SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

THE ROLE OF POLY(ADP-RIBOSE) POLYMERASE-1 (PARP-1) IN THE REGULATION OF TRANSCRIPTION

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DEBRECEN, 2011

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The Examination takes place at Department of Physiology, Medical and Health Science Center, University of Debrecen, 2011.06.29. 11:00

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The Ph.D. Defense takes place at the Lecture Hall of the 1st Department of Medicine, Institute for Internal Medicine, Medical and Health Science Center, University of Debrecen 2011.06.29. 13:00

Introduction

The enzymatic properties of poly(ADP-ribose) polymerase-1

Poly(ADP-ribose) polymerase-1 (PARP-1) belongs to the poly(ADP-ribose) polymerase (PARPs) superfamily. PARP-1 is a multidomain protein comprising a DNA binding domain at the N-terminus, an automodification domain, functioning as a target for direct covalent auto-modification and a catalytic domain at the carboxyl terminus that polymerizes ADP ribose units.

The catalytic function of PARP1 relates to its role as a DNA-damage sensor and signalling molecule. The PARP recognize single- and double-stranded DNA breaks and PARP1 catalyses covalent attachment of ADP-ribose units from donor NAD^+-molecules to a variety of target proteins resulting in linear or branched polymers of PAR.

Under physiological condition PARP-1's enzymatic activity is very low, but it is stimulated dramatically in the presence of a variety of allosteric activators such as single- and double-strand breaks, crossovers, cruciforms, and supercoils, as well as some specific double-stranded sequences, nucleosomes, and a variety of protein-binding partners. The best-characterized activator of PARP-1 is DNA damage induced by free radicals that exert several different effects in cells. Enhanced oxidative stress-provoked excessive PARP activation may deplete cellular NAD^+/ATP stores blocking apoptosis and resulting in necrosis. Moreover, reactive species can play a role in a series of other cell process such as cell signalling, genome integrity or transcription.

Reactive species evoked oxidative stress: activators of PARP-1

Oxidative stress is an imbalance between the excessive formation of ROS (**R**eactive **O**xygen **S**pecies, ROS) and/or RNS (**R**eactive **N**itrogen **S**pecies) and limited antioxidant defenses. In chemistry, molecules or elements bearing an unpaired electron are defined as free radicals. In biology free radicals are often regarded as reactive species formed *in vivo*, of which numerous bear unpaired electrons. However, some reactive species possess reactive character although lack unpaired electrons (e.g. peroxynitrite). By chemical features, free radicals and their derivatives can be subdivided as reactive oxygen species and reactive nitrogen species depending on the central atom. Derivatives are formed at the same time and

at the same place under oxidative stress, developing their biological impact on the cells together.

Among these reactive species hydroxyl radicals, hydrogen peroxide and peroxynitrite are among the most reactive. The reactivity of hydroxyl radicals is extremely high, therefore hydroxyl radical are short lived. Hydroxyl radical is a powerful oxidizing agent that can react at a high rate with most organic and inorganic molecules in the cell. Although hydrogen peroxide is not radical by definition but causes damage to the cells. Hydrogen peroxide can easily penetrate biological membranes. In contrast to superoxide radicals that are considered relatively stable and have constant, relatively low reaction rates with biological components. Superoxide can react with NO forming peroxynitrite ($ONOO^{*}$) is a powerful oxidizing agent that - upon protonation - decomposes to hydroxyl radical and nitrogen dioxide. It causes the depletion of sulfhydryl (SH) groups and oxidation of a wide variety of molecules causing damage.

Endogenous sources of reactive species

Cells are exposed to a large variety of ROS and RNS from both exogenous and endogenous sources. The exposure to endogenous sources is much more important and extensive, because it is a continuous process during the life span of every cell.

Mitochondria The most prominent ROS producing organelle is the mitochondria. Mitochondria represent the place of terminal oxidation and oxidative phosphorylation. Mitochondria may leak electrons from the respiratory chain at complex I, complex III and reduce oxygen to superoxide. Only complex III is capable of leaking electrons to the intermembrane space. As a result of this process, several different oxygen derivatives are formed (e.g. H_2O_2).

Peroxisomes Peroxisomes also possess ROS generating abilities. The main metabolic processes contributing to the generation of H_2O_2 in peroxisomes are the â-oxidation of fatty acids and the enzymatic reactions of the flavin oxidases. The occurrence of reactive species leads to lipid peroxidation, damage of the peroxisomal membrane and loss of peroxisomal functions.

