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Role of poly(ADP-ribose) polymerase (PARP)-2 in mitochondrial metabolism and in doxorubicin-induced vascular damage

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UNIVERSITY OF DEBRECEN
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The Examination takes place at the Department of Physiology, Medical and Health Science Center, University of Debrecen.
June 1, 2012., 11:00

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The Ph.D. Defense takes place at the lecture hall of Department of Dermatology, Medical and Health Science Center, University of Debrecen.
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INTRODUCTION

The PARP superfamily

Poly(ADP-ribosyl)ation (PARylation) is a transient post-translational modification of proteins carried out by the poly(ADP-ribose) polymerase (PARP) enzymes. This is a dynamic process during which the enzymes catalyze the formation of ADP-ribose polymers onto different acceptor proteins using NAD^+ as a substrate. The PARP superfamily has 17 members in humans, encoded by different genes. PARP enzymes share a conserved catalytic domain that contains the PARP signature motif, a highly conserved sequence that forms the active site. The PARylation reaction specifically occurs in response to DNA damage. The prototypical enzyme participating in this modification is poly(ADP-ribose) polymerase-1 (PARP-1). NAD^+ serves as a donor of ADP-ribose residues. The half-life of the polymer is very short because of the fast degradation by poly(ADP-ribose) glycohydrolase (PARG).

PARP-2, another DNA-damage dependent PARP

PARP-2 was discovered as a result of the presence of residual DNA-dependent PARP activity in *PARP-1*$^{-/-}$ MEFs family. So far PARP-1, PARP-2 and PARP-3 are the only enzymes whose catalytic activity is immediately stimulated by DNA strand breaks. It has been shown on numerous occasions that PARP-2 accounts for 5-15% of total PARP activity in cells depending on the model used.

The structure of the *PARP-2* gene and PARP-2 protein

PARP-2 gene is located on chromosome 14 in humans. Due to alternative splicing, two isoforms of PARP-2 exist. The sequence of *PARP-2* is highly homologous among mammalian species.

PARP-2 protein (62 kDa) consists of similar functional regions as PARP-1. The N-terminus of mouse PARP-2 contains the DNA binding domain (DBD), followed by domain E and the catalytic domain (domain F). Domain E predominantly serves as a homodimerization interface, an automodification domain and a protein-protein interaction domain as well. Auto-PARylation of PARP-2 takes place on domain E and on lysine 36 and 37. Domain F on the C-terminus of PARP-2 contains the PARP signature motif. PARP-2 and PARP-1 share a catalytic domain of 69% similarity, with the exception that PARP-2 contains an additional three amino acids insertion in the loop connecting the β-strands *k* and *l* in PARP-1.

Expression pattern of *PARP-2*

PARP-2 expression in tissues shows different pattern than that of *PARP-1*, or *PARP-3*. *In situ* hybridization was performed on fetal and newborn mice. In the fetus, *PARP-2* was expressed highly in the thymus. Liver expression of *PARP-2* was high. In the central nervous system PARP-2 content was high in the spinal ganglia and in certain parts of the brain. Apart from the previously mentioned tissues, *PARP-2* is highly expressed in the cortical region of the

kidneys, the spleen, adrenal glands, stomach, thymus and intestinal epithelium. The testis was also positive for *PARP-2* expression.

In humans slightly different expression pattern was detected. PARP-2 was very abundant in skeletal muscle, brain, heart, testis, high in pancreas, kidney, placenta, ovary, spleen and low PARP-2 expression was detected in lungs, leukocytes, gastrointestinal tract (both colon and small intestine), thymus and liver.

The interactome of PARP-2

The PARP-2 interactome was mapped in affinity-purification mass spectrometry (AP-MS) analysis that identified 42 interactors of PARP-2. These proteins covered a wide array of functions such as cell cycle, cell death, DNA repair, DNA replication, transcription, metabolism, energy homeostasis and RNA metabolism. Only a part of these interactors are unique to PARP-2, while the others are shared with PARP-1.

