Manganese superoxide dismutase (Sod2) removes mitochondrially derived superoxide (O2) at near-diffusion limiting rates and is the only antioxidant enzyme whose expression is regulated by numerous stimuli. Here it is shown that Sod2 also serves as a source of the intracellular signaling molecule H2O2. Sod2-dependent increases in the steady-state levels of H2O2 led to ERK1/2 activation and subsequent downstream transcriptional increases in matrix metalloproteinase-1 (MMP-1) expression, which were reversed by expression of the H2O2-detoxifying enzyme, catalase. In addition, a single nucleotide polymorphism has recently been identified (1G/2G) at base pair −1607 that creates an Ets site adjacent to an AP-1 site at base pair −1602 and has been shown to dramatically enhance transcription of the MMP-1 promoter. Luciferase promoter constructs containing either the 1G or 2G variation were 25- or 1000-fold more active when transiently transfected into Sod2-overexpressing cell lines, respectively. The levels of MMP-2, -3, and -7 were also increased in the Sod2-overexpressing cell lines, suggesting that Sod2 may function as a “global” redox regulator of MMP expression. In addition, Sod2+/− mouse embryonic fibroblasts failed to respond to the cytokine-mediated induction of the murine functional analog of MMP-1, MMP-13. This study provides evidence that the modulation of Sod2 activity by a wide array of pathogenic and inflammatory stimuli may be utilized by the cell as a primary signaling mechanism leading to matrix metalloproteinase expression.

Reactive oxygen species are constantly generated in aerobic organisms during normal metabolism as well as in response to both internal and external stimuli. Although low levels of reactive oxygen species are indispensable in several physiological processes ranging from cell proliferation to apoptosis, high levels of reactive oxygen species and their inefficient removal result in oxidative stress that can be potentially toxic to the cells. An imbalance in reactive oxygen species has been hypothesized to play a causative role in many disease pathologies, including cancer (1), ischemia/reperfusion injury (2), degenerative diseases such as photoaging (3), atherosclerosis (4), osteoarthritis and neurodegeneration (5). A feature often associated with these diseases is a malfunctioning of the connective tissue remodeling process due to increased activity of extracellular matrix-degrading metalloproteinases (MMPs). The matrix metalloproteinase family is composed of at least 20 zinc-dependent extracellular endopeptidases that are primarily expressed as inactive precursors orzymogens (6). MMP expression is low in normal tissues and is induced when extracellular matrix remodeling is required; in all the above-mentioned disease pathologies and during senescence, there is a coincident increase in the production of reactive oxygen species and MMPs.

The mitochondrial manganese-containing superoxide dismutase (Sod2) is one of the numerous enzymatic and non-enzymatic defenses that exist to combat oxidant injury. Sod2 catalyzes the diffusion-limited removal of superoxide to H2O2 at its site of production. It has recently been shown that the Sod2-dependent production of H2O2 leads to the expression of MMP-1 (7). In addition, Sod2 (CD11/Sod2mKO) heterozygous knockout mice develop premature interstitial fibrosis and accumulate collagen when compared with wild-type age-matched controls (8). Whether the accumulation of collagen in the Sod2−/− mice is due to increased collagen synthesis or degradation is not known. These observations have led us to evaluate the importance of Sod2 in regulating the expression of the collagen-degrading enzyme MMP-1.

This study demonstrates that the Sod2-dependent H2O2 production leads to the activation of the ERK1/2 signaling cascade and subsequent downstream transcriptional increases in MMP-1 expression. Furthermore, a specific single nucleotide polymorphism sensitizes the MMP-1 promoter to Sod2-dependent activation. Thus, the cell has evolved to utilize the mitochondria as an important source of the signaling molecule H2O2 via Sod2 and provides a mechanistic rationale for the increased expression of Sod2 and MMP in numerous disease pathologies.

