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ERα positively regulated DNMT1 expression by binding to the gene promoter region in human breast cancer MCF-7 cells

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ABSTRACT

Estrogen receptors (ER) are expressed in approximately 65% of human breast cancer. Clinical trials and retrospective analyses showed that ER-positive (ER+) tumors were more vulnerable to development of chemotherapy resistance than ER-negative (ER−) tumors. The underlying mechanism is still to be elucidated. Aberrant DNA methylation has been recognized to be associated with cancer chemotherapy resistance. Recently, steroid hormone and their receptors have been found to be involved in the regulation of methyltransferases (DNMTs) and thereby contribute to chemotherapy resistance. The purpose of this study is to explore whether ERα could directly regulate the DNMTs expression. We first analyzed the methylation alterations and its correlation with the expression levels of three types of DNMTs in our established paclitaxel-resistant breast cancer lines, MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX cell lines, using qMSP, real-time PCR and Western blot. Then we determined the function of ERα in regulation of DNMT1 using luciferase report gene systems. Our data demonstrated for the first time that ERα could upregulate DNMT1 expression by directly binding to the DNMT1 promoter region in MFC-7(ER+)/PTX cells.

1. Introduction

Breast cancer is one of the most common worldwide diseases in women, and usually treated by surgery accompanied by adjuvant endocrinotherapy or chemotherapy [1,2]. Estrogen receptor (ER) is expressed in approximately 65% of human breast cancer [3,4]. Around 40% of patients with ER-positive breast cancer inevitably relapse and these hormone refractory tumors often have a poor prognosis, although patients with ER-positive breast cancer have better prognosis than ER-negative breast cancer benefitting from endocrine therapy [5,6]. Cumulative data from clinical trials and retrospective analyses showed that ER-positive tumors were more vulnerable to resistance to chemotherapy agents including paclitaxel (PTX), a first-line chemotherapeutic drug, than ER-negative tumors [7–9]. The underlying mechanism is still to be elucidated.

ER is a member of the super family of nuclear receptors that function as transcription factors. In addition to estrogen-induced activation, it also interacts with growth factor pathways [10,11]. ER proteins have two different isoforms, ERα and ERβ. It is known that ERα rather than ERβ plays a crucial role in breast cancer development [12–14]. Recent study has revealed that expression of ERα interferes with paclitaxel-induced apoptotic cell death of MCf-7 breast cancer cells [8]. To study the functional role of ER in development of drug resistance of breast cancer, our laboratory established paclitaxel-resistant cell lines, MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX [15]. We found that expression of ERα was significantly increased in paclitaxel-resistant MCF-7 cells (MCF-7(ER+)/PTX) compared with the parent cell line (MCF-7), while ERα expression was not detective in ERα-negative paclitaxel-resistant MDA-MB-231(ER−)/PTX cells [15]. All together, these observations suggested that ERα played an important role in development of chemotherapy resistance of ER-positive breast cancer cells.

Disruption of the DNA methylation landscape is one of the most common features of human tumors. Aberrant DNA methylation has been recognized to be associated with the transcriptional inactivation of genes related to cancer drug resistance development [16–18]. Segura-Pacheco B and his colleagues have shown that the multidrug resistant (MDR) phenotype was accompanied by global DNA hypermethylation, over expression of DNMT genes, and increased DNA methyltransferase activity as compared with wild-type MCF-7 cells. Clearly, DNA hypermethylation is at least partly responsible for development of the MDR phenotype in the MCF-7

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drug resistance model [19]. Thus, evaluation of the mechanisms by which DNA methylation is disrupted is of great importance for understanding the development of drug resistant phenotype and improving cancer chemotherapy.

DNA (cytosine-5) methyltransferases (DNMTs) is divided into three subtypes, DNMT1, DNMT3a, and DNMT3b [20]. DNMT1 is primarily a maintenance methyltransferase, as it prefers to methylate hemimethylated DNA during DNA replication. DNMT3a and DNMT3b are mainly de novo methyltransferases and show similar activity on unmethylated and hemimethylated DNA [21–24]. Recently, steroid hormone and their receptors have been found to be involved in the regulation of DNMTs and thereby influence DNA methylation [25,26]. It was reported that estrogen treatment increased the expression of DNMT3b and that hormonal therapy promoted hormone resistant phenotype by increasing DNMT activity and expression in prostate cancer models [25,27]. However, whether ERα can directly regulate DNMTs expression to alter DNA methylation in drug resistance breast cancer is still unclear. The purpose of this study is to explore whether ERα influences genomic DNA methylation by directly regulating the DNMTs expression. In this study we first analyzed the methylation status and its correlation with the expression levels of three types of DNMTs in our established paclitaxel-resistant breast cancer lines, MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX as well as their parental cell lines using qMSP, real-time PCR and Western blot. Then we determined the function of ERα in regulation of DNMT1 using luciferase report gene system. Our work demonstrated that ERα could upregulate DNMT1 expression by directly binding to the DNMT1 promoter region in MFC-7(ER+)/PTX cells.

2. Materials and methods

2.1. Cell lines and cell culture

The human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from the ATCC. The paclitaxel resistance cell lines MDA-MB-231(ER−)/PTX was established by pulse selection with paclitaxel (100 nM, 10 pulses, once a week for 4 h), and MCF-7(ER+)/PTX cells (200 nM, 10 pulses, once a week for 4 h) [15]. MCF-7 and MCF-7(ER+)/PTX cells were cultured in EMEM containing 10% (v/v) fetal bovine serum and insulin (0.2 U/ml), whereas MDA-MB-231 and MDA-MB-231(ER−)/PTX cells were cultured in L-15 containing 10% (v/v) calf bovine serum.

