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Human MUC1 Carcinoma Antigen Regulates Intracellular Oxidant Levels and the Apoptotic Response to Oxidative Stress*

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The DF3/MUC1 transmembrane oncoprotein is aberrantly overexpressed by most human carcinomas. Certain insights are available regarding a role for MUC1 in intracellular signaling; however, no precise function has been ascribed to this molecule. The present results demonstrate that MUC1 expression is up-regulated by oxidative stress and that this response is mediated by activation of *MUC1* gene transcription. A role for MUC1 in the oxidative stress response is supported by the demonstration that MUC1 expression is associated with attenuation of endogenous and H_2O_2-induced intracellular levels of reactive oxygen species (ROS). MUC1-dependent regulation of ROS is mediated at least in part by up-regulation of anti-oxidant enzyme (superoxide dismutase, catalase, and glutathione peroxidase) expression. In concert with these findings, we show that the apoptotic response to oxidative stress is attenuated by a MUC1-dependent mechanism. These results support a model in which activation of MUC1 by oxidative stress provides a protective function against increased intracellular oxidant levels and ROS-induced apoptosis.

The human DF3/MUC1 mucin-like transmembrane is normally expressed on the apical borders of secretory epithelial cells (1). In carcinoma cells, polarization of MUC1 is lost with high levels of expression over the entire cell surface (1). Estimates indicate that over 70% of newly diagnosed cancers aberrantly express MUC1 (2). The MUC1 proteins consist of an N-terminal ectodomain with variable numbers of 20-amino acid tandem repeats that are extensively modified with *O*-linked glycans (3, 4). The C-terminal region includes a transmembrane domain and a 72-amino acid cytoplasmic tail. Following proteolytic cleavage, the >250-kDa ectodomain remains associated with the ~25-kDa C-terminal subunit at the cell surface. β-Catenin, a component of the adherens junction of mammalian cells, interacts directly with the MUC1 intracellular region (5). Other studies have shown that phosphorylation of MUC1 by glycogen synthase 3β, c-Src, or the epidermal growth factor receptor contributes to regulation of the interaction between MUC1 and β-catenin (6–8). More recent work has demonstrated that MUC1 colocalizes with β-catenin in the nucleus and that MUC1 induces transformation (9, 10).

Normal cellular metabolism is associated with the production of reactive oxygen species (ROS).[1] Common forms of ROS include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals, and nitric oxide. Mitogenic signals induced by certain growth factors and activated Ras are mediated by ROS production (11, 12). Under nonphysiologic conditions, increases in ROS levels above the reducing capacity of the cell can cause damage to DNA, proteins, and lipids (13, 14). To prevent damage associated with increases in ROS, aerobic cells have developed enzymatic (superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx)) and non-enzymatic (glutathione and thioredoxin) defense mechanisms to balance the reduction-oxidation (redox) state (15). In the absence of an adequate defense, cells respond to oxidative stress with the induction of apoptosis (14). Although few insights are available regarding mechanisms responsible for ROS-induced cell death, H_2O_2 has been shown to activate topoisomerase II-mediated cleavage of chromosomal DNA and thereby apoptosis (16). The $p66^{shc}$ adaptor protein (17, 18) and the p85 subunit of phosphatidylinositol 3-kinase (19) have also been implicated in the apoptotic response to H_2O_2.

The present studies demonstrate that MUC1 expression is activated by oxidative stress. The results also demonstrate that MUC1 regulates intracellular oxidant levels and attenuates the apoptotic response to oxidative stress.

MATERIALS AND METHODS

Cell Culture—Human breast (MCF-7, ZR-75-1), colon (HCT116), and cervical (HeLa) carcinoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). MCF-7, HCT116, and HeLa cells were grown in Dulbecco's modified Eagle's medium (high glucose; Cellgro) supplemented with 10% heat-inactivated fetal calf serum, and 2 mM L-glutamine. ZR-75-1 cells were cultured in RPMI 1640 medium (Cellgro) supplemented with 10% fetal calf serum and 2 mM L-glutamine. Cells were treated with H_2O_2 (Sigma).

Immunoblot Analysis—Cells were lysed in ice-cold lysis buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) for 30 min. Lysates were cleared by centrifugation for 20 min at 4 °C as described (20). Proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-DF3/MUC1 (1), anti-SOD1 (Santa Cruz Biotechnology), anti-SOD2 (Upstate Biotechnology, Inc.), anti-catalase (Sigma), anti-GPx (MBL Medical and Biological Laboratories) or anti-β-actin (Sigma). The antigen-antibody complexes were visualized by enhanced chemiluminescence (ECL, Amersham Life Sciences).

Reverse Transcription Polymerase Chain Reaction (RT-PCR)—Total cellular RNA was extracted in Trizol, dissolved in RNase-free water, and incubated for 10 min at 55 °C. MUC1-specific primers (5′-TCTACTCTGGTGCACAACGG-3′ and 5′-TTATATCGAGAGGCTGCTTCC-5′) were designed to span a region within genomic DNA that contains two introns, resulting in the amplification of a 489-bp fragment from RNA and a 738-bp fragment from genomic DNA. RNA-specific primers for human β-actin were used as a control. The RNA was reverse transcribed and amplified using SuperScript One-Step RT-PCR with Plat-

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[1] The abbreviations used are: ROS, reactive oxygen species; RT-PCR, reverse transcription PCR; Luc, luciferase; SOD, superoxide dismutase; GPx, glutathione peroxidase; HE, hydroethidine; DCF, dichlorodihydrofluorescein; DCFH-AM, 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate.

inum *Taq* (Invitrogen). Amplified fragments were analyzed by electrophoresis in 2% agarose gels.