Phagolysosomes In phagolysosomes exogenous invaders (e.g. invading bacteria) are destroyed using reactive species. Phagolysosomes are mainly present in certain white blood cells (granulocytes, monocytes and lymphocytes) and major producers of endogenous ROS

during inflammatory processes. Following stimulation, these cells undergo a respiratory burst. The consequence of the respiratory burst is that a number of reactive oxygen species are produced, which kill the engulfed bacteria.

Nitric oxide synthase enzymes Nitric oxide synthase (NOS) enzymes synthesize NO. NOS enzymes convert L-arginine to NO and L-citrulline via the intermediate N-hydroxy-L-arginine. There are three isotypes of NOS. Neuronal NOS (nNOS, NOS-1), endothelial NOS (eNOS, NOS-3) and the inducible form of NOS (iNOS or NOS-2). nNOS and eNOS are activated in response to a calcium signal, while iNOS expression is regulated at the level of expression. In contrast to nNOS and eNOS, iNOS generates high concentrations of NO. iNOS has an eminent role in inflammation-related "respiratory burst".

The harmful effects of reactive species

Reactive species can attack vital cell components like polyunsaturated fatty acids, proteins, and nucleic acids.

Lipid peroxidation influences membrane phospholipids containing polyunsaturated fatty acids. Peroxidation of lipids can disturb membrane assembly causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes

Predominantly hydroxyl, alkoxyl, and nitrogen-centered radicals cause protein damage. Proteins can undergo oxidation, damage to specific amino-acid residues or tertiary structure degradation, and fragmentation. The consequences of protein damage are loss of enzymatic activity, hence altered cellular functions.

Reactive species induce modifications of DNA such as modification of nucleotide bases, single- and double strand DNA breaks, loss of purines (apurinic sites), damage to deoxyribose, DNA-protein cross-linkage, and damage to the DNA repair system.

These reactive species-induced oxidative modifications can also play role in redox signaling. These changes may modulate gene expression through redox-sensitive transcription factors, such as NF-κB, AP-1, or ATF-2. These transcription factors are known to interact with PARP-1, although in most cases the exact molecular background of the interaction is not yet defined.

The biological functions of PARP-1

As mentioned earlier, PARP-1 has diverse biological functions covering DNA repair functions such as base excision repair, nucleotide excision repair and contributes to double strand break repair. PARP-1 also possesses non-repair functions such as regulation of cardiac remodelling, vasoconstriction, astrocyte and microglial function, long term memory, inflammation, aging, energy metabolism and transcription.

The role of PARP-1 in the regulation of transcription

The regulation of gene expression requires a wide array of protein factors that can modulate chromatin structure. PARP-1 can regulate transcription as a component of insulator, enhancer/promoter regulatory complexes, a modulator of chromatin structure and a transcriptional coregulator.

PARP-1 can act as an insulator. Insulators are DNA elements that help to organize the genome into discrete regulatory units by limiting the effects of enhancers on promoters or by preventing the spreading of heterochromatin.

The earliest characterized effects of PARP-1 on the genome were the modulation of chromatin structure and the PARylation of histones. PARP-1 binding to nucleosomes in the absence of NAD^+ promotes the compaction of nucleosomal arrays into higher order structures. In the presence of NAD^+, PARP-1 undertakes its own automodification and dispatches from chromatin, leading to decompaction and restoration of transcription *in vitro*.

Many of the early studies have described direct effects of PARP-1 on the transcriptional regulation. In this action PARP-1 binds to specific DNA sequences or structures in the regulatory regions of genes and PARP-1 acts a classical enhancer binding factor.

Finally, PARP-1 has been identified as promoter-specific coregulator (either a coactivator or a corepressor) for a number transcription factors, such as NF-ęB, nuclear receptors and others. PARP-1 can interact with enhancers and promoters of genes by direct sequence-specific binding, recruitment via DNA binding of transcription factors (e.g. NF-ęB), direct binding to DNA structures and binding to the dyad axis. The respective DNA-binding factors recruit PARP-1 to relevant target promoters, where in turn PARP-1 may promote the binding of further factors to DNA, stimulating the formation of enhanceosomes and the activation of transcription. The enzymatic activation of PARP-1 is necessary DNA binding for

the appropriate function of most transcription factors (Sp1, NFAT). DNA-binding factors or other components of the coregulatory complex are targets of PARP-1-dependent PARylation. Together, these studies highlight the diverse mechanisms of PARP-1 coregulator function, which are likely to vary in an activator-and gene-specific manner. Most of these transcription factors modulate diverse physiological and pathophysiological conditions, such as inflammation, cellular differentiation, cell proliferation, cell adhesion or metabolic regulation.