2.2. RNA extraction and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. To prepare cDNA, 1 μg of total RNA was reverse-transcribed according to TOYOBO manufacturer's instructions. Primers designed for specifically real-time PCR were listed in Table 1.

Table 1
Primers used in the present study.

Primers		Sequence (5′–3′)
qMSP	LINE1 M-F	GTCGAATAGGAATAGTTTCGG
	LINE1 M-R	ACTCCCTAACCCCTTACGCT
	Actin-F	TGGTGATGGAGGAGGTTTAGTAAG
	Actin-R	AACCAATAAAACCTACTCCTCCCTTAA
RT-PCR	DNMT1-F	CCTGAGGCCTTCACGTTCAA
	DNMT1-R	ACTTGTGGGTGTTCTCAGGC
	DNMT3a-F	CAGCGTCACACAGAAGCATATC
	DNMT3a-R	ACCACATTCTCAAAGA GCCAGA
	DNMT3b-F	GGATGAAGATCAGAGCCGAGAA
	DNMT3b-R	CAGGCACTCCACACAGAAACAC
	Actin-F	TCATGAAGTGTGACGTGGACAT
	Actin-R	CTCAGGAGGAGCAATGATCTTG

2.3. DNA extraction and quantitative methylation specific real-time PCR (qMSP)

Total DNA was extracted using Multisource Genomic DNA Miniprep Kit (Axygen) according to the manufacturer's protocol. 1 mg of genomic DNA from each sample was modified by sodium bisulfite with the CpGenome™ DNA Modification Kit (Chemicon). LINE-1 is a non-LTR class of retrotransposons in human genome and comprises approximately 18% of human genome. Therefore the level of LINE-1 methylation could be regarded as a surrogate of global DNA methylation [28,29]. β-Actin was used to normalize for input DNA, a region of β-actin devoid of any CpG dinucleotide was amplified. The primer sequences used in this study were listed in Table 1.

2.4. Plasmids

For overexpression of ERα we used the pcDNA3.0 construct containing ERα full length cDNA. For silencing of ERα oligonucleotide: sense 5′-GATCCCCGCTACTGTTTGCTCCTAACTTCAAGAGAGTTAGGAGCAAACAGTAGCTTTTTGGAAA-3′; antisense 5′-AGCTTTTCCAAAAAGCTACTGTTTGCTCCTAACTCTCTTGAAGTTAGGAGCAAACAGTAGCGGG-3′ were purchased from Invitrogen and cloned into pGC (for transient transfection).

2.5. Transient transfection

MCF-7 cells and MCF-7(ER+)/PTX cells were planted in a six-well plate at a density of 1×10^6 cells/well and incubated overnight in EMEM supplemented with 10% FBS. PcDNA3.0-ERα and pGC-ERα-shRNA plasmid were diluted in serum-free EMEM medium (250 μl) and then mixed with Lipofectamine™ 2000 (Invitrogen). Then, 6 h after transfection, culture medium was changed to normal medium. As a negative control, pcDNA3.0 and pGC-control-shRNA were used.

2.6. Western blot

Total cellular protein extracts were obtained using ice-cold lysis buffer. Twenty micrograms of total protein per sample were separated on 8% SDS–polyacrylamide gel and transferred to PVDF membranes (Bio-Rad). After blocking in 5% skimmed milk for 1 h, membranes were incubated with a primary antibody overnight at 4 °C. Membranes were washed with 3 times for 10 min in TBST and incubated with a HRP-conjugated secondary antibody (R&D) for 1 h at room temperature. After washing 3 times for 10 min in TBST, the membranes were developed by ECL detection system. Quantification was performed using the Quantity One (Bio-Rad). Antibodies against DNMT1 and DNMT3b were purchased from cell signaling technology, anti-β-actin was obtained from Sigma–Aldrich.

2.7. Cell proliferation assay

Cells were seeded at a density of 8000 cells per well for MCF-7 cells or MCF-7(ER+)/PTX cells in 96-well plates. The cells were then treated with chemotherapeutic agent paclitaxel. At the end of the culture, 10 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml) were added to each well. Then, 150 μl of dimethylsulfoxide were added to each well to lyse the cells. Absorbance was measured at 570 nm using a microplate reader.

2.8. Colony formation assay

Transfected cells were re-seeded onto 6-well plates at 400 cells per well. Two weeks later, colonies were fixed with 100% methanol for 15 min and stained with crystal violet for 20 min. After taking

photographs, the number of colonies with diameter more than 1.5 mm was counted.

2.9. Luciferase reporter assay

The promoter of human MDR1 gene was amplified by PCR using the following primers: forward; 5′-GGGGTACCCCCTTTACCAGATAATACAGTTTGC-3′, reverse; 5′-TAGGAAGCTTCGCAGACGACTTCGGAGGCTC-3′. PCR products were cloned into the multi-cloning sites of the pGL3 reporter plasmid (Ambion), obtaining the WT firefly luciferase reporter gene. To introduce mutations into the seed sequences of three ERE sites within the DNMT1 promoter and generate the DNMT1 MT1, MT2, and MT3 reporter genes, which mutated at each of three potential ERα binding sites (at positions −1336 to −1313, −734 to −714, and −114 to −92, respectively) were constructed by overlap extension PCR. The primers used were as follows: MT1 forward; 5′-CTGGCCCAATGATTTAGCTTAGG-3′, reverse; 5′-CCTAAGCTAAATCATTGGGCCAG-3′, MT2 forward; 5′-AGACACCAGTGTGCTCTGTC-3′, reverse; 5′-GACAGAGCACACTGGTGTCT-3′, MT3 forward; 5′-TGCCTCCAATGACTACGCTACC-3′, reverse; 5′-GGTAGCGTAGTCATTGGAGGCA-3′ (Fig. 3D). Cells seeded onto 24-well plates were co-transfected with firefly reporter constructs containing the MDR1 promoter, Renilla expressing plasmid, phRL-TK, and ERα plasmid or negative control using Lipofectamine 2000. Firefly luciferase activity and Renilla luciferase activity were measured 30 h after the initiation of transfection by the Dual Luciferase Asssay Systems (TOYOBO). Firefly luciferase activity was normalized to Renilla luciferase activity.

