Targeting the Intracellular MUC1 C-terminal Domain Inhibits Proliferation and Estrogen Receptor Transcriptional Activity in Lung Adenocarcinoma Cells

Carolyn M. Klinge, Brandie N. Radde, Yoannis Imbert-Fernandez, Yun Teng, Margarita M. Ivanova, Sabra M. Abner, and Alexandra L. Martin

Abstract

Mucin 1 (MUC1) is a diagnostic factor and therapy target in lung adenocarcinoma. MUC1 C-terminal intracellular domain (CD) interacts with estrogen receptor (ER) α and increases gene transcription in breast cancer cells. Because lung adenocarcinoma cells express functional ERα and ERβ, we examined MUC1 expression and MUC1–ER interaction. Because blocking MUC1 CD with an inhibitory peptide (PMIP) inhibited breast tumor growth, we tested whether PMIP would inhibit lung adenocarcinoma cell proliferation. We report that MUC1 interacts with ERα and ERβ within the nucleus of H1793 lung adenocarcinoma cells in accordance with MUC1 expression. PMIP was taken up by H23 and H1793 cells and inhibited the proliferation of H1793, but not H23 cells, concordant with higher MUC1 protein expression in H1793 cells. Lower MUC1 protein expression in H23 does not correspond to microRNAs miR-125b and miR-145 that have been reported to reduce MUC1 expression. PMIP had no effect on the viability of normal human bronchial epithelial cells, which lack MUC1 expression. PMIP inhibited estradiol-activated reporter gene transcription and endogenous cyclin D1 and nuclear respiratory factor-1 gene transcription in H1793 cells. These results indicate MUC1–ER functional interaction in lung adenocarcinoma cells and that inhibiting MUC1 inhibits lung adenocarcinoma cell viability. Mol Cancer Ther; 10(11); 2062–71. ©2011 AACR.

Introduction

Although the incidence of lung adenocarcinoma has increased in never and former smokers, women, and young adults, the role of gender in risk remains unresolved (1). Interestingly, breast cancer patients receiving antiestrogens for breast cancer had lower lung cancer mortality, indicating a possible role for estrogens in disease progression (2). We reported that lung adenocarcinoma cell lines from females respond proliferatively to estradiol (E2) and were inhibited by estrogen receptor (ER) antagonists 4-hydroxytamoxifen (4-OHT) or ICI 182,780 (ICI, fulvestrant; ref. 3). In contrast, lung adenocarcinoma cells from males were neither stimulated by E2 nor blocked by 4-OHT or ICI. Similar responses were detected when examining E2-induced cyclin D1 (CCND1) transcription (3). ERα and ERβ expression was similar among cell lines from males and females, indicating that the observed phenotype was not due to lower ER expression in cells from males. Other investigators detected E2-induced proliferation and activation of intracellular mitogen-activated protein kinase and phosphoinositide 3-kinase (PI3K) by ERα, ERβ, GPR30, and epidermal growth factor receptor (EGFR) in lung adenocarcinoma cell lines in a gender-independent manner (4, 5).

Mucin 1 (MUC1) is a high-molecular weight plasma membrane–bound protein with a heavily O-glycosylated N-terminal domain (NTD) that protrudes from the apical surface of glandular epithelial cells in lung, breast, and colon (6). MUC1 has a single transmembrane domain and a 72-amino acid C-terminal intracellular domain (CD) that is cleaved and regulated by tyrosine phosphorylation (7).

MUC1 is a marker of type II pneumocyte progenitor cell lineage (8). Its surface expression is critical for the protective function of the airway epithelium (9). A MUC1 gene polymorphism for the large MUC1 allele was associated with lung adenocarcinoma but not with squamous cell carcinoma (10). MUC1 is an oncogene (11), is increased in lung cancer (12), and is a clinical marker for lung adenocarcinoma (13). During dedifferentiation in lung adenocarcinoma progression, MUC1 is reduced and mislocalizes to the entire...
plasma membrane where it blocks cell–cell and cell–matrix adhesion, allowing tumor cell invasion (14). The NTD is secreted by tracheobronchial epithelial cells and is a mucus component (15).