Luciferase Reporter Assays—A fragment spanning the region from −1464 to +24 of the human *MUC1* gene (21) was ligated in the *Kpn*I and *Bgl*II sites of the firefly luciferase pGl3-Basic vector (Promega). The resulting plasmid was designated pMUC1-Luc. Cells were transfected with a mixture of pMUC1-Luc and SV40-*Renilla* Luc (5:1) constructs (Promega) in the presence of LipofectAMINE for 14 h. After washing and incubation for an additional 24 h, the cells were treated with H_2O_2 and then lysed in Passive Lysis Buffer (Promega). Lysates were analyzed for firefly and *Renilla* luciferase activities using the Dual Luciferase Reagent Assay Kit (Promega). Luminescence was measured in a luminometer.

Stable Transfectants—HCT116 and HeLa cells were transfected with pIRESpuro2 or pIRESpuro2-MUC1 as described (8) and selected in the presence of 0.4 μg/ml puromycin (Calbiochem-Novabiochem).

Measurement of ROS Levels—Cells were incubated with 10 μM DCFH-AM (Molecular Probes) for 30 min at 37 °C to assess H_2O_2-mediated oxidation to the fluorescent compound DCF (22). Fluorescence of oxidized DCF was measured at an excitation wavelength of 480 nm and an emission wavelength of 525 nm using a flow cytometer (BD Biosciences). For the assessment of superoxide (O_2^-) levels, cells were incubated with 10 μM hydroethidine (HE) (Polyscience Inc.) for 20 min at 37 °C. O_2^--mediated conversion of HE to ethidium was measured by excitation at 470 nm and emission at 590 nm (23).

Apoptosis Assays—Sub-G_1 DNA content was assessed by staining ethanol-fixed and citrate buffer-permeabilized cells with propidium iodide and monitoring by flow cytometry (BD Biosciences). Chromatin condensation was assessed by staining cells with ethidium bromide and counting the number of cells with bright orange areas in their nuclei as described (24).

RESULTS

Up-regulation of MUC1 Protein by Oxidative Stress—To assess the effects of oxidative stress on MUC1 expression, human MUC1-positive MCF-7 cells were exposed to 0.4 mM H_2O_2 as a source of ROS. Lysates of the H_2O_2-treated cells were analyzed by immunoblotting with anti-MUC1 (DF3 antibody). The results demonstrate that MUC1 levels increase 3-fold at 15 min of H_2O_2 treatment (Fig. 1*A*). MUC1 expression was up-regulated through 45 min and then declined at 60 min of H_2O_2 treatment (Fig. 1*A*). As a control, immunoblot analysis of the lysates with anti-β-actin demonstrated equal loading of the lanes (Fig. 1*A*). To extend these findings, MCF-7 cells were treated with different concentrations of H_2O_2 for 30 min. The results show that over a range of 0.1 to 1.0 mM, increases in MUC1 expression were apparent at 0.1–0.2 mM and maximal at 0.4–0.6 mM H_2O_2 (Fig. 1*B*). Treatment of human MUC1-positive ZR-75-1 cells with H_2O_2 was also associated with increases in MUC1 expression (Fig. 1*C*). The kinetics, however, differed somewhat from that found in MCF-7 cells with maximal increases at 2 h and down-regulation to below base-line levels at 6 h (Fig. 1*C*). By contrast, similar studies with MUC1-negative HCT116 cells demonstrated no detectable induction of MUC1 expression in response to H_2O_2 treatment (data not shown). These findings indicate that MUC1-positive cells respond to oxidative stress with increases in MUC1 expression.

Oxidative Stress Induces MUC1 Transcription—To determine whether activation of *MUC1* transcription contributes to up-regulation of MUC1 protein in the oxidative stress response, *MUC1* mRNA levels were quantitated by RT-PCR. Treatment of MCF-7 cells with H_2O_2 was associated with increases in *MUC1* transcripts at 15 min (Fig. 2*A*). Moreover, in concert with regulation at the protein level, *MUC1* mRNA levels were increased through 45 min and then declined at 60 min (Fig. 2*A*). As a control, there was little effect of H_2O_2 on β-actin mRNA levels (Fig. 2*A*). Treatment of ZR-75-1 cells with H_2O_2 was also associated with increases in *MUC1* transcripts (Fig. 2*B*). The increase in *MUC1* transcripts was maximal at 1 h of H_2O_2 exposure and was detectable in the absence of changes in β-actin mRNA levels (Fig. 2*B*). To assess the effects

