Molecular Cell *21*, 295–305, January 20, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2005.11.030

MUC1 Oncoprotein Stabilizes and Activates Estrogen Receptor α

Xiaolong Wei,[1,2] Hai Xu,[1,2] and Donald Kufe[1,*]
[1] Dana-Farber Cancer Institute
Harvard Medical School
Boston, Massachusetts 02115

Summary

The MUC1 protein is aberrantly overexpressed by most human breast carcinomas. We report that the MUC1 C-terminal subunit associates with estrogen receptor α (ERα) and that this interaction is stimulated by 17β-estradiol (E2). MUC1 binds directly to the ERα DNA binding domain and stabilizes ERα by blocking its ubiquitination and degradation. Chromatin immunoprecipitation assays further demonstrate that MUC1 (1) associates with ERα complexes on estrogen-responsive promoters, (2) enhances ERα promoter occupancy, and (3) increases recruitment of the p160 coactivators SRC-1 and GRIP1. In concert with these results, we show that MUC1 stimulates ERα-mediated transcription and contributes to E2-mediated growth and survival of breast cancer cells. These findings provide evidence that MUC1 stabilizes ERα and that this oncoprotein is of importance to the activation of ERα function.

Introduction

The MUC1 transmembrane glycoprotein is normally expressed on the apical borders of secretory mammary epithelia (Kufe et al., 1984). With transformation and loss of polarity, MUC1 is aberrantly overexpressed on the entire surface of breast cancer cells (Kufe et al., 1984; Perey et al., 1992). MUC1 is expressed as a stable heterodimer after translation of a single polypeptide and cleavage into two subunits in the endoplasmic reticulum (Ligtenberg et al., 1992). The MUC1 N-terminal subunit (MUC1 N-ter, MUC1-N) contains variable numbers of 20 amino acid tandem repeats that are extensively modified by O-linked glycans (Gendler et al., 1988; Siddiqui et al., 1988). The MUC1 C-terminal subunit (MUC1 C-ter, MUC1-C) consists of a 58 amino acid extracellular domain, a 28 amino acid transmembrane domain, and a 72 amino acid cytoplasmic tail (Merlo et al., 1989). On the cell surface, MUC1-N extends well beyond the glycocalyx and is tethered by MUC1-C to the cell membrane. MUC1-C also accumulates in the cytosol of transformed cells and is targeted to the nucleus (Li et al., 2003a, 2003b, 2003c; Wen et al., 2003) and mitochondria (Ren et al., 2004). The MUC1 cytoplasmic domain (MUC1-CD) associates with β-catenin (Huang et al., 2005; Li and Kufe, 2001; Yamamoto et al., 1997) and with the p53 tumor suppressor (Wei et al., 2005). MUC1-CD is also subject to phosphorylation by the epidermal growth factor receptor (EGFR) (Li et al., 2001b), c-Src (Li et al., 2001a), and glycogen synthase kinase 3β (GSK3β) (Li et al., 1998), supporting a role for MUC1 in both the ErbB receptor tyrosine kinase and Wnt signaling pathways (Li et al., 2003c; Schroeder et al., 2001). Other studies have shown that MUC1 overexpression is sufficient to confer anchorage-independent growth and tumorigenicity (Huang et al., 2005, 2003; Li et al., 2003b; Schroeder et al., 2004).

Most human breast cancers are estrogen dependent (Ali and Coombes, 2002). Estrogen action is mediated by two members of the nuclear receptor family, estrogen receptor α (ERα) and ERβ. Both ERs contain a central DNA binding domain (DBD), which binds to estrogen response elements (EREs), and a C-terminal ligand binding domain (LBD). Upon estrogen binding, ERα undergoes conformational changes and dimerization that confer binding to EREs. Activation of ERα-mediated transcription is regulated by activation function-1 (AF-1) in the N-terminal region and AF-2 in the LBD. AF-1 is activated by growth factors through the MAP kinase pathway (Kato et al., 1995), and AF-2 is activated by binding of estrogen (Bourguet et al., 1995). In the response to estrogen, ERα transcription complexes on target promoters recruit coactivators from the p160 family (SRC-1/NCoA-1, GRIP1/NCoA-2, and AIB1/RAC3/ACTR) (Onate et al., 1995; Shiau et al., 1998). Notably, recruitment of p160 coactivators is sufficient for ERα-mediated gene activation and for estrogen-induced growth stimulation (Shang et al., 2000). By contrast, tamoxifen competes with estrogen for binding to ERα and induces conformational changes that block recruitment of coactivators (Brzozowski et al., 1997; Shang et al., 2000). In addition, tamoxifen bound ERα recruits corepressors to estrogen-responsive promoters (Shang et al., 2000).

