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ORIGINAL ARTICLE

Application of Cell Culture System in Radiation Dose Optimization of Breast Cancer Radiotherapy

Meme Kanseri Radyoterapisinde Radyasyon Dozunun Optimizasyonu İçin Hücre Kültür Sistemlerinin Kullanılması

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Abstract

Introduction: Metastatic breast cancer causes high mortality in women. Therefore, the search for effective treatment continues. A suitable radiation dose should be determined to cause only a minimum damage to the surrounding tissue. We aimed to determine if cell culture systems can be used to demonstrate the effects of different radiation doses on breast cancer cells.

Methods: Here, MDA-MB-231 breast cancer cells were used. The antiproliferative effect of different doses of X-rays was measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide test. A wound healing test was performed to assess the metastatic potential, and flow cytometry analysis was used to evaluate the cell cycle.

Results: It is found that 2 Gy, 4 Gy, and 8 Gy radiation doses were applied to the cells. Cell proliferation was significantly decreased at all doses ($p<0.05$). However, there was no significant decrease between the different dose groups ($p>0.05$). The lateral mobilization potential of MDA-MB-231 cells was decreased significantly in radiation applied cells as compared with the control. Cell cycle analyses showed that different doses of radiation delayed the cells in G1 as compared with control cells in the S phase.

Discussion and Conclusion: In the cell culture system, a decrease in proliferation and metastatic potential of breast cancer cells by the application of different doses of radiation is found. Therefore, for effective planning of radiation therapy, additional parameters of radiation should also be assessed via cell culture systems. It has been shown that cell culture systems may be used for dosimetry studies of radiation therapy for breast cancer.

Keywords: Breast cancer; Cell biology; Cell culture; Molecular biology; Radiotherapy

Breast cancer is the second most cause of cancer-related deaths in women and is responsible for 15% of all cancer-related deaths in women.[1] Despite the development of early diagnosis and comprehensive treatment strategies, the survival rate of metastatic breast cancer is only 25%.[2] According to the clinical condition of the

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patient in breast cancer, surgical intervention, radiotherapy, chemotherapy, hormonal, and biological therapy are applied alone or in combination.[3] Radiation therapy is traditionally used to improve survival after surgical treatment in stage I and III breast cancer.[4] It has been stated that the use of cell culture systems in the investigation of the effects of radiotherapy on molecular mechanisms such as cell cycle and chemotherapeutic resistance will form the basis of radiotherapy clinical applications.[5] For radiobiology research, optimizing the precision and measurement details of radiation dosimeters is of high importance in establishing and standardizing guidelines for future work.[6] In addition, it is important to prevent dosimetry from being a source of variability to eliminate the differences in the results according to the experimental design differences in these studies.[7] Radiotherapy, which is frequently applied postoperatively in breast cancer, provides a 20% reduction in regional recurrences. It also provides a 5% reduction in the risk of death.[8] Determining the optimal radiation dose to be applied to the tumor in radiotherapy in a way that causes the least damage to the surrounding tissue is critical in the treatment.[9] The aim of this study was to determine the feasibility of using cell culture systems in planning the radiation dose to be applied in breast cancer radiotherapy.

Materials and Methods

Cells

The triple-negative breast cancer cell line MDA-MB-231 was purchased from the American Type Culture Collection (ATCC, Wesel, Germany). Cells were cultured in media containing 10% fetal bovine serum (BIOIND, Israel), 2 mM L-glutamine (Sigma), and 1% antibiotic–antimycotic solution, high glucose DMEM (Capricorn, Germany). Culture conditions were 37ºC, 5% CO_2, and 100% humidity.

Radiation

In the treatment, radiation doses were calculated according to the surface area of the tumor that was proportioned to the surface area of the T-25 culture flasks. Different radiation doses of 2 Gy, 4 Gy, and 8 Gy were applied to the cells that were 70% confluent in T-25 flasks with X-rays at 6 MV (without smoothing filter) and 15 MV energies in Elekta Versa HD linear accelerator device. Then, 8 Gy was applied to the cells in the 6-well plate for the lateral motility test. For cell cycle analysis, radiation was applied at 8 Gy dose at a rate of 10.5 Gy/min, 2.7 Gy/min, and 0.3 Gy/min, and the Cells were exposed to radiation for 10 min.

Cell Proliferation

The effect of radiation dose intensity on the proliferation of breast cancer cells was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) test. For each experimental group, 2000 cells per well were seeded in 96-well plates (Nest Scientific USA, Inc.) and incubated at 37ºC with 5% CO_2. A quantity of 10 µL of MTT final concentration 0.5 mg/mL solution (Roche, Catalog Number 11465007001) was added to each well at 0 h and 72 h. It was incubated for 4 h in the dark and at 37ºC. An amount of 100 µL of dissolution solution was added to each well to dissolve the formazan crystals. It was mixed by pipetting and left for 15 min to ensure complete dissolution. Absorbance was recorded at 570 nm in a microplate reader (BioTek Synergy H1, BioTek Instruments, Winooski, VT, USA). Three values were averaged for each group and analyzed at 0 h and 72 h.

