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Therapeutics, Targets, and Chemical Biology

Cancer Research

Differential Regulation of Estrogen Receptor α Expression in Breast Cancer Cells by Metastasis-Associated Protein 1

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Abstract

Metastasis-associated protein 1 (MTA1) is a component of the nucleosome remodeling and histone deacetylase (HDAC) complex, which plays an important role in progression of breast cancer. Although MTA1 is known as a repressor of the transactivation function of estrogen receptor α (ERα), its involvement in the epigenetic control of transcription of the ERα gene *ESR1* has not been studied. Here, we show that silencing of MTA1 reduced the level of expression of ERα in ERα-positive cells but increased it in ERα-negative cells. In both MCF7 and MDA-MB-231, MTA1 was recruited to the region +146 to +461 bp downstream of the transcription start site of *ESR1* (ERpro315). Proteomics analysis of the MTA1 complex that was pulled down by an oligonucleotide encoding ERpro315 revealed that the transcription factor AP-2γ (TFAP2C) and the IFN-γ–inducible protein 16 (IFI16) were components of the complex. Interestingly, in MCF7, TFAP2C activated the reporter encoding ERpro315 and the level of ERα mRNA. By contrast, in MDA-MB-231, IFI16 repressed the promoter activity and silencing of MTA1 increased expression of ERα. Importantly, class II HDACs are involved in the MTA1-mediated differential regulation of ERα. Finally, an MDA-MB-231-derived cell line that stably expressed shIFI16 or shMTA1 was more susceptible to tamoxifen-induced growth inhibition in *in vitro* and *in vivo* experiments. Taken together, our findings suggest that the MTA1–TFAP2C or the MTA1–IFI16 complex may contribute to the epigenetic regulation of *ESR1* expression in breast cancer and may determine the chemosensitivity of tumors to tamoxifen therapy in patients with breast cancer. *Cancer Res; 74(5); 1484–94. ©2014 AACR.*

Introduction

Estrogen receptor α (ERα) is a hormone-activated nuclear receptor that plays critical roles in breast cancer pathogenesis, progression, and treatment (1, 2). Two thirds of breast cancers overexpress ERα, and are in general sensitive to hormonal therapy and have a better prognosis than tumors with low or absent ERα expression (3). By contrast, ERα-negative tumors are resistant to hormonal therapy, which is associated with early recurrence, development of metastasis, and a high tumor grade and proliferative index with a greater probability of death (4, 5). Given the effectiveness and low number of complications associated with hormonal therapy, the possibility of inducing hormone responsiveness in ERα-negative cancer remains an attractive treatment strategy.

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Previous research has suggested multiple mechanisms at multiple levels for the generation and progression of the ERα-negative breast cancer phenotype. Although amplification of the ERα gene *ESR1* has been suggested as a common mechanism of ERα overexpression in breast cancer, the mechanism of ERα loss is not clearly understood (6). Loss of heterozygosity or mutation in the ER gene locus was demonstrated to play a minor role in loss of ERα expression, which would imply that ERα expression and its repression are controlled at the epigenetic level (7, 8). Indeed, hypermethylation of the CpG islands in the *ESR1* promoter has been found in certain ERα-negative breast cancer cell lines and tumors (9, 10). ERα expression was reactivated in ERα-negative breast cancer cells by treatment with trichostatin A (TSA) and 5-aza-2′-deoxycytidine (5-aza-dc), well-characterized pharmacologic inhibitors of histone deacetylation and DNA methylation, respectively (11–13). Moreover, several *trans*-acting factors were found to contribute to ERα loss in ERα-negative tumor cells through epigenetic mechanisms. Macaluso and colleagues (14) demonstrated that an epigenetic repressor pRB2/p130-E2F4/5-histone deacetylase 1 (HDAC1)–DNA methyltransferase 1 (DNMT1)–SUV39H1 complex occupied the *ESR1* promoter and regulated *ESR1* transcription in ERα-negative MDA-MB-231 cells. In addition, Twist basic helix-loop-helix transcription factor recruited the HDAC1 and DNMT3B repressor complex to the ER promoter, resulting in repression of ERα expression and generation of hormone resistance in breast cancer cells (15). Identification and characterization of such key elements

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in the epigenetic regulation of ERα may provide a novel therapeutic target in the treatment of breast cancer.

Metastasis-associated protein 1 (MTA1) is a cancer progression-related gene product that is overexpressed in a variety of human cancers including breast, liver, ovarian, and colorectal cancer (16). In breast cancer, *MTA1* was first identified as a candidate metastasis-associated gene that was expressed in highly metastatic mammary adenocarcinoma cell lines (17). *MTA1* was mapped to a region showing significantly lower loss of heterozygosity in primary breast cancers with metastasis compared with node-negative tumors (18). Furthermore, MTA1 overexpression was closely associated with higher tumor grade and increased tumor angiogenesis (19). MTA1 exists in coactivator or corepressor complexes containing RNA polymerase II or HDAC and functions as a transcriptional coregulator to activate or repress the transcription of target genes (20). For instance, MTA1 activates the transcription of breast cancer amplified sequence 3 (*BCAS3*), which contributes to tamoxifen-resistance of breast cancer in premenopausal patients (21). By contrast, as a component of the nucleosome remodeling and histone deacetylation complex, MTA1 interacts with HDAC1 and represses the transcription of breast cancer 1, early onset (*BRCA1*), which is known as a tumor suppressor gene, in the ERα-positive breast cancer cell line MCF7 (22). Interestingly, silencing of MTA1 by shRNA restored ERα expression in the ERα-negative cell line MDA-MB-231 (23).

Although evidence suggests a potential role for MTA1 in epigenetic control of chromatin remodeling and its involvement in ERα expression in breast cancer, a link between MTA1-induced epigenetic control and the transcriptional regulation of *ESR1*, and its significance in the progression of breast cancer, have not been addressed. Here, we report that MTA1 functions as a regulator of transcriptional expression of *ESR1* that differentially controls ERα levels in ERα-positive and ERα-negative breast cancer cells, a process that is associated with *trans*-acting factors including transcription factor AP-2γ (TFAP2C) and IFN-γ–inducible protein 16 (IFI16). We also investigated the functional interplay between MTA1, TFAP2C, and IFI16 that determines the sensitivity of breast cancer to hormonal therapy.

Materials and Methods

Cells and cell culture

Human breast adenocarcinoma cell lines, ERα positive, MCF7, ZR75-1, and T47D, and ERα negative, MDA-MB-231, and BT-20 were obtained from the American Type Culture Collection (ATCC). The ERα negative, MDA-MB-453 was obtained from the Korean Cell Line Bank (KCLB). These cells were authenticated by ATCC and KCLB, by short tandem repeat profiling and monitoring cell morphology. Cells were maintained in Dulbecco's Modified Eagle Medium containing 10% FBS at 37°C in a 5% CO_2/95% air incubator. Valproic acid (VPA), TSA, and tamoxifen were purchased from Sigma-Aldrich. MC1568 was purchased from Santa Cruz Biotechnology.

Plasmids, siRNA duplexes, and transient transfection

FLAG-tagged TFAP2C and IFI16 were constructed by inserting a PCR-amplified full-length human TFAP2C or IFI16 into p3XFLAG-CMV10 (Sigma-Aldrich). The pCDNA3–FLAG–HDAC1, pCDNA3–FLAG–HDAC4, pCDNA3–FLAG–HDAC5, and pCDNA3–FLAG–HDAC6 plasmids were kindly provided by Dr. T.-P. Yao of Duke University (Durham, NC). The ERpro315-Luc was constructed by inserting a PCR-amplified +146 to +461 bp region of the ERα promoter region into the pGL2 promoter (Promega). Sequences of siRNA duplexes used in this study are described in Supplementary Table S1. Transient transfection and reporter gene analysis were performed as described previously (24). The significance of any differences was determined using Student *t* test and was expressed as a probability value. Differences in means were considered significant at $P < 0.05$.

