**G₀ and G₂ Chromosomal Assays in the Evaluation of Radiosensitivity in a Cohort of Italian Breast Cancer Patients**

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**Breast Cancer/Individual radiosensitivity/G₀ and G₂ radiosensitivity assays.**

Breast cancer (BC) is the most common type of malignancy in female patients and radio-treatment is the conventional therapy even if a great number of studies reported that enhanced sensitivity to ionizing radiation as measured as chromosome effects is present in a significant proportion of cancer patients, including breast cancer ones. In this study we analysed whether peripheral blood lymphocytes from sporadic BC patients and healthy subjects showed a different sensitivity to ionizing radiation and whether cytogenetic radiosensitivity may serve as a breast cancer risk biomarker. To test this hypothesis, the *in vitro* radiation sensitivity was measured by using both G₀ and G₂ chromosome radiosensitivity assays, on 46 subjects (23 BC patients and 23 healthy subjects). Results show that cancer patients are more radiosensitive than healthy controls and that G₂ assay could be more appropriate to define the individual radiosensitivity if compared to G₀ assay.

**INTRODUCTION**

Breast cancer (BC) is worldwide the most common type of female cancer and the incidence rates have risen during the last century, although recent data reveal a statistically significant decline in breast cancer frequency." It is known that only a small percentage of BC patients have a strong genetic predisposition caused by the highly penetrant BRCA1 and BRCA2 genes; instead, breast cancer is principally represented by sporadic cases related to mutations in different genes than BRCA.² A substantial proportion of people may be predisposed to this pathology through mutations in low penetrance genes, such as those genes involved in the processing of DNA damage.³,⁴

Generally, a great number of studies reported that enhanced sensitivity to ionizing radiation as measured as chromosome effects is present in a significant proportion of cancer patients.³,⁵,⁶ As a consequence, the connection between cancer predisposition and radiosensitivity has led to the suggestion that chromosomal radiosensitivity has to be carefully investigated as possible marker of cancer prone-

Several experimental approaches have been applied in order to compare radiosensitivity between cancer patients and healthy subjects. The Comet assay to evaluate the presence of DNA single and double strand breaks has been extensively used with conflicting results showing both a similar background and radiation induced DNA damage in peripheral blood lymphocytes from breast cancer patients and healthy individuals⁷,⁸ and an increased basal as well as an elevated radiation induced damage in BC patients.⁹-¹¹

Our group has recently shown that BC patients have a higher extent of basal DNA damage and an higher mean value of induced damage after irradiation compared to healthy controls.¹²

Individual radiosensitivity has been also carried out in human lymphocytes by using cytogenetic biomarkers. Among these, the G₂ and G₀ assays seem to be the most relevant. Both tests are largely determined by genetic factors.¹³ In particular, the G₂ chromatid break assay can be considered as the standard measure of individual chromosomal radiosensitivity.¹⁴,¹⁵ In general the two assays are not correlated with each other because different DNA damage processing mechanisms are operating in G₀ and G₂ phases of the cell cycle.³,¹⁶,¹⁷

The aim of this study was to evaluate whether sporadic breast cancer patients and healthy subjects showed a different sensitivity to ionizing radiation and whether chromosome radiosensitivity may serve as a breast cancer risk biomarker. To test this hypothesis, the *in vitro* radiation sensitivity of peripheral blood lymphocytes obtained from 23 control sub-
jects and 23 BC patients was measured by using both G0 and G2 chromosome radiosensitivity assays.

MATERIALS AND METHODS

Study population

Sporadic breast cancer patients (23 subjects; age range 40–78 years; mean age 54 year) were recruited at Radiotherapy Unit San Camillo-Forlanini Hospital (Rome), after breast-conserving surgery and before receiving chemotherapy and/or primary radiotherapy. Healthy subjects enrolled in the study (23 subjects; age range 40–72 years; mean age 48 year) were cancer-free individuals randomly selected from women attending health clinic examination as a part of a breast cancer risk evaluation project of the same hospital. Women with a prior history of cancer and breast disease were excluded. All donors completed a written questionnaire to obtain information on their lifestyle, such as dietary habits, medical history and exposure to chemical and physical agents. Individuals having confounding factors, other than smoking (drug and alcohol consumption, recent radio-diagnostic exposure, major illness) were excluded. Overall, 20 BC and 21 control subjects were non-smokers. The remaining subjects declared to smoke 5 cigarettes/day, consequently we considered smoke not a relevant confounding factor.

