Manganese Superoxide Dismutase Modulates Interleukin-1α Levels in HT-1080 Fibrosarcoma Cells

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Reactive oxygen species of mitochondrial origin have been implicated in regulating the expression of several tumor necrosis factor (TNF)-induced genes. Manganese superoxide dismutase (Mn-SOD) is one of many genes, but only antioxidant enzyme, induced in response to tumor necrosis factor. Mn-SOD is a nuclear-encoded mitochondrial matrix protein and serves a protective function by detoxifying superoxide. To address the role of superoxide in regulating gene expression in response to TNF, we have constitutively overexpressed Mn-SOD in a human fibrosarcoma cell line and asked what effect this has on the expression of a number of TNF-responsive genes using reverse transcription-polymerase chain reaction chain reaction. Of the TNF-induced transcripts analyzed, only interleukin-1α (IL-1α) was modulated in response to Mn-SOD overexpression. In all cases of Mn-SOD overexpression, IL-1α protein and mRNA levels were lowered constitutively and in response to TNF when compared to the parental and mock-transfected cell lines. The induction of IL-1α by TNF can also be decreased by growth in 3% oxygen as compared to growth in 21% O2; in addition, growth in low oxygen lowers the basal level of IL-1α protein. The effect of Mn-SOD overexpression on IL-1α expression can be overcome by treatment with the protein kinase C activator, phorbol 12-myristate 13-acetate. Mn-SOD overexpression and low oxygen alter IL-1α mRNA levels by decreasing the stability of the IL-1α mRNA. These findings indicate that both Mn-SOD and O2 may regulate the levels of a cellular oxidant involved in both basal and TNF-induced IL-1α expression, presumably superoxide.

Manganese superoxide dismutase (Mn-SOD)1 is one of three superoxide dismutases found in mammalian cells which catalyze the dismutation reaction of superoxide to hydrogen peroxide (1). The strategic localization of this enzyme in mitochondria allows it to be readily available for removal of superoxide generated via leakage of electrons from the mitochondrial respiratory chain (2, 3). Superoxide is a highly reactive oxygen radical, which is capable of directly or indirectly oxidizing protein, lipids, and nucleic acids. Thus, superoxide has been implicated as an important pathogenic mediator in various disorders including cancer (4, 5), inflammation, and ischemic injury (6, 7). Numerous conditions enhance the generation of superoxide in cells including: UV irradiation, hyperoxia, bacterial infection, and inflammation (4–8). To combat these stimuli, the cell responds by coordinately elevating the levels of Mn-SOD, presumably to circumvent any superoxide-mediated damage (9).

A number of studies have demonstrated an increase in Mn-SOD both in vitro and in vivo in various cell lines and tissues in response to TNF (10–14). Mn-SOD levels are also modulated in response to other inflammatory stimuli including IL-1, LPS, interferon-γ, and irradiation (13, 15–17). Mn-SOD serves a protective role by detoxifying O2− and preventing superoxide-mediated damage to the mitochondria.

Exposure to high levels of reactive oxygen species is toxic to all cells, but lower levels cause extensive adaptive responses, including greatly enhanced resistance (18, 19). Reactive oxygen species, such as superoxide and hydrogen peroxide, have also been proposed to play a role in cellular signal-transduction (20, 21). Oxygen-derived radicals may mediate their effect via oxidative modifications of redox-sensitive sites on transcription factors. Oxygen intermediates have been implicated in the regulation of the transcription factors NFκB and AP-1, which lead to the transcriptional activation of a multitude of genes involved in inflammation, differentiation, and proliferation (20, 22–24). The inflammatory cytokine, TNF, is a potent activator of NFκB (20, 21). The pathway leading to TNF-mediated activation of NFκB has been shown to require oxygen-derived radicals of mitochondrial origin (25, 26). These findings prompted us to investigate what role Mn-SOD may play in regulating the levels of these oxygen-derived signal molecules of mitochondrial origin.

EXPERIMENTAL PROCEDURES

Cell Culture—Human fibrosarcoma HT-1080 cells were cultured in Minimum essential medium supplemented with 10% fetal calf serum. Cells were treated with recombinant human TNF (R & D Systems, Minneapolis, MN), phorbol 12-myristate 13-acetate (PMA), or cycloheximide (Sigma).

