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CHROMOSOMAL DAMAGES AND MUTAGENESIS IN MAMMALIAN AND HUMAN CELLS INDUCED BY IONIZING RADIATIONS WITH DIFFERENT LET

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The problem of mutagenic action of ionizing radiation on mammalian and human cells attracts especial attention in the last decade in connection with a deterioration of the environment and radioecological situation in the world. The study of the mechanisms of the mutagenesis in mammalian and human cells is a rather difficult problem because of both incompleteness of knowledge about the structure and functioning of eucariots' genome and insufficient development of the methods for a realization of such investigations. In the last decade the test-systems for a registration of some gene mutations, connected with biochemical processes in cell nuclei, and a number of molecular-genetic methods for revealing of mutation events (sequence, FISH-technique, blot-analysis) are developed.

At this time the data about the mutations of some genes (HPRT, APRT, TK and others), which cause cell resistance to the action of some toxic analogues of the purines and the pyrimidines are obtained. The interest to these agents arised in 60-th in earlier period of cancer chemiotherapy in connection with an appearance of resistant to them variants in the populations of cancer cells. Later it was shown that the resistance was conditioned by insufficiency or full loss of phosphoribosyltransferase or nucleoside kinase enzymes.

We are investigating the mutants on HPRT-locus and the data concerning this gene will be considered then. The gene in humans is in the X-chromosome. It is about 35–45 kb of genomic DNA in mammalian cells (44 kb – in human cells) and has 9 exons that are ~1.3 kb. HPRT-gene codes hypoxanthine guanine phosphoribosyltransferase, which catalyzes the condensation of 5'-phosphoribosyl-1-pyrophosphate and the purine bases of hypoxanthine and guanine in the formation of the mononucleotides, utilized by the cells during DNA synthesis.

The revealing of mutations in HPRT-locus is based on the following principle: the mammalian cells can use exogenic purines or synthesize them for construction of the nucleotides de novo. Thus they can also incorporate toxic for them exogenic purine analogues as 6-thioguanine, 8-azaguanine and others. In selective medium with purine analogue only the cells survive and

form colonies, in which the mutation in HPRT-locus has taken place, resulting in impair or absent HPRT activity. The cells have impaired or absent ability to incorporate these toxic analogues and are resistant to them. They will form colonies, that allows one to evaluate the intensity of mutation processes after the action of different physical and chemical agents and also to isolate the individual colonies and to investigate HPRT-mutant subclones.

As our investigations have shown the types of dose dependence curves for mutation induction and chromosome aberrations also, as well as survive curve of Chinese hamster cells depend on LET of radiations. After γ-irradiation and heavy ions action with LET up to 80 keV/μm they are nonlinear, at higher LET values they come nearer to linear. The RBE–LET dependence [1] on all three criteria is described by the curves with a maximum at 80–100 keV/μm (Fig. 1). All this, and also the close values of RBE factors on the number of mutations and chromosome aberrations allow one to believe, that at the basis of these changes one and the same primary damages lie, namely, double-strand breaks of DNA, and as a result both structural chromosome aberrations and microstructural damages of DNA, registered as gene mutations, are formed.

Accumulated in the literature data testify the role of structural DNA damages in the induction of gene mutations in mammalian and human cells [2-16]. The data of Table 1 allow one to judge about the nature of HPRT-mutations in these cells. The changes were not revealed at the majority of independent spontaneous mutants (up to 85%). Amongst radiation-induced (by X- and γ-rays) mutants the fraction of changed ones becomes prevailing (up to 55–100%), except only the data of Hakoda M. et al.(1989) [12] for lymphocytes *in vivo* from persons, survived after Atom bombardment: the changed mutants comprise only 15–25%, that corresponds to spontaneous mutant level for this test. At spontaneous and radiation-induced mutants the partial or total gene deletions prevail, and moreover at radiation-induced ones a fraction of mutants with total deletion of gene [13, 14] is raised. The data, received by sequence or PCR methods show diversified mutation chan-

