Autofágia, egy lehetséges kompenzációs mechanizmus a glikogén tárolási betegség I-es típusában

(Final report)

One of the major intracellular compartments is the endoplasmic reticulum (ER), which constitutes a special proteome and metabolome within the cell. Alterations of the intracellular homeostasis may provoke ER stress and initiate survival or apoptotic pathways. The glucose-6-phosphatase system of the ER is of vital importance in the maintenance of blood glucose level. The system is responsible for the hydrolysis of glucose-6-phosphate to glucose, catalyzing the ultimate common step of gluconeogenesis and glycogenolysis. Mutations of diverse components of the system are well characterized and they cause the subtypes of glycogen storage disease type I (GSD I). GSD I is an inherited metabolic disorder, where impaired carbohydrate metabolism causes severe hypoglycemia in fasting periods and glycogen accumulation in the liver.

The proposed project aimed to examine, whether GSD I is accompanied with ER stress, and whether ER stress together with the starvation-like phenotype, promotes autophagy. Autophagy is connected with ER stress and is able to catabolize accumulated macromolecules, e.g. glycogen. Thus, we supposed that autophagy may be an ameliorating factor of GSD I, which improves glucose homeostasis by the breakdown of accumulated glycogen, and producing free glucose.

In a first set up of experiments, we tested several cell lines for glucose-6-phosphatase expression by PCR reaction to pick up the optimal one for the experiments. Primers for the catalytic subunit of glucose-6-phosphatase (G6PC) were designed by NCBI primer designing tool. HepG2 human hepatocellular carcinoma cell line, FL83B mouse liver hepatocyte cell line, Hepa1c1c7 mouse liver hepatoma cell line were tested for G6PC expression. As a negative control, Hek293 human embryonic kidney cell line, as a positive control, isolated primary liver cells from mice were used. As a result, HepG2 showed the highest level of G6PC, therefore this cell line was used for any further experiments. Parallel with this, we have isolated primary hepatocytes from mice and tested the optimal conditions for their isolation and culture.

The transfection efficiency of the HepG2 cells were checked and the optimal transfection conditions were chosen by the co-transfection of Luciferase and inducible anti-luciferase silencing vector. The optimal induction protocol by Doxycycline was also controlled: different concentrations were added after transfection, and the fluorescent intensity of HepG2 cells was measured.

Cloning of silencing vectors with inducible promoters in order to silence G6PC protein, we have constructed silencing vectors containing a Doxycycline inducible promoter and 3 different short hairpin RNA sequence and one scrambled vector as a control. After transfection with Lipofectamin, stable clones were selected with Gentamycine treatment for 2 weeks, and the induction with Doxycycline was performed. Monitoring the effectiveness of silencing was performed with Western blot (with G6PC antibody) and RT-PCR analysis (with the above mentioned primers). The glucose production of HepG2, silenced HepG2 and primary hepatocyte cells was measured with the glucose-oxidase-peroxidase kit and the time-dependence of the glucose production was also determined.

The preliminary observations reported above have not been resulted in publishable data. Moreover, a publication from another lab (Farah et al, J. Hepatol. 2016) described the presence of a decreased level of autophagy in GSD Ia models, due to the inhibition of AMPK pathway and the stimulation of mTOR pathway. In the light of this paper, our hypothesis should have been changed and a new wokplan was built up for the future investigations. The new specific aims of the modified plan included:

- investigation of the presence or absence of ER stress in the GSD I models
- induction of the basic level of autophagy and the examination of its effect on glycogen homeostasis
- inhibition of autophagy and its effect on glycogen homeostasis.

The modified research plan was submitted to OTKA and it was approved. However, due to the elapsed time, specific aims could not be fully accomplished. According to the modified research plan, the crosstalk between ER stress dependent apoptosis and autophagy was investigated. ER stress signaling is constituted by the three main branches of the unfolded protein response (UPR). The primary role of UPR is to try to drive back the system to the former or a new homeostatic state by self-eating dependent autophagy, while excessive level of ER stress results in apoptotic cell death. We studied the role of PERK and IRE-1 induced arms of UPR in life-or-death decision. We confirmed that silencing of PERK extends autophagy-dependent survival, meanwhile the IRE-1 controlled apoptosis inducer is down-regulated during ER stress. The proper order of surviving and self-killing mechanisms was controlled by a positive feedback loop between PERK and IRE-1 branches. This regulatory network makes possible a smooth, continuous activation of autophagy with respect to ER stress, while the induction of apoptosis is irreversible and switch-like. Our model claims that the two arms of UPR accomplish an altered upregulation of autophagy and apoptosis inducers during ER stress [7].

