P2-047 INDUCIBLE CELL MODEL OF TAUOPATHY EXPRESSING FULL-LENGTH TAU

Yipeng Wang, Inna Khlistunova, Jacek Biernat, Marcus Pickhardt, Martin von Bergen, Eckhard Mandelkow, Eva Maria Mandelkow, *Max Planck Institute, Hamburg, Germany. Contact e-mail: wang@mpasmb.desy.de*

In the course of our studies to develop drugs that prevent tau protein aggregation and degeneration of neurons in tauopathies, we recently generated several cell models of tauopathy. They are based on the N2a neuroblastoma cell line and express different variants of the repeat domain of tau (tau_{RD}) in an inducible fashion (Tet-on): the wild-type sequence, a pro-aggregation mutant (ΔK280), and an anti-aggregation mutant, containing additional proline residues. The cells expressing K18ΔK280 mutant show robust aggregation of tau. The aggregates are toxic to cells and their removal is beneficial. We also found that fragmentation of tau_{RD} is important for initiation of aggregation and that phosphorylation in the repeat domain cannot be considered as a precursor of PHF-tau_{RD}. To find out which protease(s) are responsible for the fragments generated from K18ΔK280 in N2a *Tet-On* cells, we used different protease inhibitors against caspases, proteasome, calpain and thrombin. Only the thrombin inhibitor PPACK inhibited the fragmentation of tau_{RD} suggesting the involvement of a thrombin-like activity in tau aggregation in this cell system. We have now obtained a significantly improved N2a cell model generating tau aggregates composed of full length tau molecules. In order to evaluate the influence of the phosphorylation at SP or TP motifs (in the flanking region of the repeats, targets of proline-directed kinases) on aggregation, we generated a new cell model expressing the full-length isoform htau40ΔK280 and observed its aggregation in the N2a cell model. The aggregation was characterized by three methods: 1) on the biochemical level, the presence of aggregates was demonstrated by sarcosyl extraction of the cells and analysis of soluble and insoluble components, 2) fluorescence microscopy using ThS fluorescence which reports on the propensity of the protein to form β-structure, and finally 3) we visualized PHFs by electron microscopy after density gradient enrichment and gold labeling.

P2-048 UP-REGULATION OF IGF-II/M6P RECEPTOR AND OTHER ENDOSOMAL-LYSOSOMAL MARKERS IN CELLS SURVIVING TOXICITY

Cheryl Hawkes[1], Zelan Wei[2], Doreen Kabogo[2], Satyabrata Kar[2], *[1]University of Toronto, Toronto, ON, Canada; [2]University of Alberta, Edmonton, AB, Canada. Contact e-mail: cheryla.hawkes@utoronto.ca*

Background: The insulin-like growth factor-II/mannose-6-phosphate receptor (IGF-II/M6PR) functions primarily in the trafficking of M6P-containing lysosomal enzymes, such as cathepsin D, from the Golgi to the endosomal-lysosomal (EL) system. Given that EL dysfunction is associated with a variety of neurodegenerative disorders, it is possible that the IGF-II/M6PR may have a role in regulating neuronal viability following toxicity/injury. **Objective(s):** To determine the effect of *in vivo* ablation of basal forebrain cholinergic neurons and *in vitro* treatment of PC12 cells with beta-amyloid (Aβ) on the levels of IGF-II/M6PR, Rab5, LAMP2 and cathepsin D. **Methods:** Adult male Spague-Dawley rats received a bilateral i.c.v. injection of 192-IgG saporin (0.4 μg/μl). Animals were sacrificed at 4, 7, 14 and 28 days following surgery and brain tissues were processed for Western blotting and immunohistochemistry. For *in vitro* cell culture, PC12 cells were treated with 10 μM Aβ1-42 for 6, 12, 24, 48 or 72 hr and then assessed for cell death or changes in EL protein levels. **Results:** 192 IgG-saporin caused a selective loss of basal forebrain cholinergic neurons (and their projection fibers in the cortex and hippocampus) expressing the low-affinity neurotrophin receptor (p75NTR). This loss resulted in an increase of IGF-II/M6PR levels in the basal forebrain and frontal cortex, which were associated with surviving non-cholinergic and p75NTR-negative cholinergic neurons in the basal forebrain. This was accompanied by a time-dependent increase in cathepsin D, Rab5 and LAMP2. An increase in the levels of cathepsin D, and to some extent Rab5, was also noted in the frontal cortex of treated animals, while LAMP2 levels/expression remained unchanged. Additionally, PC12 cells treated with 10 mM Aβ1-42 also showed a time-dependent increase in levels of all EL proteins examined, which was generally not associated with dying cells. **Conclusions:** Given the role of the EL system in protein clearance and structural reorganization following injury, and the importance of the IGF-II/M6PR in lysosomal enzyme delivery, it is likely that the observed increases in IGF-II/M6PR and other EL proteins in surviving neurons/PC12 cells represent an adaptive mechanism aimed at restoring the metabolic and structural abnormalities induced by cell loss (support: Alzheimer Society of Canada).