3. Results

3.1. Genomic DNA methylation was significantly altered in paclitaxel-resistant breast cancer cells

To evaluate the alteration of genomic DNA methylation during development of paclitaxel resistance of breast cancer cells and its possible correlation with ER, we analyzed the methylation level of LINE-1 promoter in our established paclitaxel-resistant cell lines MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX as well as their corresponding parental cell lines using quantitative methylation specific real-time PCR (qMSP). By methylation analysis, we found that the level of LINE-1 methylation was dramatically up-regulated in MCF-7(ER+)/PTX cells compared with the parental MCF-7 cells, whereas the level of LINE-1 methylation was down-regulated in MDA-MB-231(ER−)/PTX cells compared with the parental MDA-MB-231 cells (Fig. 1A). These results suggested that genomic DNA methylation was significantly altered in paclitaxel-resistant breast cancer cells and ER might contribute to the genomic DNA hypermethylation.

3.2. The expression levels of DNMT1 was correlated to alterations of genomic DNA methylation in paclitaxel-resistant breast cancer cell lines

To determine the change in expression of DNMTs correlated to observed alterations of DNA methylation in the drug resistant breast cancer cell lines, we analyzed expression levels of DNMT3a, DNMT3b and DNMT1 in MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX cells and their parental cell lines with real-time PCR and Western blot. The expression of DNMT1 mRNA and protein were significantly increased in MCF-7(ER+)/PTX cells and decreased in MDA-MB-231(ER−)/PTX cells, which was consistent with the alterations of genomic DNA methylation in the cell lines (Fig. 1B and C). The expression of DNMT3a was not significantly changed in the two drug resistant cell lines, when compared with their parental cell lines. The expression of DNMT3b was increased in both MCF-7(ER+)/PTX cells and MDA-MB-231(ER−)/PTX cells (Fig. 1B and C). These data indicated that the alterations of genomic DNA methylation induced by paclitaxel in the drug resistant breast cancer cell lines was specifically correlated to expression level of DNMT1.

3.3. Ectopic expression of DNMT1 increased the resistance of MCF-7 cells to paclitaxel

In order to determine whether increased expression of DNMT1 contributes to paclitaxel-resistance of breast cancer cells, we evaluated effects of ectopic expression of DNMT1 in paclitaxel-sensitive MCF-7 cells. Western blot assay indicated that DNMT1 expression significantly increased at early phase (2 days after transfection with DNMT1) (Fig. 2A). Correspondingly, the MCF-7 cells transfected with DNMT1 were more resistant than those transfected with a control plasmid as revealed by cell proliferation assay (Fig. 2B and C). These results suggested that DNMT1 contributed to the development of PTX resistance phenotype in MCF-7 cells.

3.4. Knockdown of DNMT1 attenuated paclitaxel-resistance of MCF-7(ER+)/PTX cells

To further confirm the contribution of DNMT1 to PTX resistance, we performed DNMT1 knockdown experiments. The level of DNMT1 in PTX-resistant MCF-7(ER+)/PTX cells was knocked down with plasmid expressing DNMT1 short hairpin RNAs (shRNA). Western blot analysis conformed that shRNA targeted to DNMT1 in MCF-7(ER+)/PTX cells effectively reduced the expression level of DNMT1 (Fig. 2D). Knockdown of DNMT1 in MCF-7/PTX cells increased the sensitivity of cells to paclitaxel (Fig. 2E and F). Furthermore, we used 5-Aza-2′-deoxycytidine (5-aza-dC), a kind of nucleoside analogue that can inhibit DNA cytosine methylation, to treat PTX-resistant MCF-7(ER+)/PTX. As expected, treatment of cells with 5-aza-dC significantly increase the sensitivity of the cells to paclitxel (Fig. 2G), which was consistent with the result in Fig. 2F. Taken together, our data strongly suggested that DNMT1 was directly responsible for paclitaxel resistance in MCF-7(ER+)/PTX cells.

3.5. ERα enhanced transcriptional activity of the DNMT1 gene by directly binding to the promoter region

In order to determine the functional role of ER in regulation of DNMT1, we analyzed DNMT1 promoter region for the potential ERα binding site using the Genomatix software (http://www.genomatix.de/index.html) and found three putative ERα binding sequences. To prove that DNMT1 is a direct target of ERα, we constructed a firefly luciferase reporter construct containing the DNMT1 promoter with these potential target sites (DNMT1-WT). The paclitxel-resistant MCF-7(ER+)/PTX cells and paclitxel-sensitive MCF-7 cells were then transfected with the DNMT1-WT construct. Our data showed that the luciferase activity was higher in paclitxel-resistant MCF-7(ER+)/PTX cells that expressed high level of ERα than in paclitxel-sensitive MCF-7 cells that expressed lower level of ERα (Fig. 3A and B). To further observe the effect of ERα on promoter activity of the DNMT1 gene, MCF-7 cells were transfected with ERα expression vector. As indicated in (Fig. 3C), introduction of ERα into MCF-7 cells increased luciferase activity of DNMT1-WT compared with that of a control vactor. In contrast, knockdown of ERα in MCF-7(ER+)/PTX cells decreased the luciferase activity of DNMT1-WT compared with the control. These data suggested that ERα could upregulate the DNMT1 promoter activity.

