antibody (romosozumab-aqqg; Evenity) was recently approved by the FDA to treat patients at increased risk of fracture. However, an increased risk of cardiovascular events was reported, resulting in issue a ‘black box warning’ requirement for romosozumab. One potential solution to lower the risk of adverse events is to reduce the medication dose. Previously, we found that dual inhibition of sclerostin and Dkk1 produced extremely potent synergistic bone anabolic effects, in both genetic and pharmacological models. While Dkk1 inhibition alone has no consistent bone-building effects, combining antibodies that target sclerostin (Scl-mAb) and Dkk1 (Dkk1-mAb) at 3:1 ratio resulted in 2-3X more bone gain as Scl-mAb alone. Further, much lower total doses of dual antibody treatment, given at optimized proportions, generated equivalent bone anabolic effects as Scl-mAb alone (at much higher doses), suggesting that a combinatorial strategy has obvious translational benefits. Finally, we tested whether low-dose combination therapy can maintain the same osteogenic effect as Scl-mAb in adult (6 month) and aged (20 month) mice. Outcome measures derived from radiographic, biomechanical, and histomorphometric assays revealed that a 3:1 ratio of Scl-mAb:Dkk1-mAb at 12.5mg/kg was as efficacious as 2.5mg/kg of Scl-mAb alone, in both age groups. Moreover, cortical porosity—a significant factor contributing to skeletal fragility in the aged skeleton—was significantly reduced by both Scl-mAb and low-dose combination treatment. In conclusion, our findings suggest that optimized low-dose combinational therapy is viable strategy for improving skeletal fragility.

ORALLY ACTIVE, CLINICALLY TRANSLATABLE SENOLYTICS RESTORE A-KLOTHO IN MICE AND HUMANS

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Decreased α-Klotho, a geroprotective factor, and increased senescent cell burden are both associated with early onset of physical disability, cognitive impairment, and premature all-cause mortality. It has been demonstrated that eliminating senescent cells can enhance physical function, cognition, and survival in mice, as does overexpressing α-Klotho. Mice with low α-Klotho exhibit accelerated senescent cell accumulation, recombinant α-Klotho decreases senescent cell burden and restores lifespan in these mice, and senescent epidermal cells are reduced in mice overexpressing α-Klotho. Here, we tested the hypothesis that senescent cells cause decreased α-Klotho and hence that reducing senescent cells can increase α-Klotho. Senescent cell conditioned medium (CM) reduced α-Klotho in cultured non-senescent human umbilical vein endothelial cells (HUVECs), renal tubular endothelial cells, and astrocytes. These effects of senescent CM were partially attenuated by neutralizing antibodies against the senescence-associated secretory phenotype (SASP) factors, activin A and IL-1α. Transplanting senescent cells into younger mice caused decreased urine and brain α-Klotho. Genetically reducing highly p16INK4a-expressing cells in old INK-ATTAC mice or administering the senolytics, Dasatinib plus Quercetin (D+Q) or Fisetin (F), to young mice transplanted with senescent cells, young diet-induced obese (DIO) mice, or naturally-aged mice increased urine, kidney, and/or brain α-Klotho. Treating patients with idiopathic pulmonary fibrosis (IPF), a cellular senescence-related disease, with D+Q led to increased urinary α-Klotho. Thus, targeting senescent cells causes increases in the geroprotective factor α-Klotho, potentially amplifying the beneficial effects of senolytic drugs.

OXR1 STABILIZES THE RETROMER TO EXTEND LIFESPAN AND NEURONAL HEALTH BY DIETARY RESTRICTION

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Dietary restriction (DR) delays aging and neurodegeneration, but the mechanisms behind this remain unclear. We reared over 150 fully sequenced fly strains from the Drosophila Genetic Reference Panel under ad libitum feeding or diet-restricted conditions and measured lifespan as well as healthspan to identify new targets for DR-mediated longevity. Through genome-wide association study, we identified genetic variants associated with influencing these traits under each dietary condition. A variant in mustard (mtd, called Oxidation resistance 1, OXR1, in humans), significantly associated with DR-specific lifespan. We demonstrate that mtd/OXR1 in neurons is necessary for DR-mediated lifespan extension. Neuronal knockdown of mtd also accelerates sensory decline, arguing for a specific role of mtd/OXR1 in neuroprotection. We show that mtd is essential for stabilizing the retromer complex, which is necessary for trafficking transmembrane proteins and lipids for reuse. As a result of OXR1 deficiency, the retromer destabilizes and lysosomes become overused. Overexpression of retromer proteins or supplementation with chaperone compound R55 rescues the lifespan defects and neurodegeneration seen in mtd-deficient flies, and R55 is capable of rescuing lysosomal aggregation and OXR1-retromer co-localization in cells from humans with OXR1 deficiency. We further show through multi-omic analyses in flies and humans that mtd/OXR1 associates with accelerated transcriptomic aging and proteins involved in neurodegenerative diseases, including Alzheimer’s disease (AD). Overexpression of OXR1 and retromer proteins rescued AD-associated phenotypes in a fly model of AD. Thus, mtd/OXR1 enhances protein recycling in response to DR through the retromer, improving neuronal health and lifespan through mechanisms conserved across species.

