the selected Spiegelmer in sandwich ELISA based assays, we developed an Amplified Luminescent Proximity Homogenous Assay using our receptor and a commercial cTnI selective antibody. The obtained data corroborated our assumption; the developed Spiegelmer could detect purified human troponin complex and recombinant troponin I protein even in such a complex protein matrix as blood serum. These findings indicate that Spiegelmers could be reasonable alternatives of antibodies in diagnostics. Funding: National Research, Development and Innovation NKFIA-VKSZ_14-1-2015-0004 grant.

P57

Regulation of de- and remyelination in the central nervous system

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Despite of substantial basic and clinical research, two major questions are still elusive in the pathogenesis of multiple sclerosis (MS); (i) why susceptibility of oligodendrocyte subsets to death stimuli varies, and (ii) why oligodendrocyte precursor cells in affected areas fail to replace eliminated oligodendrocytes. To answer these questions, we performed proteomic analysis in a cuprizone-induced demyelination model, the degenerative animal model of MS, after 4 weeks of demyelination, and 2 or 14 days of remyelination. We performed nano-liquid chromatography coupled nano-electronspray ionisation mass spectrometry analysis after isobaric tags for relative and absolute quantitation labeling of lysyl endopeptidase-digested peptides from corpus callosum proteins. We were able to identify about 6000 proteins, out of which 828 changed its steady state level and/or post-translational modification status during de- and remyelination. To indicate the importance of kinase signaling mechanisms of these processes, protein phosphorylation was found to be the most substantial among the observed changes. Additionally, we found significant differences between the groups among proteins involved in apoptosis inducing factor- and caspase-mediated apoptotic processes, and negative regulation of nuclear factor kappa B. Presently we are comparing the results on the cuprizone model with liquor proteomics data of 98 patients with MS. We are performing pathway analyses on the significant proteins common in the two systems and also aim to identify pathogenesis-related biomarkers in MS. This study was supported by grants from OTKA NN-109841 and the Lundbeck Foundation.

P58

Application of SV40 large T antigen-based *in vitro* replication for monitoring translesion DNA synthesis in cellular extracts

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DNA damage tolerance via translesion synthesis is important in bypassing DNA lesions during replication and contributes to genome stability. In this study, we tested the applicability of an SV40 large T antigen-based in vitro replication system to the investigation of replicative lesion bypass. Here we show that plasmids containing synthetic cys-syn cyclobutane pyrimidine dimer photoproducts are replicated with equal efficiency to lesion-fee plasmids in vitro. By applying both Sanger- and new generation sequencing we demonstrate that translesion synthesis is a frequently used lesion bypass pathway in soluble cytosolic extracts of Hela cells, providing almost completely error-free bypass on cys-syn cyclobutane pyrimidine dimer lesions. This suggests the involvement of polymerase η in the process. Acknowledgement: Momentum Grant of the Hungarian Academy of Sciences LP2011-15.

P59

Drosophila as a new tool to study the chromatin structural changes activated by DNA damages

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In eukaryotic cells, any processes which involve DNA have to take place in the context of chromatin structure, which affects the probability of the damaging agents to cause DNA breaks and the recruitment of the repair proteins. The improper repair or persistence of breaks leads to genome instability, which could result in tumor formation. Our goal is to understand what makes cells able to recognize the appearance of DNA break and how the chromatin structure could change around the break. The answers to these questions will provide information on whether specific chromatin structures predispose sites for DNA break and whether memory of previous break is retained in the chromatin structure. We started to setup human cell culture-based and Drosophila *in vivo* experimental systems by which we could study how unique histone post-translational modifications (PTMs) could affect the DNA repair. We take advantage of the *Drosophila* model system where we delete the endogenous histone cluster

and we substitute it with mutant histones which permits or mimics unique histone PTMs. We have already started to mutate histone genes and screen 50 different histone PTMs. We will use these flies to check the DNA repair kinetics in those animals which consist of only the mutated histones. Using the Drosophila and the human cell culture based system we have already identified new H3 and H4 histone PTM candidates that play role in chromosomal rearrangement which could influence the DNA repair processes. The system developed in our laboratory would help in understanding the mechanisms, which give rise to frequent chromosomal break points often detected in tumors. Progress in integrating the chromatin dimension in DNA repair will help to understand how DNA damage may impact on genome stability. These results would also help identifying new key targets in DNA damage repair and the final goal of the project is to find potential biomarkers which could be used in anticancer therapies. Supported by OTKA-PD [112118] and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P60

