



Dossier: Superoxide dismutases: recent advances and clinical applications

## Modulation of skin tumorigenesis by SOD

Daret St. Clair<sup>a,\*</sup>, Yunfeng Zhao<sup>a</sup>, Luksana Chaiswing<sup>b</sup>, Terry Oberley<sup>b</sup><sup>a</sup> Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536, USA<sup>b</sup> Department of Pathology and VA Hospital, University of Wisconsin, Madison, WI 53705, USA

Received 26 January 2005

Available online 17 March 2005

## Abstract

Generation of reactive oxygen species (ROS) has been implicated in the development of cancer. Groundwork establishing mitochondria as a critical source of ROS generation and the role of manganese superoxide dismutase (MnSOD) in preventing mitochondria-mediated cell death have been well established. In a seemingly contradictory role, it also is well documented that increased MnSOD expression suppresses the carcinogenesis effect of ROS. Our recent studies demonstrated that overexpression of MnSOD reduced tumor incidence in the two-stage 7,12-dimethylbenz(a)-anthracene (DMBA)/12-*O*-tetradecanoylphorbol-13-acetate (TPA) skin carcinogenesis model. However, reduction of MnSOD by heterozygous knockout of the MnSOD gene (Sod 2+/-) did not lead to an increase in tumor incidence. Thus, how modulation of mitochondrial ROS levels alter the outcome of developing cancer is unclear. This review will provide background information on the sequence of ROS-mediated events in the mitochondria and evidence that suggests that the antioxidant and tumor suppressor functions of MnSOD are indeed inter-related. It also will offer insights into the mechanisms by which MnSOD modulates the outcome of early stage skin carcinogenesis.

© 2005 Elsevier SAS. All rights reserved.

## 1. Redox reactions, superoxide, and mitochondria

Redox or reduction–oxidation reactions, which involve the transfer of electrons or hydrogen atoms from one atom or molecule to another, are the major source of superoxide production from oxygen utilization. Superoxide radicals, the product of one electron reduction of molecular oxygen, are produced in mitochondria as a result of the imperfect flow of electrons through the electron transport chain. At least two sites in the electron transport chain, Complex I and ubiquinone, have been identified as primary sources of superoxide production in mitochondria [18,4]. Although superoxide radicals are not considered highly reactive in the hierarchy of reactive oxygen species (ROS), toxicity from superoxide radicals has been clearly demonstrated by the necessity that they be removed for survival of aerobic life. This unconventional relationship between their reactivity and toxicity may, in part, be due to their strategic location in the mitochondria and their effectiveness in striking critical targets in the respiratory chain.

Generation of hydroxyl radicals and formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) in mitochondrial

DNA during mitochondrial electron transfer have been demonstrated [18]. Mitochondrial DNA is highly susceptible to mutation because mitochondrial DNA is not protected by histones. Mutations in any of the genes coding for cytochrome oxidase, cytochrome *bc*<sub>1</sub>, NADH dehydrogenase, or ATPase complexes may lead to defective function of these enzymes. Additionally, ROS can directly inactivate mitochondrial enzymes leading to mitochondrial dysfunction and increased generation of ROS. It has been demonstrated that iron, sulfur-cluster (FeS) containing enzymes, which include several key components of the electron transport chain, are highly sensitive to redox-induced inactivation [13,49]. Thus, mitochondria are particularly prone to free radical-induced changes. Consequently, protecting against mitochondrial injury by increasing the mitochondrial antioxidant capability may reduce oxidative stress in cells.

In addition to an important role in energy production, mitochondria also have been shown to play a role in maintaining cellular redox status by eliminating cytosolic superoxide radicals [17]. Cytosolic superoxide radical scavenging by mitochondria is accomplished by a polarized inner mitochondrial membrane, which is positively charged during respiration. This phenomenon enhances the spontaneous dismutation of superoxide, which diffuses into the mitochondrial intermem-

\* Corresponding author. Tel.: +1 859 3956; fax: +1 859 323 1059.