Construction of Recombinant Expression Vectors—The vector pRC/ CMV was purchased from Invitrogen. The plasmid pGEM/Mn-SOD, which contains a full-length Mn-SOD cDNA (27), was a gift of Dr. David Goeddel (Genentech). The pRC/CMV was digested with HindII, and the ends were filled in with the Klenow fragment of DNA polymerase. The Mn-SOD cDNA was excised with EcoRI and HindII and the ends were filled in. The 760-base pair cDNA was then inserted into the Smal site of the pRC/CMV expression vector to obtain pRC/Mn-SOD expression vectors with the cDNA insert in either sense or antisense orientation. Restriction digests revealed the orientation of the Mn-SOD cDNA in the vector. Construction of the pCAT1.8 vector was performed by inserting the 1.8-kb PCR-amplified genomic DNA fragment into the SalI site of the pCATBASIC vector (Promega) upstream of the CAT reporter gene. The 1.8-kb base pair fragment comprised a...
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region from position 297 to 2126 of known sequence for the human IL-1α gene (accession number X03833). The IL-1α insert and orientation was confirmed by restriction digest, Southern blotting, and DNA sequencing.

Mn-SOD Transfection—HT-1080 cells were transfected with Lipofectin (Life Technologies, Inc.). Approximately 2 μg of pRC/Mn-SOD (sense or antisense) was mixed with 30 μg of Lipofectin in a total volume of 100 μl and incubated at room temperature for 5 min; 720 μl of minimal essential medium minus serum was added before transferring to a single well of a six-well cluster plate containing ~70% confluent HT-1080 cells. After 4 h, 1.2 ml of minimal essential medium supplemented with 3.3% calf serum was added. The next day, the cells were transferred to a 24-well culture plate. Selection with 1.25 units of Tau polymerase (Promega, Madison, WI) or 1.25 units of Tau polymerase (Promega, Madison, WI) was started the next day. After 10 days, resistant cells were harvested and approximately 100 cells were plated in a 75-cm² flask. The cells were allowed to grow until visible colonies were observed. Single colonies were then picked up and transferred to 24-well culture plates. This cloning protocol has been described previously (28). After cloning, cells were cultured continuously in selective medium.

Enzyme Assays—Cells were grown to confluence in 25-cm² culture flasks and incubated with TNF for 4 h. Cells were then washed twice and harvested from each flask with 2 ml of phosphate-buffered saline, pH 7.2, plus 1 mM EDTA. After a brief centrifugation, the pellet was resuspended in 200 μl of potassium phosphate buffer, pH 7.8, containing 0.1 mM EDTA, and sonicated for 15 s. The lysate was centrifuged at 10,000 × g for 10 min and the supernatant collected; this centrifugation was repeated twice. The protein concentration of the final supernatant was determined using the BCA protein reagent (Pierce). SD activity was assayed according to the method of Beauchamp and Fridovich (29).

Lysate supernatants were analyzed by electrophoresis in a discontinuous polyacrylamide gel, consisting of a 5% stacking gel (pH 6.8) and a 10% running gel. To visualize SOD activity, the gels were incubated for 15 min in the dark with 2.5 mM nitro blue tetrazolium, 30 mM TEMED, 0.028 mM riboflavine, 50 mM phosphate buffer, pH 7.8, washed twice in deionized water, and then exposed to fluorescent light until clear zones were visible. Mn-SOD activity was assayed by the method of McCord and Fridovich (30), using xanthine/xanthine oxidase as the source for superoxide radicals. One enzyme unit of superoxide dismutase is defined as the amount that inhibits cytochrome c reduction by 50%, at room temperature at pH 7.8. Cu/Zn-SOD activity was measured as the cyanide-inhibitable fraction of total SOD activity.

Detection of IL-1α—Cells were collected in phosphate-buffered saline, sonicated for 30 s, and centrifuged twice for 15 min at 10,000 × g. The supernatant was assayed for IL-1α with enzyme-linked immunosorbent assay kits purchased from R & D Systems. The sensitivity of this assay was 0.3 pg for IL-1α.