The overexpression of MUC1 in most human breast carcinomas, the correlation between MUC1 and ERα levels in breast tumors (Lundy et al., 1985), and the importance of ERα for breast cancer cell growth prompted us to investigate whether MUC1 interacts with ERα. The results demonstrate that MUC1 binds directly to the ERα DBD and stabilizes ERα. We also show that MUC1 is present in the ERα transcription complex, stimulates ERα-mediated transcription, and promotes E2-mediated growth and survival of breast cancer cells.

Results

MUC1 Associates with ERα

Human MCF-7 breast cancer cells that express endogenous MUC1 were studied to determine if MUC1 interacts with ERα. Immunoblot analysis of anti-ERα immunoprecipitates with an antibody that reacts with the MUC1 C-terminal subunit (MUC1-C) demonstrated that ERα coprecipitates with MUC1-C (Figure 1A). The results also demonstrate that the association between ERα and MUC1-C is increased by 17β-estradiol (E2) stimulation (Figure 1A). Similar results were obtained with ZR-75-1 breast cancer cells that express endogenous MUC1 (Figure 1B). A kinetic analysis of the interaction showed that MUC1-ERα complexes increase at 3 to

*Correspondence: donald_kufe@dfci.harvard.edu
[2] These authors contributed equally to this work.

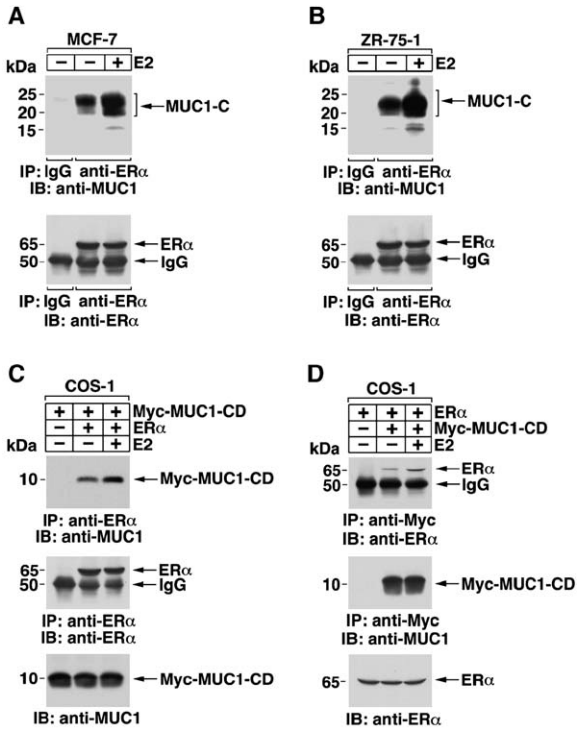

Figure 1. MUC1 Associates with ERα

(A and B) Human MCF-7 (A) and ZR-75-1 (B) breast cancer cells were grown in phenol red-free medium supplemented with 10% charcoal-dextran-stripped FBS for 3 days. The cells were then left untreated or were stimulated with 100 nM E2 for 3 hr. Lysates were subjected to immunoprecipitation (IP) with anti-ERα or a control IgG. The immunoprecipitates were analyzed by immunoblotting (IB) with anti-MUC1-C and anti-ERα.

(C and D) COS-1 cells expressing Myc-MUC1-CD and ERα were stimulated with 100 nM E2 for 3 hr. Anti-ERα (C) or anti-Myc (D) IPs were immunoblotted with anti-MUC1-C or anti-ERα. Lysates not subjected to IP were immunoblotted with anti-MUC1-C or anti-ERα (lower panels).