Table 1

Spontaneous and radiation-induced HPRT-mutations in mammalian and human cells

Types of mutations	Radiation	Cell line	Total analyzed mutants	Normal Southern patterns		Altered Southern patterns						References
						Total number		Mutation types				
				№	%	№	%	Total gene deletion	Partial gene deletion	Exon deletion	Duplication	Exon molecular weight changes	
Spontaneous mutants		Chinese hamster cells,V79	52	45	86	7	14	1	6	-	-	-	Breimer (1988) Thacker, Ganesh (1989)
			44	36	82	8	18	6	2	-	-	-	
		Human lymphoid cell line, TK-6	14	9	64	5	36	-	5	-	-	-	Liber et.al. (1987) Whaley, Little (1990) Gennett, Thilly (1988)
			35	15	43	20	57	1	19	-	-	-	
			85	52	61	33	39	10	21	-	2	-	
		Human T-limphocytes	21	9	43	12	57	-	12	-	-	-	Turner et. al. (1985) O'Neill et.al. (1987) Niclas et.al. (1989) Bradley et. al. (1987) Hakoda et.al (1989)
			164	149	91	15	9	4	11	-	-	-	
			326	278	85	48	15	8	40	-	-	-	
			90	75	83	15	17	-	15	-	-	-	
			105	91	87	14	13	-	14	-	-	-	
Radiation -induced mutants	γ-, α-	Chinese hamster cells,V79, line CHO	15	-	-	15	100	-	14[1]	-	1[2]	-	Thacker et.al. (1990) Breimer (1988) Morgan et.al. (1990)
	X: 2–4 Gy		113	35	31	78	69	46	32	-	-	-	
			89	10	11	79	89	54	25[1]	-	-	-	
	X: 1,5 Gy	Human lymphoid cell line, TK-6	28	13[4]	46	15	54	15		-	-	-	Liber et.al. (1987) Whaley, Little (1990) Whaley, Little (1990) Whaley, Little (1990)
	X, 1 Gy		33	16	48	17	52	10	7	-	-	-	
	^{125}I dUrd: 05 and 0,1 μCi/ml[5]		68	11	16	57	84	28	29[1]	-	-	-	
			21	8	38	13	62	10	3[1]	-	-	-	
	X	Human T-limphocytes	33	16	48	17	52	3	14	-	-	-	Skulimowski et.al.(1986) O'Neill et.al. (1990) Hakoda et.al. (1989) Hakoda et.al. (1989)
	γ: 3 Gy		24	6	25	18	75	-	-	10[3]	-	8	
	A-bomb survivors	2,46 Gy	61	45	74	16	26	-	16	-	-	-	
		2,15 Gy	59	51	86	8	14	-	8	-	-	-	

1) Partial deletions and rearrangements (the size of deletions- 1,5–14 kb); 2) Duplication of part of the 5'-end of the gene; 3) The size of deletions- 5–13 kb; 4) 5 mutants had partial HPRT- activity; 5) It is similar to 1 Gy of X-rays 6) In diffirent exons.

ges of gene. So Rossi A.M. et al.(1990) [17] have observed the loss of 1–2 or part of exons, the frameshifts, small deletions, the splice-mutations, the base-pairs substitutions (in exons 1, 2, 3, 8) amongst spontaneous mutants from human T-lymphocytes, and in the last case the transitions GC to AT were 40% of them. On an estimation of Niclas J.A. et al.(1989)[10], the viability of spontaneous mutants is kept at least at the deletions up to 22 kb from the 5'-end and 29 kb from the 3'-end of HPRT-gene. At radiation-induced mutants of Chinese hamster cells a duplication of a part of the 5'-end of the gene and gene deletions in all sites are marked by Thacker J. et al.(1990) [13]. There are also evidences of nonrandom character of breaks in HPRT-gene at spontaneous and radiation-induced mutants [7, 13].