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The abbreviations used are: MMPs, matrix metalloproteinases; ERK, extracellular signal-regulated kinase; MEM, modified Eagle’s medium; Mn-SOD, manganese-superoxide dismutase; MEFs, mouse embryonic fibroblasts; TNF, tumor necrosis factor; IL-1, interleukin-1; IL-1RA, interleukin-1 receptor antagonist; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DCF, dichlorofluorescein; CMV, cytomegalovirus; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase.
Sod2 Signals MMP Expression

FIG. 1. Redox modulation of MMP-1. A, RT-PCR analysis of MMP-1 in control transfectants (CMV and CMVmCAT), Sod2 overexpressors (HT15 = 15-fold increase in Sod2 levels), and cells overexpressing catalase in the cytosolic (CAT) or mitochondrial (mCAT) compartment and Sod2. GAPDH was co-amplified in the linear amplification range as a loading control. B, left panel, a representative FACScan analysis of DCF fluorescence in untreated CMV, HT15, and HT15mCAT cell lines (stippled trace) or DCF-treated CMV (gray trace), HT15 (black trace), and HT15mCAT (shaded area) cell lines; right panel, flow cytometric analysis of intracellular peroxides using DCF fluorescence in CMV, HT15, and HT15mCAT cell lines. The mean fluorescence of DCF was analyzed in a FACScan. Values are in arbitrary fluorescence units and represent the mean ± S.E. of four independent experiments for untreated and DCF-treated cell lines. * and †, p < 0.005 compared with control and HT15, respectively.

MATERIALS AND METHODS

Cell Culture and Reagents—HT-1080 human fibrosarcoma cells were cultured in MEM supplemented with 10% fetal calf serum, 1000 units/ml penicillin, 500 μg/ml streptomycin, and 1 mg/ml neomycin in a 37 °C humidified incubator containing 5% CO2. Constructions of recombinant Mn-SOD and catalase plasmids and transfections were previously described in detail (9, 10). Mouse embryonic fibroblasts (MEFs) derived from Sod2-deficient mice (CD1Sod2−/−) were kindly provided by Drs. C. J. Epstein and T. T. Huang (University of California, San Francisco, CA). The fibroblasts were cultured individually in 50% Dulbecco’s modified Eagle’s medium and 50% F-12 supplemented with 15 mM HEPES and 1-glutamine (Fisher) and 10% fetal calf serum in 25-cm2 flasks in 3% oxygen. They were split 1:4 and passaged every 5 days. Cells were treated with recombinant human TNF (R&D Systems, Minneapolis, MN), phorbol 12-myristate 13-acetate, or mouse IL-1 (R&D Systems).

Construction and Transfection of Full-length Human IL-1α Expression Vector—A full-length 2.4-kilobase pair IL-1α sequence was PCR-amplified with NolI/XbaI ends from an HT-1080 fibrosarcoma cell cDNA library. The resulting product was inserted into the pcR2.1 TA cloning vector (Invitrogen). A 2.4-kilobase pair fragment containing the full-length IL-1α sequence was then removed from the resulting plasmid by digestion with NolI and XbaI. This fragment was ligated into pCMV/Mu, precut with NolI and XbaI. The pCMV/IL-1α construct was sequenced bidirectionally using the Taq DyeDeoxy terminator cycle sequencing kit and an ABI Model 310 DNA sequencer (Applied Biosystems, Inc.). Cells were transfected with 1 μg of the plasmids and LipofectAMINE Plus reagent (Life Technologies, Inc.) according to the instructions provided by the manufacturer. HT-1080 cells were washed, and 0.8 μl of serum-free MEM was added per well. The DNA-LipofectAMINE Plus complexes were added to the medium and incubated at 37 °C. After 3 h, 0.8 μl of MEM with 20% fetal calf serum was added, and the cells were grown overnight. The next day, the cells were diluted 1:4 and transferred to a 75-cm2 flask. After incubation overnight, selective medium was added containing 10% fetal bovine serum, 1000 units/ml penicillin, 500 μg/ml streptomycin, and 1 mg/ml neomycin; 10 days later, resistant cells were harvested, and ~100 cells were plated in a 75-cm2 flask. The cells were grown until visible colonies were observed. Single colonies were transferred to 24-well plates for further characterization. IL-1α immuno blot analysis was performed using a rabbit anti-human IL-1α polyclonal antibody (R&D Systems) according to the manufacturer’s specifications.