Within cells, MUC1 CD interacts with proteins including PI3K, Shc, PLC1, c-Src, Grb-2, p53, IKKβ, IKKγ, β-catenin, hsp90, hsp70, and ERα (7). MUC1 overexpression in cancer cells results in MUC1 CD nuclear and mitochondrial localization (16). MUC1 CD interacts with ERα in the nucleus of breast cancer cells where it increased ERα transcriptional activity (17).

Because MUC1 is an oncogene, it makes a tempting therapeutic target (18). Its knockdown by short interfering RNA in A549 lung adenocarcinoma cells increased sensitivity to cisplatin (19). Similarly, stable transfection of siMUC1 inhibited H358 non–small cell lung cancer (NSCLC) tumor formation in mice (20). Treatment of breast cancer cells with a MUC1 inhibitory peptide (MIP) cloned adjacent to the protein transduction domain (PTD4) created a peptide called PMIP that is taken up by cells without transfection reagents (21). PMIP acts as a decoy for MUC1 binding partners. PMIP inhibited MUC1–β-catenin and MUC1–EGFR interactions and inhibited MDA-MB-231 breast cancer cell proliferation, migration, and invasion in vitro and tumor growth in mice (21). Similarly, a MUC1 inhibitor called GO-201 bound MUC1 CD, blocked MUC1 oligomerization, and induced necrosis in MCF-7, ZR-75-1, and MDA-MB-231 breast cancer cells (16). GO-201 was recently reported to inhibit the proliferation of lung adenocarcinoma cell lines (22).

This study tested the hypotheses that ERα and ERβ interact functionally with MUC1 in lung adenocarcinoma cells and that PMIP selectively inhibits lung adenocarcinoma, not normal human bronchial epithelial cells (HBEC), proliferation, and inhibits ER responses.

Materials and Methods

Chemicals

17-β-Estradiol (E2) and 4-OHT were obtained from Sigma. ICI 182,780 was obtained from Tocris. Sequences of the control peptide (NH₂-YARAAARQARATNPAPAATSANL-COOH) and PMIP (MIP adjacent to the PTD4): NH₂-YARAAARQARARYEKVSAGNGGSSLSCOOH, as reported in ref. 21. Fluorescein isothiocyanate (FITC)-PMIP and PMIP were purchased from New England Peptide.

Antibodies

The following antibodies were purchased: HC-20 for ERα from Santa Cruz Biotechnology; ERβ from Upstate (catalogue #06-629); α-tubulin from LabVision (Fisher Scientific); β-actin from Sigma; Armenian hamster anti-MUC1 CD (Ab-5, MUC1; CT2) from Thermo Scientific; and anti-MUC1 NTD (DF3) from Abcam. The secondary antibody for CT2 was anti-Armenian hamster (Jackson Immunoresearch).

Estrogen receptor

Recombinant human ERα and ERβ1 (long form) were prepared as described (23).

Cell culture

The 5 HBEC lines and their maintenance and characterization were described (23, 24), and HBECs were used at fewer than 8 passages. MCF-7 cells were purchased from American Type Culture Collection (ATCC) and used at fewer than 10 passages from ATCC. MCF-7 were maintained as described (3). Before treatment, cells were placed in phenol red–free media supplemented with 5% dextran-coated charcoal-stripped FBS (DCC-FBS) for 24 to 48 hours. Ethanol (EtOH) was used as the vehicle control.

MUC1 genotyping

PCR primers P1 and P2 were used to detect the MUC1 splice variants MUC1/A and MUC1/B (25). Products were analyzed on a DNA 500 chip of the Agilent 2100 Bioanalyzer.

Immunofluorescence imaging

H1793 cells were incubated with 10 μmol/L of FITC-PMIP for 1, 4, and 24 hours, or 10 μmol/L of FITC-PMIP for 24 hours plus 10 nmol/L E2 for the last 4 hours. Cells on coverslips were fixed with 4% paraformaldehyde for 15 minutes. After washing and permeabilization with 0.2% Triton X-100 in PBS and blocking with 10% bovine serum albumin in PBS, primary antibodies MUC1 (CT2), ERα (HC-20), and ERβ (H150) were added at 1:1,500, 1:1,000, and 1:500 dilution, respectively, overnight at 4°C. Cells were stained with secondary antibodies at a 1:2,000 dilution. The secondary AffiniPure Goat anti-Armenian hamster antibody was labeled with R-phycocerythrin 566 (red color; Jackson ImmunoResearch) or FITC, and secondary anti-rabbit antibody was labeled with Zenon Cy3-Conjugation Kit (green color; Invitrogen). Immunofluorescence imaging used a Zeiss Axiosvert 200 inverted microscope with a ×40 objective lens and AxioVision Release 4.3 software. Images were taken at the same exposure.