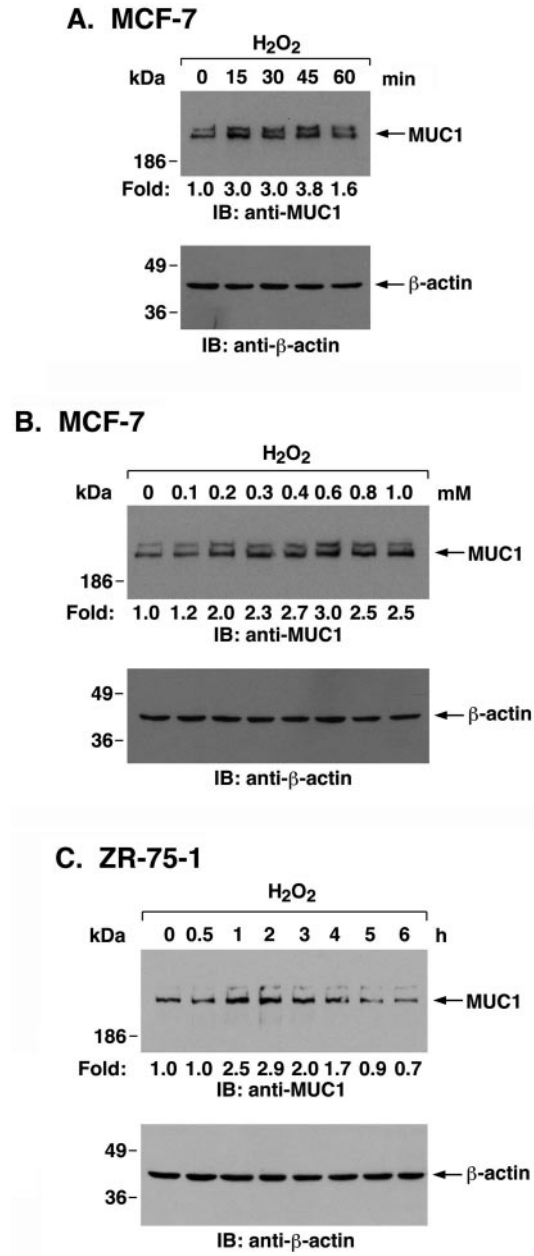

FIG. 1. **Regulation of MUC1 expression by H_2O_2.** *A*, MCF-7 cells were treated with 0.4 mM H_2O_2 for the indicated times. *B*, MCF-7 cells were treated with the indicated concentrations of H_2O_2 for 30 min. *C*, ZR-75-1 cells were treated with 0.4 mM H_2O_2 for the indicated times. Lysates were subjected to immunoblot analysis with anti-MUC1 and, as a control, anti-β-actin. The intensity of the MUC1 signals was determined by densitometric scanning, and the results are expressed as -fold change relative to the levels of MUC1 in control cells. *IB*, immunoblot.

of H_2O_2 on *MUC1* gene transcription, MCF-7 cells were transfected to express a MUC1 promoter-Luc reporter and SV40-*Renilla* Luc constructs. Treatment with H_2O_2 was associated with an increase in firefly, and not *Renilla*, luciferase activity, which was maximal at 45 min (Fig. 2*C*). In ZR-75-1 cells transfected with pMUC1-Luc and treated with H_2O_2, induction of firefly luciferase activity was maximal at 1 h (Fig. 2*D*). These findings demonstrate that H_2O_2 activates MUC1 gene transcription and thereby increases *MUC1* mRNA and protein levels.

MUC1 Regulates ROS Levels—To assess the role of MUC1 in response to oxidative stress, MUC1-negative HCT116 cells were transfected to stably express the empty vector or MUC1 (Fig. 3*A*). Expression of MUC1 in two separate isolates of stable

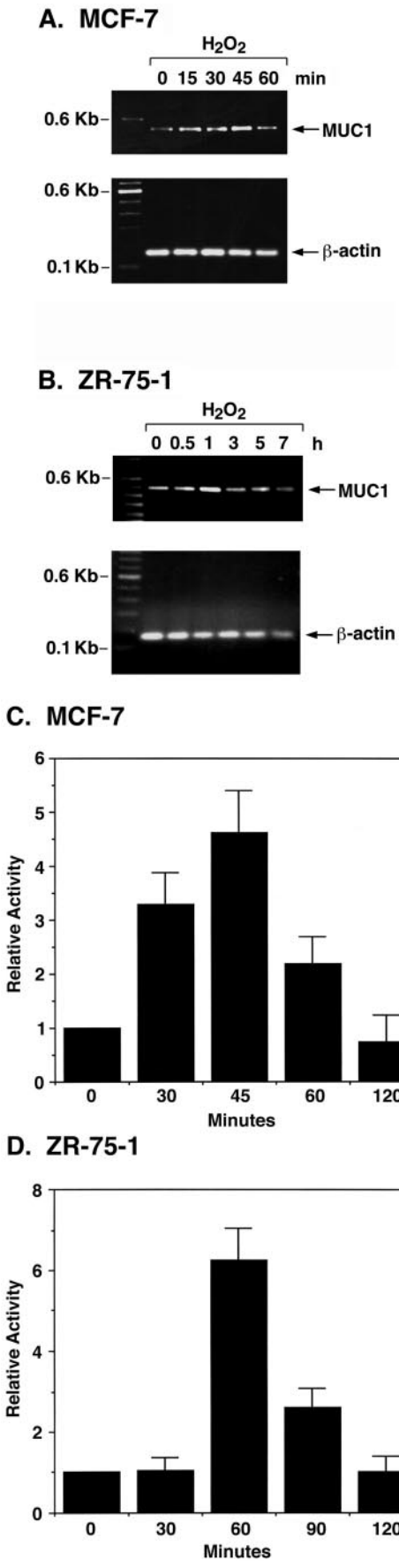

FIG. 2. **Transcriptional activation of MUC1 in response to H_2O_2.** *A* and *B*, MCF-7 (*A*) and ZR-75-1 (*B*) cells were treated with 0.4 mM H_2O_2 for the indicated times. The levels of *MUC1* and β-actin transcripts were amplified by RT-PCR and analyzed by agarose gel electrophoresis. *C* and *D*, MCF-7 (*C*) and ZR-75-1 (*D*) cells were transfected with pMUC1-Luc and SV40-*Renilla* luciferase and then exposed to 0.4 mM H_2O_2 for the indicated times. Lysates were analyzed for firefly and *Renilla* luciferase activities. The results are expressed as the mean ± S.D. of three separate experiments, each performed in triplicate, in which the ratio of firefly to *Renilla* luciferase activities is relative to that of control cells.