Cell Motility

The effect of radiation on the metastatic potential of MDA-MB-231 cells was analyzed by a wound healing test to determine the lateral mobility of cells. Cells (2×105 cells per well) were seeded in 6-well plates (Nest Scientific USA, Inc.). After 24 h, three wounds were created in each well using a P1000 pipette tip. The wells were washed once with fresh medium, and wound widths were recorded under an inverted microscope (Leica, Wetzlar, Germany). Then, an 8 Gy radiation dose was applied to these cells. The wound areas were measured at 0 h, 24 h, and 48 h by an inverted microscope (Leica, Wetzlar, Germany) at 4× magnification. Mobility was expressed as the percent reduction of wound area as compared with those of untreated cells. The closure area of the wounds was determined using the imageJ program, and the closure rate was calculated according to the control group.

Cell Cycle Analysis

MDA-MB-231 cells were seeded in 6-well plates and left for 24 h. After 24 h, it was administered at 8 Gy radiation intensity and at three different rates (high: 10.5 Gy/min, medium: 2.7 Gy/min, and low: 0.3 Gy/min). Cells were then harvested by trypsinization, washed with PBS, soaked in 70% cold ethanol overnight, and DNA was stained with propidium iodide after treatment with RNAase. Cell cycle analysis was performed by flow cytometry (BD Biosciences, USA), and cells in the G0/G1, S, G2/M, and lower G0/G1 phases were evaluated.

Statistical Analysis

All quantitative data are presented as mean±standard deviation (SD) from three independent experiments. Statistical analysis was performed using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY, USA: IBM Corp.). The Mann–Whitney U test and the Kruskal–Wallis statistical tests were performed. A value of p<0.05 was considered a significant difference.

Results

Antiproliferative Effect of Radiation on Breast Cancer Cells

The antiproliferative effect of radiation dose on breast cancer cells was evaluated by MTT analysis for 48 h after applying radiation at doses of 2 Gy, 4 Gy, and 8 Gy. Results were expressed as a percentage of control cell growth (Fig. 1). The decrease in cell growth after 24 h was 20%, 29%, and 186% in 2 Gy, 4 Gy, and 8 Gy radiation applied cells, respectively. At the 48th hour, the cell growth was decreased by 95%, 87%, and 69% in cells that received 2 Gy, 4 Gy, and 8 Gy radiation respectively. There was a significant increase in cell growth observed in all groups at 48 h compared with 24 h (p<0.005). There was a significant increase between the groups between the 24th and 48th hours (p<0.005). The proliferation of all irradiated groups decreased compared with the control group. However, the decrease in cell proliferation in 2 Gy, 4 Gy, and 8 Gy radiation groups was not found significant (p>0.005). As the 8 Gy applied group had the lowest cell count at the end of the 48th hour, lateral motility and flow cytometry analysis experiments were performed between the control and cells which received radiation at a dose of 8 Gy.

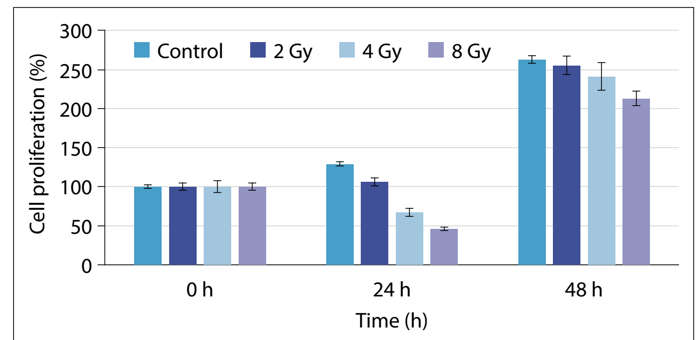

Figure 1. Effect of 2 Gy, 4 Gy, and 8 Gy radiation on the proliferation of MDA-MB-231 breast cancer cells. Bars represent the mean±SD of six experiments.

Effect of Radiation on the Metastatic Potential of Cells

A wound healing test was performed to evaluate the effect on the lateral motility of cells, which indicates their metastatic potential. Micrographs of the control and 8 Gy irradiated groups are given at 0 h, 24 h, and 48 h (Fig. 2). The area at 0 h of the control and 8 Gy irradiated groups was accepted as 100%. At all times, the wound healing was greatly impaired in irradiated groups (Fig. 2a). At 24 h, the area in the control group was closed by 53%, leaving 47% cell-free area, while the wound area in the 8 Gy group was closed by 21%, leaving 79% cell-free area. At the 48th hour, while the cell-free area in the control group was 26% and 51% in the 8 Gy group (Fig. 2b). Therefore, the metastatic behavior of irradiated MDA-MB-231 breast cancer cells is compared with untreated cells (p<0.05).