E-mail address: [dstcl00@pop.uky.edu](mailto:dstcl00@pop.uky.edu) (D. St. Clair).

brane space because of the presence of a localized proton-rich environment. Here, superoxide radicals are protonated to form the hydroperoxyl radicals, which can diffuse into mitochondrial matrices and are dismutated by superoxide dismutase (SOD) in the mitochondria. The net superoxide radical consumption in the mitochondria creates a gradient for superoxide radicals, which favors diffusion from the cytosolic to the mitochondrial space. Thus, mitochondria are not only a major site of energy production and superoxide generation but are also a source of cytosolic superoxide removal. Thus, it may not be a coincidence that mitochondria play a central role in apoptosis.

## 2. The role of MnSOD in life and death

Among the three SODs, it is the manganese containing superoxide dismutase (MnSOD) that demonstrates how toxic superoxide radicals can be in many forms of aerobic life [11]. Inactivation of MnSOD genes in *Escherichia coli* increases mutation frequency when grown under aerobic conditions [11,12]. Elimination of the MnSOD gene in *Saccharomyces cerevisiae* increases its sensitivity to oxygen [44]. This was substantiated by knockout mice lacking each of the three gene products. Homozygous knockout mice, for the cytosolic copper, zinc superoxide dismutase (CuZnSOD) and extracellular superoxide dismutase (ECSOD), are healthy unless they are stressed. The homozygous MnSOD knockout mice, however, are small at birth and die within 2–3 weeks from dilated cardiomyopathy and neurodegenerative disease [7,26,27]. Homozygous knockout mice that live longer than seven days exhibit extensive mitochondrial injury within degenerative neurons and cardiomyocytes. MnSOD heterozygous knockout mice live a normal life span and do well unless they are stressed [47,45].

In addition to being essential for survival, increased expression of MnSOD has been shown to protect against numerous agents and conditions that cause oxidative stress and/or cell death. Transfection of the MnSOD gene into cultured cervical carcinoma cells prevented cell death resulting from treatment with tumor necrosis factor [48]. Expression of the MnSOD gene by transfection with the human MnSOD cDNA resulted in cells that were resistant to paraquat-induced cytotoxicity in a mouse fibroblast cell line (C3H10T1/2) [41]. Stable expression of MnSOD in insulinoma cells prevented IL-1 beta-induced cytotoxicity and reduced nitric oxide production. Overexpression of MnSOD in neuronal cells protected the neurons from NMDA- and nitric oxide-mediated toxicity [16]. Apoptosis caused by oxidized low-density lipoprotein was shown to be MnSOD dependent [24]. Thus, MnSOD overexpression may also be important for pathological conditions where overproduction of cytokines and nitric oxide is involved. These findings pointed to the versatility of MnSOD in prevention of injury induced by both ROS and reactive nitrogen species (RNS).

The fact that increased expression of MnSOD prevents cell death induced by a broad range of agents may be due to its

unique role in regulating ROS levels in the mitochondria. Using a direct approach to generate free radicals from mitochondria by disrupting mitochondrial respiration with rotenone or antimycin, we demonstrated that MnSOD protected fibrosarcoma cells from apoptosis [23]. Cells expressing a high level of MnSOD were resistant to rotenone or antimycin-induced apoptosis. In control cells, antimycin caused the activation of caspase 3 and cleavage of poly-ADP ribose polymerase, and these changes were prevented by overexpression of MnSOD. These results clearly established that prevention of mitochondrial injury from oxidative stress plays a major role in blocking mitochondrial-mediated apoptosis.

In addition to its role in preventing apoptosis induced by intra-mitochondrial radicals, overexpression of MnSOD is effective in preventing apoptosis initiated by extra-mitochondrial sources. Using alkaline pH, a condition that reduces cytosolic proton concentration, we demonstrated that overexpression of MnSOD protected fibrosarcoma cells against apoptosis induced by alkaline conditions [29]. Cells grown under alkaline conditions developed increased levels of ROS and intracellular calcium and altered mitochondrial membrane potential. Increased levels of ROS were found both in cytosol and mitochondria. The level of a highly toxic lipid peroxidation product, 4-hydroxy-2-nonenal, bound to protein also increased in control but not in MnSOD-transfected cells. These results demonstrated a link between oxidative stress initiated in the cytosol and removal of ROS by mitochondria. This finding is consistent with the role of mitochondria as a major site of superoxide production and removal of cytosolic superoxide. Thus, in addition to its important role in energy production, mitochondrial respiration plays an important role in maintaining cellular redox status.

