

The role of manganese superoxide dismutase in skin aging

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The free radical theory of aging postulates that the production of mitochondrial reactive oxygen species is the major determinant of aging and lifespan. The skin represents an excellent and accessible model organ to study aging that is characterized by atrophy, wrinkle formation, reduced tensile strength and impaired wound healing. Oxidative stress as a consequence of an imbalance in prooxidants and antioxidants with increased ROS concentrations has been demonstrated in the aged skin *in vitro* and *in vivo*, suggesting the important role of the antioxidant balance. Here we will summarize recent data on the role of the mitochondrial superoxide dismutase 2 in skin aging.

Introduction

The free radical theory of aging, published in 1956 by Denham Harman,¹ postulates that reactive oxygen species, normally produced in organisms with cellular constituents, initiate changes associated with aging. During the last couple of years, the importance of the antioxidant system in aerobic cells, which was evolved to protect against oxidative injury, has been acknowledged. The imbalance of antioxidant enzymes, the role of reactive oxygen species as important physiological regulators of intracellular signaling pathways as well as the increased oxidative stress in senescence and aging have been a matter of increasing interest.^{1–6}

Among the multilayered and interdependent antioxidant system, which consists of non-enzymatic as well as enzymatic components, the mitochondrial superoxide dismutase 2 (SOD2, MnSOD) is a subject of particular interest, as it is located in the mitochondrial matrix where it represents the first line of antioxidant defense against superoxide anions produced as byproducts of oxidative phosphorylation. Superoxide dismutase 2 is a nuclear encoded tetrameric enzyme, which converts superoxide anion to hydrogen peroxide, which can further be removed by catalase and glutathione peroxidase.⁷ Superoxide anions are short lived, known to induce macromolecular damage—such as damage to DNA, proteins and lipids—and to react with other reactive oxygen/nitrogen species like nitric oxide to form highly

reactive peroxynitrite. Hydrogen peroxide, the product following dismutation of superoxide anion via SOD2, is more stable, can pass through membranes and has been shown to be an essential signaling molecule in a variety of signaling cascades and cell-matrix interactions.^{4,8,9} Mitochondrial-targeted overexpression of the hydrogen peroxide detoxifying enzyme catalase resulted in an extension of murine life span, showing for the first time the impact of ROS on mammalian longevity.¹⁰

In lower organisms, SOD2 was identified as part of a phylogenetically-conserved signaling pathway involved in longevity and resistance to oxidative stress.¹¹ In humans, defined polymorphisms in the SOD2 gene correlate with longevity and heart failure in elderly,^{12,13} indicating a role of the SOD2 in human aging.

Among the histogenetically and functionally different tissues and compartments in the skin—which include the epidermis, the dermal connective tissue, adnexal structures like eccrine and apocrine glands, vessels and the subcutaneous tissue—the dermal connective tissue with its principal cellular component, the fibroblasts, plays a central role to study aging, as the incidence of aging-related disorders is high in connective tissue-rich organs.

Adaptive Upregulation of SOD2 in Senescence and Aging

Skin aging is characterized by atrophy, wrinkle formation, reduced tensile strength and impaired wound healing, with loss of the structural integrity and loss of the elastic and collagen fiber network due to dysfunctional fibroblasts.^{14,15} Dermal fibroblasts have therefore been used to model senescence *in vitro*,^{16–18} not only for the dermis, but also for other connective tissue rich organs. Skin aging, among other changes, is characterized by a loss of collagen type I, collagen type III among other matrix constituents, dysregulated fibroblast-matrix interactions and impaired fibroblast interactions with organ parenchyma, mainly with organ-specific epithelial cells and muscle.^{19–25} In human senescent skin, fibroblasts which develop a growth arrest, morphological and functional changes, increased ROS concentrations have been demonstrated *in vitro* and *in vivo*^{26–29} with an adaptive upregulation of the SOD2 on mRNA and protein level^{26,30,31} (Ferchiu et al., unpublished data) providing evidence for a common response phenotype of cellular senescence.

The upregulation of the SOD2 in human fibroblasts has also been shown to be induced in a paracrine mechanism either via UV-irradiation^{32,33} and/or the release of soluble factors

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(e.g., interleukin 1 α , interleukin 1 β , and tumor necrosis factor α) from keratinocytes.³⁴ In this case, when SOD2 upregulation disturbs the balance of hydrogen peroxide (H₂O₂) level, the upregulation of H₂O₂ detoxifying enzymes ensures that an accumulation of H₂O₂ does not occur in the system. However, exclusive SOD2 overexpression *in vitro* has earlier been shown to result in enhanced H₂O₂ concentration with activation of distinct signaling pathways and transcription factors, among them the heterodimeric AP-1 which enhance the transcription and activation of matrix-metalloproteinases among other genes and gene products.²³ The family of matrix-metalloproteinases (MMP) so far consists of at least 20 members with distinct, partly-overlapping substrate specificities for extracellular matrix proteins of the skin.^{35,36} Imbalanced overexpression of SOD2 resulted in enhanced H₂O₂ accumulation with the AP-1 dependent induction of interstitial collagenase (MMP-1) and degradation of interstitial collagen in the skin—a hallmark of skin aging.^{23,37,38}

Although overexpression of SOD2 does not—or if so, only marginally does—increase life span in mice,^{39,40} reduced oxidative stress observed under caloric restriction seems to be due to SIRT3 mediated SOD2 activation.⁴¹ Until now, caloric restriction represents the most robust intervention to extend life span and delay the onset of age related diseases in mice.

