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<b>14. ABSTRACT</b> The MUC1 oncoprotein is aberrantly overexpressed in 80-90% of human breast carcinomas. Little, however, was known about the role of MUC1 in the development of breast cancer. The Specific Aims of this Idea Award were: 1) To determine if MUC1 overexpression is sufficient to induce transformation; and 2) To assess whether MUC1 localizes to the nucleus and thereby regulates gene expression. Work supported by this Award has demonstrated that MUC1 induces transformation and that the cytoplasmic domain is sufficient for this function by stabilizing $\beta$ -catenin (Task 1). Our results also demonstrate that the MUC1-C subunit is targeted to the nucleus where it interacts with p53, ER $\alpha$ and KLF4, and thereby regulates gene transcription in human breast cancer cells (Task 2). These findings have thus provided novel information that MUC1 induces transformation, localizes to the nucleus and regulates gene expression. The findings also indicate that overexpression of MUC1 by human breast tumors is of importance to the malignant phenotype and resistance to therapy.					
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## INTRODUCTION

The human MUC1 mucin-type glycoprotein is expressed on the apical borders of normal secretory mammary epithelial cells (1). MUC1 is translated as a single polypeptide and undergoes autoproteolysis into two subunits that form a heterodimeric complex (2-4). The MUC1 N-terminal subunit (MUC1-N) contains variable numbers of 20 amino acid tandem repeats that are heavily modified with *O*-linked glycans (5, 6). The MUC1 C-terminal subunit (MUC1-C) includes a 58 amino acid extracellular domain, a 28 amino acid transmembrane domain and a 72 amino acid cytoplasmic tail (7). With transformation and loss of polarity, MUC1 is expressed at high levels over the entire surface of breast carcinoma cells, a setting in which MUC1 interacts with the ErbB receptor tyrosine kinases (1, 8-10). In addition, MUC1-C accumulates in the cytosol and is targeted to the nucleus and mitochondria (11-13). Importantly, overexpression of MUC1-C, and not the MUC1-N mucin component, is sufficient to induce transformation and resistance to stress-induced apoptosis (11, 14-17). MUC1-C binds directly to and stabilizes  $\beta$ -catenin, and thereby contributes to the activation of Wnt target genes (15, 18-20). Nuclear MUC1-C also interacts with p53 and regulates p53-dependent activation of the *p21* gene in the response to genotoxic stress (21). These findings have supported a role for MUC1-C in promoting growth and survival of the 80-90% of human breast cancers that overexpress this oncoprotein.

The Kruppel-like factor (KLF) family of transcription factors is characterized by the presence of three Kruppel-like zinc fingers and includes the SP1-like proteins (22). Like certain other family members, KLF4 (GKLF/EZF) acts as both an activator and repressor of genes involved in cell-cycle regulation (23). As such, KLF4 functions as a tumor suppressor by inhibiting the proliferation of nontransformed cells. Paradoxically, KLF4 also functions as a suppressor of p53 expression by acting directing on the PE21 element in the *p53* promoter (24). In this context, KLF4 promotes transformation and resistance to DNA damage-induced apoptosis (24). The available evidence indicates that the oncogenic function of KLF4 emerges in the presence of cyclin D1 signaling or in the absence of p21 (23). KLF4 is also required for p53-

mediated induction of p21 in the growth arrest response to DNA damage (25, 26). Moreover, the demonstration that KLF4 associates with p53 has indicated that KLF4 can directly affect the p53 transactivation function (25, 27). Notably, KLF4 overexpressed in up to 70% of human breast cancers (28) and nuclear localization of KLF4 is associated with an aggressive phenotype (29). In addition, silencing of KLF4 in human breast cancer cells is associated with elevation of endogenous p53 levels and the induction of apoptosis (24), findings consistent with a KLF4 oncogenic function.

The overexpression of MUC1 and KLF4 in human breast cancers and the importance of both proteins in the regulation of p53 prompted us to investigate whether MUC1 interacts with KLF4. The results demonstrate that MUC1-C associates with KLF4 and that this interaction is of functional significance to repression of the *p53* gene.

## BODY

**MUC1 downregulates p53 mRNA and protein levels.** To determine whether MUC1 regulates p53 expression, human MCF-7 breast cancer cells that express endogenous MUC1 were stably infected with a retrovirus expressing a MUC1siRNA. Immunoblot analysis of two separately isolated MCF-7/MUC1siRNA clones demonstrated that silencing MUC1 is associated with increases in p53 as compared to that in wild-type cells and cells expressing a control siRNA (CsiRNA) (Fig. 1A). Similarly, silencing MUC1 in human ZR-75-1 breast cancer cells was associated with increases in p53 expression (Fig. 1B). In addition and to exclude off-target effects, the cells were transfected with a pool of MUC1siRNAs (Dharmacon SMARTpool Reagents). The results show that transiently silencing MUC1 increases p53 expression (Supplemental Fig. S1 and data not shown). Immunoblot analysis of purified nuclear and cytosolic fractions from the MCF-7 and ZR-75-1 cells demonstrated that silencing MUC1 is associated with increases in p53 expression in the cytoplasm and nucleus (Supplemental Figs. S2A and B). Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of the MCF-7 and ZR-75-1 cells demonstrated that p53 mRNA levels are increased by silencing MUC1 (Figs. 1C and 1D), indicating that MUC1 downregulates p53, at least in part, by a transcriptional mechanism.