Protein isolation

Whole-cell extracts (WCE) were prepared in modified radioimmunoprecipitation assay (RIPA) buffer (3). Protein concentrations were determined by the Bio-Rad DC Protein Assay (Bio-Rad Laboratories).

Western blotting

Western blot analysis was carried out as described (3). The membranes were stripped and reprobed for α-tubulin. Immunoblots were scanned with a Microtek ScanMaker VII scanner. Un-Scan-It (version 6.1; Silk Scientific) quantitated the integrated optical densities for each band, which was divided by concordant α-tubulin integrated optical densities in the same blot. For comparison...
between experiments, the MUC1 CD/α-tubulin–normalized pixel ratios for MCF-7 cells was set to 1.

**Coimmunoprecipitation**

Nuclear lysates were prepared with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce) according to the manufacturer’s protocol. Nuclear lysates (400 μg) were incubated with the indicated antibodies in RIPA buffer (20 mmol/L Tris, pH 8, 100 mmol/L NaCl, 1 mmol/L dithiothreitol, 0.2% NP40, 0.2% deoxycholate, and 0.2% Triton X-100) supplemented with protease and phosphatase inhibitors for 1 hour at 4°C. Protein G-Sepharose 4B (Zymed) was added and incubated overnight with rotation at 4°C. The beads were sedimented at 10,000 × g, washed 3 × with RIPA buffer, resuspended in 2 × Tris-Glycine buffer (Invitrogen), and incubated at 85°C for 2 minutes. Proteins in the resulting supernatant were separated on 14% Tris-Glycine gels (Invitrogen), and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked and immunoblotted. HyGLO Chemiluminescent HRP Antibody Detection Reagent (Denville Scientific) was used to detect protein bands. The membranes were visualized on a Carestream Imager using Carestream Molecular Imaging software.

**MTT assays**

MDA-MB-231, H1793, H23, HBEC2-KT, and HBEC4-KT cells were plated as described (3). Cell lines were treated with 0.1 to 20 μmol/L control peptide or PMIP, or with or without E2, 4-OHT, or ICI 182, 780 for 5 days. Treatments were changed every 24 hours. Within individual experiments, each treatment was done in quadruplicate, and experiments were carried out at least 3 times. MTT assays used Cell-Titer96 from Promega.

**Transient transfection assays**

Transient transfection of H1793 cells used FuGene6 (Roche). Each well received 5 ng of a Renilla luciferase reporter (pRL-tk; Promega) and 250 ng of pGL3-2EREc38-luciferase reporter (26). The cells were harvested 30 hours posttreatment, using Promega’s Passive Lysis buffer. Luciferase and Renilla luciferase activities were determined by Promega’s Dual Luciferase assay and calculations were carried out (3).

**RNA isolation, reverse transcriptase PCR, and quantitative real-time PCR**

RNA was extracted from cells using TRIzol (Invitrogen) or RNeasy (Qiagen). The High Capacity cDNA archive Kit (PE Applied Biosystems) was used to reverse transcribe total RNA using random hexamers. Quantitative real-time PCR (qRT-PCR) for MUC1 used ABI TaqMan primers (27) and was normalized by 18S rRNA. Analysis and fold differences were determined by the comparative C<sub>t</sub> method. Data are presented as relative to expression in EtOH-treated and control transfected cells.

**microRNA isolation and qRT-PCR**

microRNA (miRNA)-enriched total RNA was extracted from MCF-7, H1793, and H23 cells, using the miRNA isolation Kit (Exiqon). The quality and quantity of the isolated RNA were analyzed with a NanoDrop spectrophotometer and Agilent Bioanalyzer. cDNA was synthesized with the miRCURY LNA first-strand cDNA synthesis kit, and quantitative PCR was carried out with the miRCURY LNA SYBR Green master mix using the miRNA primer sets for miR-125b and miR-145 (Exiqon). 5S rRNA was used for normalization of miRNA expression. Analysis and fold change were determined by the comparative C<sub>t</sub> method. The change in miRNA expression was calculated as fold change, that is, relative to MCF-7 cells.