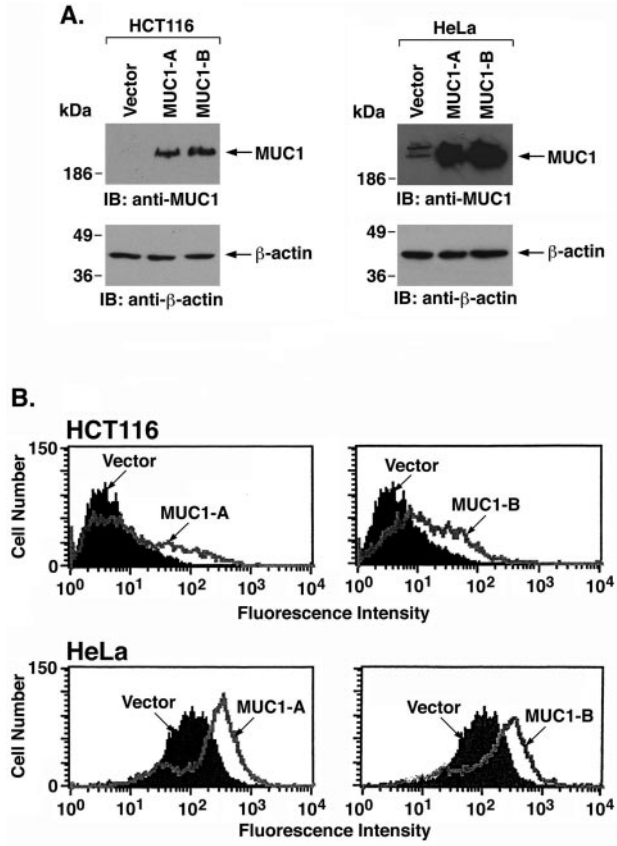

FIG. 3. **Expression of MUC1 in stable transfectants.** *A* and *B*, HCT116 and HeLa cells were transfected to stably express the empty vector or MUC1. The MUC1-A and MUC1-B cells were obtained from separate transfections. *IB*, immunoblot. *A*, cell lysates were analyzed by immunoblotting with anti-MUC1 and anti-β-actin. *B*, the indicated transfectants were analyzed for cell surface MUC1 expression by flow cytometry using the DF3 anti-MUC1 antibody.

HCT116 transfectants was somewhat lower than that found in MCF-7 cells (Fig. 3*A* and data not shown). HeLa cells, which constitutively express MUC1 (6), were stably transfected to express MUC1 at relatively higher levels (Fig. 3*A*). Analysis of the HCT116 transfectants by flow cytometry demonstrated that MUC1 is expressed on the cell surface (Fig. 3*B*). The HeLa cells stably transfected with the MUC1 vector also demonstrated an increase in cell surface MUC1 expression (Fig. 3*B*). These findings indicate that, like endogenous MUC1, transfected MUC1 is expressed as a transmembrane glycoprotein.

To determine whether MUC1 affects ROS levels, cells were incubated with DCFH-AM, and H_2O_2-mediated oxidation of the fluorochrome was assayed by flow cytometry. The results demonstrate that, compared with HCT116 cells expressing the empty vector, MUC1-positive HCT116 cells exhibited substantially lower H_2O_2 levels (Fig. 4*A*). Moreover, increased expression of MUC1 in HeLa cells resulted in marked decreases in H_2O_2 levels (Fig. 4*B*). To extend this analysis, HCT116 cells were exposed to H_2O_2 and then assayed for oxidation of DCFH-AM. Compared with HCT116/vector cells, which exhibited substantial increases in H_2O_2 levels, expression of MUC1 was associated with attenuation of this response (Fig. 4*C*). The

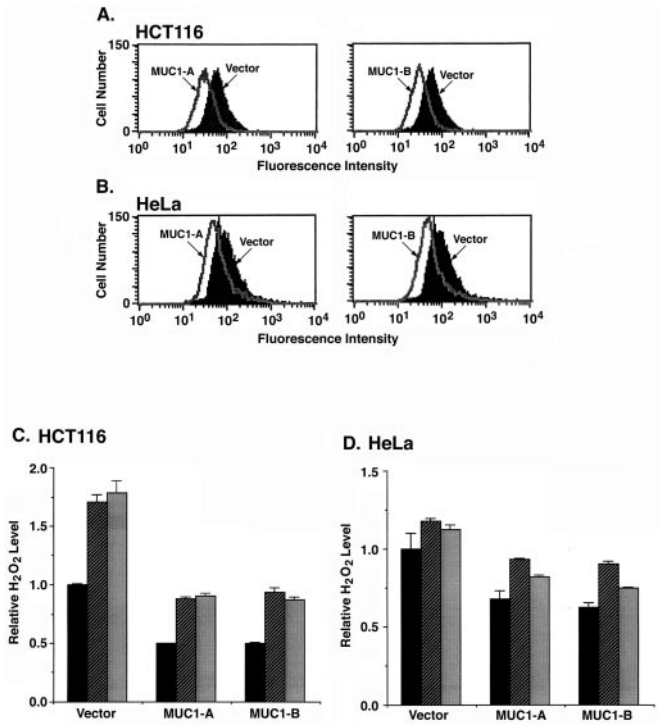

FIG. 4. **MUC1 regulates intracellular H_2O_2 levels.** *A* and *B*, HCT116 (*A*) and HeLa (*B*) cells expressing empty vector or MUC1 were incubated with DCFH-AM for 30 min. Fluorescence of oxidized DCF was measured by flow cytometry. *C* and *D*, HCT116 (*C*) and HeLa (*D*) cell transfectants were left untreated (*solid bars*) or treated with 0.3 mM H_2O_2 for 10 min (*hatched bars*) or 30 min (*shaded bars*). DCFH-AM was then added for an additional 30 min. The results are expressed as the relative H_2O_2 level (mean ± S.D. for three separate determinations) compared with the untreated vector transfectants.

HeLa/vector cells, which express endogenous MUC1, exhibited a less pronounced increase in H_2O_2 levels compared with HCT116/vector cells (Fig. 4*D*). Moreover, HeLa cells transfected to express increased MUC1 levels showed an attenuated response to H_2O_2 treatment (Fig. 4*D*). These findings demonstrate that MUC1 expression is associated with down-regulation of endogenous and induced intracellular H_2O_2 levels.

Treatment of cells with H_2O_2 is associated with mitochondrial dysfunction and thereby the generation of superoxide radicals (O_2^-) (25). To assess the effects of MUC1 on O_2^- levels, the HCT116 cell transfectants were incubated with HE and then assayed by flow cytometry. The results demonstrate that O_2^- levels increase substantially after treatment of HCT116/vector cells with H_2O_2 (Fig. 5*A*). By contrast, this response to H_2O_2 treatment was attenuated in HCT116/MUC1-A cells (Fig. 5*A*). Analysis of HE oxidation at different time points confirmed that MUC1 expression in HCT116/MUC1-A and HCT116/MUC1-B cells is associated with decreased O_2^- levels as compared with that in HCT116/vector cells (Fig. 5*B*). Treatment of HeLa/vector cells with H_2O_2 also resulted in increased HE oxidation; this response was attenuated in HeLa/MUC1-A cells (Fig. 5*C*). These findings were confirmed at different time points in the HeLa/MUC1-B cells (Fig. 5*D*). Taken together with the DCF data, the results indicate that MUC1 expression attenuates H_2O_2-induced increases in intracellular oxidant levels.