Western Analysis—20 μg of cell lysate proteins were separated in 10% denaturing polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with Blotto containing 5% nonfat dry milk, 0.1% Tween 20 in phosphate-buffered saline at room temperature for 30 min and incubated with a 1:1500 dilution of an anti-human kidney Mn-SOD polyclonal antibody (gift of Dr. Larry W. Oberley, University of Iowa) at 4°C overnight. Membranes were washed three times in Blotto for 15 min and incubated for 1 h with a 1:1000 dilution of alkaline phosphatase-conjugated secondary goat anti-rabbit antibody (Amersham). Membranes were washed three times with Blotto and developed with an alkaline phosphatase-conjugated substrate kit (Bio-Rad).

Reverse Transcription PCR (RT-PCR) mRNA Analysis—HT-1080 cells (0.1 ml of a 5 × cell suspension) were cultured in 25-cm² flasks. 24 h after treatments were performed as described in the figure legends. RNA was extracted using the RNAzol kit (Biotecx Laboratories Inc., Houston, TX), according to the manufacturer’s instructions. Following isolation, the RNA was precipitated with isopropanol alcohol, pellets washed in 70% ethanol, and air-dried. The dried pellets were resuspended in 25 μl of autoclaved water (chloroform was not used) for reverse transcribing. For reverse transcription, 2.0 μg of RNA in 10 μl was utilized to synthesize cDNA primed with olig(dT) in the following reaction mixture: 1 μl of SuperScript II reverse transcriptase (Life Technologies, Inc.) with 6 μl of 5 × reverse transcriptase buffer (250 mM Tris-Cl, pH 8.3, at 42°C, 50 mM MgCl₂, 300 mM KCl, 50 mM dithiothreitol), 0.5 μl of RNase inhibitor, 1.5 μl (1.0 mg/ml) of oligo(dT), 6 μl of 2.5 mM dNTPs (from Boehringer Mannheim), and 3.0 μl of bovine serum albumin (1 mg/ml). After a 5 min preincubation of the total RNA at 65°C and charring on ice, the reaction mix was then added. The reaction was carried out for 1 h at 42°C, then 95°C for 5 min, followed by the addition of 20 μl of H₂O and storage at –20°C until use.

For PCR amplification, the oligonucleotide primers were designed utilizing the OLIGO primer analysis software (NBI). Primers were as follows: 3′- 5′-AGAGGACATGTTGGTATGAC-3′; 5′- 5′-GTAATGCAGCAGCCGTGAAGT-3′; 5′- 5′-GAPDH, 3′- 3′-CATCATCCTCTG-5′; 3′- 5′-CATCCTG-3′. All primers were designed to span introns as to detect contaminating DNA if present and employed at 1.0 μM final concentrations. The PCR mixture (50 μl) consisted of 2.5 μl of each PCR primer (20 μM), 4.0 μl of dNTPs (2.5 mM), 5 μl of 10 × PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, 15 mM MgCl₂, 0.1% gelatin), 30.75 μl of H₂O, 5 μl of cDNA, and 1.25 units of Taq polymerase (Perkin-Elmer). Amplification was carried out in a programmable thermal cycler (Perkin Elmer, DNA Thermal Cycler 480). The amplification program was three cycles of 94°C for 60 s, 60°C for 2 min, 72°C for 2 min; followed by 27 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 15 s. Following 30 cycles, the temperature was held at 72°C for 3 min. Positive amplification was determined by agarose gel electrophoresis. All experiments utilizing the reverse transcription PCR technique to measure relative mRNA abundance were performed independently in triplicate.

Nuclear Run-off Analysis—Nuclei were prepared from 5 × 10⁴ HT-1080 cells. The nascent RNA was labeled as reported previously (31). Denatured Mn-SOD, IL-1α, and GAPDH were slot-blotted onto nitrocellulose membranes; pS2 was used as a negative control. The membranes were hybridized with 2 × 10⁶ cpm of nuclear RNA for 36 h and thoroughly washed in 1.5% SDS at 65°C before autoradiography.

Transient Transfection of HT-1080 Cells—When cultures reached 60% confluence in 25 cm² flasks, the medium was changed and 2.5 ml of serum-free Opti-MEM medium (Life Technologies, Inc.) containing 2.0 μg of pCAT1.8 or pCATBasic, 2.0 μg of pRSVgal, and 12.5 μl of Lipofectamine (Life Technologies, Inc.). Cells were incubated with the DNA/Lipofectamine mixture for 5 h, at which time treatments were begun in serum-containing minimum essential medium. Cells were treated with LPS (100 ng/ml, PMA (100 ng/ml), or TNF (10 ng/ml) for 12–18 h. Washed twice in cold phosphate-buffered saline, harvested by scraping into 600 μl of cold 100 mM Tris-HCl, pH 7.8, and subjected to the chloramphenicol acetyltransferase (CAT) and β-galactosidase assays.