To validate that ERα regulate the DNMT1 promoter activity via directly binding to the putative ERα binding sites (site 1, 2, or 3),

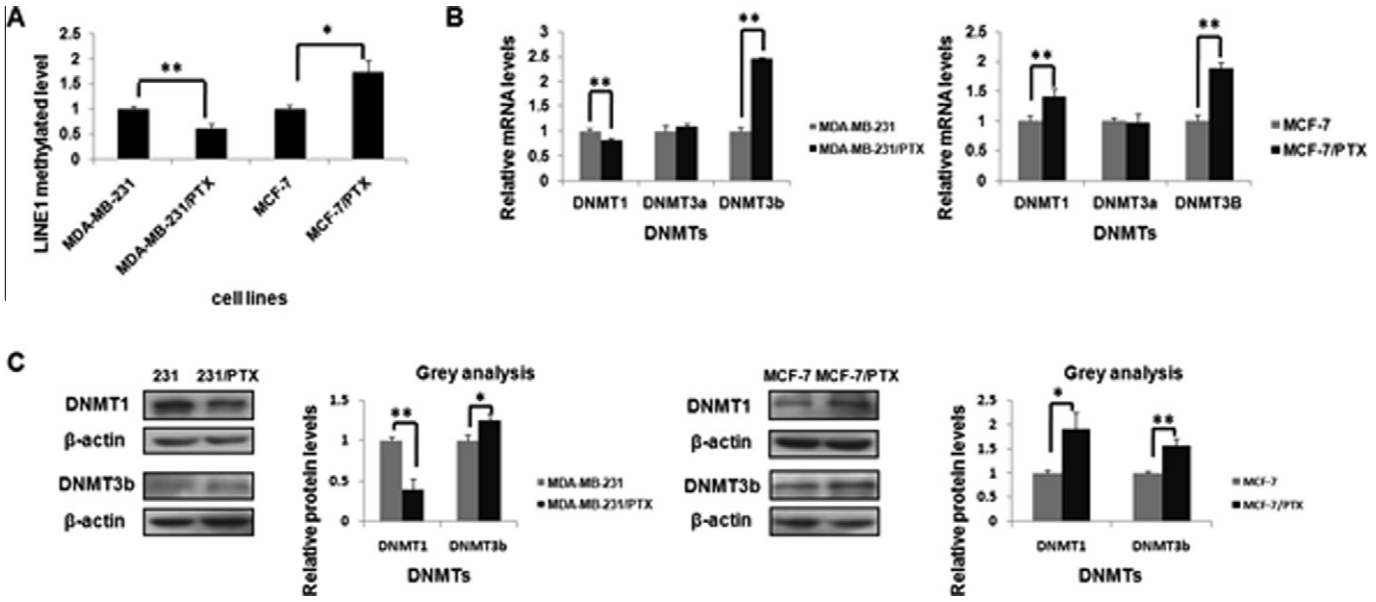

Fig. 1. The expression of DNMT1 mRNA and protein were significantly increased in MCF-7(ER+)/PTX cells and decreased in MDA-MB-231(ER−)/PTX cells, which was consistent with the alterations of genomic DNA methylation in the cell lines. (A) Quantitative methylation specific real-time PCR (qMSP) was performed to measure the genomic DNA methylation status in both paclitaxel-resistant cells and their corresponding parental control cells. (B) Real-time PCR was performed to check DNMTs transcriptional levels. (C) Western blot was performed to check DNMTs protein levels.

