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Original Contribution

Redox-sensitive gene-regulatory events controlling aberrant matrix metalloproteinase-1 expression

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ABSTRACT

Aberrant matrix metalloproteinase-1 (MMP-1) expression contributes to the pathogenesis of many degenerative disease processes that are associated with increased oxidative damage or stress. We and others have established that shifts in steady-state H_2O_2 production resulting from enforced antioxidant gene expression, senescence, or UV irradiation control MMP-1 expression. Here we establish that histone deacetylase-2 (HDAC2) protein levels and its occupancy of the MMP-1 promoter are decreased in response to enforced manganese superoxide dismutase (Sod2) expression. Inhibition of HDAC activity further accentuates the redox-dependent expression of MMP-1. Sod2-dependent decreases in HDAC2 are associated with increases in a proteasome-sensitive pool of ubiquitinylated HDAC2 and MMP-1-specific histone H3 acetylation. Sod2 overexpression also enhanced recruitment of Ets-1, c-Jun, c-Fos, and the histone acetyltransferase PCAF to the distal and proximal regions of the MMP-1 promoter. Furthermore, the Sod2-dependent expression of MMP-1 can be reversed by silencing the transcriptional activator c-Jun. All of the above Sod2-dependent alterations are largely reversed by catalase coexpression, indicating that the redox control of MMP-1 is H_2O_2-dependent. These findings identify a novel redox regulation of MMP-1 transcription that involves site-specific promoter recruitment of both activating factors and chromatin-modifying enzymes, which converge to maximally drive MMP-1 gene expression.

Matrix metalloproteinases (MMPs) are endopeptidases involved in the breakdown of extracellular matrix (ECM) in normal physiological processes, such as embryogenesis, wound healing, tissue repair, and normal matrix turnover [1]. MMP-1 or interstitial collagenase cleaves collagens types I, II, and III, and under normal physiological conditions its expression levels are extremely low and tightly regulated to maintain ECM homeostasis. In contrast, aberrant high-level MMP-1 expression is associated with numerous degenerative disease processes, such as arthritis [2], atherosclerosis [3], periodontal disease, pulmonary fibrosis, stroke injury, and metastatic disease [4–6].

High-level MMP-1 expression is commonly linked to elevations in steady-state H_2O_2 concentrations (SS-[H_2O_2]). Redox-dependent MMP-1 expression has been shown to be regulated through a juxtaposed Ets-1 and AP-1 DNA consensus binding site at −1607 of its promoter in response to oxidant-dependent activity of the mitogen-activated protein kinase (MAPK) family members ERK and c-Jun-N-terminal kinase (JNK) [7,8]. Reactive oxygen species (ROS) have been shown to be obligatory for fibronectin-stimulated MMP expression in articular chondrocytes [9] as well as UV-mediated expression in dermal fibroblasts [10]. A number of studies have established that overexpression of manganese superoxide dismutase (Sod2) is accompanied by high-level MMP-1 expression that is attributed to shifts in SS-[H_2O_2] [8,11,12].

Redox sensitivity of the MMP-1 promoter is in large part attributed to the distal promoter region that includes the Ets-1/AP-1 (c-Jun/c-Fos) consensus binding sites at position −1607 [7,13]. As illustrated in Fig. 1A, the distal promoter region spans −1663 and −1464 and encompasses the −1607 Ets-1/AP-1 (c-Jun/c-Fos) redox-responsive element and other putative Ets family member binding domains. The proximal promoter region spans bases −212 to −34 and contains two phorbol-responsive regions, one at −213 to −177, which binds JunD/c-Fos and c-Fos/c-Jun heterodimers, and the −73 AP-1 site, which binds JunD and Fra-2 [14].

In addition to transcription factor binding, posttranslational modifications of histones serve to maximally activate gene transcription. Lysine acetylation on histones H3 and H4 loosens the DNA/histone interaction and increases access of transcriptional

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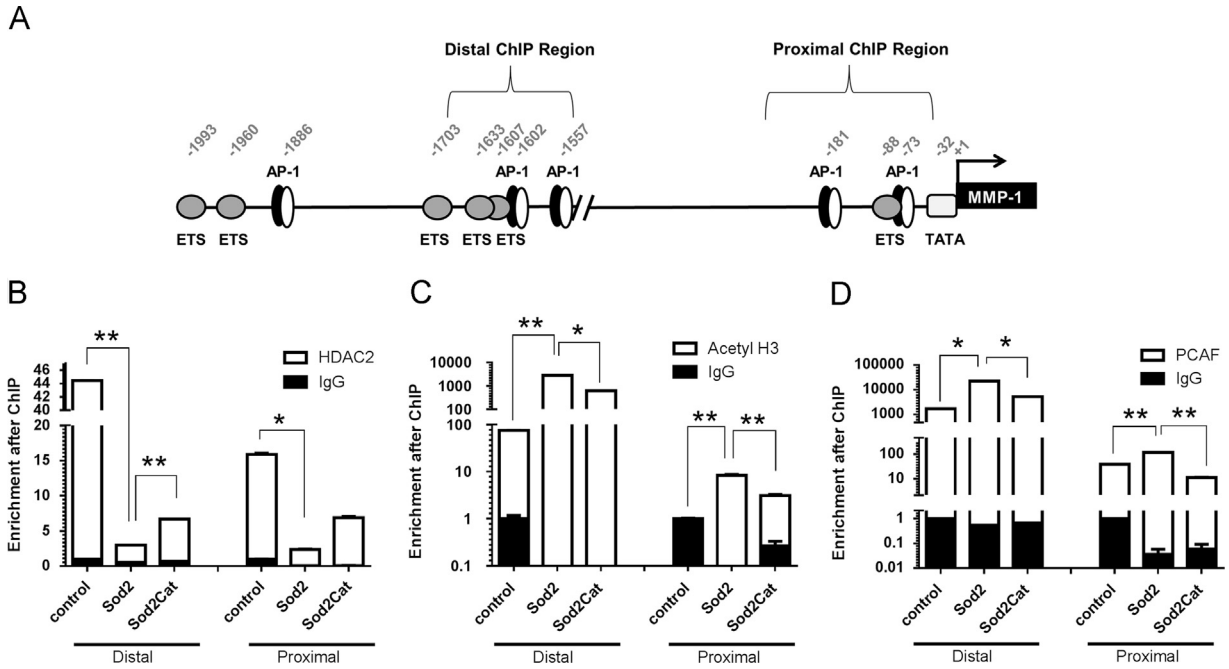