MUC1 Increases Expression of Anti-oxidant Enzymes—The predominant enzymatic mechanisms that regulate intracellular oxidant levels are mediated by SOD, catalase, and GPx (26). To determine whether MUC1 affects expression of these antioxidant enzymes, lysates from the HCT116 transfectants were subjected to immunoblot analysis with anti-SOD1 and -SOD2, anti-catalase, and anti-GPx. The results of a representative

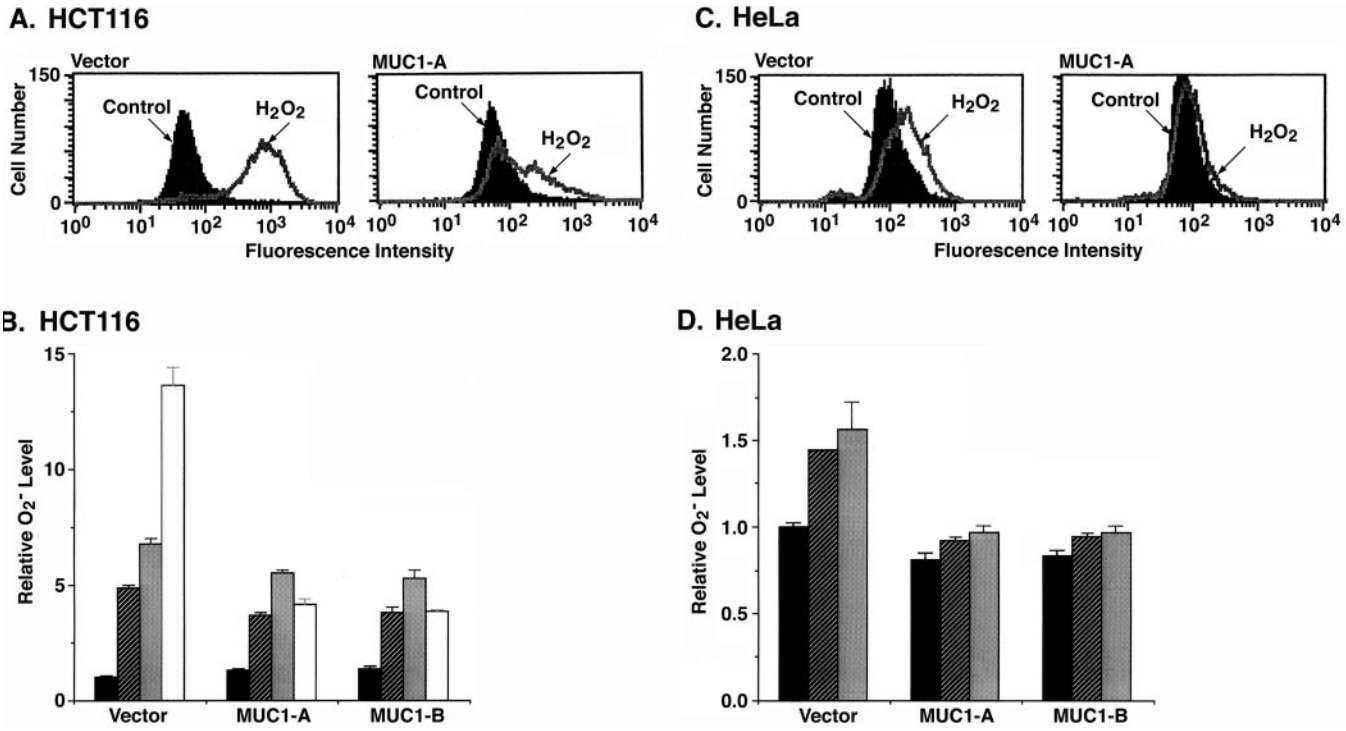

FIG. 5. **MUC1 attenuates superoxide levels.** HCT116 (*A* and *B*) and HeLa (*C* and *D*) cell transfectants were left untreated (*solid profiles* and *bars*) or treated with 0.3 mM H_2O_2 for 10 min (*hatched bars*), 30 min (*C* and *shaded bars*), 120 min (*A* and *open bars*). HE was then added for an additional 20 min. The fluorescence of oxidized HE was measured by flow cytometry. The results are expressed as the mean O_2^- level (mean ± S.D. for three separate determinations) compared with the untreated vector transfectants.

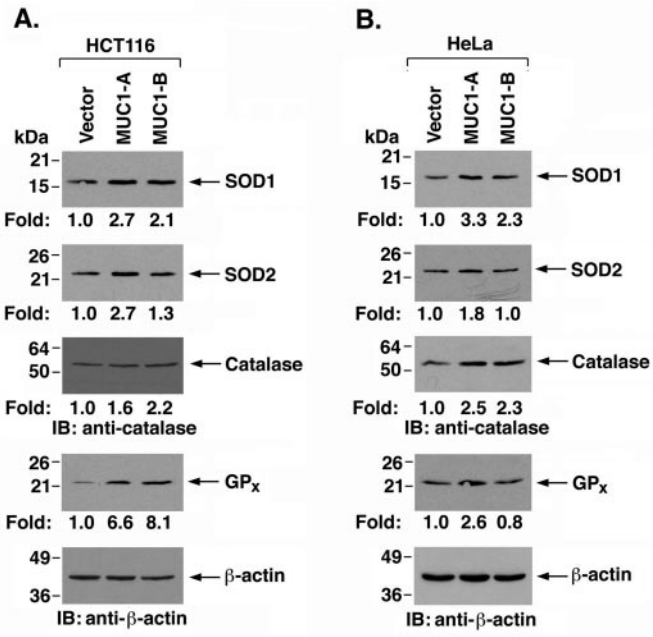

FIG. 6. **MUC1 increases expression of anti-oxidant enzymes.** HCT116 (*A*) and HeLa (*B*) cells expressing empty vector or MUC1 were subjected to immunoblotting with the indicated antibodies. The intensity of the signals as determined by densitometric scanning is expressed as -fold change relative to that in cells expressing the empty vector. Similar results were obtained in four independent experiments.

