Repletion of Nicotinamide adenine dinucleotide restores adult stem cell function and extends lifespan in mice

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Aging and aging intervention

Aging is a biological process increasingly recognized to be controlled by endogenous signaling and metabolism. It can be modulated by genetic, environmental, and pharmacological perturbations. Importantly, aging not only decreases our quality of life, but also contributes to many fatal diseases. According to the WHO report, the occurrences of many largely incurable pathological conditions, including cancer, diabetes, and neuronal degeneration, are strongly associated with aging (1).

Naturally, aging has gained significant attention and remarkable progress has been made in understanding the basic processes underlying aging. Most aging studies exploit the short lifespan of animal models, which include *Caenorhabditis elegans* (*C. elegans*), *Drosophila* and rodents. Moreover, several hallmarks have been proposed for aging, including loss of proteostasis, mitochondria dysfunction, and stem cell exhaustion (2). Similar to other biological processes, aging is strongly regulated by intrinsic signaling, transcription factors, and metabolism (3). Several highly conserved pathways, including the insulin/insulin like growth factor (IGF1), the target of rapamycin (TOR), and AMP kinase (AMPK) pathways, sirtuins, and stress-induced machinery, such as the unfolded protein response (UPR), have been identified as aging mediators (3-5). Most of these pathways are involved in nutrient sensing and/or cellular stress responses, and are tightly connected to metabolism.

Pharmacological perturbation of aging has likewise gained considerable attention. A handful of small molecules, including spermidine (targeting autophagy), metformin (modulating AMPK, NADH dehydrogenase, and bioenergetics), resveratrol (modulating SIR-2.1 and other factors), and α-KG and its analogues (inhibiting ATP synthase and TOR) have been reported as anti-aging compounds (6-10). In order to translate these compounds into clinical use, the longevity effect of these compounds must be tested more stringently in mammalian models, with comprehensive analyses carried out to explore their effects on aging-related phenotypes.

NAD$^+$ and longevity

Nicotinamide adenine dinucleotide (NAD$^+$) is a small molecule that serves as a substrate for the electron transport chain and for other post-translational modification enzymes such as the sirtuins and poly (adenosine diphosphate-ribose) polymerases. The cellular NAD$^+$ level is strongly correlated with aging. Endogenous levels of NAD$^+$ increase upon treatments which extend lifespan, such as dietary restriction, while NAD$^+$ levels decrease during aging (11). In addition, treatment with NAD$^+$ or NAD$^+$ precursors can significantly increase lifespan in yeast and *C. elegans*. The lifespan extension effect of NAD$^+$ in *C. elegans* is dependent on *sir-2.1* (worm homologue of SIRT1) as well as the mitochondria UPR^{mt} (5). Notably, the beneficial effects of NAD$^+$ are not only limited to yeast and *C. elegans*. Several

reports have shown that NAD^+ metabolism improves glucose intolerance in aged mice and exerts beneficial effects on neurodegeneration (12,13). However, whether NAD^+ can similarly increase lifespan and slow or reverse aging hallmarks in mice were largely unexplored. Recently, a study from the Auwerx group for the first time showed that NAD^+ treatment improves mitochondrial function, restores homeostasis in adult stem cell populations, and extends lifespan in mice (14).

NAD^+ attenuates aged muscle stem cell (MuSC) senescence by improving mitochondrial function

Stem cell senescence leads to reduced tissue regeneration and homeostasis and therefore is considered one of the hallmarks of aging. Age-related impairment of mitochondria, a critical regulator of cellular energetics and metabolism, is also a hallmark of aging (2,15). To investigate whether aging-induced mitochondrial dysfunction triggers stem cell senescence during aging, Zhang and colleagues first compared gene expression data sets from adult MuSCs of young and aged mice. As expected, senescence pathways were upregulated while cell cycle pathways were downregulated in the aged samples. On the other hand, tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) pathways ranked among the most downregulated pathways in the aged MuSCs. Additionally, indicators of mitochondrial function, including oxidative respiration, mitochondrial membrane potential, and the UPR^{mt}, were all reduced in aged MuSCs. These hinted at the potential role of mitochondrial dysfunction in MuSC senescence. Furthermore, NAD^+ was found to be lower in MuSCs from aged mice. Declining NAD^+ levels have been linked to aging in prior studies, and treatment with the NAD^+ precursors Nicotinamide riboside (NR) or Nicotinamide mononucleotide (NMN) can restore its level with anti-aging effects (11). In the current study, NR treatment not only reduced multiple senescence markers in aged MuSCs but also reversed the aging-related mitochondrial dysfunction described above. Consistently, NAD^+ repletion by NR treatment increased MuSC numbers though damage-induced muscle regeneration and improved MuSC functions in aged animals. In $SIRT1^{MuSC-/-}$ knockout mice, the beneficial effect of NR on muscle regeneration is largely abolished, indicating that SIRT1 is required for NAD^+ to improve mitochondrial function and prevent MuSC senescence. Together the data suggest that NAD^+ repletion alleviates MuSC senescence through rejuvenating mitochondrial function.

