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ERα directly activated the *MDR1* transcription to increase paclitaxel-resistance of ERα-positive breast cancer cells *in vitro* and *in vivo*

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ABSTRACT

Chemotherapy is commonly used to treat early-stage invasive and advanced-stage breast cancer either before or after surgery. Increasing evidence from clinical analysis and *in vitro* studies has shown that ER-positive breast cancer cells are insensitive to chemotherapy. Complete understanding of how ERα mediates drug resistance is prerequisite to improvement of the chemotherapeutic efficacy. Over-expression of P-glycoprotein (P-gp) encoded by *MDR1* gene is one of the major causes of drug resistance. The association between ERα and *MDR1* in breast cancer is still unclear and the limited reports are conflict. This study systematically explored intrinsic link between ERα and the P-gp over-expression in paclitaxel-resistant ERα(+) breast cancer cell lines and mouse model in molecular details. Our data showed that ERα activated the *MDR1* transcription in MCF-7/PTX breast cancer cells by binding to ERE1/2 and interacting with Sp1 that bridged to the downstream CG-rich element within the *MDR1* promoter. Knockdown of *MDR1* restrained the effect of ERα in MCF-7 cells and sensitized the cells to paclitaxel. Treatment of ICI 182,780 that selectively suppressed ERα significantly decreased the *MDR1* expression and increased the sensitivity of drug resistant breast cancer cells and xenograft tumors to paclitaxel. Our data strongly demonstrated that ERα was able to increase drug resistance of breast cancer cells through activating *MDR1* transcription. This novel mechanism provides new insight to how the ERα signaling regulates response of ERα(+) breast tumors to chemotherapy, which may be exploited for developing novel therapeutic strategies for breast cancer in the future.

1. Introduction

Breast cancer is a classical hormone-dependent tumor (Ding et al., 2013). Chemotherapy is usually used to treat early-stage invasive and advanced-stage breast cancer, either before surgery or after surgery (Regan and Gelber, 2005; Pruthi et al., 2007), as well as for the treatment of recurrent and metastatic breast tumors (Cardoso et al., 2012; Gampenrieder et al., 2013). However, drug resistance that either may be present *de novo* or may be raised after exposure to anticancer drugs majorly limits the success of cancer chemotherapy (Gottesman, 2002).

Estrogen receptor alpha (ERα) is expressed in approximately 65% of human breast cancer and around 40% of patients with ER-positive breast cancer inevitably relapse (Plaza-Menacho et al., 2010; Colleoni et al., 2009). Cumulative data from clinical trials and retrospective analyses have confirmed that ER-positive tumors are more resistant to chemotherapy agents including paclitaxel (PTX), an active agent used in breast cancer chemotherapy, than ER-negative tumors (Colleoni et al., 2009; Tokuda et al., 2012), although patients with ERα(+) breast cancer usually benefit from adjuvant endocrine therapy. In consistent with the clinic observations, the involvement of ERα in chemotherapy resistance has been confirmed in a number of studies in cell lines and animal model (Tokuda et al., 2012; Tabuchi et al., 2009). For example, ERα(−) breast cancer

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tissue was found to be chemosensitive *in vitro* compared with ERα(+) tissues against six anticancer drugs (Maehara et al., 1990). Expression of ERα interfered with PTX-induced apoptotic cell death of breast cancer MCF-7 cells (Sui et al., 2007) and attenuates therapeutic efficacy of paclitaxel on breast xenograft tumors (Chang et al., 2012). Evidently, ERα plays a significant role in determining the response of ERα(+) breast cancer to chemotherapeutic agents and thus influences the prognosis of patients with breast cancer. In contrast to studies of ERα functional role, investigations of the underlying mechanisms of ERα-mediated drug resistance and the potential strategies for improving chemotherapeutic efficacy are very limited, although several mechanisms that may contribute to ERα-mediated drug resistance have been proposed, including promotion of cell growth, suppression of cell apoptosis, and regulation of intracellular drug concentration (Sui et al., 2011; Ross-Innes et al., 2012).

Over-expression of ATP-binding cassette (ABC) transporter genes has been well known to cause drug resistance in both laboratory model cell lines and clinical settings (Gottesman, 2002; Zhang, 2007). The alteration of ABC transporters on cancer cell membrane endows cancer cells with drug-resistant phenotype by increasing efflux of chemotherapeutic drug (Borst and Elferink, 2002; Wesolowska, 2011). Recent studies have implied the role of ERα in regulation of ABC family genes in drug resistance of cancer cells (Zhang et al., 2006; Honorat et al., 2008). P-glycoprotein (P-gp) encoded by *MDR1* gene is one of the major ABC transporter. A variety of chemotherapeutic drugs including paclitaxel, vinca alkaloids, anthracycline, antifungals are known to be the substrates of P-gp (Wesolowska, 2011; Colabufo et al., 2010). High expression of P-gp results in increased drug exclusion from cancer cells and greatly contributes to both induced and primary chemotherapy resistance of breast cancer cells (Xia et al., 2009; Jin et al., 2010). However, few of the studies on *MDR1* regulation by ERα or estrogen have been reported and the observations are discrepant (Mutoh et al., 2006; Zampieri et al., 2002). For example, estrogen was reported to down-regulate P-gp expression without effect on the mRNA level in the *MDR1*-transduced ERα(+) breast cancer cells (Mutoh et al., 2006), while E2 expression increased the cytoplasmic concentration of P-gp in ERα(+) breast cancer cells that were resistant to doxorubicin treatment (Zampieri et al., 2002). Given the deep involvement of ERα and P-gp in chemoresistance, it is of great significance to elucidation of the intrinsic link between ERα and *MDR1* for development of more effective therapeutic strategies for breast cancer.

