Primary Radiation Damage and Disturbance in Cell Divisions

Jin Kyu Kim[a*], Yun-Jong Lee[a], Jae-Hun Kim[a], Vladislav G. Petin[b], Mohammad Nili[c]

[a]Korea Atomic Energy Research Institute, Advanced Radiation Technology Institute, Jeongeup, 580-185 Korea.

[b]Biophysical Laboratory, Medical Radiological Research Center, 249036, Obninsk, Russia.

[c]Neurophysics Division, Dawnesh Radiation Research Institute, 08007, Barcelona, Spain.

Abstract. Survived cells from a homogeneous population exposed to ionizing radiation form various colonies of different sizes and morphology on a solid nutrient medium, which appear at different time intervals after irradiation. Such a phenomenon agrees well with the modern theory of microdosimetry and classical hit-and-target models of radiobiology. According to the hit-principle, individual cells exposed to the same dose of radiation are damaged in different manners. It means that the survived cells can differ in the content of sublethal damage (hits) produced by the energy absorbed into the cell and which is not enough to give rise to effective radiation damage which is responsible for cell killing or inactivation. In diploid yeast cells, the growth rate of cells from 250 colonies of various sizes appeared at different time intervals after irradiation with 600 Gy of gamma radiation from a ^{60}Co isotopic source was analyzed. The survival rate after irradiation was 20%. Based on the analyses results, it was possible to categorize the clones grown from irradiated cells according to the number of sublesions from 1 to 4. The clones with various numbers of sublesions were shown to be different in their viability, radiosensitivity, sensitivity to environmental conditions, and the frequency of recombination and respiratory-deficient mutations. Cells from unstable clones exhibited an enhanced radiosensitivity, and an increased portion of morphologically changed cells, nonviable cells and respiration mutants, as well. The degree of expression of the foregoing effects was higher if the number of primary sublethal lesions was greater in the originally irradiated cell. Disturbance in cell division can be characterized by cell inactivation or incorrect distribution of mitochondria between daughter cells. Thus, the suggested methodology of identification of cells with a definite number of primary sublethal lesions will promote further elucidation of the nature of primary radiation damage which finally results in cell death.

KEYWORDS: ionizing radiation, sublethal damage, yeast cells, hit theory.

1. Introduction

Individual cells from both survived and inactivated parts of a homogeneous population respond differently to the same dose of radiation. Inactivated cells produce microcolonies consisting of various numbers of cells [1]. In diploid cells, effects of several primary lesions (sublethal lesions) may be summarized and inherited resulting in various mitotic disorders. These disorder effects are the higher the greater the number of primary sublethal lesions is produced in the originally irradiated cells. In the course of forming macrocolonies by diploid irradiated cells «lethal sectors» are often formed. Cell damage by radiation can be quantitatively described by the probability model based on the supposition that clone formation is a probable process and the probability for the successful division of cell is determined by a number of damages. This means that the probability model is a peculiar synthesis of hit-and-target principle and biological stochastic. According to this model, single irradiated cells are damaged in a random fashion in accordance with the hit-principle, as in classical models, and the number of damages defines the probability for the successful division [2]. Reduced probability for the successful division can remain unchanged through the successive division of cells. The mechanism of clone formation is the well-known “birth-and-death” process. It would be of interest to obtain experimentally the distribution of survived cells in accordance with the number of primary lesions and after that, taking the population of cells with a known number of sublethal lesions, to determine various biological responses in the dependence of the primary sublethal lesions.

* Jin Kyu Kim, E-mail: jkkim@kaeri.re.kr

Irradiated single cells are damaged in a random fashion in accordance with the hit-principle, and the number of damages defines the probability *P* for the successful division. If the probability of damage expression (the probability of refusal) is α, the probability for the successful division of a cell with one damage is

$$P_1 = (1-\alpha). \quad (1)$$

For independent interaction of radiation damages, the probability for the successful division of a cell with i primary damage (sublethal lesion, hit) may be presented as

$$P_i = (1-\alpha)^i. \quad (2)$$

The reduced probability for the successful division can remain unchanged through the successive division of cells [3]. Hence, the mechanism of clone formation is the well-known "birth-and-death" process. The process operates when an entity, in our case a single cell, either gives rise to progeny like itself (birth), or is removed in some way (death), and these two events occur in a random fashion. It would be of interest to obtain experimentally the distribution of survived cells in accordance with the number of primary lesions and after that, taking the population of cells with a known number of sublethal lesions, to determine various biological responses in the dependence of the primary radiation sublesions.

