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Effect of doxorubicin treatment at the expression levels of *BCL-2* and *BAX* on MCF7 cell line

Mohammed H. Awwad[1], Hayam ELSharawy[1] and Fatma Ashour[1]

[1]Department of Zoology, Faculty of Science, University of Benha, Benha, Egypt.

Abstract

Breast cancer (BC) is a chemotherapy sensitive tumor. Doxorubicin hydrochloride (DOX) is one of the most common chemotherapeutic drugs that used in breast cancer treatment. It intercalates with DNA and stops the replication process. And it was found that, following 48h of DOX treatment, cell death Increases in ER^+ breast cancer cell lines. Here we investigated the effect of DOX treatment at the antiapoptotic *BCL-2* and the proapoptotic *BAX* genes. **Methods**: MCF7 cell line was cultured in the appropriate media and treated with DOX for 48 hours. Then the expression levels of *BCL-2* and *BAX* were investigated using qPCR. **Results:** the expression of *BCL-2* showed a slight increase in its levels after treatment while *BAX* gene showed a striking increase (3.62 fold). **Conclusion:** our results was in line with previous studies showed that treatment with DOX induce apoptosis in MCF7 cells.

Introduction

Breast undergoes a lot of pathological conditions that may be non-neoplastic (e.g: lesions) or neoplastic (e.g: breast carcinoma) (Bateman and Shaw 2013). Breast carcinoma (BC) is one of the most public reasons of cancer death in women all over the world (Ferlay, Shin et al. 2010). There are different types of BC; the histological subtypes including ductal carcinoma and lobular carcinoma. Both ductal and lobular carcinoma may be either insitu or invasive (when it invades the surroundings) (Nazário, Facina et al. 2015). Also the molecular subtypes includes; ER^+ and ER^- according to the status of estrogen receptors (ER) (Sotiriou, Neo et al. 2003). The response to drugs differs from ER^+ and ER^- (Puhalla, Bhattacharya et al. 2012, Lippman and DICKSON'r 2013). Doxorubicin hydrochloride (DOX) is one of the most common chemotherapeutic drugs that used in BC treatment (Pritchard, Dillon et al. 2012). DOX has been shown to induce apoptosis (Sharma, Tyagi et al. 2004) and arrest cell cycle (Rusetskaya, Lukyanova et al. 2009).

BCL-2 is an anti-apoptotic gene which prevent cell death, Dole and Minn (1995) revealed that high expression levels of *BCL-2* makes the cancer cells resist the apoptotic effect of chemotherapeutic drugs (Dole, Jasty et al. 1995, Minn, Rudin et al. 1995). In the other hand *BAX* is a pro-apoptotic molecule that stimulate cell death, and its expression is not affected by estrogen treatment (Teixeira,

Reed et al. 1995). We study the effect of DOX treatment on the expression levels of these two genes in the ER^+ MCF7 breast cancer cell line.

Methods

Cell line and Cell growth

Human ER^+ breast cancer cell line MCF7 (VACSERA, Cairo, Egypt) was sustained in RPMI high glucose media (Lonza, Walkersville, MD, USA) complemented with 1% penicillin/streptomycin (Lonza, Walkersville, MD, USA), 10% fetal bovine serum (Seralab, West Sussex, United Kingdom) and 25 µM HEPES (Lonza, Walkersville, MD, USA). Cells then cultivated in a humid incubator at 37˚C and 5% CO_2.

DOX treatment

After reaching 70-80% confluence, cells then separated into two groups; Control (C) group: cells grown in fresh media and drug treated (D) group: cells grown in fresh media treated with DOX at final concentration of 1µM. Also we added 17-β estradiol (Sigma Aldrich, St. Louis, MO, USA) used at final concentration of 10nM to activate estrogen receptor. Cells were treated for 48h.

RNA Extraction and cDNA Synthesis

RNA was then extracted by means of iTRAZOL reagent (ITSI Biosciences, Johnstown, PA, USA) by following steps in its pamphlet. Using Revert aid first strand cDNA kit (Thermo Fisher scientific, Waltham, MA, USA) we then synthesed the cDNA also by following steps in its pamphlet.

