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Challenges in Modern Biomarker Discovery – 17th HUPO BPP Workshop, May 24–25, 2012, Sao Paulo, Brazil

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Abstract

The HUPO Brain Proteome Project (HUPO BPP) held its 17th workshop in Sao Paulo, Brazil, on May 24 and 25, 2012. The focus was on the progress on the Human Brain Proteome Atlas as well as ideas, strategies and methodological aspects in clinical neuroproteomics.

Keywords

Alzheimer; Brain; Brain Proteome Project; HUPO; Neurodegeneration; Parkinson

The 17th HUPO BPP workshop was held at the University of Sao Paulo Medical School, on May 24 and 25. The focus was to present recent clinical outcomes in the field of NDD.

The following workshops are planned:
18th HUPO BPP workshop in Boston, USA, September 12, 2012
19th HUPO BPP workshop in Essen, Germany, May 23 and 24, 2013
20th HUPO BPP workshop in Yokohama, Japan, September 2013

The HUPO BPP is an open project – thus, anyone interested in the project shall be welcomed cordially, and the latest information will always be publicly available at http://www.hbpp.org.

The authors have declared no conflict of interest.

Lea T Grinberg, HBPP co-chair, Paulo Nascimento Saldiva, Director of the Pathology Department and Wilson Jacob Filho, Director of Geriatrics, as local hosts welcomed the audience of about 95. Also Helmut E. Meyer, co-chair of the initiative gave a brief insight into the history of HUPO and its initiatives (liver, lung, brain, etc.).

The first session started with Helmut Heinsen (Würzburg, Germany): “Alzheimer’s disease as a proteinopathy and the concept of selective vulnerability: reductionism versus neurobiology?” Biochemically, the major steps in the catabolism of amyloid precursor protein (APP) and the phosphorylation of tau have been elucidated. Theoretically, each of these biochemical reactions gives an opportunity for therapeutical pharmacologic intervention. A major focus in research was directed at the prevention of amyloid accumulation and some progress was achieved in animal models. However, unwanted side effects in human trials concerning amyloid removal and neglect to prevent tau pathology remain a major challenge in Alzheimer’s disease (AD) prevention and therapy.

“Proteomic and functional analysis of an APP/APLP1/APLP2-knockdown & FE65-knockdown cell culture model – relevance for Alzheimer’s disease” was then presented by Andreas Schrötter (Bochum, Germany). A central hallmark of AD is senile plaques mainly composed of ß-amyloid, which is a cleavage product of the amyloid precursor protein (APP). The physiological function of APP and its family members APLP1 and APLP2 is poorly understood. In order to fill this gap, we established a cell-culture based model with simultaneous knock-down of all members of this family. A comprehensive proteome study of the APP/APLP1/APLP2-knockdown cell lysates vs. controls revealed significant protein abundance changes. Targeted functional analysis and validation of selected candidates supported the significant down-regulation. Our results point to a role of the APP family proteins in cellular methylation mechanisms and fit to findings of disturbed levels of S-adenosylmethionine (SAM) in tissue and cerebral spinal fluid (CSF) of AD patients vs. controls. Furthermore current AD research gives evidence that cell cyclere-entry might contribute to a central and causative hallmark in AD. Neuronal cell re-entry into the cell cycle and DNA damage are described to result in apoptosis – a central event in neurodegenerative diseases. Our work provides evidence for the underlying mechanism including two prominent proteins. Initially, we identified both proteins as differentially abundant in a proteomic study comparing a stable FE65-knockdown cell line with respective controls. However, a set of proliferation assays in this work revealed that FE65-knockdown cells demonstrate significantly less cell growth. Derived from these experiments we hypothesize that elevated nuclear FE65 levels cause a cell cycle re-entry mediated by the interaction and abundance of our protein candidates. Various confirmation experiments, co-immunofluorescence and a FE65 interaction study using human brain lysates and human cell culture revealed that elevated nuclear levels of FE65, which have been shown by others to be present in AD brain neurons, result in a stabilization of one of our identified candidates in nuclear mobile spheres. These spheres are able to grow and fuse, and potentially correspond to the nuclear domain 10. The findings from both knockdown studies result in a putative pathway which might be highly relevant for AD.

Co-Chair Lea T. Grinberg (San Francisco, USA and Sao Paulo, Brazil) finished the session’s first part giving insights into “Effects of beta amyloid regulation in cerebrovascular disease”.

Interaction of cerebrovascular changes and AD is a very sensitive issue in AD research. The resting cerebral blood flow (CBF) is reduced in selected neocortical regions even in early stages of the disease. Abeta effects on cerebrovascular regulation nespecially abeta 1–40, the common form found in cerebral amyloid angiopathy (CAA). Its effects are mediated by endothelial factors that could be modulated with drugs improving clinical symptoms in AD. This is an interesting question for neuroproteomics studies.

