Effects of X-Ray Irradiation of Female Mice in Preconceptive Period on Polymorphism of Simple Repeats in DNA of Offspring of Different Sex

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Sibs groups of F1-offspring born by non-irradiated mice and by female mice exposed to X-ray radiation in preconceptive period (50-200 cGy) were compared. Arbitrary primed PCR revealed significantly increased polymorphism of simple DNA repeats in somatic tissues of the offspring from female mice irradiated in a dose of 200 cGy. The increase in DNA polymorphism in postmitotic brain tissues and in peripheral blood was more pronounced than in proliferating spleen tissues and in the epithelium of tail tip. In the tissues of female offspring from irradiated mothers, higher increase in DNA polymorphism was observed in comparison with the tissues of male offspring from the same mothers.

Key Words: *simple sequence repeat DNA polymorphisms; AP-PCR; X-ray radiation; mice*

The possibility for induction and transmission of genome instability (GIS) from parents exposed to genotoxic factors, *e.g.* ionizing radiation (IR), to their offspring was recently demonstrated by various methods [2,3,6,7,9,11,12]. GIS manifested in increased mutation rate, frequency of somatic mutations, risk of tumors and by other pathologies in the offspring born from irradiated parents. Our previous experiments [1,5,13] revealed an increase in simple sequence repeat DNA polymorphism in somatic tissues and frequency of micronuclei in bone marrow erythrocytes in the offspring from irradiated males and females, which can also be interpreted as an evidence of transgenerational GIS transmission from irradiated parents to the offspring. Generally, induced transgenerational GIS was analyzed by examination of total offspring sample.

For extending our knowledge about biological basis of induced transgenerational GIS phenomenon, we should determine its pattern in offspring of different sexes born by mothers exposed to IR. Here we report the differences in the level of microsatellite-associated DNA repeat polymorphism in some tissues (brain, spleen, peripheral blood, and tail tip epithelium) in male and female offspring born by preconceptively irradiated females.

MATERIALS AND METHODS

Experiments were carried out on 2-month old BALB/c mice from the nursery of experimental animals of the branch of Institute of Bioorganic Chemistry, Russian Academy of Sciences (Pushino). Two-month old male and females were clearly labeled. One male and 3 females were placed in one cage. One week later, the females were transferred to individual cages to obtain control offspring. One month after birth of the control offspring, female mice were irradiated on a RUM-13 X-ray unit at radiation intensity of 100 cGy/min (voltage 200 kV, current intensity 8 mA). To synchronize ovulations, the females were treated with hormonal agents before irradiation according to common approach: the mice received menogon subcutaneously and 2 days later with chorionic gonadotropin intra-

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peritoneally (10 U). Thirty minutes after the second injection, the animals were irradiated in doses of 50, 100, or 200 cGy. Irradiated females were housed with the same males, from which the control offspring was obtained. DNA polymorphism levels were compared between the sibs groups of the offspring. Offspring from irradiated animals were sacrificed by cervical translocation at the age of 5 weeks (as well as F0-generation, parental). Samples of postmitotic tissues (brain tissues and peripheral blood) and proliferating tissues (spleen and tail tip epithelium) were obtained from the offspring of control and irradiated mothers. Tail tip samples were taken from the females after delivery of control and irradiated offspring and from male parents after the first mating. Blood DNA was extracted immediately other tissues were frozen and stored at -20°C. DNA from blood and frozen tissues was extracted by phenol-chloroform method after treatment with protein kinase K. Arbitrary primed PCR (AP-PCR) was carried out using 5'-TGG TGT TCC TGC CAC AGA AA-3' primer (synthetized at Syntol company). This oligonucleotide is the fragment of DNA sequence flanking microsatellite Atp1b2 locus in the neighborhood of *p53* gene at 11 chromosome in mice [10]. The reaction mixture for PCR (25 µl) contained: 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.02% Tween-20, 6% sucrose, 3.5 mM $MgCl_2$, 0.2 mM of each dNTP, 7.5 pmol primer and 0.5 U Taq-polymerase. Total DNA from analyzed tissues (12 ng) was used in reactions. Amplification regimen was 3 min at 94°C and then 45 cycles: 1 min at 94°C (denaturation), 1 min at 58°C (annealing), and 5 min at 72°C (elongation). AP-PCR products were separated by electrophoresis in 6% PAAG and stained with silver nitrate as described elsewhere [8]. The gels were dried and scanned. DNA fingerprints in the range of 872-271 b.p. were compared using special computer software [4]. To assess the polymorphism level, the total number of products amplified by AP-PCR was counted as the number of bands on the corresponding row. The data for a group of animals was used to calculate mean number of the products per animal. Thereafter, the number of "non-parental bands" (NPB), *i.e.* bands detected in DNA fingerprints of the offspring, but absent in both parents, was determined. NPB values averaged for the groups were presented as the frequency (% from the mean number of products) per one offspring in the sibs group. Since polymorphism can manifest in either increase or decrease in NPB frequencies in DNA of offspring from irradiated animals, the absolute values of relative normalized changes in polymorphism (*d*) were also calculated. This parameter can serve as an additional nondimensional criterion of irradiation-induced changes in polymorphism of microsatellite-associated DNA sequence repeats. The following formula was used to calculate *d* value:

