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Article

Egr-1 Enhances Drug Resistance of Breast Cancer Cells by MDR1 Dependence

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Paclitaxel (PTX) is one of the most effective drugs used in the treatment of breast cancer. Nonetheless, the appearance of MDR1 (multidrug resistance 1) in tumor cells has become a significant hindrance for efficacious chemotherapy. In this study, we show that the expression level of Egr-1 (early growth response gene-1) in cancer tissues (from paclitaxel chemotherapy failure patients) and MCF-7/PTX cells (the breast cancer cell line that was resistant to paclitaxel) was increased. Cell proliferation assay and apoptosis assay revealed that Egr-1 could promote cell growth and inhibit apoptosis in MCF-7/PTX. Mechanistic studies indicated that Egr-1 could bind to the proximal MDR1 promoter and enhance MDR1 transcription. These findings indicate that paclitaxel induced Egr-1 accumulation and upregulated the expression of MDR1, thereby inducing the drug resistance in MCF-7/PTX. Our results suggest a novel pathway by which paclitaxel induces MDR1 expression, possibly illuminating a potential target pathway for the prevention of MDR1-mediated drug resistance.

Key words: Egr-1. Breast cancer. MDR1. Drug resistance.

INTRODUCTION

Breast cancer (BC) is one of the most common causes of death of women worldwide, and the second leading cause of cancer-related mortality (Jemal *et al.*, 2009). A range of risk factors, such as genetic factors, hormones, immunity, and other lifestyles factors are related with development of BC (Dumitrescu, Cotarla, 2005). Notably, deaths due to BC have decreased because of routine treatment, including surgery, hormone therapy, radiotherapy, and chemotherapy (Berry *et al.*, 2005). Nonetheless, the clinical therapy efficacy in individuals is still unsatisfactory (Guarneri, Conte, 2004). The molecular-targeted therapy has altered the therapeutic approach for multiple tumors. With the development of the understanding of the related gene's aberrant expression in BC, it is meaningful to identify the specific oncogene and the underlying mechanism for a new biomarker and therapeutic target (Bos, 2005).

Egr-1, as an important nuclear transcription factor that is induced by mitogen and differentiation factors, was first discovered in 1987 (Milbrandt, 1987). Egr-1 is also known as NGFI-A (Jimenez-Cervantes *et al.*, 1998), zif268 (Christy, Lau, Nathans, 1988), krox24 (Lemaire *et al.*, 1988), and Tis8 (Varnum *et al.*, 1989). Egr-1 promotes cell proliferation, differentiation, and transformation (Sukhatme *et al.*, 1988), and can be transiently activated by many cytokines, growth factors, hormones, and DNA-damaging agents (Tao *et al.*, 2013). Egr-1 is expressed in many normal tissues; its absence is often found in BC. In a previous study, the expression of Egr-1 mRNA was decreased in several human BC cell lines and tumor tissues, which was in accordance with Egr-1 functioning as a tumor suppressor in breast carcinoma (Huang *et al.*, 1997).

Paclitaxel (PTX), a new anticancer agent with a novel mechanism of action, has been used in treatment of breast cancer (Mekhail Markman, 2002). A major problem in the clinical treatment of breast cancer with PTX is MDR1 (multidrug resistance 1) in tumor cells. It was found that acquisition of MDR1 phenotype in tumor cells is one of the main challenges during the treatment process, which often results in chemotherapeutic failure (Bast, Hennessy,

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Mills, 2009; Gottesman, 2002). MDR1 results from the overexpression of P-glycoprotein (P-gp), an ATP-binding cassette transporter localized to the cell membrane and the best characterized drug efflux pump (Roninson *et al.*, 1986; Wang *et al.*, 2018a). The overexpression of MDR1 could contribute to anticancer drug resistance by extruding drugs out of tumor cells.

The purpose of this study is to explore the molecular mechanism underlying the effect of Egr-1 on PTX chemotherapy failure in a MDR1-dependent manner and provide a novel target for clinical treatment in drug resistance breast cancer.

MATERIAL AND METHODS

Cell culture and reagents

MCF-7/PTX cells were obtained from Beijing Tumor Cell Bank (Beijing, China). MCF-7/PTX cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 5 nmol/L paclitaxel, 100 μg/mL penicillin, and 100 μg/mL streptomycin. Antibodies to the following epitopes and proteins were purchased from the indicated vendors: Egr-1 (Bioworld), Cleaved Caspase 3 (CST), MDR1 (Santa Cruz Biotechnology), and actin (ZSGB-BIO). CCK8 was obtained from Beyotime. The Annexin V-FITC Kit (FITC Annexin V Apoptosis Detection Kit I) was obtained from BD (USA). Paclitaxel (PTX) was purchased from Guilin Huiang Biochemistry Pharmaceutical Co. (China).

Transfection

Transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The siRNAs (Egr-1, negative control) were obtained from GenePhara. After 48 h of transfection, the efficiency of overexpression was analyzed by Western blot.

Immunoblotting

Total cell lysates were solubilized in lysis buffer. Proteins were resolved by SDS-PAGE gels and then proteins were transferred (Bio-Rad) to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% nonfat milk for 2 h at room temperature prior to incubation with indicated primary antibodies. Subsequently membranes were washed and incubated for 1 h at room temperature with peroxidase-conjugated secondary antibodies (LI-COR Biosciences). Following several washes, chemiluminescent images of immunodetected bands on the membranes were recorded on X-ray films using the enhanced chemiluminescence (ECL) system (Tanon) according to the manufacturer's instructions.

