Abstract

Short telomeres lead to pleiotropic clinical symptoms in dyskeratosis congenita (DC), including BM failure and shortened lifespan, for which no effective pharmacological treatment is available. We recently identified low nicotinamide adenine dinucleotide (NAD) levels in primary fibroblasts derived from DC patients, which leaves NAD insufficient for other important NAD metabolic processes, including mitochondrial and genome maintenance, consequently exacerbating cell growth retardation and senescence, mitochondrial impairment, telomere damage of DC fibroblasts. In addition, we found that a NAD precursor, nicotinamide riboside (NR) restored NAD metabolism and ameliorated cell deficits in DC fibroblasts and in late generation telomerase null mice. Thus, our findings provide insight on the applicability of NR to counter diseases driven by telomere shortening.

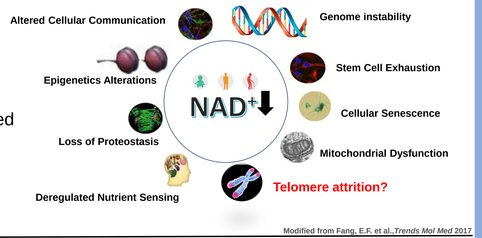
Background

Dyskeratosis congenita- a short telomere syndrome

- Patients have mutations in telomere maintenance genes
- mucocutaneous triad, pulmonary fibrosis, increased risk of cancer
- bone marrow failure- primary cause of early mortality

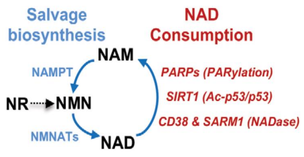
NAD-linked metabolism, aging, and telomere attrition

- **Nicotinamide adenine dinucleotide (NAD)**-an essential coenzyme for metabolic/redox reactions
- Telomere shortening and decreased NAD are key features of aging
- Link between NAD decline and telomere attrition?



Modified from Fang, E.F. et al.,*Trends Mol Med* 2017

NAD biosynthesis and consumption



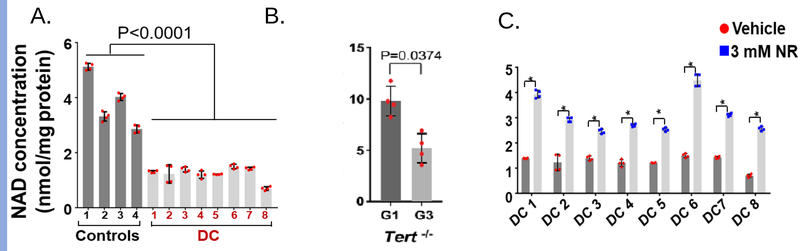
- NAD salvage pathway
  - NAD biosynthesis: NAMPT, NMNATs
  - NAD consumption: PARPs, SIRTs, CD38, SARM1

Aim

Evaluate NAD metabolism and intervention in models of telomere dysfunction

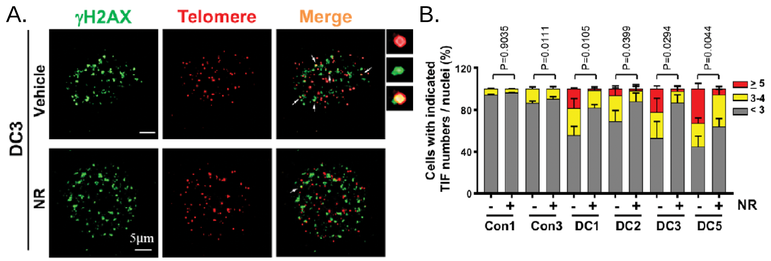
Results

Fig 1. NR restores NAD levels, which are depleted in DC models



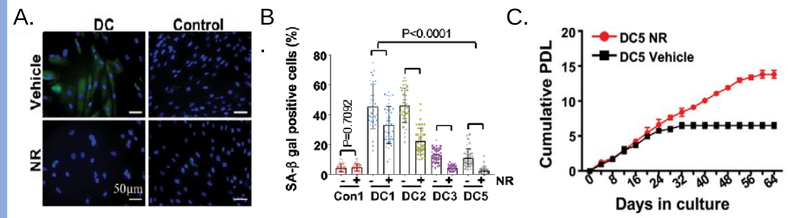
A. Intracellular NAD is decreased in DC compared to age-matched healthy control fibroblasts.
B. Intracellular NAD is decreased in G3 (short telomeres) compared to G1 *Tert-/-* (normal-length telomeres) mouse brain tissues.
C. NR restores NAD levels in DC fibroblasts

Fig 2. NR reduces telomere dysfunction-induced DNA damage foci



A. IF-FISH analysis shows colocalization (white arrows) of γH2AX DNA damage foci with telomeres (telomere dysfunction- induced DNA damage foci, TIFs) .
B. Percentage of DC and healthy control cells with indicated TIFs per nuclei.

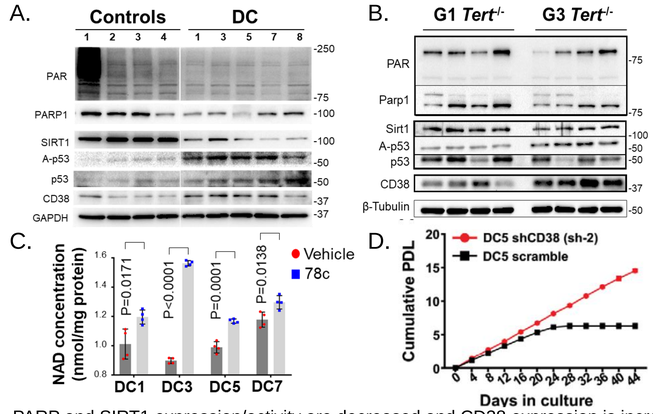
Fig 3. NR ameliorates cellular senescence in DC cells



A. SPiDER-β-gal staining shows increased senescence-associated (SA)-β-gal-positive cells in DC compared to healthy control fibroblasts.
B. % (SA)-β-gal-positive DC and age-matched healthy control fibroblasts treated with vehicle or NR. Dots represent % cells per image. NR decreased the % of (SA)-β-gal-positive DC fibroblasts.
C. Cumulative population doublings are increased with NR- indicating delayed replicative senescence.

Results continued

Fig 4. NAD depletion in DC models is driven by the CD38 NADase



A. PARP and SIRT1 expression/activity are decreased and CD38 expression is increased in DC compared to healthy control fibroblasts.
B. PARP and SIRT1 expression/activity are decreased and CD38 expression is increased in G3 compared to G1 Tert-/- mice brain tissues.
C. The CD38 inhibitor, (78c) increased NAD+ levels in DC fibroblasts.
D. Knockdown of CD38 (shCD38) improves replicative lifespan in DC fibroblasts.

Conclusions

- NAD intervention alleviates telomere dysfunction, mitochondrial impairment (published but not shown here), and cellular senescence in models with short telomeres.
- Our findings support NAD intervention as a potential strategy for treating short telomere syndromes. Additional research should be conducted.
- This work is published:

  Sun C, Wang K, Stock AJ, Gong Y, Demarest TG, Yang B, Giri N, Harrington L, Alter BP, Savage SA, Bohr VA, Liu Y. Re-equilibration of imbalanced NAD metabolism ameliorates the impact of telomere dysfunction. EMBO J. 2020 Nov 2. doi: 10.15252/embj.2019103420

Acknowledgements

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