the FEBS Journal

Volume 282 Supplement 1 July 2015

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Abstracts

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P21-015
Identification of transmembrane pseudo-phosphatase Plasticity related gene 2 as an interacting partner of PTEN

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We identified the transmembrane protein Plasticity Related Gene 2 (PRG2) as a novel binding partner of PTEN in mouse brain. PTEN is an important tumor suppressor and negative regulator of the PI3K/Akt pathway with established roles in neuronal circuit formation. PRGs belong to the family of lipid phosphatases/phosphotransferases and share high homology with bioactive lipid e.g. LPA, S-1-Pi inactivating phosphatases, influencing cell migration and neurite retraction. We hypothesize that the C-domain of PRG2, containing a unique and highly acidic polyglutamate stretch, may control PTEN membrane localization and/or activity. For example, PRG2 may sequester PTEN away from the membrane and provide an efficient 'off'-switch for PTEN-mediated inhibition of the PI3K/Akt pathway. Our work supports this idea: Overexpression of PRG2 in HEK cells antagonizes PTEN function towards decreasing PI3K/Akt signaling. Further analyses demonstrate that PTEN interacts with PRG2, whilst different regions within the C-terminal PRG2 domain, participate in PRG2-PTEN interaction. Importantly, a mutant PRG2 lacking the acidic stretch, still binds PTEN but is ineffective in relieving PTEN-dependent downregulation of pS473 Akt phosphorylation in cells. To study the role of this interaction, we established inducible ES cell clones expressing tagged versions of PRG2 and deletion variants. Following neuronal differentiation into ES cell derived motor neurons and induction, PRG2 localizes prominently to plasma membrane domains and active growth cones, increases in early axonal outgrowth and filopodia length. Our results suggest that PRG2 may regulate neuronal cell morphology and growth by fine-tuning the efficacy of the PTEN/PI3K/Akt pathway.

P21-016
Optimizing CNS-delivery by lactyl stearate-coupled liposomes

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Meningitis is the inflammation of tissues which covers brain & spinal cord. Thus lactyl stearate coupled liposomes bearing rifampicin (highly lipophilic) is used for effective management of meningitis. Brain drug targeting brings a healthy skepticism to the study of the BBB, which is the most frustrating obstacle for pharmacologists wishing to find treatments for brain disorders. Synthesized Lactyl stearate was used to prepare liposomes bearing rifampicin by Lipid cast film method. Formulations were characterized for vesicle shape by Transmission Electron Microscopy (TEM), vesicle size, drug entrapment efficiency, *in-vitro* drug release. The *in-vivo* studies the drug distribution in various organs and blood of albino rats was assessed after I.V. administration. The quantitative uptake of the formulations by the brain in albino rats was assessed by fluorescent microscopy. The % encapsulation efficiency was 41% & 34% in uncoupled & coupled liposomes. Brain uptake was increased about 2-3 times in case of uncoupled liposomes and plain drug. Accumulation was increased about 6-8 times with coupled liposomes in comparison to uncoupled and about 10–12 times higher compared to drug solution. Fluorescence study indicate that the preparation is crossing basal carotid system & accessing the nervous system. This delivery system not only increased the brain uptake of the drug but it also reduces the administered dose and toxic effect of the drug. Thus, Lactyl stearate coupled liposomes effectively delivers the drug to the brain and has great potential for brain targeting.

Mol Neu S3, Degeneration and Ageing of the Nervous System

P22-005-SP
Defective cross-talk between the ubiquitin proteasome system and the autophagy lysosomal pathway under proteasome stress in aged rat hippocampus

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Autophagy plays a key role in the maintenance of cellular homeostasis participating in essential cell-fate decisions concerning cell death and survival. Autophagy deregulation gives rise to severe disorders, such as cancer and neurodegeneration. Despite autophagy machinery is well known, many of the signaling pathways regulating autophagy under stress situations are still poorly understood. Using a model of proteasome stress in rat hippocampus, we have analyzed the age-related modifications in the cross-talk between the two major cellular proteolytic systems: the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathway (ALP). We demonstrated that under proteasome stress both autophagy activation and resolution were efficiently induced in young but not in aged rats. Protein homeostasis was rapidly restored in young animals, whereas aged animals accumulated aggregates of ubiquitinated proteins, as well as non-digested autophagic vacuoles, in pyramidal neurons. Importantly, proteasome inhibition inhibited GSK-3β in young but not in aged rats, which could have consequences on both the b-catenin stabilization and the transcription factor EB (TFEB) signaling. Moreover, the age-related difference in the GSK-3β signaling could be due to a dysfunction in the signaling pathway of the insulin growth factor-1 (IGF-1). Considering that alteration of proteostasis represents a hallmark of neurodegenerative diseases, present data highlight the role of GSK-3β as a master regulator in restoring proteostasis, representing a key molecular target in order to sort out this deleterious effect.