Cell proliferation assay

Cell proliferation was detected with a Cell Counting Kit-8 (CCK-8 kit, Beyotime). 24 h after transfection, cells were plated in a 96-well microplate (Corning Incorporated, New York, USA) in triplicate and incubated at 37ºC with 5% CO2. 10 μL CCK-8 solution with 100 μL serum-free medium (Hyclone) was added to each well at 24 h, 48 h, 72 h and 96 h, respectively, followed by incubation for 2 h. The absorbance at 450 nm (OD value) was recorded to gauge the cell viability by a multi-function enzyme-linked analyzer (Biotek Instruments, Winooski, VT, USA). 1 x 103 cells were plated into 6-cm plates. Two weeks later, cells were fixed with methanol and stained with 0.1 % crystal violet. The number of colonies, defined as ≥ cells/colony, was counted. The experiments were performed in triplicate.

Immunohistochemistry

Immunohistochemistry was performed according to the streptavidin–peroxidase (Sp) method using a standard SpKit (Zhongshan biotech, Beijing, China). The tissue slice was incubated with monoclonal mouse anti-Egr-1 antibody (1:100) (Bioworld) overnight at 4°C, and diaminobenzidine (DAB; Zhongshan Biotech, Beijing, China) was used to produce a brown precipitate. The immunoreactivity was assessed blindly by two independent observers using light microscopy (Olympus BX-51) and the image was collected by Camedia Master C-3040 digital camera.

Rhodamine-123 retention assay

P-gp activity was evaluated using a rhodamine-123 retention assay in MCF-7/PTX cells. The cells were first incubated in FBS-free DMEM for 18 h and then in Hanks' balanced salt solution for 30 min at 37ºC. The cells were then incubated with 20 mM rhodamine-123 for 90 min. Following this incubation, the medium was completely removed, and the cells were washed three times with ice-cold phosphate buffer (pH 7.0). After lysis, the rhodamine-123 content in the cell lysates was measured using excitation and emission wavelengths of 485 and 528 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to control values.

Chromatin immunoprecipitation (ChIP)

MCF-7/PTX cells was cross-linked by incubation in 1% (v/v) formaldehyde-containing medium for 10 min at 37ºC, then sonicated to form soluble chromatin. Egr-1 antibody was used to precipitate DNA fragments bound by the corresponding elements. The protein--DNA complex was collected using protein A Sepharose beads (Millipore, Darmstadt, Germany) followed by the processes of elution and reverse crosslink. After protease K treatment (Millipore), samples were extracted with phenol/chloroform and precipitated with ethanol. Recovered DNA was re-suspended in TE buffer (Millipore) and amplified by PCR. Primers designed specifically to detect if Egr-1 could bind with the gene promoter of MDR1 (Ibsbio).

Human tumor tissue samples

A total of 30 specimens of breast biopsy were obtained from Xuzhou Health Hospital of Women and Children. Four cases of cancerous tissues and four cases of paracancerous tissues collected from Xuzhou Health Hospital of Women and Children. Four cases of BC were confirmed by postoperative pathology. All patients failed with paclitaxel treatment, without serious complications of heart and lung. The material was recruited stored at -80 ºC.

Statistical analysis

The data were analyzed by SPSS 18.0 statistical software. The measured data were expressed as mean ± standard deviation (± s). The measurement data were tested by Student's t-test. Assume that the test level was determined by $\alpha = 0.05$, $P < 0.05$ was considered statistically significant. The graph was drawn using Graphpad 5.0 software.

RESULTS

Egr-1 overexpression in drug-resistant BC

Four cases of PTX chemotherapy failure breast cancer patients' tissues were collected from Xuzhou Health Hospital of Women and Children to explore the expression of Egr-1. The expression level of Egr-1 was significantly upregulated in cancerous tissues compared with paracancerous tissues (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Figure 1A). Further, immunohistochemistry (IHC) of 30 cases of PTX chemotherapy failure breast cancer tissues showed that Egr-1 was mostly located in the nucleus of BC tissues and were more positive in cancerous tissues than paracancerous ones (Figure 1B). Subsequently, we investigated the expression of Egr-1 on paclitaxel (PTX)-resistant human breast carcinoma (MCF-7/PTX, paclitaxel at 5 nmol/L concentration) cells (Figure 1C). These results suggested that the level of Egr-1 in drug resistance BC was increased.