**MUC1 suppresses p53 gene transcription.** To determine if MUC1 regulates activation of the *p53* promoter, cells without or with MUC1 silencing were transfected with a *p53* promoter-Luc reporter (-2400-p53-Luc) (Fig. 2A) and an internal control LacZ expression plasmid (pCMV-LacZ). Results of luciferase assays showed that *p53* promoter activity is decreased in MCF-7/CsiRNA expressing endogenous MUC1 as compared to that in MUC1-silenced MCF-7/MUC1siRNA cells (Fig. 2B). Similar results were obtained with the ZR-75-1/CsiRNA and ZR-75-1/MUC1siRNA cells (Fig. 2C), indicating that MUC1 represses activity of the *p53* promoter. The PE21 element in the proximal promoter of the *p53* gene has been shown to confer suppression of *p53* transcription (24, 30). To determine whether the PE21 element is required for MUC1-

mediated suppression, MCF-7 and ZR-75-1 cells without or with MUC1 silencing were transfected with *p53* promoter-Luc (-2400-*p53*-Luc) or the reporter with a mutant PE21 element (-2400-PE21-MUT-Luc) (Fig. 2A). The increase in *p53* promoter activity in MCF-7 cells silenced for MUC1 was abrogated by mutating the PE21 element (Fig. 2D). Similar results were obtained when using the -320-*p53*-Luc or -320-PE21-MUT-Luc (Fig. 2D). Activation of the *p53* promoter in ZR-75-1 cells silenced for MUC1 was also abrogated by mutating the PE21 element in both -2400-*p53*-Luc and -320-*p53*-Luc (Fig. 2E), indicating that the PE21 element is required for MUC1-mediated suppression of the *p53* promoter.

**MUC1 occupies the *p53* proximal promoter.** To study if MUC1 binds to the *p53* promoter, chromatin immunoprecipitation (ChIP) assays were performed on the *p53* proximal promoter (PP; -118 to +14) with an anti-MUC1-C antibody (Fig. 3A). MUC1 occupancy of the *p53* proximal promoter was detectable in anti-MUC1-C, and not control IgG, precipitates (Fig. 3B, left). In addition, there was no detectable MUC1 associated with a control region (CR; -6020 to -5940) upstream to *p53* proximal promoter (Fig. 3A). To determine whether MUC1 binds to the PE21 element (-79 to -59), ChIP analyses were performed using primers that cover the *p53* promoter region from -118 to -54 (designated PE21 region; Fig. 3A). MUC1 occupancy of the PE21 region was detectable in anti-MUC1-C, and not the control IgG, precipitates from MCF-7 and ZR-75-1 cells (Fig. 3B, right). These results indicate that MUC1 occupies the PE21 region and thereby contributes to suppression of *p53* gene transcription. KLF4 suppresses *p53* gene transcription by occupying the PE21 element of *p53* gene promoter (24). To determine if MUC1-C occupies the PE21 region with KLF4, Re-ChIP assays were performed using anti-MUC1-C and anti-KLF4 antibodies. Analysis of MCF-7 and ZR-75-1 cells showed that anti-KLF4 precipitates the PE21 region after their release from anti-MUC1-C, indicating that MUC1-C occupies the PE21 region with KLF4 (Fig. 3C). To determine if MUC1-C binds directly to KLF4, GST, GST-MUC1-CD or GST-MUC1-CD deletion fusion proteins were incubated with [<sup>35</sup>S]-labeled KLF4. Analysis of adsorbates to glutathione beads demonstrated that KLF4 binds to MUC1-CD(1-72)

and MUC1-CD(1-46), but not with MUC1-CD(47–72) (Fig. 3D). These results indicate that KLF4 forms complexes with MUC1-C in cells by binding directly to the MUC1-CD N-terminal region (amino acids 1-46). ChIP assays were also performed with anti-KLF4 to assess whether MUC1 affects KLF4 occupancy of the *p53* promoter. Notably, silencing MUC1 was associated with decreased occupancy of the PE21 region by KLF4 (Fig. 3E). By contrast, MUC1 silencing had no apparent effect on total cell KLF4 levels (Supplemental Fig. S3), indicating that MUC1-C increases KLF4 occupancy of the PE21 region.

