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Apoptosis Signalling Pathway in Cervical Cancer Cells Treated with Gama Radiation †

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† Presented at the 2nd International Cell Death Research Congress, Izmir, Turkey, 1–4 November 2018.

Published: 5 December 2018

Abstract: The objectives of this study are to identify changes in gene expression, which involved in apoptosis pathways induced by gamma radiation, Cervical cancer cells were exposed to various doses of a single fraction of gamma radiation. After incubation for different periods, the proliferation of C-4 I and HeLa cells were investigated by MTT assay, wile morphological features were assessed by fluorescent microscopy to measure the Apoptotic Index (AI). In addition, gene expression was evaluated using micro-array molecular processes, and signalling pathway analysis were performed. Gamma irradiation inhibits proliferation of HeLa and C-4 I cells in a time- and dose- dependent manner. From our results a significant difference was observed between HeLa and C-4 I cell lines ($p < 0.01$), whereas, HeLa cells seemed to be radio resistant, while C-4 I cells radio-sensitive. IC50 and AI dose for C-4 I and HeLa cells were 16 Gy and 32 Gy respectively. Microarray results monitored the expression of some factors that are known apoptosis activators were up regulated by gamma radiation treatment, whereas some anti-apoptosis members were down regulated. Pathway analysis identified that significant pathways related to apoptosis, WNT, cell cycle and P53 were significantly reinforced. These results give evidence that ionized radiation directly induces anti proliferative effects by converting the expression of genes related to apoptosis and cell proliferation pathways in HeLa and C-4 I cervical cancer cells.

Keywords: apoptosis; cervical cancer; gamma radiation; microarray; Gene Ontology

1. Introduction

More than half of all cancer patients receiving radiotherapy during the course of treatment, and it is the most effective therapy for cervical cancer in advanced stages [1]. Ionizing radiation leads to many cellular changes like pathways initiated at the level of the plasma membrane through activation of some receptors, while ionized radiation activates signaling pathways in the nucleus as a result of DNA damage, which result in a coordinate network of signal transduction pathways involved in apoptosis, stress response, cell cycle arrest and the activation of DNA repair processes. Ionizing radiation can also induce apoptosis via the generation of free radical oxygen species. Apoptosis is the major mode of programmed cell death, and is characterized by a series of morphological hallmarks, including nuclear DNA condensation, cell shrinkage and fragmentation, as well as plasma membrane blebbing, which formed the apoptotic bodies [2]. Understanding programmed cell death is a key to understanding the origin of tumors and to developing innovative strategies for cancer treatment [3]. Therefore, the present study aimed to determine whether gamma radiation could induce the expression of specific genes involved in apoptosis signaling pathways. Identifying upregulated or down-regulated genes helps us in better understanding the cancer dynamics, which might be used

to increase the therapeutic efficacy of radiotherapy and help identify markers and treatment targets for cervical cancer.

2. Materials and Methods

Cervical cancer C-4 I and HeLa cell lines (ATCC), maintained at 37 °C in Waymouth's MB 752/1 for C-4 I cells and Minimum Essential Medium, for HeLa cells, both mediums supplemented with 10% fatal bovine serum, 100 IU/mL penicillin, and 100 go/mL streptomycin, in a humidified atmosphere of 5% CO_2. To detach the cells from the flasks cells were "passaged" every 2 to 3 days using 0.25% trypsin [4]. Cells were plated in a 6-well plate at a density of 2.5×10^5 cells per well. After overnight incubation, cells were irradiated by different dozes of γ-^{60}Co radiation (2, 8, 16, 32 and 64 Gy) with a dose rate of 100 cGy/min. and incubated for 24, 48, 60 and 72 h. After irradiation, cell cultures were replaced and maintained at 37 °C, under 5% CO_2. For the irradiation groups, the time when the irradiation was finished was set as time 0. Cell proliferation was evaluated using MTT cell viability assay, as previously described [4,5]. In order to measure the AI, morphological features were assessed by fluorescent microscopy using DAPI stain, as previously described [4,5]. C-4 I and HeLa cells were irradiated by 16 Gy single dose of gamma radiation at a density of 2.5×10^5 cells per well. cells were harvested after incubation for 60 h and total RNA was extracted using High Pure RNA Isolation kit (Roche, Istanbul, Turkey) according to the manufacturer's instructions. RNA quantity was checked by measurement of the A 260/280 nm ratio. In order to evaluate the expression patterns of more than 48,000 transcripts in C-4 I and HeLa cells, Human HT-12 Expression Bead Chips with the Illumina Whole-Genome Gene Expression Direct Hybridization Assay system were used, according to the manufacturer's instructions. The signal was taken as the measure of mRNA abundance derived from the level of gene expression. Significance levels of differences between the groups were calculated for each probe set using FC and P-values ($p < 0.05$ and FC ≥ |2|). Differentially expressed genes were used as input for a network analyses that were performed with Gene Ontology (GO) network building tools (PANTHER). GO and pathway analysis were performed on the upregulated and down-regulated genes. Significant functions were identified based on p-value ($p < 0.05$) [5,6]. For the experimental cell proliferation and AI data sets, statistical significance was determined using one-way ANOVA and unpaired Students't test ($p < 0.05$).

