EVALUATION OF RADIOINDUCED DAMAGE AND REPAIR CAPACITY IN HUMAN BREAST CANCER CELLS, MCF-7 AND T-47D

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ABSTRACT

Ionizing radiation is an established etiologic agent for breast cancer, but on the other hand it is a therapeutic modality used in cancer treatment. Accumulation of DNA damage and deficient DNA repair are considered as factors of susceptibility that predispose individuals to breast cancer development. In the present study, genetic damage induced by gamma radiation and repair capacity in the target cells, i.e. cells originating of breast cancer, were analyzed using micronucleus test and comet assay (single-cell alkaline gel electrophoresis). So, two breast tumor cell line, MCF-7 and T-47D were irradiated in a ^{60}Co source (0.722 Gy/min) with various doses (0.5; 1.0; 2.0; 4.0 and 5.0 Gy). Cytogenetic data showed similar spontaneous damage of two cell lines, the radioinduced damage, however, was higher in T-47D, starting from 2 Gy, with a more accelerated proliferation rate than MCF-7 at all doses analyzed. Both tumor cell lines were capable to repair a considerable part of radioinduced damage within 1 hour after exposure, indicating a relative radioresistance of theses cell lines to the genotoxic action of ionizing radiation.

1. INTRODUCTION

Cancer is a multifactorial disease, resulting of a complex interaction of diverse risk factors, such as environmental, hormonal and hereditary ones. Among several types of neoplasm, breast cancer has attracted a special attention over the last years because of its high incidence among women. In industrialized countries, breast cancer represents one on three of all female neoplasm [1]. About 90% of cases are considered sporadic, attributed to somatic events and about 10% have a family history and only 4-5% of these are decurrent of hereditary factors [2].

Approximately 80-90% of the hereditary cases of breast and ovarian cancer are associated with germline mutations in BRCA1 and BRCA2 tumor suppressor genes [1, 3]. However, the molecular analysis carried out on tumor samples has failed to identify somatic BRCA1 and BRCA2 mutations, suggesting that these genes may not play a role in the genesis of common sporadic breast cancer [4]. The etiology of hereditary and sporadic breast cancer can be different [5]. Mechanisms underlying sporadic breast cancer are not fully understood, but environmental exposure may be involved [2].

On this basis, several studies were conducted in order to investigate the relation between the factor of susceptibility and the genotoxic exposure in breast cancer patients. A high sensitivity and a reduced repair capacity in peripheral blood lymphocytes from breast cancer patients when submitted to X-rays and gamma radiation [6-9], UV light [10] and various chemotherapeutic drugs have been reported [2, 11]. Higher levels of DNA damage and deficient or suboptimal DNA repair may predispose individuals to breast cancer development [12]. Impaired DNA repair can favor the malignant transformation of cells due to the accumulation of somatic mutations in target genes, consequently increasing the risk of tumor induction.

However, once established the tumorigenic process, an enhanced resistance to genotoxic agents and an enhanced repair capacity by part of neoplastic cells, may play a role in the resistance of cancer cells to therapeutic drugs and radiation and therefore can be considered as one of the main obstacles to cancer therapy [2].

In spite of many studies realized, there is, however, little information about the cytogenetic effect derived from the exposure of the target cells (cells originating from breast cancer) to mutagenic/carcinogenic agents.

Epidemiologic data point out to the ionizing radiation as an established etiologic agent for breast cancer [12], but on the other hand this is a therapeutic modality widely used in cancer treatment. In this sense, studies of damage and repair in cell lines originating from breast cancer may offer a support to a better understanding of the cellular and molecular mechanisms involved in carcinogenesis, besides being of great importance not only from an oncologic and therapeutic viewpoint but also for radiobiological purposes.