RNA Isolation and Northern Blot Analysis—Cell lines were grown to confluence in 25-cm2 culture flasks, and treatments were performed as described in the figure legends. Total cytoplasmic RNA was extracted with TRIzol reagent (Life Technologies, Inc.) according to the manufacturer’s instructions and quantified spectrophotometrically. Northern blot analysis was performed as previously described (11). To normalize for RNA loading and transfer, the membrane was hybridized to a GAPDH or actin probe.

cDNA Probes—The human MMP-1, a probe 1.3-kilobase pair fragment, was isolated from plasmid was kindly provided by Dr. G. I. Goldberg (Washington University School of Medicine, St. Louis, MO). All other Northern probes were made as described in detail by Dumin et al. (12).

Reverse Transcription-PCR (RT-PCR) mRNA Analysis—RT-PCR analyses of MMP-1 and Sod2 were carried out as described by Melendez and Davies (9) with slight modifications. GAPDH was co-amplified in the linear amplification range as a loading control. The primers used for the PCR amplification were designed by utilizing OLIGO primer analysis software (National Biosciences, Inc.). The primers were as follows: human 5′-MMP-1, 5′-GGAGAAACTCTTCTTCAAT-3′; human 3′-MMP-1, 5′-CTCAGAAAGAGCAGCATC-3′; GAPDH, 5′-CATCACCCTGCCCTACTCGG-3′; 3′-GAPDH, 5′-CTCTTTCTCTTCTGTTGCT-3′; human SOD2, 5′-TCCCCGACCTGCCCTACGAC-3′; 3′-SOD2, 5′-CATTCTCCGCAG-TTGTACCAT-3′.

Transient Transfections of Human MMP-1 Promotor Constructs—Cell lines were transiently cotransfected with the pGL3-MMP-1(1G) or pGL3-MMP-1(2G) construct (13) and pCMV.SPORT β-gal using LipofectAMINE Plus reagent according to manufacturer’s instructions. The cells were lysed 18 h post-transfection, and the luciferase reporter activity was determined using the Promega assay system.

Dichlorofluorescein Fluorescence—Construction of the expression vectors, characterization of the cell lines, and FACScan analysis of DCF fluorescence were performed as described in detail by Rodriguez et al. (14).

Detection of ERK1/2 Phosphorylation by Immunoblotting—Control (CMV) cells at 80–90% confluence in 100-mm dishes were made quiescent by incubation in serum-free MEM for 24 h. The cells were then stimulated with 20% fetal calf serum for 15 min, washed three times with ice-cold phosphate-buffered saline, and placed on ice. The cells were lysed by incubation for 10 min on ice with 400 μl of hypotonic buffer (20 mM HEPES (pH 7.9), 0.1 mM EDTA, and 10 mM HCl) (0.2% Nonidet P-40, protease inhibitors (3 μg/ml aprotinin and 1 mM phenylmethylsulfonyl fluoride), phosphatase inhibitors (0.1 mM sodium orthovanadate and 20 mM sodium fluoride), 10% glycerol, and 1 mM dithiothreitol). The solubilized proteins were centrifuged at 14,000 × g in a microcentrifuge (4 °C) for 1 min, and the supernatants were stored at −80 °C. Extracted proteins were then quantified using the BCA protein reagent (Pierce). 40 μg of denatured protein in 5% 2-mercaptoethanol was separated on a 10% polyacrylamide gel by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad) at 100 V for 1 h. The membranes were then blocked for 2 h at room temperature in Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat dry milk. The blots were then incubated overnight at 4 °C with primary antibody (mouse anti-phospho-ERK1/2 monoclonal
antibody, Santa Cruz Biotechnology) at 1:1000 in Tris-buffered saline containing 0.01% Tween 20. This was followed by incubation with secondary antibody (biotinylated anti-mouse IgG) at 150,000 for 2 h at room temperature. Detection of the phosphorylated forms of the protein was performed by the addition of the Vectastain Elite ABC reagent, followed by Pierce Supersignal chemiluminescent substrate and exposure to Kodak X-Omat radiographic film. The immunoblot was then reprobed by incubation with standard stripping buffer for 15 min at 60°C, washed twice, blocked, and then reprobed using rabbit polyclonal antibody (Promega) that recognizes ERK1/2 regardless of its phosphorylation state at a 1:1000 dilution. The blot was incubated with biotinylated antibody (Promega) that recognizes ERK1/2 regardless of its phosphorylation state at a 1:1000 dilution, and the signal was detected as described above.