Fig. 1. Redox-dependent MMP-1 expression is associated with decreased HDAC and increased HAT recruitment that is accompanied by enhanced histone acetylation. Cells were subjected to ChIP as described under Materials and methods. Protein–DNA complexes were pulled down using specific antibodies to HDAC2, PCAF, and acetylated H3. IgG antibody was used as a negative control for nonspecific binding. (A) Schematic diagram of transcription elements on the MMP-1 promoter indicating primer spanning regions used in ChIP. Distal and proximal primers span −1491 to −1696 and −245 to −37 upstream of the transcription start site, respectively. (B) HDAC2 promoter occupancy at the distal and proximal MMP-1 promoter. $^{*}p = 0.0149$ compared to control, $^{**}p < 0.0001$ control vs Sod2 or Sod2Cat, Sod2 vs Sod2cat. (C) Acetylation of histone H3 at the distal and proximal regions of the MMP-1 promoter. $^{*}p = 0.0007$ or $^{**}p < 0.0001$ compared to control. (D) Promoter occupancy of PCAF at the distal and proximal MMP-1 promoter. $^{*}p < 0.0001$ or $^{**}p < 0.05$ for Sod2 compared to control or Sod2 compared to Sod2Cat. $n = 3$ independent immunoprecipitations. For data analysis, ChIP samples were all normalized to the amount of input DNA for each sample. Data are presented as enrichment after ChIP, with the IgG control sample set to 1. Mean values are plotted with error bars representing standard error of the mean.

activators to the DNA. Thus, histone acetylation is a key determinant in regulating chromatin access after transcription factor recruitment. Epigenetic modifications around the MMP-1 loci affect MMP-1 expression. In rheumatoid arthritis synovial fibroblasts, elevated MMP-1 levels are correlated with increases in H4 acetylation in the distal MMP-1 promoter [15]. Martens et al. [16] have shown that acetylation, histone H3 lysine 4 di- and trimethylation, and histone H3 serine 10 phosphorylation are important steps in the activation of MMP-1. We have previously reported that both histone acetyl transferase P300 and histone H3 acetylation on the MMP-1 promoter are sensitive to shifts in SS-[H_2O_2] [7]. It has been suggested that histone acetylation can be affected by the presence of ROS through the activation of MAPK pathways [17–19]. Hydrogen peroxide has been shown to increase histone acetylation in alveolar epithelial cells, leading to aberrant IL-8 expression [20–24]. Alveolar macrophages derived from cigarette smokers [22] and cystic fibrosis airway epithelial cell models [25] show changes in acetylation, probably due to elevated exposure to ROS. These observations indicate that oxidants play an important role in regulating the accessibility of chromatin to remodeling factors that allow for efficient gene transcription.

The facts that ROS are known inducers of histone acetylation and that MMP-1 is redox-regulated led us to explore the participants that drive MMP-1 epigenetic regulation and transcriptional activation. Using a series of redox-engineered cell lines to modulate SS-[H_2O_2] we have identified a number of redox-responsive signaling components that regulate MMP-1 transcription. The current work identifies the various chromatin remodeling factors and transcriptional activators that control the redox-responsive MMP-1 promoter. Using chromatin immunoprecipitation (ChIP) we have determined that c-Jun, c-Fos, and Ets-1 as well as P300/CBP-associated factor (PCAF) recruitment to the MMP-1 promoter is H_2O_2-dependent. MMP-1 transcriptional initiation is also enhanced by redox-dependent, but H_2O_2-independent, modifications of histone deacetylase-2 (HDAC2). Overall, these studies demonstrate that alterations in SS-[H_2O_2] enhance kinase signaling, which modifies components of the transcription initiation complex leading to maximal MMP-1 production.

Materials and methods

Cell culture and reagents

All cell lines were maintained in 75-cm^2 flasks in minimal essential medium containing 10% fetal calf serum, 1000 U/ml penicillin, 500 μg/ml streptomycin, 50 μg/ml zeocin, and/or 1 mg/ml G418 sulfate, in a 37 °C incubator containing 5% CO_2. Cell lines, recombinant Sod2 and catalase plasmids, and transfections have been described elsewhere [26,27]. In brief, the redox-engineered cell lines were generated in our laboratory from HT1080 human fibrosarcoma cells. The cell lines generated included cells that were transfected with control plasmid (CMV) or *Sod2* targeted to the mitochondria (Sod2) and cells cotransfected with catalase directed to the cytosol (Sod2Cat). Sod2 enzyme activity and catalase levels were confirmed (Supplementary Fig. 1). MG-132 and 3-amino-1,2,4-triazole were from Sigma (St. Louis, MO, USA); trichostatin A was obtained from Upstate Biotechnology/Millipore (Billerica, MA, USA).

Antibodies

Total histone H3 antibody (06-755) was from Upstate Biotechnology/Millipore. c-Fos (sc-52x) and Ets-1 (sc-350) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). c-Jun antibody (9162) was obtained from Cell Signaling