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Review

The paradoxical role of thioredoxin on oxidative stress and aging

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ABSTRACT

In spite of intensive study, there is still controversy about the free radical or oxidative stress theory of aging, particularly in mammals. Our laboratory has conducted the first detailed studies on the role of thioredoxin (Trx) in the cytosol (Trx1) and in mitochondria (Trx2) on oxidative stress and aging using unique mouse models either overexpressing or down-regulating Trx1 or Trx2. The results generated from our lab and others indicate that: (1) oxidative stress and subsequent changes in signaling pathways could have different pathophysiological impacts at different stages of life; (2) changes in redox-sensitive signaling controlled by levels of oxidative stress and redox state could play more important roles in pathophysiology than accumulation of oxidative damage; (3) changes in oxidative stress and redox state in different cellular compartments (cytosol, mitochondria, or nucleus) could play different roles in pathophysiology during aging, and their combined effects show more impact on aging than changes in either oxidative stress or redox state alone; and (4) the roles of oxidative stress and redox state could have different pathophysiological consequences in different organs/tissues/cells or pathophysiological conditions.

To critically test the role of oxidative stress on aging and investigate changes in redox-sensitive signaling pathways, further study is required.

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Oxidative stress and aging

The free radical or oxidative stress theory of aging is one of the most popular theories in aging research and has been extensively studied over the past several decades. One consistent line of evidence supporting this theory is the large amount of data that has shown an age-related increase in oxidative damage in various cellular molecules (including lipids, proteins, and DNA) in organisms ranging from invertebrates to humans [1–10]. Another strong line of evidence comes from studies using manipulations that increase lifespan, including treatments such as calorie restriction (CR[2]) and genetic mutations. These models (including invertebrates and mice) have tended to show a correlation between extended longevity and increased resistance to oxidative stress and/or reduced oxidative damage [2,11–30]. However, because these studies are correlative, there is still a possibility that the increased longevity in these animal models could arise through other mechanisms. In fact, a study using naked mole-rats (NMR), which have a lifespan approaching 30 years, showed that these animals have higher oxidative damage than mice [31], which calls into question the significance of oxidative damage in aging.

The use of genetic mutations of antioxidant enzymes in animals [including transgenic (Tg) and knockout (KO) animals] provides investigators with a unique system to directly test the oxidative stress hypothesis of aging by altering the cellular defense against accumulation of oxidative damage and determining its effect on aging/lifespan [32–46]. Data from these studies could establish a causative role for oxidative stress/damage in aging. However,

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[2] *Abbreviations used:* AP-1, activator protein 1; ASK1, apoptosis signal-regulating kinase-1; CR, calorie restriction; Gpx, glutathione peroxidase; GSH, glutathione; KO, knockout; mCAT, catalase in mitochondria; MetO, methionine sulfoxide; NFκB, nuclear factor κB; NMR, naked mole-rat; Prx, peroxiredoxin; ROS, reactive oxygen species; SOD, superoxide dismutase; Tg, transgenic; Trx, thioredoxin.

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studies using invertebrates and mice have shown inconsistent results, raising the possibility that differences in species might affect the outcome of a genetic manipulation on lifespan.

Survival studies using various transgenic mice have shown no change in lifespan. Transgenic mice that overexpress human Cu/Zn superoxide dismutase (SOD) in various tissues [47] do not show an extension of lifespan compared to wild-type mice [48], although these transgenic mice are more resistant to cerebral ischemia [49,50]. Richardson, Van Remmen, and their colleagues have conducted survival studies using mice that overexpress Cu/ZnSOD, MnSOD, catalase (in peroxisomes), both Cu/ZnSOD and MnSOD, and both Cu/ZnSOD and catalase, all of which also showed no increase in lifespan compared to wild-type littermates [51]. Interestingly, transgenic mice that overexpress catalase in mitochondria (mCAT mice) show increased lifespan and reduced oxidative damage [52], which may indicate that altering the antioxidant defense system in mitochondria may be more important than in the cytosol. However, it is still unknown why only catalase overexpression in mitochondria showed beneficial effects on aging.

Studies with mice underexpressing antioxidant enzymes also showed little effect on aging. Richardson, Van Remmen, and their colleagues conducted a survival study with mice that had reduced expression of a major antioxidant enzyme, MnSOD. Although the $Sod2^{+/-}$ mice showed higher levels of DNA oxidation and a higher incidence of cancer, no difference in lifespan was observed between the $Sod2^{+/-}$ and wild-type mice [53]. Later, survival studies were performed with mice deficient in Glutathione peroxidase 1 (Gpx1) and MnSOD (including genotypes $Gpx1^{-/-}$, $Sod2^{+/-}$, $Gpx1^{+/-} \times Sod2^{+/-}$, and $Gpx1^{-/-} \times Sod2^{+/-}$) to further test the role of oxidative damage in aging [54]. In each case, survival was not affected by reduced antioxidant enzyme levels. These results are also inconsistent with the oxidative stress theory of aging. The only exception was a study with Cu/ZnSOD-null mice, which lack Cu/ZnSOD. These mice had a shorter lifespan compared to wild-type control mice [55]. Interestingly, the short lifespan of these knockout mice (with a C57BL/6 background) was associated with a high incidence (approximately 90%) of hepatocellular carcinoma, which is not commonly observed in C57BL/6 mice. The results from these studies indicate the importance of antioxidant enzymes in pathophysiology.

