 attomole per cell.

In our laboratory, our interests focussed on the neocortex, the part of the brain most involved in higher mental functions. The complexity of the neuronal network composed by an highly heterogeneous population of neurons led us to design an unique method to study cellular diversity at the single cell level: single cell RT-PCR after patch clamp. In brief, after electro-physiological recordings of a cell, the cytoplasm of the cell is aspirated in the recording pipette and the content analysed for the presence of few different messenger RNA species. Experimental data (quantitative PCR) showed that a relatively well expressed neuronal gene (GluR1) is represented by 1000 copies of messenger RNA per cell.

With multiplex RT-PCR, expression of 100 different genes can be monitored simultaneously. By the ongoing work of our laboratory with DNA microarrays chips it is expected that most of the transcription program of a single cell could be analysed in the future. We are also developing the use of Mass Spectrometry (LT-FT-ICR) in order to identify proteins, peptides, neurotransmitters, second messengers and products of the cellular metabolism in a single cell. These transcriptome, proteome and metabolome analysis at the single cell level represent a new frontier in biology and are essential to deciphering the heterogeneity and the function of the neuronal network of the brain.

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15.37

Human Neuronal Growth Inhibitory Factor (h-GIF) and Alzheimer's Disease (AD)—Comparative Proteomics Research

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Proteomics is essential in functional genomics research, and it possesses great potential in pathology research of serious diseases. Through comparing normal sample and pathologic sample, proteins with up-regulated or down-regulated expression could be detected which may serve as drug targets or biomarkers for disease diagnosis. Collaborated with other molecular and cell biology technologies, proteomics could be applied to further research on interaction of target proteins with other proteins.

Based on this proteomics platform, we have conducted researches on serious human diseases including Alzheimer's Disease (AD), Corticobasal Degeneration (CBD), and mechanism of brain aging, etc. The Result of Research on Alzheimer Disease are listed below:

1. Proteomics Research on frontal lobe of normal humans and of AD patients. The phenotype of AD includes degeneration of memory and cognition. Frontal lobe is the main area responsible for memory and learning. Through proteomic comparing frontal lobes of age-matched normal human and of AD patients, several interesting proteins have been identified including structure proteins, enzymes, regulatory proteins, and other AD related proteins.

2. Proteomics Research on Hippocampus of Normal Human and of AD Patients. Hippocampus is closely related to many activities including short-term memory, behavior and autonomic nerve functions. Alzheimer disease causes damages to nerve system responsible for learning and memory. Several down-regulated proteins in hippocampus have been detected: such as Amyloid Protein Precursor, oxidoreductase, etc.

3. Differential Display Proteomics Research: Transiently Transfected on SH-SY5Y or PC-12 cells with Neuronal Growth Inhibitory Factor (GIF) gene. GIF is the first identified protein that has specific neuron growth inhibitory function in nerve system. The expression of GIF is obviously down-regulated in astrocytes in the damaged brain region of neuron degenerative disease patients. Differential display proteomics research on SH-SY5Y or PC-12 cells transiently transfected with human GIF gene can study the role of GIF and the mechanism of AD. The over-expression of GIF can affect expression of several other proteins. The up-regulated proteins that have been identified include: Glutamate transporter and Zinc finger protein 1, etc. Most proteins with changed expression level are related to AD and other neuro-degenerative diseases.

4. Proteomics Research on Function of GIF in Anti-Apoptosis of PC-12 Induced by Glutamate.

5. Proteomics Research on the protective role of GIF in zinc-induced neuronal cell apoptesis and rat memory damage.

15.38

Analysis of Proteins in Mouse Brain Fractions

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The multitude of brain regions in conjunction with their different functions and presence of different cell types make protein analysis of brain tissue very complex. Investigations of the brain are therefore often carried out on the single brain regions or brain structures, e.g. synaptosomes or post synaptic densities. Using two-dimensional gel electrophoresis (2-DE), we studied protein expression in mouse brain fractions obtained by either anatomical dissection (brain regions) or structural fractionation. In our study two questions were of interest: (1) does fractionation reveal fraction-specific proteins and (2) does fractionated extraction of brain proteins reveal low-abundant proteins not detectable in total extracts. The investigated fractions were four brain regions: striatum, frontal cortex, hippocampus and olfactory bulbs, and the tissue fractions: synaptosomes, Mossy fiber synaptosomes, synaptic membranes and myelin. Comparisons were made between brain regions and single regions against total brain extracts, furthermore, between tissue fractions and single fractions against total brain extract.

