Classification: Microbiology & Infectious Disease, Biochemistry & Chemical Biology

Coronavirus Infection and PARP Expression Dysregulate the NAD Metabolome: A Potentially Actionable Component of Innate Immunity

Collin D. Heer[a,b], Daniel J. Sanderson[c,&], Yousef M.O. Alhammad[d,&], Mark S. Schmidt[b,&], Samuel A.J. Trammell[b,*], Stanley Perlman[e], Michael S. Cohen[c], Anthony R. Fehr[d,#], Charles Brenner[b,#]

[a]Free Radical and Radiation Biology Program, University of Iowa, United States,

[b]Department of Biochemistry, University of Iowa, United States

[c]Department of Chemical Physiology & Biochemistry, Oregon Health Sciences University, United States,

[d]Department of Molecular Biosciences, University of Kansas, United States,

[e]Department of Microbiology & Immunology, University of Iowa, United States,

[&]equal contributions

[#]correspondence to arfehr@ku.edu and charles-brenner@uiowa.edu

*current address: Novo Nordisk Foundation, Center for Basic Metabolic Research, University of Copenhagen, Denmark

Keywords: SARS-CoV-2, interferon, innate immunity, PARP, ADP-ribosylhydrolase, COVID-19, MHV

Abstract

Over the past several decades, multiple coronaviruses (CoVs) have emerged as highly infectious, lethal viruses in humans, most notably in the pandemic outbreak of COVID-19, the disease caused by SARS-CoV-2. To date, there are no known therapeutic or preventative agents to target CoVs. Though age and comorbidities severely increase case fatality rates, the host factors that influence resistance or susceptibility to infection with highly pathogenic human CoVs are unknown. Innate immune responses to CoVs are initiated by recognition of double-stranded (ds) RNA and induction of interferon, which turns on a gene expression program that inhibits viral replication. Here we show that COVID-19 patient samples of SARS-CoV-2 conserve the invariant sequence of the CoV macrodomain, an ADP-ribosylhydrolase domain previously shown to counteract innate immunity to both mouse hepatitis virus (MHV), a model CoV, and SARS-CoV. RNA sequence analysis of cell lines, infected ferrets, and a deceased patient's lung shows that a set of poly(ADP-ribose) polymerase (PARP) family members are induced by SARS-CoV-2 infection; these PARPs include enzymes required for the innate immune response to MHV. Further, we show that MHV infection induces an attack on host cell nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). The data indicate that overexpression of a virally induced PARP, PARP10, is sufficient to depress host cell NAD metabolism and that NAD^+ boosting strategies differ in their efficacy to restore PARP10 function. The data suggest that boosting cytoplasmic but not nuclear NAD^+ may restore antiviral PARP functions to support innate immunity to CoVs and other viruses susceptible to PARP-mediated antiviral activity.

INTRODUCTION

Disease attributed to the current novel coronavirus (CoV) outbreak (COVID-19) has rapidly spread globally, infecting 2 million people and killing more than 125,000 as of mid-April 2020 (Dong, Du, & Gardner, 2020). The causative agent, severe acquired respiratory syndrome coronavirus 2, SARS-CoV-2, is transmitted largely by lipid droplets that infect cells of the lung epithelium (Wu, Wu, Liu, & Yang, 2020). Like other positive strand RNA genome CoVs, SARS-CoV-2 replication proceeds through formation of double-stranded (ds) RNA (Fehr & Perlman, 2015), which elicits an interferon (IFN) response (Kindler, Thiel, & Weber, 2016; Totura & Baric, 2012). A component of innate immunity that is expressed broadly and does not depend on professional cells of the acquired immune system, IFN-driven gene expression elicits responses that have largely evolved to arrest protein synthesis and other aspects of host metabolism on which the virus depends (Schoggins & Rice, 2011). Specifically, CoV genomes do not encode enzymes needed for fuel oxidation, ATP generation, nucleotide, amino acid, lipid or protein synthesis, and therefore depend on exploitation of host functions to synthesize and assemble more viruses (Fehr & Perlman, 2015; Zhu et al., 2020). Accordingly, viral replication and host cell homeostasis both depend on the four nicotinamide adenine dinucleotide (NAD) coenzymes, NAD^+, NADH, $NADP^+$ and NADPH, which are the central catalysts of metabolism (Belenky, Bogan, & Brenner, 2007). These coenzymes accept and donate electrons in essential, ubiquitous processes of fuel oxidation, biosynthesis, and the generation and detoxification of reactive oxygen species.

We and others showed that CoVs elicit an IFN response in naïve cells. In this process, IFN proteins are secreted by infected cells to engage IFN receptors and, in an autocrine and paracrine manner, induce expression of a set of IFN-stimulated genes (ISGs). The collective activities of ISGs reorient cellular metabolism toward infection control (Channappanavar et al., 2019). Among the ISGs are several members of the poly(ADP-ribose) polymerase (PARP) superfamily (Alhammad & Fehr, 2020; Fehr et al., 2020).

PARPs have an absolute requirement for NAD^+ (Cohen, 2020; Gupte, Liu, & Kraus, 2017). However, rather than using NAD^+ as an electron acceptor, PARPs consume NAD^+ in order to transfer the ADP-ribose moiety to protein side chains

(Belenky et al., 2007). The best-known members of the PARP superfamily, PARP1 and PARP2, form polymers of ADP-ribose, largely in response to DNA damage. However, most other members of the PARP superfamily possess monoADP-ribosyl (MARylating) transfer activities on target proteins (Cohen, 2020). We showed that MHV infection strongly induces expression of PARP7, PARP9, PARP10, PARP11, PARP12, PARP13, and PARP14 (Grunewald et al., 2019). To determine whether these gene expression changes are incidental to MHV infection, facilitate MHV infection, or are part of an innate immune response against MHV, we treated cells with siRNAs to knock down expression of these gene and then analyzed the impact on MHV replication. Our data showed that PARP7 plays a role in facilitating replication. In contrast, PARP14 is required for full induction of IFN-β expression (Grunewald et al., 2019; Grunewald, Shaban, Mackin, Fehr, & Perlman, 2020), suggesting PARP14 is directly involved in establishing the innate immune response in CoV infected cells.

Most CoV genomes encode 16 non-structural proteins (nsps) (Fehr & Perlman, 2015; Zhu et al., 2020). nsp3 contains a macrodomain, herein termed the CoV ADP-ribosylhydrolase (CARH), that removes monoADP-ribose modifications from acidic amino acids on protein targets. Thus, CARH reverses the modification that is installed by the IFN-induced activities of MARylating PARP family members (Fehr et al., 2016). CARH activity is required for virulence *in vivo* using mouse models of both MHV and SARS-CoV (Eriksson, Cervantes-Barragan, Ludewig, & Thiel, 2008; Fehr et al., 2015; Fehr et al., 2016). Moreover, an active site mutation that ablates the ADP-ribosylhydrolase activity of CARH resulted in a virus that replicates poorly in primary bone-marrow derived macrophages (BMDMs) (Grunewald et al., 2019). We further identified PARP12 and PARP14 as CoV-induced ISGs that are required for the depressed replication of CARH mutant viruses, indicating that their activity is opposed by CARH-mediated reversal of ADP-ribosylation (Grunewald et al., 2019).

