MUC1 Oncoprotein Activates the FOXO3a Transcription Factor in a Survival Response to Oxidative Stress*

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The MUC1 transforming protein is aberrantly overexpressed by most human carcinomas. Recent studies demonstrated that MUC1 confers a protective function against oxidative stress-induced apoptosis; however, the mechanisms responsible for this response are not known. The present work demonstrates that MUC1 regulates FKHR1/FOXO3a, a member of the forkhead family of transcription factors that induces oxidant scavenging and DNA repair. We show that MUC1 attenuates activation of the phosphoinositide 3-kinase → phospho-Akt/PKB pathway in HCT116 colon carcinoma cells and thereby decreases FOXO3a phosphorylation. MUC1 is expressed as an N-terminal ectodomain that is tethered to the cell surface by a C-terminal transmembrane subunit. The results demonstrate that the MUC1 cytoplasmic domain is sufficient to induce FOXO3a activation and attenuation of oxidative stress. We also demonstrate that stable down-regulation of endogenous MUC1 in ZR-75-1 breast cancer cells inactivates FOXO3a, increases intracellular oxidant levels, and sensitizes cells to H2O2-induced necrosis. These findings indicate that MUC1 regulates the FOXO3a signaling pathway in a survival response to oxidative stress.

The secreted mucins function in the physical protection of epithelial cell surfaces. The integral membrane mucins that are tethered to the cell surface contribute to the protective mucous barrier and may also signal the presence of adverse conditions in the external environment. Human DF3/MUC1 is a heterodimeric mucin that is expressed on the apical borders of normal secretory epithelial cells (1). The >250-kDa MUC1 N-terminal ectodomain (N-ter),1 which extends well beyond the glyocalyx, consists of variable numbers of heavily glycosylated 20-amino acid tandem repeats (2, 3). The ~25-kDa MUC1 C-terminal subunit (C-ter) contains a 58-amino acid extracellular domain, a 28-amino acid transmembrane domain, and a 72-amino acid cytoplasmic tail (4). The MUC1 cytoplasmic domain (MUC1-CD) interacts with β-catenin, a component of the adherens junction and a coactivator of Wnt target genes (5, 6).

Additional studies linking MUC1 to the Wnt pathway have shown that the Wnt effector, glycogen synthase kinase 3β, phosphorylates MUC1-CD and down-regulates the MUC1-β-catenin interaction (7). MUC1 also interacts with members of the ErbB family of receptor tyrosine kinases (8–10). The epidermal growth factor receptor phosphorylates MUC1-CD and increases binding of MUC1 to β-catenin (8). Other work has shown that MUC1-CD functions as a substrate for phosphorylation by c-Src and protein kinase Cδ (PKCδ) (11, 12). Localization of MUC1 C-ter in the nucleus and mitochondria has provided further support for involvement of this subunit in transducing cell membrane signals to the interior of the cell (10, 13–16). The demonstration that MUC1 functions in protecting cells against increased intracellular oxidant levels and H2O2-induced apoptosis has further supported a role for MUC1 in signaling a defensive response to oxidative stress (17).

The FKHR1/FOXO3a transcription factor regulates reactive oxygen species (ROS) scavenging and resistance to oxidative stress (18). FOXO3a function is controlled in part by growth factor binding to specific receptor tyrosine kinases and the activation of PI3K. PI3K phosphorylates phosphatidylinositols at their 3-position and thereby converts phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate (19). The serine-threonine Akt/PKB kinase and the phosphoinositide-dependent kinase 1 localize to the cell membrane by binding of their pleckstrin homology domains to PI(3,4,5)P3 (20, 21). Phosphoinositide-dependent kinase 1 phosphorylates Akt and stimulates Akt activity (22). In turn, Akt phosphorylates FOXO3a, resulting in binding of FOXO3a to 14-3-3 proteins and retention of FOXO3a in the cytoplasm (23). Conversely, dephosphorylation of FOXO3a induces its nuclear localization and FOXO3a-mediated transactivation of gene transcription (18). Overexpression of FOXO3a induces G1 cell cycle arrest (24). Other studies have shown that FOXO3a activates the G2-M checkpoint (25). Moreover, FOXO3a-mediated activation of Gadd45a expression has indicated that FOXO3a contributes to DNA repair in the response to genotoxic stress (25). FOXO3a also contributes to the induction of apoptosis by activating expression of the Fas ligand gene (23). These findings have indicated that, under lower levels of stress, FOXO3a activates a repair and survival response, and in the presence of irreparable damage, FOXO3a contributes to an apoptotic response.