**MUC1-CD potentiates KLF4-mediated repression of *p53* transcription.** To determine if MUC1 affects activation of the *p53* promoter, ZR-75-1/MUC1siRNA cells were transfected with -2400-*p53*-Luc or -320-*p53*-Luc and MUC1-CD. Of note, the MUC1siRNA used to silence MUC1 in the ZR-75-1 cells targets the extracellular region of MUC1-C and not the cytoplasmic domain (12). Results of the luciferase assays showed that MUC1-CD suppresses *p53* gene transcription (Fig. 4A, left). Immunoblot analysis further showed that MUC1-CD downregulates *p53* levels (Fig. 4A, right). MCF-7 and ZR-75-1 cells were also transfected with -2400-*p53*-Luc, MUC1-CD and increasing amounts of KLF4. The results confirmed that MUC1-CD potentiates KLF4-mediated suppression of *p53* transcription (Fig. 4B). Histone deacetylases (HDACs) are a family of enzymes involved in transcriptional repression by catalyzing the deacetylation of core histones (31, 32). To determine if MUC1 occupies the PE21 region with HDACs, Re-ChIP assays were performed using anti-MUC1-C, anti-HDAC1 and HDAC3 antibodies. Analysis of MCF-7 and ZR-75-1 cells showed that anti-HDAC1 precipitates the PE21 region after release from anti-MUC1-C, indicating that MUC1-C occupies the region with HDAC1 (Fig. 4C). The results also demonstrate that MUC1 occupies the PE21 region with HDAC3 (Fig. 4C). Recruitment of HDACs plays an essential role in transcriptional repression by catalyzing the deacetylation of acetylated core histones (31, 32). ChIP assays from MCF-7 cells demonstrated that occupancy of the PE21 region by HDAC1 and HDAC3 is higher in MCF-7/CsiRNA cells, which express endogenous MUC1, as compared to MUC1-negative, MCF-7/MUC1siRNA cells



(Fig. 4D, left). We also found that MUC1 decreases the acetylation of histone 3 and histone 4 in MCF-7/CsiRNA, as compared to MCF-7/MUC1siRNA cells (Fig. 4D, left). Similar results were obtained in the ZR-75-1 cells (Fig. 4D, right). These findings indicate that MUC1 represses activity of the *p53* promoter by the recruitment of HDACs to the PE21 element and thereby deacetylation of histones.

**MUC1 regulates both p53 function and expression.** Previous work demonstrated that MUC1-C binds directly to p53 and coactivates p53-mediated transcription of the *p21* gene (21). MUC1-C also occupies the *Bax* proximal promoter that includes the TATA box and, in contrast to *p21*, represses *Bax* gene transcription by disrupting assembly of the basal transcription apparatus (21). The human *p53* promoter does not have a TATA or GC box (30, 33). However, the PE21 element within the *p53* proximal promoter directs bi-directional initiation activity as found with TATA and GC boxes (30, 34, 35). The PE21 element functions as a binding site for KLF4, a repressor of p53 transcription that transforms cells as a function of p21 status (23, 24, 30). The present results demonstrate that MUC1-C binds to KLF4, occupies the PE21 region with KLF4 and increases KLF4 occupancy of PE21 (Fig. 5). We also found that MUC1 contributes to the recruitment of HDAC1/3, deacetylation of core histones and repression of *p53* transcription (Fig. 5). These results indicate that, in addition to regulating the p53 transcription function, MUC1-C acts by suppressing p53 expression. Importantly, like MUC1 (1), KLF4 is overexpressed in the majority of human breast tumors (28, 29, 36). Thus, the interaction between MUC1-C and KLF4 in repressing activation of the *p53* gene may be of importance to the development of human breast cancer.

## **KEY RESEARCH ACCOMPLISHMENTS**

**1. MUC1 blocks GSK3 $\beta$ -mediated phosphorylation and degradation of  $\beta$ -catenin.** Dysregulation of  $\beta$ -catenin is of importance to the development of diverse human malignancies. The MUC1 oncoprotein is aberrantly overexpressed by most human carcinomas and

associates with  $\beta$ -catenin. However, the functional significance of the MUC1- $\beta$ -catenin interaction is not known. In this work, we demonstrate that MUC1 increases  $\beta$ -catenin levels in the cytoplasm and nucleus of carcinoma cells. Previous studies have shown that glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) phosphorylates  $\beta$ -catenin and thereby targets it for proteosomal degradation. Consistent with the upregulation of  $\beta$ -catenin levels, our results show that MUC1 blocks GSK3 $\beta$ -mediated phosphorylation and degradation of  $\beta$ -catenin. To further define the interaction between MUC1 and  $\beta$ -catenin, we identified a serine-rich motif (SRM) in the MUC1 cytoplasmic domain that binds directly to  $\beta$ -catenin Arm repeats. Mutation of the SRM attenuated binding of MUC1 to  $\beta$ -catenin and MUC1-mediated inhibition of  $\beta$ -catenin degradation. Importantly, disruption of the MUC1- $\beta$ -catenin interaction with the SRM mutant also attenuated MUC1-induced anchorage-dependent and -independent growth and delayed MUC1-mediated tumorigenicity. These findings indicate that MUC1 promotes transformation at least in part by blocking GSK3 $\beta$ -mediated phosphorylation and thereby degradation of  $\beta$ -catenin.

**2. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response.** Aberrant overexpression of the heterodimeric MUC1 protein by most carcinomas has indicated that this alteration is, like mutations of the p53 tumor suppressor, among the more commonly found in human malignancies. This work demonstrates that the MUC1 C-terminal subunit associates with p53 in diverse human carcinoma cells and that this interaction is increased by genotoxic stress. In vitro studies show that the MUC1 cytoplasmic domain binds directly to the p53 regulatory domain. Chromatin immunoprecipitation (ChIP) assays further demonstrate that MUC1 coprecipitates with p53 on the p53-responsive elements of the *p21* gene promoter. MUC1 occupancy of the *p21* promoter is p53-dependent and associated with increases in i) recruitment of the transcriptional coactivator CBP, ii) acetylation of histone H4, and iii) coactivation of *p21* gene transcription. By contrast, studies of the p53-responsive *Bax* gene demonstrate that MUC1 occupies the proximal promoter by a p53-independent mechanism and attenuates DNA damage-induced activation of *Bax* transcription by interfering with the basal

transcription complex. In concert with these results, MUC1 promotes selection of the p53-dependent growth arrest response and suppresses the p53-dependent apoptotic response to DNA damage. These findings provide the first evidence that the MUC1 oncoprotein regulates p53-responsive genes and thereby cell fate in the genotoxic stress response.