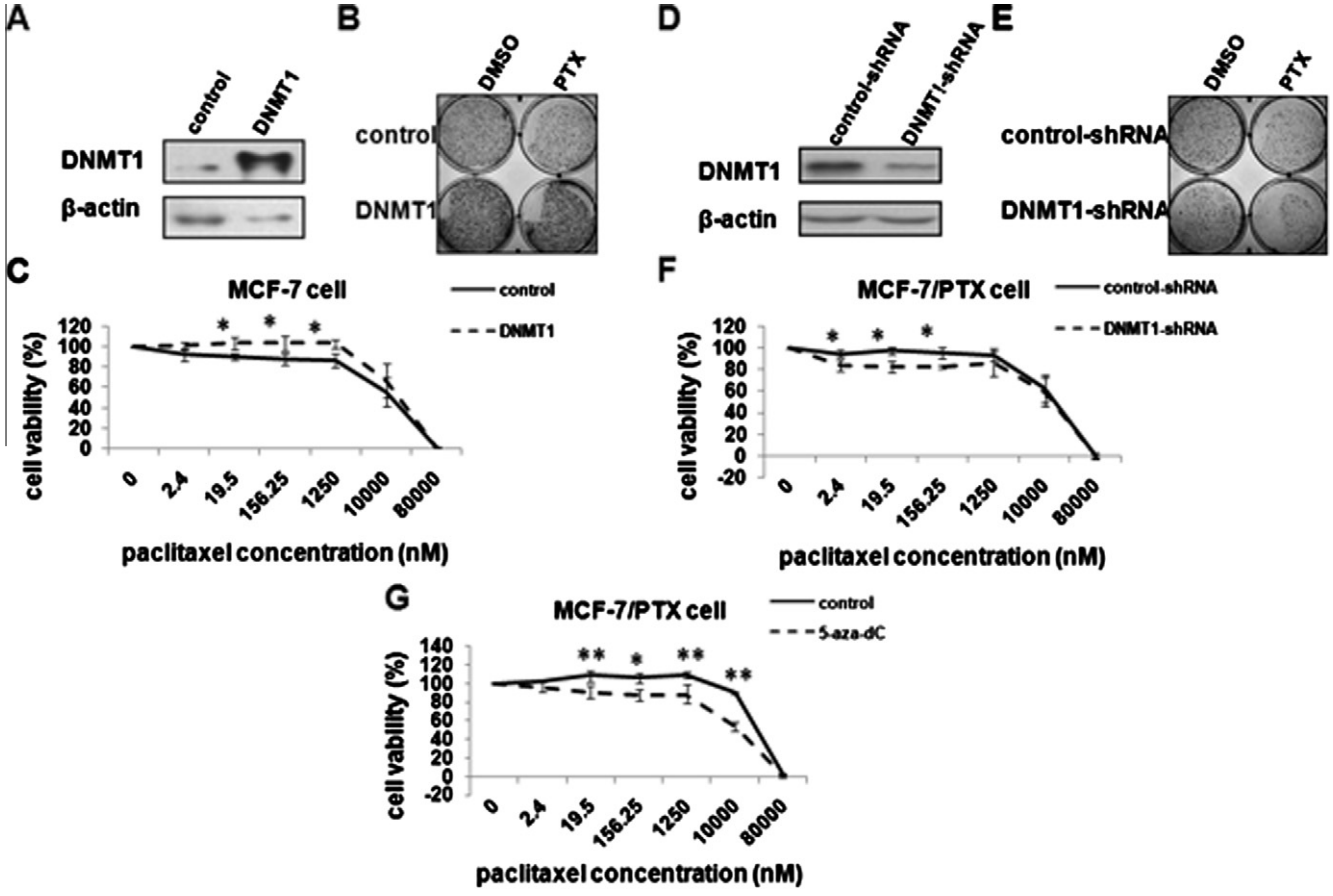

Fig. 2. The expression level of DNMT1 was responsible for paclitaxel resistance of ER-positive breast cancer. (A) Western blot was performed with lysate of MCF-7 cells transfected with cDNA of DNMT1 to confirm DNMT1 overexpression. (B, C) The number of viable of MCF-7 cells transfected with a negative control or DNMT1 was treated with the concentrations of paclitaxel using colony formation assay and MTT assay. (D) Western blot was performed to measure the amount of DNMT1 in MCF-7/PTX cells transfected with DNMT1-shRNA for 48 h. (E, F) Proliferation of MCF-7(ER+)/PTX cells transfected with a negative control or DNMT1-shRNA was determined by a cytotoxicity assay. (G) MCF-7(ER+)/PTX cells treated with DNMTs inhibitor 5-aza-dC was measured by MTT assay in present of paclitaxel.