The local Ethical Committee approved the study design and all donors gave informed consent.

A 5 ml blood sample was taken from each BC patient and healthy subject. The samples were coded to ensure anonymity.

G2 chromosome radiosensitivity assay

Peripheral blood sample from all donors was drawn into lithium heparine vacutainers (Euroclone, Milan, Italy) and cultured within 24 h from the blood collection. The G2 radiosensitivity assay was performed as described by Scott and co workers, with minor changes. In brief, 0.5 ml of heparinized blood was added to 4.5 ml of complete RPMI-1640 Dutch Modified (Euroclone, Milan, Italy) supplemented with 10% heat-inactivated foetal bovine serum (Euroclone, Milan, Italy), 1% L-glutamine (Euroclone, Milan, Italy) and 1.5% penicillin and streptomycin (5000 IU/ml and 5000 mg/ml, respectively) (Euroclone, Milan, Italy). Lymphocytes were stimulated to divide with 1% phytohemagglutinin (Gibco-Invitrogen, Carlsbad, CA) was added to the cell cultures, in order to allow stimulated lymphocytes to divide. After an incubation period of 48 h at 37°C, 100 μl of colcemid (10 μg/ml, Sigma-Aldrich) were added to the cell cultures for further 2 h. Cell cultures were then fixed and air-dried metaphases were set and stained as described for the G2 assay.

A total of 100 well-spread metaphases containing (46 ± 1) centromers were examined for each culture, on coded slides. Chromosome- and chromatid-type aberrations, but not gaps, were recorded. The CsA value is the sum of Dic + CR and excess acentric fragments (Ace). We did not observe any translocation.

In total, the G0 analysis was conducted on 19 healthy controls and 18 BC patients because in some cases (probably for technical problems related to the hypotonic treatment) metaphases were not well spread and consequently the scoring were not reliable.

Statistical analysis

To verify the distribution of G2 phase index values and chromosome aberrations in BC patients and healthy subjects, we used the Kolmogorov-Smirnov test. Assuming normal distribution, we applied the Student’s t-test to compare controls vs patients. Moreover, Pearson correlation coefficient was used to analyse the correlation between G2 and the G0 assays data both in healthy subjects and BC patients.
Statistical analyses were performed using GraphPad software Instat (GraphPad, San Diego, CA, USA).

RESULTS AND DISCUSSION

In order to verify the hypothesis of evaluating individual radiosensitivity through the measure of X ray-induced chromosomal damage, we analysed G0 and G2 induced chromosome damage in lymphocytes of a group of sporadic breast cancer patients (analysed before any kind of chemo- or radio-therapy treatment) in comparison to a group of healthy subjects. The choice of BC patients as study population was due to published observation that deficiency or impairment in DNA repair capacity can be a predisposing factor in familial and in some sporadic breast cancer cases. Genomic instability has also been described in various cancer diseases including breast cancer. Moreover, in some cases, it has been shown that sensitivity to the induction of chromosome damage by ionising radiation is higher in breast cancer patient lymphocytes than in healthy subjects. Generally, a number of studies reported that a higher sensitivity to the damage caused by ionising radiation at chromosome level is present in a significant proportion of cancer patients.

As a consequence, the connection between cancer predisposition and radiosensitivity has led to the suggestion that chromosomal radiosensitivity may be carefully investigated as it could be a marker of cancer proneness.