Only a small number of additional protein spots were detected by comparing above mentioned regions and fractions. For example, proteins specific for only one region were found exclusively in olfactory bulbs (7 protein spots). Extracts of synaptosomes showed approximately 30 protein spots not visible in total brain, while the number of specifically enriched protein spots of synaptosomes detectable in the total brain extracts was approximately 70.

The general observation was that the number of additional protein spots in the investigated fractions was rather small compared to the several thousand protein spots anyhow detected on our large-gel 2-DE brain patterns.

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15.39

Proteomic Analysis of Injured Rat Spinal Cords After Transplantation of Human Embryonic Stem Cells

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Spinal cord injury is a major medical problem worldwide, and realistic goals of functional repair have only recently been acknowledged. Recent advances in neural injury and repair, and the progress towards development of neuroprotective and regenerative interventions are basis for increased optimism. A number of potential approaches aim to optimize functional recovery after spinal cord injury. They include minimizing the progression of secondary injury, manipulating the neuroinhibitory environment of the spinal cord, replacing lost tissue with transplanted cells or peripheral nerve grafts, remyelinating denuded axons, and maximizing the intrinsic regenerative potential of endogenous progenitor cells. Embryonic stem (ES) cells can give rise to all neural progenitors and they represent an important scientific tool for approaching neural repair. However functional recovery in movement after stem cell transplantation into spinal cord have been reported from several case in laboratory animals, it still remains unclear to understand the molecular mechanism of recover after transplantation of stem cells. Selective marker expression in transplanted ES cell derived neural cells is providing new insights into transplantation and repair not possible previously. These features of ES cells will produce a predictable and explosive growth in scientific tools that will translate into discoveries and rapid progress in neural repair. Two dimensional-based Proteome analysis was employed to identify the proteins associated with functional recovery after spinal cord injury. Male Sprague-Dawley rats were anesthetized with sodium pentobarbital and subjected to spinal cord injury (SCI) model. Rats were laminectomized and SCI was induced using NYU spinal impactor at T9 spinal segment. Human embryonic stem (ES) cells were transplanted into a rat spinal cord 1 week after SCI. A behavioral test using BBB locomotor rating scaling was performed every one week for 1 months. Hindlimb performance was modestly improved in human ES cell-transplanted rats compared to vehicle-treated rats. Five proteins displayed different expression levels in the spinal cords of stem cell transplanted rats. Among these proteins, GFAP is dramatically changed in the spinal cords of stem cell transplanted rats, even though the total expression level of GFAP is not quietly changed. GFAP immunoreactivity is quietly distinct in the spinal cords of stem cell transplanted rats with immunohistochemical localization. It may be suggested that GFAP act an important role during functional recovery of movement after stem cell-trasnplant into injured rat spinal cords.

15.40

Toxicoproteomics of Solvent Neuropathy

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Sixteen Chinese reports (1996–2004) of occupational neuropathy in Fujian, Guangdong, Helongjiang, Henan, Jiangsu and Liaoning blame workplace overexposure to *n*-hexane, the γ-diketone metabolite (2,5-hexanedione, 2,5-HD) of which binds to ϵ-amino groups of lysine and cross-links proteins. We used proteomic methods to identify neuroprotein targets of 2,5-HD and its more potent aromatic cousin 1,2-diacetylbenzene (1,2-DAB), both of which induce axonal microtubule segregation and neurofilamentous (NF) axonopathy in rodents. Central and peripheral nervous tissue from rats and mice was treated in vitro and in vivo with 1,2-DAB, 2,5-HD or, as negative controls, their respective protein-non-reactive and non-neurotoxic isomers 1,3-DAB and 2,4-HD. Total lysine content correlated with protein susceptibility to γ-diketones in vitro, those rich in lysine (NF-H, NF-M, kinesin, dynein) showing greater vulnerability than those with low lysine content (NF-L, β-tubulin). Proteomic analysis of brain cytosol from mice daily dosed for one week with 1,2-DAB or 1,3-DAB was performed in triplicate using μLC-FTICR-MS. Numerous protein changes were observed, including markedly increased abundance of a ubiquitous protein (stathmin) that promotes microtubule disassembly. Since 2,5-HD increases microtubule stability in vitro, and stathmin deletion triggers axonal neuropathy, this widely expressed phosphoprotein may be a key γ-diketone target. The rapid segregation of axonal microtubules from NFs induced by direct application of 2,5-HD to sciatic nerve in vivo may reflect a failure to transport NFs, which then accumulate proximally in focal swellings.