Deficiency of SOD2 Induces Premature Aging

Interestingly, there is indirect evidence that in aged skin, fibroblasts—both superoxide anion and H₂O₂—are increased (Ferchiu et al., unpublished data).²⁹ To model the situation with an increase of superoxide anion, mice with deficiency of SOD2 were generated. Mice with a homozygous deficiency of SOD2 in all organs die within postnatal day 8 to 18 due to cardiomyopathy and neurodegeneration.^{42,43} Treatment with synthetic superoxide dismutase (SOD)-catalase mimetic (EUK) extends lifespan of mice with inactivated SOD2 by three-fold and attenuates mitochondrial defects.⁴⁴ Corresponding to naturally-aged mouse skin, where the number of senescent cells increases⁴⁵ and the mitochondrial activity decreases, constitutive lack of SOD2 correlates with an increase in senescence-associated β -galactosidase expression, impaired mitochondrial complex II activity and increased nuclear DNA damage in the skin. Cellular senescence in SOD2 nullizygous mice with decreased proliferation and increased terminal differentiation of keratinocytes may result in the observed thinning of the epidermis.²⁵

Mice with a heterozygous deficiency of the SOD2 revealed an unchanged lifespan⁴⁶ in spite of oxidative damage like lipid peroxidation, spontaneous apoptosis,⁴⁷ endothelial dysfunction with impaired vasorelaxation and mtDNA damage.⁴⁸ In the context of telomere dysfunction, heterozygous deficiency of SOD2 does not further accelerate aging.⁴⁹

To study aging as consequence of mitochondrial oxidative stress, we therefore set out to generate mice with a conditional homozygous deficiency of SOD2 in fibroblasts resident in the connective tissue. Interestingly, due to a deficiency of SOD2 in fibroblasts in the connective tissue of virtually all organs, these mice gradually developed a progeroid aging phenotype in

connective-tissue rich organs with severe skin atrophy, kyphosis, osteoporosis, muscle degeneration and finally a reduced life span (Fig. 1). In fact, a strong atrophy of the dermal connective tissue, the subcutaneous fat tissue and the muscle fibers of the panniculus carnosus were observed in skin sections of connective tissue specific SOD2 deficient mice.⁵⁰ The architecture of the collagen fibers was severely disturbed compared with control skin with thin and loosely packed collagen bundles. Procollagen I was reduced in SOD2 deficient skin compared with control skin. Mitochondria from SOD2-deficient fibroblasts revealed severe morphological damage. SOD2 deficient fibroblasts showed a severe functional impairment with delay in organization of the surrounding collagen fibers, when seeded into three dimensional collagen matrices. No increase in the rate of apoptosis in skin sections of SOD2-deficient mutant mice, compared with the corresponding control skin sections of heterozygous and wild-type mice, was found, but a significant lower number of proliferating cells is found in mutant mouse skin, indicating that the atrophy is most probably due to a decrease in proliferation in the dermis as well as in the epidermis. We found the senescence marker p16INK4a significantly increased in mutant SOD2-deficient skin, and this is particularly relevant for human skin aging as we and others have previously shown that reactive oxygen species accumulate fibroblasts in skin *in vitro* and *in vivo* and, importantly, p16INK4a increases in human skin with age and represents a robust *in vivo* marker for aging.⁵⁰⁻⁵²

Conclusion and Perspectives

Superoxide anion concentrations increase in skin fibroblasts during senescence and skin aging (Ferchiu et al., unpublished data). We here provide evidence that the lack of connective tissue specific superoxide anion detoxification at least in part mimics the situation and leads to the installment of an oxidative damage induced senescence program with the upregulation of p16INK4a not only in the skin but in all connective tissue rich organs or the stroma surrounding the parenchyma, enabling further insights into the general mechanisms of cellular, tissue as well as organismal aging. This is particularly interesting in the light of a recent publication showing that the removal of p16INK4a expressing cells rescues skin as well as organismal aging.⁵³ Further understanding of underlying mechanisms of ROS mediated premature aging will provide therapeutic approaches in pathological states to counteract accelerated skin aging in particular and organismal aging in general. It will be of clinical relevance to develop strategies to rebalance increased superoxide anions and most likely hydrogen peroxide concentrations in the connective tissue of the dermis and most likely of all organs to avoid the activation of tissue degradation and atrophy. The equipment of histogenetically distinct cells and tissues with antioxidant enzymes is not very well studied. Therefore it is dangerous to use systemic antioxidant approaches. Also it became clear that a mild oxidative stress by sport may delay aging which is abolished by the systemic application of N-acetylcysteine.⁵⁴ Therefore, it will be particularly interesting to target antioxidant rebalancing molecules to resident fibroblasts in the connective tissue.

