The control of mitochondrial biogenesis and energy homeostasis through modulating NAD+ metabolism in beige (brite) adipocytes

Final report for the NKFIH K108308 project

Beige studies

The aim of our proposal was to characterize the involvement of NAD+ metabolism (often referred as the NAD-node) in the differentiation of beige adipocytes. Beige adipocytes phenocopy white adipocytes in terms of morphology, while possess inducible heat generating capacity similarly to brown adipocytes. We modulated NAD+ levels by inhibiting NAD+-consuming enzymes (e.g. PARPs), administrating NAD+ precursors (e.g. nicotinamide riboside – NR) or by modulating the activity of enzymes proximal to NAD+-dependent regulation (e.g. AMPK). We assessed other processes of beige development too. In the in vitro studies we used human white adipose tissue-derived stem cells (stromal-vascular fraction of the white adipose tissue) that were differentiated to white and brown adipocytes.

We used a form of vitamin B3, nicotinamide riboside (NR) to directly induce cellular NAD+ levels through NR salvage. NR supplementation in 1 mM enhanced the beige-like properties of the differentiated beige and white adipocytes. Namely, NR treatment rendered the morphology of white adipocytes more beige-like that went together with the induction of the mRNA and protein expression of PRDM16 and UCP1 (both are brown/beige markers). In line with these observations, NR treatment activated AMPK and induced the NAD+/NADH ratio that points towards sirtuin activation. Finally, NR treatment turned white adipocytes hypermetabolic as reflected by increased glycolytic flux, mitochondrial oxidation, fatty acid oxidation and ATP production. We are still characterizing the effects of NR on beige adipocytes.

We also used another modality to induce cellular NAD+ levels, we inhibited PARPs by a clinically available inhibitor, olaparib (OLA) in 1 μM. OLA treatment diverted the morphology of white adipocytes to a more beige-like morphology. OLA induced the mRNA and protein expression of PRDM16, UC1 and TBX1 (all are beige/brown markers). In OLA-treated cells we observed the induction of AMPK. OLA treatment led to a slight increase in NAD+/NADH ratio. Finally, OLA treatment turned white adipocytes hypermetabolic as reflected by increased glycolytic flux, mitochondrial oxidation, fatty acid oxidation and ATP production. We are still characterizing the effects of OLA on beige adipocytes.

In good agreement with these data, we observed major changes to lipid metabolism in cells, where PARP1 or PARP2 was suppressed. We have shown that PARP-2 is the repressor of the promoter of Sterol Regulatory Element-Binding Protein (SREBP)-1. In the absence of PARP-2 the expression of SREBP-1 is induced, moreover, its nuclear translocation is enhanced culminating in higher de novo cholesterol synthesis. Our data suggest that the enzymatic activity of PARP-2 is indispensable for the repressor activity of PARP-2. The excess cholesterol is not exported from the liver, but accumulates in the liver. Importantly, the deletion of PARP-2 does not interfere with the bile acid homeostasis. In PARP-2-/- mice HDL levels and ABCA1 expression decreases that point towards hampered cholesterol traffic both from the liver towards the periphery and from the periphery to the liver (in mice HDL carries out transport in both directions). (Szántó et al. BBA-Mol Basis Dis 2014)
We performed lipidomics experiments in PARP-1+/+ and PARP-1-/- mice and showed that in the skin of PARP-1-/- mice the ration of n-3 polyunsaturated fatty acids (PUFAs) increased as compared to the n-6 PUFAs that bring about an anti-inflammatory environment that is in accordance with the anti-inflammatory properties of PARP inhibitors. Increases in the ratio of n-3 PUFAs is probably due to the increased activation of the promoter of FABP7 that is a fatty acid transporter that has preference towards n-3 PUFAs and the consequent increase in FABP7 expression in PARP-1-/- mice. (Kiss et al., Mol Med Rep 2014)

