Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging

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Van Remmen, Holly, Yuji Ikeno, Michelle Hamilton, Mohammad Pahlavani, Norman Wolf, Suzanne R. Thorpe, Nathan L. Alderson, John W. Baynes, Charles J. Epstein, Ting-Ting Huang, James Nelson, Randy Strong, and Arlan Richardson. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol Genomics 16: 29–37, 2003; 10.1152/physiolgenomics.00122.2003.—Mice heterozygous for the Sod2 gene (Sod2+/− mice) have been used to study the phenotype of life-long reduced Mn-superoxide dismutase (Mn-SOD) activity. The Sod2+/− mice have reduced MnSOD activity (~50%) in all tissues throughout life. The Sod2+/− mice have increased oxidative damage as demonstrated by significantly elevated levels of 8-oxo-2-deoxyguanosine (8oxodG) in nuclear DNA in all tissues of Sod2+/− mice studied. The levels of 8oxodG in nuclear DNA increased with age in all tissues of Sod2+/− and wild-type (WT) mice, and at 26 mo of age, the levels of 8oxodG in nuclear DNA were significantly higher (from 15% in heart to over 60% in liver) in the Sod2+/− mice compared with WT mice. The level of 8oxodG was also higher in mitochondrial DNA isolated from liver and brain in Sod2+/− mice compared with WT mice. The increased oxidative damage to DNA in the Sod2+/− mice is associated with a 100% increase in tumor incidence (the number of mice with tumors) in old Sod2+/− mice compared with the old WT mice. However, the life spans (mean and maximum survival) of the Sod2+/− and WT mice were identical. In addition, biomarkers of aging, such as cataract formation, immune response, and formation of glycoxidation products carboxymethyl lysine and pentosidine in skin collagen changed similarly in both WT and Sod2+/− mice. Thus life-long reduction of MnSOD activity leads to increased levels of oxidative damage to DNA and increased cancer incidence but does not appear to affect aging.

oxidative damage; mitochondria

A COMPLEX ANTIOXIDANT DEFENSE system, including antioxidants, antioxidant enzymes, and a variety of pathways to repair oxidative damage, has evolved to protect cells from oxidative stress. The major antioxidant enzymes found in eukaryotes are the superoxide dismutases (SODs), the glutathione peroxidases (GPxs), and catalase. The SODs play a critical role in protecting cells from oxidative stress by catalyzing the dismutation of superoxide anions to hydrogen peroxide. There are three mammalian SODs: cytosolic CuZnSOD, which is the predominant SOD in most cells/tissues (70–80% of cellular SOD activity); extracellular SOD (EC-SOD), which is a minor form of the enzyme expressed in significant amounts in only a limited number of tissues (lung, kidney, and fat tissue); and MnSOD, which is located in the mitochondrial matrix of all cells and contributes 10–20% of the total SOD activity in the cell. The cellular location of MnSOD means that it is the major antioxidant defense system involved in protecting mitochondria from superoxide anions that are produced as a byproduct of the respiratory chain.

Two independent laboratories have generated Sod2 knockout mouse models by deletion of different segments of the Sod2 gene. The Sod2+/− phenotype is lethal in both knockout models, and mice lacking MnSOD die within 1–18 days from dilated cardiomyopathy or neurodegeneration, depending on the genetic background (18, 19). The Sod2−/− mutants produced by Epstein and colleagues (19) have a distinct phenotype characterized by small size and pale appearance compared with either wild-type (WT) or Sod2+/- mice. The Sod2−/− mice are also hypotonic, hypothermic, and suffer from accumulation of lipid in liver and skeletal muscle and metabolic acidosis. At death, the Sod2−/− mice have enlarged hearts with a dilated left ventricular cavity, consistent with dilated cardiomyopathy. In contrast, the Sod2−/− mice produced by Lebovitz et al. (18) could not be distinguished from their littermate controls at birth. However, between day 2 and day 7, they showed a slowing of growth that continued until death, which usually occurred within 18 days after birth. They exhibited severe anemia and degenerative injury to large CNS neurons, particularly in the basal ganglia and brain stem, motor disturbances, and evidence of mitochondrial damage. Interestingly, only 10% of these Sod2−/− mice showed signs of cardiomyopathy.