Transcriptional events in inflammation mediated by PARP-1

Studies performed with Parp1 knockout mice revealed that pharmacological inhibition or genetic deletion of PARP-1 inhibits the propagation of inflammation. The disregulation of different transcription factors represents the root for the protection against inflammatory diseases. The dominant transcription factors activated during inflammation are NF-κB, AP-1, and ATF-2.

p65 or RelA belongs to NF-ęB family, existing in virtually all cell types, where they act as the principal regulators of inflammatory process. NF-κB, built up of p65 and p50 is the active transcription factor that due to a nuclear localization signal (NLS), is targeted into the nucleus where it binds to DNA. In non-stimulated cells NLS is masked by the action of a monomeric inhibitor protein IκB, resulting in the cytoplasmic accumulation of the transcription factor. Under cytokine stimulation NF-κB is released from IκB, followed by nuclear translocation and transcription of NF-κB-dependent genes.

ATF-2 forms complex with c-Jun or itself and binds to AP-1 sites. The heterodimeric complex AP-1 formed by the dimerization of c-Fos, c-Jun or ATF-2. The genes are induced by extracellular stress, linking AP-1 activity to the cell stress response. AP-1 can therefore be viewed as a critical intermediers in many signal transduction pathways. Under oxidative stress, activated $p38^{MAPK}$ rapidly translocates from the cytoplasm to the nucleus and phosphorylates its substrates such as ATF-2 and c-Jun or other kinases. The AP-1 transcription factor then can bind to DNA and can start the transcription of inflammatory genes.

Inflammatory cytokines such as TNFα, IL-1β are central regulators of the inflammatory process orchestrated by Th1 cells. These cytokines induce the expression of other cytokines and chemokines (chemoattractants), adhesion molecules, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 and matrix metalloproteinases. If PARP-1 is

inhibited or knocked out, the expression of inflammatory cytokines is suppressed (MIP-1α, MIP-2, IL1β, TNFα, and MCP-1). These chemokines and cytokines are NF-κB dependent. Therefore, the suppression of NF-κB activity provides a likely explanation for the reduced expression of these genes.

iNOS is responsible for NO synthesis under inflammatory conditions. iNOS is regulated via NF-κB activation, hence PARP inhibition markedly reduces iNOS expression and nitrosative stress.

MMPs are indispensable for tissular movement of cells. MMPs may be activated by free radical-induced structural changes. MMP activation during inflammation can be prevented by knocking out Parp-1 or by pharmacological PARP inhibition.

Tissue inhibitors of MMPs (TIMPs) that reduce MMP activity are inversely regulated than MMPs upon inflammation. PARP inhibition is capable of restoring the balance between MMPs and TIMPs.

PARP-1 in the transcriptional control of oxidative energy metabolism

SIRTs – homologs of the yeast sir2 (silent mating-type information regulation 2) - are NAD^+-dependent class III histone deacetylases that are induced by caloric restriction and have been implicated in influencing aging and regulating transcription, stress resistance, as well as energy efficiency. SIRT1 can be activated by fasting, exercise or low glucose availability. In all cases increases in intracellular NAD^+ levels induces SIRT1 activity. Upon activation, SIRT1 binds and deacetylates transcription factors such as PGC-1á and FOXO. Deacetylation activates these transcription factors, whereby they induce mitochondrial biogenesis in skeletal muscle, liver, brown adipose tissue and brain. Fat utilization, glycolysis, and gluconeogenesis is enhanced in these tissues. Moreover, in skeletal muscle SIRT1 activation leads to muscle fiber type switch. Muscle fiber type switch elevates the quantity of type I (oxidative) fibers characterized by a higher mitochondrial content.

