

Final report

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Investigation of the role of poly(ADP-ribose) polymerase-2 in SIRT1 activation, energy expenditure and the modulation of cellular NAD⁺ concentrations

In the frame of the project we investigated the metabolic properties of poly(ADP-ribose) polymerase enzymes.

In PARP-1^{-/-} animals we have detected an energy expenditure (EE) phenotype involving the skeletal muscle and the brown adipose tissue (BAT). Oxygen consumption was increased in the PARP-1^{-/-} mice in line with higher glucose oxidation rate (increased RQ). In parallel, in skeletal muscle and BAT mitochondrial biogenesis and the number of mitochondria was enhanced. Increased EE ameliorate the metabolic profile of the mice (e.g. better insulin and glucose tolerance, protection against high fat feeding-induced obesity).

The interplay of PARP-1 and SIRT1 lay behind the increased mitochondrial biogenesis. In PARP-1^{-/-} mice due to the lack of PARP-1 PARP activity decreases drastically, while NAD⁺ levels increase (the concentration of NAM that is the endproduct of NAD⁺ decomposition does not change). In parallel with these changes we have detected the increase of SIRT1 activity. SIRT1 activation is capable of enhancing mitochondrial biogenesis through PGC-1 α deacetylation.

In HEK293T cells where PARP-1 had been depleted by shRNA or in PARP-1^{-/-} MEF cells we obtained similar results, whereby upon the ablation of PARP-1 NAD⁺ levels and SIRT1 activity increase. SIRT1 depletion by shRNA hampers the enhancement of mitochondrial biogenesis upon PARP-1 depletion.

Since both PARP-1 and SIRT1 are NAD⁺ dependent, moreover PARP-1 is quicker and it has higher affinity towards NAD⁺ than SIRT1 it seems plausible that the common NAD⁺ substrate is the link between PARP-1 and SIRT1. We would like to emphasize however that some gene expression effects (e.g. UCP3 induction) were SIRT1 independent, hence there are probably other molecular events behind the EE phenotype in PARP-1^{-/-} mice.

Upon the utilization of PARP inhibitors we experienced similar enhancement of EE as in the case of genetic ablation of PARP-1. When C2C12 myofibers were treated with PJ34 (a PARP inhibitor) we have observed the decrease in PARP activity and the induction of SIRT1 activity and mitochondrial biogenesis. The effects evoked by PJ34 were mostly SIRT1 dependent (i.e. lost upon SIRT1 ablation). Moreover, when mice were treated with PJ34 for 4 consecutive days we were also able to detect the induction of EE. (Bai et al., 2011, Cell Metab. 13(4):461-8.)

In PARP-2^{-/-} mice we observed similar changes as in PARP-1^{-/-}. PARP-2^{-/-} mice displayed higher EE than PARP-2^{+/+}. In PARP-2^{-/-} mice we observed lower RQ values than in PARP-2^{+/+} suggesting increased oxidization of fatty acids. Increased EE involved the skeletal muscle and liver of the PARP-2^{-/-} animals and was marked by increased amount of mitochondria and increased mitochondrial biogenesis. Interestingly, while skeletal muscle was affected in both strains, the BAT was affected only in the PARP-1^{-/-}, while the liver in the PARP-2^{-/-} mice.

In the skeletal muscle of the PARP-2^{-/-} mice we have observed the induction of the SIRT1 – PGC-1 α axis. The higher level of mitochondrial biogenesis ameliorated the metabolic profile of the mice (e.g. better insulin tolerance, protection against high fat feeding). In case of SIRT1 depletion the expressional changes and the higher oxygen consumption rates that are responsible for the advantageous metabolic properties were lost. In C2C12 myofibers, where PARP-2 was depleted by shRNA we have observed similar effects as *in vivo* (higher oxygen consumption, SIRT1 activation, higher mitochondrial content etc.).

The mechanism of SIRT1 induction is radically different in PARP-2^{-/-} then in PARP-1^{-/-} mice. While in PARP-1^{-/-} mice SIRT1 induction is linked to “NAD⁺ sparing”, we did not detect increases in NAD⁺ upon the depletion of PARP-2. However, we observed the induction of SIRT1 expression upon PARP-2 ablation. Later we have shown that PARP-2 is a repressor of the SIRT1 promoter and binds to the -1 - -91 region. Moreover, the region responsible for PARP-2 binding is highly conserved from the *Xenopus* to the human promoter.

