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CHROMOSOMAL RADIOSENSITIVITY IN UKRAINIAN BREAST CANCER PATIENTS AND HEALTHY INDIVIDUALS

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Aim: **Recent studies showed that increased chromosomal damage induced by ionizing radiation is observed among patients with different tumor types. The aim of the study was evaluation of chromosomal radiosensitivity in breast cancer (BC) patients (n = 37) and healthy women (n = 44).** ***Methods:*** **Chromosomal radiosensitivity was assessed with G0 and G2 assay. For G0 assay lymphocytes were exposed *in vitro* to 1,5 Gy of X-rays before culture setting. For G2 assay lymphocytes were irradiated with 0,5 Gy of X-rays after 47 h of incubation.** ***Results:*** **Significant differences in mean scores both of G0 and G2 assay between breast cancer patients and controls were observed indicating the increased chromosomal radiosensitivity of lymphocytes of cancer patients. 11% of healthy women and 38% of BC patients were determined to be radiosensitive with G2 assay.** ***Conclusion:*** **Obtained results support the concept of association between elevated individual G2 chromosomal radiosensitivity and predisposition to BC.**
Key Words: **chromosomal radiosensitivity, breast cancer susceptibility, G_2 chromosome breakage assay, DNA repair.**

Current investigations of breast cancer (BC) development revealed new genes and their variations influencing susceptibility and the cancer risk involved in cell functional transduction pathways. At the same time it was shown that they are responsible for individual reaction of cell to genotoxic agents such as ionizing radiation and chemical mutagens, development of sensitive/resistant cell phenotype. First evidence in favor of relationships between chromosome damage induced by ionizing radiation and high cancer predisposition was obtained from examinations of patients with rare heritable chromosome breakage syndromes [1, 2]. Many of these disorders are caused by specific mutations in genes controlling DNA damage recognition, repair and elimination.

Recent studies showed that chromosome damage induced by ionizing radiation is also higher in peripheral blood lymphocytes (PBL) of cancer patients with different tumor types. Increased chromosomal radiosensitivity was observed in 40% of unselected breast, approximately 30% of colorectal, cervix and lung, head and neck cancer patients [3–5]. BC patients with family history or with known genetic predisposition, such as *BRCA1/BRCA2* mutations, demonstrate essentially higher chromosome damage than healthy individuals [6–8]. Mutations in highly penetrant *BRCA1* and *BRCA2* genes are shown to account for 15% of familial and approximately for 5% of sporadic BC cases. At the same time 40% of group of unselected BC patients have demonstrated increased chromosomal damage in G2 radiosensitivity assay. It was suggested that BC predisposition and high chromosomal radiosensitivity may be caused by other BC susceptibility genes of low penetrance involved in DNA repair [9–11]. Thus polymorphic variants of candidate genes of moderate and low susceptibility risk, their effects in combination with environmental factors on BC etiology are in focus of recent investigations. It is shown that polymorphic alterations in *ATM*, *BRIP1*, *BARD1*, *PALb2 NBS1, CYP17, NAT2, CYP1A1, FGFR2, GSTM1, GSTP1, XRCC1, XRCC3, XRCC6, hRAD51* could be essential risk factors for inherited and sporadic BC development and clinical outcome [12–15]. Many of these genes are associated with DNA damage repair systems, the defects of which contribute to increased levels of chromosomal aberrations. From this point of view high inter-individual variations of chromosomal radiosensitivity of PBL of cancer patients and healthy individuals reflect altered DNA repair efficiency, gene expression and cytogenetic parameters of radiosensitivity can serve as possible cancer risk marker.