PARP-2 in DNA repair and genomic integrity

The *PARP-2*$^{-/-}$ phenotype in mice involves hypersensitivity to ionizing radiation and alkylating agents. At the cellular level, PARP-2 deficiency leads to chromosomal breaks. PARP-1 and PARP-2 are key sensors of DNA-strand breaks which are normally repaired by the SSBR or BER pathways. It is reported that the depletion of PARP-2 in human A549 cells have only little effect on global SSBR. However, as shown in murine models, upon the loss of

PARP-2, base excision repair (BER) slows down. PARP-2 interacts with BER factors XRCC1, DNA polymerase β and DNA ligase III, that are all PARP-1 partners as well. Taken together, it is tempting to suggest that PARP-2 is an important contributor to the SSBR/BER processes, similarly to PARP-1. This is strengthened by the observed embryonic lethality of the *PARP-1*$^{-/-}$*PARP-2*$^{-/-}$ double-mutant mice. It has been reported that PARP-1 and PARP-2 have different targets both in DNA and in chromatin. Unlike PARP-1 which binds to SSB, PARP-2 has a higher affinity for gaps or flaps. There are reports in literature suggesting a role of PARP-2 also in double strand break (DSB) repair. Appropriate telomere and centromere maintenance also requires PARP-2. PARP-2 localizes to mammalian centromeres in a cell-cycle dependent manner.

These observations suggests essential role of PARP-2 in accurate chromosome segregation through the maintenance of centromeric heterochromatin structure.

PARP-2 in chromatin remodeling and genome maintenance during spermiogenesis

PARP-2 is highly expressed in human testis. PARP-2-deficient mice exhibit severely impaired spermatogenesis due to a defective meiotic prophase I. These features lead to smaller testis size and male hypofertility in *PARP-2*$^{-/-}$ mice. Probably decreased spermatogenesis has multiple roots that all trace back to insufficient maintenance of genomic integrity during spermatocyte differentiation.

The role of PARP-2 in thymopoesis and inflammatory regulation

The earliest reports on PARP-2 have described high *PARP-2* expression in the subcapsular zone of the thymus where lymphocyte proliferation is the highest. In *PARP-2*$^{-/-}$ mice thymocyte numbers were decreased by half comparing to that of wild type or *PARP-1* $^{-/-}$ mice. The reduced number of thymocytes was associated with decreased $CD4^{+}CD8^{+}$ double-positive (DP) cell survival rather than by a lower cell proliferation rate.

The lack of PARP-2 inhibits astrocyte activation and provides protection against colitis. Interestingly, the lack of PARP-2 suppresses the expression of similar genes as the lack of PARP-1 (*iNOS, IL-1β, TNFα*).

PARP-2 as a regulator of gene expression

PARP-2 acts on multiple levels on gene transcription. Apparently PARP-2 is apt to modify chromatin through regulating transcriptional intermediary factor (TIF) 1β and heterochromatin protein (HP)-1α whereby the depletion of PARP-2 altered the expression of two genes (*Mest* and *HNF4*) in a TIF1β-HP1α-dependent manner. Other studies suggested that PARylation has further roles in epigenetic control, which points towards yet uncovered actions of PARP-2.

PARP-2 can also modulate gene expression through direct DNA binding thus influence the expression of different RNA forms. PARP-2 has been shown to interact with nucleophosmin/B23 that is involved in rRNA transcription. During mRNA expression PARP-2

may act either as a positive co-factor, or a repressor of gene expression.

PARP-2 is proven to interact with members of the nuclear receptor superfamily, such as peroxisome proliferator activated receptors (PPARs). The absence of PARP-2 hampers PPARγ activation, while probably enhances PPARα and PPARδ activation.

PARP-2 interacts with thyroid transcription factor (TTF)-1 and thus PARP-2 may regulate the expression of surfactant protein-B.