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15.41

Modulation by Desensitized Nicotinic Acetylcholine Receptors on mRNA Levels and Function Expressions of Important Proteins in Rat Brain

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When rats were treated with nicotine for 14 days at doses of 2.4 mg/kg/day via two daily subcutaneous injections, the nicotinic acetylcholine receptors (nAChRs) were induced in desensitized states. The mRNA levels and function expressions of important proteins involved in neurotransmitter receptors, as well as proteins involved in signaling pathways, were observed in the brain. The results showed that (1) when nAChRs were desensitized, 224 transcripts showed altered expression (greater than twofold change; 115 increased and 104 decreased) compared with control group. The major classes of transcripts altered were neurotransmitter receptors, kinases and ion channels (92 transcripts altered). In the neurotransmitter receptor systems, there were 9 transcripts up-regulated, including NMDA receptor, GABA receptor, alpha-1A-adrenergic receptor, D1 receptors, etc, 13 transcripts were down-regulated, such as D3 receptor, beta 3-adrenergic receptor, 5HT3 receptor subunit, etc; In cholinergic receptors, mRNA levels of nicotinic receptors in nicotine-treated rats were similar to that of control rats, only mRNA levels of m2 and m3 were up-regulated. In the signaling pathways, 13 transcripts were up-regulated, involved in protein kinase C III, and 7 transcripts were down-regulated, but the transcripts involved in α, γ, ζ subunits of PKC, PKC I, cAMP phosphodiesterase, PKA II regulator, and PKA inhibitor did not change; (2) when nAChRs were in desensitized states, the responses of DA receptors to their agonists were enhanced, the inhibitory effects of clonidine, 8-OH-DPAT and diazepam on locomotor activities were weakened, but the function of opioids receptors didn't altered; And all of the muscarinic effects, including electroencephalogram seizures, behavioral convulsions, tremors, homogeneous receptor down-regulation, and inhibition of spontaneous locomotor activity induced by muscarinic agonists, could be enhanced by nAChRs desensitization; And the activities of PKA and PKC in the rat brains were inhibited when nAChRs were desensitized. These results suggested that nAChRs desensitization induced by chronic nicotine caused extensive effects on gene and function expressions of important proteins in the brain, but there were not similar trends in the changes between mRNA level and function expression of those proteins, implying that the unparallel alterations exist in gene transcriptions and function expressions of proteins induced by drugs.

15.42

Proteome Analysis of Proteins Up-regulated During Axon Outgrowth

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Neural cell has only one long axon and plural dendrites. In cultured hippocampal neuron, stage 2 neuron has several short neurites. At this stage, it is not determined which neurites will become an axon. Only one neurite rapidly extends to become an axon in stage 3 neuron. Although axon formation plays critical roles for neural circuit formation, molecular mechanisms underlying it are not well characterized. The purpose of this study is to identify proteins that change expression level or post-translational modification during axon formation by proteome analysis with two dimensional gel electrophoresis (2-DE) and mass spectrometory. 2-DE analysis detected 6,197 [^{35}S] labeled protein spots in cultured rat hippocampal neurons. During the stage 2–stage 3 transition, expression levels of 277 protein spots were up-regulated while those of 265 spots were down-regulated. We analyzed the protein spots up-regulated by MALDI-TOF/MS and 96 proteins were identified. The identified proteins included cytoskeletal, vesicle associated and signal transduction proteins which may be involved in axon formation. We also identified a novel protein, which was remarkably up-regulated during axon outgrowth. In addition, this protein was highly localized at axonal growth cones. These results suggest that this novel protein is involved in axon outgrowth during neural circuit formation.

15.43

Determining Nicotine Dose-response for the Suppression of Nitric Oxide (NO) Production of LPS-stimulated Glial Cells

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Glial cells consist of microglia, astrocytes, and oligodendrocytes. Glia act as supporting cells for neurons in many ways, for example as phagocytes for beta amyloid protein, a major player in Alzheimer's disease pathology. However, when activation is prolonged and excessive, as is the case in AD and other neurodegenerative conditions, microglia and astrocytes can be induced to produce large amounts of pro-inflammatory factors. This will result in an over-production of nitric oxide (NO), which is detrimental to neurons. Nicotine has been shown to attenuate TNF-alpha production of activated microglia. We aimed to find out, whether nicotine was able to attenuate NO production in a mixed primary rat glial culture. In our experiment the hypothalamus and the midbrain were the source of the rat glia. After cell plating the glia various concentrations (0.3 μM, 1 μM, 3 μM, 10 μM, and 30 μM) of nicotine were incubated with them. The incubation lasted for three days to replicate the chronic conditioning of nicotine in a body. The cells were stimulated with 50 ng/ml LPS (lipopolysacchride) to over-produce the nitric oxide (NO). The supernatants were collected then measured for absorbance with a spectrometer. The results indicate that 3 μM nic conc of the midbrain glia had strongest suppression (39%). Also 0.3 μM of the midbrain and the hypothalamus had the next strongest suppressions (33% and 22%). Though 0.3 μM to 1 μM are in the range of a more natural physiological conc, in vitro 3 μM could be the ideal conc for the glia. The 10 μM and 30 μM nic concs had no effect on the glia. To explain this effect more extensive research will be required. In our future experiments another stimulant, Interferon-gamma, will be added with LPS, the microglia activator, for more astro-glia activation. In the future pharmacologists could make treatments for Alzheimer's disease. Thus AD drugs that mimic nicotine and bind to nicotinic acetylcholine receptors (nAChRs).