The dependence of SIRT1 on NAD links its enzymatic activity directly to the energy status of the cell via sensing cellular NAD^+/NADH ratio. PARP-1 can influence NAD^+ levels since its activation consumes cellular NAD^+. Moreover, PARP-1 is a faster and more efficient NAD^+ consumer than SIRT1. These data suggest that PARP-1 activity may impact on gene transcription through modulating SIRT1 activity. PARP-1 and -2 have a baseline PARP activity that is initiated by constant oxidative stress stemming from mitochondrial oxidative

metabolism. Therefore, these PARP enzymes constantly turn over NAD^+ even under non-stressed conditions. Recent studies revealed that through modulating NAD^+ PARP-1 impacts on other NAD^+-dependent enzymes, such as SIRT1.

Literary overview and aims

PARP-1 has been shown to influence transcription and oxidative stress at multiple levels. We set out to investigate the effects of PARP-1 depletion on transcription in different models. On one hand, we utilized a skin inflammation model, oxazolon-induced contact hypersensitivity. In this model, we have already shown the prominent role of oxidative stress and PARP activation. However, the exact molecular mechanism though which PARP-1 or -2 influence inflammation is unknown. On the other hand, we set out to investigate how PARP-1 may act on SIRT1 and oxidative metabolism.

Our aims were the following:

I. Investigation of the role of PARP-1 in oxazolone-induced contact hypersensitivity:

- Defining the respective role of PARP-1 and -2 in oxazolone-induced CHS.
- Characterization of oxidative stress in oxazolone-induced CHS.
- Characterization of transcriptional changes during oxazolone-induced CHS (proinflammatory cytokines, chemokines, adhesion molecules and transcription factors).

II. Characterization of gene expression changes after PARP-1 depletion or inhibition in

- Skeletal muscle, brown adipose tissue (BAT), liver,
- murine embryonic fibroblasts,
- pharmacology inhibition of PARP-1 with PJ34.

Materials and Methods

Animal studies

All animal experiments were approved by the local ethical committee (9/2008/DE MÁB) and were performed according to EU and national guidelines.

In oxazolone-induced contact hypersensitivity reaction, homozygous PARP-1 and PARP-2 knockout (KO) mice and their respective wild-type (WT) littermates on C57Bl/6 background were used. Female mice were randomized into four groups (WT non-sensitized, WT-sensitized, KO non-sensitized, KO sensitized) and were sensitized and challenged.

In PMA–induced irritative dermatitis model PMA (10 µl, 0.05% w/v) was briefly smeared onto both sides of the ears of female mice (6 animals per group), ear swelling was determined together with all other biochemical measurements 24 hours later.

In metabolic studies, male PARP-$1^{+/+}$ and PARP-$1^{-/-}$ mice on a C57Bl/6 background were used. Mice were housed separately, had ad libitum access to water and standard rodent chow (10 kcal% of fat, Safe, Augy, France) and were kept under a 12h dark-light cycle.

In case of PJ34 treatment, mice received each 12h (at 7:00 and 19:00) 10 mg/kg PJ34 by intraperitoneal injection for 5 continuous days. In all studies animals were killed either CO_2 inhalation or cervical dislocation after 6h of fasting (starting at 8:00), and tissues were collected each time (between 14:00 and 15:00) and processed as specified below.

Preparation of MEF cells

After crossing heterozygous PARP-$1^{+/-}$ mice, we sacrificed the pregnant female at day 13 post coitum by cervical dislocation. Dissected out the uterine horns, then excised embryos were finely minced and digested in trypsin-EDTA. Debris were seeded into Petri dishes and fibroblasts were the only cells that attached to the dishes.

Histology and microscopy

Haematoxylin-eosine (HE) histochemistry was performed using antibodies against nitrotyrosine (Sigma), neutrophil elastase (Santa Cruz, Santa Cruz, CA, USA) and PAR (BD Biosciences, San Jose, CA, USA) on paraffin-fixed 7 µm tissue sections.

Myeloperoxidase activity assay

The activity of myeloperoxidase (MPO) was determined in ear homogenates using tetramethylbenzidine and hydrogen peroxide.

MMP activity assay

MMP activity was assayed in protein extracts of ears using fluorescein-conjugated gelatine (DQ gelatine, Invitrogen, Carlsbad, CA, USA).

8-OHdG determination

8-OHdG determination was performed using a commercial kit from Cell Biolabs (San Diego, CA, USA).

Protein carbonylation assay

Protein carbanoylation was assessed using a commercial kit (Cayman Chemicals, Ann Harbour, MI, USA).

Lipid peroxidation assay

Lipid peroxidation was measured using a commercial kit to detect HNE-protein adducts from Cell Biolabs (San Diego, CA, USA).