## 3. MnSOD as a tumor suppressor and redox modulator

Increased expression of MnSOD has been shown to suppress cancer phenotypes in a large number of established human and murine tumors. This particular aspect of MnSOD will be expertly reviewed in this same issue by Dr. Larry Oberley. Thus, this discussion will focus on the role of MnSOD in early stages of cancer development. Since expression of MnSOD is protective against agents that cause apoptosis, it is unclear how MnSOD may act to suppress carcinogenesis. As the first step toward investigating the role of MnSOD in the development of cancer, we used gene transfection approaches to determine the role of MnSOD in cellular neoplastic transformation. In this study, we transfected the human MnSOD gene into mouse C3H10T1/2 cells and verified that the transfected MnSOD was expressed and that the protein was properly targeted to mitochondria. When these cells were irradiated with gamma radiation, an agent known to generate free radicals and cause cellular transformation, the results demonstrated that expression of human MnSOD in these cells significantly reduced the frequency of radiation-induced neoplastic transformation [42]. This result provided a direct link

between mitochondrial antioxidants and cellular neoplastic transformation.

Because tumor formation may result from an increase in cell growth or a decrease in cell differentiation, we determined the effect of MnSOD expression on the differentiation of C3H10T1/2 cells and their SOD-transfected counterparts. Confluent C3H10T1/2 cells have an epithelioid appearance but do not normally differentiate. Following treatment with 5-azacytidine (5-AZA), some C3H10T1/2 cells differentiated into muscle cells, fat cells, or chondrocytes. Treatment with 3 mM 5-azacytidine induced a low frequency of parental C3H10T1/2 cells to differentiate into myoblasts but induced extensive differentiation of SOD-transfected cells into myoblasts [43]. These results suggested that expression of MnSOD promotes differentiation. A significant implication of these results was that an elevated level of MnSOD can create an environment that is permissive for cellular differentiation. It is important to recognize that elevated levels of MnSOD do not appear to directly cause differentiation, but rather promote differentiation following exposure to an agent which signals the cell to differentiate.

We further found that overexpression of MnSOD showed both anti-apoptotic and differentiation-promotion effects. Treatment with 10  $\mu$ M 5-Aza alone induced apoptosis in the control C3H10T1/2 cell line, while the MnSOD-overexpressing cell line demonstrated differentiated morphology. The levels of the myogenic transcription factor, MyoD, and the muscle specific marker,  $\alpha$ -actin, were increased over time with 5-AzaC treatment in the SOD-overexpressing cell line [50]. The nuclear transcription factor NF $\kappa$ B was activated in the MnSOD-overexpressing cell line, while inhibition of NF $\kappa$ B activation reduced the levels of MyoD and  $\alpha$ -actin. Members of the mitogen-activated protein kinase (MAPK) pathway and the Raf1/MEK/ERK cascade were also found to play a positive role in this event. Thus, overexpression of MnSOD in mitochondria modulated the activation of MAPK and NF $\kappa$ B pathway, further demonstrating the role of mitochondrial antioxidants in the regulation of cell death and cellular redox status leading to activation of signaling pathways [50]. Since mitochondrial dysfunction may lead to changes in cellular redox status and, hence, biochemical alterations in cells, it is possible that the antioxidant and tumor suppression functions of MnSOD may indeed be inter-related; i.e. expression of MnSOD suppresses tumorigenesis by controlling mitochondrial-mediated oxidative stress which leads to the biochemical and phenotypic changes observed in tumor cells.