In support of the antiviral roles of IFN-induced MARylating PARP isozymes, PARP12 was shown to promote the degradation of nsp1 and nsp3 in Zika virus infection (L. Li et al., 2018). PARP12 has also been shown to inhibit a wide variety of RNA viruses, including several alphaviruses, which also contain an nsp3-encoded
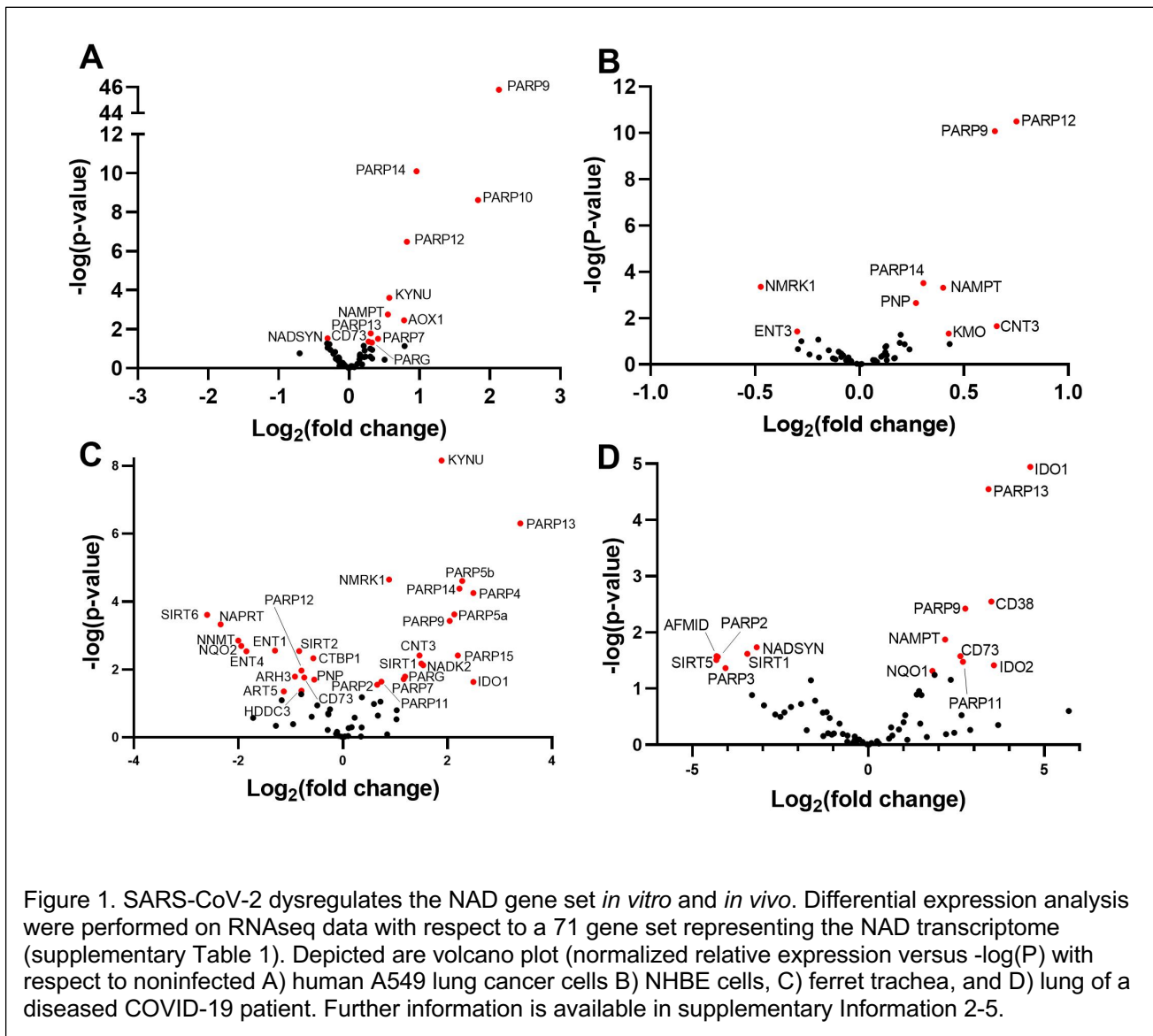
CARH activities (Atasheva, Akhrymuk, Frolova, & Frolov, 2012; Atasheva, Frolova, & Frolov, 2014). Further, the nsp10 of SARS-CoV has been identified as an inhibitor of electron transport at the NADH site of complex I in the mitochondrial electron transport chain (Q. Li et al., 2005). These observations suggest that key events in the innate immune response to viral infections are played out in the infected cell's NAD metabolome.

Here we show that SARS-CoV-2 infected tissue culture cells, ferrets and a deceased human victim of COVID-19 indicate that viral infection induces high level expression of multiple PARP isozymes including many of the same PARPs induced by MHV infection of BMDMs. We also show that MHV infection results in a significant depression of key cellular NAD metabolites and that PARP overexpression is sufficient to depress NAD metabolism in a manner that resembles MHV infection. Whereas multiple approaches exist to restore NAD, we show that NAMPT activation but not PARP1,2 inhibition supports increased PARP10 enzymatic activity. The data justify further analysis of how nutritional and therapeutic modulation of NAD status may potentially restrict viral infection by boosting innate immunity.

RESULTS

SARS-CoV-2 Infection of Human Lung Cell Lines Induces a MHV-like PARP Transcriptional Program

MHV infection in murine BMDMs launches a transcriptional program that induces transcription of PARP isozymes 7, 9, 10, 11, 12, 13 and 14 by greater than 5-fold (Grunewald et al., 2019; Grunewald et al., 2020). We utilized RNAseq data from SARS-CoV-2 infection of a human lung carcinoma cell line, A549, and normal human bronchial epithelia cells, NHBE (Blanco-Melo et al., 2020). To determine whether SARS-CoV-2 dysregulates the NAD system upon infection we assembled and analyzed a set of 71 genes that encodes the enzymes responsible for conversion of tryptophan, nicotinic acid (NA), nicotinamide (NAM), and nicotinamide riboside (NR) to NAD^+, plus the enzymes responsible for NAD(H) phosphorylation, NADP(H) dephosphorylation, NAD^+-dependent deacylation, ADP-ribosylation, cADP-ribose formation, nicotinamide methylation/oxidation, and other related functions in transport, binding, redox and regulation (Supplementary Information 1). As shown in Figure 1A, SARS-CoV-2 induces transcription of PARPs 9, 10, 12 and 14 with lesser effects on PARP7 and PARP13 in A549 cells and induces transcription of PARP9, 12 and 14 in NHBE cells.

Figure 1. SARS-CoV-2 dysregulates the NAD gene set *in vitro* and *in vivo*. Differential expression analysis were performed on RNAseq data with respect to a 71 gene set representing the NAD transcriptome (supplementary Table 1). Depicted are volcano plot (normalized relative expression versus -log(P) with respect to noninfected A) human A549 lung cancer cells B) NHBE cells, C) ferret trachea, and D) lung of a diseased COVID-19 patient. Further information is available in supplementary Information 2-5.