The goal of the present study was to analyze the response of cells originating from breast cancer (T-47D and MCF-7) when exposed to ^{60}Co gamma radiation (0.5 to 5 Gy), by using

DNA damage and repair capacity as parameters of radiosensitivity, via micronucleus and single cell gel electrophoresis (Comet assay) techniques. Micronuclei represent mutations at the chromosomal level that mainly derive from unrepaired DNA lesions, particularly double strand- breaks [13]. The alkaline version of comet assay is able to detect a wide range of DNA damage including single- and double-strand breaks, as well as alkali-labile sites, major lesions induced by ionizing radiation, at the individuals cells level. It is a relatively simple, rapid and sensitive visual technique, without need of cellular proliferation [14].

2. MATERIALS AND METHODS

2.1. Cell lines

Human breast epithelial carcinoma cell lines, T-47D and MCF-7 were purchased from American Type Culture Conditions (ATCC, Rockville, MD). The cells were maintained in culture flasks with RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (Gibco), 1% penicillin and streptomycin (Sigma) and incubated at 37C in the presence of 5% CO_2.

2.2. Irradiation

MCF-7 and T-47D cells in the exponential growth phase were exposed to ^{60}Co gamma radiation source of the panoramic type (Yoshizawa Kiko, Japan) (0.722 Gy/min) in Eppendorf tubes (1 X 10^6 cells/ml) at doses of 0.5; 1.0; 2.0; 4.0 and 5.0 Gy at room temperature. One tube was maintained as control (0 Gy).

2.3. Micronucleus test

The micronucleus test was carried out by the cytokinesis block method using cytochalasin B. Basically, after irradiation with ^{60}Co; the cells were seemed in medium contained cytochalasin B (2 μg/ml) (Sigma) to obtain binucleated cells. After 72 h of culture, cells were trypsinized, treated with isotonic solution, fixed with acetic acid and methanol (1:3) and stained with 10% Giemsa dye in phosphate buffer, pH 6.8 and examined with a Carl Zeiss microscope at a X400 magnification. The criteria adopted by Countryman and Heddle [15] were used for the identification of micronucleus. For each dose, 500 binucleated cells containing up to 5 micronuclei were analyzed for 6 independent assays for each cell line. All accompanying mononucleated and multinucleated cells were counted for the determination of the proliferation index (PI) according to the formula: PI = [(number of mononucleated cells)+ 2 (number of binucleated cells) + 3 (number of multinucleated cells)] / total number of cells X 100.

2.4. Comet assay (single-cell alkaline gel electrophoresis)

The alkaline version described by Singh et al. [16] was used. For the evaluation of initial damage, immediately after irradiation, the samples were kept on ice to avoid repair of radioinduced damage (no incubation period). For repair evaluation, the cells were kept on room temperature for 30 minutes, 1 and 3 h after irradiation. Briefly, cell suspensions (10 μl),

irradiated or not, were embedded between two layers of agarose (Sigma), normal- and low-melting, onto frosted microscopic slides (in triplicate) and allowed to gel at 4C. The slides were treated with lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% triton X-100 and 10% DMSO) for 2h at 4C. After lysis, the cells were then placed for 30 min in electrophoresis buffer containing 1 mM EDTA, 300 mM NaOH, pH > 12. Electrophoresis was performed at 25 V, 300 mA (Pharmacia) for 30 min at 4C. The slides were then neutralized with 0.4 M tris buffer, pH 7.5 and stained with ethidium bromide (20 μg/ml; Sigma). The cells were analyzed with a fluorescent microscope (Carl Zeiss) at 200 X, with a 515-560 nm exciting filter and a 590 nm barrier filter. Approximately 50 comets were analyzed for each radiation dose for 3 independent assays for each cell line. For the evaluation of radioinduced damage, the cells were assigned to 5 classes (0-4) based on the visual aspect of the comets, considering extent of DNA migration according to the criteria established by Visvardis et al. [17]. Comets with a bright head and no tail were classified as class 0 (cells with no DNA migration) and comets with a small head and a long tail were classified as class 4 (severely damaged cells). Comets with intermediate characteristics were assigned to classes 1, 2 and 3. Radioinduced DNA damage (DD) were estimated quantitatively using the equation described by Jaloszynski et al. [11]. DD values ranged from 0 to 400 arbitrary units (au), corresponding to situations ranging from no damaged comets to all comets extremely damaged:

$$DD = (n1 + 2n2 + 3n3 + 4n4)/ (\Sigma/100) \quad (1)$$

n1-n4 = number of class 1 to 4 comets
Σ = total number of scored comets, including class 0