The interaction of PARPs with SIRT1 and mitochondrial metabolism

SIRT1 is an NAD^+-dependent protein deacetylase. SIRT1 acts as a regulator of metabolic processes via sensing cellular energy status through NAD^+ availability. SIRT1 activation leads to the deacetylation and activation of numerous metabolic transcription factors such as PGC-1α or FOXOs. Their activation boosts mitochondrial biogenesis and oxidative metabolism through enhancing the expression of key mitochondrial enzymes. The ability of NAD^+ levels to control SIRT1 activity gave rise to the hypothesis that artificially modulating NAD^+ levels, probably by the inhibition of alternative NAD^+ consumer, could be effective in the regulation of SIRT1 activity. Confirming this hypothesis, several laboratories have shown that the activity of PARP-1 and SIRT1 are interrelated due to the competition for the same limiting intracellular NAD^+ pool.

However it is not known whether other PARP family members, such as PARP-2, have similar effects on SIRT1 activity and global metabolism.

PARP-2 in oxidative stress-linked pathologies

PARP-1 is activated upon oxidant-mediated DNA damage. Once activated, it catalyzes the transfer of ADP-ribose moieties from NAD^+ to target proteins. This process depletes intracellular NAD^+ and ATP pools which is a major contributor to cell dysfunction and tissue injury in conditions associated with oxidative stress. It is well understood that the depletion of PARP-1, or its pharmacological inhibition interferes in this process and provides protection against numerous oxidative stress-related diseases. Increased oxidative stress is a major factor implicated in the cardiotoxicity of doxorubicin (DOX).

PARP-2 is also known as a PARP activated upon DNA damage. Studies reported that upon the lack of PARP-2 a protective phenotype evolved against injuries caused by diseases linked with increased oxidative stress, such as focal and global cerebral ischemia, and colitis. However, the mechanism by which PARP-2 deletion provides protection is not yet known.

LITERARY OVERVIEW AND AIMS OF THE STUDY

SIRT1 has been demonstrated to enhance or restore mitochondrial activity in various tissues and reversing mitochondrial damage proved to be successful in counteracting mitochondrial injury caused by oxidative stress-related diseases. There are multiple reports that the lack of PARP-2 provides protection against oxidative stress-related injury. Moreover, the founding member of the PARP family, PARP-1 was demonstrated to be interconnected with SIRT1 in oxidatively stressed cells. These observations prompted us to investigate a possible link between PARP-2 and SIRT1.

DOX therapy is also marked by the disruption of mitochondrial membranes causing cardiovascular injury, against which *PARP-1*$^{-/-}$ mice were protected. Furthermore, SIRT1 has been shown to act as a cardiovascular protective factor. These studies furthered the examination of a possible protective role of the deletion of PARP-2 in DOX-induced vascular damage.

Our scientific aims were the followings:

1. We aimed to explore if PARP-2 interacts with SIRT1.

2. Furthermore, we investigated whether the lack of PARP-2 is protective in a model of doxorubicin-induced oxidative damage.

MATERIALS AND METHODS

Cell cultures

Murine myocytes (C2C12 cells) and aortic smooth muscle cells (MOVAS) were cultured in 5% CO_2 atmosphere at 37 °C in Dulbecco's modified Eagle's medium (4.5 g/l glucose) containing 10 % heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Medium of MOVAS was supplemented with 0,2 mg/ml genetycin.

Lentiviral infection of C2C12 and MOVAS cells

PARP-2 was depleted in C2C12 and MOVAS cells using lentiviral shRNA system. Transduced cells were selected and were then constantly maintained in media containing puromycin.

Animal studies

Homozygous female *PARP-2*$^{-/-}$ and littermate *PARP-2*$^{+/+}$ on a mixed C57/BL6J / SV129 (87.5%/12.5%) background from heterozygous crossings were used. Mice were kept under a 12/12 hours dark/light cycle with *ad libitum* access to water and food.

Mice were randomly sorted into 4 groups: *PARP-2*$^{+/+}$ and *PARP-2*$^{-/-}$ control (CTL), and *PARP-2*$^{+/+}$ and *PARP-2*$^{-/-}$ doxorubicin (DOX) treated. DOX treatment was performed by the administration of 25 mg/kg DOX or saline i.p. Two days post injection mice were sacrificed and aortae were harvested for further experiments.

