Final report

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Intervention on SIRT1 activity and energy homeostasis through poly(ADP-ribose) polymerase pharmacological inhibition

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 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-1α 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^+ then 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 then PARP-2$^{+/+}$. In PARP-2$^{-/-}$ mice we observed lower RQ values then 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-1α 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.)

The enhancement of mitochondrial function upon the depletion of PARP-2 prompted us to investigate the possible protective effect of that phenomenon in pathological states associated with mitochondrial damage, such as doxorubicin (DOX)-induced vascular damage. DOX damages aortic function short after the beginning of the treatment. PARP-2 ablation protected the aorta from DOX-induced damage to vascular smooth muscle. We performed animal and cellular models to elucidate the mechanism of protection. We have shown the ablation of PARP-2 does not follow the same pathway as PARP-1 ablation. PARP-2 is responsible for a minor part of total PARP activity, therefore its loss does not lead to marked protection of cellular NAD$^+$ levels. However we have detected the induction of SIRT1 expression upon the reduction of PARP-2 expression. Induction in SIRT1 activity has led to mitochondrial stabilization that was responsible for the protection against DOX toxicity. (Szántó et el. 2011. Cardiovasc. Res. 92(3):430-8)

We were involved in a study investigating the role of poly(ADP-ribosyl)ation in skin, eye and in different melanomas. We have shown that in normal skin poly(ADP-ribose) (PAR) is present in keratinocytes, sebocytes, hair follicles, endothelial cells and subcutaneous adipocytes. When investigating a set of melanomas we have shown a positive correlation between PAR content and different indices, such as melanoma thickness, Clark, Breslow and AJCC scores. Altogether PAR accumulation showed strong, positive correlation with the severity of malignant melanoma. We have detected PAR staining in uveal melanomas as well.

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 ablation protects against DOX-induced vascular deteriorations through SIRT1-mediated mitochondrial stabilization.
- There is background PARP activity in the skin and the eye.
- The severity of malignant melanoma show positive correlation with PARylation status.