As follows from a technique of revealing the mutations, a necessary condition for realization of arised premutation damage in a mutation, for example, after irradiation, is to do several cycles of cell division in normal physiological conditions (on the different data, from 3–5 up to 10 and more) before the cultivation in selective medium with toxic purine analogue ("the expression time "). As is known, during the period of first postirradiation mitosis the structural chromosomal aberrations are revealed. Amongst them structural gene deletions, the sizes of which exceed the microscope resolution, must also appear. The minimum values are about 1,2-1,3 kb, that coincides with the appropriate sizes, received at the blot-analysis, when it was a success to evaluate them (1,5–14 kb) [13, 14]. It is shown earlier, that the partial or total gene deletions were characterized for the majority of HPRT-mutants. The data of the analysis of such unstable chromosome aberrations, as the fragments and, especially, interstitial deletions, can also serve as indirect confirmation of a role of gene structural damages in mammalian and human cells mutagenesis. At the cytogenetic analysis the different sizes of interstitial deletions attract attention: from very small up to large circular or oval structures up to acentric rings, which are identical to such deletions on the mechanism of origin. The assumption is probable perfectly that the structural gene deletions with different sizes are also registered amongst the fine inter-

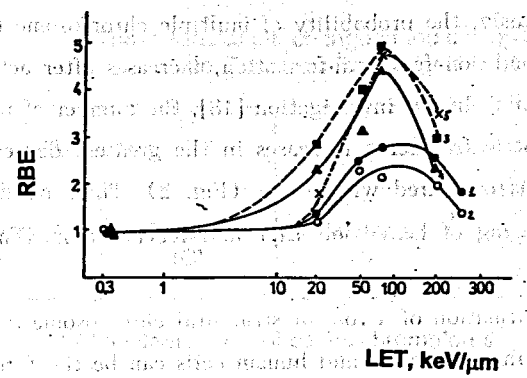


Fig.1. RBE of heavy ions as a function of LET on survival (1- 10 % level of survival, 2 - D_0), on induction of mutations (3- mutation frequency 5×10^{-5}, 4 - mutation frequency 10×10^{-5}), on induction of chromosomal aberrations (5) (Chinese hamster cells, V 79).

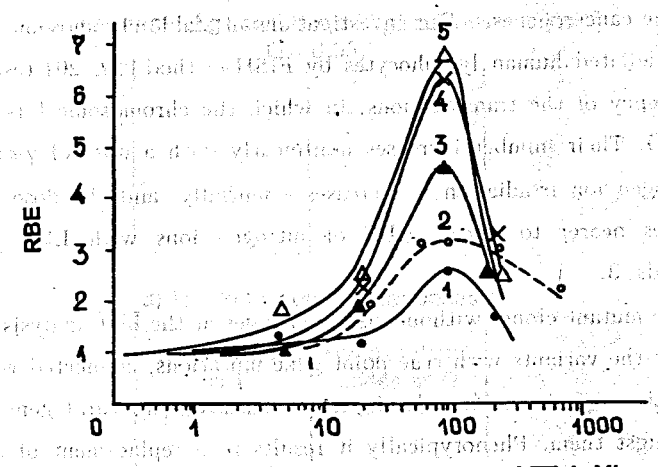


Fig.2. RBE of heavy ions as a function of LET on cytogenetical criterions: 1- dicentrics and acentric rings, 2- survival, 3- total chromosomal aberrations, 4- intersticial deletions, 5- double acentric fragments (Chinese hamster cells, V 79).

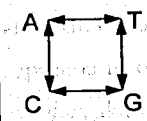
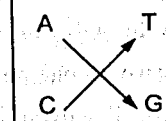
stitial deletions. Obviously, the probability of multiple chromosome breaks, that are a necessary condition for their formation, increases after action by heavy ions with high LET. In our investigation [18], the number of interstitial deletions and acentric fragments increases in the greatest degree after heavy ion irradiation as compared with γ-rays (Fig. 2). Their number increases with the increasing of heavy ion LET and reaches a maximum at about 100 MeV/μm.

Other indirect confirmation of a role of structural chromosome damages in mutagenic processes in mammalian and human cells can be the formation of stable chromosome aberrations, for example, translocations, when the exchange of parts between the chromosomes takes place. In this case the loss of genetic matter does not occur and these aberrations are kept in the population of irradiated cells for a long time. It is general recognized, that subsequently such cells can cause the development of mutation processes in the organisms and the cancerogenesis. Our investigations of stable chromosome aberrations in irradiated human lymphocytes by FISH-method [19, 20] testify the high frequency of the translocations, in which the chromosome-1 is involved (Fig. 3). Their number increases nonlinearly with a dose of γ-rays, and after nitrogen ion irradiation it increases essentially, and the dose dependence comes nearer to linear. RBE of nitrogen ions with LET ~80 keV/μm exceeds 3.

