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Research Paper

A new role for oxidative stress in aging: The accelerated aging phenotype in *Sod1*$^{-/-}$ mice is correlated to increased cellular senescence

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ABSTRACT

In contrast to other mouse models that are deficient in antioxidant enzymes, mice null for Cu/Zn-superoxide dismutase (*Sod1*$^{-/-}$ mice) show a major decrease in lifespan and several accelerated aging phenotypes. The goal of this study was to determine if cell senescence might be a contributing factor in the accelerated aging phenotype observed in the *Sod1*$^{-/-}$ mice. We focused on kidney because it is a tissue that has been shown to a significant increase in senescent cells with age. The *Sod1*$^{-/-}$ mice are characterized by high levels of DNA oxidation in the kidney, which is attenuated by DR. The kidney of the *Sod1*$^{-/-}$ mice also have higher levels of double strand DNA breaks than wild type (WT) mice. Expression (mRNA and protein) of p16 and p21, two of the markers of cellular senescence, which increased with age, are increased significantly in the kidney of *Sod1*$^{-/-}$ mice as is β-gal staining cells. In addition, the senescence associated secretory phenotype was also increased significantly in the kidney of *Sod1*$^{-/-}$ mice compared to WT mice as measured by the expression of transcripts for IL-6 and IL-1β. Dietary restriction of the *Sod1*$^{-/-}$ mice attenuated the increase in DNA damage, cellular senescence, and expression of IL-6 and IL-1β. Interestingly, the *Sod1*$^{-/-}$ mice showed higher levels of circulating cytokines than WT mice, suggesting that the accelerated aging phenotype shown by the *Sod1*$^{-/-}$ mice could result from increased inflammation arising from an accelerated accumulation of senescent cells. Based on our data with *Sod1*$^{-/-}$ mice, we propose that various bouts of increased oxidative stress over the lifespan of an animal leads to the accumulation of senescent cells. The accumulation of senescent cells in turn leads to increased inflammation, which plays a major role in the loss of function and increased pathology that are hallmark features of aging.

1. Introduction

The Free Radical or Oxidative Stress Theory of Aging postulates that reactive oxygen species (ROS) formed exogenously or endogenously from normal metabolic processes play a role in the aging process. The imbalance of pro-oxidants and antioxidants leads to an age-related accumulation of oxidative damage in macromolecules, resulting in a progressive loss in function and aging [1]. Over the past three decades, the Oxidative Stress Theory of Aging has become one of the most popular theories to explain the biological/molecular mechanism underlying aging because several lines of evidence support the theory. First, the levels of oxidative damage to lipid, DNA, and protein have been reported to increase with age in a wide variety of tissues and animal models [2]. Second, studies with animal models showing increased longevity are consistent with the Oxidative Stress Theory of Aging. Longer-lived animals show reduced oxidative damage and/or increased resistance to oxidative stress, e.g., dietary restriction in rodents and genetic manipulations that increase lifespan in invertebrates (*C. elegans* and *Drosophila*) and in mice [3]. Thus, the observations that experimental manipulations that increase lifespan in invertebrates and rodents were correlated to increased resistance to oxidative stress or reduced oxidative damage provided strong support for the Oxidative

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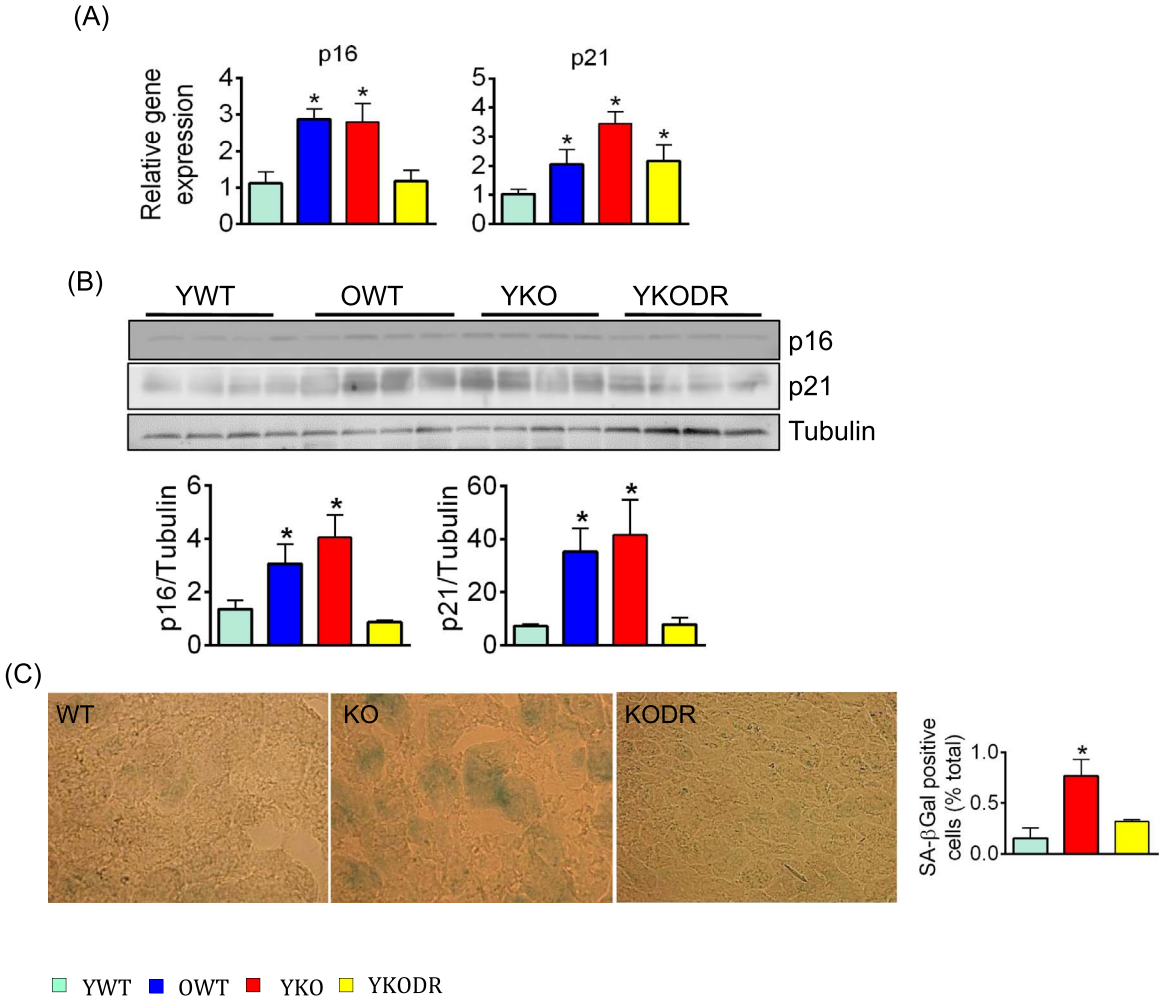