P2-049 EFFECT OF PROTEASOMAL INHIBITORS ON TAU TRUNCATION AND AGGREGATION IN A CELLULAR MODEL OF TAUOPATHY

Tadanori Hamano, Li-wen Ko, Tania Gendron Fields, Shu-Hui Yen, *Mayo Clinic Jacksonville, Jacksonville, FL, USA. Contact e-mail: Hamano.Tadanori@mayo.edu*

Background: Intracellular assembly of filamentous tau aggregates is a prominent pathological feature that Alzheimer's disease (AD) shares with many other neurodegenerative disorders known collectively as tauopathies. The feature is recapitulated in our cellular model (M1C) derived from human neuroblastoma BE(2)-M17D cells that overproduce wild-type human brain tau isoforms via distinct inducible expression mechanisms (Ko et al. 2004). **Objective:** To determine the role of proteasomal inhibition in tau degradation and aggregation. **Methods:** We seeded M1C cells in 10-cm dishes one day before initiating tetracycline (Tet) Off induction with 1ng/ml Tet. They were exposed to proteasomal inhibitors, *e.g.* epoxomicin (Epx) at 2-50nM, on day 3 after TetOff induction and harvested 2 days after such drug treatment. Cell lysates with or without further fractionation were processed for SDS-polyacrylamide gel electrophoresis, Western blotting and enzymatic assays. **Results:** Treatment with proteasomal inhibitors led to a decrease in chymotrypsin-like protease activity in a dose dependent manner, an increase in ubiquitin and β-catenin immunoreactivities and a reduction of a phopshorylated, full-length tau of 62 kDa. Such reduction in the 62kDa tau correlated with an increase in the level of monomeric and oligomeric tau species, and with caspase and calpain activation as revealed by Western blotting analysis and fluorogenic peptide substrate assays. Some of the monomeric and oligomeric truncated tau displayed immunoreactivity with antibody tau C3, which is specific to caspase-cleaved tau. The drug treatment also augmented the assembly of sarkosyl-insoluble tau aggregates containing truncated tau. Pretreatment of M1C cells with pan-caspase inhibitor was effective in reducing the level of tau C3 immunoreactive species. **Conclusion:** Exposure to proteasomal inhibitors increases the level of truncated and aggregated tau species via caspase and calpain activation.

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P2-050 PROGRESSIVE INTRANEURONAL AMYLOID-BETA 1-42 ACCUMULATION FOLLOWING CALCIUM MEDIATED HYPERPHOSPHORYLATION OF TAU AND AMYLOID PRECURSOR PROTEIN

Jean Noel Octave[1], Nathalie Pierrot[1], Marina Morel[2], Jean-Pierre Brion[2], *[1]Universite Catholique de Louvain, Brussels, Belgium; [2]Universite Libre de Bruxelles, Brussels, Belgium. Contact e-mail: octave@nchm.ucl.ac.be*