Total RNA isolation and RT-qPCR

Total RNA was prepared applying TRIzol reagent and then was transcribed into cDNA. Diluted cDNA samples were used for RT-qPCR reactions.

Mitochondrial DNA (mtDNA) analysis

Mitochondrial DNA was determined in qPCR reactions.

Histology and microscopy

Immunohistochemistry was performed on 7 µm paraffin embedded tissue sections with specific antibodies against PAR, PARP-2 and smooth muscle actin (SMA).

TUNEL assay

DNA breakage was labelled with terminal deoxyribonucleotidyl transferase (TdT) and a deoxyribonucleotide mix containing digoxigenin labelled dUTP. Peroxidase conjugated anti-digoxigenin antibody was used and peroxidase was detected by diamino-benzamide reaction.

Malondialdehyde assay

Determination of lipid peroxidation and oxidative stress in tissues was carried out by analyzing malondialdehyde formation measured as thiobarbituric acid-reactive components.

Measurement of PARP activity

PARP activity in cell lysates was determined by using the assay based on the incorporation of isotope from ^{3}H-NAD^{+} into TCA precipitable proteins.

Oxygen consumption

Oxygen consumption of cells was measured by the application of an XF96 oxymeter (Seahorse Biosciences, North Billerica, MA, USA).

Intracellular NAD^{+} measurement

The NAD^{+} content of cells was measured photometrically after an enzymatic reaction that is based upon an alcohol dehydrogenase cycling reaction in which the tetrazolium dye is reduced by NADH in the presence of PMS.

Luciferase reporter assay

Cells were transfected with luciferase reporter constructs containing deletion mutants of the SIRT1 promoter. Luciferase activity was expressed as measured luciferase activity/β-galactosidase activity.

Determination of mitochondrial membrane potential

Mitochondrial membrane potential was analyzed by TMRE (tetramethylrhodamine ethyl ester) fluorescent staining.

Determination of superoxide production

Superoxide production was measured by hydroethidine fluorescent staining.

Western blot analysis

Protein extracts were separated on SDS-polyacrilamide gel then transferred to nitrocellulose membrane. Primary antibodies against PARP-2, SIRT1 and β-actin were used, respectively. Binding of peroxidase-conjugated secondary antibodies was detected using enhanced chemiluminescence.

Chromatin immunoprecipitation (ChIP)

Chromatin was prepared from cell lysates. Chromatin-bound proteins were immunoprecipitated using antibodies against PARP-2 and matrix metalloproteinase-2 as non-specific control. Immunoprecipitated chromatin fragments were then amplified using a primer specific to the promoter region of SIRT1.

Statistical analysis

To determine statistical difference between different groups Student's t-test was applied and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

PARP-2 regulates mitochondrial metabolism by repressing the SIRT1 promoter

Our work was initiated by some intriguing metabolic features of the *PARP-2*$^{-/-}$ mice. *PARP-2*$^{-/-}$ mice were smaller and leaner than their *PARP-2*$^{+/+}$ littermates. At the same time, *PARP-2*$^{-/-}$ mice showed higher oxygen consumption rates pointing towards higher oxidation rates as compared to the wild type mice. Increased energy expenditure (EE) stemmed from higher mitochondrial content of skeletal muscle fibers and had effects on the metabolism of the *PARP-2*$^{-/-}$ mice. *PARP-2*$^{-/-}$ mice were protected against diet-induced obesity and insulin sensitivity of the knock-out animals was retained even after high fat feeding. Multiple studies reported that the activation of SIRT1 in mice results in higher whole-body EE and protection against high fat diet (HFD)-induced obesity. Taken together, *PARP-2*$^{-/-}$ mice phenocopied the effects of SIRT1 activation. Studies that have already demonstrated a link between PARP-1, the major PARP activity in most tissues, and SIRT1 activity furthered this hypothesis. PARP-1 and SIRT1 are linked through competing for the limiting nuclear NAD^+ pool. Taken together, it was logical to assume that PARP-2 depletion acts on SIRT1 activity.