Gelatin Zymography—Serum-free conditioned medium from cells at 80–90% confluence was prepared by washing two times with phosphate-buffered saline, followed by incubation with serum-free MEM. MMPs were concentrated from the serum-free conditioned medium by incubation with 250 µl of gelatin-agarose beads (Sigma) overnight at 4°C. The beads were then centrifuged at 10,000 g for 2 min and eluted in electrophoresis sample buffer (final concentration of 2.25% SDS, 9% glycerol, and 45 mM Tris (pH 6.8), and bromophenol blue) by incubation for 30 min at room temperature, followed by centrifugation at 14,000 g for 2 min. Equal volumes of the eluate were then resolved on a 10% non-denaturing SDS-polyacrylamide gel impregnated with 1 mg/ml gelatin (Sigma). MMP-2 and MMP-9 gelatinolytic activities were measured as described elsewhere (15).

**RESULTS**

**Sod2-dependent Regulation of MMP-1 Expression**—To establish whether the expression of MMP-1 is regulated by Sod2, we first measured the levels of MMP-1 in HT-1080 fibrosarcoma cell lines designed to overexpress Sod2 utilizing RT-PCR (10). Overexpression of Sod2 by 15-fold resulted in a dramatic increase in both the basal expression of the MMP-1 mRNA and the steady-state concentrations of H2O2, both of which were reversed upon coexpression of caspase in either the cytosolic or mitochondrial compartment (Fig. 1, A and B). The addition of O2 generating compounds was not required to increase the production of H2O2. The increase in the steady-state levels of H2O2 was also associated with a decrease in the ratio of reduced to oxidized glutathione from 2.01 ± 0.37 to 1.22 ± 0.16 (p < 0.05), an indicator of the oxidant-buffering capacity of the cell (data not shown). Thus, a 15-fold increase in Sod2 levels, which is well within the range of activity observed in response to cytokines and growth factors, can enhance MMP-1 expression in an H2O2-dependent fashion.

**Regulation of MMP-1 by Mn-SOD Is IL-1a-independent**—Previous studies have demonstrated that IL-1α is a potent inducer of MMP-1 in many biological systems (16) and is modulated by reactive oxygen species (9, 17). Analysis of IL-1α levels in Sod2-overexpressing cells showed a 3-fold increase in basal IL-1α levels that was reversed when caspase was coexpressed in these cell lines (Fig. 2A). To examine whether IL-1α was responsible for the Sod2-dependent changes in MMP-1 gene expression, Sod2-overexpressing cells were treated with a concentration of IL-1 receptor antagonist (IL-1RA) that has previously been shown to block the IL-1-mediated induction of MMP-1 (18). Neither acute nor chronic treatment of the HT15 cell line with IL-1RA was effective in decreasing the expression of MMP-1 (Fig. 2B). Furthermore, in contrast to many other mesenchymal cells, HT-1080 fibrosarcoma cells did not respond to IL-1α by increasing the expression of MMP-1 (Fig. 2C, upper panel). It is nevertheless possible that intracellular IL-1α modulates the expression of MMP-1 in situ by stimulating pro-MMP-1 constitutively active (19). To address this question, HT-1080 fibrosarcoma cells were transfected with an IL-1α expression vector. Cloned transfectants with levels of IL-1 mRNA and protein that were equivalent to those observed upon Sod2 overexpression showed no increase in basal MMP-1 expression (Fig. 2C, middle and lower panels). Thus, Sod2 overexpression alone is sufficient to signal MMP-1 expression.

