K123975 New connections between lipid metabolism and DNA repair – how to integrate stress into metabolism
Final report

On the course of the project we assessed multiple facets of the role of PARP enzymes in regulating the lipid metabolism and lipid homeostasis. The PARP enzyme family in humans consists of 17 members, of which PARP1, PARP2 and PARP10 were assessed. These enzymes are activated under stress conditions and disrupt (lipid) metabolism under these conditions, contributing to numerous pathologies. There are pharmacological inhibitors to these enzymes, available in clinical settings and there are ongoing developments for second generation, isoform specific inhibitors. Our results point out that these inhibitors can be used to modulate lipid metabolism highlighting that the results of the project have translational potential.

Those manuscripts are underscored where the key authors (first or senior authorship) are among the participants of the K123975 project. Fifty papers were published in the frame of the project, their overall impact factor is 268.82. There are 37 papers where the participants of the project were first authors or corresponding authors, the overall impact factor of these papers is 202.381. Four patent applications were filed on the course of the project.

The below-mentioned papers were published in high visibility journals.
Szántó et al. Genes and Development (IF: 11.631) – invited manuscript
Smolkova et al. Antioxidants and Redox Signaling (IF: 8.41) – invited manuscript
Curtin et al. British Journal of Pharmacology (IF: 8.739)
Hegedűs et al. Redox Biology (IF: 10.787)
Szántó et al. Progress in Lipid Research (IF: 14.763)
Kovács et al. Cancer and Metastasis Reviews (IF: 9.237)
Fagyas et al. Geroscience (IF: 7.595)
Wang et al. Cell Reports (IF: 9.995)
Rezen et al. Cellular and Molecular Life Sciences (IF: 9.237)
Antal et al. Journal of the European Academy of Dermatology and Venereology (IF: 9.228)

Contents

Detailed description of the papers directly related to the project

The role of PARP2 enzyme in the regulation of lipid homeostasis was suggested by us and by other groups through findings linking PARP2 to mitochondrial fatty acid oxidation and the hepatic cholesterol biosynthesis.

In the frame of this project we showed that the neutral lipid composition of the skeletal muscle fiber of mice and their cell model counterparts, C2C12 cells drastically change upon the deletion or silencing of PARP2. The silencing of PARP2 altered the expression of multiple genes involved in lipid homeostasis, among these, the genes of cholesterol homeostasis. We showed that PARP2 silencing induces the expression, activity and nuclear transport of the sterol regulatory element-binding proteins (SREBP) without impacting on protein stability. These processes lead to the enhanced synthesis of cholesterol in skeletal muscle fibers. In

fact, skeletal muscle has similar cholesterol content as the liver and *de novo* cholesterol biosynthesis has vital role in maintain skeletal muscle fiber integrity and health marked by rhabdomyolysis, a side effect of statins that are inhibitors of cholesterol biosynthesis. Membrane fluidity changed in PARP2 knockdown cell suggesting that cholesterol content changed in the membrane. Cholesterol also serves as a starting material for steroids. PARP2 silencing induced the expression of multiple steroidogenic genes and enhanced the muscular, but not the serum levels of dihydrotestosterone. Dihydrotestosterone has key functions in maintaining skeletal muscle health. In good agreement with that, skeletal muscle fibers from PARP2 knockout mice were stronger despite their muscle of origin or biochemical background (fast/slow twitch). Finally, we showed that PARP2 expression can be modulated by multiple lipid species. Altogether, these data point out that PARP2 is a constituent of a regulatory loop that cross-connects lipid homeostasis and the DNA repair system.
Márton et al. Molecular and Cell Biology of Lipids 1863:1399-1422, 2018

In the frame of the previous study, we observed electron-dense bodies in the cytoplasm of PARP2-silenced myoblasts and in PARP2-silenced HepG2 cells. These bodies proved to be autophagic vesicles verified by multiple techniques. AMPK and mTORC2 activity were reduced in the absence of PARP2, and the pharmacological modulation of these signaling protein complexes validated their involvement in PARP2-mediated autophagy. Similar, modulation of the intracellular NAD+ pool by using nicotinamide-riboside that can feed forward PARP(2) activity, counteracted the induction of autophagy induced by the silencing of PARP2. Furthermore, pharmacological PARP inhibition exacerbated the genetic silencing of PARP2. Finally, SIRT1 activation aggravated the phenotype induced by PARP2 silencing.
Jankó et al. Cells 9(2):380, 2020

