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Effect of Dose Irradiation on the Expression of BRCA1 and BRCA2Genes in MCF-10A and MCF-7 cell lines

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ABSTRACT

Introduction: Breast cancer can be caused by a mutation in its genome. Some mutations are cancer-predisposition which exist at the moment of germ cell genesis. It has been discovered that BRCA1 and BRCA2 are linked to hereditary breast cancer. BRCA1/2 are tumor suppressor genes involved in DNA repair and transcriptional regulation in response to DNA damage. Irradiation, particularly ionizing radiation used in clinical radiotherapy, causes DNA damage. This study aims to find out whether different doses of x-radiation might change the expression of BRCA1/2.

Material and Methods: Cancer and normal breast cell lines (MCF10-A and MCF7) cultured in flasks were irradiated with X- rays in different doses including 50, 100, 400, 2000, and 4000 mGy. Then, the expression of BRCA1/2 genes was measured using real-time quantitative reverse transcription PCR (RT-qPCR). Relative changes for mRNA were calculated based on the ΔΔCt method.

Results: MCF-10A cells represent a significant increase in BRCA2 expression at all irradiation doses while increasing the mRNA level for the BRCA1 gene observed after exposure to 50, 100, and 2000 mGy. This figure shows overexpression of BRCA2 gene after all irradiation doses except 100 mGy for MCF7 cells. The BRCA1 gene upregulated after exposure to 400 and 2000 mGy and downregulated at 50,100 and 4000 mGy in these cells.

Conclusion: Incidence of cancer initiation was probable in normal breast cells after low-dose radiation, with up-regulation of BRCA1 and BRCA2 gene expression. BRCA mutation may be an inadequate outcome predictor of survival rate and other factors may be involved too.

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Introduction

Breast cancer is one of the most common cancers in women. Approximately a million women worldwide are diagnosed with breast cancer every year. Women who have early menarche or late menopause are at an increased risk, as are those who have a favorable family history [1-4]. According to statistical research, about 10% of women with a history of breast cancer in first-degree relatives are prone to catch cancer, and 20 to 25 percent of these cases show underlying hereditary gene mutations [5, 6]. Breast and ovarian cancers are more prevalent among women who have mutations in the BRCA1 or BRCA2 genes, which are known as breast cancer susceptibility genes. While these genes do not solely function as tumor suppressors in breast and ovarian cancer, the breast cancer susceptibility genes BRCA1 and BRCA2 are the most frequently mutated genes in these cancers.[1, 7]. The risk of lifelong breast cancer in BRCA1 mutation carriers are up to 70% compared to 12% in non-carriers [6]. In BRCA1 mutation carriers, the basal-like subtype of breast cancer accounts for up to 75% of all breast cancers and is often linked with a poor prognosis of survival [6]. Besides, since BRCA1 is a tumor suppressor gene, women with a germline BRCA1 mutation are prone to the wild-type allele mutation [2]. Scientists identified that BRCA1 mutations are unlikely to cause male breast cancer, but BRCA2 mutations can increase the risk[2]. According to earlier research, a mutation in any copy of BRCA1 or BRCA2 causes hereditary breast and ovarian cancer (HBOC) syndrome, associated with a 50-80% lifetime risk of breast cancer [7]. One more important function of BRCA genes is their involvement in DNA damage response (DDR) mechanisms. BRCA1 is involved in DNA double-strand break (DSB) repair, which is required in all tissues, and BRCA2 is a homologous recombination (HR) mediator, an important DNA repair mechanism, that makes use of an undamaged sister chromatid to perform high-fidelity repairs on DSB [6]. BRCA1 and BRCA2 genes regulate various proteins which are known to be involved in HR [7, 8]. Evidently, in the absence of BRCAs gene regulation, HR fails to function correctly, and the replication-associated DSBs can cause chromosomal

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rearrangements. Eventually, the BRCA1–BRCA2–HR pathway that suppresses tumorigenesis may disrupt the repair process and increase the risk of cancer initiation in normal cells [2, 7]. Using a comprehensive multi-omics study to investigate the role of BRCA1/2, biologists have suggested the BRCA1/2 genes as good therapeutic targets and a predictive biomarker in breast cancer [9].