Total RNA preparation, reverse transcription and qPCR

Total RNA was prepared using TRIzol (Invitrogen) that was then transcribed into cDNA. 10X diluted cDNA were used for quantitative PCR (qPCR) reactions. The qPCR reactions.

Transcription factor transactivation studies

For nuclear protein extraction, the Transfactor Extraction kit (Clontech, Mountain View, CA, USA) was used according to the manufacturer's instructions. For the quantification of transcription factor transactivation, the TransFactor Inflammatory Profiling-1 kit was utilized (Clontech). 10 µg ear nuclear extract was loaded into each well and the measurement was performed according to the Manufacturer's instructions.

mtDNA analysis

Mitochondrial DNA was determined in qPCR reactions.

Statistical analysis

Results were expressed as means ±SEM. Statistical significance between groups was determined by Student's t-test, $p<0.05$ was considered as significant. Error bars represent +/- SEM.

Results and discussion

Several studies have proven the major role of PARP-1 in the regulation of transcription. Our aim was to elucidate the transcriptional role of PARP-1 in relation to skin hypersensitivity inflammatory reaction and to SIRT1 mediated transcription.

Role of PARP-1 in oxazolone-induced contact hypersensitivity

Characterization of the oxazolone-induced contact hypersensitivity reaction in PARP-1$^{-/-}$ and PARP2$^{-/-}$ mice

After oxazolone challenge, the first measurable physiological parameter of contact hypersensitivity (CHS) is ear swelling, indicative of the degree of edema. Ear swelling was determined 24 hours after OXA challenge. OXA challenge in PARP-1$^{+/+}$ mice caused a 4-5 fold ear swelling compared to vehicle-sensitized animals, while OXA-induced ear swelling was significantly reduced in the PARP-1$^{-/-}$ mice. In contrast, there was no significant difference between the OXA-sensitized PARP-2$^{+/+}$ and the OXA-sensitized PARP-2$^{-/-}$ mice.

To assess immune cell infiltration, myeloperoxidase (MPO) activity, indicative of the neutrophil infiltration, was evaluated. PARP-1$^{-/-}$, but not PARP-2$^{-/-}$ mice were protected against the OXA-evoked increase in MPO activity. The degree of protection provided by the PARP-1$^{-/-}$ phenotype was similar to the mice treated with the PARP inhibitor, PJ34. Histology examination revealed inflammatory cell infiltration in the ear upon OXA challenge which was markedly reduced in the PARP-1$^{-/-}$ mice in correlation with ear swelling and MPO activity. Using cell type-specific markers, we identified predominant neutrophil infiltration.

Oxazolone has strong irritative properties, therefore we set out to investigate whether PARP-1 might influence irritative dermatitis for which we applied a 12-O-tetradecanoyl-phorbol 13-acetate (PMA)-induced irritative dermatitis model. Similarly, to our previous observations with the PARP inhibitor compound PJ34, the PARP-1$^{-/-}$ phenotype also conferred partial protection against the PMA-induced irritative dermatitis both at the level of ear swelling, MPO activity and matrix metalloproteinase (MMP) activity. In contrast, genetic ablation of PARP-2 did not affect irritative dermatitis. Apparently, both the irritative and the immunological components are affected by the loss of PARP-1.

Since our data indicate that PARP-2 has functional role neither in CHS nor in irritative dermatitis we focused our investigation on the role of PARP-1 and omitted the PARP-2 knockout strain from all further studies.

The lack of oxidative/nitrosative stress in the PARP-1$^{-/-}$ mice

Leukocytic infiltration is usually accompanied by the production of reactive oxygen and nitrogen species. Moreover, these oxidants were shown to influence redox-sensitive transcription factors that prompted us to investigate oxidative stress and its related biochemical events in OXA-induced CHS.

As the endothelial and the inducible nitric oxide synthase enzymes (eNOS and iNOS, respectively) can be considered as the most important sources of NO in the ear under inflammatory conditions, their expression was assessed. Both NOS isoforms were expressed to lower levels in PARP-1-/- mice, however iNOS was induced upon OXA-induction. Apparently, iNOS appears to be the major source of NO in our model system. This is in line with other findings obtained in different models of inflammation. Nitrosative stress is indicated by the formation of protein tyrosine nitration, which could be observed in the ears of wild type but not that of PARP-1$^{-/-}$ mice.