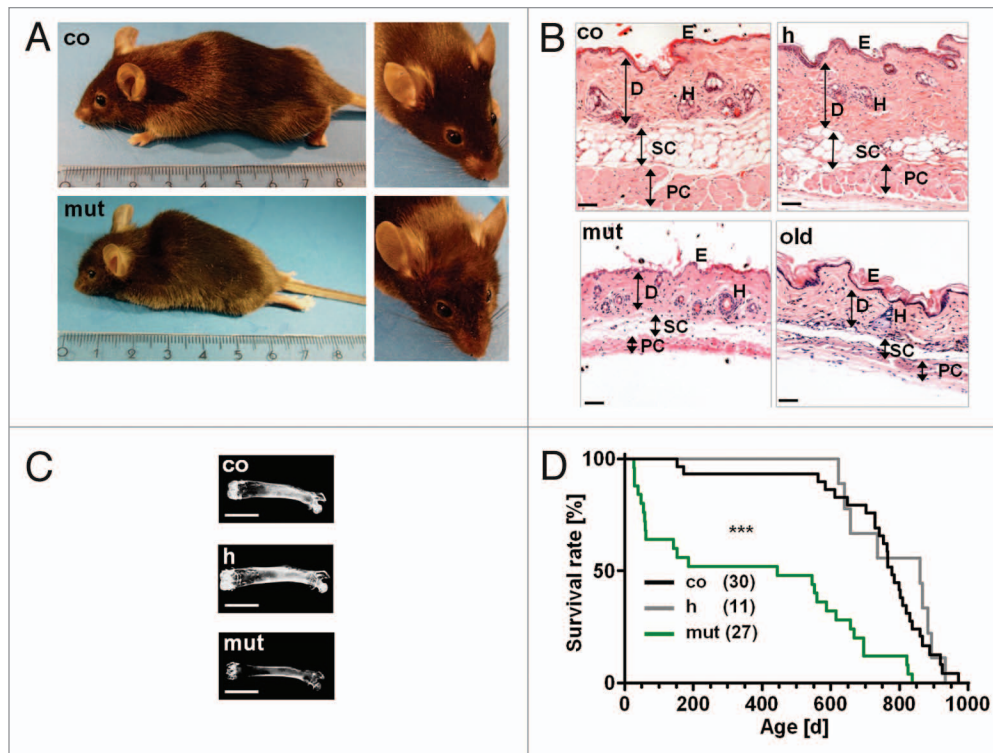


Figure 1. Accelerated aging phenotype in mice with conditional deficiency for mitochondrial superoxide dismutase in the connective tissue. **(A)** The mutant mice (mut) revealed kyphosis and a prominent progeroid forehead compared with littermates of SOD2 competent (co) mice at the age of 47 d. The intrinsically aged control mice (co) of 900 d also exhibited incidence of kyphosis similar to mutant mice. **(B)** Representative hematoxylin-eosin stained sections of the skin from control (co), heterozygous (h), mutant (mut) and intrinsically aged control (old) mice. Mutant mice at an age of 6 weeks revealed a severe atrophy of the dermis, the subcutaneous fat tissue and the muscle fibers of the panniculus carnosus compared with SOD2 competent mice (co). Only minor changes in SOD2 heterozygous mice (h) were evident compared with SOD2 competent mice (co). Intrinsically aged control mice (old) of 900 d also show skin atrophy similar to mutant mice. E, epidermis; D, dermis; H, hair follicle; SC, subcutaneous fat tissue; PC, panniculus carnosus. Scale bars, 20 μ m. **(C)** X-ray analysis from dissected femur bone of SOD2 competent mice (co), SOD2 heterozygous mice (h) and mutant mice (mut) revealed an osteoporosis-like phenotype in mutant mice. Scale bars, 0.5 cm. **(D)** Significantly reduced survival time of mutant mice (median 444 d) compared with SOD2 competent control mice (median 784 d) (** $p < 0.0001$, log-rank test stratified for gender). No statistically significant difference was found between SOD2 competent (co) and SOD2 heterozygous (h) mice ($p = 0.65$). The maximal life span for mutant mice was 837 d compared with 971 d in SOD2 competent mice and 932 d in SOD2 heterozygous mice. Figure modified from Treiber et al., 2011.⁵⁰

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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