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Deletion of PARP-2 induces hepatic cholesterol accumulation and decrease in HDL levels

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ABSTRACT

Poly(ADP-ribose) polymerase-2 (PARP-2) is acknowledged as a DNA repair enzyme. However, recent investigations have attributed unique roles to PARP-2 in metabolic regulation in the liver. We assessed changes in hepatic lipid homeostasis upon the deletion of PARP-2 and found that cholesterol levels were higher in PARP-2$^{-/-}$ mice as compared to wild-type littermates. To uncover the molecular background, we analyzed changes in steady-state mRNA levels upon the knockdown of PARP-2 in HepG2 cells and in murine liver that revealed higher expression of sterol-regulatory element binding protein (SREBP)-1 dependent genes. We demonstrated that PARP-2 is a suppressor of the SREBP1 promoter, and the suppression of the SREBP1 gene depends on the enzymatic activation of PARP-2. Consequently, the knockdown of PARP-2 enhances SREBP1 expression that in turn induces the genes driven by SREBP1 culminating in higher hepatic cholesterol content. We did not detect hypercholesterolemia, higher fecal cholesterol content or increase in serum LDL, although serum HDL levels decreased in the PARP-2$^{-/-}$ mice. In cells and mice where PARP-2 was deleted we observed decreased ABCA1 mRNA and protein expression that is probably linked to lower HDL levels. In our current study we show that PARP-2 impacts on hepatic and systemic cholesterol homeostasis. Furthermore, the depletion of PARP-2 leads to lower HDL levels which represent a risk factor to cardiovascular diseases.

1. Introduction

Poly(ADP-ribose) polymerase (PARP)-2 (also known as ARTD2) belongs to the PARP superfamily [1]. PARP-2 binds to DNA nicks and abnormal DNA structures through the SAP motif on its N-terminus [2] that activates PARP-2. Active PARP-2 cleaves NAD^+ to form poly(ADP-ribose) polymers attached to itself and to other acceptor proteins [1,3], however to date the proteins poly(ADP-ribosyl)ated by PARP-2 are poorly mapped.

PARP-2 participates in a plethora of processes such as DNA repair and genome surveillance, spermatogenesis, T cell maturation, inflammation and mediates oxidative injury [4]. PARP-2 was recently identified as a metabolic transcriptional regulator by influencing the activity of thyroid transcription factor 1, peroxisome proliferator activated receptor-γ (PPARG), pancreatic and duodenal homeobox 1 (pdx-1) and SIRT1 [5–7]. Through these transcription factors PARP-2 regulates metabolism in white adipose tissue (WAT), pancreatic beta cells, skeletal muscle and liver [5,6]. Partial deletion of PARP-2 decreases PPARG and pdx-1 activity, which hampers WAT and beta cell function [5,6]. In skeletal muscle and liver the knockdown of PARP-2 induced SIRT1 expression and activity that consequently resulted in the deacetylation of downstream SIRT1 targets such as FOXO1 (forkhead

Abbreviations: ABCA1, adenosine triphosphate-binding cassette transporter A1; ACACA, cetyl-CoA carboxylase alpha; ACLY, ATP citrate lyase; ACOX2, acyl-CoA oxidase 2; ARTD2, ADP-ribosyl transferase diphtheria toxin-like 2; cyp51A1, cytochrome P450, family 51 subfamily A, polypeptide 1; CYP39A1, cytochrome P450, family 39, subfamily A, polypeptide 1; EGR-1, early growth response protein-1; ERα, estrogen receptor α; FABP1, fatty acid binding protein-1; FADS2, fatty acid desaturase 2; FASN, fatty acid synthase; FDPS, farnesyl diphosphate synthase; FOXO1, forkhead box protein O1; HDAC, histone deacetylase; HMGCR, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase; HMGCS1, 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase 1 (cytoplasmic); HNF-4, hepatocyte nuclear factor 4; HP1α, heterochromatin protein 1; K19, keratin 19; LDLR, LDL receptor; LIPA, lipase A; LIPG, endothelial lipase; LXR, liver X receptor; ME2, malic enzyme 2; MTTP, microsomal triglyceride transfer protein; PARP, poly(ADP-ribose) polymerase; Pdx-1, pancreatic and duodenal homeobox 1; PGC-1α, peroxisome proliferator activated receptor cofactor-1α; PPARG, peroxisome proliferator activated receptor-γ; SCD, stearoyl-CoA delta-9-desaturase; sp1, specificity protein 1; SREBP, sterol regulatory element-binding protein; TIF1β, tripartite motif containing 28; WAT, white adipose tissue