DSBs can arise as a result of DNA replication or exposure to ionizing radiation or other genotoxic agents. The use of ionizing radiation (IR) in medical diagnosis and treatment has raised concerns due to the consequences of various exposure levels on human health. Despite this caution, little evidence about the long-term effects of low-dose irradiation is known. Long-term low-dose radiation exposure has been linked to negative health impacts such as genetic instability and cancer [9, 10]. Furthermore, chromosomal aberrations are highly correlated with both low and high-dose irradiations [11-14]. Considering these facts, the DSBs repair and the expression of genes related to this mechanism are essential for the survival of both normal and cancer cells.

Given that ionizing radiation plays a significant role in both cancer initiation through imperfect DSBs repair and cancer treatment through DSBs, and considering the fact that BRCA-deficient cells are more sensitive to ionizing radiation, the present study assessed potential genomic damage resulting from exposure to both low and high doses of X-rays in both cancer and normal breast cell lines: MCF-7 and MCF-10A.

Materials and Methods

Cell line and cell culture

Roswell Park Memorial Institute 1460 (Bioidea) supplemented with 10% fetal bovine serum (Gibco), 100 u/ml penicillin, and 100 g/ml streptomycin, was utilized for the cultivation of two human breast cell lines: MCF-7 (homo sapiens, epithelial cells, metastatic adenocarcinoma) and MCF-10A (homo sapiens, epithelial cells, fibrocystic disease, normal tissue). The cells were maintained at 37 degrees Celsius in a humidified atmosphere containing 5% CO_2. Subculturing of cells was performed as needed using a trypsin-0.5 mM EDTA solution, with fresh medium replenished every two days.

Irradiation Condition

Cells were plated in the 25 cm^2 tissue culture flask. When cells reached 70% confluence, were irradiated with various single dose of x-ray exposure for the time required to apply a prescribed dose (50, 100, 400, 2000, and 4000 mGy) at room temperature. The Linac accelerator (Elekta company, Stockholm, Sweden) 6mv and SSD (Source-skin distance) of 100cm and dose rate of 200 mGy/min was used. Irradiated cells were maintained at 37^0C and 5% CO_2. Cells which receive no radiation were used as the control group. The experiments were conducted at Namazi Hospital, Shiraz, Iran.

RNA Extraction

Total RNA was extracted after 6 hours from irradiated MCF-7 and MCF-10A cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufactures' instruction. The concentration, purity, and integrity of the extracted RNA were examined using an agarose gel electrophoresis and a nanodrop® spectrophotometer (Bioner, Daejeon, South Korea). As a next step before cDNA synthesis, the RNA was frozen at -80 degrees Celsius.

cDNA synthesis

Total miRNAs reverse transcriped into cDNA using Suprime Script RTase, Oligo-dT and dNTPs (Exiqon, Denmark) according to the manufacturer's instructions. cDNA samples then were stored at -20℃ until further process. The average amount of total miRNAs which were used for cDNA synthesis was 1µg based on the evaluation of the NanoDrop instrument.

Polymerase chain reaction

The Ampliqon Taq DNA polymerase Master Mix RED kit (Denmark) was used in a polymerase chain reaction to verify the accuracy of the synthesized cDNA. In this reaction, βactin primers were utilized, and the resulting products were run on an agarose gel at a concentration of 2%. These were the conditions for cycling the polymerase chain reaction: 2 minutes at 95°C (Activation), 45 cycles: 15 sec at 95°C (Denaturation), 1 min sec at 62°C (Annealing), 1 min at 72°C (Extension). The final extension step was carried out at 95 ºC for 15 sec.

Real-Time Quantitative polymerase chain reaction (RT-qPCR)

Finally, the Ampliqon SYBER Green PCR kit (Denmark) was used to perform RT-qPCR for genes of interest BRCA1 and BRCA2. To perform real-time PCR StepOne Real Time PCR system (AIB Applied Biosystems ™, USA was used). All experiments were performed in triplicates.