Here we have shown that a clear relationship exists between PARP-2 and SIRT1. However, unlike PARP-1, the impact of PARP-2 on SIRT1 activity is not necessarily based on changes in NAD^+

content, which is in line with the previous observations that PARP-2 represents only a minor PARP activity in cells, therefore probably it does not have significant influence on NAD^+ homeostasis.

In ChIP experiments PARP-2 was shown to bind directly to the SIRT1 promoter. We used reporter constructs, in which serial deletions of the SIRT1 promoter region controlled luciferase expression. These studies demonstrated that knocking down PARP-2 promoted a 2-fold increase in SIRT1 transcription through the very proximal promoter region (-91 bp), an effect that was maintained for the whole upstream regulatory region analyzed. This was strengthened by increased SIRT1 mRNA expression and protein content upon PARP-2 depletion or deletion. In line with that, expression of genes involved in mitochondrial metabolism (e.g. *MCAD, Ndufa2, Cyt C*) enhanced significantly. Taken together, our data identify PARP-2 as a repressor of the SIRT1 promoter.

This is not the first report on the transcriptional regulatory role of PARP-2. In the thymus of *PARP-2*$^{-/-}$ mice, an increased expression of the proapoptotic protein NOXA was observed. In cultured lung epithelial cells PARP-2 has been shown to interact with TTF-1 and hence PARP-2 regulates the expression of surfactant protein-B. Moreover, our group has previously reported that PARP-2 is a positive regulator of PPARγ, therefore its absence impairs fat accumulation. SIRT1 induction may provide an auxiliary mechanism to decrease lipid deposition *in vivo* since SIRT1 may inhibit PPARγ. PARP-2 depletion unites all the beneficial effects previously observed upon triggering SIRT1 activation by various means, such

as enhanced oxidative metabolism, endurance phenotype and protection against body weight gain.

In summary, hereby we provided data that expand the role of PARP-2 as a transcriptional regulator identifying PARP-2 as a suppressor of the SIRT1 promoter. Accordingly, deletion of PARP-2 has the ability to induce SIRT1 transcription and mitochondrial metabolism. The actual molecular mechanism(s) through which PARP-2 impacts on gene transcription is yet to be uncovered. It is of note though, that the interaction of PARP-2 and the promoter region of *SIRT1* gene has been detected in cultured myotubes, skeletal muscle, liver and smooth muscle, therefore it seems to be a widespread mechanism and probably of phylogenetic significance. However, the fact that in certain organs depletion of PARP-2 does not induce EE (e.g. brown adipose tissue) suggests that auxiliary mechanisms might be needed to couple SIRT1 induction to EE. Nevertheless, hereby we suggest the possible utilization of PARP-2 inhibitors to activate SIRT1 and promote oxidative metabolism which could be exploited in the management of metabolic diseases.

Effects of PARP-2 depletion on doxorubicin-induced aortic smooth muscle damage

Two days after the administration of doxorubicin (DOX) we detected the deterioration of endothelial and smooth muscle function in the vasculature of mice. However, in *PARP-2*$^{-/-}$ mice the smooth muscle, and not the endothelium, was partially protected against DOX-induced dysfunction. Moreover, after DOX-treatment smooth

muscle actin (SMA) immunoreactivity decreased in *PARP-2*$^{+/+}$ mice, while it was retained in *PARP-2*$^{-/-}$ mice, suggesting that the deletion of PARP-2 protects against vascular smooth muscle damage upon DOX treatment.

DOX-induced PARP activation is not altered upon PARP-2 depletion

Reactive oxygen species produced by mitochondrial redox cycling of DOX have previously been shown to cause DNA strand breaks hence induce PARP activation and PAR synthesis. It has also been shown that the ablation of PARP-1 resulted in suppressed PAR synthesis and protection against DOX-evoked cardiovascular damage. Since PARP-2 also possesses PARP activity and can be activated upon DNA damage, we set out to investigate the role of PARP-2 in DOX-evoked PAR synthesis.