The present studies demonstrate that MUC1 activates FOXO3a. We show that MUC1-CD is sufficient to activate FOXO3a, decrease intracellular ROS levels, and attenuate ROS-induced apoptosis. The results also demonstrate that knocking down MUC1 inactivates FOXO3a and increases the sensitivity of cells to oxidative stress.

MATERIALS AND METHODS

Cell Culture—HCT116/vector, HCT116/MUC1, and HCT116/MUC1-CD colon carcinoma cells (8, 26) were cultured in Dulbecco’s...
modified Eagle’s medium (high glucose, Cellgro) supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. ZR-75-1 breast carcinoma cells were cultured in EGM-2 (Cambrex) supplemented with EGF (20 ng/ml), insulin (10 μg/ml), and hydrocortisone (1 μg/ml). Knockdown of MUC1 was achieved by transfection of MUC1-specific (MUC1siRNA) (16) or MUC1-CD (17) into HCT116 cells with LipofectAMINE for 24 h. After washing and incubation for an additional 10 h, the cells were treated with H2O2 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).

Measurement of H2O2 Levels—Cells were transfected with pFHRE-Luc (23) and SV-40-Renilla Luc (Promega) at a ratio of 10:1 in the presence of LipofectAMINE for 24 h. After washing and incubation for an additional 10 h, the cells were treated with H2O2 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).

FIG. 1. MUC1 regulates activation of the PI3K → p-Akt → p-FOXO3α pathway. A and B, HCT116/vector and HCT116/MUC1 (two separately isolated clones, A and B) were left untreated or exposed to 0.12 mM H2O2 for 16 h. Lysates were analyzed by immunoblotting (IB) with the indicated antibodies. C and D, cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. LY294002 (40 μM) was added during the last 1 h of incubation. Lysates were immunoblotted with the indicated antibodies.

RESULTS

MUC1 Attenuates p-Akt and p-FOXO3α Signaling—To determine whether MUC1 affects activation of the PI3K → p-Akt → p-FKHR pathway, lysates from HCT116/vector and HCT116/MUC1 cells were analyzed by immunoblotting with an antibody against p-Akt. Constitutive levels of p-Akt were similar in the HCT116/vector and HCT116/MUC1 cells indicated by staining ethanol-fixed and citrate buffer-permeabilized cells with propidium iodide and monitoring by flow cytometry (BD Biosciences) as described (12). For assessment of necrosis, cells were incubated in 1 μg/ml propidium iodide/phosphate-buffered saline for 5 min at room temperature and then monitored by flow cytometry.

Clonogenic Survival—Cells were treated with 0.12 mM H2O2 for 16 h, washed, and maintained in complete medium for 10–14 days. Colonies of more than 20 cells were detected by Giemsa staining and scored as positive.

MUC1-CD Is Sufficient to Regulate ROS Levels—To define the region of MUC1 that regulates ROS levels, the cells were incubated with DCFH-DA (Molecular Probes) for 30 min at 37 °C. 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).

Immunoblot Analysis—Cells were lysed as described (17) and analyzed by immunoblotting with anti-phospho-Akt (anti-p-Akt; Cell Signaling Inc.), anti-Akt (Cell Signaling Inc.), anti-phospho-FOXO3α (anti-p-FOXO3α, Cell Signaling Inc.), anti-FOXO3α (Santa Cruz Biotechnology), anti-cytochrome c (Santa Cruz Biotechnology), anti-caspase-3 (Cell Signaling Inc.), and anti-PKCδ (Santa Cruz Biotechnology). Antibody-antibody complexes were visualized by enhanced chemiluminescence (ECL, Amersham Biosciences).

Measurement of H2O2 Levels—Cells were transfected with pFHRE-Luc (23) and SV-40-Renilla Luc (Promega) at a ratio of 10:1 in the presence of LipofectAMINE for 24 h. After washing and incubation for an additional 10 h, the cells were treated with H2O2 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).