As concerns the mutant clones without visible changes at the blot-analysis, it is obvious that the variants with true point gene mutations, connected with single substitutions of the nucleotides in the codones of structural gene of DNA, are amongst them. Phenotypically it results in a replacement of the aminoacids in the ferments, the synthesis of which is controled by this gene. But unlike bacteria, at which gene mutations are always point ones, as for them any double-strand break of DNA becomes lethal event, at mammalian and human cells it is not always possible to distinguish small chromosomal damages (for example, interstitial and terminal microdeletions, insertions) from point mutations, which inactivate this gene. Phenotypically such struc-

Table 2

The means of the change of the coding in irradiated DNA.
(In: Timofeev-Resovsky N.V. et al., 1981)

Pyrimidine		Purines		The changes in coding
Thymine (T)	Cytosine (C)	Adenine (A)	Guanine (G)	
1) The destruction of bases chromofores				It is possible all kinds of transversions 
G ≅ 2 (includes the formation of hydroperoxides, glycols and other)		G ≅ 1 (includes the distruction of pyrimidine or imidazole cycle)		
2) The break of glycoside binding and loss of base (G ≅ 0.05)				and all kinds of transitions 
3) The formation of other bases				Two kinds of transitions are possible 
Uracil codes as T G ≅ 0.02		Hypoxanthine codes as G G ≅ 0.05		

Radiation-chemical yields (G) are in molecules per 100 eV.

tural mutations are displayed in a replacement and a loss of individual aminoacids or, on the contrary, their additional occurrence in molecules of enzyme. Radiation-induced gene mutations are essentially identical to the same changes in codones of structural gene of DNA, as well as at spontaneous mutagenesis. But besides some products of radiation-chemical modification of nitrogen bases of DNA can be also a reason of single substitutions of the nucleotides. In Table 2 the damages of nitrogen bases (the purines and the pyrimidines), that result in the change of the coding in irradiated DNA [21], are shown schematically. As a result of the destruction of pyrimidine and imidoazole cycles and the loss of nitrogen bases because of break of glicoside binding, the different single transversions and transitions in codones of DNA gene must arise. As a result of base modification only single transitions arise. So in consequence of desamination the adenine can turn in the hypoxanthine, which codes as the guanine. The base-pair substitution of AT to GC will be in this place during replication. In consequence of desamination the cytosine can turn to uracil, which codes as the thymine, and in this place of DNA the base-pair GC will be substitute by a base-pair AT. The majority of transitions and some transversions have no influence on matrix activity of DNA and do not interfere in replication and transcription processes, that further aids in remaining these mutations in the cells.

As has been marked earlier, for realization of a damage in a mutation of resistance to 6-TG, several cell cycles of division in normal conditions of growth are necessary before the cells will pass on selective nutritious medium. The damaged nitrogen bases kept unrepared during the first replication cycle, will cause the base substitution in new synthesized DNA (transition or transversion) and occurrence of potentially mutagenic cell. A normal daughter-cell will appear simultaneously. After the second division cycle the first mutant cell with a changed base pair in DNA, one potentially mutant and two normal cells will appear. If all cells will be viable, the 63 mutant, 1-potential mutant and 64 normal cells will appear in the colony after the 7th divisions. During cultivating in medium with 6-TG only the cells with muta-

