Session 24: Mitochondrialand Lysosomal Diseases

Lectures

L24.1

Mechanisms and therapeutic options of lysosomal storage diseases

Grzegorz Wegrzyn, Karolina Pierzynowska, Lidia Gaffke, Joanna Brokowska, Zuzanna Cyske

University of Gdansk, Department of Molecular Biology, Gdańsk, Poland
Grzegorz Wegrzyn <grzegorz.wegrzyn@biol.ug.edu.pl>

Lysosomal storage diseases (LSDs) is a group consisting of over 50 disorders caused mostly by dysfunctions of lysosomal proteins and resultant accumulation of particular compounds inside cells and extracellular volumes in affected organisms. Genetic diseases are among the most difficult targets for medical treatment. Nevertheless, understanding of molecular bases of LSDs made it possible to develop novel procedures of treatment, employing molecular medicine. Although various therapeutic approaches have been proposed, and some of them were introduced into clinical practice, none of them was found to be effective in correcting all symptoms in treated patients. Central nervous system and skeleton appear to be the most difficult targets to be improved. Therefore, a proposal appeared that perhaps no single therapeutic procedure may be fully effective in treatment of LSD patients, and only combination of two or more approaches could be a successful therapy. Current stage of various therapeutic options and possibilities of their combinations to treat LSDs will be discussed.

L24.2

Leber's hereditary optic neuropathy

Elona Jankauskaite[1], Agata Kodron[1], Ewa Bartnik[1,2]

[1]Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland
Ewa Bartnik <ewambartnik@gmail.com>

Mitochondrial dysfunction is the basis of many diseases, and can be due to mutations both in nuclear and in mitochondrial DNA (mtDNA). For most diseases caused by the latter type of mutations the symptoms depend on the percentage of mutated mitochondrial DNA molecules in a given tissue, and in general most tissues can be affected. Leber's hereditatary optic neuropathy is unusual in that practically all cells contain 100% mutated mtDNA but only one type of cells – the retinal ganglion cells – are affected, which can lead to blindness. Mutations in three complex I genes are responsible for most of the cases, however, the mutations by themselves are not sufficient – the symptoms will appear in 50% of male but only 10% of female carriers.

We have analyzed the effects of testosterone on apoptosis and autophagy in cells from patients and controls to try and explain the above-mentioned differences.

Acknowledgements:
This work was supported by grant 2014/15/B/NZ2/02272 from National Science Centre in Poland.

Oral presentations

O24.1

Nuclear genes involved in mitochondrial diseases with mitochondrial DNA instability in adults

Joanna Rusecka[1], Biruta Kierdaszuk[2], Magdalena Kaliszewska[1], Małgorzata Rydzanicz[3], Piotr Stawiński[3], Tomasz Gambin[4], Anna Kostera-Pruszczyk[2], Ewa Bartnik[1,5], Anna Kamińska[2], Rafał Płoski[3], Katarzyna Tońska[1]

[1]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland; [2]Department of Neurology, Medical University of Warsaw, Warsaw, Poland; [3]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland; [4]Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland; [5]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Katarzyna Tońska <kaska@igib.uw.edu.pl>

Mitochondrial diseases are, by definition, caused by a deficiency of the mitochondrial respiratory chain. One subgroup are diseases characterized by mitochondrial DNA (mtDNA) instability like multiple deletions and depletion resulting from nuclear gene mutations and inherited in a mendelian way. Multiple genes involved in mtDNA maintenance are known and the products they encode for are responsible for mtDNA replication and transcription but also nucleotide metabolism and mitochondrial architecture. The full list of the genes of interest is still unknown.

In adults, mtDNA instability usually leads to progressive external ophthalmoplegia (PEO), frequently accompanied by additional symptoms typically involving the nervous system and muscles (PEO+) but also to ataxia neuropathy spectrum.

Whole exome sequencing (WES) was used as a tool to search for the genes involved in mitochondrial disease in 24 adult Polish patients with multiple mtDNA deletions and excluded *POLG* and *TWNK* coding pathogenic variants – the most frequent cause of mtDNA instability in Poland. The efficiency of WES in this group reached 16%. No new genes were found but novel variants in already known genes like *TYMP* or *RNASEH1* were detected. We can also propose the *SETX* gene as a new player in mitochondrial disease.

O24.2

The murine cellular model of mucopolysaccharidosis III, type B (MPS IIIB) – a preliminary study

Marta Kaczor-Kamińska[1], Kamil Kamiński[2], Krystyna Stalińska[3], Aleksandra Pisarek[3], Arleta Feldman[4], Maria Wróbel[1]

[1]Jagiellonian University, Collegium Medicum, Chair of Medical Biochemistry, Kraków, Poland; [2]Jagiellonian University, Faculty of Chemistry, Kraków, Poland; [3]Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Kraków, Poland; [4]'Mały Maciek i Wielcy Czarodzieje' Fundation, Kraków, Poland

Marta Kaczor-Kamińska <marta.b.kaczor@uj.edu.pl>

MPS IIIB is an autosomal recessive lysosomal storage disorder caused by homozygous or compound heterozygous mutation in the gene encoding N-alpha-acetylglucosaminidase (NAGLU). It is classified as a rare disease. The relatively low incidence of the disease as well as lack of any MPS IIIB cellular model were the limiting factor in basic research conduction. Till today studies were conducted based on cells derived from patients suffering from MPS or *in vivo* MPS mouse models. These factors have enabled us to define our research goal – creation and characterization of the murine *in vitro* model of MPS IIIB.

Primary cells were isolated from both *wild type* (WT) and Naglu$^{-/-}$ mouse tails. Cells selected to the experiments were derived from two groups of mice (KO3 and KO6) with mutation in the NAGLU gene (mice were 3 and 6 months old, respectively) and from wild type (WT3) group of mice, 3 months old. The immortalization process of murine dermal fibroblasts was carried out using a lentivirus carrying the T40 antigen. Cells were selected for the presence of green fluorescent protein (GFP). To the further study, immortalized cells derived from WT and Naglu$^{-/-}$ mice, as well as, primary cells isolated from mice of both genotypes were used.

The most important feature of the murine cellular model of MPS IIIB should be tendency to glycosaminoglycans (GAGs) accumulation. Therefore, the GAGs levels in all experimental groups were checked and differences in GAGs level between KO (Naglu$^{-/-}$) and WT cell types were revealed. It was also confirmed that the KO cells did not produce the *Naglu* enzyme (Western blot techniques, the enzyme activity measurement). The lysosomal staining pictures of these two cell types were taken using confocal microscopy. Additionally, to confirm the proper action of the obtained model, the expression and activity of three selected enzymes: cystathionine γ-lyase, rhodanese and 3-mercaptopyruvate sulfurtransferase were examined and the level of low-molecular thiols (reduced and oxidized glutathione, cysteine and cystine) in the cells of both lines was determined. The results obtained in cell lines are in accordance with the results obtained in tissues (liver, kidney, heart) of mice that were used to create these lines. All these results suggest that the KO cells might be a convenient *in vitro* model of MPS IIIB, however further studies are still necessary.

There is a need to create a cellular model of MPS IIIB disease. This action will could bring many benefits, including: enabling better understanding of the disease mechanisms, enabling the study of new, potential treatments, and what is the most important, it will allow significantly limit the number of experimental animals used for MPS research.