Assays of Apoptosis and Necrosis—Sub-G1 DNA content was assessed by staining ethanol-fixed and citrate buffer-permeabilized cells with propidium iodide and monitoring by flow cytometry (BD Biosciences) as described (12). For assessment of necrosis, cells were incubated in 1 μg/ml propidium iodide/phosphate-buffered saline for 5 min at room temperature and then monitored by flow cytometry.

Clonogenic Survival—Cells were treated with 0.12 mM H2O2 for 16 h, washed, and maintained in complete medium for 10–14 days. Colonies of more than 20 cells were detected by Giemsa staining and scored as positive.

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MUC1-CD Is Sufficient to Regulate ROS Levels—To define the region of MUC1 that regulates p-FOXO3α, we performed studies on HCT116 cells stably expressing only the MUC1 cytoplasmic domain (MUC1-CD) (Fig. 2A). Treatment of two separately isolated HCT116/MUC1-CD clones with H2O2 had no detectable effect on MUC1-CD expression (Fig. 2A). To determine whether MUC1-CD affects ROS levels, the cells were incubated with DCFH-DA, and H2O2-mediated oxidation of the fluorochrome was assayed by flow cytometry. As shown for full-length MUC1 (17) and compared with HCT116/vector cells, expression of MUC1-CD was associated with a substantial decrease in H2O2 levels (Fig. 2B). The cells were also exposed to H2O2 and then assayed for oxidation of DCFH-DA. Compared with HCT116/vector cells, H2O2 levels were attenuated in the HCT116/MUC1-CD, as compared with those in HCT116/vector cells (Fig. 2C). These findings indicate that MUC1-CD is sufficient to confer regulation of oxidant levels.

MUC1-CD Is Sufficient to Attenuate PI3K Signaling—Immunoblot analysis of lysates from the HCT116/MUC1-CD cells demonstrated a decrease in p-Akt levels compared with those in HCT116/vector cells (Fig. 3A). H2O2-induced increases in p-Akt as found in HCT116/vector cells were also attenuated by MUC1-CD expression (Fig. 3A). Similar results were obtained when the lysates from control and H2O2-treated cells were probed for p-FOXO3α and FOXO3α (Fig. 3B), indicating that, like full-length MUC1, MUC1-CD down-regulates Akt and FOXO3α signaling. In addition, treatment with LY294002 decreased constitutive and H2O2-induced levels of p-Akt and p-FOXO3α
FIG. 2. MUC1-CD decreases intracellular H$_2$O$_2$ levels. A, HCT116/vector and HCT116/MUC1-CD (two separately isolated clones, A and B) were left untreated or exposed to 0.12 mM H$_2$O$_2$ for 16 h. Lysates were analyzed by immunoblotting (IB) with anti-MUC1 C-ter and anti-β-actin. B, cells were incubated with DCFH-DA for 30 min. Fluorescence of oxidized DCF was measured by flow cytometry. C, cells were left untreated (solid bars) or exposed to 0.12 mM H$_2$O$_2$ for 10 (open bars) or 30 (shaded bars) min. DCFH-DA was then added for an additional 30 min. Cells were analyzed by flow cytometry. The results are expressed as the relative H$_2$O$_2$ level (mean ± S.D. for three separate experiments) compared with that in the untreated HCT116/vector cells.

in both the HCT116/vector and HCT116/MUC1-CD cells (data not shown). Phosphorylated FOXO3a binds to cytosolic 14-3-3 and is thereby sequestered from activating gene transcription. To assess the effects of MUC1 on FOXO3a-dependent transcription, we transfected HCT116 cells with the pFHRE-Luc reporter construct (23). Analysis of Luc activity demonstrated that activation of the reporter is ~15-fold higher in the HCT116/MUC1 compared with that in HCT116/vector cells (Fig. 3C). In addition, treatment with H$_2$O$_2$ had little effect on FOXO3a-mediated transcription (Fig. 3C). In concert with these results, the pFHRE-Luc reporter construct was activated over 10-fold in HCT116/MUC1-CD as compared with HCT116/vector cells (Fig. 3D). These findings indicate that MUC1-CD is sufficient to confer attenuation of the PI3K → p-Akt → p-FOXO3a signaling pathway and thereby activation of FOXO3a-mediated transcription.