In vitro radiation-induced chromosome aberrations in PBL have been investigated as a basis for predictive chromosomal assay for radiosensitivity evaluation since the development of G2-chromatid break assay by Sanford et al. and its modification by Scott et al. [16, 17]. Important direction of its application is determination of patients' risk to develop acute or late normal tissue responses after radiotherapy [18]. In our previous investigations G2-assay was used to estimate chromosomal radiosensitivity in group of healthy individuals (n = 113) with the purpose of primary prevention of radiation-associated cancer [19]. It was also shown that G2 chromosomal radiosensitivity was unrelated to sex and age of examined healthy donors. Another cytogenetic test — micronucleus (MN) G0 radiosensitivity assay was shown to be also relevant in assessment of *in vitro* radiosensitivity and estimations of inter-indidvidual variations of cytogenetic radiosensitivity parameters [17, 20]. This study presents evaluation of inter-individual variations of G0 and G2 chromosomal radiosensitivity of PBL obtained from BC patients and healthy individuals and correlation of its values with several clinical data.

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Abbreviations used: BC – breast cancer; PBL – peripheral blood lymphocytes.

MATERIALS AND METHODS

Patients and normal control. Blood samples were obtained from 44 healthy women, aged between 28–55 years (mean — 43 years) without cancer family history and primary 37 BC patients aged between 31–81 years (mean — 56 years) with no previous cancer history and no prior radio- or chemotherapy. The tumors were categorized as T1 or T2, lymph node status as N0 or N1, distant metastases as M0 (UICC TNM classification), grade form I–III. Additionally 8 women aged between 53–77 years (mean — 63 years) with BC recurrences (all tumors — T1N1M0 stage) were tested in chromosomal radiosensitivity assays. Parallel cultures of lymphocytes containing 0,5 ml of heparinized blood, 4,5 ml RPMI-1640 medium (Sigma-Aldrich) were established for each donor: to evaluate spontaneous level of chromosome aberrations, G0 and G2 assay scores. All donors gave informed concent for participation in the study. The experimental protocols were approved by the Bioethical Committee of the Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine.

G0 and G2 radiosensitivity assays. G0-assay was conducted for 30 healthy donors, 30 BC primary patients (all of them were tested in G2-assay). Briefly, samples were irradiated with 1,5 Gy dose of X-rays (300 kV, 10 mA, HVT 0,5 mm Cu + 1 mm Al) and 2 h later lymphocytes were stimulated with phytohemagglutinin (Gibco/Invitrogen Co). After 46 h of incubation 100 µl of Colcemid solution (10 µg/ml, Sigma-Aldrich) was added to block cells in metaphases. At 48 h cultures were harvested according to common protocol [21].

For G2 assay cell cultures were irradiated with 0,5 Gy of X-rays after 47 h of incubation. At 30 min later 100 µl of Colcemid solution (10 µg/ml) was added and cells were harvested following 1 h. Thereafter cells were treated as described in [24]. Some minor procedure changes made it possible to analyze cells in the first post-radiation mitosis. At least 100 metaphase cells were scored for each sample. Chromosome- and chromatid type aberrations were recorded in G0 assay; chromatid and isochromatid breaks were analyzed in G2 assay. G0 and G2 scores were obtained by subtracting the spontaneous aberration yield from obtained after G0 and G2 cell irradiation.

Statistical analysis. The variances in chromosomal radiosensitivity was expressed as coefficient of variation (CV) of obtained cytogenetic parameters, determined as follow: $CV = (SD/M)x100$, where *SD* is the standard deviation, *M* — mean of aberration yield.

The unpaired Student t-test was used to compare G0 and G2 scores. The proportions of G0 and G2 radiosensitive individuals were calculated using 90th percentile as described in [18]. The χ^2-test was applied to compare differences in proportion of sensitive donors of different groups. Pearson correlation was used to analyze G0 and G2 assays data. The Mann — Whitney (MW) U test was applied to compare G2 scores in subgroups of BC patients with different clinical characteristics. A significance level of $p < 0.05$ was used throughout.