2.5. Statistical analysis

The statistical analyses were performed using the Graph Pad Prism Software (version 2.0). For the comparison of the data between the two cell lines, a Student`s t-test was applied.

3. RESULTS

Table 1 shows the cytogenetic data obtained for breast cancer cell lines, irradiated with various doses of gamma radiation. In both cell lines, the frequency of micronucleus (number of micronucleus/cell), as well as the % of binucleated cells containing micronucleus, increased as a function of the dose radiation. The spontaneous damage was similar for the two cell lines, the radioinduced damage, however, was higher in T-47D cells than MCF-7 cells, starting from 2 Gy. Besides that, T-47D cells presented a higher proliferation rate in relation to MCF-7 cells ($p < 0.05$) meaning that T-47D with more radioinduced damage proliferate more quickly than MCF-7 cells.

Table 1. Frequencies, distribution of micronucleus (MN) and proliferation index in MCF-7 and T47-D binucleated cells (BNC) after exposure to ^{60}Co.

Cell lines	Dose (Gy)	Total of BNC analyzed	BNC with MN 1	2	3	4	5	% BNC with MN (mean ± SEM)	MN/BNC (mean ± SEM)	Proliferation index
MCF-7	0.0	3004	35	6	1	0	0	42 (1.4 ± 0.7)	50 (0.01 ± 0.01)	1.61 ± 0.06
	0.5	3031	57	11	0	1	0	69 (2.2 ± 1.2)	83 (0.02 ± 0.01)	1.62 ± 0.07
	1.0	3006	73	19	5	0	0	97 (3.2 ± 1.8)	126 (0.04 ± 0.02)	1.61 ± 0.06
	2.0	3000	115	23	3	0	0	141 (4.7 ± 1.7)	170 (0.05 ± 0.02)	1.59 ± 0.02
	4.0	3096	190	47	16	4	2	259 (8.3 ± 2.1)	358 (0.11 ± 0.03)	1.58 ± 0.07
	5.0	2963	208	84	25	9	3	329 (11.1 ± 3.0)	502 (0.16 ± 0.06)	1.57 ± 0.03
T-47D	0.0	3056	40	10	0	0	0	50 (1.63 ± 0.58)	60 (0.02 ± 0.01)	1.81 ± 0.13
	0.5	3150	65	10	0	0	0	75 (2.42 ± 0.90)	85 (0.02 ± 0.01)	1.82 ± 0.08
	1.0	3010	107	13	2	0	0	122 (4.07 ± 0.85)	139 (0.04 ± 0.01)	1.78 ± 0.14
	2.0	3066	201	37	6	1	0	245 (8.02 ± 1.11)	297 (0.09 ± 0.01)	1.77 ± 0.12
	4.0	3040	320	104	23	4	0	451(14.70 ± 4.52)	613 (0.20 ± 0.07)	1.77 ± 0.13
	5.0	3092	336	143	52	16	4	551(17.73 ± 1.64)	862 (0.27 ± 0.05)	1.69 ± 0.15

The results obtained with respect to DNA migration and efficiency in repair, by comet assay, analyzed at different times after irradiation, are presented in Figure 1. Basal values detected by visual classification ($p > 0.05$) showed no significant differences between the cell lines. No significant difference was also observed in basal values with respect to the time of incubation ($p > 0.05$) in both cell lines.