MUC1-CD Attenuates the Apoptotic Response to Oxidative Stress—To determine whether MUC1-CD protects against H$_2$O$_2$-induced apoptosis, we first monitored release of mitochondrial cytochrome c into the cytosol. Treatment of HCT116/vector cells with H$_2$O$_2$ was associated with increases in cytosolic cytochrome c (Fig. 4A). By contrast, this response was attenuated in the HCT116/MUC1-CD cells (Fig. 4A). H$_2$O$_2$ treatment was also associated with activation of caspase-3 in HCT116/vector but not HCT116/MUC1-CD cells (Fig. 4A). PKCδ is a caspase-3 substrate that is cleaved to a constitutively active catalytic fragment (27). In concert with the results for caspase-3 activation, full-length PKCδ was cleaved to PKCδ catalytic fragment in HCT116/vector but not HCT116/MUC1-CD cells (Fig. 4A). As an additional assessment of the apoptotic response, the HCT116 cells were assayed for H$_2$O$_2$-induced sub-G$_1$ DNA. Treatment of the HCT116/vector cells with H$_2$O$_2$ was associated with greater than 30% apoptosis (Fig. 4B). By contrast, H$_2$O$_2$ had no apparent effect on induction of sub-G$_1$ DNA in HCT116/MUC1-CD cells (Fig. 4B). Analysis of sub-G$_1$ data from three independent experiments confirmed that MUC1-CD expression substantially attenuates H$_2$O$_2$-induced apoptosis (Fig. 4C).

Down-regulation of MUC1 Increases H$_2$O$_2$ Levels—To determine whether knocking down MUC1 expression affects oxidative levels, we stably expressed a CsiRNA or a MUC1siRNA in MUC1-positive ZR-75-1 breast cancer cells. Immunoblot analysis confirmed down-regulation of MUC1 N-ter and C-ter expression in ZR-75-1/MUC1siRNA (two separately isolated clones) but not in ZR-75-1/CsiRNA cells (Fig. 5A). As assessed by oxidation of DCFH-DA, down-regulation of MUC1 was associated with an increase in intracellular H$_2$O$_2$ levels (Fig. 5B). Moreover, treatment of the ZR-75-1/MUC1siRNA cells with H$_2$O$_2$ was associated with a substantial increase in DCFH-DA oxidation as compared with the attenuated response in H$_2$O$_2$-treated ZR-75-1/CsiRNA cells (Fig. 5C). Analysis of three separate experiments confirmed that constitutive H$_2$O$_2$ levels are increased by knocking down MUC1 and that the response to H$_2$O$_2$ treatment is attenuated in ZR-75-1/CsiRNA as compared with ZR-75-1/MUC1siRNA cells (Fig. 5D). These results indicate that endogenous MUC1 functions in the regulation of H$_2$O$_2$ levels.

Knocking Down MUC1 Increases Sensitivity to Oxidative Stress—Expression of MUC1 in HCT116 cells attenuates the apoptotic response to oxidative stress (17). To assess the effects of knocking down MUC1, ZR-75-1 cells were exposed to H$_2$O$_2$ and monitored for sub-G$_1$ DNA. There was no detectable induction of sub-G$_1$ DNA in H$_2$O$_2$-treated ZR-75-1/CsiRNA or ZR-75-1/MUC1siRNA cells (data not shown). We also found no significant effect of H$_2$O$_2$ on annexin-V staining (data not shown), indicating that ZR-75-1 cells positive or negative for MUC1 expression are null for an apoptotic response to oxidative stress. To determine whether ZR-75-1 cells exhibit a necrotic
response, we monitored uptake of propidium iodide as a measure of loss of cell membrane integrity. Flow cytometric analysis of control and H2O2-treated ZR-75-1/CsiRNA cells demonstrated a modest necrotic response to oxidative stress (Fig. 7A). By contrast, propidium iodide staining was increased substantially following H2O2 treatment of ZR-75-1/MUC1siRNA cells (Fig. 7A). Similar results were obtained in 3 separate experiments (Fig. 7B), indicating that MUC1 protects against H2O2-induced necrosis of ZR-75-1 cells. To extend these results, we assayed control and H2O2-treated ZR-75-1 cells for colony formation as a measure of survival. H2O2 treatment of ZR-75-1/CsiRNA cells was associated with a partial decrease in colony formation (Fig. 7C). However, in contrast with the propidium iodide staining data, survival of ZR-75-1/MUC1siRNA cells was substantially decreased by H2O2 exposure (Fig. 7C). These findings indicate that MUC1 protects ZR-75-1 cells from oxidative stress-induced loss of survival.