#### 4. Oxidative stress in the multi-stage mouse skin carcinogenesis model

The polycyclic aromatic hydrocarbon 7,12-dimethylbenz(*a*)-anthracene (DMBA) can act as a complete carcinogen or an initiator of mouse skin carcinogenesis [9,40]. A single large dose of DMBA is capable of inducing skin tumors

in mice in which papillomas appear after a relatively short latency period (10–20 weeks), with carcinomas developing after a much longer period (20–60 weeks). The sequential application of a subthreshold dose of DMBA, followed by repetitive treatment with a promoter such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or benzoyl peroxide, will also induce skin tumors [35,39]. In the latter protocol, initiation is achieved by a single treatment of the skin with a chemical carcinogen. This is considered to result in a population of initiated cells that remain dormant until treatment with the promoter stimulates clonal expansion, which leads to the formation of benign well-differentiated papillomas. A portion of these papillomas will progress to squamous carcinomas and eventually invasive undifferentiated carcinomas.

Initiators of carcinogenesis have long been associated with an ability to cause DNA mutation leading to activation of proto-oncogenes. Members of the *ras* gene family have been found to be one of the most frequently detected oncogenes in human tumors and experimental animals [2,7]. Mutations are specific for the initiating agent but not the promoting agent [6]. Most papillomas analyzed in the DMBA-initiated/TPA-promoted mouse skin tumor model contained a mutation in codon 61 of the *Ha-ras* oncogene. In greater than 90% of these tumors, the activating mutation of the *Ha-ras* gene was an A-T transversion and the remainder of the papillomas contained an A-G transition. Using a sensitive PCR amplification technique, it has been demonstrated that epidermal cells with *Ha-ras* mutation appeared one week after initiation with DMBA, before the mouse skin was promoted by TPA and before benign tumors developed [6,8]. Furthermore, transgenic mice expressing the *ras* oncogene developed hyperkeratosis and papillomas [1]. Thus, mutation of codon 61 in the *Ha-ras* gene is considered to be a critical step in the formation of mouse skin tumors and a marker for the occurrence of the initiating event in the DMBA-treated skin carcinogenesis model.

It is well established that promotion with TPA produces oxidants and oxidatively damaged macromolecules [5,6]. On the other hand, the activity of xanthine oxidase, an enzyme capable of generating superoxide radicals, was increased in mice treated with TPA [36,34]. The activity of antioxidant enzymes, such as SOD and catalase, was decreased in mouse skin treated with TPA [37,38]. These results indicated that application of TPA to mouse skin led to an increase in cellular pro-oxidants and a decrease in antioxidant capability. Interestingly, single or multiple applications of TPA to mouse skin did not lead to a significant decrease in the level of GSH or GSH/GSSG ratio. In contrast, the GSH content increased in papillomas and carcinomas. Thus, the activity of antioxidant enzymes may play a more prominent role for the regulation of cancer development than the level of GSH. However, given the known antioxidant property of GSH, these results could also suggest that oxidative stress was present but confined to a specific subcellular compartment such as mitochondria [37].

ROS have been implicated as second messengers in regulating gene expression. It has been shown that oxidative stress

can modulate the activity of protein kinases, which in turn phosphorylate a wide range of cellular proteins [15]. The structure of this group of proteins contains a tandem repeat of cysteine-rich amino acids in their regulatory domains [33]. These amino acids are the target for oxidation. It is interesting to note that while excessive oxidation inactivates protein kinase activity, mild oxidation selectively modifies the regulatory domain of protein kinases causing persistent activation of kinases and subsequent phosphorylation of downstream targets such as *fos* and *jun* [15]. Activation of protein kinase activity has been widely demonstrated in mouse skin promoted with TPA [31,46,14]. Since *fos* and *jun* form a heterodimeric complex that interacts with the DNA regulatory element known as the AP1 binding site and transcriptional activation by AP1 is a result of the cooperative action of these proteins [21], the tumor promoter activity of TPA may in part be due to its ability to generate mild oxidative stress with resultant activation of protein kinases leading to increased phosphorylation of *fos* and *jun* proteins.

