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EDITORIALS: CELL CYCLE FEATURES

NAD^+ in DNA repair and mitochondrial maintenance

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ATM is a kinase and major master regulator of the DNA damage response cascade. Ataxia telangiectasia (A-T) patients have mutations in *ATM* and suffer from telangiectasia, radiosensitivity, cancer, and neurologic features.[1] Some features of A-T are readily explained by the loss of DNA repair capacity, i.e. radiosensitivity and cancer, but not the neurologic deficits. Therefore, we initiated studies to explore an alternative hypothesis to explain the neurologic findings.

Prior literature revealed mitochondrial deficits in A-T.[2] Since there are well-established links between DNA repair defects, neurodegeneration and mitochondrial dysfunction,[3,4] we focused our attention on mitochondria. In 2 other DNA repair-deficient syndromes, Cockayne Syndrome (CS),[4,5] and Xeroderma Pigmentosum group A (XPA),[3] we reported that persistent nuclear DNA damage created a signal to mitochondria, which we called nuclear to mitochondrial (NM) signaling.[6] Nicotinamide adenine dinucleotide (NAD^+), an important metabolite in all human cells, was the signaling molecule. Emerging evidence strongly suggest NAD^+ is a key player in aging, neurodegeneration and metabolic diseases.

In NM signaling, we proposed that persistent activation of poly(ADP-ribose) polymerase1 (PARP1), an NAD^+-dependent enzyme that poly(ADP-ribosylates) (PARylates) proteins, induces a loss of intracellular NAD^+ whereby other NAD^+-dependent enzymes, like the sirtuins, experience a loss of activity.[6] PARP1 is a major abundant nuclear protein, which serves as a sensor of DNA damage. In response to DNA damage, the rate of PAR synthesis increases rapidly up to 500-fold which can consume a significant amount of NAD^+.[7] In the NM signaling cascade, the pathways critically affected by PARP1-dependent depletion of intracellular NAD^+ are SIRT1-dependent protein deacetylation, mitochondrial oxidative phosphorylation and mitophagy, the selective clearance of damaged or defective mitochondria. SIRT1 is a multifunctional protein deacetylase which plays important roles in cellular pathways including: longevity, metabolism, cellular senescence, genome maintenance, DNA repair, and inflammation. Thus, loss of NAD^+ has direct and indirect consequences on multiple cellular endpoints. Ultimately, depletion of intracellular NAD^+ alters the NAD^+/SIRT1 axis and leads to defects in mitochondrial homeostasis, ROS production, DNA repair and cell survival.[6]

To test whether increased NAD^+ might ameliorate some of the features noted above we activate the NAD^+/SIRT1 pathway using 3 strategies: NAD^+ supplementation with an NAD^+ precursor nicotinamide riboside (NR), PARP1 inhibition with olaparib or SIRT1 activation by SRT1720. Consistently, all 3 treatments improved the mitochondrial phenotypes, and decreased PARylation. We have consistently seen that loss of activity from the NAD^+/SIRT1 axis also inhibits mitophagy. Thus, we interrogated whether NR could improve mitophagy in the *ATM*-knockdown cells, and as expected replenishment of NAD^+ stimulated mitophagy and improved mitochondria morphology. Using a worm model of A-T, *atm*-1, we corroborated our results and showed that the mitochondrial improvements were the result of increased mitophagy. Further, we demonstrated that NR-dependent enhancements in mitophagy were dependent upon expression of SIR-2.1 (the worm homolog of SIRT1), DAF16, PINK-1 and DCT-1 (homolog of mammalian NIX/BNIP3L). On a functional level, *atm*-1 worms treated with NR, SRT1720 or olaparib all experienced improved lifespan, mitochondrial morphology, swimming and memory. Our data suggest that stimulating the NAD^+/SIRT1 axis improves healthspan in part through re-establishment of mitophagy.

As ATM is a central DNA repair protein, we also interrogated whether NAD^+ supplementation could improve double strand break (DSB) repair. Using ATM and SIRT1 depleted cells, we showed that ATM-deficient cells are significantly impaired in Non-homologous end joining (NHEJ) and that NR treatment can partially restore NHEJ activity. These results were conditional upon both SIRT1 and SIRT6 expression. We corroborated that NR promoted NHEJ DSB repair in *atm*-1 worms and showed that NR treatment dramatically improved genomic stability in *atm*-1 worms. Together, these results suggest that part of the DNA repair defect exhibited by ATM-deficient cells may not be a directed effect of ATM loss, but rather a consequence of loss of SIRT1s ability to stimulate NHEJ repair.

Importantly, we extended our NR supplementation to Atm-deficient mice and documented a profound extension of lifespan. Although we do not know the precise mechanism of action yet, our findings underscore how metabolites, like NAD^+, provide the cell with a gauge to respond to a changing cellular environment. Under normal conditions,

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when a cell is damaged and PARP1 catabolizes NAD^+, the extent of NAD^+ depletion likely correlates with the extent of the damage. If the damage is too excessive, loss of NAD^+ and mitochondrial homeostasis induces cell death. Under conditions of persistent DNA damage which elicits a PARP1 response, chronic NAD^+ catabolism may outstrip the cellular capacity to replenish it, compromising other NAD^+-dependent enzymatic activities, mitochondrial fitness and rendering cells more sensitive to exogenous stress.

Finally, while it remains to be seen how well NAD^+ supplementation translates to the clinical treatment of A-T patients, our findings suggest NR and other NAD^+ supplementation strategies hold the potential to at least partially offset some of the damaging effects of ATM loss and may improve the quality of life for these individuals. Additionally, since diagnosis of mitochondrial defects are notoriously difficult, more DNA repair syndromes or conditions that provoke catabolism of NAD^+ should be evaluated for NAD^+ supplementation.

Disclosure of potential conflicts of interest

We declare that we receive funding from ChromaDex, a company that may be affected by the research reported in the enclosed paper.

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