In contrast to the null mice, mice heterozygous for the Sod2 gene (Sod2+/-) appear normal but have ~50% of the MnSOD activity of WT mice in all tissues (42). Because mitochondria are a primary site of reactive oxygen species (ROS) production, reduced antioxidant protection in this cellular compartment would be predicted to contribute significantly to oxidative damage/stress in cells and tissues. In previous studies, we have
shown that the Sod2+/− mice exhibit alterations in mitochondrial function and increased mitochondrial oxidative damage as early as 2–4 mo of age (43, 46). For example, the activities of aconitate and NADH-oxidoreductase, mitochondrial enzymes that are sensitive to inactivation by oxidative stress, are significantly decreased in mitochondria from liver and heart. In addition, mitochondrial respiration is altered in the young Sod2+/− mice as shown by a significant decrease in the respiratory control ratio for substrates metabolized by complexes I, II, and III for liver mitochondria and complex I and II for heart mitochondria isolated from Sod2+/− mice compared with WT mice (43, 46). Thus mitochondria from young Sod2+/− mice show alterations that are consistent with increased oxidative stress in cells/tissues of Sod2+/− mice, even though the mice appear normal. In this series of experiments, we studied the Sod2+/− mice over their entire life span to determine whether they show any phenotype that would be predicted to arise from life-long increased oxidative stress, e.g., an increase in the incidence of disease or accelerated aging.

METHODS

Sod2+/− knockout mice. Sod2+/− mice, designated Sod2tm1Cre, were originally produced in the CD1 strain of mice (19). The mice were maintained under barrier conditions in a temperature-controlled environment and fed a commercial mouse chow (Teklad Diet LM485) ad libitum and age-matched with WT mice (43, 46). Thus mitochondria from young Sod2+/− mice were determined using concentrations of the tissue extracts in which the activities of CuZnSOD and MnSOD were measured in tissue extracts from liver, heart, brain, and spleen. These tissues were collected from female WT and Sod2+/− mice euthanized at 26–28 mo of age analyzed without knowledge of the animal’s identity. Tissues were fixed with 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin–eosin as described previously (32). A tumor profile was constructed for each mouse. The severity of lymphoma was assessed based on histological grading systems similar to that developed for Fischer 344 rats (20, 35, 36). The severity of lymphoma was graded as follows: grade 1 (primary site only); grade 2 (2–3 organs); grade 3 (multiple organs, more than 3 organs); and grade 4 (grade 3 with additional pathological changes, e.g., effusion, hemorrhage). The level of cell proliferation in lymphoma from the Sod2+/− and WT mice was compared by measuring the percentage of proliferating cell nucleic antigen (PCNA)-positive cells using immunohistochemistry. Sections 5-μm thick of liver, spleen, and lymph nodes that showed neoplastic lymphocytes were placed on glass slides coated with poly-l-lysine (Sigma, Deisenhofen, Germany), then examined immunohistochemically using a PCNA monoclonal antibody purchased from DAKO (Carpinteria, CA). The samples were analyzed by the avidin–biotin complex (ABC) method (13) using VECTOR MOM immunodetection Kit (Vector Laboratories, Burlingame, CA). The image analysis system used to quantify the number of immunopositive cells consisted of a SPOT cooled color digital camera (Diagnostic Instruments, Sterling Heights, MI), an Olympus AX 70 True Research System Microscope (Olympus America, Lake Success, NY), a Dell Dimension XPS M166x (Dell Computer, Round Rock, TX), a FlashPoint video graphics card (Integral Technologies, Indianapolis, IN), and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The image analysis was accomplished by a double-blind procedure without knowledge of the genotype of animals. The frequency of a lesion or grade of lesion was analyzed with a chi-square test (37). When the expected frequencies were too small for the chi-square test, the data were analyzed with Fisher’s exact test for 2 × 2 tables (37).