The deletion of PARP-2 did not alter the DNA breakage - PARP-1 activation - NAD^+ depletion - cell death pathway, nor did it decrease DOX-induced free radical production and the consequent PARP-1 activation. Taken together, PARP-2 depletion or deletion results in vascular protection without affecting DOX-induced general PARP activation.

PARP-2 depletion and consequent SIRT1 overexpression counteracts DOX toxicity

The above described results suggested that the protection provided by the depletion of PARP-2 is based upon a different mechanism than the one responsible for the protective effect of PARP-1 inhibition during DOX treatment. It is well understood that mitochondrial function and structure is deteriorated upon DOX treatment. It has also been reported that the preservation of mitochondrial function is associated with protection against DOX toxicity. Therefore we intended to investigate pathways that modulate mitochondrial function.

SIRT1 activation promotes mitochondrial biogenesis. As we have described, PARP-2 has been identified as a repressor of SIRT1 expression. Therefore, it is tempting to assume that the induction of SIRT1 provoked by PARP-2 depletion might be capable of counteracting DOX-evoked mitochondrial dysfunction.

Indeed, the aortae of *PARP-2*$^{-/-}$ mice and PARP-2 knock-down aortic smooth muscle cells displayed increased SIRT1 expression and SIRT1 content which was the consequence of the decreased occupancy of the SIRT1 promoter by PARP-2. Accordingly, we have examined mitochondrial functions. Mitochondrial DNA content increased in *PARP-2*$^{-/-}$ mice and in PARP-2 depleted smooth muscle cells. Besides, increased expression of genes involved in biological oxidation (such as FOXO1, ATP5g1, ndufa2, ndufb5) also pointed towards increased mitochondrial biogenesis. The boost in oxidative gene expression was maintained

in *PARP-2*$^{-/-}$ mice even after DOX treatment compared with the wild type animals. To further support our hypothesis on increased mitochondrial activity we set out investigating mitochondrial membrane potential and oxygen consumption in aortic smooth muscle cells upon DOX treatment. In the absence of DOX PARP-2 depleted cells had a tendency towards higher oxygen consumption rate, as compared to the control cell line. DOX treatment induced a gradual decrease in cellular oxygen consumption, indicative of DOX-induced mitochondrial dysfunction. DOX treatment for 7 hours accentuated the difference between control and PARP-2 knock-down cells. Mitochondrial membrane potential in control cells increased in line with DOX concentration, pointing to mitochondrial hyperpolarization that has been described as an early event in apoptosis, further supporting impaired mitochondrial biogenesis. The PARP-2 depleted cells were protected against mitochondrial hyperpolarization that equally points towards retained mitochondrial function upon DOX treatment.

Augmented SIRT1 activity boosted mitochondrial biogenesis which probably contributed to the protective phenotype against vascular DOX toxicity that evolved upon the lack of PARP-2. Enhanced mitochondrial biogenesis, induced by PARP-2 depletion, may be able to counterbalance the DOX-induced loss of mitochondrial activity and provides a new approach to counteract the oxidative stress-evoked damage in blood vessels. Indeed, SIRT1 induction has been shown to act as a cardiovascular protective factor, but this is the first time when it was achieved by direct

transcriptional induction through depleting PARP-2. Based on these data, it is tempting to speculate that SIRT1-mediated induction of mitochondrial biogenesis may also have contributed to the protective phenotype upon PARP-2 depletion in oxidative stress-mediated diseases such as colitis or cerebral ischemia and could be exploited in other oxidative stress-related diseases.

Our data also spotlight the importance of developing PARP-2 specific inhibitors and raises the question about the applicability of such inhibitors in therapy as a vascular protective agent. In current therapeutic protocols, dexrazoxane hydrochloride (Cardioxane, Novartis) is used to circumvent DOX-induced cardiovascular damage. However, in July 2011 the US Food and Drug Administration released a statement restricting use only in adult patients with breast cancer who have received >300 mg/m^2 doxorubicin and general approval for use for cardiovascular protection was withdrawn since clinical trials showed higher rates of secondary malignancies and acute myelogenous leukemia in pediatric patients treated with dexrazoxane. The use of PARP-2 specific inhibitors might therefore offer an alternative solution for the effective protection of the vasculature in DOX-treated patients.