Western blot analysis, immunoprecipitation, and immunofluorescence

Western blotting and immunoprecipitation were carried out as described previously using specific antibodies against ERα, MTA1, TFAP2C, and IFI16 (Santa Cruz Biotechnology) or α-tubulin (Calbiochem; ref. 24). For immunocytochemistry, MCF7 or MDA-MB-231 cell lines were cultured on glass slides. Cells were fixed and stained with antibody targeting MTA1, TFAP2C, or IFI16, and Alexa Fluor 555- or 568-conjugated secondary antibody (Invitrogen). Images were acquired using confocal microscopy.

Reverse transcription PCR and chromatin immunoprecipitation assay

Reverse transcription (RT)-PCR was carried out using specific primers as described previously (24). The chromatin immunoprecipitation (ChIP) assay was performed as described previously using specific antibodies against MTA1, p300, N-CoR, TFAP2C, IFI16, HDAC4, HDAC5, HDAC6 (Santa Cruz Biotechnology), and AcH3K9 (Abcam). Bound target DNA fragments were detected using PCR. The primers used to amplify DNA fragments are described in Supplementary Table S1.

DNA pull-down assay and liquid chromatography/tandem mass spectrometry

Biotinylated ERpro315 DNA fragments were prepared by PCR using biotinylated specific primers. Preparation of nuclear extract and DNA pull down were performed as described previously (24). After protein–DNA complexes were obtained, the complexes were subjected to SDS-PAGE followed by Western blotting using anti-MTA1, anti-TFAP2C, and IFI16 (Santa Cruz Biotechnology). Pulled down proteins fractionated by SDS-PAGE were subjected to liquid chromatography/tandem mass spectrometry analysis, and analyzed as described previously (24).

Establishment of stable cell lines expressing shMTA1 and shIFI16

pLKO.1-shMTA1 and pLKO.1-shIFI16 were constructed by annealing primers and cloning the product into the *Age*I-*Eco*RI site of the pLKO.1-TRC vector. The lenti-shRNA targeting MTA1, IFI16, or GFP, lentiviral packaging plasmids (psPAX2), and envelope plasmid (pMD2.G) were cotransfected into HEK293T packaging cells using lipofectamine 2000 (Invitrogen). After 60-hour incubation, the lentivirus in the supernatant

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was collected and centrifuged and used to infect MCF7 or MDA-MB-231 cells with hexadimethrine bromide at a final concentration of 8 μg/mL. After puromycin selection (1.5–2.0 μg/mL) for 2 to 4 weeks, stable clones were obtained and subsequently confirmed by Western blotting.

Clonogenic survival assays

MDA-MB-231 shRNA stable cell lines, shGFP, shMTA1, and shIFI16, were seeded at 1,000 cells/plate in triplicate into 35-mm plates. After 48-hour incubation, cells were treated with 10 μmol/L tamoxifen for 12 days. At the end of treatment, colonies were fixed with methanol and stained with 0.5% crystal violet (Sigma-Aldrich). Colonies that composed of greater than 50 cells was counted. Statistical significance was evaluated by 2-way ANOVA followed by Bonferroni posttest.

Xenograft experiments

Animal experiments were performed in accordance with guidelines of Seoul National University Animal Care and Use Committee. Female 6-week-old athymic (nu/nu) BALB/c mice were obtained from Orient Bio Inc. and housed in an air-conditioned room at a temperature of 22°C to 24°C and a humidity of 37% to 64%, with a 12-hour light/dark cycle. After 1 week of acclimatization, tumor inoculation was performed. Each 5×10^6 cells of MDA-MB-231 shGFP, shMTA1, and shIFI16 stable cell lines were mixed at a 1:1 ratio with Matrigel (BD Biosciences) and inoculated subcutaneously into the flanks of mice. When the tumor volume reached approximately 100 mm^3, mice were randomly divided into 2 groups. The experimental groups received a 21-day release tamoxifen pellet (25 mg/pellet) or a placebo pellet (Innovative Research of America) for 2 weeks. Tumor diameter was measured with caliper 2 times a week and tumor volumes were estimated using the following formula: tumor volume (cm^3) = (length × $width^2$) × 0.5. Statistical significance was evaluated by 2-way ANOVA followed by Bonferroni posttest.

Gene expression analysis based on public datasets

The public datasets were obtained from ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) with accession numbers E-TABM-157 (25) and E-TABM-158 (26), and from Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) with accession series GSE7390 (27) and GSE21921 (28). E-TABM-157, E-TABM-158, and GSE7390 datasets were obtained using the Human Genome U133A Array (Affymetrix), and the GSE21921 dataset using the DASL HumanRef-8 Whole Genome v3.0 (Illumina). The processed data including normalization procedures are available at the above websites. The data for individual probe IDs were averaged and analyzed as log 2 expression of the gene using Microsoft Excel. Significance of differences was determined by a 2-tailed unpaired *t* test.

Results

Differential regulation of ERα expression by MTA1 in ERα-positive and ERα-negative breast cancer cells

To address the role of MTA1 in transcriptional control of *ESR1*, we first examined the level of ERα expression in the ERα-positive MCF7 cells under conditions in which *MTA1* was silenced or overexpressed. When *MTA1* was suppressed by transfection of siRNA, the protein and mRNA levels of ERα in MCF7 cells were both decreased (Fig. 1A). Consistent with this, the levels of ERα protein and mRNA in MCF7 cells were enhanced after overexpression of MTA1 (Fig. 1B). By contrast, the repression of *MTA1* expression by siMTA1 restored the levels of ERα protein and mRNA in ERα-negative MDA-MB-231 cells (Fig. 1C). Similar results were obtained in other breast cancer cell lines, such as ERα-positive ZR75-1 and T47D, and ERα-negative BT-20 and MDA-MB-453 (Fig. 1). These results indicate that MTA1 differentially regulates transcription of ERα in ERα-positive and ERα-negative breast cancer cells.

To gain insight into the MTA1-induced differential regulation of ERα transcription, we searched the *ESR1* promoter regions for the region to which MTA1 was recruited. Transcription of *ESR1*

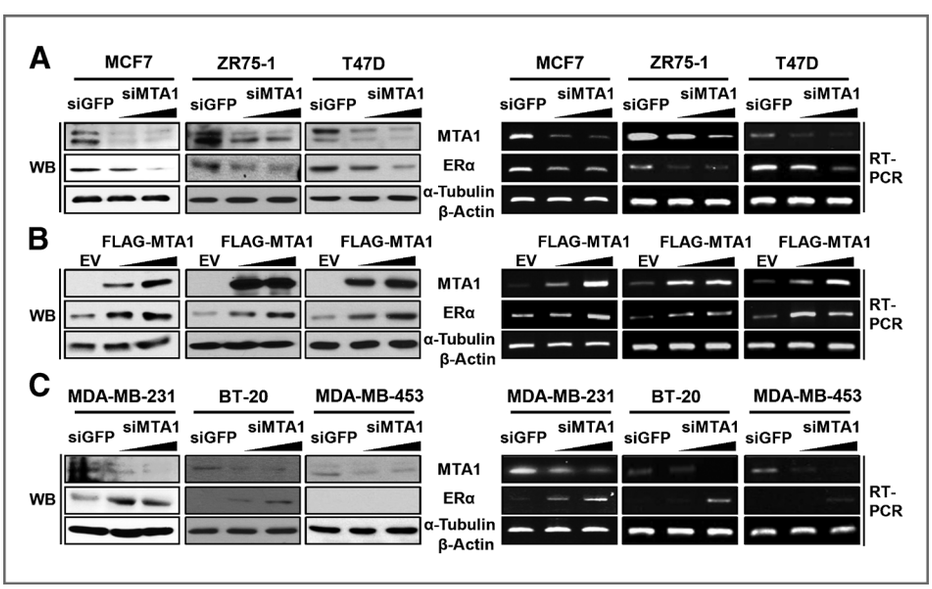

Figure 1. Differential regulation of ERα expression by MTA1 in ERα-positive and ERα-negative breast cancer cells. A, MCF7, ZR75-1, and T47D cells were transfected with siMTA1 (100 and 200 pmol) for 48 hours. B, MCF7, ZR75-1, and T47D cells were transfected with FLAG-MTA1 (2 and 4 μg) for 24 hours. C, MDA-MB-231, BT-20, and MDA-MB-453 cells were transfected with siMTA1 (100 and 200 pmol) for 48 hours. Expression levels of protein or mRNA of MTA1 and ERα were analyzed by Western blotting (left) or RT-PCR (right), respectively.