Table 1. List of primer sequences and their product

Primer name	Primer Sequence (5' to 3')	product size
βactin- forward	GGGAAATCGTGCGTGACATTAAGG	183
βactin -reverse	GGAAGGAAGGCTGGAAGAGTGC	
BRCA1 -forward	GCTCTGGGTAAAGTTCATT	116
BRCA1- reverse	CAGACAGATGGGACACT	
BRCA2-forward	TGTTTCCTGTCCACTTCTAA	97
BRCA2-reverse	ACATACCACTGACTTATCTCTT	

Allele ID7 (Premier Biosoft International; Palo Alto, CA, USA) was used to create the primers. Bioneer Corporation (South Korea) designed and synthesized primers in the exon regions to remove genomic DNA contamination. The specific primer sequences were listed in table 1. The β-actin gene was used as the endogenous reference to measure the relative expression quantity of the target genes. Beta-actin is one of the housekeeping genes widely used in various cell and molecular biology studies, such as evaluating changes in gene expression due to constant expression in different cells to normalize data and as an internal standard. Humans only have one of the six known actin isoforms. One of the two Actins found in the cytoskeleton but not in muscle. Actins are a family of highly conserved proteins that play critical roles in cell movement, structure, and function. Each RT-qPCR reaction contained 10 µl of 2x SYBR Green qPCR Master Mix (Yekta Tajhiz Azma, Iran), 2 µl of primers (each 2 µl was 10 mol), 6 ul of deionized water, and 2 µl of cDNA. Two minutes at 95 degrees Celsius (Activation), followed by 45 cycles of 15 seconds at 95 degrees Celsius (Denaturation), one minute at 62 degrees Celsius (Annealing), one minute at 72 degrees Celsius (Extension), and 15 seconds at 95 degrees Celsius (Final Extension). Ct values for the BRCA1 and BRCA2 genes in each sample, normalized to βactin. PCR efficiency was measured according to data obtained from each gene using serial dilutions of the PCR product. Relative changes in the amount of mRNA were calculated based on the ΔΔCT method where ΔCT is the difference in threshold cycle between the target and reference genes and CT is a threshold cycle generated by the qPCR system and.

Statistical analysis

The data were statistically analyzed using Graph Pad Prism version 5.0 (Graph Pad Software, Inc., La Jolla, CA, USA). Normality of the quantitative data was checked by Shapiro-Wilk test of normality. Analysis of the difference in gene expression profile was performed using one-way ANOVA tests. All results were expressed as mean± SD, and P value< 0.05 was considered as significant value. All experiments were performed in triplicates.

Results

BRCA1/2 gene expression in MCF-10A cells after exposure

As is depicted in figure1, expression of BRCA1 changed differently in normal breast cells (MCF-10A) after exposure to various dose radiation. Expression of BRCA1 changed in normal breast cells (MCF-10A). There was an up-regulation between 0 to 100 mGy, with significant differences between 0 to 50 mGy and 50 to 100mGy ($p<0.001$). A down-regulation was seen in 400 mGy. A significant difference was found between 50 and 400 mGy ($p<0.001$). Following that, an up-regulation was observed in 2000 mGy again, while a down-regulation at dose of 4000 mGy was noticed. Although the expression of BRCA2 changed in breast normal cells (MCF-10A) at all radiation doses, it was not statistically significant.