RESULTS AND DISCUSSION

Recent studies have reported a significant proportion of sporadic BC patients with high chromosomal radiation sensitivity of PBL according to the results of G0 and G2 chromosomal radiosensitivity assays compared to healthy individuals. The main idea of these assays in contrast to spontaneous level estimation is the analysis of chromosomal damage after low dose radiation exposure of lymphocytes and estimation of inter-individual variations of obtained parameters. In G2-assay test-irradiation is applied during G2/M — the most radiosensitive cell cycle point. Chromatid breaks observed in this case are seemed to be the result of radiation induced DNA double strand breaks and markers of DNA repair capacity [22, 23]. To test the hypothesis whether cytogenetic parameters of radiation sensitivity associated with BC risk and to estimate inter-individual variations in chromosomal radiosensitivity of PBL of healthy women and sporadic BC patients we used both G0 and G2 assays.

Results of cytogenetic examination of BC patients and healthy controls are shown in Fig. 1. The mean value of spontaneous frequency of chromosomal aberrations in group of BC patients (1.9 ± 0.64) was not significantly higher than that of the healthy controls (1.1 ± 0.32; unpaired t-test; $p>0.05$). There was low but significant difference in mean G0 scores between BC patients and controls (t-test, $p<0.05$). Coefficients of variations (CV) of total yield of chromosome aberrations in G0 assay were 10% for controls and 12% for BC patients. To indicate the proportion of normal and sensitive response to radiation, 90th percentile of control values as cut-off point for chromosomal radiosensitivity was used as described in [18]. Go chromosomal radiosensitivity was increased in 13% BC patients and in 7% individuals in control group with insignificant difference ($\chi^2 = 0.66$; $p=0.04$) (Table 1).

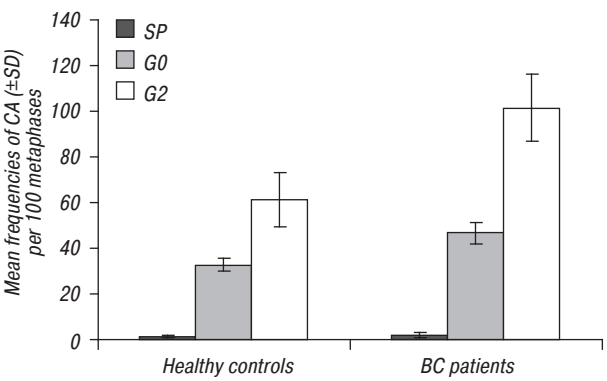

Fig. 1. Spontaneous (SP), G0 and G2 mean frequencies (±SD) of chromosome aberrations observed in lymphocytes of healthy controls and BC patients

The mean overall G2 score was essentially higher in BC group than in controls — 100.3±12.3 and 61.2±8.0 respectively (unpaired t-test, $p < 0.001$). CV of chromatid break yields induced in G2 phase in group of healthy individuals and BC patients were

higher than for G0 scores — 22 and 26%. 11.4% from healthy women (5/44) and 38% among BC patients (14/37) were determined as sensitive showing a significant difference between proportions ($\chi^2 = 4.03$, $p < 0.001$). Fig. 2 and 3 show the distributions of G0 and G2 scores for healthy donors and BC patients. At the same time there was no correlation between G0 and G2 scores in both groups: Pearson correlation coefficient was 0.05 for controls and 0.11 for cancer patients. There were no individuals with simultaneously increased G0 and G2 scores in control group and only 1 cancer patient had increased G0 and G2 chromosomal radiosensitivity values.