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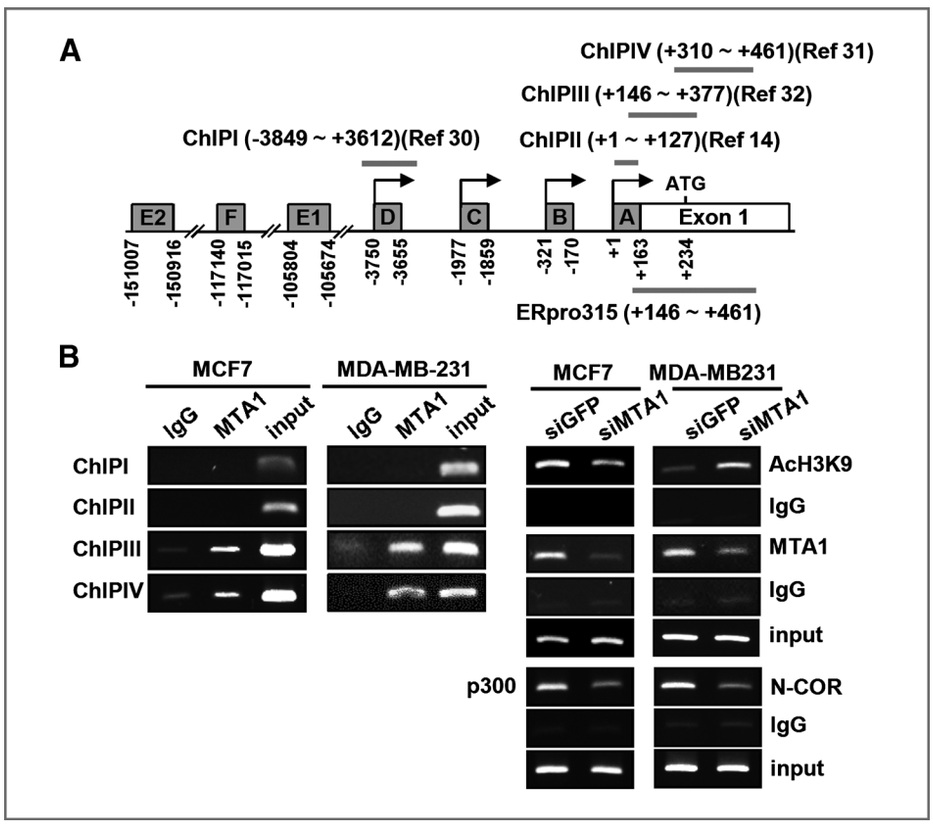

Figure 2. MTA1 binds to the proximal region of ERα promoter. A, schematic representation of seven different human ERα promoters (A–F) with primers for ChIP experiments (29). Four regions that confer distinct ERα transcriptional activity in ERα-positive and ERα-negative cells were shown with references. Numbers indicate the nucleotide number relative to transcription initiation site of ERα gene. B, DNA binding of MTA1 on the four different promoters of ERα gene. DNA fragments that were immunoprecipitated by anti-MTA1 antibody were amplified by PCR using primers for ChIPI, ChIPII, ChIPIII, or ChIPIV. C, epigenetic control of ERpro315 region by MTA1. MCF7 or MDA-MB-231 cells were transfected with siMTA1 for 48 hours. DNA fragments that were immunoprecipitated by anti-H3K9Ac, anti-MTA1, anti-p300, or anti-NCoR antibodies were amplified by PCR using primers for ERpro315.

is directed by at least seven different promoters, the usage of which varies among tissues and cell types (29). We selected four possible promoter regions of *ESR1* with which MTA1 may be associated (Fig. 2A). According to the literature, these regions are strongly regulated in breast cancer cells, and show distinctly different transcriptional activity between ERα-positive and ERα-negative breast cancer cells (14, 30, 31, 32). ChIP assays were performed with four sets of primers, one specific for each putative region (Fig. 2A). In both MCF-7 and MDA-MB-231, MTA1 was recruited to regions III (+146 to +377) and IV (+310 to +461), but not to regions I (−3849 to −3612) and II (+1 to +127; Fig. 2B). Thus, we named these two closely associated regions the ERpro315 (+146 to +461). Knockdown of MTA1 in both MCF7 and MDA-MB-231 decreased the binding of MTA1 to ERpro315, confirming the specific association of MTA1 with this promoter region. When the binding of MTA1 was decreased, the acetylation level of lysine 9 in histone 3 (AcH3K9) and coactivator p300 binding were decreased in MCF7, indicating that loss of MTA1 binding to ERpro315 decreased transcriptional activity of *ESR1* in MCF7. By contrast, binding of AcH3K9 was increased and binding of corepressor N-CoR was decreased in MDA-MB-231, suggesting that loss of MTA1 binding to the ERpro315 increased the transcriptional activity of *ESR1* in MDA-MB-231. Consistently, DNA binding levels of other epigenetic markers, the activation marker H3K4me3 and the repression marker H3K27me3, were altered after silencing of MTA1 in MCF-7 and MDA-MB-231 (Supplementary Fig. S1). Together these results indicate that the *ESR1* promoter +146 to +461 region is important for the MTA1-induced differential regulation of *ESR1* expression in ERα-positive and ERα-negative breast cancer cells (Fig. 2B).

TFAP2C and IFI16 are associated with the MTA1-mediated transcriptional regulation of ESR1

To characterize further the MTA1-mediated regulation of *ESR1* expression, the MTA1 complex that bound to ERpro315 was pulled down from nuclear extracts of MCF7 or MDA-MB-231 and subjected to proteomics analysis. Among the proteins identified, TFAP2C and IFI16 recorded notable hits with high-probability scores in MCF7 and MDA-MB-231, respectively. To confirm the binding of TFAP2C and IFI16 to ERpro315, a DNA pull-down assay followed by Western blotting was performed. Binding of TFAP2C was observed only in MCF7 while binding of IFI16 was seen only in MDA-MB-231 (Fig. 3A). Binding of TFAP2C and IFI16 to ERpro315 *in vivo* was verified by ChIP assays (Fig. 3B). Reporter gene analysis using a reporter encoding ERpro315 showed that exogenously introduced TFAP2C activated the reporter in MCF7, whereas IFI16 repressed it in MDA-MB-231, indicating that the binding of TFAP2C and IFI16 functionally modulates the *ESR1* promoter (Fig. 3C). Surprisingly, we found that TFAP2C was highly expressed in MCF7, whereas IFI16 was highly expressed in MDA-MB-231 (Fig. 3D). MTA1 was physically associated with TFAP2C in MCF7 and with IFI16 in MDA-MB-231, especially in the nucleus (Fig. 3E and F).