For the CAT assay cells were lysed by four freeze/thaw cycles and centrifuged for 15 min at 4°C. A portion of the supernatant was saved for β-galactosidase analysis, while the remainder of the supernatant was heated for 5 min at 65°C. Equivalent amounts of protein sample were subjected to the CAT assay by using [3H]acetyl-CoA (0.5 μCi/reaction) and chloramphenicol (1 mM/reaction) and the release of lipid-soluble [3H]chloramphenicol was monitored for 1–3 h at 37°C by using scintillation spectrophotometry. β-Galactosidase activity was used to normalize transfections, using a chemiluminescent assay.

RESULTS

Mn-SOD Activity and Protein—A pRC/Mn-SOD expression vector containing the complete sense coding region of the human Mn-SOD gene, under the control of a cytomegalovirus promoter, was transfected into HT-1080 fibrosarcoma cells. This vector also contained a neomycin resistance gene driven by the SV40 early promoter, which allowed selection for resistance to G418. Mn-SOD activity of stable transfectants was checked utilizing a Mn-SOD PAGE activity assay, Western blot analysis and a spectrophotometric assay (Fig. 1). The level of Mn-SOD in the HT-1080 and control transfectants (CMVA and CMV2) were similar and equally induced in all three cell lines in response to TNF (Fig. 1A, compare – and + TNF treatments). Mn-SOD activity levels were increased in all the Mn-SOD transfectants when compared to the control cell lines. Western blot analysis closely reflected both the spectrophotometric and PAGE activity assay results (Fig. 1B). In all cases, transfection with the vector alone had no effect on the constitutive or TNF-inducible Mn-SOD activity or immunoreactive protein levels. Furthermore, the overexpressed Mn-SOD level was higher than the level of Mn-SOD in the HT-1080 or control transfectants in response to TNF.

To establish that the overexpressed Mn-SOD was associated with mitochondria, subcellular fractionation was performed as described by Vokely and Fahimi (32). SD activity gels on
cytosolic, nuclear, mitochondrial, and peroxisomal fractions demonstrated that the Mn-SOD activity resided in the mitochondria (data not shown).

Mn-SOD Overexpression and IL-1α Protein—Utilizing RT-PCR a number of TNF-induced transcripts including the endogenous Mn-SOD, IL-1α, IL-1β, IL-6, and plasminogen activator inhibitor type-2 were analyzed, of which only IL-1α was modulated in response to Mn-SOD overexpression. The effect of Mn-SOD overexpression on IL-1α protein levels was also determined utilizing a specific enzyme-linked immunosorbent assay in cell extracts (Fig. 2). In response to TNF, IL-1α protein levels are increased in the HT-1080 and control transfectants. However, all the cell lines with increased Mn-SOD activity have a reduced level of IL-1α protein both constitutively and in response to TNF. In addition, Mn-SOD overexpression does not affect the cytotoxicity of TNF, which implies that the TNF-receptor complex has not been affected.

The ability of Mn-SOD overexpression to alter both the constitutive and TNF-induced IL-1α protein levels was also detected at the mRNA level by utilizing RT-PCR for detection of IL-1α mRNA abundance (Fig. 3). Fig. 3A shows the relative abundance of the IL-1α RT-PCR product of the cell lines characterized in Fig. 2. The RT-PCR results closely mimic the enzyme-linked immunosorbent assay results (Fig. 2) and demonstrate that Mn-SOD overexpression is involved in preventing the increase in IL-1α mRNA in response to TNF. The mRNA levels of the housekeeping gene GAPDH were also monitored as an internal control for PCR-amplification. In all cases GAPDH levels were found to be equal except for a slight elevation in HT-3.6 (Fig. 3A), which accounts for the apparent increase in IL-1α mRNA level in that cell line. This increase in IL-1α mRNA levels was not observed in two other independent experiments and was not observed at the the protein level (Fig. 2).