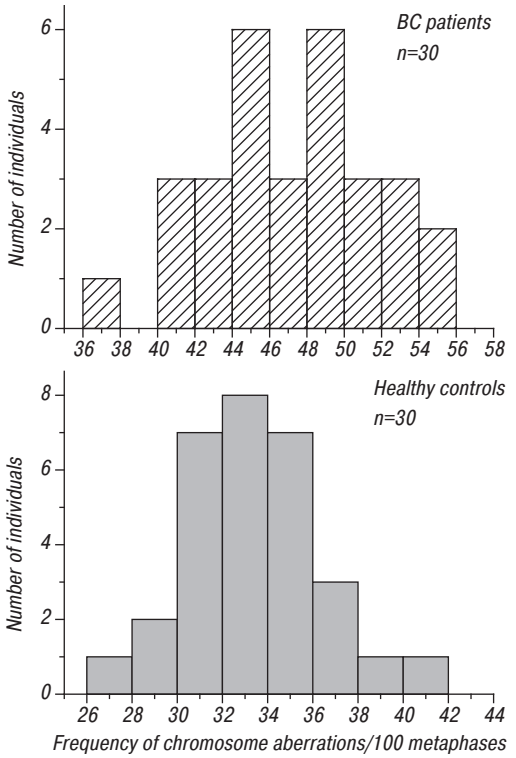

Fig. 2. Distribution of radiation-induced G0 chromosome aberrations in group of healthy women and BC patients

According to clinical data all of the examined BC patients had invasive adenocarcinoma, 2 cases were classified as mixed ductal lobular adenocarcinoma. Analysis has not revealed any significant correlations between G2 scores and several specific clinical characteristics such as tumor size (T-category, MW test, $p = 0.11$), lymph node involvement (N-category, MW test, $p = 0.45$), tumor grade (G, MW test, $p = 0.08$) and menopausal status (Table 2, MW test, $p = 0.06$). Due to small number of patients we can only notify the tendency of increased G2 score association with tumor grade III and pre-menopausal cancer development. There is contradictory literature data on the influence of hormonal status on chromosomal radiosensitivity of BC patients. Riches et al. [24] demonstrated that oestrogen receptor positive subpopulation of BC patients had demonstrated increased G2 radiosensitivity . It was also shown that high G2 scores identifies a sub-population of patients with distinctive tumor characteristics and with a predicted improved prognosis as compared with those in the low radiosensitivity group.

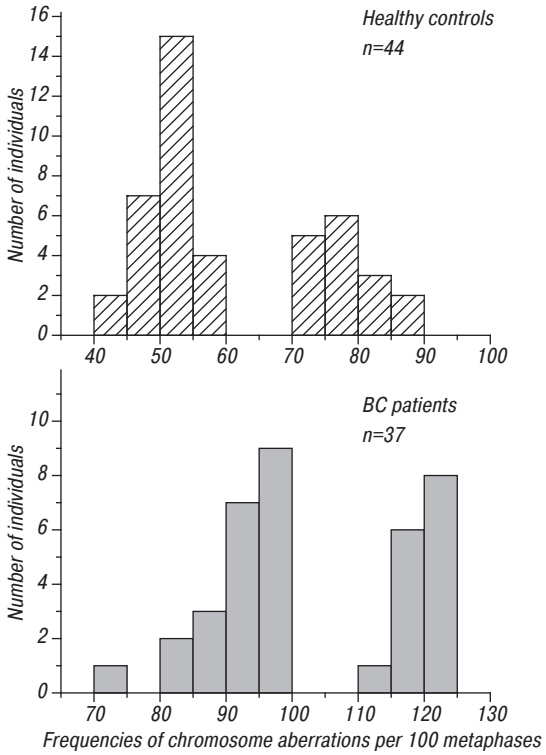

Fig. 3. Distribution of radiation-induced G2 chromatid breaks in group of healthy women and BC patients

Table 1. Comparison of G0 and G2 scores in healthy controls and BC patients

	Healthy individuals				BC patients				
Assay	n	M±SD	CV, %	% of sensitive individuals (n)	n	M±SD	CV, %	% of sensitive individuals (n)	P^2
G0	30	32.8±3.0	10	7 (2)	30	46.6±4.5	12	20 (6)[1]	<0.05
G2	44	61.2±8.0	22	11 (5)	37	100.3±12.3	26	38 (14)[1]	<0.001

Notes: 1 – significant difference in proportions ($p < 0.05$, χ^2-test); 2 – significant difference from control (unpaired t-test)