Background: Alzheimer's disease (AD), the most frequent cause of dementia, is characterized by the presence of both senile plaques and neurofibrillary tangles in the brain. The amyloid core of senile plaques contains Aβ peptide, which is generated by the sequential cleavage of the amyloid precursor protein (APP), performed by BACE and the γ-secretase complex containing presenilin 1, nicastrin, PEN-2 and APH-1. Paired helical fila-

ments, found in neurofibrillary tangles, contain the hyperphosphorylated protein tau. Both senile plaques and neurofibrillary tangles have to coexist in the human brain to confirm the clinical diagnosis of AD. Nevertheless, the neuronal metabolism of their constitutive proteins, which could explain the formation of both amyloid deposits and paired helical filaments, remains poorly understood. In AD however, many serine and threonine residues of tau protein are phosphorylated by glycogen synthase kinase-3β (GSK3β) and cyclin-dependent kinase-5 (Cdk5). Interestingly, GSK3β and Cdk5 are also able to phosphorylate APP on Thr668. **Results:** In rat cultured neurons expressing human APP, depolarization by 35 mM KCl induced an important increase in cytosolic calcium concentration. In these experimental conditions, a transient increased phosphorylation of human APP on Thr668 was observed, which was inhibited by nimodipine, a specific antagonist of L-type calcium channels. Roscovitine, a Cdk5 inhibitor, was also able to partly inhibit Thr668 phosphorylation. Concomitant with this increase in APP phosphorylation, a transient increased phosphorylation of endogenous tau was also demonstrated on serine 396/404, using the PHF1 antibody. These residues are known to be phosphorylated by GSK3β. Following these transient hyperphosphorylations of both tau and APP, a progressive intraneuronal accumulation of Aβ1-42 was measured. When human APP carrying the Thr668Ala mutation was expressed in rat cultured neurons, KCl-induced depolarisation did not allow the production of intraneuronal Aβ1-42. Therefore, calcium mediated phosphorylation of APP on Thr668 leads to intraneuronal production of Aβ1-42. **Conclusions:** In primary cultures of rat neurons, we conclude that high cytosolic calcium concentrations, induced by neuronal depolarization, trigger both tau hyperphosphorylation and the production of intraneuronal Aβ1-42 resulting from APP phosphorylation on Thr668. Therefore, hyperphosphorylation could represent a biochemical link between the two main pathological lesions found in AD.

P2-051 CATHEPSIN D, B AND L ARE DOWN-REGULATED IN FIBROBLASTS FROM ALZHEIMER'S DISEASE PATIENTS

Aldo Orlacchio[1], Lorena Urbanelli[1], Carlo Massini[1], Simona Mencarelli[1], Giuseppe Pelicci[2], Sandro Sorbi[3], Andrei Hasilik[4], Giorgio Bernardi[5,6], Antonio Orlacchio[5,6], Carla Emiliani[1], *[1]Università di Perugia, Perugia, Italy; [2]Instituto Europeo di Oncologia (IEO), Milan, Italy; [3]Università di Firenze, Florence, Italy; [4]Philipps-University Marburg, Marburg, Germany; [5]Centro Europeo di Ricerca sul Cervello (CERC) - Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Santa Lucia, Rome, Italy; [6]Università di Roma 'Tor Vergata', Rome, Italy. Contact e-mail: orly@unipg.it*

Background: Previous studies established that the population of neurons that frequently degenerate in Alzheimer's Disease (AD) exhibit robust up-regulation of the lysosomal system. **Objective(s):** We investigated the expression of lysosomal proteases cathepsin D, B and L at peripheral level, using as cell model skin fibroblasts from AD patients affected either by sporadic or familial forms of the disease. **Methods:** Cell cultures, enzymatic assays, Western Blotting analysis. **Results:** By enzymatic assays, we observed a down-regulation of cathepsin D in 50% of AD patients. These decreased levels were consequent to regulation at transcriptional level. Western Blotting analysis confirmed the presence of decreased content of cathepsin D in AD fibroblasts. We extended the analysis to cathepsin B and cathepsin L and observed decreased content of these proteins in the same cells. A parallel increase of ras transcript and ras protein had been previously revealed in AD fibroblasts. To investigate the role of ras in the expression of cathepsin D, B and L, we over-expressed in primary fibroblasts a ras mutant (rasV12) known to induce premature senescence. Fibroblasts infection with a retrovirus encoding a constitutively active ras decreased cathepsin D levels but increased levels of cathepsin L and, to a lesser extent, of cathepsin B. **Conclusions:** These data demonstrate that decreased levels of cathepsins in AD fibroblasts are not correlated with ras activation. Overall data provide evidence that lysosomal dysfunction is not confined to CNS. In addition, decreased cathepsin levels and activities could lead to a decreased ability of proteolysis by the cell and might be related to the pathogenesis of the disease.