### 5. Mitochondria and p53-induced apoptosis

Although it has been called the guardian of the genome, activation of apoptosis is an important mechanism in p53 tumor suppression [10]. Apoptosis mediated by p53-dependent transcriptional activation of its target genes has been extensively studied [32]. However, the requirement of transcriptional activation of p53-target genes in apoptosis remains controversial. Studies in which p53 levels were overexpressed to induce apoptosis in cultured cells demonstrated exceptions to this requirement. For example, in HeLa cells apoptosis occurred in the absence of Bax translocation to the mitochondria, but ROS were generated, which in turn caused the collapse of the mitochondrial membrane potential [28]. Ectopic expression of a p53 mutant inactive as a transcription factor strongly induced apoptosis in HCT116 colon carcinoma cells [25]. Deliberate targeting of ectopic p53 to mitochondria via fusion with the mitochondrial import leader peptide bypassed the nucleus and was sufficient to induce apoptosis in three different p53-deficient tumor cell lines [30]. P53 may induce apoptosis by forming complexes with mitochondrial proapoptotic proteins such as p53AIP1 [12], Bcl-2/Bcl-x<sub>L</sub> [20], and translocation of p53 to mitochondria requires Bax, a product of p53 transcription. These data suggested that p53 may mediate apoptosis by mechanisms that are both dependent and independent of its transcription activity, thereby amplifying its apoptosis potency.

Mutation in the p53 tumor suppressor gene has been found frequently in many types of cancer, including skin carcinomas [19,20]. High levels of p53 gene mutations and immunoprecipitable mutated p53 protein have been reported in the UV-radiation mouse skin carcinogenesis model [3]. However, loss of heterozygosity on chromosome 11, where the mouse p53 gene is located, was not detected in papillomas resulting from the DMBA/TPA skin carcinogenesis model,

suggesting that the p53 mutation is not associated with initiation or promotion but rather is associated with malignant progression. Direct support for this hypothesis is the finding that reduction of the p53 gene in p53 knockout mice did not increase initiation or promotion but enhanced malignant progression of cancer in the multi-stage skin carcinogenesis model [22]. In this model, the number, size, and growth rate of benign papillomas were not increased in the p53 heterozygous knockout mice in comparison with the wild-type mice. Furthermore, the p53 null mice showed a reduced yield of papillomas with increasing rate of malignant progression [22]. Thus, the role of p53 in early stages of skin carcinogenesis is unclear.

### 6. Mechanisms of tumor suppression by MnSOD

To determine how expression of MnSOD may suppress the development of cancer, we employed the well-established skin carcinogenesis model consisting of sequential application of a sub-threshold dose of the mutagenic chemical initiator DMBA, followed by repetitive treatments with the tumor promoter TPA using MnSOD-overexpressing and MnSOD-deficient mice. In a MnSOD-overexpressing mice model, 78% of non-transgenic mice developed papillomas, averaging 2.1 papillomas per mouse, while 50% of MnSOD-overexpressing mice developed papillomas, averaging 0.75 papillomas per mouse after 14 weeks of TPA treatment, following a single application of DMBA [51]. Expression of MnSOD modulated tumor formation by inhibition of AP-1 signaling, consistent with the reduction of tumors in MnSOD-overexpressing mice. Interestingly, in a MnSOD-deficient mouse model, there was no difference in the incidence and frequency of papillomas when comparing the MnSOD-deficient mice with their wild type littermates [52]. The numbers of proliferating cells in DMBA/TPA-treated mouse skin were higher in the MnSOD-deficient mice. However, histological examination also demonstrated a concurrent increase in the number of apoptotic cells in the MnSOD-deficient mice after DMBA/TPA treatment. Additionally, MnSOD deficiency enhanced TPA-induced oxidative stress markers, AP-1 and p53 levels, consistent with the increase in both proliferation and apoptosis events observed histologically. These results suggested that increased apoptosis may negate increased proliferation in the MnSOD-deficient mice during an early stage of tumor development. Based upon the kinetics of cell death and proliferation, we performed a timed administration of a catalytic antioxidant (MnTE-2-PyP<sup>5+</sup>) in order to inhibit proliferation without interfering with apoptosis in the MnSOD-deficient mice. These results indicated that application of a catalytic antioxidant prior to apoptosis suppressed cell death and cell proliferation, results consistent with those obtained in MnSOD-overexpressing mice. Application of the catalytic antioxidant after apoptosis did not interfere with the apoptosis process but significantly reduced cell proliferation and the incidence of papillomas. These results dem-

onstrated a proof-of-principle for a mechanistic-based model for modulation of cancer development by SOD.