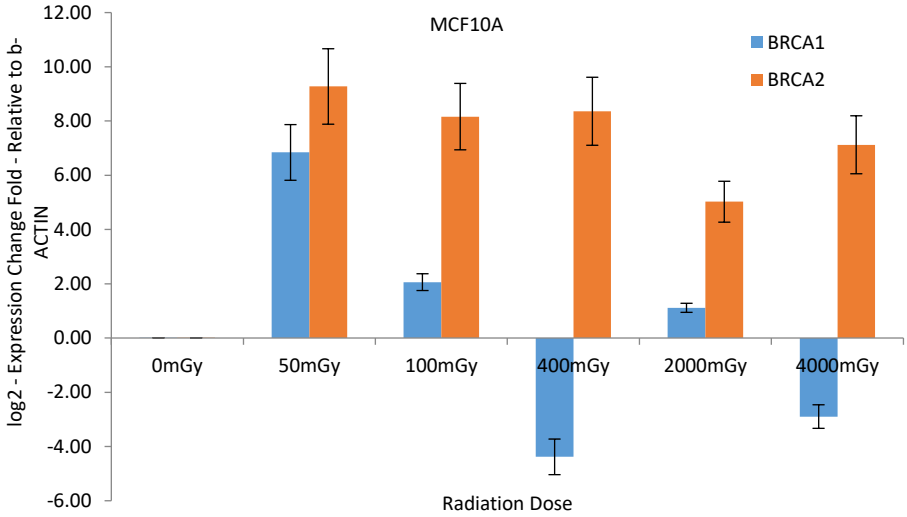

Figure 1. The relative expression of BRCA1 and BRCA2 in MCF-10A cell line after irradiation in 50, 100, 400, 2000, 4000mGy of x–rays. The expression level was assessed after 6 hours

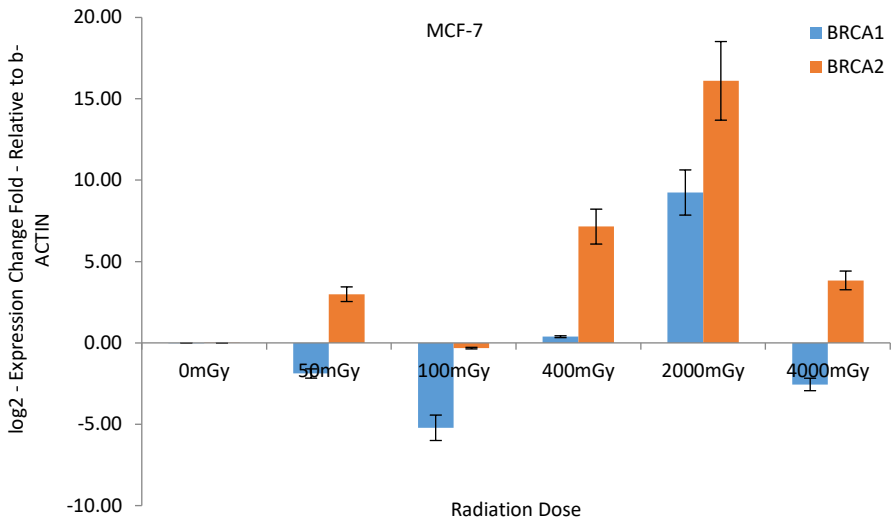

Figure 2.The relative expression of BRCA1 and BRCA2 in MCF-7 cell line after irradiation in 50, 100, 400, 2000, 4000mGy x–rays. The expression level was assessed after 6 hours

BRCA1/2 gene expression in MCF7 cells after exposure

As illustrated in Figure 2, a down-regulation in the expression of BRCA1 was observed at radiation doses of 50, 100, and 4000 mGy in MCF7 cells. However, the levels of BRCA1 gene expression increased at doses of 400 and 2000 mGy. Additionally, the use of a multiple comparison test revealed a significant difference ($p < 0.001$) between the doses of 2000 and 4000 mGy.

Discussion

A BRCA mutation changes the BRCA1 or BRCA2 gene expression, which are tumor suppressors. Hundreds of mutations in these genes have been discovered, some of which are detrimental [15]. As an illustration, for women who carry deleterious mutations in either BRCA1 or BRCA2, a five-fold increased risk of breast cancer has been reported [16]. Moreover, BRCAs gene mutations that block a crucial error-free DNA repair pathway dramatically raise the probability of breast cancer initiation and other malignancies [17]. BRCA1 has a role in various biological activities, including cell cycle regulation, transcriptional regulation, chromatin remodeling, DNA double-strand breaks (DSB), and apoptosis. BRCA2's functions, on the other hand, are essentially confined to DSB repair via homologous recombination (HR) promotion [7, 18, 19]. DSB is one of the deadly effects of ionizing radiation, which is implicated in the development of cancer and must be repaired [20]. A connection between BRCA1/2 gene expression and their crucial role in DNA damaged repair mechanisms on one side and the role of dose irradiation in inducing DNA damage on the other side revealed that there might be a correlation between BRCA1/2 gene expression and dose irradiation and therefore it necessitates more extensive research. Since DSBs can occur due to irradiation, the present study will look at the impact of low, medium, and high doses of irradiation on the expression of BRCA1/2 genes in cancer and normal breast cell lines (MCF-7 and MCF-10A).