Curiously, the glucose tolerance of the PARP-2^{-/-} mice was impaired after high fat feeding suggesting the dysfunction of pancreas. Indeed, the size of the Langerhans islands was smaller in PARP-2^{-/-} mice (the islets were unable to undergo hyperplastic response). Moreover, we detected lower pancreatic insulin content in PARP-2^{-/-} mice in accordance with an expression pattern indicative of beta cell dysfunction. Pancreas dysfunction in PARP-2^{-/-} mice is related to SIRT1 induction that apparently impairs beta cell regeneration. (Bai et al, 2011, Cell Metab. 13(4):450-60.)

In our *in vitro* experiments we used C2C12 and HepG2 cell where PARP-2 was knocked down using lentiviral vectors containing a 21 nucleotide long interfering sequence specific for PARP-2 (shPARP-2 sequence). We designed another sequence of the same length and nucleotide composition that had no human or murine homolog (scrPARP-2). A549, C2C12 and HepG2 cells were transduced with the above viruses. A549 cells served as controls of transduction as their capabilities were determined earlier (Erdélyi et al. FASEB J, 2009). Data on the PARP-2 silenced C2C12 cells were detailed above.

In shPARP-2 HepG2 cells we have observed the induction of the EE phenotype marked by increased mitochondrial number and mitochondrial membrane potential. Consequently the level of stored lipids and glycogen decreased. Currently we are examining the carbohydrate metabolism of the shPARP-2 HepG2 cells.

The phenotyping of these cells began with a comprehensive analysis of gene expression using microarray. For the analysis we used Affymetrix GeneChip Human Gene 1.0 ST and GeneChip Mouse Gene 1.0 ST microarray slides (n=3/3 scr/shPARP-2). Low values (<500) were omitted as no, or low expression genes and the rest was normalized. The respective values for each gene was compared (sh vs. scr) using a unpaired t-test after Bonferroni correction and p<0.05 was considered as significant. In HepG2 cells 653, while in C2C12 cells 757 genes were dysregulated when comparing sh and scr cells. These genes were analysed using the BINGO module of the Cytoscape software in order to classify the genes into biochemical pathways and functions. The results of the microarray have been validated in RT-qPCR assays.

Among multiple others we observed the induction of cholesterol biosynthesis enzymes. Upon examining the transcription factors driving the expression of the genes induced we presumed that the activation of LXR and/or SREBP-1 and -2 are

responsible for the above detailed effect. Validation suggested that the expression of SREBP-1 and -2 are induced leading to consecutive induction of HMG-CoA synthase and HMG-CoA reductase expression and higher intracellular cholesterol levels. Higher cholesterol levels were observed in shPARP-2 HepG2 cells and in PARP-2^{-/-} mice. These investigations are currently being continued.

We investigated the role of PARP enzymes in gene expression in a murine model of contact hypersensitivity (CHS). On the course of the hypersensitivity reaction of the skin considerable amount of free radicals are produced that – as our previous studies indicated – may induce PARP activation. We have shown that PARP-1 is responsible for the poly(ADP-ribosyl)ation in such situations and its ablation reduces inflammation. In contrast, PARP-2 did not influence the CHS reaction. PARP-1 depletion reduced neutrophil infiltration, proinflammatory cytokine production and the expression of cellular adhesion molecules and MMP9 through influencing the redox-sensitive NFκB and ATF-2 transcription factors. Reducing inflammation blunts oxidative and nitrosative stress and hence PARP-1 activation. Our results identify oxidative stress and PARP-1 activation as major actors in the intensification of skin inflammatory processes. Furthermore we have shown that PARP-1 depletion has partial protective effect on irritative dermatitis. (Brunyánszki és mtsai, 2010. J Invest Derm 130(11):2629-37)

Our group was involved on the tenure of the grant in a study investigating the role of PARP-1 and poly(ADP-ribose) glycohydrolase (PARG) in oxidative stress-induced cell death. In flow cytometry experiments we have shown that both PARP-1 and PARG knockdown cells are protected against hydrogen-peroxide – induced membrane permeabilisation and cell death. (Erdélyi és mtsai, 2009. FASEB J 23(10):3553-63)

Our major findings are as follows:

- The depletion of PARP-1 and -2 enhance biological oxidation in skeletal muscle through inducing SIRT1 activation.
- The depletion of PARP-1 and -2 provide protection against obesity induced by hypercaloric feeding.
- PARP inhibitors evoke similar EE phenotype as PARP-1 depletion.
- PARP-2 depletion leads to enhanced cholesterol biosynthesis and hypercholesterinaemia through the induction of SREPB-1 and -2 and/or LXR activation.