PARP inhibition initiates the formation of mitochondria-associated mTOR-phospho-ATM NEMO-Akt cytoprotective signalosome in oxidative stress

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Cytoprotection of inhibition of poly-ADP-ribose polymerase 1 (PARP1) in oxidative stress is mediated by preservation of NAD^+ and ATP level of the cell. Previously, we proved that PARP1 inhibition also activates phosphorylation of Akt, which effect contributes to the cytoprotective effect of PARP inhibition. However, it has not yet been revealed how the nuclear signal of PARP inhibition might be transported to the cytoplasm leading to Akt activation. Here, we demonstrate that another DNA break sensor ATM has a pivotal role in the signal translocation and cytoprotection induced by PARP inhibition in oxidative stress. Several studies have proved that ATM is a substrate for PARP and here we establish that the interaction of ATM and PARP is increased in oxidative stress. It is also proved that ATM is exported from the nucleus to the cytoplasm by interacting with NEMO/IKKγ in a Ca^{2+} dependent mechanism. This study establishes first that interaction of ATM and NEMO is increased by PARP inhibition; furthermore, phospho-ATM migrates to cytosol in NEMO dependent manner and forms a mitochondria-associated mTOR-phospho-ATM-NEMO-Akt cytoprotective signalosome in oxidative stress. However, the protective effect of this signalosome induced by PARP inhibition in oxidative stress was completely attenuated by sequenced suppression of ATM NEMO or mTOR, respectively, by siRNA. Taken together, these data suggest that PARP inhibition initiates the formation of mitochondria-associated mTOR-p-ATM-NEMO-Akt cytoprotective signalosome in oxidative stress.

P61

Flotillin-1 is an interacting partner of protein phosphatase 2A

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Protein phosphatase 2A (PP2A), one of the main phospho-Ser/Thr specific phosphatases in mammalian cells plays an important role in the regulation of cell cycle, signal transduction, cell differentiation, cytoskeletal remodeling and even cellular dysfunction. A typical PP2A holoenzyme contains a scaffold A subunit, a catalytic C subunit and one of the many possible regulatory B subunits, which are assigned into B, B′, B″ and B″′ families. The variable B subunit(s) influences substrate specificity and/or subcellular localization of a given PP2A holoenzyme. Our goal was to identify new interacting partners or substrate proteins of the PP2A–B55α holoenzyme in endothelial cells. The coding sequence of B55α was amplified using specific primers and cloned into pGEX-4T-2 vector. Recombinant protein expression was optimized and to identify new interacting partner of PP2A-B55 in endothelial cells, GST pull-down assay was performed. Flotillin-1 protein was identified by LC-MS/MS analysis and the interaction was confirmed by Western blot analysis of the pull-down samples using anti-flotillin-1 antibody. Bacterial expression construct of flotillin-1 was also created and interaction of GST-flotillin-1 with the A, C and B55 subunit of PP2A holoenzyme was shown by pull down assay. Immunoprecipitation experiments were utilized to verify the interaction of the endogenous proteins in endothelial cells. Immunofluorescent staining of flotillin-1 and B55 showed co-localization of the proteins in the cytosol of the cells. Interestingly, the co-localization pattern suggests that the proteins are associated with the intermedier filaments of the endothelial cells. Accordingly, we showed that both PP2A and flotillin-1 interact with vimentin. Our further plans are to characterize the PP2A – flotillin-1- vimentin interaction and to test whether flotillin-1 is a substrate of the PP2A. This work was supported by grant PD116262 from the Hungarian Scientific Research Fund.