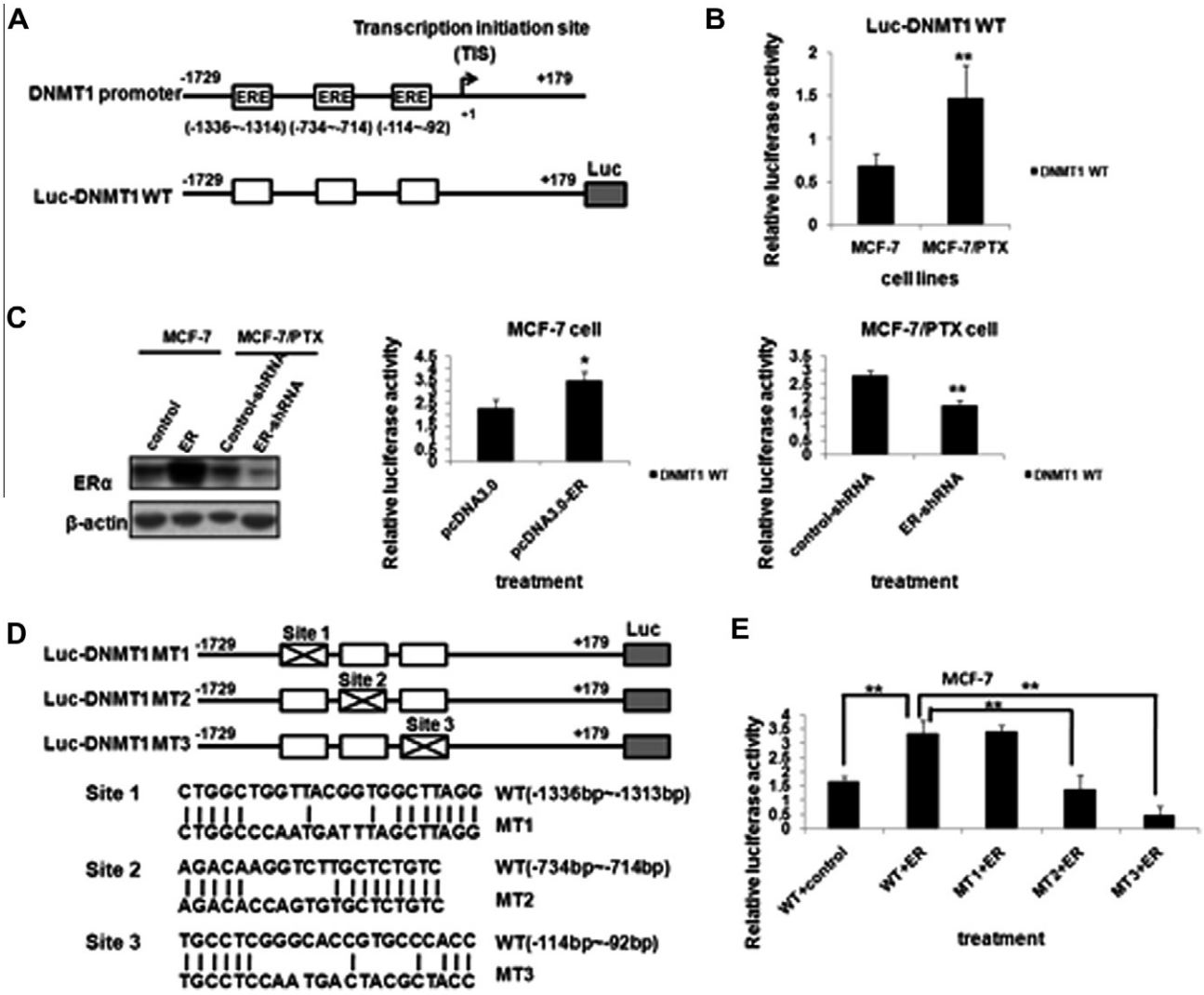

Fig. 3. ERα enhanced transcriptional activity of the DNMT1 gene by directly binding to the promoter region. (A) ERα binding sites in human DNMT1 gene promoter and the construct of luciferase report gene DNMT1-WT. (B) Luciferase reporter assay was done to measure the activity of DNMT1-WT. (C) Luciferase reporter assay was performed to detcet that ERα can regulate the activity of DNMT1 promoter region. (D) Mutations were introduced into the seed regions of all three ERα binding sites. (E) Luciferase reporter assay showed that ERα could enhance DNMT1 transcriptional activity by directly binding to the sites 2 and 3 of the DNMT1 promoter.

we generated DNMT1 promoter firefly luciferase reporter gene constructs in which one of the putative ERα binding sites was mutated (DNMT1-MT1, MT2, or MT3) (Fig. 3D). MCF-7 cells were then transfected with each reporter gene construct together with the ERα expression vector. We found that mutation in MT2 or MT3 significantly decreased luciferase activity (Fig. 3E), while mutation in DNMT1-MT1 had no significant effect. These data clear indicated that ERα could enhance DNMT1 transcriptional activity by directly binding to the sites 2 and 3 of the DNMT1 promoter.

4. Discussion

Accumulative evidence has associated disruption of DNA methylation with chemotherapy resistance of cancer cells. Recently influence of steroid hormones and their receptors on DNA methylation status have begun to draw attention. In this study, we explored whether ERα influenced genomic DNA methylation through regulation of the DNMTs expression in our established paclitaxel-resistant breast cancer cells. We confirmed that paclitaxel induced opposite alterations of global DNA methylation in ER-positive and ER-negative breast cancer cells. The alterations were correlated to DNMT1 expression levels. Furthermore, over expression of DNMT1 expression decreased the sensitivity of MCF-7(ER+) cells to paclitaxel, while knockdown of DNMT1 expression increased the sensitivity of the cells to paclitaxel. By bioinformatic analysis and reporter gene system with the selected mutations in putative ER binding sites within DNMT1 promoter, we confirmed that ERα could enhance the transcription activity of DNMT1 gene through directly binding to ERE sequence (sites 2 and 3) of DNMT1 promoter in ER-positive MCF-7 cell lines.

Aberrant DNA methylation was the first epigenetic mark to be associated with drug resistance, while DNMTs control the degree of global genomic methylation [30,31]. DNMT3 is the first DNMT that has been recognized to be associated with the aberrant DNA methylation related to cancer development and cell chemotherapy resistance [32,33]. For example, DNMT3b gene amplification has been shown to positively related to resistance to DNA demethylating drugs of embryonal carcinoma cells [34]. In neuroblastoma cells DNMT3b7, a truncated DNMT3b, modifies the epigenome to induce changes in gene expression, inhibit tumor growth, and increase sensitivity to ATRA [35]. Subset of primary breast cancers and breast cancer cell lines express a hypermethylation defect (characterized by DNMT hyperactivity and DNMT3b over

expression) which contributes to chemotherapy resistance and provides a target for development of new treatment strategies [36]. In this study we verified that DNMT1, instead of DNMT3b, was specifically associated with global genomic methylation in two paclitaxel resistant breast cancer cell lines, MCF-7(ER+)/PTX and MDA-MB-231(ER−)/PTX that display hyper- and hypomethylation, respectively, although paclitaxel-induced expression of DNMT3b was observed in these two PTX-resistant breast cancer cell lines. These observations imply that DNA methylation machinery is widely involved in drug resistance of cancer cells. The base for the regulation DNA methylation may vary, depending on cell type or subtype and stress that cells are facing. In the case of breast cancer cells, the ER expression level should be a critical factor for response of cellular DNA methylation machinery to environmental stress. This notion is supported not only by our observation that DNMT1 expression was specifically correlated with ERα expression of the two paclitaxel resistant cell lines, but also by our finding that ERα could directly bind to ER response elements in DNMT1 promoter and regulate DNMT1 transcription.