SARS-CoV-2 Infection of Ferrets Strongly Dysregulates the NAD Gene Set

Cell lines such as A549 and NHBE are adapted to grow on plastic and clearly may lack key aspects of host-viral biology. Ferrets have been shown to be permissive to SARS-CoV-2 infection (Shi et al., 2020) and are currently being used as a system to probe host responses as well as potential preventative and therapeutic agents. We probed RNAseq data from the tracheas of control and 3-day SARS-CoV-2 infected ferrets (Blanco-Melo et al., 2020) and showed that the PARP induction program is conserved in this relevant animal model (Figure 1C). Specifically, in the ferret, PARPs 4, 5, 9, 13, 14 and 15 were all more than 4-fold induced with significant but lesser induction of PARPs 7 and 11. In ferret tracheas, it was also easy to discern

other significant alterations to the NAD gene set. The data indicate that transcription of   NMRK1 and concentrative nucleoside transporter CNT3 go up, suggesting increased capacity for conversion of nicotinamide riboside to NAD^+ and $NADP^+$ (Bieganowski & Brenner, 2004). Notably, in mouse models of damaged brain and heart, upregulation of NMRK gene expression is associated with therapeutic efficacy of NR (Vaur et al., 2017) (Diguet et al., 2018). Additionally, the ferret data show strongly depressed NNMT expression—by decreasing NAM methylation, this gene expression change could promote NAM salvage (Neelakantan et al., 2019) and represent a homeostatic attempt of virally infected cells to maintain their NAD metabolome (Brenner, 2014).

COVID19 Patient Lung Samples Recapitulate the PARP Induction Program Seen in Ferrets and *In Vitro*

Finally, though ferrets are susceptible to infection by SARS-CoV-2, they do not progress to the serious disease seen in people (Shi et al., 2020). We therefore examined the NAD gene set in RNAseq data from the lung of a person who died of COVID-19 (Blanco-Melo et al., 2020). Though lacking the replicates and the synchrony of the extremely high quality ferret RNAseq data and, with the caveat that tissue from a deceased patient is less likely to produce the highest quality RNA, the human data were informative.
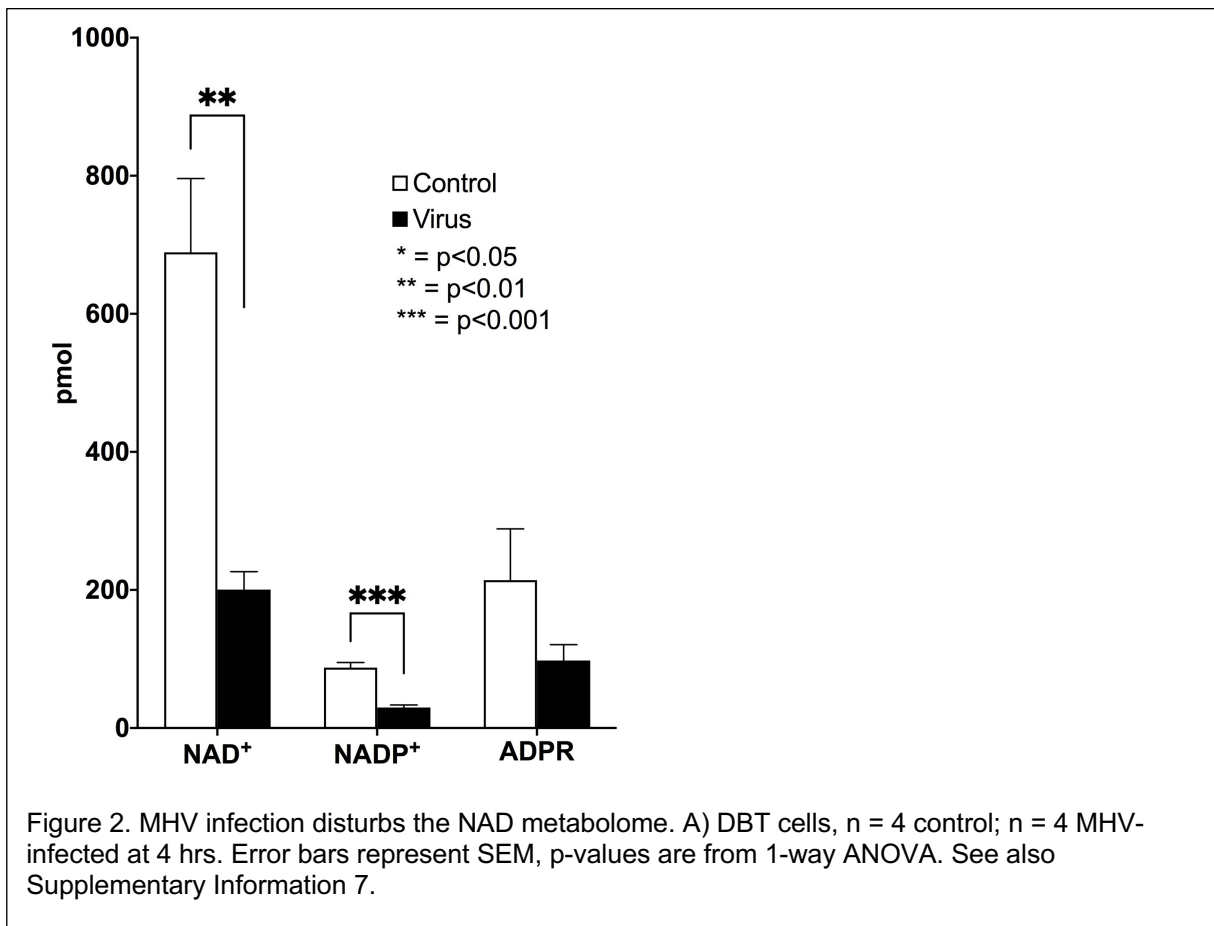
As shown in Figure 2D, PARP9, 11 and 13 were upregulated in the deceased person's lung. Thus, with stringent statistical standards, PARP9 was upregulated in every SARS-CoV-2 transcriptome while PARP9, PARP11 and PARP13 were upregulated in both *in vivo* analyses. Additional MARylating PARP genes met statistical significance in cell lines.

NAD biosynthetic gene changes were also conserved *in vivo*: NMRK1 expression, indicating capacity for NR salvage, was elevated with statistical significance in ferret trachea, and NAMPT expression, indicating capacity for NAM salvage, was elevated with statistical significance in human lung. NMRK1 and NMRK2 transcripts were additionally elevated in the human patient data set and NAMPT transcript was elevated in the ferret infection data set without meeting