Mitochondria is the main site for fatty acid oxidation in cells. The absence of PARP2 leads to mitochondrial biogenesis. In the frame of the project we showed, although the silencing of PARP2 leads to mitochondrial biogenesis the mitochondria appear fragmented. In view of the above detailed induction of autophagy, we assessed whether mitochondrial fragmentation is due to mitophagy, however, the mitochondrial fragments did not co-localize with autophagic vesicles ruling out mitophagy as an explanation for mitochondrial fragmentation. We have not detected consistent changes in the mRNA or protein expression of the genes involved in regulating mitochondrial dynamics. We have detected increases in oxidative stress in C2C12 cells in which PARP2 was silenced and consequently, we showed that neutralizing reactive oxygen species with antioxidants as reduced glutathione or N-acetyl-cysteine reverted mitochondrial fragmentation, as well as, mitochondrial biogenesis and fatty acid oxidation. These point out that reactive species production is a key signaling element in inducing mitochondrial biogenesis. Furthermore, we showed that MitoTEMPO, a mitochondrial reactive species scavenger can also block mitochondrial biogenesis and mitochondrial fragmentation. Altogether, mitochondrial reactive species production is an element of the signaling pathway inducing mitochondrial biogenesis in the absence of PARP2.
Jankó et al. Cells 10(6):1387, 2021

In addition, we showed PARP2 can poly(ADP-ribosyl)ate NRF2, a key transcription factor of genes with antioxidant function. PARP2-dependent PARylation NRF2 is important to maintain nuclear localization of NRF2, in the absence of PARP2 the nuclear fraction of NRF2 decreases and the expression of antioxidant genes decrease. Low NRF2 activity can also contribute to reactive oxygen species production and mitochondrial fragmentation.
Unpublished data, under revision at Scientific Reports

Recently, the dichotomy of white and brown adipose tissue was revisited with the discovery of beige adipocytes. Brown and beig adipocytes store neutral lipids and have high capacity for lipid oxidation. Using human adipose-derived mesenchymal stem cells (hADMSCs) we assessed the role of major PARP enzymes in regulating the differentiation of hADMSCs to different adipocyte types. Olaparib, a pan-PARP inhibitor was applied on cell

differentiating towards the white lineage. Olaparib on one hand reduced the differentiation rate of white adipocytes and reduced the size of intracellular lipid droplets that is a morphological feature similar to beige/brown adipocytes. Olaparib induced mitochondrial biogenesis in white adipocytes, again, rendering these cells similar to beige/brown adipocytes. Olaparib treatment induced AMPK activation and the rearrangement of NAD+ metabolism that is the PARP-mediated primary events leading to the shift of the white adipose tissue differentiation program to the brown/beige direction.
Nagy et al. Biochemical Pharmacology 167:76-85., 2019

Importantly, as PARPs are NAD+-dependent enzymes, replenishing NAD+ using nicotinamide-riboside phenocopied the changes elicited by olaparib. NAD+ supplementation induced reactive species production that was key to induce fatty acid oxidation and uncoupled respiration. These findings are similar to our results on the role of PARP2 in mitochondrial structure.
Nagy et al. Frontiers Cell and Developmental Biology 10:979330, 2022

Finally, we have participated in multiple studies assessing the elements of the signaling driving the differentiation of beige adipocytes.
Kristof et al. BBA-Experimental Cell Research 377(1-2):47-55., 2019
Lénárt et al. International Journal of Molecular Medicine 23(9):5175, 2022

We identified PARP10 as a regulator of mitochondrial biogenesis. PARP10 is a minor PARP enzyme, contributing only a negligible fraction of total cellular PARP activity, however, there are advanced specific inhibitors to PARP10. In fact, the selective inhibition of PARP10 may circumvent elements of the side effect profile of the currently available pan-PARP inhibitors. Silencing of PARP10 by siRNA induced mitochondrial oxidative metabolism, including fatty acid oxidation in multiple unrelated cell lines. In addition, markers of glycolysis were also induced suggesting that in the absence of PARP10, cells turn hypermetabolic. In contrast to the silencing of PARP2, the silencing of PARP10 reduced reactive species production through inducing the mRNA expression of antioxidant genes, pointing towards a different mechanism to induce mitochondrial biogenesis. In fact, the absence of PARP10 induced AMPK activity and PGC1a expression that are known inducers of mitochondrial biogenesis. These features can be regarded as anti-Warburg elements of metabolism, and indeed, the silencing of PARP10 reduced cell proliferation in multiple cellular carcinoma models.
Márton et al. PLoS One, 13(1):e0187789, 2018