IL-1α increases in response to TNF have been shown to occur via enhancement of mRNA stability, mediated by protein kinase C (33). Phorbol esters activate transcription of IL-1α mRNA and enhance IL-1α mRNA stability (33, 34). The present data demonstrate that Mn-SOD overexpression reduces the TNF-mediated induction of IL-1α. Therefore, we tested if Mn-SOD overexpression affects the protein kinase C-mediated increase in IL-1α mRNA levels. IL-1α induction by PMA was not altered by Mn-SOD overexpression in any of our cell lines (Fig. 3B).

Oxygen-dependent Regulation of Basal and TNF-induced IL-1α Levels—To address the involvement of oxygen-derived reactive oxygen metabolites in regulating IL-1α levels, we chose to alter the relative levels of oxygen in the cell. Consistent estimates suggest that 1–2% of the oxygen consumed by the cell is converted to O₂ in electron transport. Therefore, by reducing the amount of available oxygen, one would predict a concomitant decrease in mitochondrial superoxide production. Cells were incubated in 3% O₂, and then the effects of TNF on IL-1α expression were tested. As shown in Fig. 4, there was a decrease in both the basal and TNF-induced levels of the IL-1α protein in cells incubated at 3% as compared to 21% O₂. Mn-SOD overexpression also caused a further reduction in IL-1α protein levels alone and in response to TNF. The effects observed between the respective groups (i.e., control + TNF at 3% O₂ versus control + TNF at 21% O₂) were significant (p < 0.005) using an unpaired Student’s t test with unequal variances.

Modulation of IL-1α mRNA Levels Occurs Post-transcriptionally—Previous studies have demonstrated that IL-1α mRNA abundance can be regulated both transcriptionally or post-transcriptionally. The above data suggest that IL-1α levels are dramatically altered by growth in 3% O₂ or by overexpression of Mn-SOD. Nuclear run-off experiments were performed to establish whether the reduction of IL-1α mRNA in
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3% oxygen was due to a decreased rate of transcription. Both IL-1α and Mn-SOD mRNA were actively transcribed in control cells grown in 21% oxygen, but growth in 3% oxygen did not appear to decrease the rate of transcription of the IL-1α mRNA with only a slight decrease in Mn-SOD mRNA (Fig. 5, lower panel). The nuclear run-off experiments indicate that 3% oxygen does not decrease IL-1α mRNA by decreasing its rate of transcription.

To determine whether the increase in Mn-SOD levels negatively regulate IL-1α promoter activity, we transiently transfected the parental, control cells (CMV) or Mn-SOD-overexpressing cell lines with the plasmid pCAT1.8 containing the CAT reporter gene linked to a 1.8-kilobase pair region of the human IL-1α promoter. Mn-SOD overexpression had no effect on the basal IL-1α promoter activity when compared to the control (CMV) cell lines (Fig. 5, upper panel). To assess the activity of the promoter, a number of known inducers of IL-1α were used, of which only LPS was shown to stimulate promoter activity in these cell lines. These results confirm that the plasmid pCAT1.8 is active in response to LPS treatment, but is not affected by overexpression of Mn-SOD. Both the promoter studies and the nuclear run-off analysis suggest that Mn-SOD overexpression or low oxygen do not alter the transcription rate of IL-1α mRNA. Alternatively, Mn-SOD overexpression and low oxygen may alter the stability of IL-1α mRNA.

Gorospe et al. (33) have previously demonstrated that the increase in IL-1α levels in response to TNF is due to an increase in the TNF-induced IL-1α mRNA. Experiments were designed to test the effect of oxygen and Mn-SOD overexpression on IL-1α mRNA stability. The protein synthesis inhibitor cycloheximide is commonly used to detect unstable mRNAs by increasing their half-life. HT-1080 cells were treated with cycloheximide for 4 h in low (3%) or high (21%) oxygen. Following treatment with cycloheximide, the cells were washed and the decrease in mRNA levels was checked utilizing RT-PCR (Fig. 6, inset). Quantitation of RT-PCR band intensity showed that the apparent half-life of IL-1α mRNA was 2-fold greater in high oxygen than in low oxygen. Mn-SOD overexpression was also shown to specifically decrease IL-1α mRNA half-life with no effect on IL-1β, IL-6 or GAPDH mRNA half-lives (data not shown). Therefore, the decrease in IL-1α levels observed in cell lines overexpressing Mn-SOD or in the presence of low oxygen results from an accelerated decay of IL-1α mRNA.