2. Materials and Methods

Saccharomyces ellipsoideus (vini), strain Megri 139-B, and *S. cerevisiae*, strain 5*a*3b*α* that is heterozygous for the *ade2* mutation. Aliquots with 10^6 cells/ml from incubated cells for 3-5 days at 30°C were exposed to gamma radiation (^{60}Co, Gammacell 220, Atomic Energy Canada Ltd.). The survival was assessed by counting colonies in platings. Primary colonies, which appear after irradiation, were used to obtain subclones. Colonies, subclones of which grew simultaneously with a control and did not differ from the control phenotypically, were identified as stable (normal cell clones). Colonies, which formed slowly growing subclones and/or subclones that differed in morphology, were believed to be unstable clones.

To determine the content of mitotic recombinants in diploids that are heterozygous for the *ade1* and *ade2* mutations, colonies were replated on nutrient media YEPD and the number of white, red and sectored colonies was counted. The quantitative evaluation of respiratory mutants was performed as described in Ogur *et al*. [4], and the content of nonviable cells was determined by detecting, under a microscope, budding and nonbudding cells on the surface of the nutrient agar after growing for one day. Other details were described in the literatures [5,6].

3. Experimental Results and Discussions

The various macrocolonies produced from the irradiated diploid yeast cells with morphological changes may be attributed to the example of the expression of primary sublethal lesions. In case of the strain Megry 139-B irradiated with 600 Gy, the content of morphologically changed cells with two and three sublesions was significantly greater than that for clones without or with one primary sublesion (hit). Among the colonies produced after replating, saltant colonies were about 1% for clones without sublethal lesions, while they were higher than 80% for clones with two or three sublethal lesions.

Table 1. The content of nonviable cells in clones produced from the diploid yeast cells survived after exposure to ionizing radiation

No. of primary sublethal lesions (hits)	No. of tested clones	The content of nonviable cells, %	
		Optimal condition	Suboptimal condition
0	28	17 ± 6	24 ± 7
1	50	20 ± 5	43 ± 11
2	22	27 ± 9	54 ± 15
3	28	36 ± 13	87 ± 13

The increased probability of unsuccessful division (refusal) should result in the existence of nonviable cells in clones produced by the single cells survived after irradiation. Experimental data concerning the content of cells incapable to proliferation at optimal (30°C, standard nutrient media) and suboptimal (37°C, standard nutrient media + 7% NaCl) conditions for distant progenies of diploid yeast cells (strain Megry 139-B, 600 Gy) are presented in **Table 1**.

It was shown before[4)] that for diploid strains Megry-139B and 5*a*3Bα the probability of refusal α = 0.12. Hence, the following probabilities for the successful division of cells with 0, 1, 2, 3 and 4 damages can be obtained: P_o = 1, P_1 = 0.88, P_2 = 0.77, P_3 = 0.68 and P_4 = 0.60. It turned out that the reduced probability for the successful division of damaged cell is retained for a large number of cell generation[4,5)]. It leads to the decrease of clone-formation rate immediately after irradiation and upon the successive plating.