**Statistical analysis**

Statistical analyses were conducted by the Student t test or one-way ANOVA followed by the Student–Newman–Keuls or Dunnett post hoc tests, using GraphPad Prism.

**Results**

**MUC1 in lung adenocarcinoma cell lines and HBEC lines**

MUC1 expression was examined in WCE prepared from 8 lung adenocarcinoma cell lines, 5 immortalized HBEC lines with similar phenotypic and genotypic properties as primary HBECs (24), and MCF-7 breast (28) cancer cells (Fig. 1). In agreement with other reports (29), MCF-7 cells express high MUC1 protein. Interestingly, H2073 and H1793 cells, derived from female patients, also express high MUC1 protein. All 4 lung adenocarcinoma cell lines from males (H1792, H1299, A549, and H23) have lower MUC1 than MCF-7 protein expression, as do H1944 and H1395 cell lines from females. MUC1 was not detected in HBECs (Fig. 1B and Supplementary Fig. S1).

**MUC1 splice variants in lung adenocarcinoma cells and HBECs**

Although MUC1 is used as a clinical marker for lung adenocarcinoma (13, 30), to our knowledge, no one has examined MUC1 splice variants in lung adenocarcinomas or cell lines derived from lung tumors. Reverse transcriptase PCR was carried out to detect MUC1/A and MUC1/B splice variants (31). The results indicate no apparent gender-dependent difference in MUC1/A or MUC1/B splice variant expression (Supplementary Table S1), although a larger population would be needed to confirm these data.

Total MUC1 mRNA expression was evaluated with a primer set that recognizes all variants of MUC1 (Fig. 1D) and a primer set that recognizes only MUC1/B (Fig. 1E). Previous investigators reported that E2 increased MUC1 in MCF-7 cells (28); however, these results were based on semiquantitative PCR that detected 2 MUC1 bands, one of which the authors called a precursor form, and no
quantitation of the single-point experiment was carried out. Here \( E_2 \) had no significant effect on MUC1/B or MUC1 mRNA expression in MCF-7, H1793, H23, H1944, H2073, HBEC2-KT, or HBEC3-KT cells. The expression of total MUC1 and MUC1/B correspond to the MUC1 genotyping in Supplementary Table S1.

miR-125b and miR-145 expression does not correspond to differences in MUC1 protein between H23 and H1793 cells

MUC1 is downregulated by miR-125b (32) and miR-145 (33), which interact with the 3’ untranslated region (UTR) of MUC1 mRNA and reduce MUC1 protein in ZR-75-1 breast cancer cells and HEK-293T cells, respectively. To determine whether the levels of miR-125b and miR-145 corresponded inversely with MUC1 protein in H23 and H1793 cells, miR-125b and miR-145 expression was evaluated with MCF-7 cells used as a positive control. Given that MCF-7 and H1793 have approximately 8-fold more MUC1 protein than H23; we anticipated lower miR-125b and miR-145 expression in MCF-7 and H1793 cells than in H23 cells. MCF-7 has higher miR-125b and miR-145 expression than either H1793 or H23 cells (Fig. 2A and B). The lower miR-145 expression in H1793 than in H23 cells suggests that miR-145 may reduce MUC1 protein in H23 cells. To test this idea, H23, H1793, and MCF-7 cells were transfected with control antisense (AS) and AS-miR-145 and MUC1 protein was examined by Western blot (Fig. 2C). Supplementary Fig. S2 shows that AS-miR-145 reduced miR-145 and that neither the AS control nor mock transfection affected miR-145 in any cell line. However, AS-miR-145 had no effect on MUC1 protein, indicating that the miR-145 is not responsible for the lack of MUC1 in H23 cells and does not regulate MUC1 in H1793 or MCF-7 cells.

MUC1 interacts with ER\( \alpha \) and ER\( \beta \) in lung adenocarcinoma cell lines

Because MUC1 interacts with ER\( \alpha \) in the nucleus of MCF-7 cells (17), we evaluated the interaction of ER\( \alpha \) and ER\( \beta \) with MUC1 in NE prepared from H1793 and H23 cells and used MCF-7 cells as a positive control (Fig. 3). ER\( \alpha \) and ER\( \beta \) are located in the nucleus, cytoplasm, and...
mitochondria of H1793, H23, and MCF-7 cells (34). MUC1 interacted with nuclear ERα and ERβ in H1793 cells in accordance with higher basal MUC1 expression. The molecular weight estimation of the 2 ERα bands detected in the immunoprecipitation was 66 and 46 kDa. Notably, only the 46-kDa band (ERα46) was detected in H23 cells (also see input lane), despite the detection of full-length ERα using another antibody (Supplementary Fig. S3). These data reflect the expression of ERα variants in lung adenocarcinoma cells as reported by us (3, 23, 34) and others (35). Low MUC1–ERα46 and MUC1–ERβ interaction was seen in H23 cells, reflecting low MUC1 protein.