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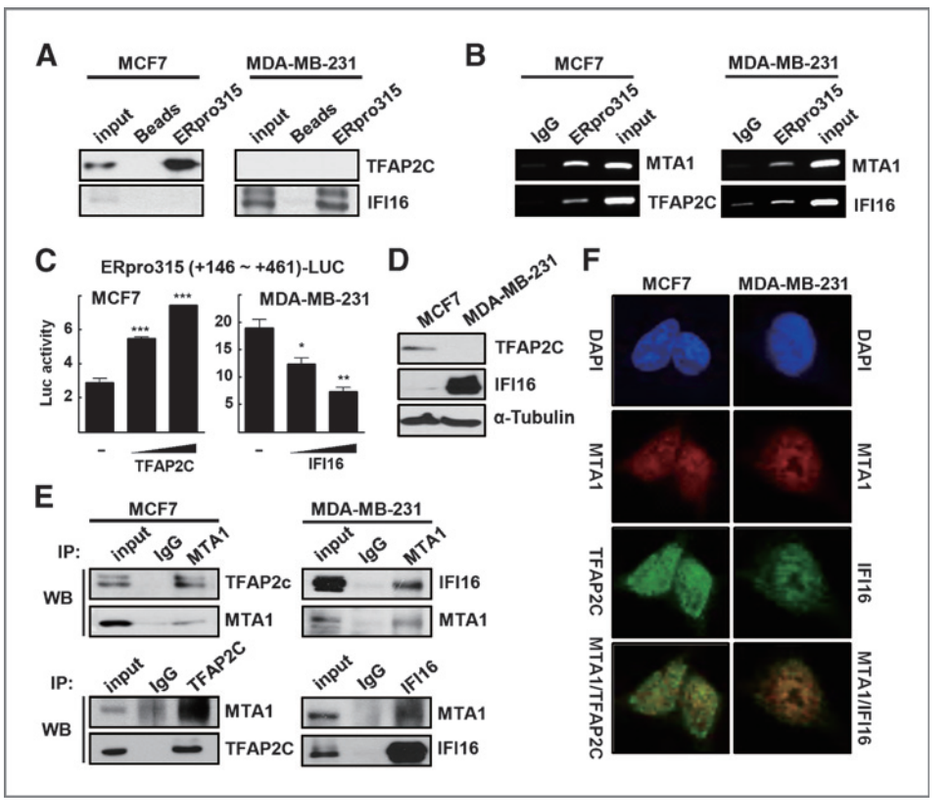

Figure 3. Differential binding of TFAP2C and IFI16 on ERpro315 of the ERα promoter. A, DNA binding of TFAP2C and IFI16 on ERpro315 was examined by DNA pull-down assays. Nuclear extract obtained from MCF7 or MDA-MB-231 cells were incubated with the PCR amplified double-stranded biotin end-labeled oligonucleotide probe for ERpro315. Pull-downed mixtures fractionated by SDS-PAGE were proved by Western blotting using an anti-TFAP2C or anti-IFI16 antibody. B, DNA binding of TFAP2c or IFI16 on the ERpro315 region was analyzed by ChIP analysis. DNA fragments that were immunoprecipitated by anti-TFAP2c or anti-IFI16 antibodies were amplified by PCR using primers for ERpro315. C, MCF7 or MDA-MB-231 cells were transfected with the ERpro315-Luc reporter with real amounts of FLAG-TFAP2c or FLAG-IFI16. Experimental values are expressed as the mean ± SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ versus empty vector transfected. D, expression of TFAP2C and IFI16 in MCF-7 and MDA-MB-231 cells was examined by Western blotting. E, whole cell lysates were immunoprecipitated (IP) with normal immunoglobulin G (IgG), anti-MTA1, anti-TFAP2C, or anti-IFI16 antibodies, and immunoprecipitates were fractionated and probed by Western blotting (WB) using an anti-MTA1, anti-TFAP2C, or anti-IFI16 antibody. F, subcellular localization of TFAP2C and IFI16 was examined by immunocytochemistry and the images were overlaid. Nuclei were visualized by staining with 4′,6-diamidino-2-phenylindole (DAPI).

Next, we tested whether TFAP2C and IFI16 were associated with the MTA1-mediated differential regulation of *ESR1* in ERα-positive and ERα-negative breast cancer cells. The expression level of ERα was decreased when TFAP2C was knocked down by siRNA, whereas it was significantly upregulated at both protein and mRNA levels by overexpression of TFAP2C (Fig. 4A and B; ref. 33). TFAP2C-mediated activation of the ERpro315 reporter was further enhanced by addition of MTA1 (Fig. 4C), but the TFAP2C-induced ERα expression disappeared when MTA1 was knocked down in MCF7 (Fig. 4D). In the case of MDA-MB-231, repression of IFI16 using siIFI16 restored ERα expression (Fig. 4E). IFI16-mediated repression of the ERpro315 reporter was further suppressed by coexpression of MTA1 (Fig. 4F).

Class HDAC II family is associated with the differential regulation of ESR1

Earlier observations that HDAC inhibitors such as TSA and VPA differentially regulate transcription of *ESR1* suggest that HDACs may be involved in MTA1-mediated ERα regulation (12, 34, 35). Indeed, TSA treatment altered the levels of AcH3K9 bound to the ERpro315 region in MCF7 and MDA-MB-231 in opposite ways, indicating that ERpro315 is involved in the differential regulation of ERα by TSA (Fig. 5A). Although TSA inhibits both class I and class II HDACs with similar potency (IC_{50} values ranging from 100 to 300 nmol/L), VPA inhibits class I HDACs with IC_{50} values of 0.7 to 1 mmol/L and class II HDACs with IC_{50} values greater than 1.5 mmol/L (36). Interestingly, the half-maximal effects of the VPA-induced alterations of ERα levels in MCF7 and MDA-MB-231 were estimated at approximately 6 to 7 mmol/L, which may suggest that the class II HDACs are involved in ERα regulation (Fig. 5B). As expected, knockdown of the class II HDACs such as HDACs 4 to 6 altered ERα levels more efficiently than knockdown of HDAC1 in both MCF7 and MDA-MB-231 (Fig. 5C and D). Consistent with this, we found that HDACs 4 to 6 bound to ERpro315 in both breast cancer cell types (Fig. 5E). These observations suggest that the class II HDACs are involved in

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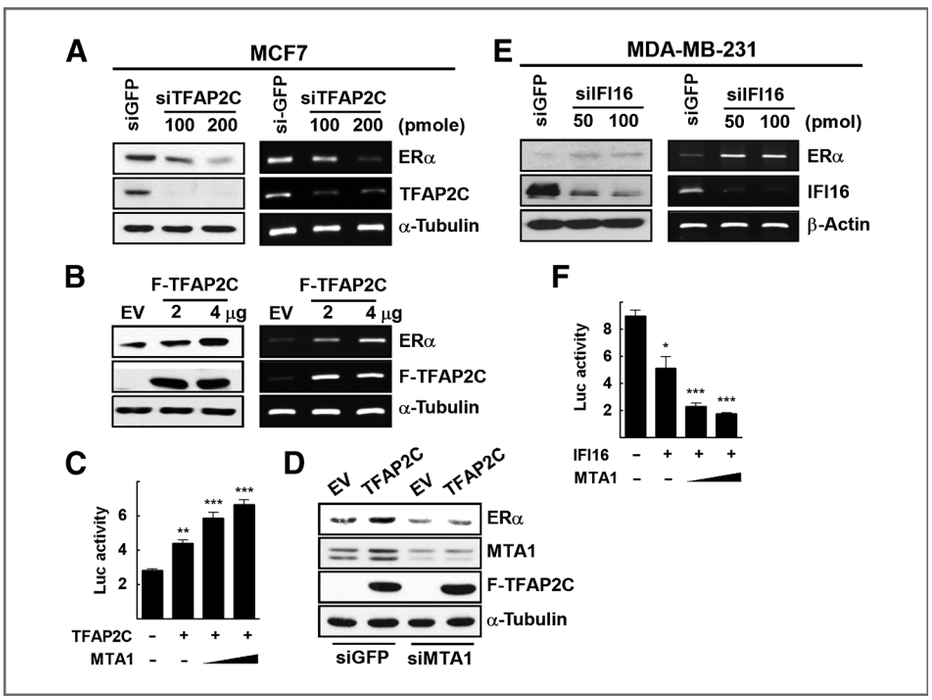

Figure 4. TFAP2C or IFI16 collaborates with MTA1 in differential regulation of ERα expression in ERα-positive and ERα-negative breast cancer cells. A, MCF7 cells were transfected with the indicated amount of siMTA1 for 48 hours. B, MCF7 cells were transfected with empty vector (EV) or real amounts of FLAG-TFAP2C for 24 hours. Expression levels of protein or mRNA of ERα and TFAP2C were analyzed by Western blotting (left) or RT-PCR (right), respectively. C, MCF7 cells were transfected with the human ERpro315-Luc reporter and FLAG-TFAP2C with increasing amounts of real amounts of FLAG-MTA1. Experimental values are expressed as the mean ± SD ($n = 3$). **, $P < 0.01$ and ***, $P < 0.001$ versus control. D, MCF7 cells were transfected with siGFP or siMTA1. After 24 hours of transfection, cells were transfected with EV or FLAG-TFAP2C for another 24 hours. Expression of the indicated proteins was analyzed by Western blotting. E, MDA-MB-231 cells were transfected with siIFI16 for 48 hours. Expression levels of protein or mRNA of MTA1 and ERα were analyzed by Western blotting (left) or RT-PCR (right), respectively. F, MDA-MB-231 cells were transfected with the human ERpro315-Luc reporter and FLAG-IFI16 with real amounts of FLAG-MTA1. Experimental values are expressed as the mean ± SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ versus control.

the differential regulation of *ESR1* expression that is mediated through the ERpro315 region.