Biomarkers of aging. We measured several chemical and physiological indicators that have previously been shown to be altered during aging [e.g., carboxymethyl lysine (CML) and pentosidine in ice-cold lysis solution, and the heart was homogenized in a ground-glass homogenizer in ice-cold lysis solution. Nuclei were collected by centrifuging the homogenate at 10,000 g for 20 s, and the nuclear pellets were resuspended in the enzyme reaction solution and proteinase K (10 μg/ml) provided with the kit. RNase cocktail (Ambion, Austin, TX) was then added to a final concentration of 20 μg/ml. Mitochondrial DNA (mtDNA) was isolated using mtDNA Extractor CT Kit (Wako Chemicals). Briefly, mitochondria were isolated from livers pooled from five mice or brains from nine mice. The homogenates were centrifuged at 1.2 g for 20 min. The supernatants were collected and centrifuged at 10,000 g for 10 min. The pellets, which contained mitochondria, were treated with the solutions in the mtDNA extractor kit.

Quantities of 50–75 μg of nDNA and mtDNA were hydrolyzed as described by Kasai et al. (15). The 8-oxo-2-deoxyguanosine (8oxodG) and 2-deoxyguanosine (2dG) were resolved by high-pressure liquid chromatography (HPLC) and quantified by electrochemical detection as described by Hamilton et al. (9) using a CoulArray electrochemical detection system (model 5500/5600; ESA, Chelmsford, MA) and a C-18 column (YMC, Wilmington, MA). The data were expressed as the ratio of nanomoles of 8oxodG to 107 nanomoles of 2dG.

For comprehensive pathological analysis, brain, pituitary gland, heart, lung, trachea, esophagus, stomach, small intestine, colon, liver and gall bladder, pancreas, spleen, urinary bladder, thyroid/parathyroid gland, adrenal gland, psoas muscle, sternum, spinal cord, vertebra, knee joint, nasal passage, thymus, ventral abdominal skin, eyes, and gonadal tissue including the ovary, oviduct, uterus, and vagina were collected from 26- to 28-mo-old mice and fixed with 10% neutral buffered formalin. The incidence of tumors in female WT and Sod2+/− mice euthanized at 26–28 mo of age was analyzed without knowledge of the animal’s identity. Tissues were fixed with 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin–eosin as described previously (32). A tumor profile was constructed for each mouse. The severity of lymphoma was assessed based on histological grading systems similar to that developed for Fischer 344 rats (20, 35, 36). The severity of lymphoma was graded as follows: grade 1 (primary site only); grade 2 (2–3 organs); grade 3 (multiple organs, more than 3 organs); and grade 4 (grade 3 with additional pathological changes, e.g., effusion, hemorrhage). The level of cell proliferation in lymphoma from the Sod2+/− and WT mice was compared by measuring the percentage of proliferating cell nucleic antigen (PCNA)-positive cells using immunohistochemistry. Sections 5-μm thick of liver, spleen, and lymph nodes that showed neoplastic lymphocytes were placed on glass slides coated with poly-l-lysine (Sigma, Deisenhofen, Germany), then examined immunohistochemically using a PCNA monoclonal antibody purchased from DAKO (Carpinteria, CA). The samples were analyzed by the avidin–biotin complex (ABC) method (13) using VECTOR MOM immunodetection Kit (Vector Laboratories, Burlingame, CA). The image analysis system used to quantify the number of immunopositive cells consisted of a SPOT cooled color digital camera (Diagnostic Instruments, Sterling Heights, MI), an Olympus AX 70 True Research System Microscope (Olympus America, Lake Success, NY), a Dell Dimension XPS M166x (Dell Computer, Round Rock, TX), a FlashPoint video graphics card (Integral Technologies, Indianapolis, IN), and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The image analysis was accomplished by a double-blind procedure without knowledge of the genotype of animals. The frequency of a lesion or grade of lesion was analyzed with a chi-square test (37). When the expected frequencies were too small for the chi-square test, the data were analyzed with Fisher’s exact test for 2 × 2 tables (37).

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levels in skin collagen, cataract formation, and immune function]. CML and pentosidine levels residues were measured in insoluble skin collagen by isotope dilution chromatography mass spectrometry and HPLC, respectively, as previously described (27, 33). To assess cataract formation, cataracts were read blindly using a hand-held slit lamp at a 30 degree angle after dilation with 1% tropicamide. Both eyes were scored on an opacity scale of 0, 1, 2, 3, or 4+, with 4 representing complete lens opacity as described previously (47, 48).

