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Original Article

Development of radioresistance in drug resistant human MCF-7 breast cancer cells

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Abstract

Background and purpose: Radiotherapy is used for the treatment of malignant tumours, and may be used as the primary therapy. It is also common to combine radiotherapy with surgery, chemotherapy, hormone therapy or some combination of them. Even if the tumour is treated intensively, women diagnosed with breast cancer may develop a recurrence. Most recurrences may be in the form of distant metastases, development of multi-drug resistance phenotype or both together. This study demonstrated that some of the multi-drug resistant cancer cells may also become radioresistant.

Materials and Methods: Chemoresistance in paclitaxel (MCF-7/Pac), docetaxel (MCF-7/Doc), vincristine (MCF-7/Vinc), doxorubicin (MCF-7/Dox) and zoledronic acid (MCF-7/Zol) resistant MCF-7 cells were demonstrated by XTT assay. *MDR1* gene expression was detected by real-time PCR in human MCF-7 breast cancer cells. Drug resistant and sensitive cells were exposed to γ-radiation and development of radio-resistance was investigated.

Results: Results have indicated that paclitaxel, docetaxel, vincristine, doxorubicin and zoledronic acid–selected cells gained varying degrees of resistance to their selective drugs when compared with original MCF-7/S. MCF-7/Pac, MCF-7/Doc, MCF-7/Vinc and MCF-7/Dox cells have all acquired *MDR1* expression. Among the resistant sub-lines, MCF-7/Pac and MCF-7/Doc cells were significantly cross-resistant to irradiation compared to the sensitive cells.

Conclusion: MCF-7/Pac and MCF-7/Doc cell lines were found radioresistant to γ-radiation. On the contrary, doxorubicin, vincristine and zoledronic acid resistant cancer cells were still sensitive to radiation.

Keywords

MDR1; MCF-7; radioresistance; Real-time PCR

INTRODUCTION

Breast cancer remains the most common cancer diagnosed in women and the second leading cause of female cancer death.[1] Treatment of

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breast cancer may involve local removal of tumour, surgical removal of breast, radiation therapy, chemotherapy and hormone therapy.[2] Tumour size, stage and other characteristics in addition to patient preference are important factors during selection of treatment type and combination therapy.

Despite aggressive initial treatment, about one-third of all women diagnosed with breast cancer will develop a recurrence.[3] Bone metastasis is one of the complications of solid tumours including breast. As bone lesions progress, patients frequently suffer from skeletal complications.[4] Along the other treatment strategies, radiotherapy plays an important role in the management of bone metastases by inducing damage to the DNA and cell cycle check points. Various cell cycle damage check points and DNA damage repair pathways have been demonstrated.[5]

The other cause of disease recurrence is multi-drug resistance,[6] a complex phenotype whose predominant feature is resistance to wide range of structurally unrelated anti-cancer agents.[7] Intrinsic and/or acquired drug resistance are fundamental reasons for the clinical failure of chemotherapy, but the clinical relevance of the various known resistance mechanisms still remains unclear.[8] There are several mechanisms by which cancer cells develop resistance to cytotoxic agents. Cellular drug efflux associated with transporter proteins such as P-glycoprotein and the multi-drug resistance–associated protein are the most studied forms of drug resistance in breast cancer. Important roles are also attributed to other active or passive mechanisms in resistance to chemotherapeutic agents.[9]

Paclitaxel and docetaxel are taxoid group chemotherapy drugs that are given as a treatment for some types of cancer. They are most commonly used to treat ovarian, breast and non-small cell lung cancer. These drugs, by binding to β-tubulin subunits prevent microtubule depolymerisation.[10,11] Vinca alkaloid vincristine is delivered via intravenous infusion for use in various types of chemotherapy regimens. Its main uses are in non-Hodgkin's lymphoma as part of the chemotherapy regimen. Vincristine is a microtubule inhibitor and prevents microtubule polymerisation.[12] Doxorubicin, trade name Adriamycin, is a drug used in cancer chemotherapy. It is an anthracycline antibiotic, closely related to the natural product daunomycin, and like all anthracyclines it intercalates DNA. It is commonly used in the treatment of a wide range of cancers, including haematological malignancies, many types of carcinoma, and soft tissue sarcomas. Applications of the so-called drugs were shown to cause development of MDR in several tumours.[13,14] Zoledronic acid (marketed by Novartis under the trade names Zometa, Zomera, Aclasta and Reclast) is a bisphosphonate (BP). Zometa is used to prevent skeletal fractures in patients with cancers such as multiple myeloma and prostate cancer. It can also be used to treat hypercalcaemia of malignancy and can be helpful for treating pain from bone metastases. BPs are analogues of endogenous pyrophosphates in which a carbon atom replaces the central atom of oxygen. In vivo, BPs bind strongly to hydroxyapatite on the bone surface and are preferentially delivered to sites of increased bone formation or resorption.[15] There is also extensive preclinical evidence that BPs have anti-tumour activity, as evidenced by reduced proliferation and viability of tumour cell lines in vitro and reduced skeletal tumour.[16,17]