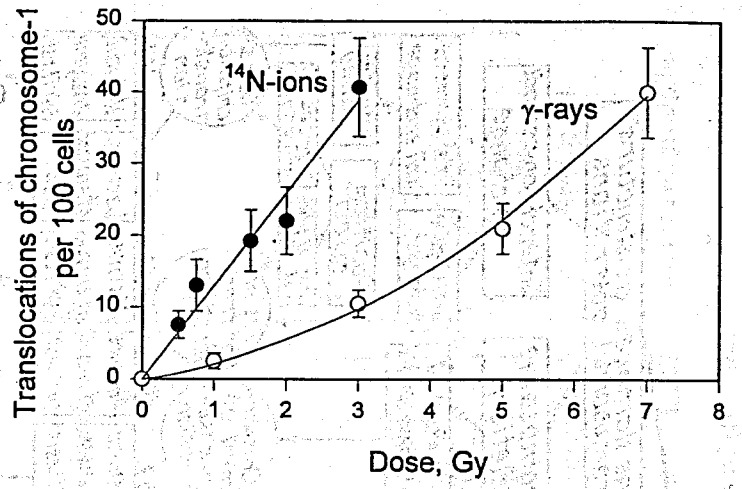


Fig.3. The frequency of translocations of chromosome-1 in human lymphocytes as a function of γ- ray and heavy ion radiation doses.

tion of HPRT-gene, resulting in impaired or absent ability to incorporate this toxic analogue have the chance to survive and to form colony. Depending on the types of base damage, the appearance of the cells with different kinds of HPRT-gene mutations in arising populations is not excluded. In case of base modification all cells will have the same mutation. But in a case of degradation of the bases or break glicozide binding with a loss of the bases, when different transversions and transitions are possible, the daughter-cells with different HPRT-gene mutations can arise from the same potentially mutant cell, and nonuniform cell population on mutagenic properties will generate.

Thus, in a basis of mutagenesis of mammalian and human cells the events lay, which are connected with chromosomes, whether it concerns their chemical organization or impair of structural integrity. It is obvious, that the realization of gene damages on a cell level is complicated. Possible consequences of HPRT-gene damages are considered on the scheme (Fig. 4). They can be connected with intragenic changes ("point" mutations, microdeletions, inversions, addition of a gene sites) and with total loss of gene or its