A large variety of proteins were reported to interact with DNMT1, ranging from DNA methyltransferases, DNA binding proteins, chromatin modifiers and chromatin binding proteins to tumor suppressors, cell cycle regulators and transcriptional regulators [37]. However, the functional role of ER in regulation of DNMT1 is not evaluated. Our data for the first time linked ER with the regulation of DNMT1 expression and provided the direct evidence that ERα could promote genomic DNA methylation through upregulation of DNMT1 in ER-positive breast cancer cells. Combining this finding with our observations that paclitaxel significantly induced ER expression in breast cancer cells and DNMT1 expression level was positively correlated to drug resistance of ER-positive breast cancer, we proposed that drug-induced ER expression might be one of the important mechanisms of chemotherapy resistance in breast cancer cells. This implies that the inhibitor of ERα may be helpful both in sensitizing ER-positive breast cancer cells to chemotherapy and in overcoming chemotherapy resistance of breast cancer. Therefore, our primary study revealed a novel possibility for improving chemotherapy of breast cancer and other steroid hormone dependent tumors. Extensive study in this area deserves carrying out.

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References

[1] B. Szekely, G. Szentmartoni, J. Kulka, A.M. Szasz, Z. Langmar, M. Dank, Primary systemic therapy in breast cancer—an update for gynecologic oncologists, Eur. J. Gynaecol. Oncol. 32 (2011) 636–641.
[2] J.H. Lee, A. Nan, Combination drug delivery approaches in metastatic breast cancer, J. Drug. Deliv. 2012 (2012) 915375.
[3] J. Liu, C. He, K. Zhou, J. Wang, J.X. Kang, Coptis extracts enhance the anticancer effect of estrogen receptor antagonists on human breast cancer cells, Biochem. Biophys. Res. Commun. 378 (2009) 174–178.
[4] I. Plaza-Menacho, A. Morandi, D. Robertson, S. Pancholi, S. Drury, M. Dowsett, L.A. Martin, C.M. Isacke, Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance, Oncogene 29 (2010) 4648–4657.
[5] C.H. Barrios, C. Sampaio, J. Vinholes, R. Caponero, What is the role of chemotherapy in estrogen receptor-positive, advanced breast cancer?, Ann Oncol. 20 (2009) 1157–1162.
[6] V.F. Semilgazov, A.G. Manikhas, V.V. Semiglazov, A.A. Bozhok, G.A. Dashian, V.G. Ivanov, R.M. Paltuev, A.G. Vasil'ev, D.E. Shchedrin, A.M. Ermachenkova, I.V. Nikitina, K.D. Pen'kov, A.A. Bessonov, T.T. Tabagua, V.V. Kolar'kova, Long-term results of adjuvant endocrine therapy for hormone receptor-positive breast cancer, Vopr. Onkol. 57 (2011) 567–577.
[7] M. Sui, H. Zhang, W. Fan, The role of estrogen and estrogen receptors in chemoresistance, Curr. Med. Chem. 18 (2011) 4674–4683.
[8] M. Sui, Y. Huang, B.H. Park, N.E. Davidson, W. Fan, Estrogen receptor alpha mediates breast cancer cell resistance to paclitaxel through inhibition of apoptotic cell death, Cancer Res. 67 (2007) 5337–5344.
[9] Y. Tabuchi, J. Matsuoka, M. Gunduz, T. Imada, R. Ono, M. Ito, T. Motoki, T. Yamatsuji, Y. Shirakawa, M. Takaoka, M. Haisa, N. Tanaka, J. Kurebayashi, V.C. Jordan, Y. Naomoto, Resistance to paclitaxel therapy is related with Bcl-2 expression through an estrogen receptor mediated pathway in breast cancer, Int. J. Oncol. 34 (2009) 313–319.
[10] I. Barone, L. Brusco, S.A. Fuqua, Estrogen receptor mutations and changes in downstream gene expression and signaling, Clin. Cancer Res. 16 (2010) 2702–2708.
[11] S. Massarweh, R. Schiff, Resistance to endocrine therapy in breast cancer: exploiting estrogen receptor/growth factor signaling crosstalk, Endocr. Relat. Cancer 13 (Suppl. 1) (2006) S15–24.
[12] G. Arpino, L. Wiechmann, C.K. Osborne, R. Schiff, Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance, Endocr. Rev. 29 (2008) 217–233.
[13] S.I. Hayashi, H. Eguchi, K. Tanimoto, T. Yoshida, Y. Omoto, A. Inoue, N. Yoshida, Y. Yamaguchi, The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application, Endocr. Relat. Cancer 10 (2003) 193–202.
[14] C.K. Osborne, R. Schiff, S.A. Fuqua, J. Shou, Estrogen receptor: Current understanding of its activation and modulation, Clin. Cancer Res. 7 (2001) 4338s–4342s;
discussion 4411s–4412s
[15] W. Ying, S. Wang, J. Shi, Y. Sun, ER−/ER+ breast cancer cell lines exhibited different resistance to paclitaxel through pulse selection, Med. Oncol. 29 (2012) 495–502.
[16] M. Boettcher, F. Kischkel, J.D. Hoheisel, High-definition DNA methylation profiles from breast and ovarian carcinoma cell lines with differing doxorubicin resistance, PLoS One 5 (2010) e11002.
[17] A. Stone, F. Valdes-Mora, J.M. Gee, L. Farrow, R.A. McClelland, H. Fiegl, C. Dutkowski, R.A. McCloy, R.L. Sutherland, E.A. Musgrove, R.I. Nicholson, Tamoxifen-induced epigenetic silencing of oestrogen-regulated genes in anti-hormone resistant breast cancer, PLoS One 7 (2012) e40466.
[18] R.H. Wilting, J.H. Dannenberg, Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance, Drug Resist. Updat. 15 (2012) 21–38.
[19] B. Segura-Pacheco, E. Perez-Cardenas, L. Taja-Chayeb, A. Chavez-Blanco, A. Revilla-Vazquez, L. Benitez-Bribiesca, A. Duenas-Gonzalez, Global DNA hypermethylation-associated cancer chemotherapy resistance and its reversion with the demethylating agent hydralazine, J. Transl. Med. 4 (2006) 32.
[20] M.G. Goll, T.H. Bestor, Eukaryotic cytosine methyltransferases, Annu. Rev. Biochem. 74 (2005) 481–514.
[21] F. Chedin, The DNMT3 family of mammalian de novo DNA methyltransferases, Prog. Mol. Biol. Transl. Sci. 101 (2011) 255–285.
[22] R.Z. Jurkowska, T.P. Jurkowski, A. Jeltsch, Structure and function of mammalian DNA methyltransferases, Chembiochem 12 (2011) 206–222.
[23] Z.M. Svedruzic, Dnmt1 structure and function, Prog. Mol. Biol. Transl. Sci. 101 (2011) 221–254.
[24] E.L. Walton, C. Francastel, G. Velasco, Maintenance of DNA methylation: Dnmt3b joins the dance, Epigenetics 6 (2012).
[25] M. Cui, Z. Wen, Z. Yang, J. Chen, F. Wang, Estrogen regulates DNA methyltransferase 3B expression in Ishikawa endometrial adenocarcinoma cells, Mol. Biol. Rep. 36 (2009) 2201–2207.
[26] Z. Zhao, L. Fan, K.M. Frick, Epigenetic alterations regulate estradiol-induced enhancement of memory consolidation, Proc. Natl. Acad. Sci. USA 107 (2010) 5605–5610.
[27] G.L. Gravina, F. Marampon, M. Piccolella, M. Motta, L. Ventura, R. Pomante, V.M. Popov, B.M. Zani, R.G. Pestell, V. Tombolini, E.A. Jannini, C. Festuccia, Hormonal therapy promotes hormone-resistant phenotype by increasing DNMT activity and expression in prostate cancer models, Endocrinology 152 (2011) 4550–4561.
[28] K. Kawakami, A. Matsunoki, M. Kaneko, K. Saito, G. Watanabe, T. Minamoto, Long interspersed nuclear element-1 hypomethylation is a potential biomarker for the prediction of response to oral fluoropyrimidines in microsatellite stable and CpG island methylator phenotype-negative colorectal cancer, Cancer Sci. 102 (2011) 166–174.
[29] J. Roman-Gomez, A. Jimenez-Velasco, X. Agirre, F. Cervantes, J. Sanchez, L. Garate, M. Barrios, J.A. Castillejo, G. Navarro, D. Colomer, F. Prosper, A. Heiniger, A. Torres, Promoter hypomethylation of the LINE-1 retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia, Oncogene 24 (2005) 7213–7223.
[30] C. Zeller, W. Dai, N.L. Steele, A. Siddiq, A.J. Walley, C.S. Wilhelm-Benartzi, S. Rizzo, A. van der Zee, J.A. Plumb, R. Brown, Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling, Oncogene (2012).
[31] X. Zhang, W. Li, H. Li, Y. Ma, G. He, G. Tan, Genomic methylation profiling combined with gene expression microarray reveals the aberrant methylation mechanism involved in nasopharyngeal carcinoma taxol resistance, Anticancer Drugs 23 (2012) 856–864.
[32] Y.Y. Qiu, B.L. Mirkin, R.S. Dwivedi, Differential expression of DNA-methyltransferases in drug resistant murine neuroblastoma cells, Cancer Detect. Prev. 26 (2002) 444–453.