3. Results

MTT assay showed that gamma irradiation inhibits proliferation of C-4 I and HeLa cells in a dose- and time- dependent manner. A significant difference was observed between C4-1 and HeLa cell lines ($p < 0.01$). From our results C-4 I cells seemed to be radio-sensitive, while HeLa cells were radio resistant. Our results showed that C-4 I and HeLa cell survival rates of all irradiated groups at 0-hour post-irradiation time were nearly the same at more than 70%, while the cell survival at 24 h post-irradiation time in the 16, 32 and 64 Gy groups were much lower, less than 70%. Our experiments showed that 16 Gy and 32 Gy were identified as the IC50 dose for C-4 I and HeLa cells respectively (Figure 1).

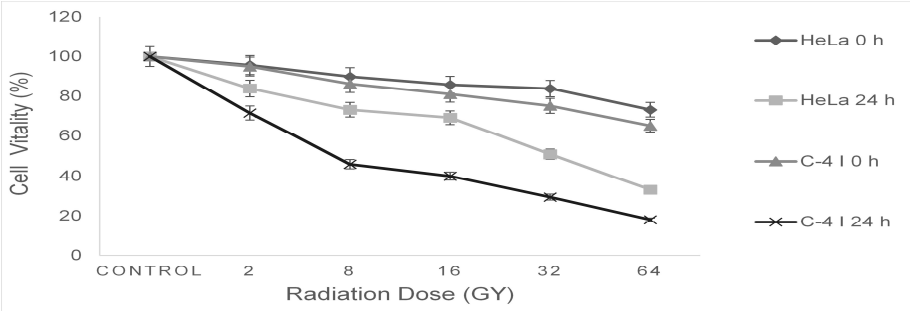

Figure 1. Effect of gamma radiation on survival of C-4 I and HeLa cells at 0 to 24 h post-irradiation time.

Fluorescent images of the nuclear morphology of C-4 I and HeLa cells in control and in all irradiated groups at 60 h post-irradiation time after DAPI staining were determined. The nuclei were normal in the control group; whereas the nuclei became condensed or fragmented in the all irradiated groups (Figure 2).

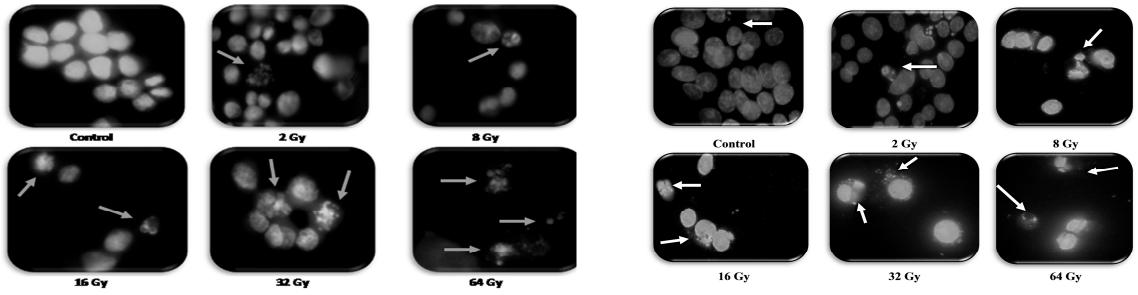

Figure 2. Representative fluorescent images of the nuclear morphology of C-4 I (**left**) and HeLa cells (**right**) in the control and all irradiated (2, 8, 16, 32 and 64 Gy) groups at 60 h post irradiation time, following DAPI staining and observed by a fluorescence microscope (X1000). Arrows represent apoptotic cells.

AI was induced by gamma radiation, and increased in a radiation dose and time-dependent manner. The highest AI values (at 60 and 72 h post-irradiation time) were reached more than 50% for 64 Gy and 32 Gy. While, at 0- and 24-h post-irradiation time the lowest AI values, less than 20% for all irradiated groups. 16 Gy and 32 Gy were determined as gamma radiation cytotoxicity and optimum AI for C-4 I and HeLa cells respectively.