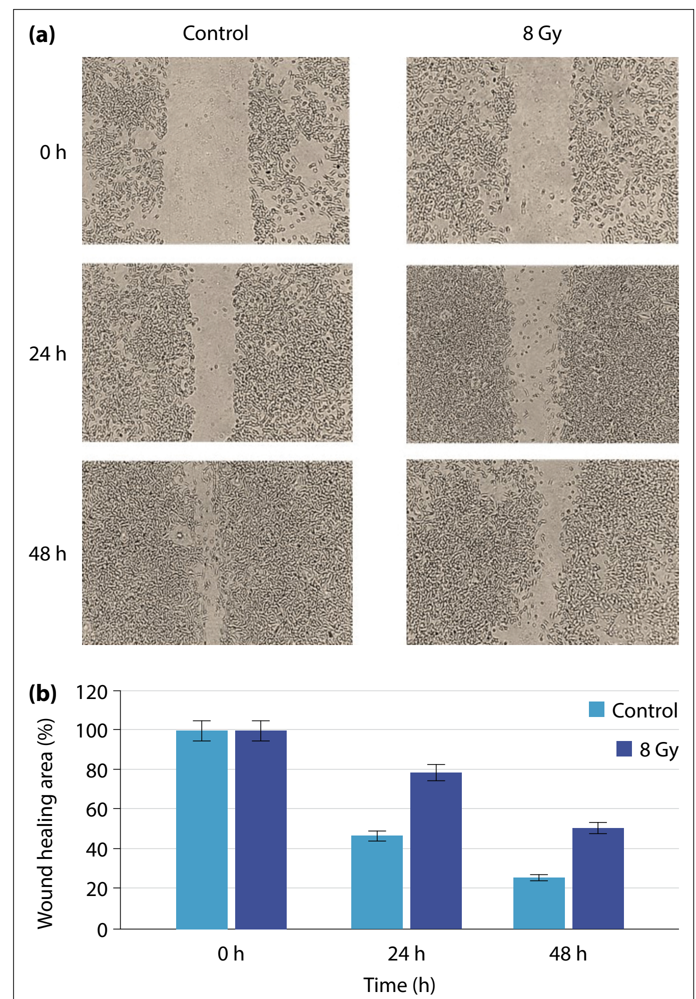

Figure 2. Effects of radiation on lateral mobility of MDA-MB-231 cells. Cells were treated with 8 Gy of radiation **(a)** and the plot of the average wound area is given **(b)**.

Radiation Prevents Transition from the G1 Phase to the S Phase

Radiation at a dose of 8 Gy was applied at different rates, and the cell cycle analysis of MDA-MB breast cancer cells

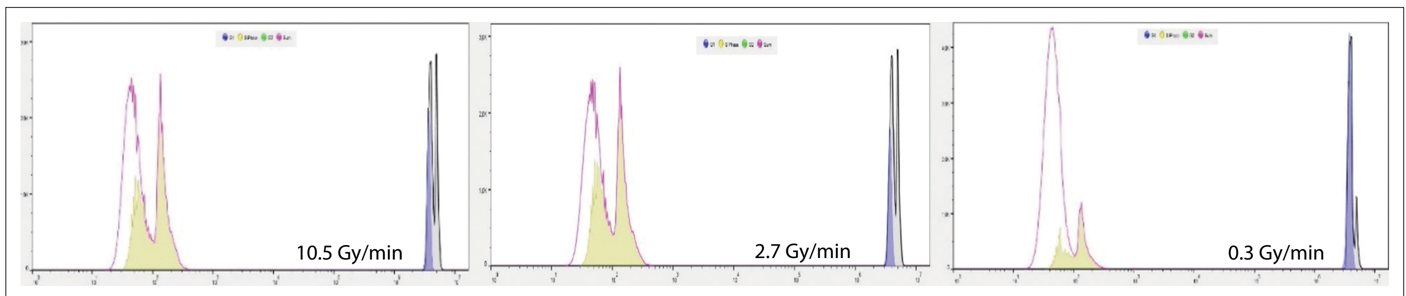

Figure 3. Cell cycle flow cytometry analysis of MDA-MB breast cancer cells irradiated at different rates after 8 days.

was carried out. As shown in Figure 3, the cell cycle analysis of irradiated MDA-MB-231 breast cancer cells has shown the delay in the cycle. The irradiation was given at rates of 10.5 Gy/min, 2.7 Gy/min, and 0.3 Gy/min for 8 days. It is found that the irradiated cells mostly remained in the G1 and S phases (Fig. 3). At 0.3 Gy/min, the number of cells in the G1 phase is higher than the number of cells in the S phase. No difference was found between 10.5 Gy/min and 2.7 Gy/min irradiation groups (Fig. 3).