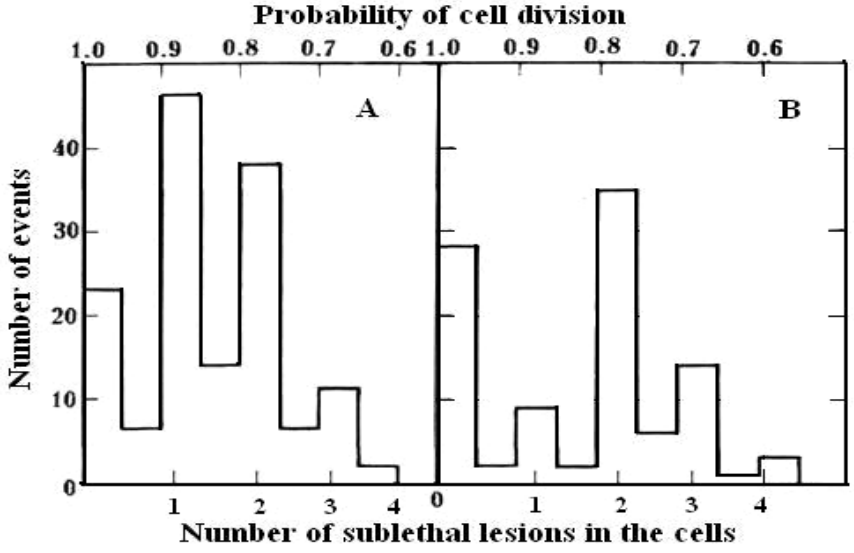
If the reduction of growth rate is related with the decreasing of the probability P_i for the successful division, the specific growth rate of cells μ_i with i sulethal lesions may be given as

$$\mu_i = \mu_c(2P_i - 1), \tag{3}$$

where μ_c is the specific growth rate of control cells. We determined the specific growth rate of cells from 150 (Megry-139B) and 100 (5A x 3B) colonies produced by the survived irradiated diploid yeast cells (^{60}Co, 600 Gy, survived ≈20%).

Knowing μ_i and μ_c, we calculated P_i for these clones. Experimental results are presented in **Figure 1**. It is of interest that the maxima of the distribution almost coincide with theoretical values of P_i predicted for cells with 0, 1, 2, and 3 primary damage (sublethal lesion). It would be of interest to test whether clones with various values of damages differ in their radiosensitivity, viability, sensitivity to environmental conditions, frequency of recombination and respiratory-deficient mutations.

Figure 1. Distribution of clones grown from irradiated cells according to the probability for successful division (upper scale of the abscissa), corresponding to the number of primary damage (sublethal lesion) in the original cell (lower scale of the abscissa); strains*:* Megry 139-B (panel A) and 5*a*3b*a* (panel B)



The relative yield of nonviable cells in clones produced by survived diploid yeast cells increases with the number of inherited primary radiation sublethal lesions. Due to this fact, the growth effectiveness of cells under suboptimal conditions of culture was 76, 46 and 13% correspondingly to clones with 0, 2, and 3 primary sublethal lesions. Some unstable clones differ by a high segregation rate of respiratory mutants. Although respiratory mutants are virtually not encountered among primary colonies of irradiated diploid, these mutants can constitute more than 30% of all subclones in plating

of some unstable clones. Diploid yeast cells (strain Megry 139-B) were irradiated in the stationary phase of growth (600 Gy). The data concerning the yield of respiratory mutants in clones with various numbers of primary sublethal lesions are presented in **Table 2**. With the tetrazolium overlag technique [4], cells with normal respiratory ability were colored in red color while clones consisting of respiratory mutants stayed white. It is obvious that irradiation resulted in an increased content of respiratory mutants in clones produced by irradiated cells. The effect was particular expressed for clones with a greater number of primary sublethal lesions (hits).

Table 2. The content of respiratory mutants in clones produced from the survived diploid yeast cells exposed to ionizing radiation

No. of primary sublethal lesions	Clones containing respiratory mutants (%)			
	0-2	>2-10	>10-50	>50-100
0	100	-	-	-
1	80	16	2	2
2	53	12	20	15
3	32	16	28	14

3. Conclusions

The primary radiation lesion is not absolutely lethal for the diploid cell. With a certain probability which depends on the total number of primary sublethal lesions and conditions of culture, they result in the disturbance of cell division. Such a disturbance revealed by cell inactivation (nonviable cells among clones produced by irradiated cells), incorrect distribution of mitochondria between daughter cells (respiratory mutants). Cells from unstable clones are characterized by the enhanced radiosensitivity. The degree of expression of the foregoing effects was the higher the greater the number of primary sublethal lesions was in the originally irradiated cell.

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