UVB radiation induces PARP activation in keratinocytes that leads to cell death. Cell death can be countered by pharmacological PARP inhibition. Survival upon PARP inhibition coincided with the induction of mitochondrial biogenesis and mitochondrial oxidation. Autophagy was also induced upon PARP inhibition that can support the removal of damaged cellular components and can feed mitochondrial oxidation. Mitochondrial biogenesis and autophagy induction upon PARP inhibition was dependent on sirtuin activation and the activation of cellular energy stress sensors as mTORC1, mTORC2, PGC1a or AMPK. It is important to note that as PARP1, PARP2 and PARP3, the major targets of the currently available pharmacological PARP inhibitors are key members of cellular DNA damage response, therefore, cell surviving UVB damage through PARP inhibition are likely to accumulate and carry over mutations to daughter cells that increases the risk of malignancies.
Hegedűs et al. Cancers 12(1):5, 2020

As discussed above, we showed that PARP1 is needed for UVB-induced DNA damage and the inhibition of PARP1 facilitates metabolic adaptation to survive UVB irradiation in keratinocytes. We extended these observations by showing that PARP1 is needed for the effective repair of cyclobutane pyrimidine dimers (CPDs). We showed that, a yet uncharted, signaling pathway stemming from UVB-induced CPDs lead to mitochondrial biogenesis,

mitochondrial fusion, and the enhancement of OXPHOS. Enhanced OXPHOS leads to the enhanced consumption of pyruvate, glutamine and fatty acids. We detected the induction in lipophagy upon UVB irradiation. These metabolic adaptations likely support UVB-irradiated cells to survive UVB irradiation. This adaptive pathway involves mitochondrial production of reactive species and a coordinated activation of sirtuins, ATM, p53, AMPK Akt, p60-S6K and elements of NAD+-signaling. Furthermore, these properties not only support the survival of keratinocytes, but contributed to the UVB-induced differentiation of keratinocytes. Our findings indicate that CPD-dependent signaling acutely maintains skin integrity by supporting keratinocytic energy metabolism.
Hegedűs et al. Redox Biology 38:101808, 2021

We participated in a landmark study assessing mechanism of the antitumor activity of PARP inhibitors. Despite numerous dedicated studies, the intricate mechanism of PARP inhibitors are not fully characterized. We showed, pharmacological PARP inhibitor treatment induces the number of tumor infiltrating macrophages that improved survival in murine models. PARP inhibition rendered macrophages hypermetabolic marked by increased mitochondrial oxidation and glycolytic flux with a dominant role of the increase of the glycolytic flux. This led to reactive oxygen species production through mitochondrial retrograde electron transport and reactive species production played key role in inducing cytostatic and cytotoxic effects in cancer cells. We showed that the rearrangement of nicotinamide metabolism plays key role in inducing the metabolic changes. These findings suggest that PARP inhibitors can support anticancer biological therapies.
Wang et al. Cell Reports, 41(2):111462., 2022

PARP1 and PARP2 were considered as proinflammatory proteins in Th1 and Th2-mediated processes. We had a very surprising discovery that in a murine model of psoriasis the genetic deletion of PARP1 exacerbated the features and symptoms of the disease. The more severe symptoms were due to the blockade of keratinocyte terminal differentiation that is dependent on the inhibition of keratinocyte cell death and due to changes in the inflammatory response that is dependent on changes to calcium signaling. We participated in a review assessing the pathways regulating antigen presentation in psoriasis.
Kiss et al. Experimental Dermatology 29(1):79-85., 2020
Antal et al. Life 12(2):234, 2022

The heavy involvement of PARP enzymes in lipid metabolism is a conceptual novelty. We published two comprehensive reviews on the topic in high visibility journals (Progress in Lipid Research IF: 14.673 and in Genes and Development IF: 11.631). We were the first to show lipid species inducing PARP enzymes and to point out the involvement of PARP enzymes in steroid metabolism. The Genes and Development review was an invited review.
Szántó et al. Progress in Lipid Research 84:101117, 2021
Szántó M and Bai P Genes and Development doi: 10.1101/gad.334284.119., 2020

We were invited to contribute to a methodology book on PARPs and PARP-related enzymes to discuss the methodological aspects of PARP enzymes and metabolic regulation.
Kovács et al. Methods in Molecular Biology, in press. Ed: Alexei Tulin, 2023