The proliferative response of splenocytes was measured as described previously (24) in the presence of either T cell mitogens [concanavalin A (5 μg/ml) or anti-CD3 antibody (2 μg/ml)] or B cell mitogen lipopolysaccharide (LPS, 10 μg/ml), anti-CD3 antibody (2 μg/ml), or B cell mitogen LPS (10 μg/ml).

RESULTS

We have previously reported that the activity of MnSOD in tissues of young Sod2+/− mice is decreased 30–60% (42). To establish that MnSOD activity is reduced throughout the life span, i.e., that no compensatory upregulation or further decline occurs as the mice age, we measured the activity of MnSOD in WT and Sod2+/− mice at 6 mo and 26 mo of age. As shown in Fig. 1, the activity of MnSOD in Sod2+/− mice is reduced in the old animals to a level similar to that observed in young animals. In addition, we also measured the activities of the other major antioxidant enzymes (e.g., CuZnSOD, GPx, and catalase) to determine whether the reduced activity of MnSOD initiates a compensatory increase in other major antioxidant enzymes. No significant alteration in the activity of any of these enzymes was observed in any of the tissues from either young or old mice (Fig. 2).

To determine whether the tissues of the Sod2+/− mice have increased oxidative damage, we measured the level of 8oxodG in DNA isolated from various tissues of young and old Sod2+/− and WT mice. The data in Fig. 3 show 8oxodG levels in nDNA from several tissues and mtDNA from liver and brain of young and old Sod2+/− and WT mice. The levels of 8oxodG in nDNA and mtDNA increased significantly with age in both the Sod2+/− and WT mice. The levels of 8oxodG in nDNA increased more than 30% with age in liver and nearly 300% in brain in WT mice. In the Sod2+/− mice, 8oxodG levels increased more than 30% in liver and more than 40% in brain with age. In liver mtDNA, an ~130–170% increase with age was observed in DNA isolated from both Sod2+/− and WT mice. More important, we observed significantly higher 8oxodG levels in the nDNA and mtDNA of both young and old Sod2+/− mice, compared with WT mice, i.e., the levels of 8oxodG were consistently higher in the Sod2+/− mice compared with the WT mice throughout the entire life span of the mice. For example, 8oxodG levels were ~50–70% higher in the nDNA and mtDNA in the livers of young and old Sod2+/− mice compared with young and old WT mice. A similar trend was observed for 8oxodG levels in nDNA and mtDNA in brain and nDNA in heart and spleen (Fig. 3).

To test whether the decrease in MnSOD activity resulted in an increased sensitivity of the Sod2+/− mice to exogenous oxidative stress, we exposed the mice to paraquat, a compound that generates superoxide anions (1, 5). As shown in Fig. 4, the young WT mice survived this dose of paraquat, whereas more than 30% of the young Sod2+/− mice died within 4 days. The sensitivity of the old WT and Sod2+/− mice to paraquat was significantly greater than young mice. The old Sod2+/− mice tended to show a greater sensitivity to paraquat than the old WT mice (over 90% of the old Sod2+/− mice were dead by 5 days), but this difference was not statistically significant with the number of mice we used in this experiment.

The data in Table 1 show the incidence of tumors in the Sod2+/− and WT mice. More than 80% of the Sod2+/− mice had neoplastic lesions at 26–28 mo of age, compared with only 41% for their WT littermates. It is important to note that the neoplastic lesions observed in the Sod2+/− mice (potentially fatal tumors, e.g., lymphoma, pituitary adenoma, hemangiomata, adenocarcinoma, and benign or occult tumors such as adenoma in thyroid and lung, and granulose-theca cell tumor in the ovary) have been reported to occur with age in C57BL/6 mice (28, 44), i.e., we observed no unusual neoplastic lesions in the Sod2+/− mice. The incidence of lymphoma was significantly higher in Sod2+/− mice (61%) compared with the WT mice (22%). The incidences of hemangiomata and adenocarcinoma were also higher in the Sod2+/− mice; however, these differences were not significantly different because of the low incidence of these tumors. Both WT and Sod2+/− mice showed similar incidences of pituitary adenoma. In addition to the increased incidence of lymphoma, the number of animals that had multiple types of tumors was greater in the Sod2+/− mice compared with the WT mice, at 66.6% compared with 18.5% (Table 1).