4.11. Real time PCR

Using Quantitect SYBR green PCR kit (QIAGEN, Hilden, Germany), the reactions were prepared and carried out using Real time PCR machine (MX3005P Stratagene, San Diego, CA, USA) with the following cycling conditions: 40 cycles of denaturation at 94˚C, annealing at temperatures mentioned in table1 depending on the gene and final extension at 72˚C. Using GAPDH as housekeeping, we detected changes in gene expression with relative quantification method (ΔΔCt) with these equations:

Gene expression (amount of target) = $2^{-\Delta\Delta Ct}$

$\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{calibrator}}$

$\Delta Ct = Ct_{\text{tested gene}} - Ct_{\text{house keeping}}$

Table 1: primers of genes under study

Gene	Forward primer(5' to 3')	Reverseprimer(5 'to 3')	Melting temperature
GAPDH	TGATGACATC AAGAAGGTGG TGAAG	TCCTTGGAGGC CATGTGGGCC AT	52˚c
BAX	GCCCTTTTGCT TCAGGGTTTC	CTGATCAGTTC CGGCACCTT	62 ˚c
BCL-2	GAACTGGGGG AGGATTGTGG	CATCCCAGCCT CCGTTATCC	56 ˚c

Results

Expression levels of *BCL-2*, *BAX* increase in the ER^+ MCF7 cells after DOX treatment.

We found that DOX treatment increased the expression levels of *BCL-2* and *BAX* after 48h of treatment. *BCL-2* showed 1.71 fold change in its expression after 48h of DOX treatment. Similarly *BAX* showed 3.63 fold change in its expression after 48h of treatment (figure 1).

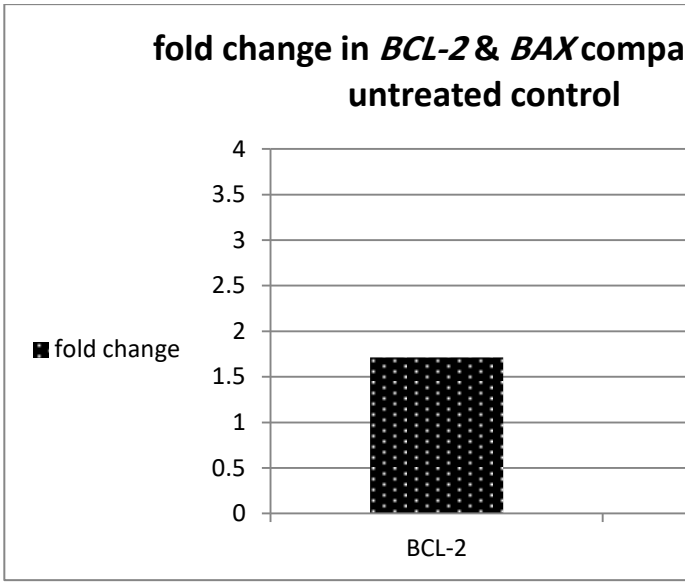

Figure 1: Gene expression of *BCL-2* and *BAX* in MCF7 cells after 48h DOX treatment. Cells were incubated with 1μM DOX for 48 h. Changes in gene expression of *BCL-2* and *BAX* after treatment were detected. They showed increase in their expression after 48h of treatment.

Discussion

Doxorubicin hydrochloride is one of the most commonly used chemotherapeutic agents in BC management(Pritchard, Dillon et al. 2012). In BC cell lines a dose of $\geq$ 1μM of DOX decreases cell viability, promotes apoptosis and stops the cell cycle(Sharma, Tyagi et al. 2004, Lüpertz, Wätjen et al. 2010). Here we tested the effect of DOX treatment on ER^+ MCF7 cell lines and how it alters the mRNA *BCL-2* and *BAX*.

When the cells were exposed to the drug for 48h, we have found that the expression of BCL-2 in was increased. But this contrast with other studies revealed that DOX down regulates *BCL-2* mRNA levels (Mcgahon, Costa Pereira et al. 1998, Leung and Wang 1999)**.** The difference between our finding and these studies could be because those studies revealed the action of DOX alone but in our study we treated the cells with estrogen to activate estrogen receptors. Indeed estrogen was shown to reverse the action of DOX alone and increase *BCL-2* levels (Teixeira, Reed et al. 1995). And this agrees with Lacroix and Leclercq (2004) who showed that active ERα prevents apoptosis of breast cancer cells via increasing the expression levels of BCL-2(Lacroix and Leclercq 2004).

We also found that the expression of BAX increased tremendously after treatment with DOX. Our BAX data is in line with other studies showed that BAX is a pro-apoptotic molecule motivates cell death and is overexpressed in MCF7 cells after treatment with DOX(Leung and Wang 1999, Sharifi, Barar et al. 2015) and

its expression is not affected by estrogen treatment(Teixeira, Reed et al. 1995).

It was found that, following 48h of DOX treatment, cell death Increases in ER^+ breast cancer cell lines (Sharma, Tyagi et al. 2004) and although we showed that the mRNA expression levels of *BCL-2* and *BAX* are increased in ER^+ MCF7 cells, it is not clear whether this change may lead to apoptosis or not. More studies investigating the gene expression profiles and apoptotic and viability assays of MCF7 are recommended to identify the final fate of the cells after DOX treatment.

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