6 hr of E2 stimulation and then decline at 12 and 24 hr (see Figures S1A and S1B in the Supplemental Data available with this article online). Densitometric scanning of the MUC1 signals obtained from whole-cell lysates as compared to that after immunoprecipitation of the lysates with anti-ERα indicate that ~3% and 5% of the total MUC1-C associates with ERα in control and E2-stimulated MCF-7 cells, respectively (Figure S1C). In ZR-75-1 cells, ~4% and 6% of total MUC1-C associated with ERα in the absence and presence of E2, respectively (Figure S1D). As a control, there was no detectable MUC1-C in anti-PCNA precipitates from MCF-7 (Figure S1E) or ZR-75-1 (Figure S1F) cells. To define the region of MUC1-C responsible for the interaction, Myc-tagged MUC1 cytoplasmic domain (Myc-MUC1-CD) was coexpressed with ERα in COS-1 cells. Immunoblot analysis of anti-ERα precipitates with anti-MUC1-CD demonstrated that MUC1-CD is sufficient for the association with ERα (Figure 1C). Moreover, stimulation of the COS-1 cells with E2 increased binding of ERα and Myc-MUC1-CD (Figure 1C). This association was confirmed in the reciprocal experiment in which anti-Myc immunoprecipitates were immunoblotted with anti-ERα (Figure 1D). These findings indicate that MUC1 associates with ERα constitutively and that this interaction is increased in the response to E2.

MUC1-CD Binds Directly to ERα

To define the regions of MUC1-CD (72 amino acids) and ERα (595 amino acids) that are responsible for the interaction (Figure 2A), GST or a GST-MUC1-CD fusion protein was incubated with ^{35}S-labeled ERα. Analysis of adsorbates to glutathione beads demonstrated binding of full-length ERα(1–595) to GST-MUC1-CD and not GST (Figure 2B). By contrast, there was no detectable binding of MUC1-CD to ERα(1–185) that contains the AF1 domain (Figure 2B). Moreover, the demonstration that MUC1-CD binds to ERα(1–282) indicated involvement of the DNA binding domain (DBD) (Figure 2B). Consistent with these results, binding of MUC1-CD was found with ERα(185–595), but not ERα(282–595) or ERα(Δ186–281) that are devoid of the DBD (Figure 2B). Binding was also observed with ERα(186–281), confirming that MUC1-CD interacts directly with the ERα DBD (Figure 2C). The results demonstrate that ^{35}S-labeled ERα binds to both full-length MUC1-CD and MUC1-CD(1–51), indicating that the N-terminal region of MUC1-CD is sufficient for the interaction (Figure 2D). Consistent with those results, deletion of MUC1-CD amino acids 9 to 46 abrogated the association with ERα (Figure 2D). The results also show an E2-dependent increase in the binding of MUC1-CD and full-length ERα (Figure 2E). By contrast, 4-hydroxytamoxifen (TAM) had no apparent effect on the formation of MUC1-CD-ERα complexes (Figure 2E). These findings indicate that MUC1-CD binds directly to the ERα DBD and that this interaction is stimulated by E2.

MUC1 Stabilizes ERα

To assess the effects of MUC1 on ERα expression, MCF-7 cells were stably infected with a retrovirus expressing MUC1siRNA. Immunoblot analysis of two separately isolated clones demonstrated partial (~80%–90%) and complete downregulation of MUC1 in MCF-7/MUC1siRNA-A and MCF-7/MUC1siRNA-B cells, respectively, as compared to that in cells expressing a control siRNA (CsiRNA) (Figure S2A). Assessment of ERα levels in the MCF-7, MCF-7/CsiRNA, and MCF-7/MUC1siRNA cells demonstrated that knocking down MUC1 is associated with decreases in ERα expression (Figure 3A, left). Densitometric scanning of the ERα signals in independent experiments and at different levels of exposure demonstrated that MUC1 increases ERα levels by 2.9 ± 0.5-fold (mean ±SD of three experiments) (Figure S2B). Compared with MCF-7 cells, ERα expression was lower in ZR-75-1 cells (Figure S2C). Moreover, silencing MUC1 in ZR-75-1 cells (Figure S2D) was associated with decreases in ERα expression (Figure 3A, right). Quantification of the ERα signals in independent experiments and at different levels of exposure showed that MUC1 increases ERα levels by 4.0 ± 0.6-fold (mean ±SD of three experiments) in ZR-75-1 cells (Figure S2E). Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of the MCF-7 and ZR-75-1 cells demonstrated that ERα mRNA levels are similar in the presence and absence of MUC1 (Figures S3A and S3B), indicating that MUC1 regulates ERα by a posttranslational mechanism. Stability of the ERα