In this study, we found a considerable increase in the expression of BRCA1 in MCF-10A in 50 mGy and 100 mGy doses (low doses). This result clearly shows the possibility of mutation and susceptibility to breast cancer in normal cells due to low dose exposure. Following these radiation doses, a non-significant up-regulation at 400 mGy, and a significant up-regulation at 2000 mGy were observed. As is expected, these results could depict a higher probability of breast cancer in higher dose irradiation. In terms of BRCA2 gene expression, an increasing trend was observed in all irradiation doses; however, it was not statistically significant.

Wiggins GA et al. demonstrated gene expression variability analysis to identify breast cancer risk-associated genes in two breast cell lines (MCF-10A, MCF-7). In their study, MCF-10A BRCA1mut/+ cells displayed less gene expression variability compared to the wild type MCF-10A cells. In another study, Jeng YM and colleagues demonstrated that BRCA1 heterozygous mice were prone to cancer with low dose ionizing radiation (IR) treatment [21]. However, our data represent that normal cells which are non-carrier of BRCA1 mutation may alter a BRCA1 gene expression and increase the risk of breast cancer even in low dose radiation. Moreover, our data confirmed that MCF7 cancer cells represented an overexpression in the mRNA level of the BRCA2 gene while being exposed to different radiation doses (50, 400, 2000, and 4000 mGy of X-ray),; however, 100 mGy of X-radiation led to downregulation of this gene expression. The trend was different for the BRCA1 gene. Breast cancer cell lines showed an upregulation in BRCA1 gene expression after exposing to 400 and 2000 mGy of dose radiation, while they downregulate at 50,100 and 4000 mGy dose irradiation. It has reported that BRCA1 knockdown in MCF-7 cells associated with a decrease in luminal and

an increase in basal marker's expression [22]. Comparing BRCA1 and BRCA2 gene expression revealed different alteration frequencies. It may be explainable with the different mutation sites of each gene. It should be mentioned that BRCA mutations frequently can occur even before irradiation [22]. Previous studies demonstrate interesting results about the role of BRCA mutations in developing breast cancer. Trainer et al.concluded that germline BRCA testing has the potential to improve the outcomes and quality of life of patients with breast cancer and their families, but it requires careful implementation and evaluation.[23]., Templeton et al. found that BRCA mutations are not associated with worse overall survival compared to non-carriers. However, the article also finds that estrogen receptor (ER) expression is an effect modifier in patients with BRCA1 mutations, meaning that ER-negative patients have worse survival than ER-positive patients. No such effect is observed for BRCA2 mutation carriers or for progesterone receptor expression. [24]. Similarly, a retrospective cohort study at the University of Texas involving women with breast cancer who underwent BRCA1/2 evaluation after undergoing definitive surgery and RT found that oncologic outcomes were similar in patients with a germline BRCA1/2 PV mutation and in patients with no mutation [25]. Since the over expression of BRCA1/2 has a significant correlation with overall survival and was related to clinicopathological characteristics such as lymph nodes, estrogen, and progesterone receptors [1], it is of great importance to being concerned about the consequences of low dose irradiation on any individual, especially those who are more susceptible to dosage radiation, such as radiographers or radiation workers. The most likely incidence of cancer initiation was detected in normal breast cells after low-dose irradiation, with up-regulation of both BRCA1 and BRCA2 gene expression.

Conclusion

These findings, in conjunction with previous research, offer a promising approach to comprehending the intricate effects of radiation on the expression of BRCA1 and BRCA2. This research holds great potential in advancing our understanding of how these genes respond to varying radiation doses and exposure conditions. Moreover, considering that BRCA1 and BRCA2 expression levels have practical applications in breast cancer diagnosis, treatment planning in radiotherapy, and assessment of radiation-related accidents, these insights could pave the way for more precise and personalized interventions in the fields of oncology and radiology, ultimately improving patient care and safety.

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