$$d=|(A_{irr}-A_{contr})/A_m|,$$

where $A_m=(A_{irr}+A_{contr})/2$ and A_{contr} and A_{irr} are NPB values for the groups of male and female offspring born before and after irradiation of female parents.

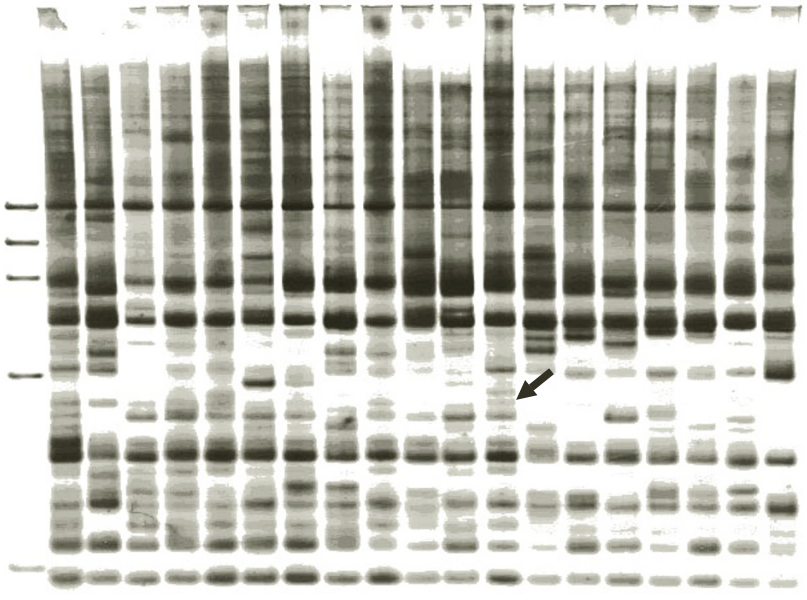

Fig. 1. Electrophoregram of AP-PCR products on blood DNA of a mouse family. *1-9*: offspring born before dam irradiation; *10-11*: parent couple (F0-generation); *12-19*: offspring born after dam irradiation. An arrow shows NPB at 12 row. *M*: size marker of AP-PCR products (DNA ϕX174/BsuRI, range 1353-271 b.p.).

For evaluation of the length of PCR amplification products, GeneRuler 50 bp DNA Ladder and φX174 DNA/BsuRI markers (UAB Fermentas) were used. Taq DNA-polymerase was obtained from Isogen company.