Nitrosative stress is accompanied by the enhanced production of reactive oxygen species. In CHS, increased level of ROS was indicated by increased lipid, protein and DNA base oxidation that was all reduced in the PARP-1$^{-/-}$ mice.

Oxidative and nitrosative stress can induce DNA breakage and PARP activation. Therefore, we set out to investigate DNA strand breakage by TUNEL assay. DNA strand breaks appeared in keratinocytes, endothelial cells and leukocytes in PARP-1$^{+/+}$ mice. The number of TUNEL-positive cells was reduced in the PARP-1$^{-/-}$ subjects. DNA strand breaks lead to PARP-1 activation resulting in the formation of PAR that followed the same pattern as DNA strand breaks.

PARP-1 mediated gene expression in OXA induced CHS

The infiltration and migration of polymorphonuclear leukocytes is facilitated by appearance of proinflammatory cytokines and chemokines. On the course of the CHS reaction, we observed the induction of MIP-1α, MIP-2, IL-1β, MCP-1 and TNFα that was blunted in the PARP-1$^{-/-}$ mice. Moreover, we observed similar changes in the expression of

different cell adhesion molecules such as I-CAM, L-CAM, V-CAM and E-Selectin. Finally,we have observed the induction of MMP-9 upon OXA challenge in the PARP-$1^{+/+}$ mice that was impaired in the PARP-$1^{-/-}$ mice. The expression of the tissue inhibitor of metalloproteinase-2 (TIMP-2) - that counterbalance MMP activity - was down-regulated in PARP-$1^{+/+}$ mice that may support higher MMP-9 activity. On the contrary, TIMP2 expression was not reduced in PARP-$1^{-/-}$ mice.

The concerted changes of iNOS, chemokines, adhesion factors, MMP-9 and TIMP-2 suggest common roots at the level of gene transcription. PARP-1 interacts with a plethora of transcription factors and modulates their activity, hence gene expression. Therefore we assessed the activation of a number of transcription factor in the ears. We have observed the strong activation of two redox-sensitive transcription factors, p65, a member of the NF-κB family and activating transcription factor-2 (ATF-2) upon OXA-sensitization in the PARP-$1^{+/+}$ mice. The activation of p65 was completely absent in the PARP-$1^{-/-}$ mice, while ATF-2 activity was only partially reduced in the PARP-$1^{-/-}$ mice.

The defective p65 activation is in line with the reduced expression of numerous NF-ęB target genes such as iNOS, adhesion factors and cytokines. The direct molecular interaction between PARP-1 and NF-ęB has already been described. However, the exact molecular mechanism through which PARP-1 supports NF-ęB, is not clear yet. It seems that PARP-1 affects NF-ęB DNA binding, but it is of question whether PARP-1 activation is crucial for NF-ęB transcriptional activity.

DNA breakage may also contribute to the transcriptional regulation through PARP-1. Estrogen receptor activation has been reported to lead to DNA nicking. These DNA nicks need to be repaired for the effective ER–related transcription. PARP-1 activation is crucial for the resolution of these nicks. Therefore it is tempting to speculate that a similar mechanism may also participate in the activation of NF-κB or ATF-2.

PARP-1 has a central role in the control of oxidative stress in the elicitation phase of CHS, and its inhibition or genetic deletion disrupts the self-intensifying propagation of inflammation. Altogether, these results provide further support for the possible therapeutic applicability of PARP inhibitors in CHS.

Transcriptional changes affected by the PARP-1 SIRT1 interdependence

Brown adipose tissue and muscle from PARP-1$^{-/-}$ mice display higher expression of genes of oxidative metabolism.

Multiple groups have identified links between SIRT1 and PARP-1, where activation of SIRT1 suppresses PARP-1 activity. Physiological observations revealed that PARP-1$^{-/-}$ mice have induced oxidative metabolism marked by higher O_2 consumption, lower amount of white adipose tissue, better glucose tolerance and lower fasting blood glucose levels despite similar insulin levels. These observations were affirmed upon high fat feeding experiments.