criteria for statistical significance. Further data on transcriptomic alterations to NAD synthesis in all four data sets are provided in Supplementary Information 6.
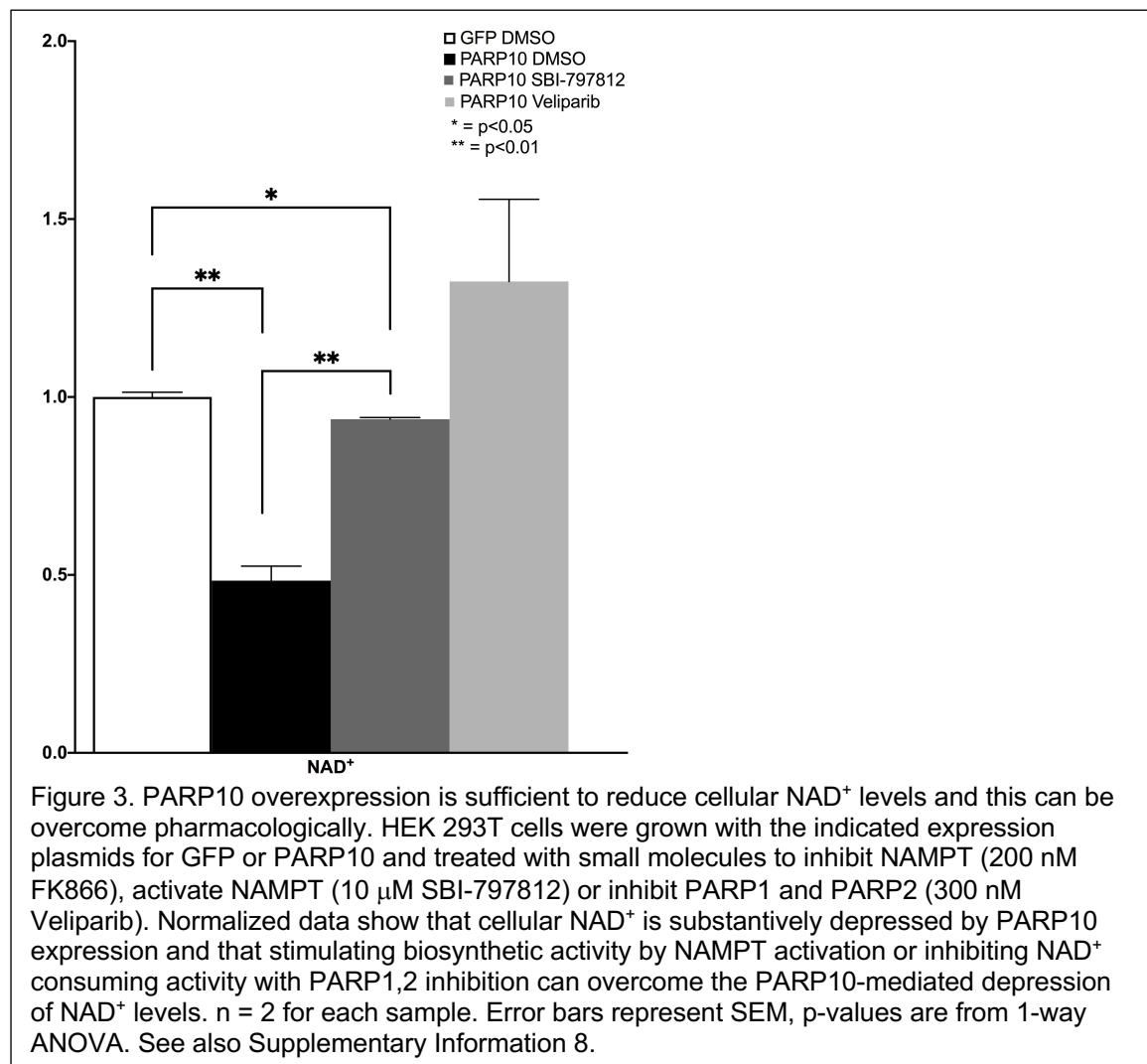
MHV Infection Drives Down Cellular NAD$^+$ and NADP$^+$ in Infected Cells

Given that divergent CoVs consistently induce members of the PARP superfamily at the mRNA level, we asked whether MHV infection alters the NAD metabolome. We infected delayed brain tumor (DBT) cells with MHV-A59 at a multiplicity of infection (MOI) of 3 PFU/cell and subjected the cells to quantitative targeted analysis of the NAD metabolome by LC-MS (Trammell & Brenner, 2013). As shown in Figure 2, infection led to a significant depression of cellular NAD$^+$ and NADP$^+$ within 4 hrs.

Figure 2. MHV infection disturbs the NAD metabolome. A) DBT cells, n = 4 control; n = 4 MHV-infected at 4 hrs. Error bars represent SEM, p-values are from 1-way ANOVA. See also Supplementary Information 7.

PARP10 Overexpression is Sufficient to Depress NAD$^+$ levels and Can be Overcome Pharmacologically
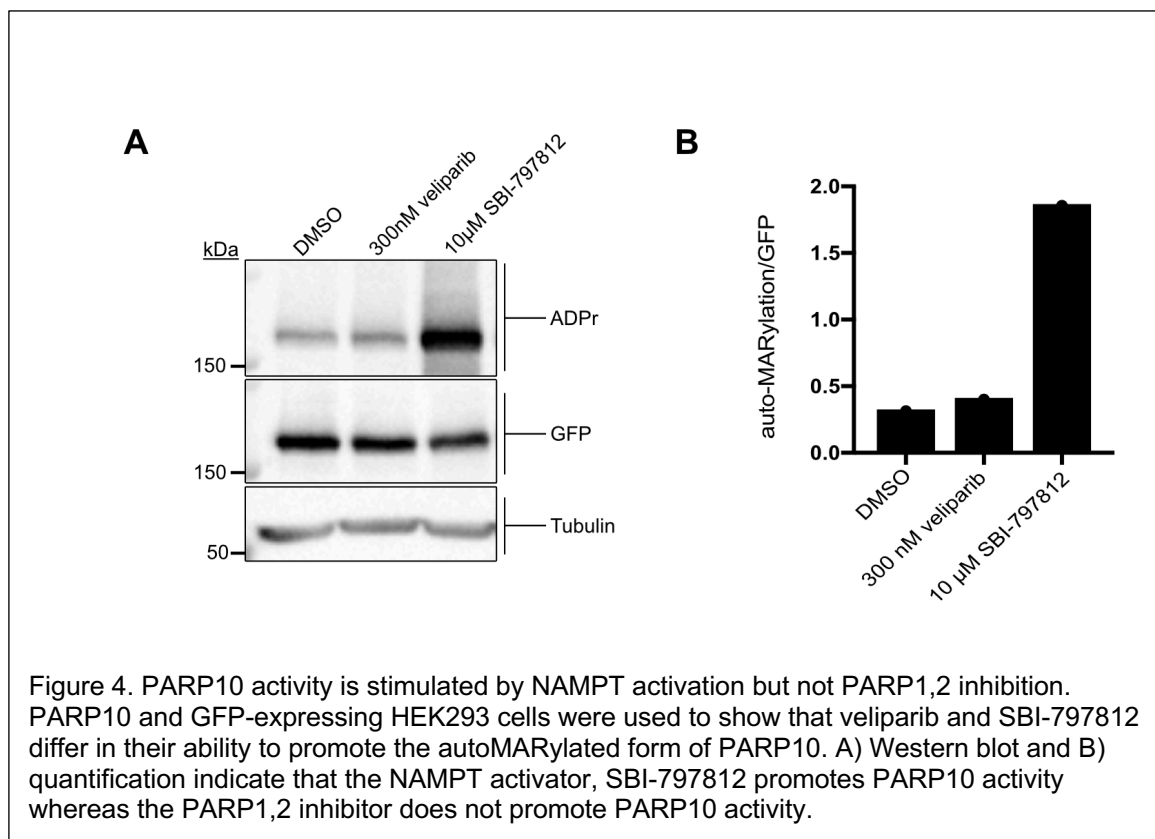
It is well known that PARP1 activation by DNA damage greatly increases its catalytic activity, leading to depression of cellular NAD^+ and ATP (Cohen, 2020). It is less clear whether transcriptional induction of the MARylating enzymes such as PARP10—that are induced substantially by viruses—might disturb cellular NAD^+. To address, this, we overexpressed PARP10 in HEK 293T cells and measured changes in NAD metabolites using LC-MS/MS. We found that overexpression of GFP-PARP10 significantly depressed NAD^+ compared to overexpression of GFP alone (Figure 3). We next determined if the PARP10-mediated loss in NAD^+ could be restored by boosting NAD^+ levels, either by increasing synthesis or decreasing consumption. To increase NAD^+ synthesis we treated cells with SBI-797812 (Gardell et al., 2019), a small molecule allosteric activator of NAMPT, which promotes NAM salvage. To decrease NAD^+ consumption, we treated cells with veliparib (Donawho et al., 2007), a selective inhibitor of PARP1 and PARP2, which are major NAD^+

Figure 3. PARP10 overexpression is sufficient to reduce cellular NAD^+ levels and this can be overcome pharmacologically. HEK 293T cells were grown with the indicated expression plasmids for GFP or PARP10 and treated with small molecules to inhibit NAMPT (200 nM FK866), activate NAMPT (10 μM SBI-797812) or inhibit PARP1 and PARP2 (300 nM Veliparib). Normalized data show that cellular NAD^+ is substantively depressed by PARP10 expression and that stimulating biosynthetic activity by NAMPT activation or inhibiting NAD^+ consuming activity with PARP1,2 inhibition can overcome the PARP10-mediated depression of NAD^+ levels. n = 2 for each sample. Error bars represent SEM, p-values are from 1-way ANOVA. See also Supplementary Information 8.

consumers in cells. We found that both NAMPT activation and PARP1/PARP2 inhibition restored cellular NAD$^+$ under these conditions.