We joined the international efforts to respond to COVID19 crisis. We published two papers, in which we provided evidence for the repurposing of PARP inhibitors. Clinically approved PARP inhibitors (PARPi) have a mild adverse effect profile and are well-tolerated as continuous daily oral therapy. We reviewed the evidence that justifies the repurposing of PARPi to block the proliferation of SARS-CoV-2 and combat the life-threatening sequelae of COVID-19 by several mechanisms. PARPi's can effectively decrease IL-6, IL-1 and TNFα levels (key interleukins in SARS-CoV-2-induced cytokine storm) and can alleviate subsequent lung fibrosis, as demonstrated in murine experiments and clinical trials. PARPi can tune macrophages towards a tolerogenic phenotype. PARPi's may also counteract SARS-CoV-2-

induced and inflammation-induced cell death and support cell survival. PARPi's had beneficial effects in animal models of acute respiratory distress syndrome (ARDS), asthma and ventilator-induced lung injury. PARPi's may potentiate the effectiveness of Tocilizumab, Anakinra, Sarilumab, Adalimumab, Canakinumab or Siltuximab therapy.

We showed experimentally that a PARP inhibitor, rucaparib can efficiently block the proliferation of the SARS-CoV-2 virus that was dependent on the binding of rucaparib to the Spike protein of SARS-CoV-2. We provided experimental evidence for the binding of rucaparib by multiple complementary methods and identified the amino acids responsible for binding. The binding pocket was highly conserved among the variants of SARS-CoV-2 including the variants currently spreading. Unfortunately, rucaparib bound to Spike in concentrations exceeding the established therapeutic range of rucaparib. Furthermore, we showed that rucaparib can efficiently block the immune response elicited by the Spike protein of SARS-CoV-2 in the established therapeutic range of rucaparib with similar efficiency as dexamethasone, the current standard of care. We published these results on the prepub server MedRXiv and the manuscript is under revision at Cell Chemical Biology. Although, that line of research could have led to the submission of a patent application, due to the severity of the pandemic crisis we felt that it would not be ethical.

We participated in two other COVID-19 related studies. We showed the applicability of PUFAs in mediating the inflammatory component of COVID-19 disease and we identified cardiac autoantibodies in critically ill COVID-19 patients.

Curtin et al. British Journal of Pharmacology 177(16):3635-3645., 2020
Papp Het al. MedRXiv https://doi.org/10.1101/2022.06.30.22277079, 2022 (under revision in Cell Chemical Biology)
Szabó Z et al. Frontiers in Physiology 11:752., 2020
Fagyas et al. Geroscience 16:1-14., 2022

Main findings and their echo in the scientific community

- On the course of the project we firmly linked lipid metabolism to cellular PARP activity, namely, to the activity and expression of PARP1, PARP2 and PARP10.
- These enzymes regulate fatty acid oxidation, cholesterol and steroid biogenesis in multiple tissues.
- PARPs mediate mitochondrial biogenesis and mitochondrial morphology in which reactive species production-mediated retrograde signaling plays key role.
- We identified regulatory loops, where PARP enzymes were activated or inhibited by lipid species and, in turn, PARP enzymes regulated biochemical pathways that generated or degraded lipid species. PARPs were often involved in negative feedback loops in this context.
- We provided evidence that PARP1 and PARP2 are regulators of autophagy and autophagy likely supply stressed cells with vital nutrients.
- We identified a novel signaling pathway in UVB-irradiated keratinocytes that stems from the UVB-elicited CPDs in DNA and induce mitochondrial biogenesis and mitochondrial oxidation to support cell survival. This mechanism likely support the carryover of mutations in UVB-irradiated keratinocytes and, hence, enhance the risk of malignancies.
- The COVID-19 pandemic interspersed the project and we enlarged our studies to join the international effort to tackle the pandemic. We identified rucaparib, a registered PARP inhibitor that can be repurposed for COVID-19 as a potent anti-inflammatory drug.

Other studies

Microbiome – tumor studies

We identified a set of bacterial metabolites with cytostatic properties in breast cancer. Namely, lithocholic acid, indoldepropionic acid, indoxylsulfate and cadaverine. These metabolites act in a hormone-like fashion. The metabolites are generated in the gastrointestinal tract and are then transported to the tumors through the circulation. The metabolites have cell surface and nuclear receptors. Through these receptors, the metabolites induce multi-pronged responses in cells that culminate in cytostasism, but not cytotoxicity. These individual responses include

anti-Warburg rearrangement of metabolism and enhanced production of reactive species that in turn inhibit EMT, cell migration, reduce the number of cancer stem cells, inhibit metastasis formation and induced antitumor immunity. We established an online tool (TAXAMAT) for the handling and curation of metagenomic data. Based on these results we submitted three patent applications.