15.44

The Impact of Blood Contamination on the Proteome of Cerebrospinal Fluid

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Human cerebrospinal fluid (CSF) is in direct contact with the brain extracellular space. Beside the secretion of CSF by the choroid plexus the fluid also derives directly from the brain by the ependymal lining of the ventricular system and the glial membrane and from blood vessels in the arachnoid. Therefore, biochemical changes in the brain may be reflected in the CSF. CSF is a potential source of protein molecular indices of central nervous system function and pathology. However, various amounts of blood contamination in CSF may arise during sample acquisition. The concentration of protein in the CSF is only 0.2 to 0.5% that of blood. Minor contamination of CSF with blood during collection of the fluid may dramatically alter the protein profile confounding the identification of potential biomarkers. We have analyzed CSF and CSF spiked with increasing amounts of whole blood using proteomic techniques. We detected at least four blood specific highly abundant proteins including hemoglobin, catalase, peroxiredoxin and carbonic anhydrase I. These proteins can be used as blood contamination markers for proteomic analysis of CSF. Proteins in blood contaminated CSF samples were less stable compared to neat CSF at 37°C. Suggesting that blood borne proteases may induce protein degradation in CSF during sample acquisition. This analysis was aimed at identification of proteins found primarily in CSF, those found primarily in blood and assessment of the impact of blood contamination on those proteins found in both fluids.

15.45

Using Proteomic Approach to Detect Stroke-related Proteins

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Stroke is a serious disease to many people. Although many works have been done, it is still difficult to know the key genes and key proteins in the disease. In this study, we would like to study the stroke-related proteins in order to understanding the mechanism on stroke and to finding potential targets for clinical usage using the proteomics approach. Both the gerbil and mouse were used as model animals for the analysis. The stroke model was prepared following standard method by blocking the internal and external carotid arteries in both sides, while the blood pressure and other features of the animals were recorded for quality control on the model. After different time of blocking the arteries and reperfusion, the whole brains were cut off immediately. Different part of the proteins from hippocampus and cerebral cortex were prepared for proteomics analysis using the 2D coupled with MALDI-TOF/MS identification. Some interesting proteins were identified. A bioinformatic analysis would help us to determine the major proteins involved in the stroke. A following study on the analysis of interested proteins in details will be done to confirm the potential key proteins related with stroke.

15.46

Profiling Brain Vascular Proteins from Alzheimer's Disease and Non-Demented Patients Using Laser-Capture Microdissection Followed by ICAT-MS/MS Analyses

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Peri-cerebrovascular deposition of β-amyloid peptides (Aβ) has been found in 82–98% of Alzheimer's disease (AD) patients, which results in not only vascular insufficiency but also inflammatory response. This Aβ-associated pathology contributes to progressive neuronal loss and cognitive decline in AD patients. We have developed a proteomic method using ICAT-MS/MS analysis for profiling brain vascular proteins of AD vs. non-demented age-matched patients (ND) in small quantity of sample isolated by laser- capture microdissection (LCM). Brain samples (2 AD and 2 ND) were frozen-sectioned at 8–12 μm and Aβ deposits were visualized by staining with an Aβ antibody or/and thioflavin S staining. For ICAT-MS/MS analyses, brain vessels were stained with fluorescein-labelled lectin Ulex Europeaus Agglutinin I and captured by LCM. Approximately 4000 vascular cells were captured from each sample, from which proteins were extracted. AD and ND proteins were labelled with cleavable heavy and light ICAT reagents, respectively. Both samples were mixed, tryptic digested and affinity purified. The samples were first analyzed by nanoLC-MS survey scan, and all the differentially expressed ICAT pairs (up-or down-regulated by $>$1.5 folds) were identified. These pairs were subsequently targeted for peptide sequencing using nanoLC-MS/MS analysis. Preliminary analyses show that a number of proteins were commonly upregulated [tubulin β-1 chain, CD27BP, CAB45755, RNF18, PRKDC, MCAD, FALP (B27), and ACAD-8] or down-regulated (IL-6R, Vacuolar proton pump-α subunit 2, fibrinogen γ-A chain precursor, RGS8, ATP8B3) in the two AD samples against the two ND samples. This study demonstrates the feasibility of an ICAT-MS/MS-based proteomic method to extract useful information from minute quantity of material isolated by LCM from AD/ND vasculature.