Table 2. Association between clinical data and G2-scores of BC patients

Clinical characteristics		n	G2-scores[1], M±SD	% (n) of sensitive donors	P^2
T-category	T1	27	97.8±10.2	37.0 (10)	0.42
	T2	10	107±14.8	40 (4)	
N-category	N0	25	99.3±13.5	36 (9)	0.24
	N1	13	102±14.3	38.5 (5)	
Grade[3]	I	4	103.6±16.1	2	
	II	28	98.8±13.7	8	
	III	5	108.4±11.6	2	
Menopause status	*Pre*	12	104.1±9.6	50 (6)	0.08
	Post	25	98.48±12.6	32 (8)	
All patients		37	100.3±12.3	37.8 (14)	
Recurrence cases[3]		8	119.3±15.4	62.5 (5)	

Notes: 1 – mean aberration frequency/100 metaphases; MW U test was applied to compare G2 scores in subgroups of BC patients with different clinical characteristics; 2 – χ^2-test for differences in the proportion of sensitive donors; 3 – unappropriate cohort for statistical analysis

To indicate correlation between G2 chromosomal radiosensitivity and age of BC patients, they were separated into 2 subgroups — with high (n = 14) and normal (n = 23) G2-chromosomal radiosensitivity. Mean age of BC patients with high G2 chromosomal radiosensitivity was 47 ± 7.0 years and that in healthy controls — 66 ± 10 years (p = 0.02). Correlation be-

tween high G2 chromosomal radiosensitivity and early age of cancer onset could be caused by possible putative predisposition of young patients. Large proportion of patients with increased G2 scores was observed among recurrence BC cases (5%). Obviously, these preliminary data need more accurate examinations in selected representative cohorts of cancer patients.

The results obtained in our study confirm investigations showing high G2 chromosomal radiosensitivity among BC patients with no family cancer history compared to healthy women. Cytogenetic parameters obtained in G2 assay are more appropriate to estimate chromosomal radiosensitivity compared to G0 assay in BC group, supporting concept of association between elevated G2 chromosomal radiosensitivity and genetic predisposition to BC, impact of G2/M checkpoint on genomic stability and cancer risk [25]. As it was suggested that abrogation of G2/M checkpoint increases chromosomal breakage [26], level of chromatid breaks observed after G2 irradiation depends on DSB number and efficiency of their repair, thus inter-individual variations in G2 scores can be caused by differences in DNA repair capacity related to different mechanisms. Thus data also testify for different DNA repair mechanisms (homological recombination or non-homological end joining) responsible for chromosome damage during different cell cycle phases that could be associated with BC development. Lack of correlation between G0 and G2 scores is reported by other research groups who also speculated to different mechanisms of chromosomal radiosensitivity involving not only DNA repair efficiency but other cell cycle control checkpoints or chromatin conformations [27]. Taking into consideration these findings special attention should be paid to individuals from healthy controls with increased G2 chromosomal radiosensitivity (11% in our study), to determine if they are in increased BC or radiation-associated cancer risk.

REFERENCES

1. Distel L, Neubauer S, Keller U, ***et al.*** Individual differences in chromosomal aberrations after *in vitro* irradiation of cells from healthy individuals, cancer and cancer susceptibility syndrome patients. Radiotherapy Oncol 2006; **81**: 257–63.

2. Leong T, Borg M, McKay M. Clinical and cellular radiosensitivity in inherited human syndromes. Clin Oncol 2004; **16**: 206–9.

3. Baria K, Warren C, Roberts SA, ***et al***. Chromosomal radiosensitivity as a marker of predisposition to common cancers? BJC 2001; **84**: 892–6.

4. Papworth R, Slevin W, Roberts SA, ***et al.*** Sensitivity to radiation-induced chromosomal damage may be a marker of genetic predisposition in young head and neck cancer patients. Br J Cancer 2001; **84**: 776–82.

5. de Ruyck K, Gelder V, van Eijkeren M, ***et al.*** Chromosomal radiosensitivity in head and neck cancer patients: evidence for genetic predisposition? BJC 2008; **98**: 1723–28.

6. Turnbull C, Mirugaesu N, Eeles R. Radiotherapy and genetic predisposition to breast cancer. Clin Oncol 2006; **18**: 257–67.