MCF-7 cell line used in this study is a human breast carcinoma model cell line that retains several characteristics of differentiated mammary epithelium.[18] In this study, parental sensitive MCF-7 (MCF-7/S) cell line and previously developed paclitaxel, docetaxel, doxorubicin, vincristine and zoledronic acid resistant MCF-7 sub-lines (MCF-7/Pac, MCF-7/Doc, MCF-7/Dox and MCF-7/Zol) were used as models for drug resistant breast cancer. *MDR1* gene expression levels were detected by real-time PCR in cell lines. The MCF-7 cell lines were exposed to γ-radiation. The results that show the cytotoxicity of irradiation on the drug resistant cell lines and developed cross-resistance may be used to suggest clinical applications for the usage of γ-radiation on drug resistant breast carcinoma.

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MATERIALS AND METHODS

Chemicals

Paclitaxel (Sigma) and docetaxel (Fluka) were dissolved in dimethyl sulfoxide to prepare stock solutions. Vincristine and doxorubicin were obtained from Gülhane Military Academy, School of Medicine, Ankara, Turkey. Vincristine and doxorubicin were diluted in deionised water. Zoledronic acid was kindly provided as the hydrated disodium salt by Novartis, Pharma AG, Switzerland and dissolved in deionised H_2O.

Cell lines, development of resistant sub-lines

MCF-7 cell line, which is a model cell line for human breast carcinoma, was used as parental cell line. The cell line exhibits some features of differentiated mammary epithelium and was donated by ŞAP Institute, Ankara, Turkey. MCF-7 cells were maintained as an attached type monolayer culture in RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum, L-glutamine and gentamicin, and incubated at 37 °C in humidified atmosphere of 5% CO_2. Paclitaxel, docetaxel, doxorubicin, vincristine and zoledronic acid were applied separately in dose increments to MCF-7 cell line for stepwise selection of resistant cells. The resistant sub-lines to 400 nM paclitaxel (MCF-7/Pac), 120 nM docetaxel (MCF-7/Doc), 120 nM vincristine (MCF-7/Vinc), 1,000 nM doxorubicin (MCF-7/Dox) and 8,000 nM zoledronic acid (MCF-7/Zol) from parental MCF-7 cell line (MCF-7/S) were developed by increasing the doses, stepwise and maintained as previously described.[19,20] Cells capable of growing in selection concentrations became resistant.

Assay for cell proliferation

Anti-proliferative effects of drugs (paclitaxel, docetaxel, vincristine, doxorubicin and zoledronic acid) on MCF-7 and drug-selected sub-lines were evaluated using the Cell Proliferation Kit (Biological Industries, Israel) as previously described.[21] Average of viable cell numbers at different drug concentrations were expressed as percentage of the control. Percent cell proliferation versus log (drug concentration) curves represent anti-proliferative effects of the drugs on cells and inhibitory concentration 50 (IC_{50}) values were calculated from these curves.

RNA isolation and quantitative real-time PCR for *MDR1* gene

RNA isolation was performed from parental and resistant MCF-7 cells using Tri Reagent RNA Isolation Reagent (Sigma, Germany). cDNA synthesis was performed with 5 μg of total RNA, 0.5 μg specific primer and 40 units of M-MuLV Reverse Transcriptase according to the manufacturer's instructions (MBI Fermentas, Lithuania). For quantitative real-time PCR (qPCR) analysis, LightCycler® (Roche, Germany) and The LightCycler® Taqman® Master Kit (Roche) was used according to manufacturer's instructions. In brief, 20 μL reaction mix contained 7 μL cDNA, 0.5 μM from each forward and reverse primers and 0.2 μM hydrolysis probe. The following specific primers for amplification of *MDR1* gene and probe (Universal probe library) were supplied by Roche; *MDR1* forward: 5′-AAGGCATTTACTTCAAACTT GTCA-3′ and *MDR1* reverse: 5′-GGATTCATCAGCTGCATTTTC-3′ ($T_{annealing}$: 56 °C). Each sample run was performed in duplicates with non-template controls. The automated system run and the software analysis with calculations were performed according to instructions supplied by the instrument (Roche LightCycler®2.0). In brief, for quantification of RT-PCR results, fluorescent signal intensities (640 nm) were plotted against the number of PCR cycles on a semi-logarithmic scale. Crossing point cycles (C_P) were determined for all samples in parallel with a standardisation series of known dilutions of *MDR1* positive cDNA as template. The C_P value of each sample was then compared to those in the standardisation series, and the calculated concentration values corresponded to the expression level of the *MDR1* gene.