P2-052 CHICKEN PRIMARY NEURONAL CELLS AS A POWERFUL MODEL FOR STUDYING TAU PHOSPHORYLATION, APP PROCESSING AND Aβ GENERATION

Juan Manuel Maler[1], Hans W. Klafki[1], Hermann Esselmann[1], Nikolaus Kunz[2], Sabine Paul[1], Johannes Kornhuber[1], Jens Wiltfang[1], *[1]University of Erlangen-Nuremberg, Erlangen, Germany; [2]University of Ulm, Ulm, Germany. Contact e-mail: Manuel.Maler@psych.imed.uni-erlangen.de*

Background: Neuritic Plaques containing aggregated beta-amyloid (Aβ) peptides and neurofibrillary tangles composed of hyperphosphorylated tau protein represent the classical neuropathological hallmarks of Alzheimer's disease (AD). **Objectives:** Understanding the cellular biology of key molecules such as APP, Aβ peptides, tau and related proteins may help to devise novel therapeutic strategies against AD. **Methods:** To address this question, a cell culture model based on primary chicken neurons was established. It allows the simultaneous assessment of Aβ peptide release from endogenous APP and the analysis of the phosphorylation state of tau protein under different experimental conditions. Aβ peptides were analysed by quantitative SDS-PAGE/immunoblot and surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), tau proteins were assessed by Western blot. **Results:** Aβ peptides Aβ1-40/42 plus three additional C-truncated species, namely Aβ1-37/38/39 were constitutively released into the culture medium, strongly resembling the highly conserved pattern of 5 Aβ peptides present in human cerebrospinal fluid. Importantly, the chicken Aβ amino acid sequence is identical to human Aβ, therefore, various commercial anti Aβ antibodies can be used for detection. Treating the cells with sulindac sulfide (100 μM) selectively decreased Aβ1-42. In parallel, Aβ1-38 and, to a lesser extent, Aβ1 37 were increased, while Aβ1-40 and the total amount of secreted Aβ peptides remained unchanged. Tau proteins prepared directly from embryonic chicken brains were highly phosphorylated at several sites such as Ser202/Thr205 (AT8 epitope), Ser422, Ser262. In contrast, tau proteins from primary chicken neurons (E8, 6th day in vitro) were unphosphorylated or showed only low levels of phosphorylation at these sites suggesting a significant change under cell culture conditions. Treating the cells with the phosphatase inhibitor okadaic acid induced PHF-like tau hyperphosphorylation as indicated by phosphospecific tau antibodies and a shift in electrophoretic mobility. **Conclusion:** Our results strongly support primary chicken neurons as a powerful experimental model for studying AD related proteins such as APP, Aβ peptides and tau simultaneously.

P2-053 INCREASE OF THE PRODUCTION OF AMYLOID BETA-PEPTIDE BY LITHIUM CHLORIDE IS INDEPENDENT FROM ITS INHIBITION OF GSK3

Christine Feyt[1], Pascal Kienlen-Campard[1], Karelle Leroy[2], Franscisca N'Kuli[1], Bernadette Tasiaux[1], Pierre J. Courtoy[1], Jean-Pierre Brion[2], Jean-Noel Octave[1], *[1]Universite Catholique de Louvain, Bruxelles, Belgium; [2]Universite Libre de Bruxelles, Bruxelles, Belgium. Contact e-mail: christine.feyt@farl.ucl.ac.be*

Background: Alzheimer's disease is characterized by the presence of senile plaques and neurofibrillary tangles in the brain. Senile plaques contain an amyloid core of Aβ peptide, while neurofibrillary tangles contain the hyperphosphorylated protein tau. Glycogen synthase kinase 3 (GSK3) is able to phosphorylate tau at many sites which are found to be phosphorylated in paired helical filaments in Alzheimer's disease. Another substrate of GSK3 is β-catenin. The phosphorylation of β-catenin by GSK3 targets β-catenin for proteasomal degradation and prevents its translocation in the nucleus. Lithium chloride (LiCl) efficiently inhibits GSK3 and was reported to also decrease the production of amyloid-β peptide (Aβ) from its precursor APP. Therefore, lithium has been proposed as a