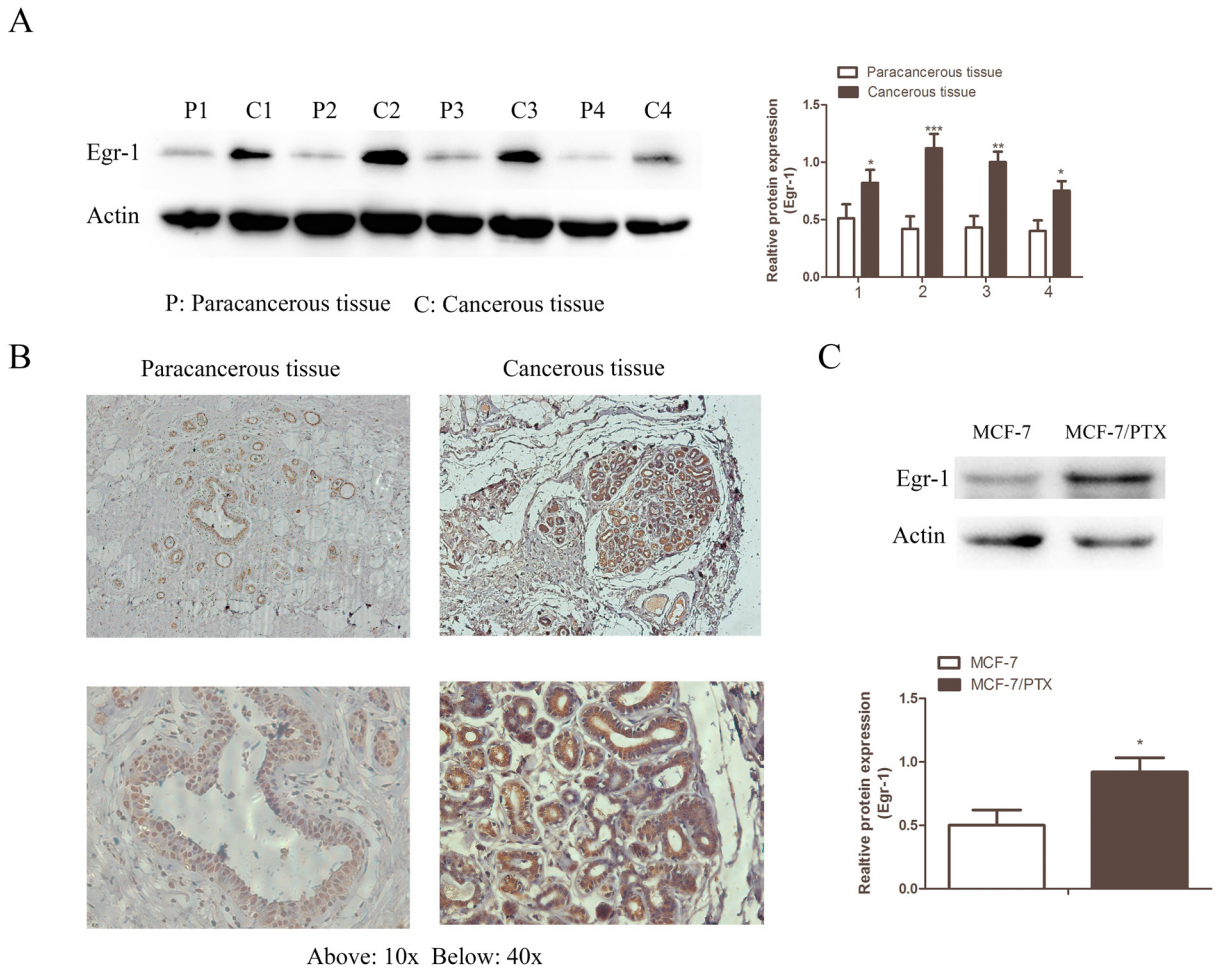


FIGURE 1 - Egr-1 was overexpressed in drug resistance BC. A. The expression levels of Egr-1 in cancerous tissues and paracancerous tissues of PTX chemotherapy failure BC by Western blot. Comparison of Egr-1 in PTX chemotherapy failure BC cancerous tissues and paracancerous tissues, *P < 0.05, **P < 0.01, ***P < 0.001. B. The expression of Egr-1 in PTX chemotherapy failure BC cancerous tissues and paracancerous tissues of BC by immunohistochemistry. (Upper figure 10x, lower figure 40x, n = 30). C. The expression of Egr-1 in MCF-7 and in MCF-7/PTX (paclitaxel at 5 nmol/L concentration) by Western blot, *P < 0.05, n = 3.

Down-regulating Egr-1 inhibited cell proliferation of MCF-7/PTX

To investigate the effect of Egr-1 on cell proliferation in MCF-7/PTX, we performed the loss-of-function experiments. A cell counting Kit-8 (CCK8) assay showed that, with paclitaxel at 5 nmol/L concentration, MCF-7/PTX cell proliferation was suppressed after transfecting siEgr-1 compared with siNC group (Figure 2A). Furthermore, clone formation assay also showed that knocking down Egr-1 inhibited MCF-7/PTX (paclitaxel at 5 nmol/L concentration) cell proliferation (Figure 2B). Taken together, these results showed that downregulating Egr-1 inhibited the cell proliferation of MCF-7/PTX.