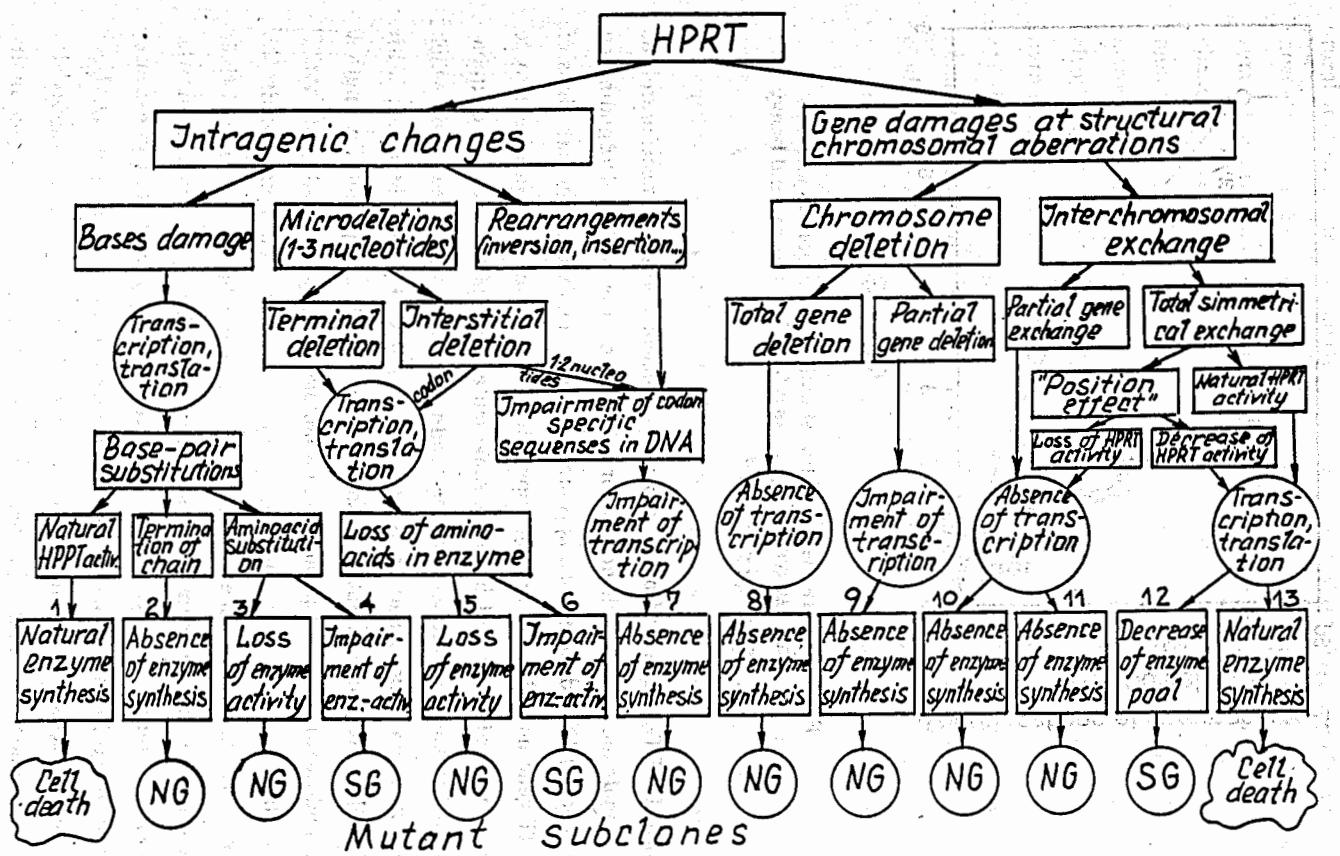


Fig.4. The consequences of HPRT gene damages.