Discussion

It was determined that the radiation applied to breast cancer cells at 2 Gy, 4 Gy, and 8 Gy dose intensities in the cell culture system caused a decrease in the proliferative and metastatic properties of the cells compared with the untreated cells. It was observed that at 8 Gy radiation intensity and 0.3 Gy/min dose rate, the cells remained mostly in the G1 phase. In a study conducted applying 9 Gy and 23 Gy doses of radiation to MCF-7 human breast cancer cells, it was observed that a typical aging phenotype was revealed by stopping cell proliferation.[10] In a study in which low-dose radiation (50 µGy/h) was applied to fibroblasts, it was shown that as compared with the control group, proliferation initially stopped, then increased, and the cells in the experimental group remained mostly in the G0/G1 phase.[11] This may be due to the emerging of mutations in genes that regulate the cell cycle such as cyclins or cyclin-dependent kinases. This in turn may lead to disruption of G1 to S transition and a decrease in proliferation.[12] It was observed that lung cancer cells cultivated as 3D culture in a 96-well plate spheroid model inhibited proliferation at a high rate in the application of 20 Gy for 4 min. In the same study, it was emphasized that 3D spherical cell colonies can be cultured longer than 2D cultures after a 20-day observation and that the 3D model is more suitable for radiobiological studies. It was stated that the use of 3D cell culture systems would be efficient for the optimization of individually planned radiotherapy treatment.[13] However, further studies are required to prove the efficiency of 3D tumor models. Mackonis et al.[14] stated that there was a scattering problem in the cells in which they planted a 24-well plate and that they sent 6 MV clinical photon irradiation and that it changed the number of adherent cells, and argued that in order to solve this problem, the cells should be surrounded by a phantom material equivalent to water. It may also help prove the efficiency of cell culture system assessment of therapy if other parameters of radiation are tested via cell culture systems.

Conclusions

It has been demonstrated that cell culture systems can be used for dosimetry studies in the radiation therapy of breast cancer. Detailed investigation of different molecular mechanisms by working mostly in cancer cell lines will contribute to the planning of radiotherapy.

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Conflict of Interest: None declared.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69(1):7–34. [CrossRef]
2. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011;147(2):275–92.
3. Akyolcu N, Kandem D. Meme Kanserinde Güncel Gelişmeler. Archives of Health Science and Research 2019;6(3):583–94.
4. Jutzy JMS, Lemons JM, Luke JJ, Chmura SJ. The evolution of radiation therapy in metastatic breast cancer: from local therapy to systemic agent. Int J Breast Cancer 2018;2018:4786819.
5. Hirst DG. The importance of radiobiology to cancer therapy: current practice and future perspectives. Clin Oncol (R Coll Radiol) 2007;19(6):367–9. [CrossRef]

6. Desrosiers M, DeWerd L, Deye J, Lindsay P, Murphy MK, Mitch M, et al. The importance of dosimetry standardization in radiobiology. J Res Natl Inst Stand Technol 2013;118:403–18.
7. Sjostedt S, Bezak E. Non-targeted effects of ionising radiation and radiotherapy. Australas Phys Eng Sci Med 2010;33(3):219–31. [CrossRef]
8. Zurrida S, Veronesi U. Milestones in breast cancer treatment. Breast J 2015;21(1):3–12. [CrossRef]
9. Senkus-Konefka E, Jassem J. Complications of breast-cancer radiotherapy. Clin Oncol (R Coll Radiol) 2006;18(3):229–35.
10. Bravatà V, Minafra L, Russo G, Forte GI, Cammarata FP, Ripamonti M, et al. High-dose Ionizing Radiation Regulates Gene Expression Changes in the MCF7 Breast Cancer Cell Line. Anticancer Res 2015;35(5):2577–91.
11. Truong K, Bradley S, Baginski B, Wilson JR, Medlin D, Zheng L, et al. The effect of well-characterized, very low-dose x-ray radiation on fibroblasts. PLoS One 2018;13(1):e0190330. [CrossRef]
12. Teyssier F, Bay JO, Dionet C, Verrelle P. Cell cycle regulation after exposure to ionizing radiation. [Article in French]. Bull Cancer 1999;86(4):345–57.
13. Tesei A, Sarnelli A, Arienti C, Menghi E, Medri L, Gabucci E, et al. In vitro irradiation system for radiobiological experiments. Radiat Oncol 2013;8:257. [CrossRef]
14. Claridge Mackonis E, Suchowerska N, Naseri P, McKenzie DR. Optimisation of exposure conditions for in vitro radiobiology experiments. Australas Phys Eng Sci Med 2012;35(2):151–7.