Our results for both G0 and G2 assays obtained on healthy subjects and BC patients are shown in Fig. 1. The mean basal frequency of aberration yield in BC patients was not significantly higher than that observed in healthy controls. When we compare the results obtained after X ray treatment, we always found higher mean values in BC patients compared to healthy controls. In particular comparing the mean frequency of chromatin breaks we found a statistically significant (p < 0.0001) higher mean value in BC patients (2.1 vs 1.19) (Fig. 1). The same significant p value was also found in comparing the mean frequency of total amount of chromosome aberrations (CsA tot) between the two groups (Fig. 1). As far as the mean frequency of dicentric chromosomes and centric rings, BC patients showed a significantly higher value, with p = 0.02 (Fig. 1).

These data indicate that lymphocytes of our BC patients are more sensitive to ionizing radiation compared to those of healthy subjects when we consider both G2 and G0 radiosensitivity assay values. This result is in agreement with our previous paper where the same subjects (both patients and healthy controls) were analysed by the Comet assay. In that study we also found a higher basal DNA damage and an impairment of DNA repair capacity associated with

![Fig. 1.](image-url)  
*Fig. 1. G2 and G0 mean frequencies (± SD) observed in lymphocytes of healthy subjects (HS) and BC patients before (white columns) and after X ray treatment (black columns). CtA = Chromatid Aberrations; CsA tot = sum of Dicentric + Rings + excess acentric fragments; DIC + CR = Dicentrics + Rings.  
**p < 0.0001 *p = 0.02 at Student’s t test
radiotherapy-related side effects in BC patients.

Furthermore, other data in literature showed enhanced chromosomal radiosensitivity in sporadic breast cancer patients.\textsuperscript{3,16,24-26} More recently, Lou and co-workers found a significant difference in lymphocyte radiosensitivity between breast cancer patients and healthy subjects by using three different assays: Comet assay, cytokinesis-block micronucleus assay and 6-TG-resistant cells scored assay.\textsuperscript{27}

Moreover, we found a higher but not statistically significant individual variability in G\textsubscript{2} assay values respect to G\textsubscript{0} assay data (data not shown).

We also analysed the correlation between G\textsubscript{2} and G\textsubscript{0} radiosensitivity assays within healthy subjects and BC patients. When Pearson correlation coefficient was calculated no significance was observed neither in the control group nor in the patient group.

The lack of correlation we observed is in agreement with Borgmann and co-worker data\textsuperscript{17} who analysed the presence of ionizing radiation-induced chromosome damage in 15 monozygotic twin pairs finding an excellent correlation among twins for G\textsubscript{2} assay values and any correlation between the G\textsubscript{0} and G\textsubscript{2} radiosensitivity assays. The lack of correlation between G\textsubscript{0} and G\textsubscript{2} chromosomal radiosensitivity has been also reported by other Authors\textsuperscript{3,16} who speculated this finding to the different mechanisms of chromosomal radiosensitivity operating at specific and different stages of the cell cycle including cell cycle checkpoint control,\textsuperscript{5} defects in DNA repair and difference in chromatin structure which influence the conversion of DNA damage into chromosome breaks.

Moreover, the chromosomal radiosensitivity as an indicator of individual radiosensitivity may be a useful strategy to identify hypersensitive patients and to “tailor” a specific radiotherapy regimen for each single patient. An effective evaluation of individual radiosensitivity may be addressed through multiple measurements from single blood samples.\textsuperscript{18,28} For ethical and practical reasons, it is really difficult to obtain multiple blood samples from cancer patients before therapy starting, so we could analyse just a single blood sample for each subject. In the future, it will be appropriate to analyse a wider cohort of subjects in order to define the role of DNA damage and repair and to confirm the application of cytogenetic assays in radiosensitivity estimation.

ACKNOWLEDGMENTS

We are grateful to Tommaso Cornetta for statistical assistance. The authors thank all patients and healthy subjects who helped to make our study possible and the personnel of the Radiotherapy Unit of San Camillo-Forlanini Hospital (Rome).

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Received on April 24, 2010
Revision received on July 15, 2010
Accepted on July 19, 2010