ERα functions as a transcriptional factor that accesses various target genes either by directly binding to specific estrogen response element (ERE) within the promoter, or by interacting with other transcriptional factors binding to the promoter (Barone et al., 2010; Mason et al., 2010; Ross-Innes et al., 2012; McDonnell and Norris, 2002). Additionally, ERα regulates target genes *via* interaction with other transfactors, such as Sp1, c-Fos/c-JunB and NF-kappa B, through ERE-independent mechanism (Wang et al., 2011; Pradhan et al., 2010; Marconett et al., 2011). The *MDR1* promoter contains both inverted CCAAT BOX (−79 to −75 bp) and a GC-rich element (−56 to −43 bp) that interacts with members of Sp family, especially Sp1 (Labialle et al., 2002; Huo et al., 2010; Sundseth et al., 1997). Upstream within the *MDR1* promoter (−110 to −103 bp) lies another GC-rich element that incapable of interacting with Sp1 (Cornwell and Smith, 1993; Labialle et al., 2002). A half-ERE sequence located between the two GC-rich elements, *i.e.* the *MDR1* promoter contains (half-ERE)-(N)x-(GC rich) motif as revealed by our bioinformatic analysis.

Based on the previous studies we proposed that ERα could increase the expression of the *MDR1* gene through activation of the gene transcription and thereby contribute to drug resistance in ERα(+) breast cancer. To test the hypothesis questions whether and how ERα regulated transcriptional activity of the *MDR1* gene and whether *MDR1* over-expression was directly involved in ERα-mediated drug resistance of breast cancer were systematically investigated based on MCF-7 drug resistance cell lines and mouse model in the present study.

2. Materials and methods

2.1. Cell culture and reagents

Human breast cancer MCF-7 and ZR-75-1 cells were obtained from ATCC. MCF-7 cells were cultured in MEM medium supplemented with 10% fetal bovine serum (FBS). ZR-75-1 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). Paclitaxel-resistant cancer cells, MCF-7/PTX and ZR-75-1/PTX, were established from parental MCF-7 and ZR-75-1 cells by stepwise of paclitaxel treatment. ICI 182,780 was purchased from Tocris (Ballwin, MO, USA). Cell culture medium and supplements were obtained from GIBCO (Invitrogen).

2.2. Cell proliferation assay

Cells were seeded at a density of 8×10^3 per well in 96-well plates containing growth media with 10% FBS. On the following day, the media was changed and cells were incubated in medium in presence or absence of paclitaxel for 72 h. Then the cells were harvested for evaluation of cell proliferation by MTT assay. Cells were incubated with MTT reagent at the final concentration of 0.5 mg/ml for 4 h. After the medium was aspirated, the cells were dissolved in DMSO and Optical density was read in a microplate reader at 570 nm.

2.3. Rhodamine-123 retention assay

At 80–90% confluence, the cells were incubated in serum-free MEM medium for 18 h. The culture medium was changed with 10 μM Rhodamine-123 (R-123) for 90 min, then the cells were washed three times with an ice-cold phosphate buffer (pH 7.0) and lysed in EBC lysis buffer. The R-123 fluorescence in the cell lysates was measured using excitation and emission wave lengths of 480 nm and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to controls.

2.4. RNA extraction and quantitative reverse transcriptase-PCR

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA integrity was assessed by visualizing the ribosomal bands on a 1% agarose gel. Finally, cDNA was synthesized from total RNA (1 μg) using High-Capacity cDNA Reverse Transcription Kits according to the manufacturer's instructions (Applied Biosystems, Life Technologies). The mRNA level of ERα or *MDR1* was normalized to β-actin endogenous control. The primers for ERα mRNA were 5′-AGACACTTTGATCCACCTGA-3′ (forward) and 5′-CAAGGAATGCGATGAAGTAG-3′ (reverse), the primers for *MDR1* mRNA were 5′-ATTTGACACCCTGGTTGGAG-3′ (forward) and 5′-ACCACTGCTTCGCTTTCTGT-3′(reverse), and the primers for β-actin mRNA were 5′-TCATGAAGTGTGACGTGGACAT-3′ (forward) and 5′-CTCAGGAGGAGCAATGATCTTG-3′ (reverse).

2.5. Western blot analysis

Western blot analysis of whole cell extracts was performed as previously described (Ying et al., 2012), using primary anti-ERα and anti-Sp1 antibodies (1:3000 dilution; Millipore, USA),