**MMP Expression is Sod2-dependent**—To further establish the linkage between Sod2 and the expression of MMP-1, MEFs derived from Sod2-deficient mice (CD1 Sod2tm1Cje) were analyzed for the expression of MMP-13 mRNA (a mouse functional analog of human MMP-1) in response to numerous stimuli (20). As analyzed by Northern blotting, the wild-type MEFs responded to the induction of MMP-13 by TNF, whereas the heterozygotes did not. This response was also shown to be specific for MMP-13 since the induction of IL-6 and IL-1 by TNF was unaffected by the loss of Sod2 activity (Fig. 3C). This suggests that Mn-SOD may be required for the TNF-mediated induction of MMP-13. To determine if Mn-SOD was also involved in signaling the expression of MMP-13 by other stimuli, Sod2+/− and Sod2−/− MEFs were treated with either IL-1 or phorbol 12-myristate 13-acetate. The induction of MMP-13 by IL-1α and phorbol myristate acetate was severely impaired in the Sod2−/− fibroblasts compared with the Sod2+/− fibroblasts (Fig. 3, A and B). To validate the genetic background of the
MEFs, RT-PCR analysis was performed to monitor Mn-SOD mRNA content (Fig. 3D). RT-PCR analysis produced the expected size product in the Sod2^{+/+} MEFs, whereas the Sod2^{-/-} MEFs produced two products. The lower size band reflects the targeted disruption of exon 3 in one of the Mn-SOD alleles. Mn-SOD activity levels also reflect the genetic background of each of the cell lines (data not shown). Furthermore, Mn-SOD content had no effect on the induction of IL-6 by both TNF and IL-1α or on the levels of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (Fig. 3D). Our data indicate that Mn-SOD plays an obligatory role in the induction of interstitial collagenase and that this is specific for MMP-13, as the induction of IL-1 and IL-6 in response to TNF was not altered by the levels of Mn-SOD. Furthermore, MEFs respond to IL-1α by signaling MMP-13 expression and, in such a setting, require Sod2. Taken together, these findings indicate that Sod2 is important for the IL-1-, TNF-, and phorbol 12-myristate 13-acetate-mediated induction of MMP-13.

**Sod2 Enhances MMP-1 Promoter Activity**—Control at the level of transcription is a major factor determining MMP-1 expression, and a single nucleotide polymorphism (1G/2G) at base pair -1607 that creates an Ets site adjacent to an AP-1 site at base pair -1602 has been shown to dramatically enhance transcription (13). Accordingly, luciferase promoter constructs containing either the 1G or 2G variation were transiently transfected in control and HT15 cell lines, and the effect on basal transcription was analyzed. The activities of the 1G and 2G promoter constructs in the HT15 cell line were increased -20- and 1000-fold, respectively, compared with the control cell line (Fig. 4A). The increase in MMP-1 promoter activity of both constructs by Sod2 overexpression was reversed by coexpression of catalase, indicating an H_2O_2-dependent regulation. An increase in the 2G frequency in tumor cell lines has been postulated to increase their invasive behavior. In such a setting, the level of Sod2 may well be a determinant of the metastatic potential of tumor cell lines by regulating the expression of MMP-1.

**Sod2 Signals MMP-1 Expression by Activating ERK1/2**—Both AP-1 and Ets transcription factors are regulated by MAPK signaling pathways (21). The three most well characterized MAPK pathways include the ERK1/2, JNK/SAPK, and p38 pathways. In general, ERK1/2 is activated by mitogens and phorbol esters, whereas JNK/SAPK and p38 are mainly stimulated by environmental stress and inflammatory cytokines. All three pathways have been implicated in the expression of MMPs, and the specific MAPK pathway that regulates MMP expression can vary depending on the inducing agent and cell line. To test which of the MAPK pathways are involved in the induction of MMP-1 by Sod2, control and Sod2-overexpressing cells were treated with the specific inhibitors of ERK1/2 (PD98059) and p38 (SB203580) (Fig. 4B, inset). Northern blotting demonstrated that the ERK1/2 inhibitor blocked basal MMP-1 expression and dramatically decreased MMP-1 mRNA levels in the HT15 cell lines. The p38 inhibitor increased MMP-1 expression in both control and HT15 cell lines. The ERK1/2 inhibitor also blocked the induction of MMP-1 by TNF. The effect of the MAPK inhibitors on MMP-1 expression was also observed with the MMP-1 promoter constructs (Fig. 4B). Furthermore, the inductive properties of SB203580 could be blocked by PD98059. It was thought possible that the ability of PD98059 to inhibit MMP-1 expression may be a property of its ring structure and potential antioxidant properties. To test this hypothesis, an alternative ERK1/2 inhibitor structurally unrelated to PD98059 was tested on MMP-1 promoter activity. The specific ERK1/2 inhibitor U0126 blocked the Sod2-dependent increase in both 1G and 2G promoter activities, whereas its non-inhibitory analog (U0124) had no comparable effect (Fig. 4C). The active forms of both ERK1/2 and p38 can be distinguished from their inactive forms by phospho-specific antibodies. Immunoblot analysis of extracts from control and HT15 cells showed an increase in the activated forms of ERK1/2 in the Sod2-overexpressing cells that was reversed upon coex-