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

**4. MUC1 oncoprotein blocks nuclear targeting of c-Abl in the apoptotic response to DNA damage.** The nonreceptor c-Abl tyrosine kinase binds to cytosolic 14-3-3 proteins and is targeted to the nucleus in the apoptotic response to DNA damage. The MUC1 oncoprotein is overexpressed by most human carcinomas and blocks the induction of apoptosis by genotoxic agents. Using human carcinoma cells with gain and loss of MUC1 function, we show that nuclear targeting of c-Abl by DNA damage is abrogated by a MUC1-dependent mechanism. The results demonstrate that c-Abl phosphorylates MUC1 on Tyr-60 and forms a complex with MUC1 by binding of the c-Abl SH2 domain to the pTyr-60 site. Binding of MUC1 to c-Abl attenuates phosphorylation of c-Abl on Thr-735 and the interaction between c-Abl and cytosolic 14-3-3. We also show that expression of MUC1 with a mutation at Tyr-60 i) disrupts the

interaction between MUC1 and c-Abl, ii) relieves the MUC1-induced block of c-Abl phosphorylation on Thr-735 and binding to 14-3-3, and iii) attenuates the MUC1 anti-apoptotic function. These findings indicate that MUC1 sequesters c-Abl in the cytoplasm and thereby inhibits apoptosis in the response to genotoxic anti-cancer agents.

**5. MUC1 oncoprotein represses transcription of the p53 tumor suppressor gene.** The MUC1 heterodimeric protein is aberrantly overexpressed in human breast cancers and induces transformation. The MUC1 C-terminal subunit (MUC1-C) is targeted to the nucleus of transformed cells, where it interacts with p53 and regulates p53-mediated transcription. These studies demonstrate that MUC1 represses activation of the p53 gene and that MUC1-C occupies the PE21 element in the p53 proximal promoter. Previous work has shown that the Kruppel-like factor 4 (KLF4) transcription factor represses p53 transcription by binding to the PE21 element. Our results demonstrate that MUC1-C binds to KLF4, occupies PE21 with KLF4 and enhances KLF4 occupancy of PE21. The results also demonstrate that MUC1-C increases recruitment of histone deacetylases 1/3, deacetylation of core histones and repression of p53 transcription. These findings indicate that overexpression of MUC1 as found in human breast cancer cells is of functional importance to repression of the p53 gene.

## **REPORTABLE OUTCOMES**

1. Huang L, Chen D, Liu D, Yin L, Kharbanda S, Kufe D. MUC1 oncoprotein blocks GSK3 $\beta$ -mediated phosphorylation and degradation of  $\beta$ -catenin. *Cancer Res.* 2005; 65:10413-10422.
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  6. The results obtained regarding MUC1-mediated repression of the p53 gene in human breast cancers have been integrated into a manuscript that is being submitted for publication.

## CONCLUSIONS

We conclude that MUC1 induces transformation and that the cytoplasmic domain is sufficient for this function by stabilizing  $\beta$ -catenin (Task 1). We also conclude that the MUC1-C subunit is targeted to the nucleus where it interacts with p53, ER $\alpha$  and KLF4, and thereby regulates gene transcription in human breast cancer cells (Task 2). These findings have thus provided a novel understanding that MUC1 induces transformation, localizes to the nucleus and regulates gene expression. The findings also indicate that overexpression of MUC1 by human breast tumors is of importance to the malignant phenotype and resistance to therapy.

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## FIGURE LEGENDS

**Figure 1. MUC1 downregulates p53 at protein and mRNA levels. A and B.** Lysates from the indicated MCF-7 (A) and ZR-75-1 (B) cells were immunoblotted with anti-p53, anti-MUC1-C and anti- $\beta$ -actin. **C and D.** Semiquantitative RT-PCR for p53 and anti- $\beta$ -actin mRNA levels was performed on the indicated MCF-7 (C) and ZR-75-1 (D) cells.

**Figure 2. MUC1 suppresses p53 gene transcription. A.** Schematic depiction of the human *p53* gene promoter and the mutants used in this study. **B-E.** MCF-7/CsiRNA cells (solid bars) and MCF-7/MUC1siRNA (open bars) (B and D), or ZR-75-1/CsiRNA cells (solid bars) and ZR-75-1/MUC1siRNA (open bars) (C and E) were transfected with the -2400-p53-Luc reporter, -2400-PE21-MUT-Luc reporter, -320-p53-Luc reporter, -320-PE21-MUT-Luc reporter and an internal control LacZ expression plasmid (pCMV-LacZ). At 40 h after transfection, the cells were assayed for luciferase activity. The results are expressed as the fold activation (mean $\pm$ SD of 3 separate experiments) compared to that obtained with wild-type cells (assigned a value of 1).