experiment show that, compared with HCT116/vector cells, SOD1 and SOD2 levels were increased up to 2.7-fold in the MUC1 transfectants (Fig. 6*A*). MUC1 expression was also associated with a 1.6–2.2-fold increase in catalase levels (Fig. 6*A*). Notably, GPx levels were increased 6–8-fold in the HCT116/MUC1 as compared with HCT116/vector cells (Fig. 6*A*). Immunoblotting for β-actin demonstrated equal loading of the lanes (Fig. 6*A*). Increased expression of MUC1 in HeLa cells was also associated with similar increases in SOD1, SOD2, catalase, and GPx levels (Fig. 6*B*). These findings demonstrate that MUC1 expression is associated with increases in anti-oxidant enzyme levels.

MUC1 Inhibits the Apoptotic Response to Oxidative Stress—To determine whether MUC1 regulates the response to oxidative stress, H_2O_2-treated HCT116/vector and HCT116/MUC1 cells were assayed for induction of apoptotic cells with sub-G_1 DNA. The results demonstrate that H_2O_2-induced apoptosis is significantly attenuated in MUC1-positive as compared with MUC1-negative HCT116 cells (Fig. 7, *A* and *B*). The apoptotic response to H_2O_2 was also attenuated by increased expression of MUC1 in HeLa cells (Fig. 7, *C* and *D*). As confirmation of the induction of apoptosis, ethidium bromide staining of H_2O_2-treated HCT116/vector (Fig. 8, *A* and *B*) and HeLa/vector cells (Fig. 8, *C* and *D*) further demonstrated bright orange areas of condensed chromatin in nuclei, which distinguishes late apoptotic from necrotic cells. Notably, there was little if any detectable ethidium bromide staining of untreated control cells or H_2O_2-treated MUC1 expressing cells (Fig. 8). These findings