DISCUSSION

MUC1-CD Activates FOXO3a—MUC1 is aberrantly overexpressed by carcinomas of the breast, prostate, lung, pancreas, and other epithelia (1). In association with loss of polarity, MUC1, which is normally found on apical borders, is expressed at high levels over the entire surface of carcinoma cells. MUC1 C-ter is also found in the nucleus and mitochondria of transformed cells (10, 13–16). Importantly, overexpression of full-length MUC1 is sufficient to confer transformation (14, 26). Moreover, MUC1 confers resistance to genotoxic and oxidative stress (16, 17). MUC1-dependent activation of the oxidative stress response is mediated at least in part by up-regulation of anti-oxidant enzyme (superoxide dismutase, catalase, and glutathione peroxidase) expression (17). The present results extend these observations by demonstrating that MUC1 regulates FOXO3a activation. FOXO3a protects cells from oxidative stress by increasing H2O2 scavenging and inducing DNA repair (25, 28). Moreover, forkhead-induced resistance to oxidative stress has been associated with prolongation of survival (18). The demonstration that FOXO3a is also activated in HCT116/MUC1-CD cells indicates that the MUC1 cytoplasmic tail is sufficient for stimulation of this pathway. In concert with these results, we found that transcription of the pFHRE-Luc reporter was substantially increased in HCT116/MUC1-CD cells compared with that obtained for untreated HCT116/vector cells (designated as 1).
MUC1-CD Regulates PI3K

3p-Akt Signaling—FOXO3a is inactivated by p-Akt-mediated phosphorylation and binding of p-FOXO3a to cytosolic 14-3-3 proteins (23). The present results demonstrate that, like p-FOXO3a, levels of p-Akt are decreased in MUC1-positive HCT116 cells. We also found that p-Akt is down-regulated in HCT116/MUC1-CD cells, indicating that the cytoplasmic tail is sufficient to activate signals that control Akt.

FIG. 4. MUC1-CD attenuates H2O2-induced apoptosis. A–C, HCT116/vector and HCT116/MUC1-CD cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. A, lysates were analyzed by immunoblotting (IB) with the indicated antibodies. B and C, cells were analyzed for sub-G1 DNA by flow cytometry (B). The results are expressed as the percentage (mean ± S.D. of three separate experiments) of control (solid bars) and H2O2-treated (hatched bars) cells with sub-G1 DNA (C). cyt c, cytochrome c; FL, full-length; CF, catalytic fragment.

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Recent studies have shown that MUC1 activates the p-Akt pathway in rat 3Y1 fibroblasts (30). MUC1 expression was also associated with increases in phospho-BAD and Bcl-xL, and thereby attenuation of stress-induced apoptosis (30). These findings collectively indicate that MUC1 regulates the Akt pathway in different cell types and that the effects of MUC1 are dependent on cell context. Activation of Akt is mediated by PI3K-dependent formation of PI(3,4,5)P3 and phosphorylation of Akt by phosphoinositide-dependent kinase 1 (22). Other work has shown that p66shc functions upstream of p-Akt and FOXO3a signaling in the response to oxidative stress (18). In the present studies, the demonstration that LY294002 blocks constitutive and H2O2-induced phosphorylation of Akt and FOXO3a indicates that PI3K is responsible for activating the p-Akt → p-FOXO3a pathway in HCT116 cells. Assessment of PI3K activity in HCT116 cells expressing MUC1 showed no apparent difference from that in HCT116/vector cells (data not shown). Thus, MUC1 may down-regulate p-Akt levels in HCT116 cells by stimulating phosphatases that regulate Akt phosphorylation.

Knocking Down Endogenous MUC1 inactivates FOXO3a and Increases Oxidant Levels—

FIG. 6. Knocking down endogenous MUC1 activates PI3K → p-Akt → p-FOXO3a signaling. A and B, ZR-75-1/CsiRNA and ZR-75-1/MUC1siRNA cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. Lysates were analyzed by immunoblotting (IB) with the indicated antibodies. C, cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. LY294002 (40 μM) was added during the last 1 h of incubation. Lysates were immunoblotted with the indicated antibodies.