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**Fig. 3.** *Sod2 is required for expression of the mouse functional analog of human MMP-1, MMP-13.* A, confluent cultures of passage 6 cells derived from Sod2^{+/+} and Sod2^{-/-} mouse fibroblasts were treated with IL-1α (10 ng/ml), TNF-α (30 ng/ml), or phorbol 12-myristate 13-acetate (PMA; 100 nM). Cells were lysed 24 h post-treatment, and total RNA was isolated and subjected to Northern analysis. MMP-13 mRNA levels were assessed by hybridization with a 32P-labeled MMP-13 probe for rat collagenase. After autoradiography, the same membrane was stripped and hybridized with a 32P-labeled actin probe. B, quantitative evaluation of data from the Northern blot in A after actin normalization was performed using a STORM PhosphorImager (Molecular Dynamics). C, Northern blot analysis of MMP-1, IL-6, IL-1α, and GAPDH from passage 4 Sod2-deficient fibroblasts treated for 18 h with or without TNF (10 ng/ml) The values were obtained after normalizing to β-actin. D, RT-PCR analysis of Sod2 mRNA levels in mouse embryonic fibroblasts. Sod2 and GAPDH mRNA levels were measured in control and IL-1 (10 ng/ml)- and TNF (30 ng/ml)-treated cells.
expression of catalase (Fig. 5A). The constitutive activation of ERK1/2 in the HT15 cell lines was also blocked by PD98059 (Fig. 5B). Interestingly, SB203580 activated ERK1/2 in both the control and HT15 cell lines, which may explain its ability to induce MMP-1 expression. The ability of SB203580 (20–50 μM) to activate ERK1/2 has also recently been reported in erythroleukemic cell lines and was not observed when lower concentrations of the drug were used (22). However, in the present study, both low and high doses of SB203580 were capable of activating MMP-1 promoter activity (Fig. 4D). The above findings indicate that the Sod2-dependent generation of H2O2 leads to the activation of ERK1/2 and are in agreement with studies demonstrating that reactive oxygen species can activate MAPK pathways (23).

The proximal AP-1 element located near position −70 plays a major role in the transcriptional regulation of MMP-1 gene expression and is found near this position in each of the inducible human MMP promoters (21). Because of the conservation of the promoter regions between MMPs, it is possible that other MMPs respond to Sod2 overexpression. In support of this hypothesis, the gelatinolytic activity of MMP-2 and the mRNA concentrations of MMP-3 and MMP-7 were increased in the Sod2 overexpressors compared with control cell lines (Fig. 5C, left panel). The observed decline in MMP-9 activity in the HT15 cell line may represent a negative regulatory aspect of Sod2 in MMP expression. Thus, Sod2 may regulate a broad spectrum of MMPs and function as a “global” redox regulator of metalloproteinases.

**DISCUSSION**

Under normal physiological conditions, the steady-state concentrations of H2O2 are well within the buffering capacity of the mitochondrial glutathione redox system. However, when Sod2 levels increase, the glutathione-buffering capacity of the mitochondria may be overwhelmed by H2O2. We propose that it is this excess mitochondrial H2O2 that signals the downstream events that lead to the expression of MMP-1 and possibly other MMP family members. The expression of catalase in either the cytosolic or mitochondrial compartment can block Sod2-dependent H2O2 production and decrease MMP-1 expression (Fig. 4C).
Fig. 5. Sod2 regulates MMP-1 transcription by activating ERK1/2. A, increased phosphorylation of ERK1/2 (p42/p44) by Sod2 overexpression. Cell lysates from confluent cells grown in complete medium (CMV, HT15, and HT15mCAT), quiescent CMV cells in serum-free medium (CMV sfm), and quiescent cells stimulated with fetal bovine serum (20%) for 15 min (CMV + FBS) were analyzed for ERK1/2 by Western blotting. B, spontaneous up-regulation of ERK1/2 (p42/p44) phosphorylation by SB203580. Control and Sod2-overexpressing cells were treated for 24 h with either PD98059 (PD; 50 μM) or SB203580 (SB; 20 μM). Cells were lysed and analyzed for phosphorylated ERK1/2 as described under “Materials and Methods.” C, enhanced levels of MMP-2, -3, and -7 in Sod2 overexpressors. Left panel, gelatin zymography; right panel, RT-PCR. D, Sod2 is activated by a variety of stimuli, leading to an increase in the steady-state production of H2O2. H2O2 activates ERK1/2, leading to transcriptional activation of the MMP-1 promoter and subsequent extracellular matrix (ECM) degradation.