EXTENDING A HEALTHY LIFESPAN WITH 3-HYDROXYANTHRANILIC ACID

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Metabolism of tryptophan by the kynurenine pathway is increasingly linked to aging. Kynurenine pathway enzymes
and metabolites influence a range of molecular processes critical to healthy aging, including regulation of inflammatory and immune responses, cellular redox homeostasis, and energy production. Aberrant kynurenine metabolism occurs during normal aging and is implicated in many age-associated pathologies including chronic inflammation, atherosclerosis, neurodegeneration, and cancer. We and others previously identified three kynurenine pathway genes—kynu-1, tdo-2, and acid-1—for which decreasing expression extends lifespan in invertebrates. More recently we discovered that knockdown of haa0-1, a fourth kynurenine pathway gene encoding the enzyme 3-hydroxyanthranilic acid dioxygenase (HAAO), extends lifespan by ~30% and delays age-associated health decline in Caenorhabditis elegans. Lifespan extension is mediated by increased physiological levels of the HAAO substrate 3-hydroxyanthranilic acid (3HAA). Aging mice fed a diet supplemented with 3HAA are similarly long-lived. The mechanism of action liking 3HAA to aging is complex and partially overlaps with multiple pathways previously implicated in aging. We recently identified activation of the Nrf2/SKN-1 oxidative stress response and alterations to iron homeostasis as key players in the benefits 3HAA. Ongoing work explores the relationship between 3HAA, Nrf2/SKN-1, and iron in C. elegans and mammalian aging, age-associated immune decline, and cancer. This works provides a foundation for detailed examination of the molecular mechanisms underlying the benefits of 3HAA, and how these mechanisms interact with other anti-aging interventions. We anticipate that these findings will bolster growing interest in developing pharmacological strategies to target tryptophan metabolism to improve health aging.

LEVERAGING THE NDUFS4-/- MOUSE AS A PLATFORM FOR TESTING LONGEVITY INTERVENTIONS

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Mitochondrial dysfunction is one of the hallmarks of biological aging, as well as the driving factor for mitochondrial diseases. Up to 30% of mitochondrial disorders are due to mutations affecting the activity of Complex 1 in the electron transport chain. Loss of the Complex 1 subunit Ndufs4 recapitulates symptoms of Leigh Syndrome, a pediatric mitochondrial disease, in mouse. Ndufs4-/- mice suffer developmental delays, early onset of neurological symptoms and extremely reduced lifespan. Several studies have now shown that Ndufs4-/- mice are exquisitely responsive to treatments and interventions of interest in the biology of aging, such as rapamycin, NAD+ precursors, reduced oxygen tension, alpha-keto-glutarate precursors, and the antidiabetic drug acarbose. These results point to common mechanisms underlying both aging and mitochondrial disorders. To put this hypothesis to the test, we show that Ndufs4-/- mice are responsive to a wide range of longevity interventions previously tested in worms, mice, and by the National Institute on Aging’s Intervention Testing Program. These observations support the hypothesis that mitochondrial and metabolic dysfunction induced by Complex I deficiency may be a key component of biological aging as well as mitochondrial disease. Furthermore, we propose that the Ndufs4-/- mice provide an affordable testing ground for candidate longevity interventions.

DELETION OF THROMBOSPONDIN-1 PRESERVES HEMATOPOIETIC STEM CELL HEALTHSPAN DURING AGING

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Aging is associated with defects within blood stem cells, termed hematopoietic stem cells (HSC), including a loss of their self-renewal potential and a skewed differentiation towards myeloid lineages at the expense of lymphoid cells. Collectively, these HSC defects manifest as anemias, poor response to vaccines and an increased incidence of myeloid neoplasms in older adults. Unlike other somatic stem cells, aged HSCs have been shown to be refractory towards established anti-aging interventions including caloric restriction, exercise, parabiosis and plasma transfer. Thrombospondin-1 (TSP1) was initially discovered as an anti-angiogenic molecule, and recent studies have identified that TSP1 promotes age-related pathologies including chronic inflammation, reactive oxygen species (ROS) generation, and mitochondrial dysfunction. Notably, each of these TSP-1 regulated processes have been shown to critically influence HSC biology, particularly in the context of aging. However, whether TSP-1 directly regulates HSC activity remains unexplored. Here, we sought to determine whether TSP-1 is essential for HSC development, and whether blocking TSP1 signaling could ameliorate age-related HSC defects. Utilizing murine models, we demonstrate that TSP-1 is dispensable for normal HSC development and hematopoiesis. We show that deletion of TSP-1 is sufficient to preserve HSC fitness during aging, as evidenced by preservation of youthful self-renewal potential and balanced lineage reconstitution during serial HSC transplantation assays. Mechanistically, we identify that TSP-1 adversely impacts mitochondrial metabolism within HSCs, and show that loss of TSP-1 prevents the age-related decline in HSC mitochondrial membrane potential. Our findings identify TSP-1 as a pro-geronic factor that can be targeted to preserve HSC healthspan.

A FAT-PROMOTING BOTANICAL EXTRACT ARTEMISIA SCOPARIA EXERTS GEROPROTECTION IN C. ELEGANS

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Like other biological processes, aging is not random but subject to molecular control. Natural products that modify core metabolic parameters, including fat content, may provide entry points to extend animal lifespan and promote healthy aging. Here, we show that a botanical extract from Artemisia scoparia (SCO), which promotes fat storage and metabolic resiliency in mice, extends the lifespan of the nematode Caenorhabditis elegans by up to 40%. Notably, this lifespan extension depends significantly on SCO’s effects on fat; SCO-treated worms exhibit heightened levels of unsaturated fat, and inhibiting Δ9 desaturases, which oversee biosynthesis of monounsaturated fatty acids, prevents SCO-dependent fat accumulation and lifespan extension. At an upstream signaling level, SCO prompts changes to C. elegans fat regulation by stimulating nuclear translocation of transcription factors.