“Proteomic analysis in neuromelanin granules in Parkinson’s disease (PD)” was then presented by Renata Paraizo Leite (Sao Paulo, Brazil). She first summarized the cooperation between MPC and BABBSG regarding PD, focusing on protein identification via mass spectrometry. The interaction is a unique opportunity for understanding the structure and function of the neuromelanin and also to identify proteins that are altered in PD. Also, the characterization of proteins in the substantia nigra is of interest.

Dirk Woitalla (Bochum, Germany) presented “Biomarker in Parkinson’s Disease: The ParkCHIP-Project”. Most of the neurodegenerative disorders are defined through pathological changes, which are out of reach during lifetime. For instance PD is defined by a lesion of the substantia nigra accompanied by the appearance of Lewy-Bodies in circumscript areas of the brain. Nevertheless, even with this sine qua non for the diagnosis of the disease, PD is more than just loss of midbrain dopamine neurons in association with Lewy Bodies. There is an ongoing discussion about the progression of the disease and the sequence of events, leading finally to the damage of substantia nigra. With the hypothesis of a pathological progression, starting from the dorsal motor nucleus of the vagus, finally reaching cortical areas. Braak and colleagues introduced a concept which motivates clinicians and scientists to study the timeframe of the disease in more detail. Actual hypothesis for the starting point include: mitochondrial dysfunction, oxidative stress, altered protein handling including the seeding theory, and inflammatory changes, finally leading to cell dysfunction and death by apoptosis or autophagy. For all these models a spread of disease could be demonstrated. The genetic model, the exotoxic model with rotenone, the infectious model with H5N1 viruses, all models demonstrate a dynamic involvement of neuronal structures staring at the enteric nervous system of the gut. With this dynamic change in mind, the question of the pathological mechanisms, leading to this upstream of events is still not answered. Even more, it is unclear whether these pathological changes are identical in all brain areas involved and in all patients with clinical symptom fulfilling the definition of PD. Neuronal death is always followed by microglial reactions, which occur recently after the cell damage. The infiltration of T-cells follows as a sequel, finally leading to astrocytosis as a cumulative endpoint. Inflammatory changes are currently not in the focus of the scientific world dealing with PD, but all models demonstrate pathological changes in conclusion with this inflammation-based concept. Activated microglia could be demonstrated in exotoxic induced parkinsonism via rotenone, and the spread of disease is always accompanied by these changes. Of course the infectious model using 5H1N viruses activate the innate immune systemand demonstrate a long lasting activation of microglia and significant loss of substantia nigra dopaminergic neurons after virus eradication. And finally the genetic mouse model demonstrates inflammatory reactions as well. The role of the key players in immunology has still to be elucidated. Mutations in some genes, regulating the immune response are associated with a higher incidence for PD. The question of

mechanisms leading to spread and concurrently cell death in the next neuron is not answered yet. Activation of immune mechanisms secondary to pathological changes and cell loss are obvious in all models so far and give raise to the hope that these changes may induce an antibody reaction reflecting the spread of the disease. These immunological mechanisms primarily involving the adaptive immune system are in the focus of the ParkCHIP-Project, which currently reached the first milestone of development. Antibodies induced primarily or secondary, could function as a “bottleneck”, mirroring the spread of the disease through different cell populations of the enteric and central nervous system.

“Clinical Study for Characterization of Autoantibodies in Parkinson’s disease by Protein arrays” was presented by Caroline May (Bochum, Germany). PD is the second most common neurodegenerative disorder in the elderly. Because no biomarker is available, the diagnosis and also the monitoring of disease progression are still based on clinical criteria. Especially in early stages, several conditions can mimic PD, which leads to false diagnosis. The trigger for the neurodegeneration is still unknown; neuroinflammatory processes seem to play a crucial role. Besides general mechanisms activating and amplifying neuroinflammation, such as generation of proinflammatory cytokines by local glial cells, a neuronal labeling in the substantia nigra pars compacta with Parkinson’s disease derived immunoglobulins was described. Therefore antibodies may play a critical role in the pathological process. Antibodies are highly stable, accessible e.g. in blood or also saliva and can be easily measured with different techniques with e.g. protein microarrays, ELISA, or other methods. Assuming the immunological mechanisms involved in PD and the requirements to an optimal biomarker, antibodies are good biomarkers candidates for an early diagnosis, with a high sensitivity and specificity and perhaps the ability to monitor disease progression. We performed a case-control study using human protein microarrays to identify human autoantibodies in serumsamples for discriminating PD patients from two other reference groups, one with neurodegenerative/autoimmune diseases and a second with non-diseased controls. We identified autoantibodies a panel that classify patients from healthy and diseased controls. The identified autoantibodies are partially directed against proteins, which are already described in literature in the context with the disease pathology. These findings suggest that a panel of autoantibodies may function as a specific biomarker for PD.