Because the incidence of lymphoma was dramatically increased in the Sod2+/− mice, we measured the severity of lymphoma in the Sod2+/− and WT mice using the grading system described in the METHODS. The data in Fig. 5 show there was no evidence for a difference in the severity of the lymphoma.

Fig. 1. Mn-superoxide dismutase (MnSOD) activity in tissues of Sod2+/− and wild-type (WT) mice. The activity of MnSOD was measured in tissue homogenates isolated from liver, heart, brain, and kidney of 6-mo-old (solid bars) and 26-mo-old (open bars) female WT and Sod2+/− mice using native gels as described in the METHODS. The data shown are the means ± SE calculated from values measured in 4–6 mice. The MnSOD activity is significantly decreased in both young (6 mo) and old (26 mo) Sod2+/− mice compared with age-matched WT mice (P < 0.05) for all tissues. Differences between values obtained from WT and Sod2+/− mice were determined using Student’s t-test.
phoma between the Sod2+/− and WT mice, even though the incidence of lymphoma was greater in the Sod2+/− mice. We also compared the proliferative activity of the lymphoma from the Sod2+/− and WT mice by measuring the PCNA-positive cells. As shown in Fig. 5, the percent of PCNA-positive cells was similar for lymphoma from the Sod2+/− and WT mice.

To determine whether the age-related accumulation of oxidative damage to DNA is important in the aging process, we measured the survival of the WT and Sod2+/− mice under barrier conditions. There was no significant difference in body weights or food consumption of the Sod2+/− and WT mice over their life spans (data not shown). As shown in Fig. 6, the life spans of WT and Sod2+/− mice were indistinguishable, e.g., the mean survival was ~30 mo and the maximum survival was 40 mo. We also measured several age-sensitive chemical and physiological biomarkers of aging (e.g., CML and pentosidine levels in skin collagen, cataract formation, and immune function) to determine whether aging was altered in the Sod2+/− mice. These markers have been shown to change with age in a variety of animal models (25, 31, 47, 48) and to be reversed by dietary restriction (30, 47, 48). The data in Fig. 7 show that each of these parameters has the expected change with age in both the Sod2+/− and WT mice, i.e., an increase in CML and pentosidine levels in skin collagen, an increase in cataract formation, and a decline in splenocyte proliferation. However, none of these indices of aging were significantly different in the Sod2+/− and WT mice at any of the ages studied.

DISCUSSION

Sod2+/− mice, which have reduced MnSOD activity in all tissues throughout their life, exhibit no overt physical phenotype, e.g., their body weight, food consumption, and fecundity are similar to WT littermates. However, the Sod2+/− mice show increased sensitivity to the superoxide anion generator, paraquat, and tissues of the Sod2+/− mice showed higher levels of oxidative damage (8oxodG) to nDNA and mtDNA over their life span. This later observation, i.e., increased levels of 8oxodG, is of particular interest because 8oxodG is known to be a premutagenic lesion in mammalian cells, which could play a role in the initiation of cancer (17), and because an age-related increase in 8oxodG is a universal phenomenon in rodents (10) that has been proposed to be important in aging (2).

Our study demonstrates that the primary phenotype resulting from reduced MnSOD activity in the Sod2+/− mice is an increased incidence of cancer. It should be stressed that we observed no unusual age-related pathology for this particular
strain of mice, i.e., the neoplastic lesions that normally occur with age in C57BL/6 mice were higher in the Sod2/H11001/H11002 mice. More than 80% of the Sod2/H11001/H11002 mice had neoplastic lesions at 26–28 mo of age, compared with only 41% for their WT littermates, and the incidence of lymphoma, the major neoplastic lesion, was significantly higher in Sod2/H11001/H11002 mice (61%) compared with the WT mice (22%). We also compared growth/development of lymphoma in the Sod2/H11001/H11002 and WT mice by measuring the severity and the cell proliferative activity of the lymphoma in these mice. The severity and percentage of PCNA-positive cells were similar for lymphoma from the Sod2/H11001/H11002 and WT mice, suggesting that the progression/growth and cell proliferation of the lymphoma were not