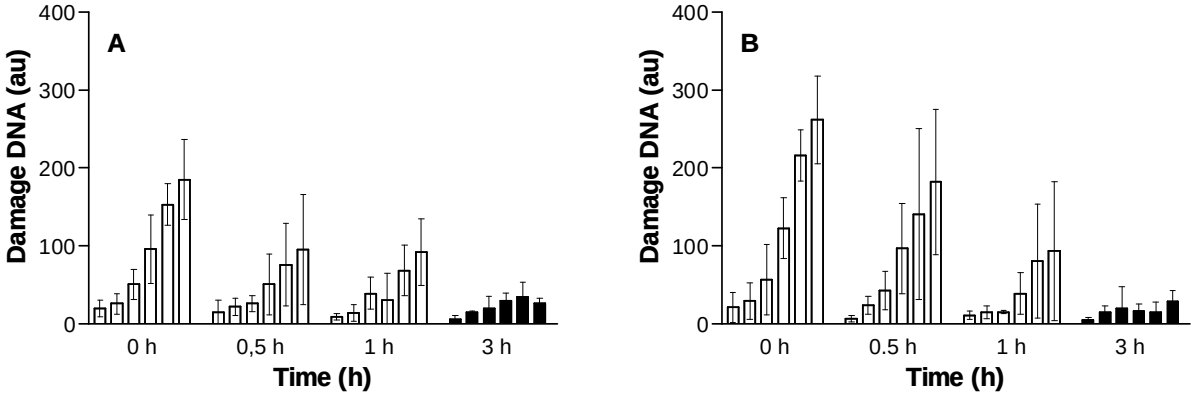

Figure 1. Radioinduced DNA damage and repair obtained for tumor cell lines, MCF-7 (A) and T-47D (B), analyzed immediately (0h), 0.5, 1 and 3h with control and a dose range of 0.5 to 5.0 Gy after in vitro exposure to ^{60}Co.

The quantitative estimation of radioinduced DNA damage based on visual classification by comet assay is presented in Table 2. In both cell lines, the DNA migration rate increased as a function of dose, with a tendency to decrease with time after exposure. DD values showed a considerable reduction after 1h when compared with the values obtained immediately after exposure (0h). T-47D cells presented a greater amount of damage than MCF-7 cells, i.e. the radioinduced damage was less efficiently repaired. However, after 3h of exposure, apparently, both cell lines showed a similar quantity of residual damage.

Table 2. Mean values of DNA damage obtained for tumor cell lines originating from human breast, processed 0 or 0.5, 1 and 3 h after exposure to ^{60}Co.

MCF-7				T-47D			
Time after irradiation	Dose (Gy)	Number of cells	DNA damage (au)	Time after irradiation	Dose (Gy)	Number of cells	DNA damage (au)
0h	0.0	150	20.0 ± 10.5	0h	0.0	150	21.3 ± 19.6
	0.5	150	26.0 ± 13.1		0.5	150	29.3 ± 23.1
	1.0	150	50.6 ± 19.4		1.0	150	56.6 ± 45.0
	2.0	150	96.0 ± 43.8		2.0	150	122.6 ± 38.8
	4.0	150	153.3 ± 26.6		4.0	150	216.0 ± 32.9
	5.0	150	185.3 ± 51.0		5.0	150	261.3 ± 56.0
0.5 h	0.0	150	15.3 ± 15.0	0.5h	0.0	150	6.6 ± 4.1
	0.5	150	22.0 ± 11.1		0.5	150	24.0 ± 11.1
	1.0	150	26.0 ± 10.0		1.0	150	42.6 ± 24.6
	2.0	150	50.6 ± 39.1		2.0	150	96.6 ± 57.4
	4.0	150	76.0 ± 53.0		4.0	150	140.6±109.8
	5.0	150	95.3 ± 70.4		5.0	150	182.0 ± 92.9
1h	0.0	150	9.3 ± 4.1	1h	0.0	150	11.3 ± 5.0
	0.5	150	14.0 ± 10.3		0.5	150	15.3 ± 8.3
	1.0	150	39.3 ± 20.5		1.0	150	15.3 ± 2.3
	2.0	150	30.6 ± 34.0		2.0	150	39.3 ± 26.4
	4.0	150	68.6 ± 32.3		4.0	150	80.6 ± 73.3
	5.0	150	92.0 ± 42.7		5.0	150	93.3 ± 88.8
3 h	0,0	150	6.0 ± 5.2	3h	0,0	150	5.3 ± 3.0
	0,5	150	14.6 ± 2.3		0,5	150	14.6 ± 8.3
	1,0	150	20.0 ± 15.6		1,0	150	20.0 ± 27.7
	2,0	150	29.3 ± 10.0		2,0	150	16.6 ± 9.2
	4,0	150	34.6 ± 18.9		4,0	150	15.3 ± 12.8
	5,0	150	26.6 ± 6.4		5,0	150	28.6 ± 14.0