Fig. 1. Cellular senescence is increased in kidney of *Sod1*$^{-/-}$ mice. (A) Transcript levels of p16INK4a and p21 in kidney measured by qRT-PCR and normalized to GAPDH. (B) The level of p16INK4a and p21 protein in kidney as measured by Western blot (top panel). Quantification of p16INK4a and p21 normalized to β-tubulin is shown in the bottom panel. (C) Images of SA β-Gal positive staining cells in kidney is shown in the left panel (arrow points to β-Gal positive cells). Percentage of SA β-Gal positive cells is quantified and graphically represented in the right panel. Four groups of mice were studied: young (4–6 month-old) WT (YWT, turquoise bar); old (24 month-old) mice (OWT, blue bar); young (4–6 month-old) *Sod1*$^{-/-}$ mice (YKO, red bar); young (6-month-old) *Sod1*$^{-/-}$ mice on DR (YKODR, yellow bar). The data are the mean ± SEM of 4 mice per group and were statistically analyzed by one-way ANOVA followed by student T-test. The asterisk (*) indicates a significance ($P < 0.05$) difference between either young WT mice or young *Sod1*$^{-/-}$ mice on DR and old WT mice or young *Sod1*$^{-/-}$ mice on DR. There were no significant differences between the old WT and young *Sod1*$^{-/-}$ mice or the young WT and young DR young *Sod1*$^{-/-}$ mice. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Stress Theory of Aging. However, all of the experimental manipulations that increase lifespan also alter processes other than oxidative stress/damage; therefore, the increase in longevity in these animal models could arise through another mechanism.

Over the past two decades, our group has directly tested the role of oxidative damage/stress in aging by genetically manipulating the antioxidant status of a wide variety of antioxidant genes to increase or reduce the level of oxidative stress/damage and determine what affect these manipulations had on lifespan. Our research with 18 different genetic manipulations in the antioxidant defense system shows that only the mouse model null for Cu/Zn-superoxide dismutase (*Sod1*) had an effect on lifespan (in this case a decrease in lifespan) as predicted by the Oxidative Stress Theory of Aging [4]. Because Elchuri et al. reported that more than 70% of *Sod1*$^{-/-}$ mice developed liver hyperplasia and hepatocellular carcinoma later in life, it was initially believed that the 30% decrease in the lifespan of *Sod1*$^{-/-}$ mice was not due to accelerated aging but was the result of a dramatic increase in hepatocellular carcinoma, which is rare in C57BL/6 mice [5]. In a more recent study, we found a similar 30% decrease in lifespan of the *Sod1*$^{-/-}$ mice; however, in our study, only about 30% of *Sod1*$^{-/-}$ mice developed hepatocellular carcinoma later in life [6]. In addition, we showed that dietary restriction (DR), which is a manipulation that retards aging in rodents, increased the lifespan of the *Sod1*$^{-/-}$ mice to that of normal, wild type (WT) mice. These data combined with studies showing that *Sod1*$^{-/-}$ mice exhibited various accelerated aging phenotypes [e.g., muscle atrophy and loss of fat mass, hearing loss [7], cataracts [8], skin thinning and delayed wound healing [9] lead us to conclude that the *Sod1*$^{-/-}$ mice exhibit accelerated aging. This then raised the question of why we observed a significant decrease in lifespan and accelerated aging in only the *Sod1*$^{-/-}$ mice and not in other mouse models with compromised antioxidant defense systems that showed changes in oxidative stress/damage.

Sod1$^{-/-}$ mice show a much higher level DNA oxidation (i.e., 8-oxo-dG levels) in tissues than any of the mouse models we have studied, which all have deficiencies in one or more of the antioxidant genes [4]. In addition, DNA mutations have been reported to increase significantly in several tissues in *Sod1*$^{-/-}$ mice [10]. Because the DNA damage response has been shown to play a central role in the generation of senescent cells [11] and because Van Deursen's laboratory has shown that clearance of senescent cells delays aging-associated disorders and increases lifespan in a progeroid mouse model [12] as well as normal, WT mice [13], we hypothesized that the increased oxidative damage to DNA in tissues of *Sod1*$^{-/-}$ mice could activate the DNA damage response and drive cells into becoming senescent. To test