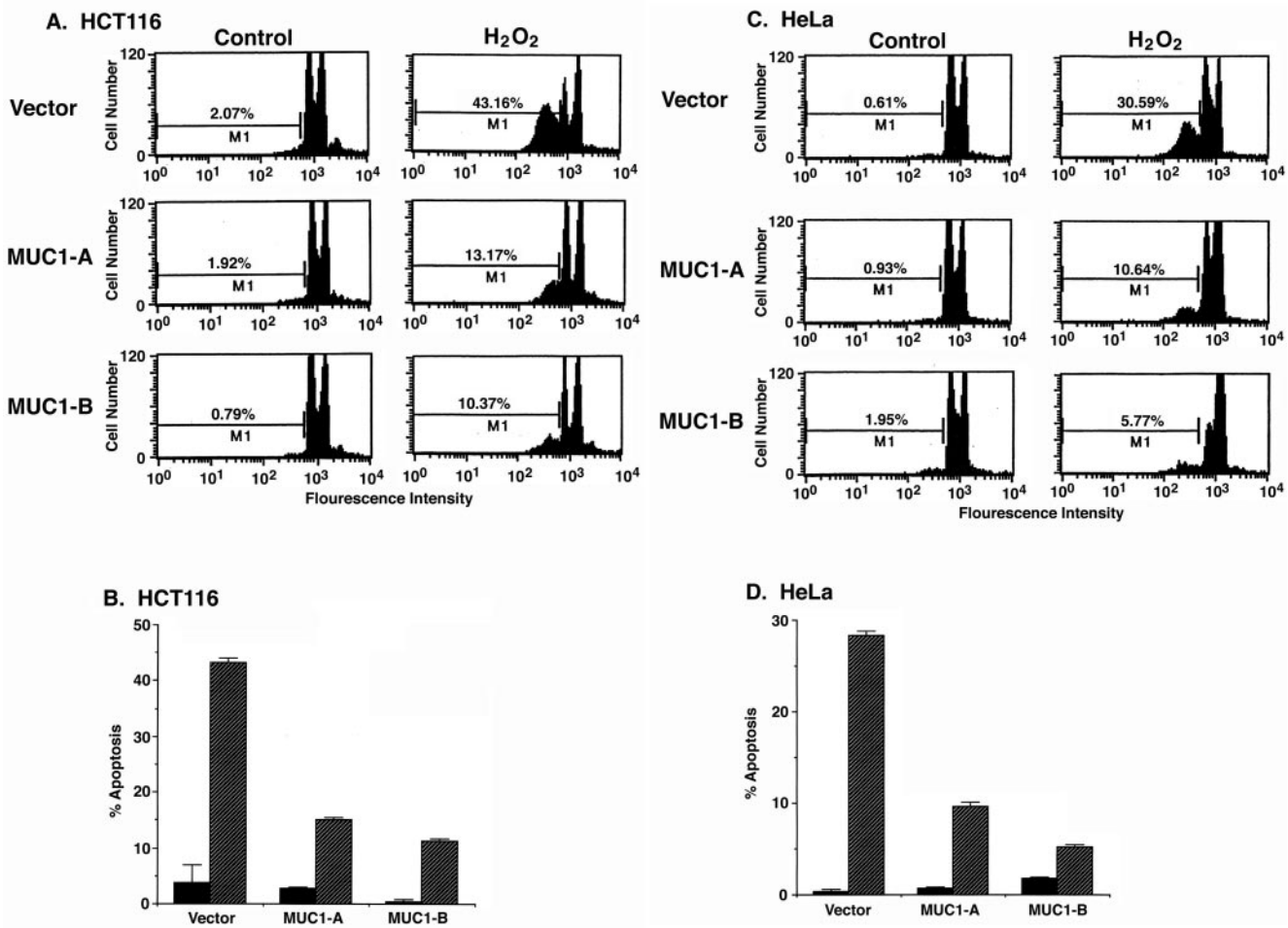

FIG. 7. **MUC1 attenuates induction of cells with sub-G_1 DNA by oxidative stress.** HCT116 (*A* and *B*) and HeLa (*C* and *D*) cells expressing the empty vector or MUC1 were treated with 0.3 mM H_2O_2 for 18 h. *A* and *C*, the percentage of cells with sub-G_1 DNA was determined by flow cytometry. *B* and *D*, the results are expressed as the percentage (mean ± S.D. of three separate experiments each performed in duplicate) of control (*solid bars*) and H_2O_2-treated (*hatched bars*) cells with sub-G_1 DNA.

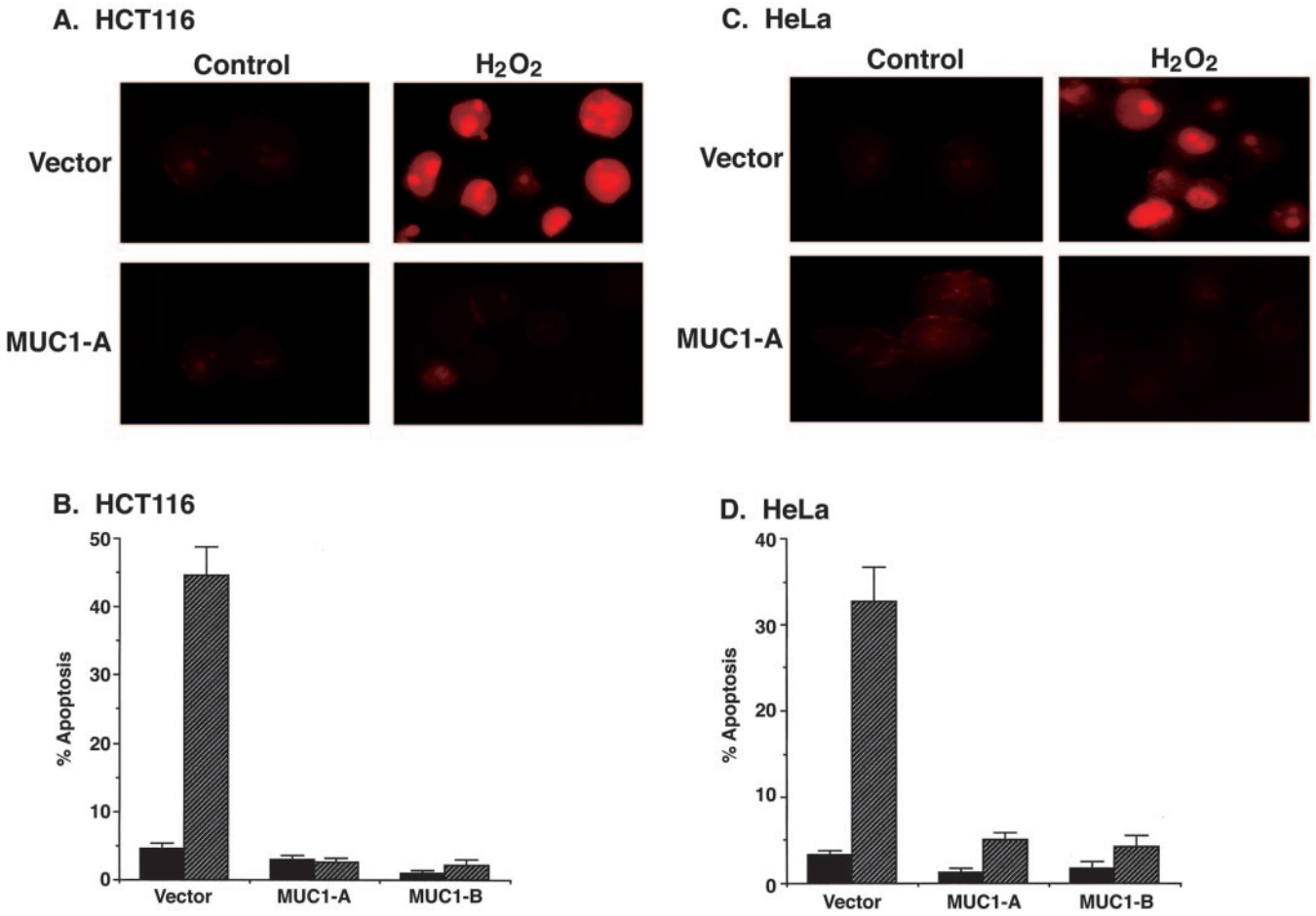

FIG. 8. **MUC1 attenuates the apoptotic response to oxidative stress.** HCT116 (*A* and *B*) and HeLa (*C* and *D*) cells expressing the empty vector or MUC1 were treated with 0.3 mM H_2O_2 for 18 h. The cells were stained with ethidium bromide to assess nuclear morphology. *A* and *C*, chromatin condensation as detected by the presence of bright orange areas in the nuclei distinguishes late apoptotic from necrotic cells. *B* and *D*, the results are expressed as the percentage of apoptotic cells (mean ± S.D. of three determinations) for control (*solid bars*) and H_2O_2-treated (*hatched bars*) cells.

collectively demonstrate that MUC1 expression is associated with an attenuated apoptotic response to oxidative stress.