PARP10 Activity Can be Restored by Support of Cytosolic but Not Nuclear NAD

The NAD metabolome is not only modulated by gene expression changes, enzyme activity and precursor availability, but also by subcellular localization (Cambronne et al., 2016). Thus, restoration of total cellular NAD$^+$ does not necessarily equate to restoration of specific enzyme activities (Ryu et al., 2018). To determine whether PARP10 activity is modulated by NAMPT activation or PARP1/PARP2 inhibition, we took advantage of the auto-MARylation activity of PARP10 as a readout of its activity in cells. PARP10 auto-MARylation activity was stimulated by SBI-797812. In contrast, treatment with veliparib did not affect PARP10 auto-MARylation (Figure 4). These results suggest that boosting NAD$^+$ levels by augmenting cytosolic synthesis rather than reducing nuclear NAD$^+$ consumption may be a preferable approach to supporting PARP10 activity in cells.

Figure 4. PARP10 activity is stimulated by NAMPT activation but not PARP1,2 inhibition. PARP10 and GFP-expressing HEK293 cells were used to show that veliparib and SBI-797812 differ in their ability to promote the autoMARylated form of PARP10. A) Western blot and B) quantification indicate that the NAMPT activator, SBI-797812 promotes PARP10 activity whereas the PARP1,2 inhibitor does not promote PARP10 activity.

Discussion

SARS-CoV-2 is a highly infectious agent that constitutes one of the greatest dangers to public health of the past century (D. Wu et al., 2020). Morbidity and mortality data make it clear that age, smoking status and multiple preexisting conditions greatly increase the frequency of serious illness and death (Yang et al., 2020). There is an abundance of data from model systems and humans that age and conditions of metabolic stress including obesity and type 2 diabetes (Trammell, Weidemann, et al., 2016), smoking (Kunzi & Holt, 2019), heart failure (Diguet et al., 2018), nerve damage (Liu et al., 2018) and central brain injury (Vaur et al., 2017) challenge the NAD system in multiple affected tissues. Those data and the work establishing the significance of PARPs and CARH in CoV infection prompted us to consider whether NAD status might be a component of innate immunity.

Our data demonstrate the PARP transcriptional induction program is conserved between MHV and SARS-CoV-2 *in vitro* and *in vivo*. Further, NAD$^+$ and NADP$^+$, the two key hydride accepting coenzymes required for fuel oxidation, the pentose phosphate pathway and—in reduced forms—oxidative phosphorylation and biosynthetic programs have cellular levels that are highly sensitive to CoV infection. While the degree of depression of the NAD metabolome is surely sensitive to time and MOI, the >3-fold depression seen in this study is precedented in human immunodeficiency virus and Herpes virus infections (Grady, Hwang, Vastag, Rabinowitz, & Shenk, 2012; Murray, Nghiem, & Srinivasan, 1995)

Nucleotide, protein and lipid synthesis are fundamental cellular processes in which the virus exploits access to cellular NAD$^+$, NADH, NADP$^+$ and NADPH. For example, the nucleotides that the virus uses to make RNA are generated via the pentose phosphate pathway and host purine and pyrimidine biosynthetic programs. Protein synthesis depends on copious supplies of GTP, whose charge depends on fuel oxidation, while lipid synthesis is linked to the pentose phosphate pathway's generation of reducing equivalents. Our data indicate that viral infection significantly depresses the NAD metabolome, which is likely to invoke significant stress on cells as CoVs commandeer nucleotides for their use despite lower levels of the critical coenzymes for their synthesis.

Multiple studies have shown that CARH is a prominent virulence factor and is critical to prevent PARP-mediated inhibition of virus replication and induction of a robust innate immune response (Eriksson et al., 2008; Fehr et al., 2015; Fehr et al., 2016; Grunewald et al., 2019). Due to the importance of NAD^+ levels for maximal PARP activity, we suggest that pharmacological targeting of CARH along with protecting the cytosolic NAD metabolome to promote PARP functions are potentially useful preventative approaches.

In Box 1, we list major approaches to boost and/or maintain NAD coenzymes when the NAD system is under attack.

---

1) Increase activity of the *de novo* biosynthetic pathway by inhibiting α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) (Katsyuba et al., 2018)

2) Increase the Preiss Handler biosynthetic pathway by supplementing with NA (DiPalma & Thayer, 1991)

3) Increase the NAM salvage pathway by supplementing with nicotinamide (A. C. Chen et al., 2015)

4) Increase the NR salvage pathway by supplementing with NR (Bieganowski & Brenner, 2004)

5) Increase the NAM salvage pathway by activating NAMPT (Gardell et al., 2019)

6) Increase NAM salvage by inhibiting NNMT (Neelakantan et al., 2019)

7) Inhibit PARP1,2-dependent NAD^+ consumption (Donawho et al., 2007)

8) Inhibit CD38-dependent NAD^+ consumption (Aksoy, White, Thompson, & Chini, 2006)

Box 1

Based on ferret and human expression data in response to SARS-CoV-2 infection (Fig. 1), approaches 1 and 2 are unlikely to be effective because they require expression of genes, such as QPRT, NADSYN and NAPRT (Bogan & Brenner, 2008), that appear to be depressed by SARS-CoV-2 infection. NADSYN, which catalyzes the final step in NAD synthesis from trp and NA (Bieganowski, Pace, & Brenner, 2003), is significantly depressed in the human lung while NAPRT is significantly depressed in the ferret trachea. It was interesting to note that the IDO1 gene and both the IDO1 and IDO2 genes are upregulated in the ferret and human infection samples, respectively. Without expression of the entire *de novo* biosynthetic pathway, these data suggest that infected cells are engaging kynurenine synthesis in

an inflammatory or immunomodulatory response to viral infection (Bessede et al., 2014).

Based on *in vivo* expression data, approaches 3 and 4 have strong potential with the caveat that at pharmacological doses, NAM has the potential to function as a PARP inhibitor (Rankin, Jacobson, Benjamin, Moss, & Jacobson, 1989). In the ferret, NMRK1 and NAMPT expression were both up about 2-fold (NMRK1 achieved statistical significance). In the deceased human lung sample, NAMPT was up about 4-fold while NMRK1 and NMRK2 were up 2- and 4-fold, though neither of the NMRK gene expression differences exceeded the significance cut off. The actual efficacy of NR and NAM will need to be assessed in ferret and other animal trials because dose and formulation could affect delivery to the airways.

Proof of concept for approach 5, NAMPT activation, was demonstrated herein (Fig. 4). This should be pursued further with the caveat that NAMPT is considered a driver of pulmonary vascular remodeling and potentially a target to be inhibited to maintain lung health of some people at risk for COVID-19 (J. Chen et al., 2017).