Fig. 3. Oxidative damage to nuclear DNA (nDNA, top) and mitochondrial DNA (mtDNA, bottom) in tissues of Sod2/mice and WT mice. The levels of 8-oxo-2-deoxyguanosine (8oxo-dG) in nDNA from liver, brain, heart, and spleen and mtDNA from brain and liver from 3- to 6-mo-old (open bars), 15- to 17-mo-old (gray bars), and 26-mo-old (solid black bars) female WT and Sod2/+ mice are shown. The data are expressed as the ratio of nanomoles of 8oxoG to 10^5 nanomoles of 2-deoxyguanosine (2dG). The values for the nDNA represent the means ± SE for 8 mice. For the liver mtDNA, the means ± SE were obtained from 3 samples with each sample pooled from the livers of 5 animals. For the brain mtDNA, the mean for 2 samples is given with each sample pooled from brain tissue collected from 9 animals. All values represent the means ± SE, and the data were analyzed using a one-way ANOVA with a Bonferroni test to show significance. Statistically significant differences (P < 0.05) within a given tissue are indicated by different lower case letters (a, b, c, d).

Table 1. Incidence of tumors in old Sod2/+ and wild-type mice

<table>
<thead>
<tr>
<th>Tumor-bearing mice</th>
<th>WT</th>
<th>Sod2/+</th>
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</thead>
<tbody>
<tr>
<td>No. of mice with multiple tumors</td>
<td>5 (18%)</td>
<td>12 (67%)*</td>
</tr>
<tr>
<td>Incidence of potentially fatal tumors</td>
<td></td>
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</tr>
<tr>
<td>Lymphoma</td>
<td>6 (22%)</td>
<td>11 (61%)*</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>0</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>9 (33%)</td>
<td>5 (28%)</td>
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</tbody>
</table>

The number of tumor-bearing mice (i.e., mice with one or more tumors) and the number of mice with multiple tumors (i.e., mice with two or more different types of tumors) were determined in for Sod2/+ mice (n = 18) and wild-type (WT, n = 27) mice as described in the METHODS. The data were analyzed by the chi-square test, and those values that are statistically different (*P < 0.01) for Sod2/+ mice when compared with the WT mice are shown.

Fig. 4. Sensitivity of Sod2/+ and WT mice to oxidative stress. Young (6 mo) and old (26–28 mo) female mice (12 mice per group) were injected intraperitoneally with 50 mg/kg paraquat dissolved in saline. Following injection, mice were monitored twice daily for 2 wk for deaths. Data shown are for the first 10 days (no deaths occurred between 10 and 14 days). Data were analyzed using the Cox-Mantel log rank test. The survival of the young Sod2/+ mice (long-dashed line) following paraquat treatment was statistically different from the young WT mice (solid line, at top) (P < 0.05). Survival in the two groups of old mice was different from the young mice (P < 0.05). Old WT mice are indicated by small-dotted line; old Sod2/+ mice are indicated by large-dotted line.
altered in the Sod2+/− mice. Therefore, the enhanced lymphoma in the Sod2+/− mice appears to be primarily due to early stages (initiation, promotion, or both) in the development of lymphoma, which is consistent with the increased levels of endogenous oxidative damage to DNA in the Sod2+/− mice playing an important role in the increased incidence of tumors in these mice.