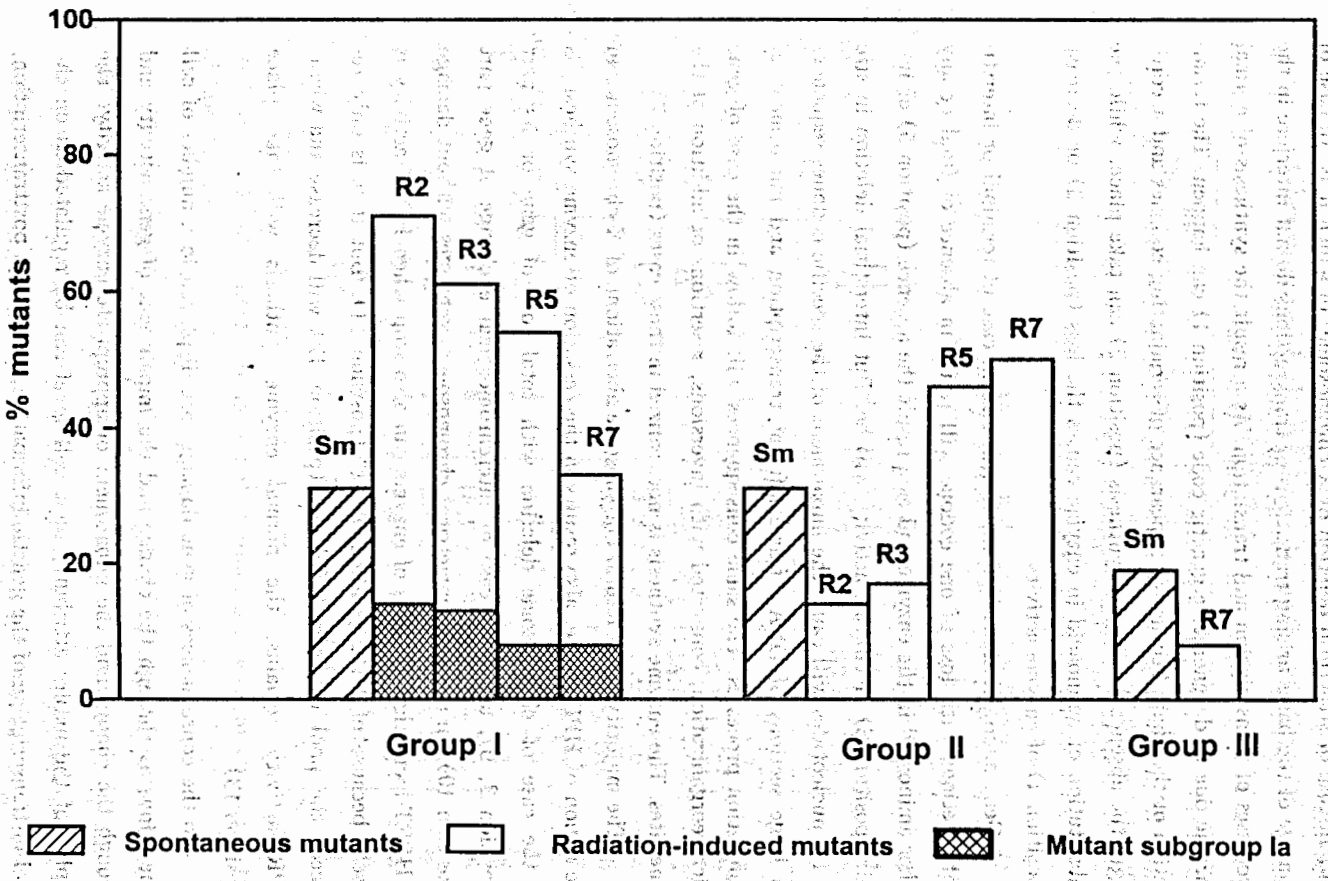


Fig.5. Persentage of diploid mutants in groups.

part in case of structural deletion of X-chromosome, in which it is. In case of damage of the bases arising transitions or transversions do not interfere in the processes of transcription and translation. As a result the synthesis of normal enzyme due to degenerative genetic code (position 1) can happen. The termination of the chain because of appearance of terminating codones and accordingly the absence of enzyme synthesis (position 2) can take place, while the substitution of aminoacid in enzyme causes the loss (position 3) or decrease (position 4) of enzyme activity.

Gene microdeletions in 3 nucleotide size in case of terminal or interstitial deletions with loss of one codone will result in absence of final or any other aminoacid in the enzyme and as a result the absence (position 5) or impair (position 6) of enzyme activity will be. But interstitial deletion in size of 1-2 nucleotides will cause the impairment of the specific codones sequences on large sections of DNA, "frameshift" at transcription and impairment of translation processes. The same consequences will follow in the case of gene microreorganization, connected with inversions, insertions or addition of the gene sites. The enzyme synthesis is not realized in these cases (position 7).

The majority of structural chromosome aberrations of X-chromosome, in the region of HPRT-gene, also results in the cessation of enzyme synthesis as in the case of chromosome deletion with total loss of gene or its part (position 8, 9), as in the case of interchromosomal exchange of gene part (position 10). In case of symmetrical chromosome exchanges without damage of HPRT-locus the synthesis of enzyme can also stop when HPRT-activity is absent because of " the position effect" (position 11). But if it is only reduced, the pool of normal ferment (position 12) will decrease and when HPRT-activity remains, the normal enzyme synthesis will take place (position 13).

So the consequences of gene damages are displayed in a number of cell generations as through the change and the impairment of gene activity and through the change and the impairment of appropriate enzyme activity. The display of the nature attributes on a cell level can be regulated as on the level of transcription of the genetic information, as through posttranscription, translation and posttranslation processes.

Table 3

Chromosomal aberrations in cells of diploid mutants

Types of mutants	Dose Gy	Group	Number of mutants	Cells with chromosomal aberrations, %	Total number of aberrations per 100 cells	Fraction of mutants, %
Control	--	--	14	8.7 ± 0.8	9.9 ± 0.8	78
Spontaneous mutants	--	I b	5	8.6 ± 1.3	10.4 ± 1.4	31
		I l	5	16.8 ± 1.8	21.2 ± 2.1	31
		III	3	39.7 ± 3.7	63.4 ± 4.6	19
Radiation - induced mutants	2	I a	1	4.0 ± 2.0	5.0 ± 2.2	14
		I b	4	9.8 ± 1.6	10.3 ± 1.6	57
		II	1	29.0 ± 5.4	44.0 ± 6.6	14
	3	I a	3	3.0 ± 1.0	3.0 ± 1.0	13
		I b	11	9.0 ± 0.9	10.2 ± 1.0	48
		II	4	19.8 ± 2.2	28.9 ± 2.3	17
	5	I a	1	4.0 ± 2.0	4.0 ± 2.0	8
		I b	6	7.8 ± 1.1	9.5 ± 1.3	46
		II	6	18.7 ± 1.8	22.3 ± 1.9	46
	7	I a	1	2.0 ± 1.4	2.0 ± 1.4	8
		I b	3	7.0 ± 1.5	7.3 ± 1.6	25
		II	6	17.5 ± 1.7	23.7 ± 1.9	50
		III*	1	88(18) ± 9.4	110(22)±10.5	8