**PMIP localizes to the nucleus of lung adenocarcinoma cell lines**

PMIP (10 μmol/L) was taken up by BT-20 cells without any transfection reagent after 4-hour incubation (21). H23 and H1793 cells were incubated with 10 μmol/L FITC-PMIP for 1, 4, and 24 hours (Fig. 4A). FITC-PMIP was distributed throughout the cell with greater nuclear uptake at 4 and 24 hours than at 1 hour, and no apparent difference in immunofluorescence signal between 4 and 24 hours was observed.

**PMIP inhibits H1793 cell proliferation**

To determine whether PMIP inhibits cell proliferation, H23, H1793, HBEC2-KT, and HBEC3-KT cells were treated with control peptide or PMIP (Fig. 4B). Because PMIP inhibited MDA-MB-231 breast tumor xenograft growth in mice (21), MDA-MB-231 cell viability was determined in parallel and was inhibited in a concentration-dependent manner (IC50 = 1.5 μmol/L). Notably, H1793 cells, but not H23, HBEC2-KT, or HBEC3-KT cells, were selectively inhibited by PMIP (Fig. 4B and C; IC50 = 0.1 μmol/L). These data correspond to the relative MUC1 protein expression in each cell line (Fig. 1). E2 reduced the PMIP inhibition of cell viability (Fig. 4D) but not completely. PMIP did not synergize with inhibition of cell viability by ER antagonists 4-OHT or ICI 182,780 (Fig. 4D).

**PMIP inhibits ER-activated gene transcription in H1793 cells**

Because MUC1 was reported to be an ERα coactivator in MCF-7 cells (17), we examined whether PMIP inhibited ER-mediated transcription in H1793 cells (Fig. 5A). PMIP did not affect basal luciferase activity but significantly inhibited E2-induced ERE-driven luciferase activity, indicating that endogenous MUC1 plays a role in ER-mediated transcription in H1793 cells.

E2 increases CCND1 (23, 34) and nuclear respiratory factor-1 (NRF-1; ref. 36) transcription in H1793 cells. Here, we found that 10 μmol/L PMIP inhibited E2-induced CCND1 and NRF-1 transcription (Fig. 5B and C), a result in agreement with PMIP inhibition of cell viability (Fig. 4B).
Intracellular MUC1 Peptide Inhibits NSCLC Proliferation

PMIP affects MUC1, ERα, and ERβ intracellular location in a time-dependent manner

To examine how PMIP affected the intracellular localization of MUC1 in H1793 cells, cells were treated with 10 μmol/L PMIP for 1, 4, or 24 hours (Fig. 6A). Treatment with PMIP increased MUC1 nuclear localization with 1- and 4-hour treatment. After 24 hours of PMIP, MUC1 was detected in the nucleus and cytoplasm. Notably, colocalization of ERα and ERβ with MUC1 is seen in the merged images (Fig. 6A and B). We also examined MUC1 and PMIP intracellular localization (Supplementary Fig. S4). MUC1 and PMIP were colocalized at all time points.

We examined how PMIP affects ERα and ERβ cellular distribution in H1793 cells (Fig. 6A and B). ERα was predominantly cytoplasmic in the absence of any treatment, and there was an increase in nuclear ERα with 4-hour PMIP treatment, followed by a return to the cytoplasmic localization at 24-hour PMIP treatment (Fig. 6A). In contrast, ERβ was primarily nuclear in the absence of PMIP treatment and 1 hour of PMIP treatment increased ERβ nuclear density (Fig. 6B). However, 4 and 24 hours of PMIP treatment resulted in ERβ translocation from the nucleus to the cytoplasm (Fig. 6B). Treatment of H1793 cells with 10 nmol/L E2 for 4 hours increased nuclear MUC1 (Supplementary Fig. S4B). E2 did not alter the reduction in nuclear ERα seen in H1793 cells treated with PMIP (Fig. 6C). These data are in agreement with the inhibition of E2-activated CCND1 and NRF-1 transcription by PMIP (Fig. 5B and C). Interestingly, 75 of 107 H1793 cells analyzed after PMIP + E2 treatment showed ERβ retention in the nucleus (Fig. 6C). These data indicate that E2 reduces the PMIP-mediated distribution of ERβ from the nucleus to the cytoplasm in approximately 70% of cells. ERα and ERβ colocalize with PMIP (Fig. 6C and D), reflecting the colocalization of ERα and ERβ with MUC1 (Fig. 6A and B).