If the high quality ferret trachea data are an indication of what happens in human infection, approach 6 might not be particularly helpful as NNMT gene expression is already being repressed. However, the observation that NNMT expression is depressed by infection might help approaches 3-5 be efficacious as it is known that both NAM and NR supplementation increase NAD turnover and resuls in increased formation of NAM and methylated NAM (Trammell, Schmidt, et al., 2016).

Failure of veliparib to stimulate PARP10 auto-MARylation activity despite restoring NAD$^+$ levels in this preclinical study (Figs. 3-4) can be considered less promising for approach 7, though additional models and molecules should be assessed. It is striking, however, that depressed NAD$^+$ In herpes virus infection is attributed to PARP1,2 activation (Grady et al., 2012) and this apparently is not the case for coronaviruses.

No data are available to assess the feasibility of approach 8, CD38 inhibition though CD38 was notably overexpressed in the lung of a deceased patient (Fig. 1D).

Further studies will be required to test the activity of NA, NAM, NR and SBI-797812 in cellular and animal models of CoV infection and prevention. While caution should be exercised with respect to any preventative measure, NAD boosting approaches have the potential to support the innate immune system and address the age-, smoking- and comorbid conditions associated with worse SARS-CoV-2 outcomes (Yang et al., 2020). Coupled with good hygiene, the potential societal benefit of a safe and readily available molecule to support prevention and public health is hard to overstate, especially as people emerge from sheltering in place and re-enter public spaces with potentially substantive viral contamination.

A potentially important insight into COVID-19 is provided by the knowledge that PARP induction is secondary to interferon secretion (Grunewald et al., 2019; Grunewald et al., 2020) and that PARP overexpression can drive down cellular NAD. Interferon therapy is notoriously toxic (Jonasch & Haluska, 2001) and an interferon storm has been proposed to mediate some of of SARS pathology (Huang et al., 2005). We suggest the testable hypothesis that PARP induction and NAD metabolome disturbance occur in noninfected tissues in coronavirus-infected animals and people, and that interferon-mediated collateral tissue damages may be prevented by nutritional or therapeutic support to the NAD metabolome.

Though the focus of this preclinical work is prevention, we note that innate immune responses to CoV infection, like other inflammatory responses, are potentially pathological if infection is not controlled. COVID-19 patients with acute respiratory distress syndrome experience a cytokine storm that features high level circulation of inflammatory cytokines (Mehta et al., 2020). Interestingly, in a small placebo-controlled clinical trial designed to address the oral safety and activity of Niagen NR in older men, it was discovered that 1 gram of NR per day depresses levels of IL-6, IL-5, IL-2 and tumor necrosis factor alpha (Elhassan et al., 2019), suggesting the possibility that Niagen and other NAD boosters may also be tested for safety, control of cytokine storm, and modulation of COVID-19 disease in patients.

Materials and Methods

RNASeq analysis

RNAseq data from A549 and NHBE cells, ferrets, and human subjects infected with SARS-CoV-2 were obtained from (Blanco-Melo et al., 2020). For A549 and NHBE cells, fold change and p-values for 71 pre-selected, NAD-related genes were obtained from Supplementary Material Tables 2 and 6 of (Blanco-Melo et al., 2020). Genes with "Status = Low" or "Status = Outlier" were not considered for analysis, leaving 56 genes from the A549 dataset and 52 genes from the NHBE dataset for analysis. Data can be found in Supplementary Material 2 and 3.

Ferret and human data were obtained from data set GSE147507 deposited by Benjamin tenOever. Data were analyzed using DESeq2 to calculate fold change and p-values (Supplementary Material 4 and 5). Ten genes did not have ferret orthologs in Ensembl and one gene exhibited undetectable expression (*TDO2*) leaving 60 ferret genes for analysis. Three genes were not present in the human dataset, leaving 68 human genes for analysis. Genes with $p > 0.05$ ($-\log(p) > 1.30$) were considered statistically significant. Graphs were generated using GraphPad Prism v8.

Cell culture. DBT, 17Cl-1, HEK293T, and HeLa cells expressing the MHV receptor carcinoembryonic antigen-related cell adhesion molecule 1 (a gift from Dr. Thomas Gallagher, Loyola University, Chicago, IL) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), HEPES, sodium pyruvate, non-essential amino acids, L-glutamine, penicillin and streptomycin. For analysis of the NAD metabolome and PARP10 MARylation status, HEK293T cells were transfected with 1µg GFP or GFP-PARP10 using CalPhos Mammalian Transfection Kit (Takara Bio).

Virus infection. MHV-A59 (Yount, Denison, Weiss, & Baric, 2002) was propagated on 17Cl-1 as previously described (Grunewald 2019). DBT cells were infected at an MOI of 3 PFU/cell. Prior to infection DBT cells were washed with PBS and replenished with serum-free DMEM with no supplements except penicillin and

streptomycin. DBT cells were maintained in serum-free media throughout the infection.

Quantitative NAD metabolomics

Cell pellets were extracted in batches of four by adding all internal standards followed by 400 uL of hot buffered ethanol (75% EtOH/25% 10 mM HEPES pH 7.1). Samples were placed on ice until up to 24 had been processed. After further heating in a shaker block at 55 °C for 3 minutes, samples were microcentrifuged at 4 °C. Supernatants were transferred to clean tubes and solvents removed under vacuum. All internal standards were added to tubes containing known amounts of both sets of standards. 400 uL of hot buffered ethanol was added and solvent removed under vacuum as above. At the start of the analysis all samples were reconstituted in 98% 10 mM ammonium acetate: 2% acetonitrile. Two separate LC-MS/MS runs were used to quantify the NAD metabolome as described (Trammell & Brenner, 2013).

Western Analysis of PARP10 MARylation

6 hours after HEK293T transfections, GFP-PARP10 transfectants were treated with chemical treatments in DMEM + 10% FBS in 37°C 5% CO_2 overnight. Cells were washed in PBS and lysed in 50 mM HEPES pH 7.4, 150 mM NaCl, 1mM $MgCl_2$, 1 mM TCEP, 1% Triton X-100 with the addition of Protease Inhibitors (Roche), 30 μM rucaparib (Selleck), and 10 μM PDD0017273 (Sigma). Lysates were microcentrifuged for 15 min at 4°C and supernatants were transferred to new tube with 4x SDS sample loading buffer (0.2M Tris-HCl pH 6.5, 0.4 M DTT, 8% w/v SDS, 6 mM Bromophenol Blue 6mM, Glycerol 4.3M). Samples were resolved via SDS-PAGE and transferred to nitrocellulose. Blots were blocked with 5% Milk-PBST for 30 min, incubated O/N in primary antibody (Rabbit Pan-ADPr 1:1000, Cell Signaling E6F6A; Rabbit GFP 1:1000, Chromotek PABG1-100; Mouse Tubulin 1:1000; Cell Signaling DM1A). Primary incubation was followed with HRP-conjugated secondary antibodies (Rabbit-HRP 1:10000, Jackson Laboratories 111-035-144; Mouse-HRP 1:5000, Invitrogen 62-6520). Blots were developed by chemiluminescence and imaged on a ChemiDoc MP system (Bio-Rad). Blot analysis was performed in Image-Lab (Bio-Rad).