In extenso papers
Mikó et al. BBA – Bioenergetics 1859(9):958-974., 2018
Vida et al. Molecular Medicine Reports, 18(5):4335-4341, 2018
Kovács et al. Cancers 11(9):1255, 2019
Kovács et al. Scientific Reports 9(1):1300, 2019
Mikó et al. Cells 8:293, 2019
Szántó et al. Experimental Dermatology 28(11):1210-1218., 2019
Kiss et al. Cancers, 12(5), 1068, 2020
Sári et al. Physiology International 107(2), 349-358, 2020
Sári et al. Cancers 12(10), 2915., 2020
Sári et al. Cancers, 12(9):2411., 2020
Vida et al. Physiology International 1, 12-17., 2020
Kovács et al. Cancer and Metastasis Reviews 40(4):1223-1249, 2021
Sipos et al. Molecular Medicine 27(1):33, 2021
Kiss et al. Gasztroenterológiai szemle (Central European Journal of Gastroenterology) 7(2):57-65, 2021
Rezen et al. Cellular and Molecualr Life Sciences 79(5):243, 2022
Smolková et al. Antioxidants and Redox Signaling 22(13):966-997, 2020

Book chapter
Kovács et al. Tumor Microenvironment – Novel Concepts. Advances in Experimental Medicine and Biology (ISSN:0065-2598). Springer-Nature Ed.: Alexander Birbrair, 2020

Patent applications
Bai P (23%), Goedert JJ (4%), Kovacs T (23%), Miko E (23%), Sebő É (2%), Tóth J (2%), Vida A (23%): ***Treatment and diagnosis of breast cancer***, WO2020/025989
Bai P (29%), Goedert JJ (7%), Kovacs T (6%), Miko E (29%), Vida A (29%): ***METHODS FOR DIAGNOSING BREAST CANCER***, WO2018/229519
Bai P (23%), Kovács T (23%), Mikó E (23%), Sári Zs (23%), Goedert JJ (4%), Tóth J (42%), Sebő É (2%): ***TREATMENT AND DIAGNOSIS OF BREAST CANCER.***, submitted for PCT.

Other cancer-related publications
We participated in a set of oncology-related studies.
Lódi et al. Journal of Translational Medicine 18(1):470, 2020
Méhes et al. International Journal of Molecular Sciences 21(14):E5001., 2020
Smolkova and Bai Frontiers in Oncology 10:628664., 2021
Curtin N and Bai P Cancers 12(12):E3494., 2020

Chemical biology studies
In collaboration with the Department of Organic Chemistry at the University of Debrecen we conducted chemical biology studies. We characterized a set of compounds with glycogen phosphorylase inhibitor activity and identified insulin-releasing signaling pathways stemming from the surface of glycogen particles. Furthermore, we charactzerized set of organic bidentate complexes with central ions belonging to the platinum group (ruthenium, osmium, iridium and rhodium). The complexes exerted cytostatic properties in a large set of carcinoma, sarcoma and lymphoma cells, but not in primary, non-transformed cells. The best complex had submicromolar IC50 value. In addition, the complexes were bacteriostatic on clinical multiresistant vancomycin-resistant *Enterococcus* and methicillin-resistant *Staphylococcus aureus* isolates. The apolar nature of the complexes was pivotal for their biological activity.

Furthermore, the complexes induced cytostasis through the production of reactive species and only vitamin E, an apolar membrane-specific antioxidant, was able to block the cytostatic activity of the complexes, but not polar antioxidants. These findings make it plausible that the complexes probably act through changing the biochemical and biophysical properties of biomembranes.
A patent application was submitted on the synthesis and the biological activity of the complexes.
Nagy et al. PLOS One 15(9):e0236081., 2020
Kacsir et al. International Journal of Molecular Medicine 23(2):813, 2021
Kacsir et al. International Journal of Molecular Medicine 22:10454, 2021
Balázs et al. Frontiers in Chemistry 10:868234, 2022
Bai P (15%), Balázs B (9%), Bokor E (13%), Buglyó P (9%), Kacsir I (9%), Kardos G (9%), Kiss GA (5%) Sipos A (9%), Somsák L (13%), Tóth Z (9%), Half sandvich transition metal complexes and uses thereof. submitted for PCT evaluation.

Animal husbandry-related studies
We participated in a set of animal husbandry-related studies.
Simon et al. Journal of Animal Physiology and Animal Nutrition, 102(1):286-296, 2018
Simon et al. Acta Biochimica Polonica 65(2):251-258, 2018
Simon et al. PEERJ 7:e6588, 2019
Csernus et al. Animals 10(2), 347, 2020