In collaboration with Pal Pacher we showed that the inhibition or deletion of PARP-1 is protective against alcoholic and non-alcoholic fatty liver. The protective effect of PARP-1 inhibition/deletion was due to the concurrent activation of SIRT1 that enhances mitochondrial biogenesis. (Mukhopadhyay et al. J. Hepatology 2018)

We identified that another member of the PARP family, PARP10 possesses metabolic regulatory function, the silencing of PARP10 enhances AMPK activity and boosts mitochondrial oxidation, within that fatty acid oxidation together with glycolysis. Importantly, the effects of PARP10 are cell type-dependent. Reduction in PARP10 expression enhanced antioxidant defense and reduced free radical production. Finally, in fasting PARP10 expression is enhanced in the brown adipose tissue, liver and skeletal muscle that points out the possible involvement of PARP10 in beige/brown adipocyte differentiation. (Marton et al. Plos One 018)

We have published seminal review papers on PARPs (Bai, Mol Cell 2015, Bai et al. Trends Endocrin Metab 2015, Xu et al. Med Res Rev 2014, Vida et al. Semin Cell Dev Biol 2017). It is of note the review in Trends in Endocrinology and Metabolism is the first review that is dedicated to discuss the links between PARPs and mitochondrial function and the review in Mol Cell was an invited review. We have described the method we applied in the above-mentioned articles in Mikó et al. Meth Mol Biol 2018. We published a review paper on the role of PARPs on aging (Vida et al. Current Protein and Peptide Science, in press) and a book chapter on the relationship between PARPs and mitochondria (Szántó és Bai, 2016, Bai, Biokémia 2017, Bai, Debreceni Szemle 2015).

To study the role of AMPK in beige differentiation, we differentiated pluripotent stem cells to white adipocytes, beige adipocytes and in a direction where the white protocol was supplemented by AICAR that is an AMPK activator. AICAR induced morphological changes in white adipocytes that are characteristic for beige cells (higher number of smaller fat droplets shown by laser scanning cytometry). Furthermore, we found that AICAR treatment led to a more fused mitochondrial network that suggested a functionally more active mitochondrial network. However, these observations were not supported by oxymetry and gene expression studies, but in contrast, AICAR treatment did not induce mitochondrial oxygen consumption and none of the beige markers (UCP1, CIDEA, PRDM16, TMEM26, TBX1) were induced by AICAR. Our data suggest that either AMPK activation is too distant to induce fully beige-like properties or the model system that we utilized is not a good responder to AICAR. (Abdul-Rahman et al. PLOS One 2016)

We conducted other collaborative studies on NAD+-dependent enzymes. In collaboration with Suowen Xu we studied another NAD+-dependent protein SIRT6 and showed that SIRT6 can protect against endothelial dysfunction and atherosclerosis. (Xu et al. Aging 2016, Xu et al. Trends in Endocrin Metab 2016.)

We were involved in collaborative studies aiming to understand beige physiology. We were involved in setting up a phenotyping platform to study beige and white adipogenesis (Kristóf et al. Sci Rep 2015). In the frame of the same collaboration we showed that Clozapine can induce browning of white adipocytes that was dependent on serotonin receptors (Kristof et al., Transl. Psych 2016). We provided evidence that transglutaminase-2 is a positive regulator of beige and brown adipogenesis, its genetic deletion hampers the function of these cells (Mádi et al, BBA – MCBL 2017). Finally, together with Krisztán Kvell (University of Pécs) we showed that upon the involution of the thymus the adipose tissue filling up the thymus has beige properties (this study is being published).