Discussion

Because MUC1 is an oncogene and its overexpression is a marker for reduced survival of patients with lung adenocarcinoma (37), inhibiting MUC1 function has become an important target for therapeutic intervention (18). At the same time, MUC1, when appropriately expressed on the apical surface, is important for normal lung function and protective mucus production (38). Inhibition of MUC1 reduced MDA-MB-231 xenograft tumor growth (16, 21). Here, we used the MUC1 inhibitory peptide PMIP created by Bitler and colleagues to be taken up by cells without any sort of transfection (21). Similar to that study, we found that H1793 and H23 cells took up PMIP without transfection within 1 hour of treatment. We observed that PMIP inhibited only the proliferation of H1793 cells that expressed significantly higher levels of MUC1 protein than did H23 cells and not HBECs that are MUC1 null. While this article was in preparation, Kufe and colleagues reported that MUC1 inhibitors, called GO-201, -202, and -203 that bind the MUC1 CD, inhibited the proliferation of lung adenocarcinoma cell lines including A549 and H1795 without affecting normal human lung epithelial cells (22). Thus, this is the second report that inhibition of MUC1 function reduces lung adenocarcinoma cell proliferation. At present, there is no clear way to compare the efficacy of GO-201, -202, and -203 with PMIP because these peptides were not tested in the same cell lines, but the IC50 of PMIP for H1793 reported here is 0.1 μmol/L whereas 5 μmol/L GRO-202 and GRO-201 inhibited H1975 lung cancer cell proliferation by 50% (no IC50 values were provided; ref. 22). Because MUC1 interacts with many proteins and has multiple roles in cells, the precise mechanisms by which inhibiting MUC1 with PMIP reduces H1793 proliferation remains to be defined. We showed that PMIP inhibited E2-induced transcriptional responses, indicating that ER is one target of blockade of MUC1 action by PMIP. Indeed, PMIP treatment resulted in retention of ERα,
but not ERβ, in the cytoplasm. Additional MUC1 targets may also be involved in PMIP activity in H1793 cells. One such MUC1-interacting partner is EGFR, as indicated in recent MUC1 inhibitor studies in NSCLC with EGFR and Ras mutation (22). EGFR is overexpressed and mutated in lung adenocarcinoma. Both H1793 and H23 cell lines have wild-type EGFR and mutant p53 (39). A focus of future studies will be to identify the interacting protein partners of intracellular MUC1 in lung adenocarcinoma cell lines and how PMIP affects those interactions.

Estrogens induce differentiation and maturation of the lung, but the role of estrogens in NSCLC is controversial. Some studies (reviewed in ref. 40) indicate a role for estrogen in lung cancer risk, but recent epidemiologic data indicate a minor role for estrogens in lung cancer (41). We and others reported ERα and ERβ expression in lung adenocarcinoma cells and tumors (reviewed in ref. 3). Because ER was transcriptionally active in some lung adenocarcinoma cell lines (3) and MUC1 is an ERα coactivator in MCF-7 cells (17), here, we examined MUC1–ER interaction and inhibition by PMIP in lung adenocarcinoma cells.