Loss of IFI16 provides susceptibility to tamoxifen-induced cell growth inhibition for ERα-negative breast cancer cells

To examine whether the loss of MTA1 or IFI16 restored the tamoxifen sensitivity of breast cancer cells, we established stable MDA-MB-231-derived cell lines expressing shMTA1 or shIFI16 using the lentiviral delivery shRNA system (Fig. 6A). As shown in Fig. 6B, the MDA-MB-231 stable cell lines lacking either MTA1 or IFI16 showed restored ERα expression. Tamoxifen treatment of these cells significantly reduced cell growth, whereas this hormone dependency was not seen in the shGFP control cells (Fig. 6C). Clonogenic viabilities of both shIFI16 and shMTA1MDA-MB-231 stable cells were significantly lower compared with shGFP control cells. Further survival fractions in response to tamoxifen treatment were dramatically reduced in shIFI16 and shMTA1MDA-MB-231 stable cells (Fig. 6D). To examine the *in vivo* susceptibility of the MDA-MB-231 stable cells to tamoxifen treatment, the shRNA MDA-MB-231 stable cells were inoculated to grow xenografts in athymic nude mice. We found significant differences in tumor growth of shIFI16 and shMTA stable cells after tamoxifen treatment, whereas no difference in shGFP control cells, strongly supporting the role of MTA1 in the regulation of ERα expression (Fig. 6E). Taken together, our results indicate that the MTA1 complex including TFAP2C and class II HDACs enhances *ESR1* transcription in MCF7, whereas the MTA1 complex including IFI16 and class II HDACs represses *ESR1* transcription in MDA-MB-231, both by binding to the ERpro315 region of the *ESR1* promoter (Fig. 6F).

Finally, we compared the expression levels of MTA1, TFAP2C, and IFI16 in ERα-positive and ERα-negative breast carcinomas using the public datasets obtained from ArrayExpress and GEO sites. Expression of MTA1 showed no significant correlation with ERα mRNA expression level in four datasets that contain gene chip profiles classified by ERα status of breast carcinoma tissues (25–28). Although the expression level of TFAP2C was not consistently correlated with that of ERα in these four datasets, in all datasets the IFI16 mRNA levels were clearly higher in ERα-negative than in ERα-positive breast carcinomas (Fig. 7). These results raise the possibility that overexpression of IFI16 in ERα-negative breast cancer cells mediates ERα negativity and hormone resistance during breast carcinogenesis.

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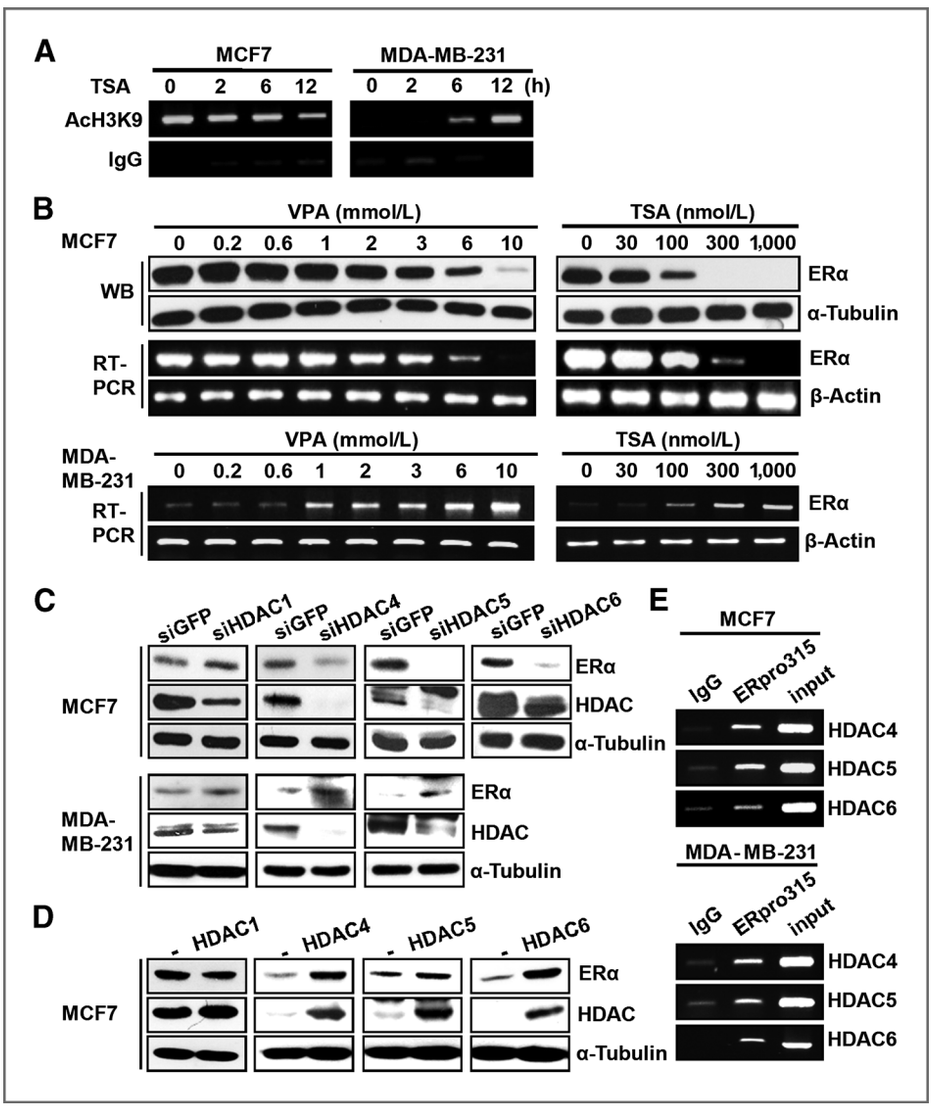

Figure 5. Class II HDAC family is involved in the differential regulation of ERα. A, epigenetic control of ERpro315 region by TSA in MCF7 and MDA-MB-231. Cells were treated with 300 nmol/L TSA for the indicated periods. DNA fragments that were immunoprecipitated by normal IgG or anti-AcH3K9 antibodies were amplified by PCR using primers for ERpro315. B, cells were treated with the indicated concentrations of VPA or TSA for 24 hours. Expression levels of protein or mRNA of ERα were analyzed by Western blotting (WB) or RT-PCR, respectively. C, MCF7 or MDA-MB-231 cells were transfected with the indicated siHDACs for 48 hours. Expression of ERα and HDACs was analyzed by Western blotting. D, MCF7 cells were transfected with FLAG-HDACs for 24 hours. Expression levels of protein of ERα and HDACs were analyzed by Western blotting. E, binding of class II HDACs to ERpro315. DNA fragments that were immunoprecipitated by anti-HDAC4, anti-HDAC5, or anti-HDAC6 antibodies were amplified by PCR using primers for ERpro315.