[33] Y.Y. Qiu, B.L. Mirkin, R.S. Dwivedi, Inhibition of DNA methyltransferase reverses cisplatin induced drug resistance in murine neuroblastoma cells, Cancer Detect. Prev. 29 (2005) 456–463.
[34] M.J. Beyrouthy, K.M. Garner, M.P. Hever, S.J. Freemantle, A. Eastman, E. Dmitrovsky, M.J. Spinella, High DNA methyltransferase 3B expression mediates 5-aza-deoxycytidine hypersensitivity in testicular germ cell tumors, Cancer Res. 69 (2009) 9360–9366.
[35] K.R. Ostler, Q. Yang, T.J. Looney, L. Zhang, A. Vasanthakumar, Y. Tian, M. Kocherginsky, S.L. Raimondi, J.G. Demaio, H.R. Salwen, S. Gu, A. Chlenski, A. Naranjo, A. Gill, R. Peddinti, B.T. Lahn, S.L. Cohn, L.A. Godley, Truncated DNMT3B isoform DNMT3B7 suppresses growth, induces differentiation and alters DNA methylation in human neuroblastoma, Cancer Res. (2012).
[36] R. Sandhu, A.G. Rivenbark, W.B. Coleman, Enhancement of chemotherapeutic efficacy in hypermethylator breast cancer cells through targeted and pharmacologic inhibition of DNMT3b, Breast Cancer Res. Treat. 131 (2012) 385–399.
[37] W. Qin, H. Leonhardt, G. Pichler, Regulation of DNA methyltransferase 1 by interactions and modifications, Nucleus 2 (2011) 392–402.