The UPR^{mt} and prohibitin pathway are activated by NAD^+ to prevent stem cell senescence and aging

The prohibitin proteins are mitochondrial stress sensors and modulators of cell senescence (16-18). Their expression was observed to decrease during aging, and NR treatment was sufficient to increase their expression in both C2C12 myoblasts and in mouse MuSCs along with markers of UPR^{mt} and the cell cycle. Interestingly, overexpression of prohibitins alone was sufficient to increase expression of UPR^{mt} and cell cycle proteins, and knockdown of prohibitin expression both prevented NR treatment from increasing expression of UPR^{mt} and cell cycle proteins as well as blocked the NR-induced increase of MuSC number *in vivo*. Together these results are consistent with an important role for both the prohibitins and UPRmt in the ability of NAD^+ to rejuvenate muscle stem cells.

NAD^+ repletion provides a new avenue to modulate mammalian lifespan

Mitochondrial dysfunction has been shown to be associated with aging, partially due to NAD^+ depletion (5). NAD^+ repletion by treatment with NAD^+ precursors can reverse aging-related mitochondrial failure and extend lifespan in multiple animal models (13,19). Zhang and colleagues found that in addition to MuSCs, NR treatment also showed beneficial effects in aged neural and melanocyte stem cells, which decline with aging (20,21). In isolated neural stem cells (NSC) from aged mice, NR can induce NSC proliferation and neurogenesis blunted by aging. Similarly, hair follicles from NR-treated aged mice showed higher levels of stemness and proliferation. An increased survival rate of old mice treated with NR beginning at the age of 700 days further demonstrated the anti-aging effects of NAD^+.

Stem cell exhaustion and senescence cause tissue dysfunction by limiting tissue regeneration, a direct cause of the aging-related decline in healthspan in mammals. This paper linked the molecular crosstalk between mitochondrial stress and stem cell senescence during aging and demonstrated that NAD+ repletion prevents senescence by regulating the mitochondrial response. Stem cells from several tissues can be rejuvenated by treatment with NAD^+ precursors, allowing them to self-renew and thereby

enhance longevity. Additionally, in senescence-prone MuSCs from *mdx* mice (a model of muscular dystrophy), NR treatment also increased MuSC numbers, reduced senescence markers, and improved their self-renewal capacity. Together these results provide strong support for the application of NAD^+ precursors as a treatment for senescence induced stem cell depletion and age-related tissue dysfunction.

NAD^+ homeostasis related longevity study

An increasing number of studies have revealed the link between NAD^+ metabolism and aging and the impressive pro-longevity effects from supplementation with NAD^+ precursors. Moreover, the beneficial effect of boosting NAD^+ levels seems not to be limited to one hallmark of aging. The current study showed that boosting NAD^+ levels significantly improves adult stem cell and mitochondrial function, and also provided evidence that the two are interconnected. Indeed, although mitochondria are not the major energy producer in adult stem cells, dysfunction of mitochondria is at least partially responsible for the adult stem cell pool decline during aging, and restoring mitochondrial function can help restore adult stem cell homeostasis. Although this opens up promising avenues for treating the aging-related decline in tissue function, much remains to be discovered regarding the detailed molecular network between mitochondria function and adult stem cell homeostasis.

Importantly, the lifespan extension achieved by treatment with NAD^+ precursors suggests that the role of NAD^+ in regulating aging is more generalizable and not likely organ specific. In agreement with this idea, a separate study carried out by the Imai group showed that treatment with another NAD^+ intermediate, NMN, significantly mitigates multiple aging-related physiological phenotypes, including loss of insulin sensitivity, dysregulated mitochondrial function, impaired eye function, as well as decreased bone density (22). However, the detailed molecular mechanisms need to be further explored, and it is plausible that NAD^+ regulates aging through multiple mechanisms. There are several NAD^+ intermediates available for lifespan analysis. It would be interesting to perform a detailed pharmacokinetics study on these small molecules and explore potential combinations of them to achieve more significant anti-aging effects. Moreover, it has not been conclusively demonstrated that NAD^+ itself is the critical lifespan-extending metabolite, or whether it is just a marker whose level is correlated with the activity of another active player.

Aging is the biggest risk factor for several age-related diseases. Prior studies have shown that NAD^+ precursor treatment can alleviate several aging-related diseases, such as diabetes and neurodegeneration (12,13). This study demonstrates that boosting NAD^+ can protect muscle stem cells from physiological stress, further supporting the beneficial effects of treatment with NAD^+ precursors in age-related disease intervention. Therefore, development of NAD^+ and related intermediates as interventions for aging and age-related diseases is a very promising area of study.

In conclusion, the Zhang *et al.*, study showed that boosting NAD^+ levels exerts remarkable anti-aging effects, including improvement of mitochondrial function, restoration of adult stem cell homeostasis, and lifespan extension in mice. Many NAD^+ precursors, such as NMN, can be obtained from various food sources including vegetables and meat, suggesting potential natural means of boosting endogenous NAD^+ levels. It will be interesting to test means of boosting NAD^+ in the clinic and examine whether this endogenous compound can also serve as an intervention for age-associated hallmarks and diseases in humans.

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