mRNA levels, and miR-222 and SOD2 mRNA levels. Hydroxytyrosol-induced increase in SOD2 activity was associated with: (a) decrease in cellular superoxide levels, and (b) protection of the proliferative capacity of quiescent NHFs. These results suggest that a redox-sensitive biogenesis of microRNAs regulates the proliferative capacity of quiescent normal cells.
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Elevated Mitochondrial Superoxide Generation Causes Early Onset of Age-associated Disorders and Adult Stem Cell Defects

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Mitochondrial ROS are proposed to play a central role in aging and age-associated disorders although direct *in vivo* evidence is needed. We recently generated a mouse mutant with a mutation in the *Inner Mitochondrial Membrane Peptidase 2-like* (*Immp2l*) gene, which impaired the signal peptide sequence processing of mitochondrial proteins cytochrome c1 and glycerol phosphate dehydrogenase 2. The mitochondria from mutant mice generated elevated level of superoxide and caused erectile dysfunction in adult males and damaged spermatogenesis at older age (Biol. Reprod. 78:601–610, 2008). Here we show that elevated mitochondrial superoxide generation increased oxidative stress in multiple organs such as the testis, the brain and the kidney, although it caused adaptive increased expression of superoxide dismutases in multiple tissues and the expression did not decrease with the increase of age. The mutants showed multiple aging-associated phenotypes not observed in age-matched normal control mice, including wasting of bodyweight, loss of subcutaneous fat, kyphosis and ataxia, starting from the age of 16 months. The loss of bodyweight and fat does not seem to be caused by decreased food intake since the mutants consumed no less food before or after showing the abnormities. Cells from stromal vascular fraction (SVF) of white adipose tissues, where the adipose progenitor/stem cells can be isolated from, formed significantly less and smaller colonies in colony formation assays, although they retained the in vitro adipogenic differentiation capability. Senescence of in vitro cultured SVF cells was not increased in the mutants, suggesting that the impaired colony formation capability of SVF cell from mutant mice most likely reflected the result of reactive oxygen species (ROS) damage on adipose stem cells in vivo rather than effects arisen during the in vitro culturing. This mutant model could be a valuable tool to study the role of ROS in the aging of adult stem cells.

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Effects of Aging on Sperm Function and Oxidative Stress

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It is known that the OS (OS) results from a decrease in the antioxidative potential upon aging. Mature sperm present at low temperatures and the low oxygen levels in testicle and epididymis will be exposed to OS during the fertilization process. Flagellar swimming depends on the ATP supply from mitochondria in the sperm, and sperm's susceptibility to OS is higher than that of any other cells. We examined male Fischer344 rat (age 15 wk -70 wk) to clarify the effects of aging on sperm function and OS. Sperm number increased until 25 wk, but decreased at 50 wk and 70 wk. Sperm movement rate tended to decrease with aging, and at 70 wk, it had decreased to approximately 60% of the 25 wk. Total number and the DNA synthetic potential of the sperm increased until 25 wk, but decreased at 50 wk and 70 wk. The HNE modified protein of the epididymidis increased with aging until 50 wk. These results suggest that age-associated increase in OS influence the sperm function.

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Nrf2 Deficiency Impairs ARE-Dependent Cardiac Antioxidant Mechanisms in Aged Mice

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Antioxidants and cytoprotective mechanisms are crucial for maintaining the intracellular redox state and mitigating free radical accumulation with aging. Presently, the transcriptional mechanisms that regulate the antioxidant defense system in the myocardium are poorly understood. Nuclear Erythroid 2 p45 related factor-2 (Nrf2) regulates basal/inducible expression of numerous cytoprotective/antioxidant genes. We hypothesize that the disruption of Nrf2 modulates the cardiac antioxidant defense mechanisms that are associated with aging.
Methods: Age-matched wild-type (WT) and Nrf2$^{-/-}$ (KO) mice at 2 months (young) and >18 months (old) were subjected to acute exercise stress (AES), and we then assessed the activation of Nrf2/ARE-dependent antioxidants in the heart. Protein/mRNA expression of antioxidant enzymes were determined by Western blot/qPCR analysis, respectively. Total ROS was measured by electron paramagnetic resonance (EPR) spectroscopy.
Results: At 2 months (young) age, total ROS levels were similar among the WT and KO mice under basal conditions, but upon AES, the KO mouse had 50% lesser glutathione with significantly increased ROS when compared with WT indicating the Nrf2-deficiency is coupled with impaired redox potential. Interestingly, the WT mice at 2 months age were able to tolerate the AES induced ROS by activating compensatory cytoprotective pathways including upregulation of antioxidants. However, the aged mice (WT & KO) developed elevated oxidative stress in response to AES. We observed an oxidative stress response that was several fold higher in the aged Nrf2-KO mice when compared with WT, suggesting an important age dependent function for Nrf2 in the myocardium. Protein and mRNA analysis revealed significant down regulation of major antioxidants in KO mice, while WT mice exhibited potential compensatory antioxidant response to the AES-induced ROS in young, when compared to the aged mice.
Conclusions: Disruption of Nrf2 increases susceptibility of myocardial tissue to AES-induced oxidative stress in aged Nrf2-KO versus young mice, suggesting a potential role for Nrf2 in aging. The Nrf2/ARE signaling might be a therapeutic target to protect the heart tissue from age dependent cardiac diseases.

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Discovering Signatures of Healthy Brain Aging with Aptamer Proteomic Technology

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SomaLogic's highly multiplexed proteomic platform currently