15.47

Preparation of Antibodies Against SPIR Unknown Proteins Discovered in Comparative Proteomics

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Using proteomic techniques, we had investigated the changes of protein expression after rat spinal cord injury and found 173 proteins to be involved in the processes of spinal cord injury and regeneration (SPIR). Database and Q-TOF analysis showed that at least 4 proteins are unknown. proteins among those SPIR-related proteins. Information of this unknown proteins we got in experiments were limited to short peptides about 13–19 amino acids. Moreover, these proteins usually are trail proteins. How to study their function thoroughly is quite difficult question. Preparation corresponding antibodies of these short peptides is likely a good approach to research their function of 4 unknown proteins. We have inserted coding sequences of 5 short peptides of these unknown proteins into pGEX-4T-2 respectively. The recombinant vector was identified by restriction endonuclease digestion analysis. These fusion proteins were expressed in *E. coli* BL21 at 37°C via 0.1mM IPTG induction. The rabbit antibodies against these unknown proteins were prepared by using fusion proteins as antigen. The antibody affection was evaluated with ELISA. When the titer of rabbit serum against these purified short peptides were all above 1:10000, animal blood was collected and spleen cells were isolated. The specificity of polyclonal antibody was further identified by Western blot. Results showed that the polyclonal antibodies specifically bound to corresponding purified short peptides. Immunohistochemistry analysis also showed that labels are all showed in perikaryons, dendrites and axons of neurons. No labels were found in neuron nuclei and Glia. All labeled cells were located in different areas of spinal cord grey matter. No small neurons and glial cells are found to be labeled. In this study, we have successfully obtained polyclonal antibodies of 4 known proteins discovered in comparative proteomics investigation and provided useful reagent for further functional investigations. This study provides a new approach and a successful sample of research of new proteins found in comparative proteomics.

15.48

Comparative Proteomic Analysis of the Hippocampus in Senescence-Accelerated Mice (SAM)

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Senescence-accelerated mouse (SAM) are unique and useful animal models for studies of functional genomics in regard to senescence or age-associated disorders, for they show accelerated senescence as an inherited phenotype. The typical feature of SAM/prone8 (SAMP8), a substrain of SAM, is the early onset of learning and memory deficit during its aging process, so SAMP8 has been considered a good animal model for the study of age-related diseases, such as Alzheimer's disease. The aim of this study was to perform a preliminary proteomic analysis of the hippocampus of SAM in order to investigate the differential proteins involved in the process of aging and accelerated impairment of learning and memory in SAMP8. The proteins from the hippocampus of SAM at different age were separated by two-dimensional electrophoresis (2DE) and visualized by Coomassie Brilliant Blue (CBB) staining, and the differential expression of the hippocampal proteins between SAMP8 and SAMR1 (SAM-resistance/1), a senescence/resistant substrain of SAM were compared by the image analysis, then some interested proteins were digested by trypsin and identified by MALDI-TOF/MS. About 600 protein spots were visualized, in which more than 50 proteins differentially expressed between SAMP8 and SAMR1. The results also showed that a 26-kDa unidentified protein expression was significantly decreased, whereas the expression of adenylate kinase isoenzyme 4 (AK4), a key enzyme implicated in mitochondrial ATP energy transfer, was increased in SAMP8. By comparing the protein expression maps (PEMs) of the hippocampus from SAMP8 at 2, 6 and 12 moths of age, an up-regulated protein and a down-regulated protein during the course of aging were detected and identified. In addition, two important proteins involved in signal transduction or protein degradation, Rho GDP-dissociation inhibitor alpha (GDI α) and ubiquitin carboxyl-terminal hydrolase L-1 (UCH L-1) were also detected, showed evidence of undergoing post-translational modifications in the progression of aging. All these results indicate that comparative proteomic analysis can be effectively used to monitor alterations in the protein expression profiles of brain tissue during the course of aging, and can further shed light on the mechanism underlying the aging process.

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