As PARP-1 is a major NAD^+-consumer, we speculated that the lack of PARP-1 activity might increase NAD^+-content, in turn activating SIRT1 that may explain the above changes. To gain insight into these changes we assessed the expression genes responding to SIRT1 activation in different *in vivo* and cellular models. Increased mitochondrial DNA content was observed in the BAT of PARP-1$^{-/-}$ mice. This higher mitochondrial DNA content in PARP-1$^{-/-}$ mice was in line with increased expression of genes involved in mitochondrial uncoupling (UCP1 and UCP3), fatty acid oxidation (MCAD) and respiration (Ndufa2, Ndufa3, Ndufb5, Cyt C, COX17 and Deiodinase-2 (Dio2). Expression analysis of muscle fiber isotype genes (Troponin I (Trop I), Myosin heavy chain I (MHCI) indicated an increase in the number of oxidative fibers.

We investigated the expression of a large set of genes is the liver: ERRα, PPARα, PGC-1α, SREBP1, Ndufa2, Ndufa3, cyt c, ATP5g1, MCAD, ACO, ACC1, ACC2, malic enzyme, PEPCK, GK and G6Pase. The lack of significant changes in the expression of this metabolic gene set suggested that the absence of PARP-1 has only a minor metabolic impact in the liver, potentially explained by the very low expression of PARP-1 in the liver relative to skeletal muscle or BAT.

Reduced PARP-1 activity in murine embryonic fibroblasts enhances oxidative matebolism.

Given the relation between PARP-1 and SIRT1 upon the somatic ablation of PARP-1 we next evaluated whether reducing PARP-1 activity in murine embryonic fibroblasts (MEF) cells could equally bring about the improvement of energy metabolism. The reduction of

PARP activity MEFs perfectly recapitulated all the above expression changes marked by higher expression of PGC-1α, Ndufb5, Cyt C, COX17, UCP-2, mCPT-1 and ACO.

Pharmacological inhibition of PARP activity enhances oxidative metabolism via SIRT1.

Since genetic ablation of PARP-1 increased the expression of SIRT1-related genes, we turned to investigate whether *in vivo* pharmacological inhibition of PARP-1 could have similar effects. Mice were injected with PJ34 (10 mg/kg) BID for 5 days. In muscle, the increase in mitochondrial gene expression (Ndufa2, Ndufb5, UCP2 and UCP3) induced by PJ34 was accompanied by an increase in myoglobin mRNA levels. All these data indicate that PJ34 treatment phenocopies the oxidative features induced by PARP-1 gene deletion.

The theoretical prediction that PARP-1 deletion would allow NAD^+ levels to increase and potentiate SIRT1 activation is supported by our experimental observations. Moreover, our results indicate that the interplay between both proteins could be exploited pharmacologically in metabolic diseases. Since several SIRT1 substrates, such as PGC-1á, FOXOs or p53, are crucial metabolic regulators, it is not surprising that the activation of SIRT1 by increased NAD^+ availability enhances mitochondrial biogenesis and oxidative metabolism. In this manner PARP-1 plays an indirect role in the regulation of gene expression for cell energy homeostasis by controlling SIRT1 activation, however we cannot rule out the possibility that PARP inhibition also affects energy metabolism through SIRT1-independent mechanisms.

Our work implicates the PARP-1 inhibition as a possible way for interfering with oxidative metabolism by riveting link between PARP-1 and SIRT1. The interplay between PARP-1 and SIRT1 might have implications in a number of additional areas, ranging from circadian rhythm, cellular proliferation and longevity, which warrant future investigation.

Conclusion

Role of PARP-1 in oxazolone-induced contact hypersensitivity

1. PARP-1 is the main regulator in the elicitation phase of CHS.
2. PARP-1 play important role in the production of ROS/RNS.
3. PARP-1, through redox-sensitive transcription factors NF-κB and ATF-2, regulates the inflammatory process in CHS.
4. Based on these observations PARP-1 inhibition can be exploited in the treatment of CHS or other skin inflammatory processes.

Transcriptional changes affected by the PARP-1 SIRT1 interdependence

1. In skeletal muscle PARP-1 depletion induce NAD^+ content and cause fiber isotype switch and increased mitochondrial biogenesis due to increased SIRT1 activation.
2. In the BAT, PARP-1 depletion induce NAD^+ levels that is associated with increased mitochondrial biogenesis and SIRT1 activation.
3. In liver, upon PARP-1 depletion we did not observed significant alterations in gene expression.
4. The *in vivo* gene expression changes were verified in MEF cells *in vitro*.
5. Pharmacological inhibition of PARP-1 phenocopies somatic PARP-1 depletion.

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Candidate: Attila Brunyánszki

Neptun ID: I0MOPN

Doctoral School: Doctoral School of Molecular Medicine

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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.

03 May, 2011