Other studies

We performed another study to assess how AICAR-mediated induction of AMPK together with methotrexate (MTX) that blunts de novo nucleotide synthesis can modulate cancer cell metabolism. None of the drugs was toxic or modulated the proliferation of MCF-7 breast cancer cells. However in combination these drugs (10 uM MTX+100 uM AICAR, termed later AICAR+MTX) slowed the proliferation of MCF-7 (in addition 4T1 and SKBR3) breast cancer cells. AICAR+MTX treatment induced mitochondrial activity (increases in oxygen consumption, mitochondrial hyperpolarization, increased expression of marker genes) and reduced glycolytic flux that is a typical anti-Warburg rearrangement. In line with these, the G1 to S and the G2 to M transition slowed down elongating the time for the completion of the cell cycle. Finally, in shRNA studies and through screening online databases we have shown that AMPK and FOXO1 are the key players in the observed anti-Warburg changes that have applicability in humans too. (Fodor et al. PLOS).

In collaboration with László Somsák (University of Debrecen, Department of Organic Chemistry) in the frame of which we studied the metabolic effects of glycogen phosphorylase (GP) inhibitors (GPi's). Our first observation was that when a GPi called KB228 was injected into mice, not only blood glucose levels dropped, but also glucose-induced insulin secretion and Langerhans islet size was enhanced. To study these effects we took MIN6 cells that is a cell model for beta cells and treated them with structurally different GPi's (KB228, BEVA335, CP316819 and BF142 that fall into three structural classes). GPi's induced pdx-1 expression (a key factor for beta cell proliferation), insulin receptor expression and insulin expression and secretion. As a side effect we showed that glucose based GPi's (KB228 and Beva335) are also capable of inducing mitochondrial biogenesis that contributes to insulin secretion. We tried to uncover the molecular mechanism of the process. We performed an in silico search for glycogen-binding proteins that identified the constituents of the insulin receptor signalling pathway – these were not verified in wet chemistry experiments yet. We performed experiments with an inhibitor of tyrosine kinase receptors (Gleevec) and Wortmannin (that in the concentration we used inhibits IP3 kinase). Both inhibitors blunted the enhancement of expression of insulin, insulin receptor and pdx-1 induced by GPi's. The currently available data suggest that insulin receptor can be found bound to the surface of the glycogen particles, therefore changes in the number of surface of glycogen particles represent a novel signal for insulin synthesis, secretion and beta cell proliferation. We commenced the patenting of these findings (Bai et al. 117099US-1423 - 62/207,929), but finally we did not begin PCT due to the low number of countries accepting the grace period. (Nagy et al. Br J Pharm 2018).

We participated in a study that assessed the involvement of TLR signalling in tooth germ development. (Papp et al. Acta Odontol Scand. 2016 May;74(4):307-14)

We have shown that the actication of the adenosine receptor A2A and A2B provides protection against obestiy via mmodulating the activation of the resident macrophages in the adipose tissue. (Csóka et al. Diabetes 2014, Csóka et al. FASEB J 2017)

We showed that the silencing of TASK-3 channel leads to mitochondrial dysfunction and cell death in melanoma cells. (Nagy el al. Arch Dermatol Res 2014)

We were involved in the testing of melanin/melanoma-specific PET tracers. (Trencsény et al. J Pharm Biomed Imaging 2017, Kertész et al. J Cancer 2017, Trencsényi et al. Eur J Nucle Med Mol Imag 2016)

We were involved in studying the cellular effects of zinc supplementation. (Emri et al. Metallomics 2015)

We provided evidence for the involvement of free fatty acid receptor 2 in the differentiation of human adipose tissue differentiation. (Stem Cell Dev 2017)

We have shown that activation of PARP-1 and the consequent mitochondrial damage and cell death has central role in bone development. (Free Rad Bio Med 76: 69-79, 2014)

We provided evidence that the absence of PARP-1 slows down wound healing. (El-Hamoly és mtsai. Mol Med 20: 363-371, 2014)

We published a standpoint paper on the translational aspects of the microbiome (Miko et al. Cell Biol Toxicol

In the frame of a method development project, we were involved in standardizing references genes for animal husbandry and poultry studies (Simon et al. J Animal Phys Animal Nutr 2018)

The studies initiated in the frame of K108308 are continued in the NKFIH K123975 project.