Irradiation of cells

Sensitive and resistant MCF-7 were seeded to 96-well microtiter plates (5,000 cells/well) and after 24-h incubation in standard culture conditions irradiated with doses of 200 and 800 cGy

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by a Theratron 780 Cobalt 60 Teletherapy Unit (AECL Medical, Ontario, Canada). After irradiation, all plates were incubated at 37 °C in a 5% CO_2 atmosphere for an additional 24 h. Then cell proliferation was evaluated using Cell Proliferation Kit (Biological Industries). IC_{50} values represent inhibitory doses of irradiation in cGy to evaluate anti-proliferative effects of irradiation on cells and were calculated from cell proliferation curves as described above.

Statistical analysis

The results of XTT cytotoxicity assay and qPCR results were subjected to two-tailed Student's *t*-test by using SPSS Software (SPSS Inc., Illinois, USA) to determine significant difference between means of groups ($\alpha = 0.05$).

RESULTS

Anti-proliferative effects of selective anti-cancer drugs on cells

According to the results obtained from cytotoxicity tests, paclitaxel, docetaxel, vincristine, doxorubicin and zoledronic acid selected cells gained varying degrees of resistance to their selective drugs, 150-, 47-, 30-, 160- and 4-fold, respectively, when compared with sensitive MCF-7 cells. IC_{50} values of sensitive and resistant sub-lines (MCF-7/Pac, MCF-7/Doc, MCF-7/Vinc, MCF-7/Dox and MCF-7/Zol) to their respective drugs are summarised in Table 1.

Expression analysis of MDR1 gene

According to real-time PCR results (Figure 1), MCF-7/Pac, MCF-7/Doc, MCF-7/Vinc and MCF-7/Dox cells have acquired *MDR1* expression. As previously described by our group MCF-7/Zol cells did not acquire *MDR1* expression, however, over-expressed other drug-resistant genes such as *BCRP* and *LRP* genes.[20] Results indicated that the cells gained resistance

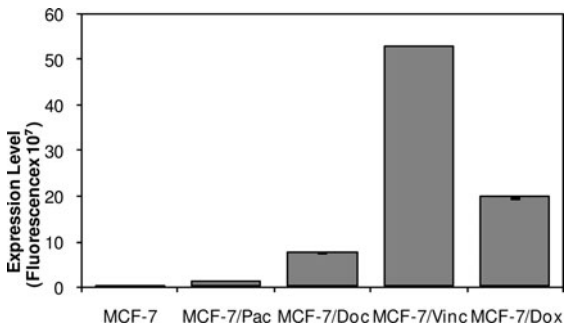

Figure 1. Fluorescent values obtained from real-time PCR for MDR1 *gene expression.*

Table 1. *Anti-proliferative effects of anti-cancer drugs and irradiation on drug selected cells*

Cells	Application	Mean $IC_{50} \pm$ SEM[a]	R[b]
MCF-7	Paclitaxel	2.12 ± 0.23 μM	–
	Docetaxel	3.49 ± 1.55 μM	–
	Vincristine	5.45 ± 0.66 μM	–
	Doxorubicin	1.14 ± 0.38 μM	–
	Zoledronate	92.20 ± 2.46 μM	–
	Irradiation	967 ± 63.98 cGy	–
MCF-7/Pac	Paclitaxel	317.94 ± 0.20 μM	149.98[c]*
	Irradiation	1534 ± 13.43 cGy	1.59*
MCF-7/Doc	Docetaxel	163.21 ± 11.19 μM	46.7*
	Irradiation	1858.33 ± 51.94 cGy	1.92*
MCF-7/Vinc	Vincristine	162.29 ± 2.19 μM	29.78*
	Irradiation	967 ± 82.77 cGy	1
MCF-7/Dox	Doxorubicin	183.11 ± 23.63 μM	160.62*
	Irradiation	974.67 ± 21.4 cGy	1.01
MCF-7/Zol	Zoledronate	340.36 ± 12.64 μM	3.69*
	Irradiation	1079.33 ± 83.22 cGy	1.12

[a] SEM (standard error of the mean) was derived from three independent experiments. [b]R (Resistance index) = IC_{50} resistant cells/IC_{50} sensitive cells. [c]* Represents significant difference between groups (resistant and sensitive cells) with $p < 0.05$.