DISCUSSION

Activation of MUC1 in Response to Oxidative Stress—The heavily glycosylated mucins are believed to function in the protection of epithelial surfaces. Secreted mucins and the transmembrane mucins that are tethered at the cell surface form a protective mucous barrier. The transmembrane mucins may also function in signaling the presence of adverse conditions in the extracellular environment. MUC1 is expressed at the cell surface as a heterodimer of the >250-kDa glycosylated N-terminal ectodomain and the ~25-kDa transmembrane C-terminal subunit. The extensive *O*-glycosylation of the MUC1 ectodomain and the resulting rod-like structure that extends beyond the glycocalyx probably contributes to the mucous barrier. Shedding of the ectodomain may also contribute to mucous formation. The available information, however, provides few if any insights into the function of MUC1 in stress-induced signaling mechanisms.

The present results indicate that MUC1 is involved in the response of cells to oxidative stress. As a consequence of prooxidant conditions in the extracellular milieu, ROS can damage DNA, RNA, proteins, and lipids (13, 14). Moreover, the presence of excessive ROS-induced damage can result in the activation of cell death mechanisms (14). Our results demonstrate that MUC1 expression is activated by exposure of cells to H_2O_2 as a form of ROS. As evidenced by use of a luciferase reporter construct under control of the MUC1 promoter, ROS increase transcription of the MUC1 gene. In concert with this finding, ROS exposure was also associated with increases in *MUC1* transcripts and MUC1 protein. Activation of MUC1 expression was transient in response to ROS exposure and returned to constitutive levels within 1–3 h depending on the cell type. These findings indicate that carcinoma cells respond to oxidative stress with a transient activation of MUC1 expression.

MUC1 Regulates Oxidant Levels—Expression of MUC1 in the oxidative stress response could reflect the activation of pathways to increase protection of the mucous barrier and/or function of MUC1 as an intracellular signaling molecule. To define the potential role of MUC1, we stably expressed MUC1 in carcinoma cells that otherwise exhibit undetectable (HCT116) or low (HeLa) MUC1 levels. Analysis of the oxidation of DCFH-AM led to the unexpected finding that MUC1 decreases endogenous intracellular H_2O_2 levels. Moreover, increases in H_2O_2 levels in response to H_2O_2 treatment were attenuated in independent MUC1 transfectants of both the HCT116 and HeLa cells. In concert with the demonstration that increases in H_2O_2 levels cause mitochondrial dysfunction and the generation of O_2^- (25), we found that oxidation of HE is increased in H_2O_2-treated cells. Importantly, MUC1 expression was associated with the attenuation of H_2O_2-induced O_2^- levels. Whereas H_2O_2 is readily diffusible across cell membranes, expression of the glycosylated MUC1 ectodomain seemed unlikely to decrease intracellular ROS levels. An alternative explana-

tion is that MUC1, particularly the transmembrane subunit, contributes to a signaling cascade that regulates levels of ROS.

MUC1 Increases Expression of Anti-oxidant Enzymes—The present results demonstrate that MUC1 increases expression of the three predominant anti-oxidant enzymes that regulate intracellular H_2O_2 levels. In mammalian cells, O_2^- is metabolized to H_2O_2 by the 32-kDa dimeric Cu,Zn-SOD (SOD1) in the cytosol and the 80-kDa tetrameric Mn-SOD (SOD2) in mitochondria. Increases in both SOD1 and SOD2 were found in the MUC1 transfectants. H_2O_2 is converted to H_2O and O_2 in peroxisomes by the tetrameric catalase (27), whereas GPx converts H_2O_2 to H_2O in a reaction that oxidizes glutathione to its disulfide form. The present results demonstrate that MUC1 expression is also associated with increases in catalase and GPx levels. Thus, it is likely that increased expression of these anti-oxidant enzymes in MUC1-positive cells contributes, at least in part, to the attenuation of endogenous and H_2O_2-induced oxidant levels.

The present studies do not exclude the possibility that MUC1 regulates other enzymes or the non-enzymatic mechanisms involved in maintaining redox balance. Moreover, the specific MUC1-mediated signals that regulate expression of SOD, catalase, and GPx as major enzymatic effectors of the ROS response are presently not known. Indeed, little is known about the signaling mechanisms that control intracellular ROS levels. Recent studies have shown that $p66^{shc}$ regulates oxidant levels in mammalian cells (18, 28). In addition, the forkhead FKHRL1 protein increases H_2O_2 scavenging and resistance to oxidative stress by increasing catalase expression (18). Other work has demonstrated that the c-Abl and Arg tyrosine kinases are activated by oxidative stress and that these proteins regulate intracellular oxidant levels (29–31). Further experiments will be needed to determine whether MUC1 signaling interacts with the $p66^{shc}$, FKHRL1, or c-Abl/Arg pathways.

Does MUC1 Expression by Human Carcinomas Confer a Survival Advantage?—MUC1 is normally expressed at the apical borders of glandular epithelial cells (1). By contrast, the polarization of MUC1 expression is lost in carcinoma cells that aberrantly overexpress the protein in the cytoplasm and over the entire cell surface (1, 32). MUC1 is also expressed in the nucleus in a complex with β-catenin (9, 10) or α-catenin (33). Based on the present results, positioning of MUC1 along the apical borders of the normal ductal epithelium could provide a defense against ROS generated, for example, during inflammatory conditions. Conversely, carcinoma cells may have exploited this mechanism by overexpressing MUC1 to achieve a survival advantage under conditions of oxidative or other forms of stress. In this context, the present studies show that MUC1 confers resistance to oxidative stress. Our results support a model in which expression of MUC1 by carcinoma cells decreases oxidant levels and thereby attenuates the apoptotic response to oxidative stress. Alternatively, MUC1 may attenuate ROS-induced apoptosis by a mechanism in addition to or independent of its effects on oxidant levels. MUC1 may also contribute to survival by blocking the necrotic response to oxidative and other types of stress. In this regard, overexpression of MUC1 is sufficient to confer transformation as assessed by anchorage-independent growth and tumorigenicity (10). Whether the survival advantage attributable to MUC1 expression by carcinoma cells *in vitro* applies to human MUC1-positive tumors is not yet clear. The present findings, however, provide the first evidence that links a protective function of a mucin to regulation of intracellular oxidant levels and the apoptotic stress response.

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