Discussion

Therapeutic strategies for ERα-positive breast cancers include ovarian ablation or anti-estrogen drug treatment. However, target-directed therapies for ERα-negative breast cancers are lacking, even though they are more clinically aggressive and their prognosis is poor. Thus, reactivation of *ESR1* in ERα-negative breast cancer has been targeted as a potentially successful breast cancer therapy. Earlier studies showed that treatment with TSA and 5-aza-dc, two well-characterized pharmacologic inhibitors of histone deacetylation and DNA methylation, reactivated ERα expression in ERα-negative breast cancer cells (11, 13). Here we show that MTA1 is a transcriptional regulator that is associated with the epigenetic control of ERα expression. The *ESR1* promoter region in which MTA1 was recruited, the ERpro315 (+146 to +461), was previously reported to be epigenetically controlled: in ERα-negative breast cancer cell lines this region was methylated and recruited methyl CpG binding protein and DNMTs, resulting in repression of *ESR1* transcription (31, 32). We found that TSA treatment decreased the level of H3K9 acetylation in the ERpro315 (+146 to +461) in MCF7 cells but increased it in MDA-MB-231 cells, indicating that histone acetylation is also involved in the epigenetic control of this promoter region (Fig. 5A). Class II HDACs such as HDACs 4 to 6 may have important roles in the differential regulation of the promoter as they bind to ERpro315 (Fig. 5E). Our observations, together with others, may suggest that MTA1 is a key regulator that coordinates histone acetylation and DNA methylation, which provide differential regulation of ERα expression during progression of breast cancer.

We identified two putative transcription factors, TFAP2C and IFI16, which are involved in the MTA1-mediated transcriptional regulation of *ESR1*. IFI16 is a member of the HIN-200 family of IFN-inducible genes that is associated with cell-cycle regulation and differentiation (37). Although genetic alterations in the coding region of IFI16, 1q21-23,

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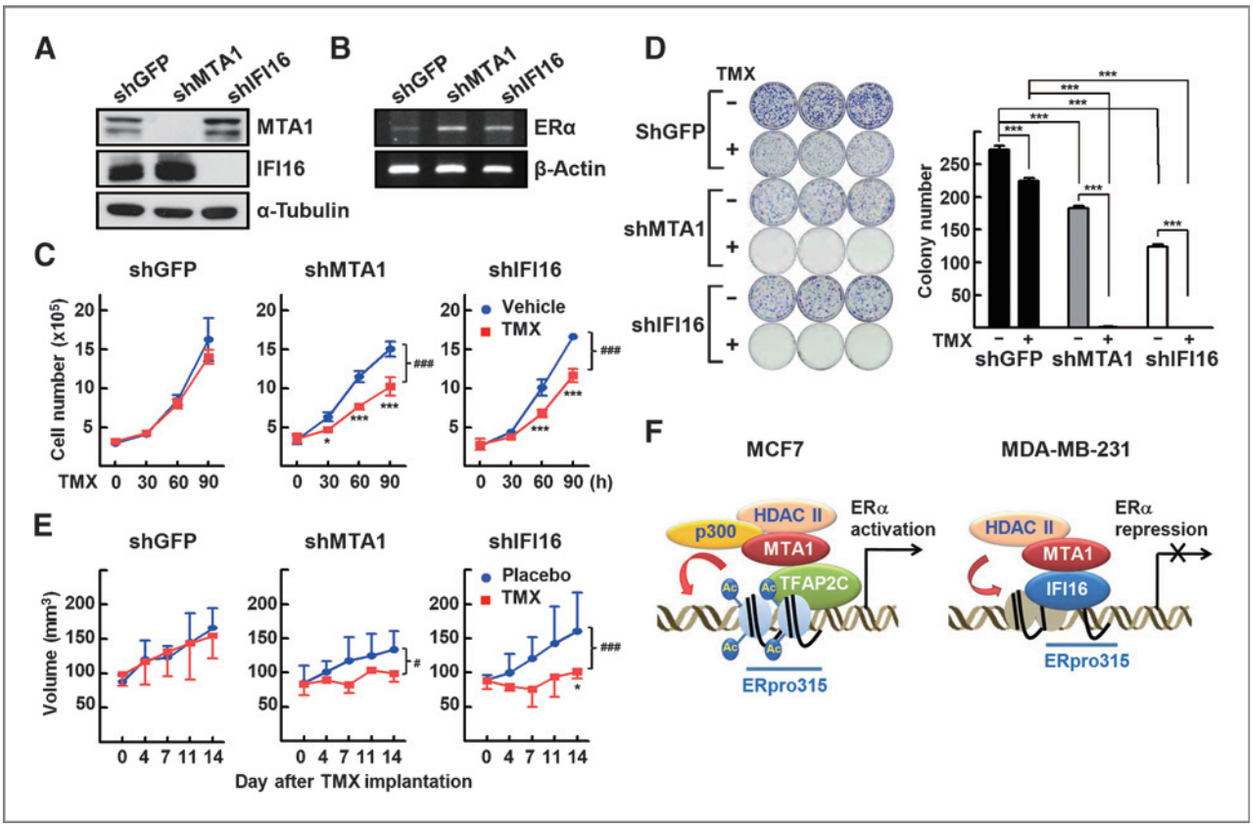

Figure 6. Silencing of MTA1 or IFI16 sensitizes MDA-MB-231 cells to tamoxifen (TMX)-induced inhibition of cell growth. A, establishment of the MDA-MB-231 stable cell lines that express shGFP, shMTA1, or shIFI16 using lentiviral-delivered shRNA system. Expression levels of the corresponding proteins were analyzed by Western blotting. B, mRNA level of ERα in the MDA-MB-231 stable cells was measured by RT-PCR. C, the MDA-MB-231 stable cells were treated with the 10 μmol/L TMX and the number of cells was counted using hematocytometer. Experimental values are expressed as the mean ± SD of three independent experiments. Statistical significance was evaluated by 2-way ANOVA followed by Bonferroni posttest. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ versus vehicle at each time; $^{\#\#\#}$, $P < 0.001$ versus vehicle group. D, the MDA-MB-231 stable cells were treated with 10 μmol/L TMX for 12 days. Colony that was composed of more than 50 cells was counted. Experimental values are expressed as the mean ± SD of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. E, female athymic nude mice were inoculated with the shRNA MDA-MB-231 stable cells. When tumor volume reached approximately 100 mm^3, placebo or TMX pellet (25 mg/pellet with 21 days release) was implanted subcutaneously. Tumor volume was measured during 2 weeks of treatment. Experimental values are expressed as the mean ± SD. The number of specimen of the experimental groups was as follows: shGFP-placebo ($n = 4$), shGFP-TMX ($n = 5$), shMTA1-placebo ($n = 3$), shMTA1-TMX ($n = 3$), shIFI16-placebo ($n = 6$), and shIFI16-TMX ($n = 4$). *, $P < 0.05$ versus placebo at that time; $^{\#}$, $P < 0.05$ and $^{\#\#\#}$, $P < 0.001$ versus placebo group. F, schematic illustration of the molecular mechanism of MTA1-mediated transcriptional regulation of ERα in ERα-positive and ERα-negative breast cancer.

in human breast cancer and E_2-induced downregulation of IFI16 in breast cancer cells have been reported, the function of IFI16 in estrogen signaling has not been studied (38, 39). Here we report a novel molecular mechanism of IFI16 action in the epigenetic control of ERα expression in breast cancer. Furthermore, knockdown of IFI16 in MDA-MB-231 cells enhanced their sensitivity to tamoxifen-induced tumor cell growth inhibition, indicating that the MTA1–IFI16 repressor complex may contribute to loss of ERα expression and acquisition of hormone resistance in ERα-negative breast cancer. Interestingly, exogenously introduced IFI16 resulted in decreases in the mRNA levels of androgen receptor and inhibited cell proliferation of the prostate cancer cell line LNCaP (40). As the role of androgen receptor in breast cancer development and treatment has attracted attention, understanding of the function of IFI16 in hormone resistance and transcriptional regulation of hormone receptors may contribute to development of a novel strategy to treat breast cancer (41).