**Figure 3. MUC1 occupies the p53 gene proximal promoter with KLF4. A.** Schema depicting the structure of *p53* gene promoter. **B.** Soluble chromatin from MCF-7 and ZR-75-1 cells was immunoprecipitated with anti-MUC1-C or a control IgG. The final DNA extractions were amplified by PCR using primers that cover a control region (CR; -6020 to -5940), the proximal promoter (PP; -118 to +14), or the region that contains the PE21 element (PE21; -118 to -54) in the *p53* gene promoter. **C.** In Re-ChIP experiments, soluble chromatin from MCF-7 or ZR-75-1 cells was immunoprecipitated with anti-MUC1-C, eluted with DTT, diluted with Re-ChIP buffer, reimmunoprecipitated with anti-KLF4, and analyzed for *p53* PE21 sequences. **D.** GST, GST-MUC1-CD (1-72), or the indicated GST-MUC1-CD deletion mutants were bound to glutathione agarose and incubated with [ $^{35}$ S]-labeled KLF4. The adsorbates were analyzed by SDS-PAGE and autoradiography. Input of the GST and GST-MUC1-CD fusion proteins was assessed by Coomassie blue staining. **E.** Soluble chromatin from MCF-7 and ZR-75-1 cells was

immunoprecipitated with anti-MUC1-C or a control IgG. The final DNA extractions were amplified by PCR using primers that cover a control region (CR; -6020 to -5940) or the region that contains the PE21 element (PE21; -118 to -54) in the *p53* gene promoter.

**Figure 4. MUC1 suppresses *p53* gene transcription by recruiting HDACs to its promoter.** **A.** ZR-75-1/MUC1siRNA cells were transfected with the -2400-*p53*-Luc reporter, -320-*p53*-Luc reporter, the indicated amounts of MUC1-CD and an internal control LacZ expression plasmid (pCMV-LacZ). At 40 h after transfection, the cells were assayed for luciferase activity. The results are expressed as the fold activation (mean $\pm$ SD of 3 separate experiments) compared to that obtained with empty vector-transfected cells (assigned a value of 1) (left). ZR-75-1/MUC1siRNA cells were transfected with the indicated mounts of pCMV or pCMV-MUC1-CD vectors. At 24 h after transfection, lysates from the indicated ZR-75-1/MUC1siRNA cells were immunoblotted with anti-*p53*, anti-MUC1-C and anti- $\beta$ -actin (right). **B.** MCF-7 (left) or ZR-75-1 (right) cells were transfected with the -2400-*p53*-Luc reporter, the indicated amounts of KLF4, 0.5  $\mu$ g MUC1-CD and an internal control LacZ expression plasmid (pCMV-LacZ). At 40 h after transfection, the cells were assayed for luciferase activity. The results are expressed as the fold activation (mean $\pm$ SD of 3 separate experiments) compared to that obtained with empty vector-transfected cells (assigned a value of 1). **C.** In Re-ChIP experiments, soluble chromatin from MCF-7 or ZR-75-1 cells was immunoprecipitated with anti-MUC1-C, eluted with DTT, diluted with Re-ChIP buffer, reimmunoprecipitated with anti-HDAC1 or anti-HDAC3, and analyzed for *p53* PE21 sequences. **D.** In ChIP experiments, soluble chromatin from MCF-7 and ZR-75-1 cells was immunoprecipitated with the indicated antibodies. The final DNA extractions were analyzed for *p53* PE21 sequences by the amplification of PCR.

**Fig. 5.** Schema depicting the downregulation of *p53* gene transcription by MUC1.

## **SUPPLEMENTAL FIGURE LEGENDS**

**Figure S1. Transient MUC1 silencing upregulates p53 protein levels in MCF-7 cells.** MCF-7 cells were transfected with a pool of non-specific control siRNA or MUC1siRNA (Dharmacon SMARTpool Reagents) in the presence of Lipofectamine. Lysates were subjected to immunoblotting with anti-p53, anti-MUC1-C and anti- $\beta$ -actin.

**Figure S2. MUC1 allows the distribution of p53 in breast cancer cells. A and B.** Nuclear and cytosolic fractions from MCF-7 (left) and ZR-75-1 (right) cells were purified and subjected to immunoblotting with anti-p53, anti-MUC1-C, anti-PCNA, and anti-I $\kappa$ B $\alpha$ . WCL: whole cell lysates.

**Figure S3. MUC1 has no effect on KLF4 levels. A and B.** Lysates from the indicated MCF-7 (A) and ZR-75-1 (B) cells were immunoblotted with anti-KLF4 and anti- $\beta$ -actin.