Two important conclusions can be drawn from this study. First, we demonstrated that Sod2+/− mice, which have increased oxidative damage to DNA, show an increase in the incidence of spontaneous tumors, which occur normally with age. Although chemicals in the environment that generate oxidative damage to DNA are known to be potentially carcinogenic (16), we provide the first direct evidence that an increase in endogenously generated ROS, which arises from a defect in the mitochondrial antioxidant defense system, can increase oxidative damage to nDNA and the incidence of cancer. Thus our data point to the potential importance of endogenously generated ROS (in particular, those of mitochondrial origin) in spontaneous cancers that occur with age. These data are consistent with a recent study by Samper et al. (29) showing that increased production of ROS in mouse embryonic fibroblasts isolated from Sod2−/− mice is correlated to an increase in chromosomal alterations and genomic instability. We envision that the reduction MnSOD in the mitochondrial matrix could lead to oxidative damage in nDNA and cancer in two ways. First, increased superoxide anions in the mitochondrial matrix that arise from the reduced MnSOD activity leads to damaged mitochondria, which in turn release more ROS, increasing oxidative damage to the mitochondrial, cytosolic, and nuclear compartments (41). Second, the increased superoxide anion levels in the matrix generate other ROS species, e.g., hydrogen peroxide, that are able to transverse the mitochondrial membrane and generate oxidative damage in the nucleus.

Our data also suggest that mutations in the human MnSOD gene, which lead to reduced MnSOD activity, could increase the risk of cancer in the human population. Polymorphisms and mutations in the MnSOD gene have been reported (11, 34). For example, the Ile58Thr mutation in the human MnSOD gene has been shown to lead to an alteration in the structure of the Thr58 polypeptide, resulting in MnSOD occurring primarily as a dimer in solution with less activity and decreased thermostability compared with the MnSOD formed from the WT Ile58 polypeptide (4). In addition, polymorphisms in the Sod2 gene, resulting in a substitution of alanine for valine at the −9 codon position in the mitochondrial targeting sequence, have been reported to be associated with increased risk of breast, lung, and colon cancer in humans (22, 38, 45). These studies support our observations with Sod2+/− mice, suggesting that a reduction in MnSOD activity would lead to an increased incidence of cancer.

The second conclusion that can be drawn from our study is that an increase in oxidative damage to DNA over the life span of the Sod2+/− mice does not accelerate aging, as would be predicted from the oxidative stress theory of aging. This theory, which is based on the tenet that damage caused by ROS plays a critical role in determining life span, has been one of the most popular theories to explain the deterioration in biochemical and physiological processes that occur during the aging process. A large number of studies have produced correlative data in support of this theory, e.g., an increase in oxidative damage to lipid, protein, and DNA with age has been demonstrated in a variety of tissues and organisms (2), and dietary restriction, which has been shown to retard aging, has been shown to reduce the age-related increase in oxidative damage (8, 10, 50). Thus far, the most direct evidence for the oxidative stress theory of aging has come from studies with Drosophila, in which the life span of the flies has been increased by overexpressing CuZnSOD (26, 39); yet even these data do not prove that the extension in life span was due to altered oxidative damage. Therefore, conclusive data in
The aging of the Sod2 maintained. In other words, our life span data accurately re-
constructed the excellent conditions under which these mice were
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oxidative stress and increased oxidative damage (two phe-
notypes which are commonly associated with alterations in
aging) does not lead to altered aging in the Sod2 mice. Thus
our data with the Sod2 mice do not support the oxidative
stress theory of aging.

Support of this theory, linking alterations in oxidative damage
with life span in mammals, are still lacking.

Surprisingly, our data show that the life span characteristics
of the WT and Sod2 mice are nearly identical even though
the Sod2 mice show an increase in oxidative damage and an
increased incidence of cancer. A disconnect between the inci-
dence of major pathological lesions, e.g., cancer, and life span
is not unusual. For example, Garcia-Cao et al. (7) observed that
transgenic mice with multiple copies of p53 show a major
reduction in cancer but no change in life span. It should also be
noted that the life spans of our Sod2 and WT mice are
exceptionally long for C57BL/6 mice (3, 28, 44), which
demonstrates the excellent conditions under which these mice were
maintained. In other words, our life span data accurately reflect
the aging of the Sod2 and WT mice and are not due to other
complicating factors that arise from poor housing conditions,
e.g., infectious disease or stress. In addition to the life span
data, several measures of age-sensitive biomarkers, e.g., CML
and pentosidine residues in skin collagen, cataract formation,
and immune function, do not differ in the Sod2 and WT mice.
Thus our data demonstrate that a reduction in MnSOD
activity in the Sod2 mice that results in increased sensitivity
to oxidative stress and increased oxidative damage (two phe-
notypes which are commonly associated with alterations in
aging) does not lead to altered aging in the Sod2 mice. Thus

Support of this theory, linking alterations in oxidative damage
with life span in mammals, are still lacking.