“The non-coding transcriptome in Parkinson’s disease” was presented by Stefano Gustincich (Trieste, Italy). He focused on the transcriptional landscape of the Mammalian Genome as well as the transcriptional landscape of dopaminergic neurons.

Nadja Lardner (Sao Paulo, Brazil) referred to “Alterations in base excision repair activity in brains from MCI and AD patients”.

Session 2, Neuropsychiatry disorders and other neurodegenerative diseases – was chaired by Helena Brentani (Sao Paulo, Brazil). RogérioPanizzutti (Sao Paulo, Brazil) presented his talk “Amino acid neurotransmitters in neuropsychiatry disorders”. He stressed the importance of abeta peptide and d-serine in ND. Increased D-serine was found in the hippocampus of an animal model. The injection of abeta oligomers increases d-serine levels in rats. “Schizophrenia gene networks” was the topic of Helena Brentani (Sao Paulo, Brazil).

She pointed out that altered oxygen metabolism is associated with neurogenesis. Comparing one region of the frontal lobe form brain tissue from the Stanley Foundation series (25 schizofrenic and 25 controls), they found 637 genes differentially expressed. Luciana Haddad (Sao Paulo, Brazil) presented her recent work on “Trinucleotide CGG repeats and alternative splicing of FMR1 transcripts in the brain”. The regulator of synapsis-mediate mRNA translation important for cerebral synaptic plasticity and increased expression of FMR1 exon 12 in the cerebral cortex and cerebellum upon birth.

Session 3, Normal Aging/Methods, was chaired by HBPP general secretary Katrin Marcus. The first talk was on “The Human Brain in Numbers: from Ancient Ages to Old Age”, presented by Roberto Lent (Rio de Janeiro, Brazil). One question to be asked is whether the human brain is an outlier in evolutionary terms. The answer is no, we are just a lucky species to have an effective brain. Severely demented people have lost a great number of their neurons.

Erich Fonoff (Sao Paulo, Brazil) gave his presentation about “Development of a dimensional brain atlas applied to functional neurosurgery”. He mentioned that it is important that brain slices have the right size, as they need to be analyzed correctly. Furthermore, the correlation with functional and postoperative data is important.

Helmut Heinsen (Würzburg, Germany) presented his second talk “A strategy to discover early biochemical changes in vulnerable neurons during aging and Alzheimer’s disease”. Knowledge of start and spread of AD-changes are mandatory for the early diagnosis of AD, the development of biomarkers and an effective therapy that intends to prevent irreversible neuron loss. At present, we are experiencing a shift in early diagnosis from cortical to subcortical regions including the brainstem. At closer examination we find in all vulnerable regions neurons that are highly vulnerable close to other neurons that are less vulnerable or not at all affected. Therefore, a single neuron proteomic analyses of vulnerable and less vulnerable neurons appears to be the most parsimonious strategy to separate unwanted confounding factors including agonal and post-mortem changes inevitably associated with human post-mortem studies.

Edson Amaro (USP Medical School) then presented his talk “Neuroimaging post-mortem: challenges and opportunities”. He stressed the fact that virtual autopsy is possible and more informative and that traditional autopsy does not detect all alterations. Virtual autopsy can guide more efficient tissue sampling.

Session 4, Biomarkers, was chaired by Young Mok Park (Ochang, Korea). The first talk was given by Jens Wiltfang (Essen, Germany). His talk was entitled “Translational dementia biomarker research: From predictive molecular diagnosis to novel preventive therapies”. He stressed that the current needs in neurodegenerative diseases are that treatment of neurodegenerative diseases starts too late and critical time windows are missed. Second, that there is need for novel preventive treatments, and third, that we need to identify sub-phenotypes of multi-genetic neurodegenerative diseases responding differentially to treatment interventions. Therefore translational biomarker research in neurodegenerative diseases has to establish biomarker-guided diagnostics of preclinical neurodegenerative

diseases which is mandatory for targeted application of novel preventive treatments. It also has to identify novel therapeutic targets and sub-phenotypes of multi-genetic neurodegenerative diseases which is mandatory for sub-phenotype specific therapies and to monitor therapy efficacy, to establish responder prediction. He presented current neuromic biomarker discovery & validation strategies as well as genome-wide Association Studies (GWAS) guided neuromic backward strategies for the identification of novel treatment targets. In summary, Wiltfang stated that Neuroproteomic biomarker discovery established CSF guided neurochemical dementia diagnostics which has entered national and international diagnostic guidelines and that neuroproteomic biomarker research becomes increasingly important for reliable early and differential diagnostics. So far biomarker guided predictive molecular diagnostics identifies high risk patients within prodromal states of neurodegenerative diseases which is mandatory for the development of novel preventive therapies and multiplex blood assays for early (predictive) diagnosis of AD seem feasible, but have to be validated.