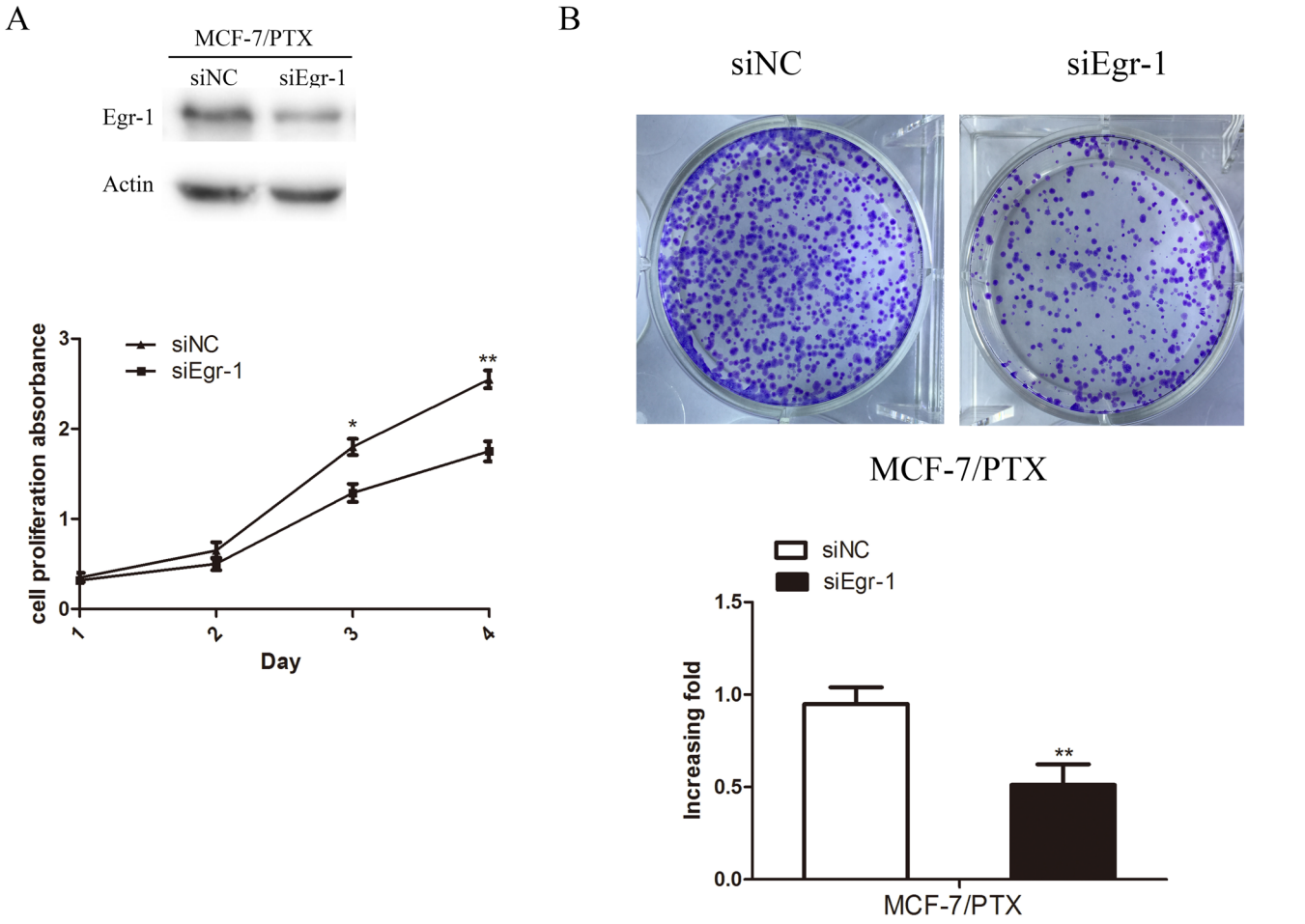


FIGURE 2 - Downregulating Egr-1 inhibited the cell proliferation of MCF-7/PTX. A. Western blot of the expression of Egr-1 protein in BC cell lines MCF-7/PTX (paclitaxel at 5 nmol/L concentration) transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC). CCK8 analysis was performed to examine the cell proliferation of MCF-7/PTX cells transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC). The cell proliferation absorbance was detected in 24 h, 48 h, 72 h and 96 h, *P < 0.05, **P < 0.01, n = 3. B. Knocking- down Egr-1 suppressed clone formation in MCF-7/PTX (paclitaxel at 5 nmol/L concentration), **P < 0.01, n = 3.

Down-regulating Egr-1 promoted apoptosis of MCF-7/PTX

An apoptosis assay was used to explore the effect of Egr-1 on MCF-7/PTX, paclitaxel at 5 nmol/L concentration. An annexin V-FITC binding assay revealed that the apoptotic rate in siNC groups was higher compared with siEgr-1 groups in MCF-7/PTX (Figure 3A). Similar effects were observed in Western blots (Figure 3B). These data indicated that downregulating Egr-1 could promote the apoptosis of MCF-7/PTX.