SUPPLEMENTARY INFORMATION

1. NAD gene list
2. Differential gene expression for Figure 1A
3. Differential gene expression for Figure 1B
4. Differential gene expression for Figure 1C
5. Differential gene expression for Figure 1D
6. NAD synthesis gene expression summarized for all experiments
7. NAD metabolomics for Figure 2
8. NAD metabolomics for Figure 3

AUTHOR CONTRIBUTIONS

ARF, MSC and CB designed the experiments. YMOA and DJS performed experiments with ART and MSC, respectively. SAJT and MSS obtained NAD metabolomic data with CB. SP provided expertise. CDH performed informatic analyses with CB. Data were analyzed by all authors. The manuscript was written by CB with assistance of ARF, MSC and CDH.

ACKNOWLEDGMENTS

Competing Interests

CB is chief scientific adviser of ChromaDex and owns shares of ChromaDex stock. Other others declare no competing interests.

REFERENCES

Aksoy, P., White, T. A., Thompson, M., & Chini, E. N. (2006). Regulation of intracellular levels of NAD: a novel role for CD38. *Biochem Biophys Res Commun, 345*(4), 1386-1392. doi:10.1016/j.bbrc.2006.05.042

Alhammad, Y. M. O., & Fehr, A. R. (2020). The Viral Macrodomain Counters Host Antiviral ADP-Ribosylation. *Viruses, 12*(4). doi:10.3390/v12040384

Atasheva, S., Akhrymuk, M., Frolova, E. I., & Frolov, I. (2012). New PARP gene with an anti-alphavirus function. *J Virol, 86*(15), 8147-8160. doi:10.1128/JVI.00733-12

Atasheva, S., Frolova, E. I., & Frolov, I. (2014). Interferon-stimulated poly(ADP-Ribose) polymerases are potent inhibitors of cellular translation and virus replication. *J Virol, 88*(4), 2116-2130. doi:10.1128/JVI.03443-13

Belenky, P., Bogan, K. L., & Brenner, C. (2007). NAD+ metabolism in health and disease. *Trends in Biochemical Sciences, 32*(1), 12-19. doi:10.1016/j.tibs.2006.11.006

Bessede, A., Gargaro, M., Pallotta, M. T., Matino, D., Servillo, G., Brunacci, C., . . . Puccetti, P. (2014). Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature, 511*(7508), 184-190. doi:10.1038/nature13323

Bieganowski, P., & Brenner, C. (2004). Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a preiss-handler independent route to NAD+ in fungi and humans. *Cell, 117*(4), 495-502. doi:10.1016/S0092-8674(04)00416-7

Bieganowski, P., Pace, H. C., & Brenner, C. (2003). Eukaryotic NAD+ synthetase Qns1 contains an essential, obligate intramolecular thiol glutamine amidotransferase domain related to nitrilase. *Journal of Biological Chemistry, 278*(35), 33049-33055. doi:10.1074/jbc.M302257200

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W.-C., Moller, R., Panis, M., Sachs, D., . . . tenOever, B. R. (2020). SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems. *Cell, in press*. doi:10.1016/j.cell.2020.04.026

Bogan, K. L., & Brenner, C. (2008). Nicotinic acid, nicotinamide, and nicotinamide riboside: A molecular evaluation of NAD + precursor vitamins in human nutrition. *Annual Review of Nutrition, 28*, 115-130. doi:10.1146/annurev.nutr.28.061807.155443

Brenner, C. (2014). Metabolism: Targeting a fat-accumulation gene. *Nature, 508*(7495), 194-195. doi:10.1038/508194a

Cambronne, X. A., Stewart, M. L., Kim, D., Jones-Brunette, A. M., Morgan, R. K., Farrens, D. L., . . . Goodman, R. H. (2016). Biosensor reveals multiple sources for mitochondrial NAD(+). *Science, 352*(6292), 1474-1477. doi:10.1126/science.aad5168

Channappanavar, R., Fehr, A. R., Zheng, J., Wohlford-Lenane, C., Abrahante, J. E., Mack, M., . . . Perlman, S. (2019). IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *J Clin Invest, 130*, 3625-3639. doi:10.1172/JCI126363

Chen, A. C., Martin, A. J., Choy, B., Fernandez-Penas, P., Dalziell, R. A., McKenzie, C. A., . . . Damian, D. L. (2015). A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. *N Engl J Med, 373*(17), 1618-1626. doi:10.1056/NEJMoa1506197

Chen, J., Sysol, J. R., Singla, S., Zhao, S., Yamamura, A., Valdez-Jasso, D., . . . Machado, R. F. (2017). Nicotinamide Phosphoribosyltransferase Promotes Pulmonary Vascular Remodeling and Is a Therapeutic Target in Pulmonary Arterial Hypertension. *Circulation, 135*(16), 1532-1546. doi:10.1161/CIRCULATIONAHA.116.024557

Cohen, M. S. (2020). Interplay between compartmentalized NAD(+) synthesis and consumption: a focus on the PARP family. *Genes Dev, 34*(5-6), 254-262. doi:10.1101/gad.335109.119

Diguet, N., Trammell, S. A. J., Tannous, C., Deloux, R., Piquereau, J., Mougenot, N., . . . Mericskay, M. (2018). Nicotinamide Riboside Preserves Cardiac Function in a Mouse

Model of Dilated Cardiomyopathy. *Circulation, 137*, 2256-2273. doi:10.1161/CIRCULATIONAHA.116.026099

DiPalma, J. R., & Thayer, W. S. (1991). Use of niacin as a drug. *Annu Rev Nutr, 11*, 169-187.

Donawho, C. K., Luo, Y., Luo, Y., Penning, T. D., Bauch, J. L., Bouska, J. J., . . . Frost, D. J. (2007). ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. *Clin Cancer Res, 13*(9), 2728-2737. doi:10.1158/1078-0432.CCR-06-3039

Dong, E., Du, H., & Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis*. doi:10.1016/S1473-3099(20)30120-1

Elhassan, Y. S., Kluckova, K., Fletcher, R. S., Schmidt, M. S., Garten, A., Doig, C. L., . . . Lavery, G. G. (2019). Nicotinamide Riboside Augments the Aged Human Skeletal Muscle NAD(+) Metabolome and Induces Transcriptomic and Anti-inflammatory Signatures. *Cell Rep, 28*(7), 1717-1728 e1716. doi:10.1016/j.celrep.2019.07.043

Eriksson, K. K., Cervantes-Barragan, L., Ludewig, B., & Thiel, V. (2008). Mouse hepatitis virus liver pathology is dependent on ADP-ribose-1"-phosphatase, a viral function conserved in the alpha-like supergroup. *J Virol, 82*(24), 12325-12334. doi:10.1128/JVI.02082-08

Fehr, A. R., Athmer, J., Channappanavar, R., Phillips, J. M., Meyerholz, D. K., & Perlman, S. (2015). The nsp3 macrodomain promotes virulence in mice with coronavirus-induced encephalitis. *J Virol, 89*(3), 1523-1536. doi:10.1128/JVI.02596-14

Fehr, A. R., Channappanavar, R., Jankevicius, G., Fett, C., Zhao, J., Athmer, J., . . . Perlman, S. (2016). The Conserved Coronavirus Macrodomain Promotes Virulence and Suppresses the Innate Immune Response during Severe Acute Respiratory Syndrome Coronavirus Infection. *mBio, 7*(6). doi:10.1128/mBio.01721-16

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol, 1282*, 1-23. doi:10.1007/978-1-4939-2438-7_1

Fehr, A. R., Singh, S. A., Kerr, C. M., Mukai, S., Higashi, H., & Aikawa, M. (2020). The impact of PARPs and ADP-ribosylation on inflammation and host-pathogen interactions. *Genes Dev, 34*(5-6), 341-359. doi:10.1101/gad.334425.119