RESULTS

Typical electrophoregram of DNA amplification products (DNA fingerprint) for offspring and their parents (Fig. 1) is presented. A band corresponding to NPB-products amplified on offspring DNA, but absent among products of PCR on DNA of both parents is shown by an arrow. The results of analysis of NPB frequencies and calculations of relative normalized differences *d* in brain tissue, blood, spleen, and tail tip epithelium in F1 males and females, offspring born by female mice exposed to IR in doses of 50, 100, or 200 cGy during the preconceptive period are presented in Table 1. The calculated NPD frequencies for each family were averaged by groups with consideration for IR dose in F0 females. The levels of simple DNA repeat polymorphism detected by AP-PCR for various tissues in offspring of the control and irradiated animals were different. No significant changes in NPD frequency on DNA fingerprints from tissue preparations of the offspring from irradiated dams were observed after irradiation in the dose of 50 cGy. An insignificant increase in NPB frequency was noted for the spleen in female offspring. In the offspring born by females irradiated in a dose of 100 cGy, significant differences in polymorphism level in male offspring was detected in the brain. An insignificant increase in polymorphism level for the tail tip epithelium was observed in female offspring. In male offspring born by females irradiated in doses of 50 and 100 cGy an insignificant decrease in NPB frequency was noted. It can be assumed that this results from the loss of parental bands. The loss of parental bands may appear in the result of deletions and translocations. It should be noted that both decrease and increase in NPB frequencies equally reflect increased polymorphism level of microsatellite repeats and eventually indicative for the genome destabilization.

Significant increase in NPB frequencies was detected in DNA of all four tissues from both male and female offspring born by F0 females irradiated in a dose of 200 cGy. Changes in all tissues were more pronounced in female offspring. This was seen from both NPB frequency (%) and absolute values of nondimensional parameter *d*. Maximum changes in polymorphism induced by X-ray irradiation of female parents were detected in offspring in DNA of resting tissues (brain and blood) in comparison with proliferating tissues (spleen and tail tip epithelium).

TABLE 1. Polymorphism of AP-PCR Products Evaluated by NPB Frequency for Tissues of Male and Female Offspring from Female Mice before and after Iirradiation (*M*±*m*)

Irradiation dose for female parents	Offspring sex	Offspring gender	Mean NPB per one offspring from the sibs group, %							
			brain		blood		spleen		tail	
			NPB, %	*d*	NPB, %	*d*	NPB, %	*d*	NPB, %	*d*
Before irradiation (control)	males	60	13.2±0.9		16.6±0.9		12.7±0.7		14.2±0.9	
	females	57	11.7±0.7		15.2±0.9		11.4±0.8		13.6±0.9	
After irradiation, 50 cGy	males	71	12.5±0.8	0.05	14.7±0.9	0.12	13.9±0.8	0.09	13.3±0.8	0.07
	females	73	12.1±0.7	0.03	15.1±0.9	0.07	13.4±0.7	0.16	14.2±0.9	0.04
Before irradiation (control)	males	34	14.0±0.9		16.5±0.9		14.5±1.0		15.2±0.8	
	females	41	11.8±1.0		14.9±1.3		15.1±1.0		12.2±1.1	
After irradiation, 100 cGy	males	58	16.5±0.8*	0.16	15.8±0.8	0.04	15.7±0.9	0.07	14.9±0.9	0.02
	females	46	12.7±0.8	0.07	15.5±1.1	0.04	16.5±1.3	0.08	14.6±0.8	0.17
Before irradiation (control)	males	58	11.1±0.7		12.2±0.9		10.3±0.7		11.8±0.7	
	females	43	10.7±0.8		11.4±0.8		9.8±0.7		11.5±0.9	
After irradiation, 200 cGy	males	55	15.9±0.8**	0.35	19.4±0.9**	0.46	13.9±0.8**	0.29	14.1±0.9*	0.18
	females	52	18.0±1.1**	0.51	20.9±1.4**	0.57	14.2±1.0**	0.36	15.5±0.9**	0.29

Note. *$p<0.05$, **$p<0.01$ in comparison with the corresponding control (Student's *t* test).

Thus, AP-PCR showed that the level of simple DNA sequence repeat polymorphism in various somatic tissues of the offspring of irradiated female mice significantly increased with increasing the irradiation dose for female mice in preconceptive period from 50 to 200 cGy (not impairing reproduction capacity). DNA polymorphism is more pronounced in postmitotic brain tissues and peripheral blood than in proliferating tissues of the spleen and in tail tip epithelium. In tissues of female offspring obtained from mothers irradiated in a dose of 200 cGy, higher increase in DNA polymorphism level was revealed in comparison with the tissues of male offspring from the same mothers.

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