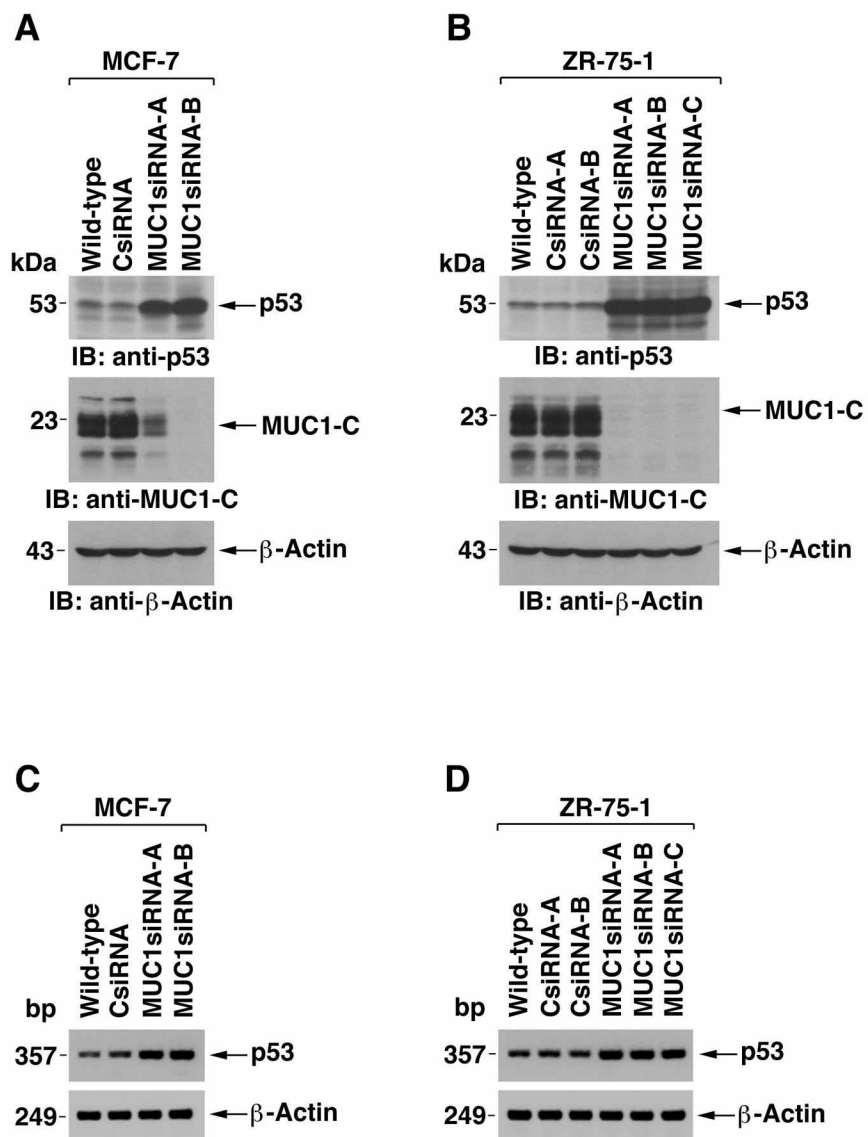


Fig. 2

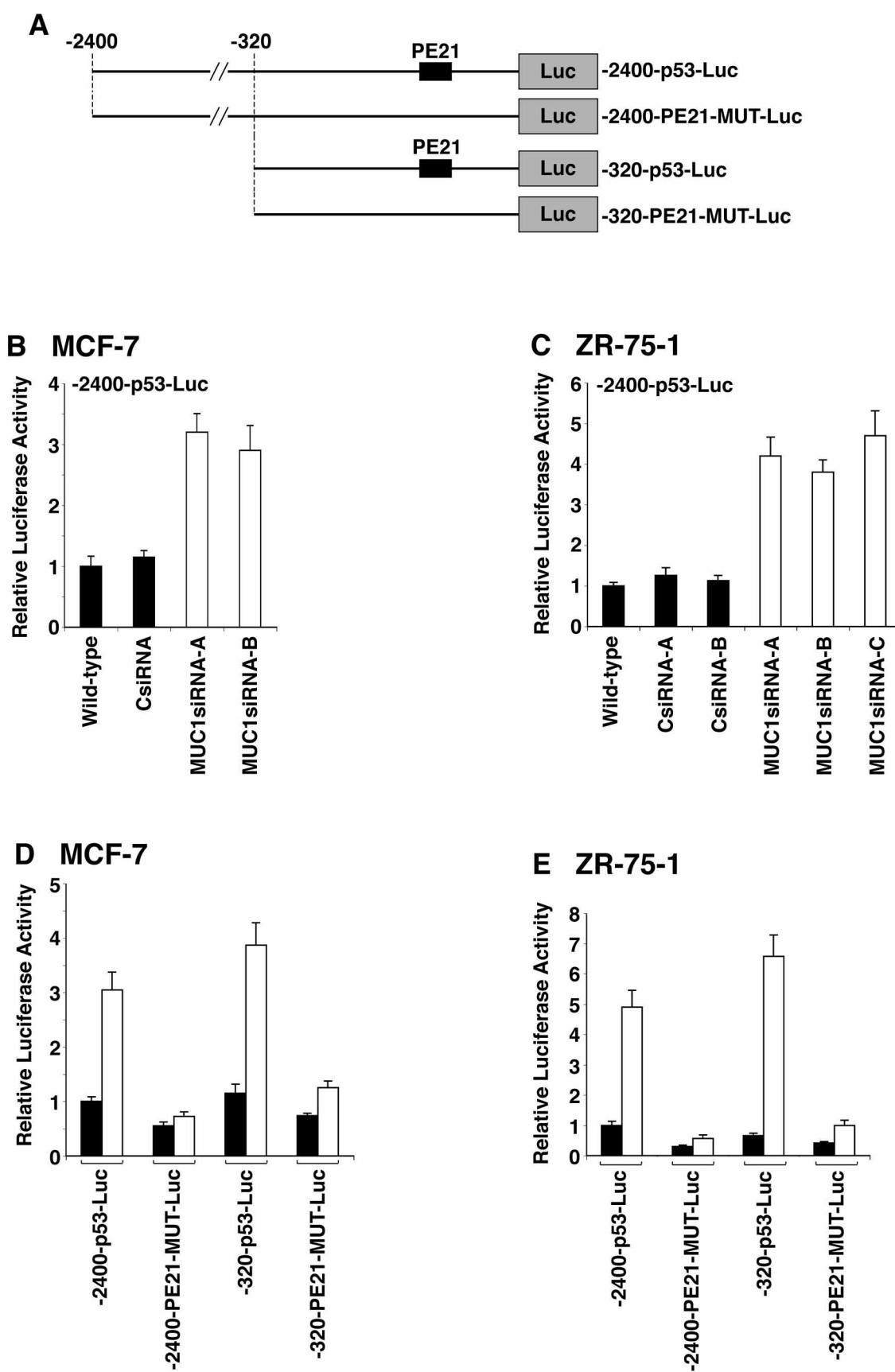