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box protein 1) or peroxisome proliferator activated receptor cofactor-1α (PGC-1α). Deacetylation of these cofactors by SIRT1 leads to increased expression of genes involved in mitochondrial biogenesis leading to enhanced fatty acid oxidation [5].

Although several PARP isoforms were shown to influence metabolic processes, only PARP-2 was identified to regulate hepatic metabolism [8] (hepatic fatty acid accumulation upon the deletion of PARP-1 does not seem to stem from changes in hepatic metabolism [8–10]). The unique hepatic action of PARP-2 prompted us to assess in detail the role of PARP-2 in the regulation of lipid metabolism in the liver.

2. Materials and methods

2.1. Chemicals

All chemicals, including UPF1069, were from *Sigma-Aldrich* (St. Louis, USA) unless stated otherwise.

2.2. Cell culture

HepG2 human hepatocarcinoma cells were obtained from ATCC and were cultured in DMEM (1 g/L glucose, 10% FCS and for the selection of transduced cells 0.25 μg/mL puromycin). PARP-2 silencing was performed using the same lentiviral constructs as in [5]. For silencing we employed constructs harboring a PARP-2 specific shPARP-2 small hairpin sequence or an unspecific scPARP-2 (scrambled) shRNA. The constructs were delivered to HepG2 cells via lentiviral particles (*Sigma*) using 40 MOI lentiviruses, and then puromycin-resistant cells were selected giving rise to PARP-2 silenced shPARP-2 HepG2 and control scPARP-2 HepG2 cell.

2.3. Animal studies

All animal experiments were carried out conforming to the national, EU and PHS ethical guidelines and were authorized by the Institutional Animal Care and Use Committee at the University of Debrecen (7/2010 DE MÁB). Homozygous male *PARP-2*$^{-/-}$ and littermate *PARP-2*$^{+/+}$ mice [11] derived from heterozygous crossings were kept in a 12/12 h dark–light cycle with ad libitum access to water and food (10 kcal% of fat, *SAFE*, Augy, France). Animals were sacrificed after 6 h of fasting (always in the same time, 12:00 p.m.), and tissues were collected and processed as specified.

2.4. Biochemical assays

Cholesterol and phospholipids in HepG2 cells, in liver and in fecal samples were determined by biochemical techniques after Floch extraction using kits from *Diagnosztikum* (Budapest, Hungary) and *WAKO* (Richmond, VA, USA). Serum cholesterol, LDL and HDL were determined using commercial kits from *Diagnosztikum*.

2.5. SDS-PAGE, Western blotting

Protein extraction, SDS-PAGE and Western blotting were performed as in [5]. Blots were probed with the following antibodies: SREBP1, SREBP2 (both 1:1000, *Santa Cruz*, Santa Cruz, CA, USA), HMGCS1, HMGCR, ABCA1 (1:1000, Abcam, Cambridge, UK), anti-poly(ADP-ribose) (mouse monoclonal antibody, 10H, Axxora, Lausen, Switzerland), actin (1:1000, *Sigma*) and PARP-2 (1:1000, *Alexis*, Lausen, Switzerland). Blots were quantified using the Image J software, then densitometry data were analyzed by statistical methods.