The glucose-6-phosphatase system of the ER includes a putative glucose transporter. However, the molecular background of the transport has not been identified; GLUT-type transporters have been the most probable candidates. GLUTs are known also as dehydroascorbic acid (DAA) transporters [1]. DAA transport into/from the ER is an important factor in the redox homeostasis of the ER lumen. Loss-of-function mutations in the gene encoding GLUT10 are responsible for arterial tortuosity syndrome (ATS), a rare connective tissue disorder. We investigated GLUT10-mediated DAA transport, supposing its involvement in the pathomechanism. GLUT10 protein produced by in vitro translation and incorporated into liposomes efficiently transported DAA. Silencing of GLUT10 decreased DAA transport in immortalized human fibroblasts whose plasma membrane was selectively permeabilized. Similarly, the transport of DAA through endomembranes was markedly reduced in fibroblasts from ATS patients. Re-expression of GLUT10 in patients' fibroblasts restored DAA transport activity. The results demonstrated that GLUT10 is a DAA transporter and DAA transport is diminished in the endomembranes of fibroblasts from ATS patients. These results identify GLUT10 as the first glucose transporter of ER localization, which suggest that the protein can be a part of the glucose-6-phosphatase system [6]. In a forthcoming paper we could show that the protein is present in the ER, beside the proviously reported perinuclear localization. The mitochondrial localization suggested by a previous paper was excluded both experimentally and by in silico search [8].

During the reported period I contributed to four review papers [2-5]. I was the first author of one of them [3]. One of the five was closely related to the topic of the project [5], which was published in Autophagy.

In conclusion, although the original hypothesis of the project has been invalidated, the modified workplan resulted in remarkable observations concerning the connections between ER stress and autophagy and revealed a component of the glucose-6-phosphatase system within the restricted time frame.

References

1. Bánhegyi G, Benedetti A, Margittai E, Marcolongo P, Fulceri R, Németh CE, Szarka A. Subcellular compartmentation of ascorbate and its variation in disease states. Biochim Biophys Acta. 2014 Sep;1843(9):1909-16.
2. Csala M, Kardon T, Legeza B, Lizák B, Mandl J, Margittai É, Puskás F, Száraz P, Szelényi P, Bánhegyi G. On the role of 4-hydroxynonenal in health and disease. Biochim Biophys Acta. 2015 May;1852(5):826-38.
3. Margittai É, Enyedi B, Csala M, Geiszt M, Bánhegyi G. Composition of the redox environment of the endoplasmic reticulum and sources of hydrogen peroxide. Free Radic Biol Med. 2015 Jun;83:331-40.
4. Appenzeller-Herzog C, Bánhegyi G, Bogeski I, Davies KJ, Delaunay-Moisan A, Forman HJ, Görlach A, Kietzmann T, Laurindo F, Margittai E, Meyer AJ, Riemer J, Rützler M, Simmen T, Sitia R, Toledano MB, Touw IP. Transit of H2O2 across the endoplasmic reticulum membrane is not sluggish. Free Radic Biol Med. 2016 May;94:157-60.
5. Klionsky DJ, ... Margittai E, ...et al.: Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1-222.
6. Németh CE, Marcolongo P, Gamberucci A, Fulceri R, Benedetti A, Zoppi N, Ritelli M, Chiarelli N, Colombi M, Willaert A, Callewaert BL, Coucke PJ, Gróf P, Nagy SK, Mészáros T, Bánhegyi G, Margittai É. Glucose transporter type 10-lacking in arterial tortuosity syndrome-facilitates dehydroascorbic acid transport. FEBS Lett. 2016 Jun;590(11):1630-40.
7. Márton M, Kurucz A, Lizák B, Margittai É, Bánhegyi G, Kapuy O. A Systems Biological View of Life-and-Death Decision with Respect to Endoplasmic Reticulum Stress-The Role of PERK Pathway. Int J Mol Sci. 2017 Jan 5;18(1).
8. Gamberucci A, Marcolongo P, Németh CE, Zoppi N, Szarka A, Chiarelli N, Hegedűs T, Ritelli M, Carini G, Willaert A, Callewaert BL, Coucke PJ, Benedetti A, Margittai É, Fulceri R, Bánhegyi G, Colombi M. GLUT10 - Lacking in Arterial Tortuosity Syndrome - Is Localized to the Endoplasmic Reticulum of Human Fibroblasts. Int J Mol Sci. 2017 Aug 22;18(8).