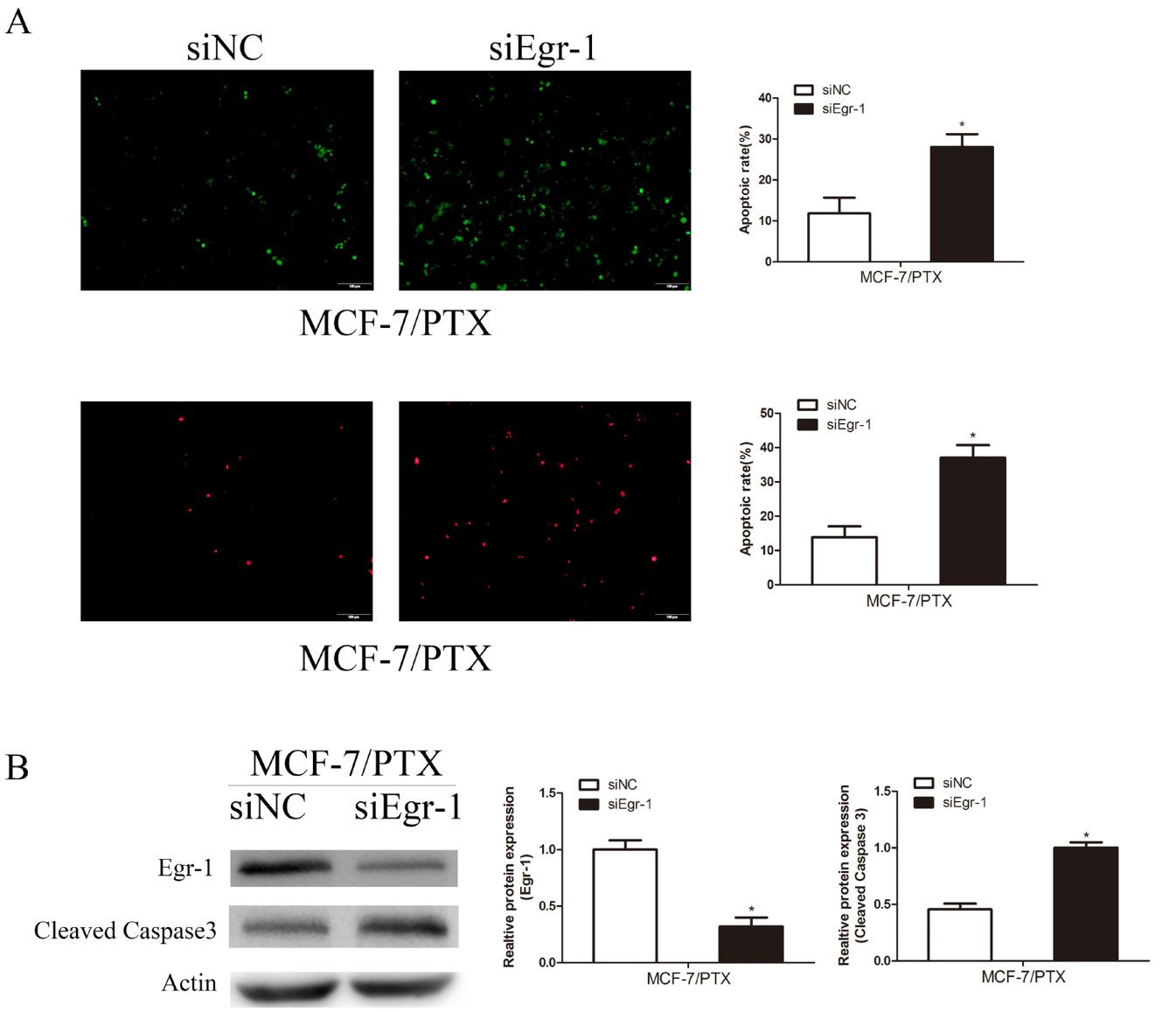


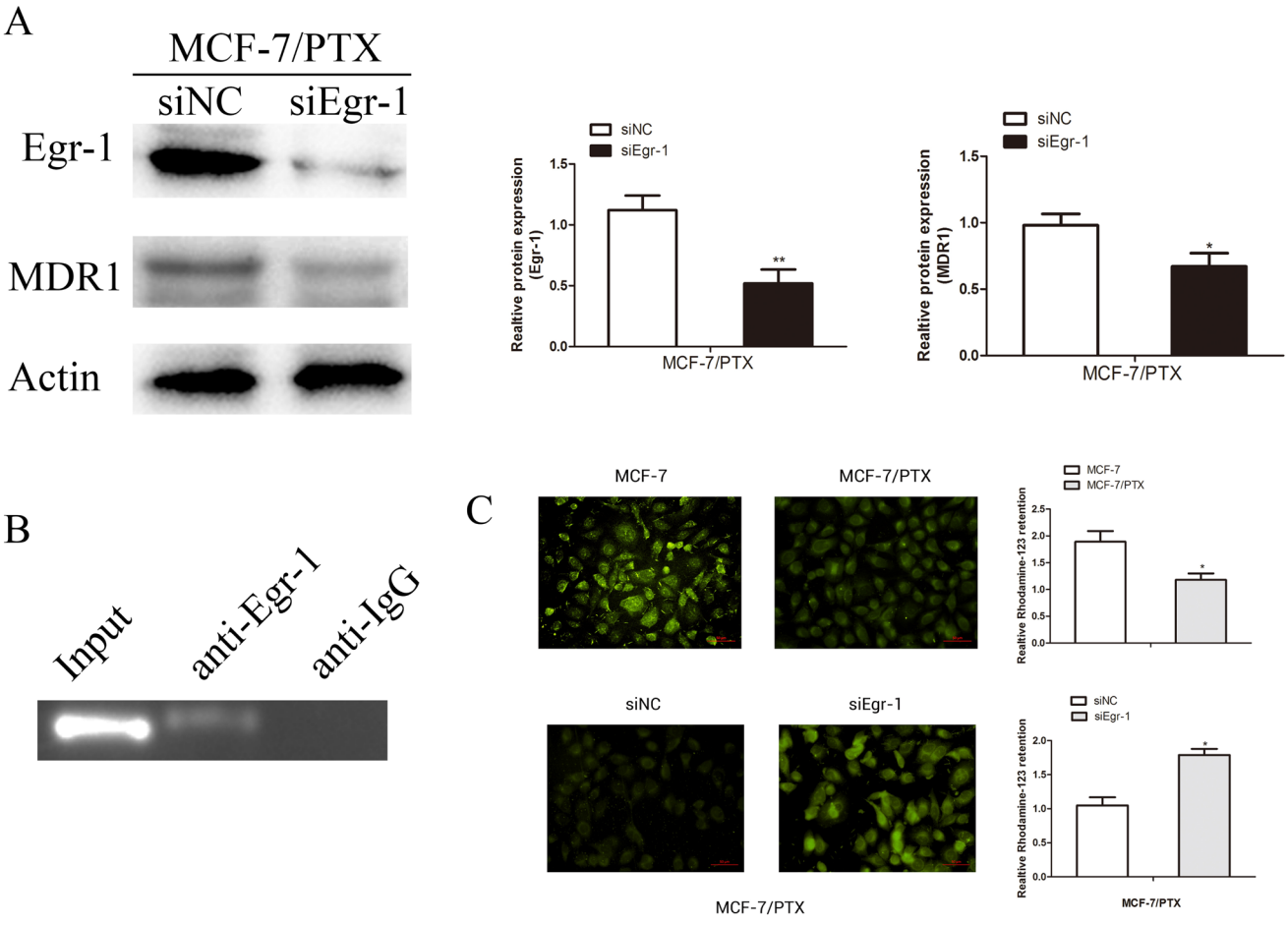
FIGURE 3 - Downregulating Egr-1 promoted the apoptosis of MCF-7/PTX. A. Annexin V-FITC binding assay was used to observe apoptotic cells by fluorescence microscope in MCF-7/PTX cells (paclitaxel at 5 nmol/L concentration) transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC). Prophase apoptotic cells were recognized by binding with FITC on the membrane (cell membrane displays green). Anaphase apoptotic cells were recognized by binding with FITC and PI on the nuclei (nuclei displays red). Data shown were from a typical experiment performed in triplicate. B. Western blot analyzed the expression of Egr-1 and cleaved caspase 3 protein in BC cell lines MCF-7/PTX (paclitaxel at 5 nmol/L concentration) transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC). *P < 0.05, n = 3.