2.6. Cell fractionation

scPARP-2 and shPARP-2 HepG2 cells were pelleted by mild centrifugation at 4 °C at 1500 rpm for 3 min. The pellets were homogenized with five volumes of homogenization buffer (0.5 M sucrose, 20 mM HEPES pH 7.5, 1 mM EDTA, 1 mM EGTA and protease inhibitors) on ice and then Nonidet P-40 was added to a final concentration of 0.5%. The lysates were kept on ice and vortexed several times. Lysates were centrifuged at 8000 ×*g* at 4 °C for 15 min. The supernatants were considered as cytosolic fractions. The pellets containing the nuclei were resuspended in four volumes of a buffer containing 0.35 M sucrose, 10 mM HEPES pH 7.9, 3.3 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT and protease inhibitors. The suspensions were then sonicated on ice for 30 s, and the sonicated suspensions were used as nuclear fractions.

2.7. Immunocytochemistry and confocal microscopy

Confocal microscopic imaging was carried out at the Molecular Cell Analysis Core Facility at the University of Debrecen. For immunodetection of SREBP1 and SREBP2 5×10^5 cells were seeded in each well of u-Slide 8 well chamber (ibidi, Munich, Germany). Cells were stained with an anti-SREBP1 or an anti-SREBP2 antibody (both in 1:50, *Santa Cruz*, Santa Cruz, CA, USA) antibody using the protocol described in [12]. Streptavidin-Alexa 488 conjugated secondary antibody was used at a dilution of 1:300 (60 min at room temperature). Nuclei were counterstained with propidium iodide (1 μg/ml). An Olympus FV1000 confocal laser scanning microscope equipped with an UPLSAPO 60× oil immersion objective (NA 1.35) was used to collect stacks of 512 × 512 pixel optical slices with a z-step size of 500 nm. Alexa 488 (marking SREBP1 and SREBP2) and PI (labeling the nucleus) were excited at 488 and 543 nm, and detected between 500–300 and 555–655 nm, respectively. The pinhole was set to 120 μm. The ratio of SREBP1 and SREBP2 concentrations within the nucleus and the cytoplasm was estimated from fluorescence intensities in these compartments. For analysis the brightest optical slice was selected from each cell. Separate regions of interest containing the nucleus and cytoplasmic areas were drawn by using the FluoView 3.0 software, and mean fluorescence intensities per pixel within the regions of interest were calculated. Background fluorescence was determined from cells incubated with the secondary antibody alone. The ratio of background-corrected intensities of nuclear to cytoplasmic intensities was calculated for ~10 cells in each sample. This ratio is proportional to the ratio of antibody (i.e. SREBP1/SREBP2) concentrations within these cellular compartments. Ratios measured for control and knockdown samples were compared by Student's t-tests.

2.8. DNA constructs and luciferase activity measurement

pGL2-SREBP1c-2600luc SREBP1 promoter was described previously [13], the luciferase reporter plasmid harboring the promoter of ABCA1 (pLightSwitch_Prom-ABCA1) was from Switchgear Genomics (Menlo Park, CA, USA). PARP-2 mediated transactivation was determined in reporter assays as in [5]. Briefly, 1×10^5 scPARP-2 and shPARP-2 HepG2 cells were seeded in 6 well plates. The following day cells were transfected with 2.5 μg pGL2-SREBP1c-2600luc/pLightSwitch_Prom-ABCA1 and 0.5 μg β-galactosidase expression plasmid (pCMV-βgal) using JetPEI (*PolyPlus*, Strasbourg, France). After 24 h cells were washed with PBS, scraped and stored at −80 °C. Luciferase assay was carried out by standard procedures. Luciferase activity was normalized to β-galactosidase activity.

2.9. Microarray experiments and validation

Total RNA was extracted from HepG2 cells using the RNeasy Mini Kit (*Qiagen*). RNA integrity was checked on Agilent Bioanalyser 2100 (*Agilent Technologies*), RNA samples with >9.0 RIN value were used in