* 88 % of cells have interchromosomal symmetrical exchange (other types of aberrations).

The future of irradiated cells during cultivating on selective medium will be various. The cells, avoided of damages, and also mutant cells, in which there is normal enzyme synthesis will die (position 1,13). Others will be capable to form colonies (position 2-12). But their phenotypical properties will

be not the same. It is possible to assume that those, which although synthesize the enzyme, but it appears less active or smaller amount of normal enzyme is synthesized(position 4,6,12), will form colonies slower than other mutant cells. In these cases a viability of mutant population is provided at the expense of the cells, which have no time to incorporate purine analogue during cell cycle. We observed the delay of mutants' growth (almost by a factor of two as compared with the control) during HPRT-mutants revealing from Chinese hamster cell culture. The share of mutants with slower growth increased from 25 up to 85% at increasing of radiation-induced dose from 2 up to 7 Gy. The appearance of the spontaneous mutants on TK-loci with slow growth, that were isolated from human limphoid cells line TK-6, had been also noted by Yandell et al.(1986) [22].

Moreover the consequences of mutation events can be displayed and were displayed in a rise of chromosome instability in population of mutant subclones [23].The mutants were highly heterogeneous on a frequency of aberrant cells and a number of chromosomal aberrations (Table 3). When we systematized them by these parameters we distinguished arbitrarily several groups of diploid mutants: the first ones did not differ essentially as compared to control, but amongst radiation-induced mutants the single ones of this group were found with the frequency of aberrations from 2 to 4 times less. The other mutants had higher level of chromosomal aberrations and were highly heterogeneous on these parameters. At mutants of the group II their level was about 2 to 4 times higher than in control. The mutants of the group III have very high level of chromosomal damages (more than 30% of aberrant cells). Percentage of radiation-induced mutants of the group I decreases from 70 to 33% but the group II increases from 14 to 50% when doses increased from 2 to 7 Gy (Fig. 5). The mutants of the group III were in fact revealed only amongst spontaneous ones. The radiation-induced by dose of 7 Gy mutant was only one and differed from all others in the chromosomal aberrations spectrum: the stable translocation was found in 88% of its cells. The level of other types of aberrations in this mutants did not differ from the level of the mutants of the group II.

Moreover 15-20% tetraploid mutants were observed also. They are characterized by the higher level of chromosomal aberrations as compared to diploid control. The frequency of chromosomal aberrations in the cells of radiation tetraploid mutants, induced by the doses of 2 and 3 Gy, was a few times higher. The single tetraploid mutant, induced by 7 Gy dose, differs from all others: the stable translocation was observed in almost 90% of the cells, the level of other types of aberrations was also high enough. Extremely high level of chromosomal aberrations is revealed at spontaneous tetraploid mutants (about 50% of aberrant cells).

So the heterogeneity on the cytogenetical properties and the increase of chromosomal instability of the mutants were a result of HPRT-mutations in the mammalian cells. The data reflect a phenomenon of the reproductive instability of chromosomes in many generations of mutant cell. The higher level of chromosome aberrations in cells of many mutants can testify that the probability of impairment of chromosome integrity raises in mutants inevitably, possibly, as a stage of the appropriate genome reorganization, that is adequate to changed vital conditions.

Thus, the analysis of aforesaid literature and proper data confirms an essential role of structural damages of chromosomes (and accordingly structural damages of genes) in radiation mutagenesis of mammalian and human cells.

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