TFAP2C, the expression of which decreases during hormone therapy, is considered an independent predictor of poor survival in patients with breast cancer (33). In an earlier study, it was reported that TFAP2C could be purified from a protein complex bound to a region of the *ESR1* promoter including the ERpro315 (+146 to +461) region (42). Subsequently, TFAP2C was shown to upregulate *ESR1* expression in breast cancer cells by alteration of chromatin structure (32, 33). Here, we demonstrated that MTA1 binds to TFAP2C and is required for TFAP2C-induced *ESR1* activation (Figs. 3 and 4), which explains the epigenetic mechanism of TFAP2C function. We speculate that MTA1 may recruit class II HDACs to the ERpro315 region occupied by TFAP2C. In this regard, MTA1 was shown previously to bind HDAC4 (43). Interestingly, HDAC4 and HDAC5 interacted with p300 in MCF-7 but not

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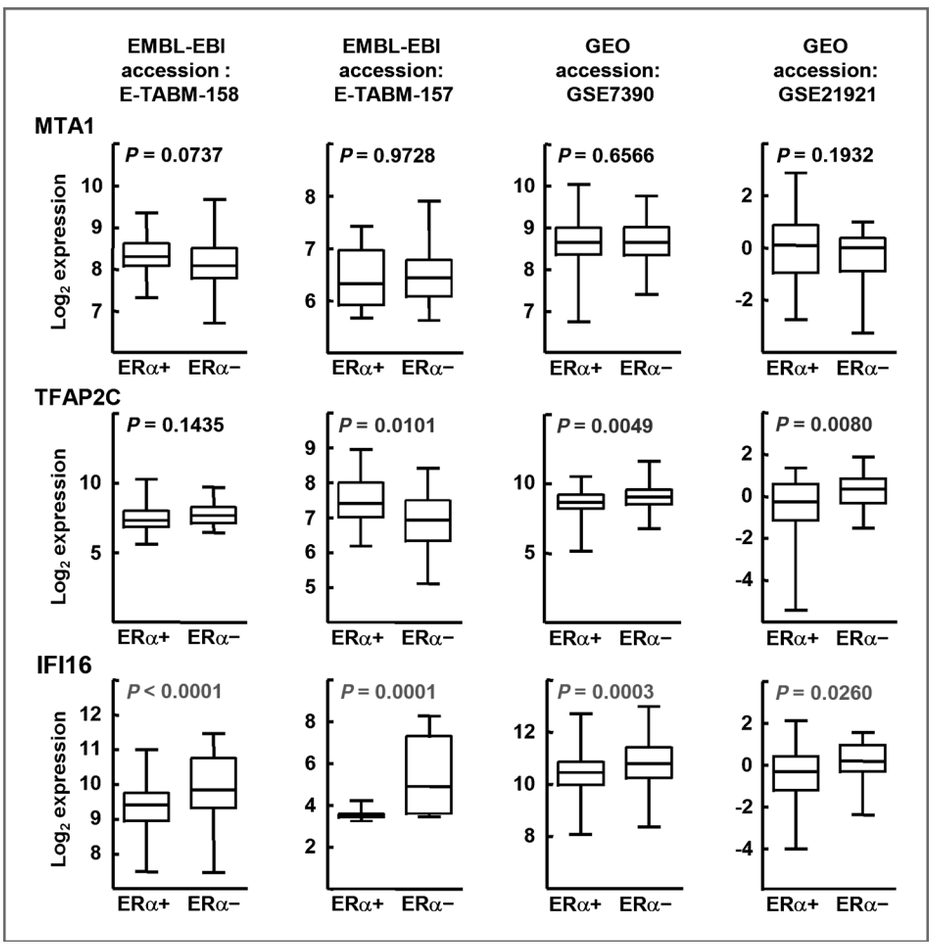

Figure 7. Expression of MTA1, TFAP2C, and IFI16 in human breast carcinoma: database-based gene expression analysis. The public datasets were obtained from ArrayExpress and GEO sites. The data processed as $\log_2$ expression value without further transformation were analyzed using Microsoft Excel tool. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

in MDA-MB-231 (Supplementary Fig. S2A). Furthermore, the binding between these HDACs and p300 was abolished when expression of TFAP2C was silenced by siRNA (Supplementary Fig. S2B). These data indicate that MTA1 complex including TFAP2C and class II HDACs recruits p300 for transcriptional activation of ERα in MCF7 cells. However, we compared target genes of TFAP2C reported by Woodfield and colleagues (44) and that of MTA1 (varied by $2 \geq$ fold, $0.05 \leq P$-value) profiled by Ghanta and colleagues (45), and found that expression of 26 genes are altered by MTA1 and TFAP2C in common (Supplementary Table S2). Among these genes, Depdc6 and Svil were identified as primary target genes of ERα, according to the global mapping of ChIP-seq analysis for ERα-controlled gene network in luminal-like breast cancer cells (46). These results strongly support the link between TFAP2C and MTA1 in regulation of luminal target genes of ERα in breast cancer cells.

Our results, together with previous reports, show that epigenetic regulation is one of the most important molecular mechanisms that results in the absence of ERα in hormone-resistant breast cancer cells. Indeed, in the clinic, reactivation of *ESR1* enhanced the chemosensitivity to tamoxifen in ERα-negative breast carcinoma. For example, in a recent phase II trial, combined therapy with tamoxifen and an HDAC inhibitor, vorinostat, for patients with ERα-positive breast cancer who had undergone prior hormonal therapy or chemotherapy was well tolerated and reversed hormone resistance (47). Currently, a clinical trial is underway of a combined treatment with tamoxifen, decitabine (5-aza-dc), and LHB589, an HDAC inhibitor, for triple-negative breast cancer patients (ClinicalTrials.gov identifier: NCT01194908). In this study, we found that class II HDACs were more efficient than class I HDACs in the restoration of ERα in MDA-MB-231 cells, suggesting that a combination therapy including selective class II HDAC inhibitors may provide a better therapeutic index for sensitizing breast cancers to systemic treatments involving tamoxifen-based chemotherapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.-J. Kang, M.-H. Lee, H.-L. Kang, S.-H. Kim, H. Na, T.-Y. Na

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.-H. Lee, H.-L. Kang, H. Na, Y.N. Kim, J.K. Seong, M.-O. Lee

Writing, review, and/or revision of the manuscript: H.-J. Kang, M.-H. Lee, H.-L. Kang, H. Na, J.K. Seong, M.-O. Lee
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.-J. Kang, M.-H. Lee
Study supervision: J.K. Seong, M.-O. Lee