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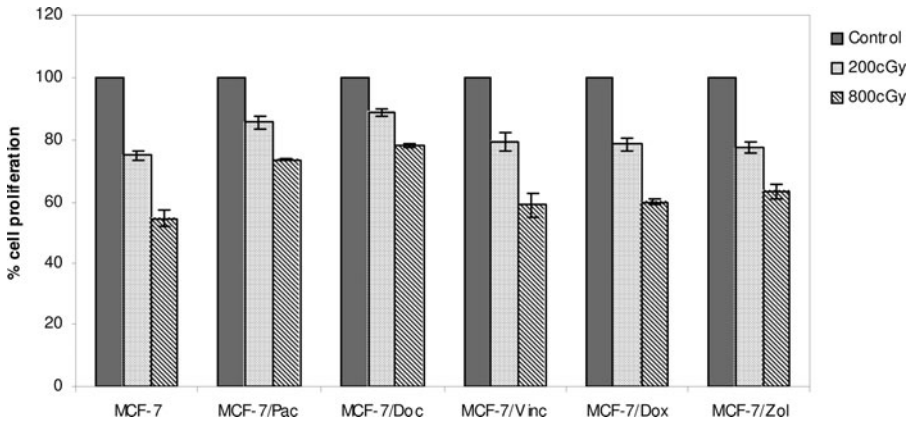

Figure 2. Anti-proliferative effects of irradiation on sensitive and resistant cells.

to their selective agents partially due to over-expression of ABC transporter genes at least.

Irradiation of cells

Cells were irradiated with doses of 200 and 800 cGy. These irradiation doses caused significant reduction in cell proliferation of sensitive and resistant MCF-7 cells (Figure 2). Inhibitory doses of radiation in cGy for sensitive and resistant cells were calculated to evaluate anti-proliferative effects of irradiation on cells and express resistance indices for irradiation. IC_{50} values and resistance indices are summarised in Table 1. According to the table, MCF-7/Pac and MCF-7/Doc cells were significantly cross-resistant to irradiation (1.6- and 2-fold, respectively) compared to sensitive cells.

DISCUSSION

Breast cancer is the most common and frequently diagnosed cancer at a median age in women.[22] Even though substantial advances in therapy and diagnosis have enhanced the survival rate of patients with breast cancer, late recurrences of the disease account for deaths from breast cancer.[23] So, further studies are needed to optimise therapeutic applications in patients with metastatic breast cancer.

The results are consistent with literature that, the paclitaxel, docetaxel, vincristine and doxorubicin resistant MCF-7 cells have *MDR1* over-expression.[13,14,24] However, zoledronic acid–resistant MCF-7 cell line does not have *MDR1* expression as it was previously reported.[20] Although resistance indices of MCF-7/Vinc are lower than MCF-7/Pac and MCF-7/Dox, *MDR1* gene expression level is the highest in this cell line. In MCF-7/Pac and MCF-7/Dox cell lines other drug resistance mechanisms may be more active than P-gp.

In clinical applications, development of cross-resistance affect the success of chemotherapy and some patients become refractory to treatment.[25,26] Our study demonstrates dose-dependent cytotoxic effect of γ-radiation on drug-resistant breast cancer cells and development of cross-resistance in MCF-7/Pac and MCF-7/Doc cell lines to irradiation. Although doxorubicin resistant MCF-7 cell line is more chemoresistant than other resistant cell lines it did not develop radioresistance. Some of the genes that are involved in DNA repair and/or cell cycle regulation may have been induced in MCF-7/Pac and MCF-7/Doc. In a previous report, levels of *DDB2* (involved in DNA repair) and *CDKN1A* (cell cycle regulator) genes were significantly induced by irradiation in breast cancer tissue samples.[27] The respective study proposed that these genes were modulated by p53 and alter the radiosensitivity/resistance profiles in tissues following radiotherapy. In addition, both *DDB2* and *CDKN1A* were previously identified as radiation-induced in peripheral white blood cells[28] and fibroblasts.[29]

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According to the results, MCF-7/Pac and MCF-7/Doc cell lines are resistant to γ-radiation and therefore the treatment of the paclitaxel and docetaxel resistant breast cancer with radiotherapy may not be useful. On the other hand MCF-7/Dox, MCF-7/Vinc and MCF-7/Zol sub-lines did not develop cross-resistance to irradiation. Therefore the γ-radiation may show its cytoreductive effect on doxorubicin, vincristine and zoledronic acid resistant breast cancer cells. A previous finding also represents that zoledronic acid and irradiation act synergistically toxic to MCF-7 cell line,[30] which is parallel to the results here. If it was considered that doxorubicin, vincristine and zoledronic acid–resistant breast cancer cell lines are models to clinically resistant breast carcinoma, it may be stated that γ-radiation may be a therapeutic protocol for metastatic and resistant breast cancer patients. However, this finding should be confirmed with drug-resistant breast cancer patients who were exposed to radiation after chemotherapy.

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Conflict of Interest Statement

There is no actual or potential conflict of interest in relation to this article.

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