Over the past five years, three other laboratories have used
transgenic or knockout mice to test the oxidative stress theory
of aging, and the results of these studies have been contradic-
tory. For example, Epstein and colleagues (14) measured the
life span of transgenic mice that overexpressed CuZnSOD.
Although these mice show increased resistance to a variety of
oxidative stresses (6, 12, 49), the increase in expression of
CuZnSOD did not result in an extension of life span in the
transgenic mice compared with WT controls. Migliaccio et al.
(21) reported that mice null for p66shc , which showed increased
resistance to oxidative stress, had a 30% increase in life span.
These data, in contrast to the studies with CuZnSOD transgenic
mice, support the oxidative stress theory of aging; however,
neither Epstein et al. (14) nor Migliaccio et al. (21) measured
the effect of their genetic manipulation on the age-related
accumulation of oxidative damage to macromolecules. More
recently, Moskovitz et al. (23) produced mice mutated in the
MsrA gene (MsrA ), which encodes methionine sulfoxide reductase, the enzyme responsible for reducing methionine
sulfoxide residues in proteins. The MsrA mice showed
increased sensitivity to oxidative stress (hyperoxia) and in-
creased protein oxidation (carbonyl groups) in kidney under
normoxia and in liver, kidney, and lung in response to hyper-

Fig. 7. Changes in biomarkers of aging in WT and Sod2 mice. Three indices of
aging were measured in female WT and Sod2 mice, and differences were deter-
mined using Student’s t-test. A: carboxymethyl lysine (CML) and pentosidine levels.
Data are results for skin collagen taken from 7–9 mice in the three age groups shown. The
increase in CML or pentosidine with age for the WT and Sod2 mice was significant at
the P < 0.01 level, but not different between the two groups. B: cataract formation; each
value is the mean ± SE for 7–14 mice. The increase in cataracts with age for both WT
and Sod2 mice is significant at the P < 0.05 level. C: splenocyte proliferation; each
point is the mean ± SD of data obtained from 6 spleens per group, and the decrease in
proliferation with age for the three mitogens for both WT and Sod2 mice is significant
at the P < 0.01 level. ConA, concanavalin A; LPS, lipopolysaccharide; Hyp, hy-
droxyproline.

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oxia. Moskowitz et al. (23) reported that the MsrA<sup>−/−</sup> mice had a shorter life span under both normal and hypoxic conditions compared with WT mice. However, the studies by both Migliaccio et al. (21) and Moskowitz et al. (23) have two important limitations. First, the life spans of the mouse colonies used in the survival studies were relatively short (WT mice in both colonies lived only 800 to 850 days, compared with nearly 1,200 days for WT mice in our colony), suggesting that the animal colonies were not maintained under optimal conditions. Thus it is possible that the alterations in life span reported in these studies occurred because of the interaction of the genetic manipulations with the stressful conditions under which the mice were housed. Second, the only information on whether these genetic manipulations altered aging is the survival data; no data were presented on whether the genetic manipulations altered physiological processes that changed with age. Although survival is the ultimate endpoint for assessing aging, in this study, we also measured several age-sensitive chemical and physiological biomarkers of aging (e.g., CML and pentosidine levels in skin collagen, cataract formation, and immune function) to ask whether the characteristics of the aging process were altered in the Sod<sup>2<sup>−/−</sup></sup> mice.

In summary, we have shown that tissues from the Sod<sup>2<sup>−/−</sup></sup> mice show increased oxidative damage to DNA throughout the life span compared with tissues of their WT littermates. The increased levels of oxidative damage to DNA in the Sod<sup>2<sup>−/−</sup></sup> mice were associated with increased incidence of cancer. We observed no difference in the life span of the Sod<sup>2<sup>−/−</sup></sup> mice compared with their WT littermates and no alteration in three independent indices of aging. Thus we show in this study that the increased levels of oxidative damage to DNA throughout the life span of the Sod<sup>2<sup>−/−</sup></sup> mice lead to increased incidence of cancer but do not appear to accelerate aging as would be predicted by the oxidative stress theory of aging.

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