**DISCUSSION**

Our findings suggest that Mn-SOD plays a role in the TNF signal pathway leading to increased IL-1α expression. Mn-SOD overexpression alters both basal and TNF-induced IL-1α mRNA levels, but has no effect on the PMA-mediated increase in IL-1α gene expression. PKC inhibitors have been shown to block the TNF-mediated increase in IL-1α mRNA levels (33). The Mn-SOD overexpression in contrast to PKC inhibitors does not block but abates the increase in IL-1α and suggests that both PKC activation and mitochondrial reactive oxygen species may be required for the maximal increase in IL-1α levels in response to TNF. However, Mn-SOD overexpression has no effect on the induction of IL-1α by the potent protein kinase C activator PMA. PMA stimulates a 30-fold increase in IL-1α levels as compared to the 10-fold increase observed with TNF.
to alter the levels of newly transcribed RNA for IL-1α also substantiates the promoter analyses.

Both basal and TNF-induced IL-1α levels are lowered under 3% O₂ (Fig. 4), while the levels of either IL-1β or IL-6 are not affected. Mn-SOD overexpression decreases the induction of IL-1α by TNF, while under low O₂ this difference is more pronounced. Both low O₂ and Mn-SOD overexpression would serve to directly decrease superoxide levels in the mitochondria. This suggests that the redox signal leading to expression of IL-1α in response to TNF may be of mitochondrial origin. Mitochondrial electron transport chain functions are, of course, quite sensitive to damage and disruption by superoxide, hydrogen peroxide, and related oxygen species (37). Schulze-Osthoff et al. have also shown that TNF-mediated activation of NFκB involves a reactive oxygen species of mitochondrial origin (26).

IL-1α is a primary response cytokine whose levels are modulated under a variety of pathological conditions. Our data suggest that IL-1α expression may be redox-regulated. Mn-SOD may modulate IL-1α expression by altering the levels or activity of a putative redox factor involved in signal transduction. Mn-SOD overexpression also mimics growth in low oxygen in terms of IL-1α expression and indicates that physiological redox events also regulate IL-1α mRNA stability. The primary function of Mn-SOD is to remove toxic superoxide generated as a consequence of normal metabolism. The induction of Mn-SOD by TNF may not only serve to decrease the toxicity of superoxide, it may also regulate the levels of a superoxide-reactive intermediate involved in the regulation of IL-1α. The induction of Mn-SOD following TNF treatment lags behind that of IL-1α. Studies in HT-1080 fibrosarcoma have shown that IL-1α levels reach maximal in 4–8 h after TNF treatment, followed by a decrease to half of maximal after 18 h of TNF treatment (33). This time frame would fit perfectly with the maximal induction of Mn-SOD, which occurs between 12 and 18 h (38). Therefore, it is plausible that Mn-SOD may indeed serve to regulate reactive oxygen species involved in the TNF signal pathway leading to IL-1α expression. The finding that Mn-SOD overexpression alters IL-1α mRNA levels suggests that a superoxide-reactive intermediate of mitochondrial origin may be involved in regulating IL-1α mRNA stability both constitutively and in response to TNF.