We report that MUC1 protein expression is higher in 4 lung adenocarcinoma cell lines from females than in 4 cell lines from males. MUC1 is in the top 10% of overexpressed genes in lung cancer (www.oncomine.org). The factors regulating MUC1 expression seem to be cell-type specific. E2 and tamoxifen increased the secreted MUC1 isoform transcription via ERα activation in breast cancer cells (29), but E2 did not increase MUC1 in human endometrial cancer cells (42). We did not detect E2 regulation of MUC1 mRNA in any lung adenocarcinoma cells, HBECs, or MCF-7 cells and conclude that E2 does not regulate MUC1 in these cells. The difference between our results and those reported earlier in MCF-7 cells (29) is that we used quantitative PCR rather than a MUC1

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Figure 4. PMIP uptake by H23 and H1793 cells and inhibition of H1793 proliferation. A, H23 and H1793 cells were incubated with 10 μmol/L FITC-PMIP for 1, 4, and 24 hours. Cells were washed and fixed, and images were captured at the same exposure setting. B, MTT assays were carried out and values are an average ± SEM of 2 to 6 experiments. MDA-MB-231 breast cancer cells, H1793 and H23 lung adenocarcinoma cells (B), and HBEC2-KT and HBEC4-KT (C) were incubated with the indicated concentrations of control peptide (CP) or PMIP for 5 days, with fresh PMIP added every 24 hours. D, H1793 cells were treated with the indicated concentrations of control peptide or PMIP alone with or without E2, 4-OHT, or ICI 182,780 for 5 days. Significantly different (P < 0.05) from control (a); the same concentration of PMIP alone (b); and 10 nmol/L E2 alone (c).
promoter-reporter and nonquantitative PCR. Although the sample size is small, we observed no significant difference in MUC1/A and MUC1/B splice variant expression between lung adenocarcinoma cell lines.

Figure 5. PMIP inhibits E2-induced transcription in H1793 cells. A, H1793 cells were transfected with an ERE-luciferase reporter, a Renilla luciferase reporter, and treated with 10 μmol/L control peptide (CP), 1 μmol/L PMIP, or 10 μmol/L PMIP and with EIOH, 10 nmol/L E2, or 100 nmol/L 4-OHT for 24 hours. Values are firefly luciferase/Renilla luciferase normalized to untreated/EtOH. *, significantly different from *, EIOH or **, 10 nmol/L E2/untreated (Utx); P < 0.05. B and C, H1793 cells were treated with 10 μmol/L control peptide or 10 μmol/L PMIP and with EIOH or 100 nmol/L E2 for 4 or 24 hours. Quantitative PCR for CCND1 (B) and NRF-1 (C) mRNA expression. *, significantly different from control peptide + E2; P < 0.05. In all panels, values are the average ± SEM of 3 separate experiments.

Figure 6. PMIP affects MUC1, ERα, and ERβ subcellular location in H1793 cells. Immunofluorescent microscopic imaging of H1793 cells. A and B, H1793 cells were treated with 10 μmol/L PMIP for the indicated time and incubated with MUC1 antibody (green), ERα (HC-20; red in A), and ERβ (H150; red in B). C and D, H1793 cells treated with 10 μmol/L FITC-PMIP (green) for 20 hours and then 10 nmol/L E2 was added for the last 4 hours. MUC1 antibody (green), ERα (HC-20; red in C), and ERβ (H150; red in D). Nuclei are stained with Hoechst (blue color). Bars (gold), 20 μm. The merged image is indicated.
derived from female versus male patients. Exploring gender- or disease-specific associations with MUC1 splice variant expression requires a large sample size and is a separate project.

As reported for MCF-7 cells (17), ERβ interacts with MUC1 in H1793 cells. Although H23 has lower MUC1 protein expression, MUC1 interacted with the ERα46 band detected in H23 cells. The role for ERα46 in lung cancer is unknown and not the focus of this study, but in breast cancer cells, it is a dominant-negative effector of ERα66 (43). We report for the first time that nuclear ERβ interacts with MUC1 in H1793 cells. The role of ERβ is not fully understood (44). ERβ is considered antiproliferative in breast cancer, but its role in lung adenocarcinoma remains undefined. Because the gene targets of ERβ in breast cancer cells, it is a dominant-negative effector of breast cancer cells, indicating that other cell-specific factors regulate MUC1 protein. miR-183 is upregulated in squamous cell lung cancer compared with normal lung (50). Despite not being identified as a high-scoring candidate, miR-145 reduced MUC1 in MDA-MB-231 cells (33). Likewise, miR-125b regulated MUC1 in BT-549 breast cancer cells (32). Although miR-145 expression was lower in H1793 than in H23 cells, transfection of miR-145 AS did not increase MUC1 expression in H23 cells. Further studies are needed to determine the mechanisms regulating MUC1 protein expression in H23 cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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