7. Burill W, Barber JBP, Roberts SA, ***et al.*** Heritability of chromosomal radiosensitivity in breast cancer patients: a pilot study with lymphocyte micronucleous assay. Int J Radiat Biol 2000; **76**: 1617–9.

8. Bayens A, Thierens H, Claes K, ***et al.*** Chromosomal radiosensitivity in BRCA1and BRCA2 mutation carriers. Int J Radiat Biol 2004; **80**: 745–56.

9. Scott D. Chromosomal radiosensitivity and low penetrance predisposition to cancer. Cytogenet Genome Res 2004; **104**: 365–70.

10. Roberts SA, Spreadborough AR, Bulman B, ***et al.*** Heretability of cellular radiosensitivity: A marker of low-penetrance predisposition genes in breast cancer? Am J Hum Genet 1999; **65**: 784–94.

11. Shadan FF, Koziol J. Induced genome instability as a potential screening test for cancer susceptibility? Med Hypoth 2000; **55**: 69–72.

12. Ripperger T, Gadzicki D, Meindl A, ***et al.*** Breast cancer susceptibility — current knowledge and implications for genetic counseling. Eur J Hum Genet 2009; **17**: 722–31.

13. Pharoah PD, Antoniou AC, Easton DF, ***et al.*** Polygenes, risk prediction, and targeted prevention of breast cancer. New Engl J Med 2008; **38**: 1239–41.

14. Easton DF, Pooley KA, Dunning AM, ***et al.*** Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; **447**: 1087–93.

15. Lerebours F, Lidereau R. Molecular alterations in sporadic breast cancer. Crit Rev Oncol Hematol 2002; **44**: 121–41.

16. Sanford K, Parshad R, Price F, ***et al.*** Enhanced chromatid damage in blood lymphocytes after G2 phase x-irradiation, a marker of the ataxia-telangiectasia gene. J Natl Cancer Inst 1990; **82**: 1050–55.

17. Scott D, Barber JB, Spreadborough A R, ***et al.*** Increased chromosomal radiosensitivity in breast cancer patients: a comparisson of two assays. Int J Radiat Biol 1999; **75**: 1–10.

18. Borgman K, Hoeller U, Nowack S, ***et al.*** Individual radiosensitivity measured with lymphocytes may predict the risk of acute reaction after radiotherapy. Int J Radiat Oncol Biol Phys 2008; **71**: 256–64.

19. Dyomina EA, Ryabchenko NM. Increased individual chromosomal radiosensitivity of human lymphocytes as a parameter of cancer risk. Exp Oncol 2007; **29**: 217–20.

20. Mozdarani H, Mansuori Z, Haeri SA. Cytogenetic radiosensitivity of G0-lymphocytes of breast and esophageal cancer patients as determined by micronucleus assay. J Radiat Res 2005; **46**: 11–16.

21. Cytogenetic Analysis for Radiation Dose Assessment. Technical Report series. IAEA. Vienna, 2001; **405**.

22. Buchholz TA, Wu X. Radiation-induced chromatid breaks as a predictor of breast cancer risk. Int J Radiat Oncol Biol Physics 2001; **49**: 533–7.

23. Parshad R, Sanford K. Radiation-induced chromatid breaks and deficient DNA repair in cancer predisposition. Crit Rev Oncol Hematol 2001; **37**: 87–96.

24. Riches AC, Bryant PE, Steel CM, ***et al.*** Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characterisitics. Br J Cancer, 2001; **85**: 1157–61.

25. Lobrich M, Jeggo PA. The impact of negligent G2/M checkpoint and cancer induction. Nat Rev Cancer, 2007; **7**: 861–9.

26. Terzoudi GI, Manola KN, Pantelias GE, ***et al.*** Checkpoint abrogation in G2 compromises repair of chromosomal breaks in ataxia telangiectasia cells. Cancer Res 2005; **65**: 11292–6.

27. Borgmann K, Haeberle D, Doerk T, ***et al.*** Genetic determination of chromosomal radiosensitivities in G0 and G2-phase human lymphocytes. Radiother Oncol, 2007; **83**: 196–202.