All known PARP inhibitors are understood to be able to inhibit both PARP-1 and -2. This is not surprising since the catalytic domains of PARP-1 and PARP-2 are very similar and most PARP inhibitors bind there. Seeking highly PARP-2 selective compounds have given rise to inhibitors that have 10-60 fold preference towards PARP-2 compared to PARP-1. UPF-1069, that inhibits PARP-2 with

60 fold higher affinity than it does PARP-1, was effective in protecting against cerebral ischemia. Unfortunately, at the moment such selectivity is the highest achievable. It is possible that in cells or in *in vivo* settings these inhibitors may partially inhibit PARP-1 too. Therefore the development of highly PARP-2 specific inhibitors is of current interest and of great significance. Since PARP-2 is a minor PARP isoform and accounts for only 5-15% of total PARP activity, its loss probably does not hamper PARP-driven DNA repair drastically. Thus using highly PARP-2 specific inhibitors may counteract certain drawbacks of pan-PARP inhibition or PARP-1 specific inhibitors.

In summary, we propose that modulation of the PARP-2-SIRT1 axis to enhance mitochondrial activity may be a new therapeutic approach to revert mitochondrial hypofunction in the cardiovascular system or in other organs.

CONCLUSIONS

1. PARP-2 regulates SIRT1 activity by direct interaction of the proximal promoter region of the *SIRT1* gene.
2. PARP-2 is a transcriptional repressor of SIRT1.
3. The deletion or depletion of PARP-2 results in the induction of mitochondrial biogenesis through enhanced SIRT1 activity.
4. The deletion or depletion of PARP-2 has advantageous metabolic effects due to increased SIRT1 activity.
5. Enhanced mitochondrial biogenesis, induced by PARP-2 depletion, provides a new approach in counteracting the oxidative stress-evoked damage.
6. Our work raises the possibility of using PARP-2 specific inhibitors in the management of metabolic diseases, and also to alleviate DOX-induced cardiovascular damage.

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APPENDIX

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Register Number: DEENKÉTK /103/2012.
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Doctoral School: Doctoral School of Molecular Medicine

List of publications related to the dissertation

1. Bai, P., Cantó, C., Brunyánszki, A., Huber, A., **Szántó, M.**, Cen, Y., Yamamoto, H., Houten, S.M., Kiss, B., Oudart, H., Gergely, P., Menissier-de, M.J., Schreiber, V., Sauve, A.A., Auwerx, J.: PARP-2 regulates SIRT1 expression and whole-body energy expenditure.
Cell Metab. 13 (4), 450-460, 2011.
IF:18.207 (2010)

*2. **Szántó, M.**, Rutkai, I., Hegedűs, C., Czikora, Á., Rózsahegyi, M., Kiss, B., Virág, L., Gergely, P., Tóth, A., Bai, P.: Poly(ADP-ribose) polymerase-2 depletion reduces doxorubicin-induced damage through SIRT1 induction.
Cardiovasc. Res. 92 (3), 430-438, 2011.
DOI: http://dx.doi.org/10.1093/cvr/cvr246
IF:6.051 (2010)

*The article dually serves as the basis of dissertation. First writer to cite the article was Ibolya Rutkai (HDBA73). Statement on th epartial use of article was issued on Dec 8, 2011

H-4032 Debrecen, Egyetem tér 1. e-mail: publikaciok@lib.unideb.hu

List of other publications

3. Brunyánszki, A., Hegedűs, C., **Szántó, M.**, Erdélyi, K., Kovács, K., Schreiber, V., Gergely, S., Kiss, B., Szabó, É., Virág, L., Bai, P.: Genetic ablation of PARP-1 protects against oxazolone-induced contact hypersensitivity by modulating oxidative stress.
J. Invest. Dermatol. 130 (11), 2629-2637, 2010.
DOI: http://dx.doi.org/10.1038/jid.2010.190
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The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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