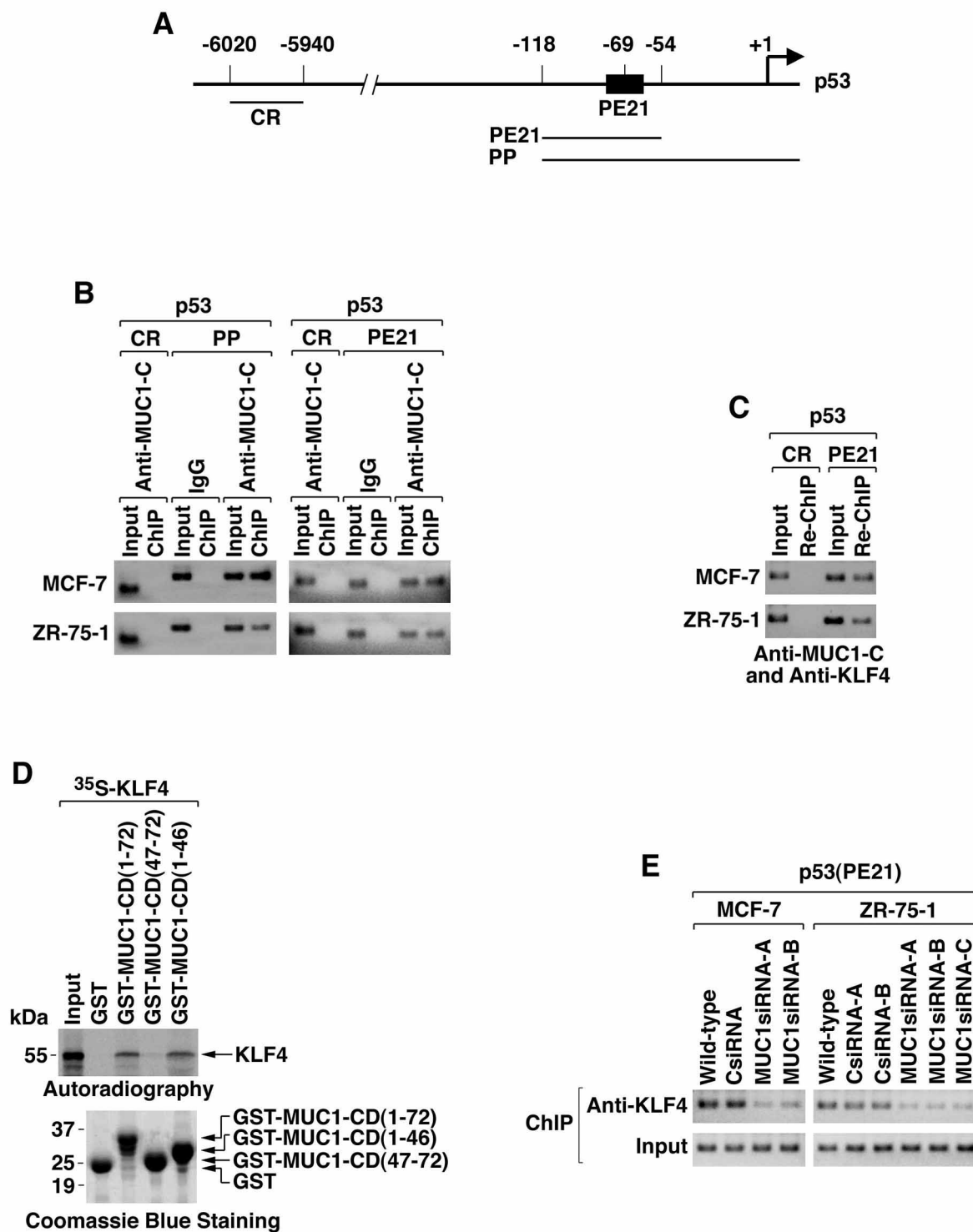
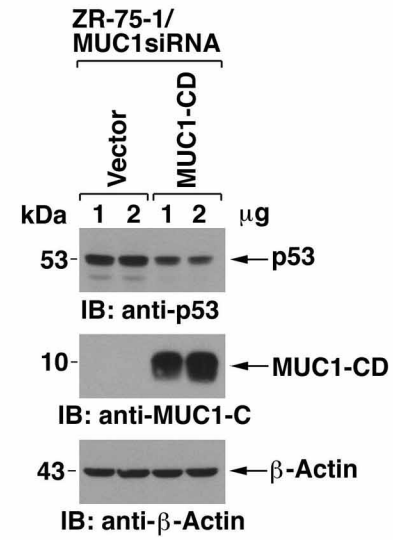
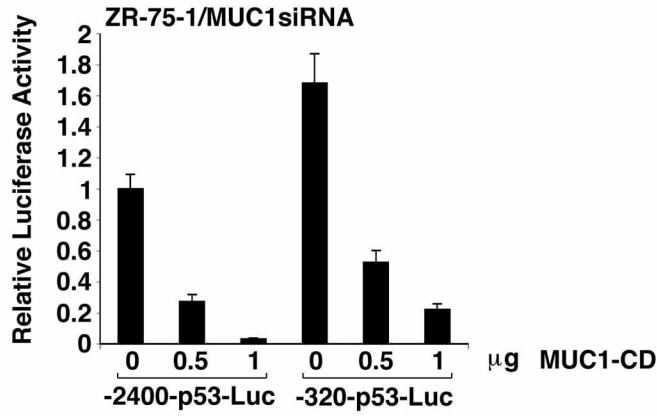