In summary, accumulating data have suggested that MnSOD constitutes one of the major cellular defense mechanisms against the toxic effects of agents that cause oxidative stress and that MnSOD functions as a tumor suppressor gene in several experimental systems. The antioxidant and anti-apoptosis effect of MnSOD is linked to its strategic location in mitochondria. Using the well-established multi-stage skin carcinogenesis model, we have demonstrated that overexpression of MnSOD reduced tumor incidence and multiplicity. However, reduction of MnSOD by heterozygous knockout in the MnSOD gene mice did not lead to an increase in tumor incidence, because a reduction of MnSOD contributed to increased levels of both cell proliferation and apoptosis. Application of a catalytic antioxidant at the window between apoptosis and proliferation significantly reduced the incidence and multiplicity of papillomas. Thus, an antioxidant approach that suppressed cell proliferation without interfering with apoptosis resulted in suppression of tumor development.

## References

- [1] Bailleul B, Surani MA, White S, Barton SC, Brown K, Blessing M, et al. Skin hyperkeratosis and papilloma formation in transgenic mice expressing a *ras* oncogene from a suprabasal keratin promoter. *Cell* 1990;62:697–708.
- [2] Balmain A, Brown K. Oncogene activation in chemical carcinogenesis. *Adv Cancer Res* 1988;51:147–82.
- [3] Berg RJW, van Kranen HJ, Rebel HG, Bries AD, Van Vloten WA, Van Kreijl CF, et al. Early p53 alterations in mouse skin carcinogenesis by UVB radiation: immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. *Proc Natl Acad Sci USA* 1996;93:274–8.
- [4] Boveris A, Cadenas E. Production of superoxide radicals and hydrogen peroxide in mitochondria. In: Oberley LW, editor. *Superoxide dismutase*. Boca Raton, FL: CRC Press, Inc.; 1982. p. 15–30 vol. 2.
- [5] Bowden GT, Nelson MA, Levy JP, Finch J, Krieg P. Molecular mechanisms of skin carcinogenesis induced by chemicals and ionizing radiation. In: Hecker, Jung, Marks, Tilgen, editors. *Skin carcinogenesis in man and in experimental models*. New York: Springer-Verlag; 1993. p. 309–21 vol. 128.
- [6] Bowden GT, Finch J, Domann F, Krieg P. Molecular mechanisms involved in skin tumor initiation, promotion, and progression. In: Mukhtar H, editor. *Skin cancer: mechanisms and human relevance*. CRC Press; 1995. p. 99–111.
- [7] Bremner R, Balmain A. Genetic changes in skin tumor progression: correlation between presence of a mutant *ras* gene and loss of heterozygosity on mouse chromosome 7. *Cell* 1990;61:407–17.
- [8] Brown K, Buchmann A, Balmain A. Carcinogen-induced mutations in the mouse *c-Ha-ras* gene provide evidence of multiple pathways for tumor progression. *Proc Natl Acad Sci USA* 1990;87:538–42.
- [9] Brown K, Balmain A. Transgenic mice and squamous multistage skin carcinogenesis. *Cancer Metastasis Rev* 1995;14:113–24.
- [10] Burns PA, Kemp CJ, Gannon JV, Lane DP, Bremner R, Balmain A. Loss of heterozygosity and mutational alterations of the p53 gene in skin tumors of interspecific hybrid mice. *Oncogene* 1991;6:2363–9.
- [11] Carlioz A, Touati D. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO J* 1986;5:623–30.
- [12] Farr SB, D'Ari R, Touati D. Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. *Proc Natl Acad Sci USA* 1986;83:8268–72.