For statistical analysis of microarray data, the study began with 47,231 probes, which filtered to 47,071 probes. Hierarchical clustering was performed on these filtered prob. For C-4 I there were 312 genes for which the expression changed significantly. From these 104 genes up-regulated, while 208 genes down-regulated. However, for HeLa cells there were 340 genes for which the expression changed significantly. From these 147 genes were up-regulated, while 193 genes were down-regulated.

We performed pathway analyses of differentially expressed genes in C-4 I and HeLa cells at 60 h after exposure to 16 and 32 Gy of gamma radiation respectively. Pathway analysis were performed on upregulated genes; 15 pathways are listed as best results. From these P53, WNT, PDGF, interleukin, chemokine and oxidative stress through the response signaling pathways were selected. Wile, pathway analysis was performed on down-regulated genes, 19 pathways were listed as best results. In particular, apoptosis, inflammation, the chemokine and cytokine signaling pathway, PDGF, glycolysis, RAS, TGF-β, WNT, PDGF, MAPK, Oxidative stress response and P53 signaling pathways were observed as a significant down-regulation pathways.

4. Discussion

In the present study, C-4 I and HeLa cell growth was depressed by large doses of gamma radiation (8, 16, 32 and 64 Gy), but not by low doses 2 Gy at 24 h post-irradiation time. In addition to decreasing cell proliferation and viability, a single dose of gamma radiation induced apoptosis in C-4 I and HeLa cells. From our results the percentage of condensed or fragmented nuclei increased with time and dose of radiation [6]. AI assay results was showed that the level of apoptosis increased with gamma radiation dose. These results indicate that irradiation primarily affects the C-4 I and HeLa cells proliferation by inducting cell apoptosis. These results are consistent with those of previous studies [3,6]. In this study ITPR1 gene expression triggers the death of C-4 I cells by WNT and PDGF signaling pathways [7]. In a study conducted by Davies et al. [8] PERP was originally identified as a novel candidate effector in the P53-dependent apoptotic pathway, being a transcriptional target gene of P53 that is specifically induced during apoptosis and not during cell cycle arrest.

This in vitro study has identified genes alterations by gamma radiation in human cervical cancer C-4 I and HeLa cells. Radiation-induced conversional genes and gene-related pathways in these cells

may provide the theoretical basis for the treatment of radiation therapy in human cervical carcinoma. Identification of specific genes may be beneficial in novel treatment strategy to increase the cancer cell sensitivity to radiotherapy by modulation of many genes expression.

References

1. Nair, S.; Nair, R.R.K.; Srinivas, P.; Srinivas, G.; Pillai, M.R. Radiosensitizing effects of plumbagin in cervical cancer cells is through modulation of apoptotic pathway. *Mol. Carcinog.* **2008**, *47*, 22–33.
2. Wyllie, A.H. A Brief Review of Apoptosis Biology. *Mol. Neurobiol.* **2010**, *42*, 4–9, doi:10.1007/s12035-010-8125-5.
3. Bian, J.; Wang, X.; Yun, J.; Cao, R.; Cao, Y.; Liang, J.; Ma, B.F. Single-fraction γ–^{60}Co radiation induces apoptosis in cultured rat C6 cells. *Ann. Saudi Med.* **2012**, *32*, 269–275.
4. Özcan, G.; Dağdeviren, Ö.; Akman, G.; Khalilia, W.; Tezel Yetiz, B.; Karagöz, A.; Melikoglu, G.; Anil, S.; Kultur, S.; Sutlupinar, N. Screening for antitumour activity of various plant extracts on HeLa and C 4-I cell lines. *J. Buon* **2016**, *21*, 1552–1560.
5. Khalilia, W.M.; Özcan, G.; Karaçam, S. Gene Expression and Pathway Analysis of Radiation-Induced Apoptosis in C-4 I Cervical Cancer Cells. *J. Autoimmun. Res.* **2017**, *4*, 1014.
6. Mi, H.; Muruganujan, A.; Casagrande, J.T.; Thomas, P.D. Large-scale gene function analysis with the PANTHER classification system. *Nat. Protocols* **2013**, *8*, 1551–1566.
7. Elkoreh, G.; Blais, V.; Béliveau, E.; Guillemette, G.; Denault, J.B. Type1inositol-1,4,5 trisphosphate receptor isalate substrate of caspases during apoptosis. *J. Cell. Biochem.* **2012**, *113*, 2775–2784.
8. Davies, L.; Gray, D.; Spiller, D.; White, M.R.H.; Damato, B.; Grierson, I.; Paraoan, L. P53 apoptosis mediator PERP: Localization, function and caspase activation in uveal melanoma. *J. Cell. Mol. Med.* **2009**, *13*, 1995–2007.