1. A and B). The inability of the endogenous catalase to effectively detoxify the mitochondrial generated H2O2 may be due to its restricted presence in the peroxisomal compartment. An increase in H2O2 production leads to the phosphorylation of ERK1/2 by a yet unknown mechanism, as well as the subsequent activation of the MMP-1 promoter. This signaling cascade may be utilized under a variety of conditions in which Sod2 levels are elevated. Sod2, IL-1, and MMP-1 expression are elevated in aging systems both in vitro and in vivo. The increased expression of collagenase may result from an elevation in Sod2 levels in response to increasing levels of IL-1 or other inflammatory cytokines. Increases in the levels of H2O2 that result from the enhanced dismutase activity in aged cells may explain the ability of antioxidant strategies to reduce the severity of UV-induced premature skin aging (3) and to extend life span in experimental models of aging (24). Reactive oxygen species have also long been implicated in mitogenic signaling and cancer. Recently, the ability of estrogen derivatives to selectively kill human leukemia cells has been attributed to the specific inhibition of SOD activity (25). Several independent groups have also reported that the elevated levels of Sod2 may correlate with aggressiveness in gastric and colorectal adenocarcinomas and are reflective of a poor overall survival of these patients (26–28). The finding that Sod2 overexpression can dramatically enhance MMP-1 promoter activity may explain the above observations, as excessive MMP-1 production is a major contributor to the stromal degradation involved in tumor invasion.

H2O2 has been implicated as one of the critical signaling molecules leading to the expression of MMP-1 in dermal fibroblasts (29). H2O2 activates AP-1, an essential transcription factor required for the induction of MMP-1 (17, 29). The expression of MMP-1 is also effectively blocked by antioxidants such as N-acetyl-L-cysteine, a precursor of glutathione (7, 30), or by treatment with catalase (17). Agents that decrease the H2O2-detoxifying capacity of the cell such as buthionine sulfoximine (a glutathione synthesis inhibitor) and amionitrazole (a catalase inhibitor) enhance basal MMP-1 production (7). The retinoid acid analog tretinoin, which effectively blocks the increase in expression of MMPs following low-dose UV irradiation (3), also blocks AP-1 activation (31). The ability of tretinoin to antagonize the process of photoinduced skin aging has been attributed to its antioxidant properties. Recent studies in cultured renal mesangial cells demonstrated that tretinoin protects from H2O2-induced cytotoxicity by increasing both cellular reduced glutathione and catalase levels (32). Thus, H2O2 has been shown to be important in the signaling events that lead to the expression of MMP-1. We (14) and others (7, 33, 34) have shown that overexpression of Mn-SOD can increase the levels of intracellular peroxides in a number of cell lines. In addition, highly metastatic MCF-7 adriamycin-resistant breast cancer cells and gastric tumors both show high levels of MMP-1 (35, 36) and Sod2 (37, 38) compared with their non-metastatic counterparts. Furthermore, many of the agents that induce the expression of MMP-1 also increase Mn-SOD levels. These agents include TNF (39, 40), IL-1 (40, 41), UV irradiation (42, 43), phorbol esters (44), and in vitro replicative senescence (45).

Sod2 is the only antioxidant enzyme that is modulated in response to pathogenic stimuli, including lipopolysaccharides, growth factors, and cytokines (40); ionizing radiation (42, 43); phorbol esters (44); and redox-cycling drugs and in vitro replicative senescence (45). These findings indicate that cells have evolved to utilize the mitochondria as a major source of the potent intracellular signaling molecule H2O2 via Sod2. The MMPs may represent just one family of genes whose expression is tightly regulated by Sod2-derived H2O2.

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