Mitiko Saiki (Sao Paulo, Brazil) focused on the “Determination of trace elements in human brain”. She reported her recent results from analyzing brain tissue. To determine elements in brain tissues from different brain regions it is important to make comparisons among these data as well as our data with values in literature. A gamma-ray spectrum and dendrogram obtained in cluster analysis for different brain regions were shown, as well as mean element concentrations in different regions of brain from normal individuals.

“A combined laser microdissection and mass spectrometry approach reveals new disease relevant proteins accumulating in aggregates of filaminopathy patients” was presented by Katrin Marcus (Bochum, Germany). She presented actual results of studies in filaminopathy using laser microdissection (LMD) in combination with lable-free mass spectrometric (MS) analysis. Filaminopathy is a subtype of myofibrillar myopathy (MFM) caused by mutations in FLNC, the gene encoding filamin C (FLNc), and histologically characterized by pathologic accumulation of several proteins within skeletal muscle fibers. With the aim of gaining new insights in aggregate composition, aggregates and control tissue from skeletal muscle biopsies of six MFM patients harboring three different FLNC mutations were collected by LMD and analyzed by a label-free MS approach. A total of 390 proteins were identified, and 31 of those showed significantly higher spectral indices in aggregates compared to patient controls. The findings suggest that aggregates in filaminopathy have a largely organized structure of proteins also interacting under physiological conditions. Different FLNC mutations seem to lead to almost identical aggregate compositions. The finding that FLNc was detected as highly abundant protein in aggregates in filaminopathy indicates that our proteomic approach may be suitable to identify new candidate genes among the many MFM patients with so far unknown mutation.

Young Mok Park (Ochang, Korea), presented the “Human Brain Proteome Atlas.” The concept of Human Brain Proteome Atlas (HBPA) was well-timed, not only because of major accomplishments of HBPP pilot phase I + II but also establishment of SOPs for experimental protocols, such as sample preparation, MS and MS/MS data analysis criteria, data Collection Center and establishment of the Brain Bank of the Brazilian Aging Brain Study Group. The HBPA is designed to be an international, multidisciplinary initiative that

was launched by HUPO BPP in 2011. The overarching goal of HBPA is to analyze the proteomic composition of distinctive areas in healthy human brain along with aging and the same areas in subjects with specific diseases, such as AD and PD. Since the launch of HBPA in HUPO congress 2011 Geneva, much effort has been put into overcoming methodological challenges. We discussed the SOPs in detail and established how to make the HBPA project successful. Five laboratories participated: Helmut Heinsen (Universität Wurzburg), Helmut E. Meyer (Ruhr-Universität Bochum), Bonghee Lee (GachonUniv, Medicine Science), Lea T. Grinberg (Univ Sao Paulo) and Young Mok Park (Korea Basic Science Institute). In terms of sample preparation, whole brain, brain regions, cell layers, neurons/glia cells, different type of neurons, human brain vs. mouse brain have been analyzed. The detailed project plan is to compare wild type vs. mutant mice at different age stages, preparation of whole brain, serial sectioning, staining, identifying different regions of interest (CA1), laser capture micro-dissection, whole CA1 vs. single (neuron) cells, and MS analysis.

Rivka Ravid (Amsterdam, The Netherlands) explained “How to run a brain bank”. The overall structure and interfaces of brain/tissue/biobanking is of great importance. Of significance also is the pH-value in the liquor as biomarker for brain tissue (post-mortem time, ante-mortem status). She explained the seven golden standards of brain banking: well-established local donor system, rapid autopsy system and a fresh dissection, compatibility of protocols for tissue procurement, generally accepted diagnostic criteria, quality control of the disseminated samples, ethical and legal aspects and monitoring proper safety procedures. Also, matching factors in tissue banking (post-mortem and ante-mortem) are important. Bankers should never give away the whole part of a brain area.

After the meeting it was decided that Lea T. Grinberg will swap with Young Mok Park and take over the chair of the HBPP project. Young Mok Park will continue his outstanding support for the initiative together with Helmut E. Meyer as co-chair. The workshop closed with final remarks from Young Mok Park and Lea T. Grinberg.

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Figure 1.
The participants at the 17th HUPO Brain Proteome Workshop in São ~ Paulo, Brazil.