Egr-1 was responsible for MDR1 activation

A previous report that Egr-1 was downregulated in BC was contrary to our findings (Ronski *et al.*, 2005). In the present study, we used BC cells that were resistant to PTX. MDR1, also known as ATP-binding cassette transporter B1 (ABCB1) or P-glycoprotein (P-gp), contributed to the formation of drug resistance (Wang *et al.*, 2018b). In this regard, we explored the interaction of Egr-1 and MDR1. Western blots were used to detect the expression of MDR1 after Egr-1 knocking down in MCF-7/PTX, paclitaxel at 5 nmol/L concentration (Figure 4A). The expression level

of MDR1 was significantly decreased in siEgr-1 group compared with siNC group. To certify the underlying mechanisms, we performed ChIP experiment. The results showed that Egr-1 could interact with MDR1 and result in Egr-1-mediated MDR1 increase (Figure 4B).

We next examined MDR1 transport activity with a rhodamine-123 retention assay. The rate of intracellular accumulation of rhodamine-123, a substrate of MDR1, was lower in MCF-7/PTX cells than MCF-7 cells. The rate of rhodamine-123 in MCF-7/PTX was higher in siEgr-1 group than siNC group (Figure 4C). These results suggest that paclitaxel, at 5 nmol/L concentration, increased activity of MDR1 in the MCF-7/PTX cells and contributed to their chemoresistance. Collectively, these findings indicated that Egr-1 might bind to MDR1 and then activate MDR1 expressing.



[FIGURE 4 - Egr-1 was responsible for the activation of MDR1. A. Effects of knocking down Egr-1 on protein expression of MDR1 in MCF-7/PTX cells (paclitaxel at 5 nmol/L concentration) by Western blot, *P < 0.05, **P < 0.01, n = 3. B. MCF-7/PTX cells (paclitaxel at 5 nmol/L concentration) were transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC). Lysates were immunoprecipitated with anti-Egr-1 antibody and immunoblotted with anti-MDR1 and anti-Egr-1 antibody. Chromatin immunoprecipitation (ChIP) analysis in MCF-7/PTX cells. The results verified that Egr-1 could bind with the gene promoter region of MDR1. The input DNA sample was 10% of the sample used for ChIP and anti-IgG was used for negative control. C. MCF-7/PTX cells (paclitaxel at 5 nmol/L concentration) incubated with 20 mM rhodamine-123 for 90 min. MCF-7/PTX cells were transfected with siRNA of Egr-1 (siEgr-1) and siRNA of negative control (siNC) for 48 h. Rhodamine-123 florescence in cell lysates was measured at excitation and emission wavelengths of 485 and 528 nm, respectively. The fluorescence values were normalized to the total protein content of each sample. *P < 0.05, n = 3.

DISCUSSION

Breast cancer (BC) remains the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide (Chen *et al.*, 2018). Chemotherapy plays an important role in the comprehensive treatments of BC. The role of paclitaxel in the chemotherapy of BC was officially confirmed in the 1980s (Seidman *et al.*, 1995). The combination of surgery and adjuvant chemotherapy has become the main choice of treatment for the BC patients and has already proven clinical efficacy (Morris, Fornier, 2009). Additionally, chemotherapy has become the mainstay of systemic treatment for advanced patients, although they typically respond poorly and rapidly progress, especially triple-negative breast cancer (TNBC) patients (Crown, O'Shaughnessy, Gullo, 2012). Finding targeted therapies in the area of BC chemotherapy failure is of great importance.