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REFERENCES
1. Fridovich, I. (1987) Adv. Enzymol. 58, 61–97
2. Boveris, A., and Chance, B. (1973) Biochem. J. 134, 707–716
3. Boveris, A., Oshino, N., and Chance, B. (1972) Biochem. J. 128, 617–630
4. Ames, B. N. (1989) Mutat. Res. 214, 41–46
5. Ames, B. N. (1983) Science 221, 1256–1264
6. McCord, J. M., and Roy, R. S. (1982) Can. J. Physiol. Pharmacol. 60, 1348–1352
7. McCord, J. M. (1985) FASEB J. 1586–15869
8. Ku, H. H., Brunk, U. T., and Sohal, R. S. (1993) Free Radical Biol. Med. 15, 621–627
9. Wong, G. H. W., Kamb, A., Tartaglia, L. A., and Goeddel, D. V. (1992) in Molecular Biology of Free Radical Scavenging Systems (Scandallios, J. G., ed) pp. 69–96, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Wong, G. H. W., and Goeddel, D. V. (1988) Science 242, 941–944
11. Wong, G. H. W., Elwell, J. H., Oberley, L. W., and Goeddel, D. V. (1989) Cell 58, 923–931
12. Ash, K., Watanebe, Y., Mizoguchi, H., Matawar, M., Ono, M., Kudo, N., and Kowano, M. (1989) Biochem. Biophys. Res. Commun. 162, 794–801
13. Visner, G. A., Dougall, W. C., Wilson, J. M., Burr, I. A., and Nick, H. S. (1990) J. Biol. Chem. 265, 2856–2864
14. Melendez, J. A., and Baglioni, C. (1992) Free Radical Biol. Med. 12, 151–159
15. Masuda, A., Longo, D. L., Kobayashi, Y., Appella, E., Oppenheim, J. J., and Matsushima, K. (1988) Free Radical Biol. Med. 2, 1007–3001
16. Harris, C. A., Derbin, K. S., Hunte-McDonough, B., Krauss, M. R., Chen, K. T., Smith, D. M., and Epstein, L. B. (1993) Nature 291, 314–319
17. Akashi, M., Hachiya, M., Paquette, R. L., Osawa, Y., Shimizu, S., and Suzuki, G. (1995) J. Biol. Chem. 270, 15864–15869
18. Davies, J. M. S., Lowry, C. V., and Davies, K. J. A. (1995) Arch. Biochem. Biophys. 317, 1–6
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| Author(s)                                      | Year(s) | Journal/Volume | Pages/DOI |
|------------------------------------------------|---------|----------------|-----------|
| Wiese, A. G., Pacifici, R. E., and Davies, K. J. A. | 1995    | Arch. Biochem. Biophys. 318 | 231-240   |
| Schreer, R., Rieber, P., and Baueuerle, P. A.     | 1991    | EMBO J. 10      | 2247-2258 |
| Schreer, R., Albermann, K., and Baueuerle, P. A.  | 1992    | Free Radical Res. Commun. 17 | 221-237  |
| Meyer, M., Schreer, R., and Baueuerle, P. A.     | 1993    | EMBO J. 12      | 2005-2015 |
| Suzuki, Y. J., Mizuno, M., and Packer, L.        | 1994    | Immuno. 153     | 5008-5015 |
| Suzuki, Y. J., Agarwal, B. B., and Packer, L.    | 1992    | Biochem. Biophys. Res. Commun. 189 | 1709-1715 |
| Schulze-Osthoff, K., Bakkers, A. C., Vanhaesebroeck, B., Beyaert, R., Jacob, W. A., and Fiers, W. | 1992 | J. Biol. Chem. 267 | 5317-5323 |
| Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G., and Fiers, W. | 1993 | EMBO J. 12 | 3095-3104 |
| Beck, Y., Oren, R., Amit, B., Levanon, A., Gorecki, M., and Hartman, J. R. | 1987 | Nucleic Acids Res. 15 | 9076     |
| Waldman, A. S.                                  | 1991    | BioTechniques 10 | 138       |
| Beauchamp, C., and Fridovich, I.                 | 1971    | Anal. Biochem. 44 | 276-287  |
| McCord, J. M., and Fridovich, I.                | 1969    | J. Biol. Chem. 244 | 6049-6055 |
| Groudine, M., Peretz, M., and Weintraub, H.     | 1981    | Mol. Cell. Biol. 1 | 281-288  |
| Volkyl, A., and Fahimi, H. D.                    | 1994    | Cell Biology: A Laboratory Handbook (Célis, J. E., ed) pp. 550-556, Academic Press Inc., San Diego |
| Gorospe, M., Kumar, S., and Baglioni, C.         | 1993    | J. Biol. Chem. 268 | 6214-6220 |
| Lee, W. Y., Butler, A. P., Loomiskar, M. F., and Fischer, S. M. | 1994 | J. Biol. Chem. 269 | 17971-17980 |
| Kolesnick, R., and Golde, D. W.                 | 1994    | Cell 77         | 325-328   |
| Carter, B. Z., and Maiter, J. S.                | 1991    | Lab. Invest. 65  | 610-621   |
| Zhang, Y., Mardillat, O., Giulivi, C., Ernster, L., and Davies, K. J. A. | 1990 | J. Biol. Chem. 265 | 16330-16336 |
| Melendez, J. A., and Baglioni, C.                | 1993    | Free Radical Biol. Med. 14 | 601-608  |