P22-006-SP
Molecular links between aberrant protein oligomers and neurodegeneration in Alzheimer's disease

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Aberrant protein oligomers have been identified as the primary pathogenic agents in many protein deposition disorders including

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Alzheimer's disease. The same polypeptide sequence can assemble into different types of oligomer displaying similar morphologies yet with different abilities to cause cellular dysfunction. The pathogenic nature of oligomeric species stems from their ability to diffuse through biological fluids and to interact with cell membranes. Thus, the role of lipid rafts and their ganglioside (notably GM1) content have attracted increasing attention. Here, we quantify the contribution of GM1 content to the cytotoxic effect of two different types of oligomers, grown from the $A\beta_{42}$ peptide associated with Alzheimer's disease or the model protein HypF-N. We found a quantitative relationship between membrane GM1 content in neuroblastoma cells and oligomer binding. In particular, it appears that toxic $A\beta_{42}$ oligomer binding to the cell membrane occurs with high affinity and apparent saturation kinetics whereas the GM1-dependence of non-toxic $A\beta_{42}$ oligomer binding follows linear kinetics and displays low affinity. Similar trends for membrane permeabilization, Ca^{2+} influx and cell viability were also found in cells with different GM1 content exposed to the oligomers, confirming that the observed cytotoxicity is direct related to oligomer affinity to the membrane. Overall, we provided a robust molecular basis of the role performed by membrane GM1 not only as aggregation promoter but also as key aggregate binding site and hence as initiator of different responses eventually resulting in neurodegeneration. This study was supported by the Fondazione Cassa di Risparmio di Pistoia e Pescia (2014.0251).

P22-007-SP
The dysfunction of retrograde transport is sufficient to disrupt Aβ clearance in astrocytes via distorted endosome trafficking

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We previously showed that aging attenuates the interaction between dynein-dynactin complex, which mediates intracellular retrograde transport system, in cynomolgus monkey brain and that dynein dysfunction reproduces age-dependent endocytic pathology such as intracellular accumulation of abnormally enlarged endosomes. Accumulating evidences suggest that endocytic disturbance is involved in Alzheimer's disease (AD) pathogenesis, and we also demonstrated that dynein dysfunction-mediated endocytic disturbance causes the accumulation of intracellular Amyloid protein (Aβ), the key factor for AD pathogenesis. Thus dynein dysfunction would be one of the causative factor for age-related endocytic disturbance leading to AD pathogenesis. On the other hand, it remains unclear whether such age-dependent endocytic disturbance also occurs in glial cells. Here, we show that intracellular accumulation of enlarged endosomes occurs even in astrocytes of aged monkey brains. Moreover, we found that Aβ accumulates in these enlarged endosomes. RNA interference studies demonstrated that dynein dysfunction reproduces astroglial endocytic pathology and disrupts Aβ clearance in astrocytes via disturbed endosome trafficking. Interestingly, dynein dysfunction did not affect Aβ uptake itself. These findings suggest that endocytic disturbance in astroglial cells may also be involved in age-dependent Aβ pathology.

P22-008-SP
Label free quantitative proteomic analysis of astrocytes directly converted to neurons

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Mesodencephalic dopaminergic (mdDA) neurons play a key role in motor control, cognition and arousal. Their dysfunction or loss is known to cause Parkinson's disease (PD). To date, only pharmacological treatment and deep brain stimulation (DBS) is able to retard the progression of PD. Here, we investigate future options of cell replacement therapies for the treatment of PD. In order to avoid immunological rejection application of autologous transplants is the preferable method. Since efficient reprogramming of patient-derived fibroblasts to neurons is still under debate, we here propose to use astrocytes as a predominant cell type in the CNS that is more prone to generate neurons. By applying cDNA transfections with the transcription factors Sox2, Mash1, Lmx1a and Nurr1 we describe a method to convert astrocytes directly into mdDA neurons. For characterization of the conversion we used label free quantitative proteomic analysis. A number of neural and pro-neuronal specific proteins were newly expressed such as Calm1, Gpm6b, Pacsin2 and Tubb6. Expression was verified at the level of downstream target mRNAs using quantitative real-time PCR and key candidate genes were identified by immunocytochemistry. Unfortunately, above methods cannot exclude unwanted side effects caused by insertional mutagenesis of foreign nucleic acids. Here, we depict the functional transduction of membrane permeable HTN-Mash1 and HTN-Lmx1a proteins, which was validated by Rhodamine-labeling and analysis of downstream target mRNA. Taken together these results provide first insights into proteome profiles during the direct conversion of astrocytes to neurons.

P22-009
Rosmarinic acid redirect lysozyme from its normal amyloid formation pathway into nontoxic amorphous aggregates and reduces cellular toxicity

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Misfolding and aggregation of various proteins and peptides is associated with a growing list of diseases, including neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases and peripheral disorders such as systemic amyloidosis and type II diabetes. Consequently, inhibition of protein misfolding and amyloid fibril formation might provide a feasible therapeutic approach for preventing amyloid-related diseases. A promising strategy is to identify compounds that inhibit amyloid fibril formation. In this study, using a range of techniques including Thioflavin T (ThT) and ANS fluorescence assays, electron microscopy and circular dichroism, we describe the efficacy of rosmarinic acid (RA), on the inhibition of fibrillogenesis and hindering cytotoxicity induced by amyloid fibrils of hen egg white lysozyme (HEWL). Our data demonstrated that