A previous study suggested that the cause of paclitaxel chemotherapy failure was the production of multidrug resistance (Gottesman, Fojo, Bates, 2002). Multidrug resistance was one of the major obstacles in BC chemotherapy and was influenced by several important factors including drug efficacy, cellular response to the treatments, and tumor microenvironment (Han *et al.*, 2007). The MDR1 gene encoded the cellular efflux protein ABCB1 (P-glycoprotein, P-gp), one of the ATP-binding cassette superfamily, which led to resistance to various chemotherapeutic drugs (Kovalev, Tsvetaeva, Grudinskaja, 2013; Vaidyanathan *et al.*, 2016; Chen *et al.*, 2017). As an efflux pump, MDR1 exported chemotherapy drugs from intracellular to extracellular compartment of the cell plasma membrane. Consequently, the intracellular drug concentration was not enough to kill tumor cells (Wang *et al.*, 2018b). As the essential role of MDR1 in inducing the multidrug resistance during chemotherapy, it is pivotal to overcome the MDR1-mediated efflux activities and improve the anti-cancer drug delivery.

In this study, the relationship of Egr-1 and MDR1 was investigated. We found that the expression level of Egr-1 in PTX chemotherapy failure breast cancer was increased and the similar change was observed in Western blots. Our results indicated that Egr-1 could bind to the promoter of MDR1 and activate MDR1 expression. Hence, Egr-1 could promote MCF-7/PTX cell proliferation and inhibit apoptosis, resulting in PTX drug resistance.

Previous studies demonstrated that the Egr-1 considered as a tumor suppressor and was downregulated in BC (Huang *et al.*, 1997). Some authors were of the view that Egr-1 was downregulated in BC cells and inhibited cell proliferation via GPER/EGFR/ERK signaling and then participated in the transcription of genes regulating in cell proliferation (Pupo *et al.*, 2012; Vivacqua *et al.*, 2012). Recent promising research pointed out that Egr-1 functions as a repressor of matrix metalloproteinase-2 (MMP-2) through downregulating MMP2 promoter activity, then markedly inhibit BC cells' invasive and proliferative capacities (Zcharia *et al.*, 2012). In the MDA-MB-231 cell line, the expression level of Bim (BH3-only protein) was increased through the upregulation of Egr-1 which induced apoptosis (Yamaguchi *et al.*, 2010). The view of these findings was that Egr-1 was associated with anti-cancer activities through multiple molecular mechanisms that were inconsistent with our study. Our results showed that Egr-1 could enhance the PTX resistance in MCF7/PTX by binding to the promoter of MDR1. Hence, the role of Egr-1 in PTX resistance breast cancer might be an oncogene via promoting cell proliferation and inhibiting apoptosis. Conversely, in breast cancer, Egr-1 was reported as a tumor inhibitor by regulating downstream genes. The differingt roles of Egr-1 in breast cancer and drug resistant-breast cancer need further elucidation. The expression of MDR1 might cause this inconsistency in the occurrence and development of tumors. Therefore, the relationship between Egr-1 and the expressions of MDR1 need more exploration.

In summary, our study confirmed that Egr-1 was increased in PTX chemotherapy failure BC tissues and PTX drug resistance cells. We also found that Egr-1 could affect the cell proliferation and apoptosis of MCF-7/PTX. In addition, Egr-1 could bind to the promoter of MDR1 and further activate the transcription activity of MDR1. Taken together, Egr-1 could provide a theoretical basis for the development of tumor therapy target drug by regulating the drug resistance in BC.

CONCLUSIONS

We have demonstrated that the expression of Egr-1 was upregulated and could be combined with MDR1 to lead to the chemotherapeutic drug resistance of breast cancer cells. This study provides a new method for improving the clinical efficacy of chemotherapy and benefit the masses of tumor patients.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Bast RC Jr., Hennessy B, Mills GB. The biology of ovarian cancer: New opportunities for translation. Nat Rev Cancer. 2009;9(6):415-28.

Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. New Engl J Med. 2005;353(17):1784-92.

Bos JL. Linking Rap to cell adhesion. Curr Opin Cell Biol. 2005;17(2):123-8.

Chen J, Ren Q, Cai Y, Lin T, Zuo W, Wang J, et al. Mesenchymal stem cells drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells via paracrine of neuregulin 1. Biochem Biophys Res Commun. 2018;501(1):212-9.

Chen JJ, Liu SP, Zhao J, Wang SC, Liu TJ, Li X. Effects of a novel photoactivated photosensitizer on MDR1 over-expressing human breast cancer cells. J Photochem Photobiol B Biol. 2017;171:67-74.

Christy BA, Lau LF, Nathans D. A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences. Proc Natl Acad Sci USA. 1988;85(21):7857-61.

Crown J, O'Shaughnessy J, Gullo G. Emerging targeted therapies in triple-negative breast cancer. Ann Oncol. 2012;23(Suppl 6):vi56-65.

Dumitrescu RG, Cotarla I. Understanding breast cancer risk: Where do we stand in 2005? J Cell Mol Med. 2005;9(1):208-21.

Gottesman MM. Mechanisms of cancer drug resistance. Ann Rev Med. 2002;53:615-27.

Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: Role of ATP-dependent transporters. Nat Rev Cancer. 2002;2(1):48-58.