However, based on data obtained from transgenic and knockout mice, changing oxidative damage levels by altering major antioxidant enzymes does not seem to have a significant impact on lifespan. Therefore, these data call into question the role of oxidative damage/stress in the aging process, i.e., accumulation of oxidative damage alone may not be a major contributing factor in longevity. Thus, significant modifications to the oxidative stress theory of aging are required if we are to understand the relationship between oxidative stress and aging.

Transgenic mice overexpressing thioredoxin in the cytosol (Trx1)

Thioredoxin (Trx) was first recognized in the early 1960s as the major reductant for a variety of enzymes. Two forms have been identified in humans, one cytosolic (Trx1) [56] and one mitochondrial (Trx2) [57]. A major role of Trx is to donate a hydrogen atom to enzymes involved in reductive reactions [e.g., ribonucleotide reductase, which reduces ribonucleotides to deoxyribonucleotides for DNA synthesis; peroxiredoxin (Prx), which reduces peroxides [58-60]; and methionine sulfoxide (MetO) reductase, which reduces MetO in proteins and provides protection against oxidative stress [61–63]]. By maintaining a reduced environment in cells through thiol-disulfide exchange reactions, Trx protects cells and tissues from oxidative stress [64]. In 1999, transgenic mice overexpressing Trx1 were generated by Yodoi and colleagues using a transgene containing human *TRX1* cDNA fused to the β-actin promoter [Tg(act-*TRX1*)$^{+/0}$] [65]. Their study demonstrated that Tg(act-*TRX1*)$^{+/0}$ mice had an extended lifespan compared to wild-type littermates [66,67], making Tg(act-*TRX1*)$^{+/0}$ mice one of the few mouse models to support the oxidative stress theory of aging. In spite of this exciting observation, the study was conducted under conventional housing conditions, and the lifespan of wild-type C57BL/6 mice in their colony was shorter than C57BL/6 mice raised under barrier conditions. The median lifespan of control mice in the study by Yodoi was approximately 23 months-of-age, which is much shorter than the median lifespan of C57BL/6 mice in aging colonies under optimal conditions (29–30 months-of-age). Because of this, our laboratory conducted an aging study with the same line of Tg(act-*TRX1*)$^{+/0}$ mice to examine the effects of increased levels of Trx1 on oxidative stress and aging under optimal housing conditions. Our study demonstrated that young and adult mice overexpressing Trx1 had increased resistance to oxidative stress and reduced oxidative damage to proteins and lipids [68]. However, our survival study showed a significant increase in the survival of male Tg(act-*TRX1*)$^{+/0}$ mice compared to wild-type mice only during the first half of their lifespan. Male Tg(act-*TRX1*)$^{+/0}$ mice had a 25% increase in lifespan in the early part of life (75% survival), a 13% increase in lifespan in the median part of life (50% survival), only a 5.5% increase in lifespan in the later part of life (25% survival), and no increase thereafter (10% survival). This result was further confirmed by another survival study using both male and female mice [68]. These survival results are partially consistent with the previous studies conducted by Yodoi and colleagues [66,67] because they showed that overexpression of Trx1 increased lifespan in the early part of life in males, but we did not see an extension in maximum lifespan. These results led us to question why Trx1 overexpression extends lifespan only in the early part of life. Our data demonstrated that the levels of overexpression significantly decreased with age, which was correlated with less reduction in protein oxidation levels [68], possibly due to the β-actin promoter driving expression of the transgene, which could cause an age-related decrease in expression of the transgene.

To investigate whether continuous overexpression of Trx1 extends maximum lifespan, our laboratory conducted a survival study using another line of Trx1 transgenic mice (Trx1Tg). We generated these mice using a fragment of the human genome containing the *TRX1* gene [a BAC clone (RP11-427L11) from the Children's Hospital Oakland Research Institute's (CHORI) BACPAC Resources Center (BPRC), Oakland, CA] with 8.3 kb and 12.3 kb of the 5′- and 3′-flanking sequences, respectively. The levels of Trx1 were significantly higher (approximately 20%–40%) in all tissues examined in this line of Trx1Tg mice compared to their wild-type littermates, and overexpression of Trx1 was maintained (up to at least 28–30 months-of-age), i.e., the levels of Trx1 overexpression in Trx1Tg mice did not show any decrease or increase during aging. We also found no compensatory changes in the levels of Trx2, glutaredoxin, glutathione, or other major antioxidant enzymes. The survival study with Trx1Tg and wild-type mice demonstrated that the early part of lifespan (75% survival) in Trx1Tg and wild-type mice was 707 and 665 days, respectively. The difference (6.3%) is not significant ($p > 0.05$) as determined by the Generalized Wilcoxon test. The mortality rate after 700 days appeared to be higher in Trx1Tg than in wild-type mice, and no life-extension was observed afterward [69]. Therefore, our data showed that continuous overexpression of Trx1 in mice had similar effects as observed in the Tg(act-*TRX1*)$^{+/0}$ mice, i.e., Trx1 overexpression showed some benefits on lifespan only in the early part of life.

To seek further explanation for the benefits of Trx1 overexpression early in life, we conducted pathological analyses of Tg(act-*TRX1*)$^{+/0}$ and wild-type mice. Our pathology data from Tg(act-*TRX1*)$^{+/0}$ mice showed a significantly reduced incidence of