Knocking down endogenous MUC1 sensitizes cells to H2O2-induced death. A, ZR-75-1/CsiRNA and ZR-75-1/MUC1siRNA cells were left untreated or exposed to 0.12 μM H2O2 for 16 h. The cells were stained with propidium iodide and analyzed by flow cytometry. B, the data represent the percentage (mean ± S.D.) of necrotic cells obtained from three separate experiments. Untreated cells, solid bars; H2O2-treated cells, hatched bars. C, cells were left untreated (solid bars) or exposed to 0.12 μM H2O2 for 16 h (hatched bars), washed, and then incubated in the absence of H2O2 for 10–14 days. Clonogenic survival is expressed as the percentage (mean ± S.D. of three separate experiments) of H2O2-treated cells that form colonies (>20 cells) compared to that obtained without H2O2 exposure.

FIG. 7. Knocking down endogenous MUC1 activates PI3K → p-Akt → p-FOXO3a signaling. A and B, ZR-75-1/CsiRNA and ZR-75-1/MUC1siRNA cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. Lysates were analyzed by immunoblotting (IB) with the indicated antibodies. C, cells were left untreated or exposed to 0.12 mM H2O2 for 16 h. LY294002 (40 μM) was added during the last 1 h of incubation. Lysates were immunoblotted with the indicated antibodies.
MUC1-CD in HCT116 cells represents one approach to assessing the effects of MUC1 on the oxidative stress response. Knocking down MUC1 in carcinoma cells that endogenously overexpress this protein was also used to assess the role of MUC1 on oxidative stress-induced apoptosis. In concert with the results in HCT116 cells, knocking down MUC1 in ZR-75-1 cells was associated with increases in p-Akt and Akt levels. Thus, the effects of MUC1 on p-Akt in ZR-75-1 cells can be attributed, at least in part, to lower levels of Akt protein. In this regard, further studies are needed to determine whether MUC1 regulates Akt levels by transcriptional or post-transcriptional mechanisms. Knocking down MUC1 also increased levels of p-FOXO3a and FOXO3a. These results, like those in HCT116 cells, are not attributable to clonal variation as similar findings were obtained in two separately isolated ZR-75-1/MUC1siRNA clones. Moreover, as found in HCT116 cells, constitutive and more pronounced increases in intracellular oxidant levels following H2O2 exposure were observed in the MUC1-negative ZR-75-1 cells. In concert with these results, we also found that knocking down MUC1 is associated with decreases in catalase expression (data not shown). Notably, like Sod2, the catalase gene is responsive to FOXO3a-induced activation. Thus, down-regulating endogenous MUC1 in ZR-75-1 cells results in inactivation of FOXO3a, decreases in catalase levels, and increases in oxidant levels. The results obtained by knocking down MUC1 are thus in agreement with the effects of stably expressing MUC1 or MUC1-CD in HCT116 cells.

Overexpression of MUC1 by Human Carcinomas May Protect against Oxidative Stress-induced Cell Death—Full-length MUC1 attenuates H2O2-induced apoptosis of HCT116 cells (17). These findings suggested that MUC1 transduces stress-induced signals from the cell membrane that protect cells from oxidative damage and death. Indeed, the present work indicates that the MUC1 cytoplasmic tail, which localizes to the nucleus and mitochondria (10, 13, 14, 16, 26), is sufficient to confer this protection. Positioning of MUC1 along the apical borders of the normal ductal epithelium could function in sensitizing the external environment. In turn, targeting of MUC1-CD to the cytosol or nucleus may contribute to the activation of effectors such as FOXO3a that confer a survival response. Moreover, mitochondrial localization of MUC1 is associated with attenuation of the intrinsic apoptotic pathway (16). Human carcinoma cells may have exploited this physiologic mechanism by overexpressing MUC1 to achieve a survival advantage under conditions of oxidative and other forms of stress. In this regard, overexpression of MUC1 has also been shown to confer resistance to genotoxic stress (16). Importantly, knocking down endogenous MUC1 sensitizes cells to stress-induced apoptosis and necrosis. Thus, down-regulating MUC1 expression or function may represent a potential approach for sensitizing tumors to therapeutic agents, such as ionizing radiation, that increase ROS levels. However, it is presently not known whether the survival advantage attributable to MUC1 expression by carcinoma cells in vitro applies to primary and metastatic MUC1-positive carcinomas in man.

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