Guarneri V, Conte PF. The curability of breast cancer and the treatment of advanced disease. Eur J Nucl Med Mol Imaging. 2004;31(Suppl 1):S149-61.

Han W, Li L, Qiu S, Lu Q, Pan Q, Gu Y, et al. Shikonin circumvents cancer drug resistance by induction of a necroptotic death. Mol Cancer Ther. 2007;6(5):1641-9.

Huang RP, Fan Y, de Belle I, Niemeyer C, Gottardis MM, Mercola D, et al. Decreased Egr-1 expression in human, mouse and rat mammary cells and tissues correlates with tumor formation. Int J Cancer. 1997;72(1):102-9.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics. 2009. CA Cancer J Clin. 2009;59(4):225-49.

Jimenez-Cervantes C, Pichon B, Dumont JE, Maenhaut C. Activation by thyroid stimulating hormone of nerve growth factor-induced gene-B expression in thyrocytes in culture: relation with proliferation and specific gene expression. Biochim Biophys Acta. 1998;1403(3):232-44.

Kovalev AA, Tsvetaeva DA, Grudinskaja TV. Role of ABC-cassette transporters (MDR1, MRP1, BCRP) in the development of primary and acquired multiple drug resistance in patients with early and metastatic breast cancer. Exp Oncol. 2013;35(4):287-90.

Lemaire P, Revelant O, Bravo R, Charnay P. Two mouse genes encoding potential transcription factors with identical DNA-binding domains are activated by growth factors in cultured cells. Proc Natl Acad Sci USA. 1988;85(13):4691-5.

Mekhail TM, Markman M. Paclitaxel in cancer therapy. Exp Opin Pharmacother. 2002;3(6):755-66.

Milbrandt J. A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor. Science. 1987;238(4828):797-9.

Morris PG, Fornier MN. Novel anti-tubulin cytotoxic agents for breast cancer. Exp Rev Anticancer Ther. 2009;9(2):175-85.

Pupo M, Pisano A, Lappano R, Santolla MF, De Francesco EM, Abonante S, et al. Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. Environ Health Perspect. 2012;120(8):1177-82.

Roninson IB, Chin JE, Choi KG, Gros P, Housman DE, Fojo A, et al. Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinoma cells. Proc Natl Acad Sci U S A. 1986;83(12):4538-42.

Ronski K, Sanders M, Burleson JA, Moyo V, Benn P, Fang M. Early growth response gene 1 (EGR1) is deleted in estrogen receptor-negative human breast carcinoma. Cancer. 2005;104(5):925-30.

Seidman AD, Tiersten A, Hudis C, Gollub M, Barrett S, Yao TJ, et al. Phase II trial of paclitaxel by 3-hour infusion as initial and salvage chemotherapy for metastatic breast cancer. J Clin Oncol. 1995;13(10):2575-81.

Sukhatme VP, Cao XM, Chang LC, Tsai-Morris CH, Stamenkovich D, Ferreira PC, et al. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell. 1988;53(1):37-43.

Tao W, Shi JF, Zhang Q, Xue B, Sun YJ, Li CJ. Egr-1 enhances drug resistance of breast cancer by modulating MDR1 expression in a GGPPS-independent manner. Biomed Pharmacother 2013;67(3):197-202.

Vaidyanathan A, Sawers L, Gannon AL, Chakravarty P, Scott AL, Bray SE, et al. ABCB1 (MDR1) induction defines a common resistance mechanism in paclitaxel- and olaparib-resistant ovarian cancer cells. Br J Cancer. 2016;115(4):431-41.

Varnum BC, Lim RW, Kujubu DA, Luner SJ, Kaufman SE, Greenberger JS, et al. Granulocyte-macrophage colony-stimulating factor and tetradecanoyl phorbol acetate induce a distinct, restricted subset of primary-response TIS genes in both proliferating and terminally differentiated myeloid cells. Mol Cell Biol. 1989;9(8):3580-3.

Vivacqua A, Romeo E, De Marco P, De Francesco EM, Abonante S, Maggiolini M. GPER mediates the Egr-1 expression induced by 17beta-estradiol and 4-hydroxitamoxifen in breast and endometrial cancer cells. Breast Cancer Res Treat. 2012;133(3):1025-35.

Wang Y, Liu M, Zhang J, Liu Y, Kopp M, Zheng W, et al. Multidrug resistance protein 1 deficiency promotes doxorubicin-induced ovarian toxicity in female mice. Toxicol Sci. 2018b;163(1):279-92.

Wang YH, Imai Y, Shiseki M, Tanaka J, Motoji T. Knockdown of the Wnt receptor Frizzled-1 (FZD1) reduces MDR1/P-glycoprotein expression in multidrug resistant leukemic cells and inhibits leukemic cell proliferation. Leuk Res. 2018a;67:99-108.

Yamaguchi H, Chen CT, Chou CK, Pai A, Bornmann W, Hortobagyi GN, et al. Adenovirus 5 E1A enhances histone deacetylase inhibitors-induced apoptosis through Egr-1-mediated Bim upregulation. Oncogene. 2010;29(41):5619-29.

Zcharia E, Atzmon R, Nagler A, Shimoni A, Peretz T, Vlodavsky I, et al. Inhibition of matrix metalloproteinase-2 by halofuginone is mediated by the Egr1 transcription factor. Anticancer Drugs. 2012;23(10):1022-31.

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