- [13] Gardner PR, Nguyen DH, White CW. Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs. *Proc Natl Acad Sci USA* 1994;91:12248–52.
- [14] Fischer SM, Jasheway DW, Klann RC, Butler AP, Patrick KE, Baldwin JK, et al. Correlation of phobol ester promotion in the resistant C57BL/6J mouse with sustained hyperplasia but not ornithine decarboxylase or protein kinase C. *Cancer Res* 1989;49:6693–9.
- [15] Gopalakrishna R, Anderson WB. Ca<sup>2+</sup>- and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. *Proc Natl Acad Sci USA* 1989;86:6758–62.
- [16] Gonzalez-Zulueta M, Ensz LM, Mukhina G, Lebovitz RM, Zwacka RM, Engelhardt JF, et al. Manganese superoxide dismutase protects nNOS neurons from NMDA and nitric oxide-mediated neurotoxicity. *J Neurosci* 1998;18:2040–55.
- [17] Guidot DM, Rapine JE, Kitlowski AD, Flores SC, Nelson SK, Wright RM, et al. Mitochondrial respiration scavenges extramitochondrial superoxide anion via a nonenzymatic mechanism. *J Clin Invest* 1995;96:1131–6.
- [18] Halliwell B, Gutteridge JMC. In: *Free radicals in biology and medicine*, 2nd ed. Oxford: Clarendon Press; 1989. p. 86–123.
- [19] Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancers. *Science* 1991;253:49–53.
- [20] Kanjilal S, Ananthaswamy HN. The role of oncogenes and tumor suppressor genes in ultraviolet-induced carcinogenesis. In: Mukhtar H, editor. *Skin cancer: mechanisms and human relevance*. CRC Press; 1995. p. 305–16.
- [21] Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 1995;270:16483–6.
- [22] Kemp CJ, Donehower LA, Bradley A, Balmain A. Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* 1993;74:813–22.
- [23] Kiningham KK, Oberley TD, Lin SM, Mattingly CA, St. Clair DK. Overexpression of manganese superoxide dismutase (MnSOD) protects against mitochondrial-initiated poly (ADP-ribose) polymerase-mediated cell death. *FASEB J* 1999;13:1601–10.
- [24] Kinscherf R, Claus R, Wagner M, Gehrke C, Kamencic H, Hou D, et al. Apoptosis caused by oxidized LDL is manganese superoxide dismutase and p53 dependent. *FASEB J* 1998;12:461–7.
- [25] Kokonis JM, Wagner A, O'Leary M, Liao S, Hay N. A transcriptional activation function of p53 is dispensable for and inhibitory of its apoptotic function. *Oncogene* 2001;20:659–68.
- [26] Lebovitz RM, Zhang H, Vogel H, Cartwright Jr. J, Dionne L, Lu N, et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci USA* 1996;93:9782–7.
- [27] Li Y, Huang T-T, Carlson EJ, Melov S, Ursell PC, Olson JL, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995;11:376–81.
- [28] Li P-F, Dietzi R, von H. p53 regulates mitochondria membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. *EMBO J* 1999;18:6027–36.
- [29] Majima HJ, Oberley TD, Furakawa K, Mattson MP, Yen HC, Szweda LI, et al. Prevention of mitochondrial injury by manganese superoxide dismutase reveals a primary mechanism for alkaline-induced cell death. *J Biol Chem* 1998;273:8217–24.
- [30] Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria: a potential role in apoptotic signaling. *J Biol Chem* 2000;275:16202–12.
- [31] McSwine-Kennick RL, McKeegan EM, Johnson MD, Morin MJ. Phorbol diester-induced alterations in the expression of protein kinase C isozymes and their mRNAs. *J Biol Chem* 1991;266:15135–43.