Fig. 3

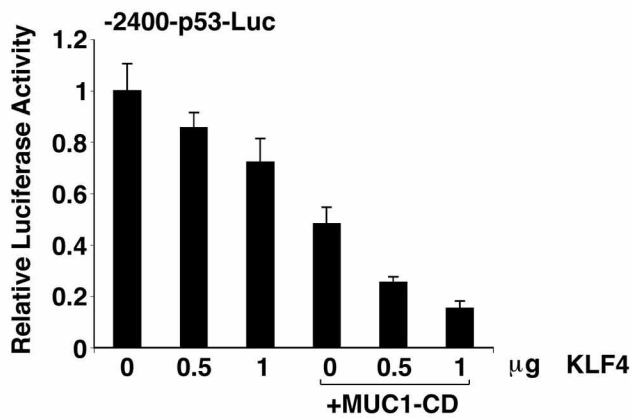


**A**

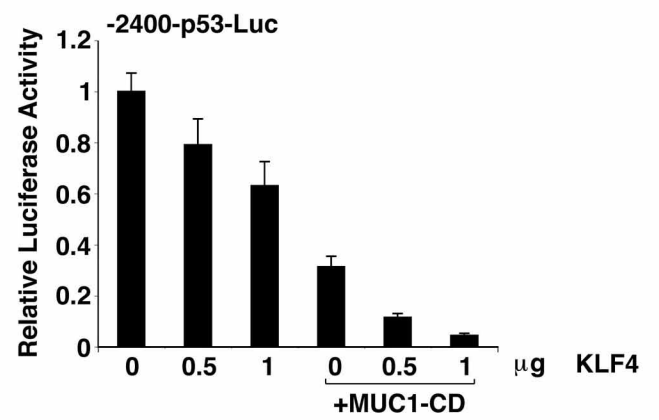


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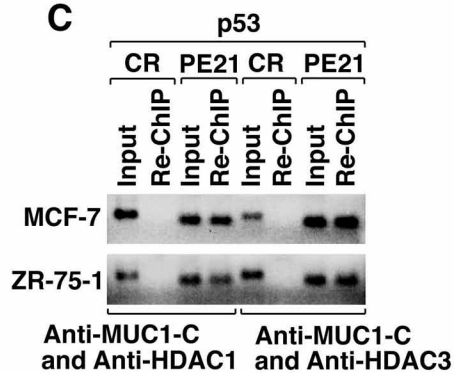
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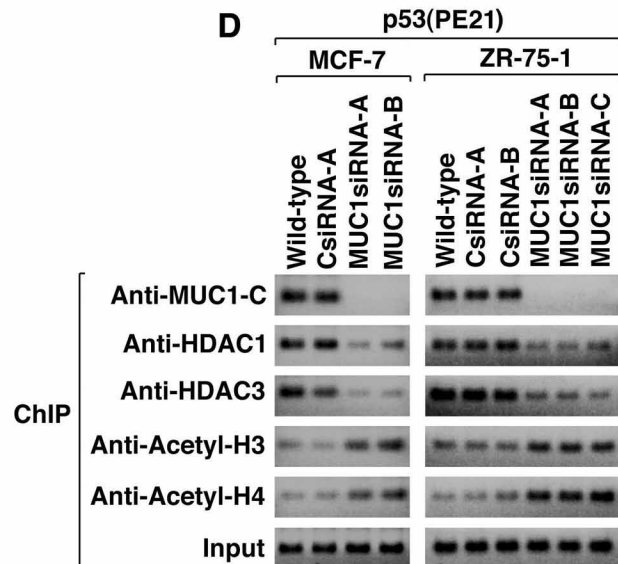
**ZR-75-1**



**C**



**D**





**Fig. 5**