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References

1. Katzenellenbogen BS, Katzenellenbogen JA. Estrogen receptor transcription and transactivation: estrogen receptor α and estrogen receptor β: regulation by selective estrogen modulators and importance in breast cancer. Breast Cancer Res 2000;2:335–44.
2. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al. Estrogen receptors; how do they signal and what are their targets. Physiol Rev 2007;87:905–31.
3. Lower EE, Glass EL, Bradley DA, Blau R, Heffelfinger S. Impact of metastasic estrogen receptor and progesterone receptor status on survival. Breast Cancer Res Treat 2005;90:65–70.
4. Knight WA, Livingston RB, Gregory EJ, McGuire WL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 1977;7:4669–71.
5. Schairer C, Mink PJ, Carroll L, Devesa SS. Probabilities of death from breast cancer and other causes among female breast cancer patients. J Natl Cancer Inst 2004;96:1311–21.
6. Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, et al. Estrogen receptor α (ESR1) gene amplification is frequent in breast cancer. Nat Genet 2007;39:655–60.
7. Iwase H, Greenman JM, Barnes DM, Bobrow L, Hodgson S, Mathew CG. Loss of heterozygosity of the oestrogen receptor gene in breast cancer. Br J Cancer 1995;71:448–50.
8. Herynk MH, Fuqua SA. Estrogen receptor mutation in human disease. Endocr Rev 2004;25:869–98.
9. Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res 1994;54:2552–5.
10. Pinzone JJ, Stevenson H, Strobl JS, Berg PE. Molecular and cellular determinants of estrogen receptor α expression. Mol Cell Biol 2004; 24:4605–12
11. Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson NE. Synergistic activation of functional estrogen receptor (ER)-α by DNA methyltransferase and histone deacetylase I inhibition in human ER-α-negative breast cancer cells. Cancer Res 2001;61: 7025–29.
12. Sharma D, Saxena NK, Davidson NE, Vertino PM. Restoration of tamoxifen sensitivity in estrogen receptor-negative breast cancer cells: tamoxifen-bound reactivated ER recruits distinctive corepressor complexes. Cancer Res 2006;66:6370–78.
13. Fan J, Yin WJ, Lu JS, Wang L, Wu J, Wu FY, et al. ER alpha negative breast cancer cells restore response to endocrine therapy by combination treatment with both HDAC inhibitor and DNMT inhibitor. J Cancer Res Clin Oncol 2008;134:883–90
14. Macaluso M, Cinti C, Russo G, Russo A, Giordano A. pRb2/p130-E2F4/5-HDAC1-SUV39H1-p300 and pRb2/p130-E2F4/5-HDAC1-SUV39H1-DNMT1 multimolecular complexes mediate the transcription of estrogen receptor-alpha in breast cancer. Oncogene 2003;22: 3511–7.
15. Vesuna F, Lisok A, Kimble B, Domek J, Kato Y, van der Groep P, et al. Twist contributes to hormone resistance in breast cancer by down-regulating estrogen receptor-α. Oncogene 2012;31:3223–34.
16. Toh Y, Nicolson GL. The role of the MTA family and their encoded proteins in human cancers: molecular functions and clinical implications. Clin Exp Metastasis 2009;26:215–27.
17. Toh Y, Pencil SD, Nicolson GL. A novel candidate Metastasis-associated gene, mta1, differentially expressed in highly metastatic mammart adenocarcinoma cell lines. cDNA cloning, expression, and protein analysis. J Bio Chem 1994;269:22958–63.
18. Martin MD, Fischbach K, Osborne CK, Mohsin SK, Allred DC, O'Connell P. Loss of heterozygosity events impeding breast cancer metastasis contains the MTA1 gene. Cancer Res 2001;61:3578–80.
19. Jang KS, Paik SS, Chung H, Oh YH, Kong G. MTA1 overexpression correlates significantly with tumor grade and angiogenesis in human breast cancers. Cancer Sci 2006;97:374–9.
20. Manavathi B, Singh K, Kumar R. MTA family of coregulators in nuclear receptor biology and pathology. Nucl Recept Signal 2007;5:e010.
21. Gururaj AE, Holm C, Landberg G, Kumar R. Breast cancer-amplified sequence 3, a target of metastasis-associated protein 1, contributes to tamoxifen resistance in premenopausal patients with breast cancer. Cell Cycle 2006;5:1407–10.
22. Molli PR, Singh RR, Lee SW, Kumar R. MTA1-mediated transcriptional repression of BRCA1 tumor suppressor gene. Oncogene 2008;27: 1971–80.
23. Jiang Q, Zhang H, Zhang P. ShRNA-mediated gene silencing of MTA1 influenced on protein expression of ER alpha, MMP-9, cyclin D1 and invasiveness, proliferation in breast cancer cell lines MDA-MB-231 and MCF-7 *in vitro*. J Exp Clin Cancer Res 2011;30:60.
24. Lee MH, Na H, Kim EJ, Lee HW, Lee MO. Poly(ADP-ribosyl)ation of p53 induces gene-specific transcriptional repression of MTA1. Oncogene 2012;31:5099–107.
25. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 2006;10:515–27.
26. Chin K, DeVries S, Fridlyand J, Spellman PT, Roydasgupta R, Kuo WL, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. Cancer Cell 2006;10:529–41.
27. Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. Clin Cancer Res 2007;13:3207–14.
28. Waddell N, Cocciardi S, Johnson J, Healey S, Marsh A, Riley J, et al. Gene expression profiling of formalin-fixed, paraffin-embedded familial breast tumours using the whole genome-DASL assay. J Pathol 2010;221:452–61.
29. Kos M, Reid G, Denger S, Gannon F. Mini review: genomic organization of the human ERα gene promoter region. Mol Endocrinol 2001;15: 2057–63.
30. Tang Z, Treilleux I, Brown M. A transcriptional enhancer required for the differential expression of the human estrogen receptor in breast cancers. Mol Cell Biol 1997;17:1274–80.
31. Sharma D, Blum J, Yang X, Beaulieu N, Macleod AR, Davidson NE. Release of methyl CpG binding proteins and histone deacetylase 1 from the estrogen receptor α (ER) promoter upon reactivation in ER-negative human breast cancer cells. Mol Endocrinol 2005;19: 1740–51.
32. Woodfield GW, Hitchler MJ, Chen Y, Domann FE, Weigel RJ. Interaction of TFAP2C with the estrogen receptor-α promoter is controlled by chromatin structure. Clin Cancer Res 2009;15:3672–9.
33. Woodfield GW, Horan AD, Chen Y, Weigel RJ. TFAP2C controls hormone response in breast cancer cells through multiple pathways of estrogen signaling. Cancer Res 2007;67:8439–43.
34. Reid G, Métivier R, Lin CY, Denger S, Ibberson D, Ivacevic T, et al. Multiple mechanisms induce transcriptional silencing of a subset of

Published OnlineFirst January 10, 2014; DOI: 10.1158/0008-5472.CAN-13-2020

genes, including oestrogen receptor alpha, in response to deacetylase inhibition by valproic acid and trichostatin A. Oncogene 2005;24: 4894–907.
35. Fortunati N, Bertino S, Costantino L, De Bortoli M, Compagnone A, Bandino A, et al. Valproic acid restores ERα and antiestrogen sensitivity to ERα-negative breast cancer cells. Mol Cell Endocrinol 2010;314:17–22.
36. Gurvich N, Tsygankova OM, Meinkoth JL, Klein PS. Histone deacetylase is a target of valproic acid-mediated cellular differentiation. Cancer Res 2004;64:1079–86.
37. Asefa B, Klarmann KD, Copeland NG, Gilbert DJ, Jenkins NA, Keller JR. The interferon-inducible p200 family of proteins: a perspective on their roles in cell cycle regulation and differentiation. Blood Cells Mol Dis 2004;32:155–67.
38. Chen LC, Dollbaum C, Smith HS. Loss of heterozygosity on chromosome 1q in human breast cancer. Proc Natl Acad Sci U S A 1989; 86:7204–7.
39. Harvell DM, Richer JK, Allred DC, Sartorius CA, Horwitz KB. Estradiol regulates different genes in human breast tumor xenografts compared with the identical cells in culture. Endocrinology 2006; 147:700–13.
40. Alimirah F, Chen J, Xin H, Choubey D. Androgen receptor auto-regulates its expression by a negative feedback loop through upregulation of IFI16 protein. FEBS Lett 2006;580: 1659–64.
41. Hickey TE, Robinson JL, Carroll JS, Tilley WD. Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? Mol Endocrinol 2012;26:1252–67.
42. McPherson LA, Baichwal VR, Weigel RJ. Identification of ERF-1 as a member of the AP2 transcription factor family. Proc Natl Acad Sci USA 1997;94:4342–7.
43. Reddy SD, Pakala SB, Molli PR, Sahni N, Karanam NK, Mudvari P, et al. Metastasis-associated protein 1/histone deacetylase 4-nucleosome remodeling and deacetylase complex regulates phosphatase and tensin homolog gene expression and function. J Biol Chem 2012;287: 27843–50.
44. Woodfield GW, Chen Y, Bair TB, Domann FE, Weigel RJ. Identification of primary gene targets of TFAP2C in hormone responsive breast carcinoma cells. Genes Chromosomes Cancer 2010;49:948–62.
45. Ghanta KS, Li DQ, Eswaran J, Kumar R. Gene profiling of MTA1 identifies novel gene targets and functions. PLoS One 2011;6: e17135.
46. Cicatiello L, Mutarelli M, Grober OM, Paris O, Ferraro L, Ravo M , et al. Estrogen receptor alpha controls a gene network in luminal-like breast cancer cells comprising multiple transcription factors and microRNAs. Am J Pathol 2010;176:2113–30.
47. Munster PN, Thurn KT, Thomas S, Raha P, Lacevic M, Miller A, et al. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. Br J Cancer 2011;104:1828–35.

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Differential Regulation of Estrogen Receptor α Expression in Breast Cancer Cells by Metastasis-Associated Protein 1

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