4. DISCUSSION

In the present study, the behavior of two breast cancer cell lines submitted to the action of ionizing radiation was investigated with the main focus on DNA damage and repair capacity. By cytogenetic technique as well as comet assay, T-47D cells showed higher DNA damage compared to MCF-7 cells. The results indicate that T-47D cells responded to the genotoxic action of ionizing radiation with a higher proliferative potential. Both cell lines showed efficient repair capacity, once that 1h after the irradiation a considerable part of the radioinduced damage was repaired. After 3h, a similar amount of residual damage was presented by both cell lines when compared with the initial damage, determined immediately after exposure.

In a previous paper [8] we demonstrated that most of the radioinduced damage in peripheral lymphocytes from healthy individuals was repaired within 3h, while breast cancer patients still presented more residual DNA lesions even after 24h. However, these described groups (healthy and breast cancer patients) presented quantitatively similar radioinduced damage, immediately after exposure, that were significantly higher in comparison with the two tumoral cell lines, MCF-7 and T-47D cells at all doses and at different times after irradiations.

It is well known that T-47D cells present a mutated p53 gene [18], while MCF-7 cells are carriers of wild-type p53 [19]. This tumor suppressor gene participate in various cellular processes, including cell repair, apoptosis and cell cycle checkpoints [20]. However, T-47D cells showed a relatively efficient repair capacity and more non-repaired double-strand breaks than MCF-7 cells, producing a higher number of micronucleus.

The double-strand breaks are considered, among various types of DNA lesions induced by ionizing radiation, to be the most difficult to repair and most relevant from the standpoint of biological effects, i.e. the lack of repair or misrepair of double-strand breaks underlie effects such as cell killing, mutation and cancer [21]. Taken together these observations, it can be suggested that repair enzymes, other than those mediated by p53 or other double-strand breaks repair pathways, can be active in these cells.

Deficient DNA repair appears to be a predisposing factor in the development of cancer. On the other hand, the mechanism by which cancer cells become resistant to ionizing radiation and chemotherapy drugs is not clear, but enhanced DNA repair of the lesions is a possible factor [22]. So, several plausible mechanisms responsible for increasing cell resistance were proposed. These include DNA repair status, altered oncogene expression and compensatory defense mechanisms that involve elevated expression of antioxidant enzymes [23].

Nevertheless, further investigations are necessary for a better analysis of the nature of radioinduced lesions in DNA and of cell repair, and also for a better understanding of the radiobiological phenomena involved.

5. CONCLUSIONS

In summary, the results obtained showed relative radioresistance of the human breast cancer cells, MCF-7 and T-47D to the genotoxic action of gamma radiation. T-47D cells presented a higher radiosensitivity and a higher proliferative rate compared to MCF-7 cells. The next steps of the study will include the evaluation of cell death in these tumor cell lines and a comparison with non tumorigenic human breast cells, MCF-10A.

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