Gardell, S. J., Hopf, M., Khan, A., Dispagna, M., Hampton Sessions, E., Falter, R., . . . Pinkerton, A. B. (2019). Boosting NAD(+) with a small molecule that activates NAMPT. *Nat Commun, 10*(1), 3241. doi:10.1038/s41467-019-11078-z

Grady, S. L., Hwang, J., Vastag, L., Rabinowitz, J. D., & Shenk, T. (2012). Herpes simplex virus 1 infection activates poly(ADP-ribose) polymerase and triggers the degradation of poly(ADP-ribose) glycohydrolase. *J Virol, 86*(15), 8259-8268. doi:10.1128/JVI.00495-12

Grunewald, M. E., Chen, Y., Kuny, C., Maejima, T., Lease, R., Ferraris, D., . . . Fehr, A. R. (2019). The coronavirus macrodomain is required to prevent PARP-mediated inhibition of virus replication and enhancement of IFN expression. *PLoS Pathog, 15*(5), e1007756. doi:10.1371/journal.ppat.1007756

Grunewald, M. E., Shaban, M. G., Mackin, S. R., Fehr, A. R., & Perlman, S. (2020). Murine Coronavirus Infection Activates the Aryl Hydrocarbon Receptor in an Indoleamine 2,3-Dioxygenase-Independent Manner, Contributing to Cytokine Modulation and Proviral TCDD-Inducible-PARP Expression. *J Virol, 94*(3). doi:10.1128/JVI.01743-19

Gupte, R., Liu, Z., & Kraus, W. L. (2017). PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes Dev, 31*(2), 101-126. doi:10.1101/gad.291518.116

Huang, K. J., Su, I. J., Theron, M., Wu, Y. C., Lai, S. K., Liu, C. C., & Lei, H. Y. (2005). An interferon-gamma-related cytokine storm in SARS patients. *J Med Virol, 75*(2), 185-194. doi:10.1002/jmv.20255

Jonasch, E., & Haluska, F. G. (2001). Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. *Oncologist, 6*(1), 34-55. doi:10.1634/theoncologist.6-1-34

Katsyuba, E., Mottis, A., Zietak, M., De Franco, F., van der Velpen, V., Gariani, K., . . . Auwerx, J. (2018). De novo NAD(+) synthesis enhances mitochondrial function and improves health. *Nature, 563*(7731), 354-359. doi:10.1038/s41586-018-0645-6

Kindler, E., Thiel, V., & Weber, F. (2016). Interaction of SARS and MERS Coronaviruses with the Antiviral Interferon Response. *Adv Virus Res, 96*, 219-243. doi:10.1016/bs.aivir.2016.08.006

Kunzi, L., & Holt, G. E. (2019). Cigarette smoke activates the parthanatos pathway of cell death in human bronchial epithelial cells. *Cell Death Discov, 5*, 127. doi:10.1038/s41420-019-0205-3

Li, L., Zhao, H., Liu, P., Li, C., Quanquin, N., Ji, X., . . . Cheng, G. (2018). PARP12 suppresses Zika virus infection through PARP-dependent degradation of NS1 and NS3 viral proteins. *Sci Signal, 11*(535). doi:10.1126/scisignal.aas9332

Li, Q., Wang, L., Dong, C., Che, Y., Jiang, L., Liu, L., . . . Ma, S. (2005). The interaction of the SARS coronavirus non-structural protein 10 with the cellular oxido-reductase system causes an extensive cytopathic effect. *J Clin Virol, 34*(2), 133-139. doi:10.1016/j.jcv.2004.12.019

Liu, H. W., Smith, C. B., Schmidt, M. S., Cambronne, X. A., Cohen, M. S., Migaud, M. E., . . . Goodman, R. H. (2018). Pharmacological bypass of NAD(+) salvage pathway protects neurons from chemotherapy-induced degeneration. *Proc Natl Acad Sci U S A, 115*(42), 10654-10659. doi:10.1073/pnas.1809392115

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., & Hlh Across Speciality Collaboration, U. K. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet, 395*(10229), 1033-1034. doi:10.1016/S0140-6736(20)30628-0

Murray, M. F., Nghiem, M., & Srinivasan, A. (1995). HIV infection decreases intracellular nicotinamide adenine dinucleotide [NAD]. *Biochem Biophys Res Commun, 212*(1), 126-131. doi:10.1006/bbrc.1995.1945

Neelakantan, H., Brightwell, C. R., Graber, T. G., Maroto, R., Wang, H. L., McHardy, S. F., . . . Watowich, S. J. (2019). Small molecule nicotinamide N-methyltransferase inhibitor activates senescent muscle stem cells and improves regenerative capacity of aged skeletal muscle. *Biochem Pharmacol, 163*, 481-492. doi:10.1016/j.bcp.2019.02.008

Rankin, P. W., Jacobson, E. L., Benjamin, R. C., Moss, J., & Jacobson, M. K. (1989). Quantitative studies of inhibitors of ADP-ribosylation in vitro and in vivo. *J Biol Chem, 264*(8), 4312-4317.

Ryu, K. W., Nandu, T., Kim, J., Challa, S., DeBerardinis, R. J., & Kraus, W. L. (2018). Metabolic regulation of transcription through compartmentalized NAD(+) biosynthesis. *Science, 360*(6389). doi:10.1126/science.aan5780

Schoggins, J. W., & Rice, C. M. (2011). Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol, 1*(6), 519-525. doi:10.1016/j.coviro.2011.10.008

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., . . . Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science*. doi:10.1126/science.abb7015

Totura, A. L., & Baric, R. S. (2012). SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. *Curr Opin Virol, 2*(3), 264-275. doi:10.1016/j.coviro.2012.04.004

Trammell, S. A., & Brenner, C. (2013). Targeted, LCMS-based Metabolomics for Quantitative Measurement of NAD(+) Metabolites. *Comput Struct Biotechnol J, 4*, e201301012. doi:10.5936/csbj.201301012

Trammell, S. A., Schmidt, M. S., Weidemann, B. J., Redpath, P., Jaksch, F., Dellinger, R. W., . . . Brenner, C. (2016). Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun, 7*, 12948. doi:10.1038/ncomms12948

Trammell, S. A., Weidemann, B. J., Chadda, A., Yorek, M. S., Holmes, A., Coppey, L. J., . . . Brenner, C. (2016). Nicotinamide Riboside Opposes Type 2 Diabetes and Neuropathy in Mice. *Sci Rep, 6*, 26933. doi:10.1038/srep26933

Vaur, P., Brugg, B., Mericskay, M., Li, Z., Schmidt, M. S., Vivien, D., . . . Duplus, E. (2017). Nicotinamide riboside, a form of vitamin B3, protects against excitotoxicity-induced axonal degeneration. *FASEB J, 31*(12), 5440-5452. doi:10.1096/fj.201700221RR

Wu, D., Wu, T., Liu, Q., & Yang, Z. (2020). The SARS-CoV-2 outbreak: what we know. *Int J Infect Dis*. doi:10.1016/j.ijid.2020.03.004

Yang, J., Zheng, Y., Gou, X., Pu, K., Chen, Z., Guo, Q., . . . Zhou, Y. (2020). Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *Int J Infect Dis*. doi:10.1016/j.ijid.2020.03.017

Yount, B., Denison, M. R., Weiss, S. R., & Baric, R. S. (2002). Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59. *J Virol, 76*(21), 11065-11078. doi:10.1128/jvi.76.21.11065-11078.2002

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., . . . Research, T. (2020). A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med, 382*(8), 727-733. doi:10.1056/NEJMoa2001017