- [32] Moll UM, Zaika A. Nuclear and mitochondrial apoptotic pathways of p53. *FEBS Lett* 2001;493:65–9.
- [33] Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* 1988;334:661–5.
- [34] Pence B, Reiners JJ. Murine epidermal xanthine oxidase activity: correlation with degree of hyperplasia induced by tumor promoters. *Cancer Res* 1987;47:6388–92.
- [35] Reiners JJ, Nesnow S, Slaga TJ. Murine susceptibility to two-stage carcinogenesis is influenced by the agent used for promotion. *Carcinogenesis* 1984;5:301–7.
- [36] Reiners JJ, Pence BC, Barcus MCS, Cantu A. 12-*O*-Tetradecanoylphorbol-13-acetate-dependent induction of xanthine dehydrogenase and conversion to xanthine oxidase in murine epidermis. *Cancer Res* 1987;47:1775–9.
- [37] Reiners JJ, Thai G, Rupp T, Cantu AR. Assessment of the antioxidant/prooxidant status of murine skin following topical treatment with 12-*O*-tetradecanoylphorbol-13-acetate and throughout the ontogeny of skin cancer. Part I: quantitation of superoxide dismutase, catalase, glutathione peroxidase and xanthine oxidase. *Carcinogenesis* 1991;12:2337–43.
- [38] Reiners JJ, Kodari E, Cappel RE, Gilbert HF. Assessment of the antioxidant/prooxidant status of murine skin following topical treatment with 12-*O*-tetradecanoylphorbol-13-acetate and throughout the ontogeny of skin cancer. Part II: quantitation of glutathione and glutathione disulfide. *Carcinogenesis* 1991;12:2345–52.
- [39] Reiners JJ, Pavone A, Maldve R, Fisher SM. 12-*O*-Tetradecanoylphorbol-13-acetate-mediated systemic co-promotion in the murine skin multistage carcinogenesis protocol. *Carcinogenesis* 1993;14:411–5.
- [40] Slaga TJ, Fischer SM, Weeks CE, Klein-Szanto AJP, Reiners J. Studies on the mechanisms involved in multistage carcinogenesis in mouse skin. *J Cell Biochem* 1982;18:99–119.
- [41] St. Clair DK, Oberley TD, Ho YS. Overproduction of human Mn-superoxide dismutase modulates paraquat-mediated toxicity in mammalian cells. *FEBS Lett* 1991;293:199–202.
- [42] St. Clair DK, Wan XS, Oberley TD, Muse KE, St. Clair WH. Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. *Mol Carcinog* 1992;6:238–42.
- [43] St. Clair DK, Oberley TD, Muse KE, St. Clair WH. Expression of manganese superoxide dismutase promotes cellular differentiation. *Free Rad Biol Med* 1994;18:275–82.
- [44] van Loon APGM, Pesold-Hurt B, Schatz G. A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hypersensitive to oxygen. *Proc Natl Acad Sci USA* 1986;83:3820–4.
- [45] Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics* 2003;16:29–37.
- [46] Verma AJ, Pong RC, Erickson D. Involvement of protein kinase C activation in ornithine decarboxylase gene expression in primary culture of newborn mouse epidermal cells and in skin tumor promotion by 12-*O*-tetradecanoylphorbol-13 acetate. *Cancer Res* 1986;46:6149–55.
- [47] Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J Biol Chem* 1998;273:28510–5.
- [48] Wong GHW, Elwell JH, Oberley LW, Goeddel D. Manganese superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* 1989;58:923–31.
- [49] Zhang Y, Marcillat O, Giulivi C, Ernster L, Davies KJ. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J Biol Chem* 1990;265:16330–6.
- [50] Zhao Y, Kiningham KK, Lin S-M, St. Clair DK. Overexpression of MnSOD prevents murine fibrosarcoma (FsaII) cells from apoptosis and promotes a differentiation program upon treatment with 5-azacytidine: involvement of MARK and NF- $\kappa$ B pathways. *Antioxid Redox Signal* 2001;3:375–85.
- [51] Zhao Y, Xue Y, Oberley TD, Kiningham KK, Lin S-M, Yen H-C, et al. Overexpression of MnSOD suppresses tumor formation by modulation of AP-1 signaling in a multistage skin carcinogenesis model. *Cancer Res* 2001;61:6082–8.
- [52] Zhao Y, Oberley TD, Chaiswing L, Lin S-M, Epstein CJ, Huang T-T, et al. Manganese superoxide dismutase deficiency enhances cell turnover via tumor promoter-induced alteration in AP-1 and p53-mediated pathways in a skin cancer model. *Oncogene* 2002;21:3836–46.