ONCOLOGY

Higher Efficacy of Cloning in Semisolid Medium of Transformed Cells Survived after High-Dose γ-Exposure

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The capacity of descendants from cells exposed to high-dose γ-radiation to form colonies irrespective of the substrate is studied. Clonogenic activity of descendant cultures was several times higher than that of the initial cell line both on solid substrate (glass) and in semisolid medium.

Key Words: *descendants from γ-irradiated cells; high dose; semisolid medium; clonogenic activity; proliferative potential*

The rate of recovery of tumor cell population after irradiation depends on many factors: size of growth fraction (count of clonogenic cells survived after exposure), their proliferative activity, and time of generation. Combination of high radioresistance due to which the growth fraction can be retained in the exposed population and high clonogenic activity of these cells can promote rapid recovery of the tumor cell population after high-dose irradiation.

Previously we showed that the descendants from cells irradiated in doses of 10 and 20 Gy (DIC-10 and DIC-20) were several times more radioresistant than the initial reference cells [3]. The proportion of highly radioresistant cells in DIC groups was 17-20%. These experiments revealed another interesting fact: the efficacy of inoculation (EI) is sharply increased in descendants from irradiated cells in comparison with the initial culture.

Clonogenic activity is commonly characterized by the EI of viable cells on a solid substrate (glass). Another indicator of proliferative activity typical of transformed cells is the so-called true clonogenic activity, or capacity of cells to form colonies in a semisolid medium. The efficacy of cloning on a solid substrate and in semisolid medium can differ by several orders of magnitude [2].

A drastic increase of the proliferative activity of DIC can be associated with increase of their malignancy, therefore, we investigated the capacity of these cells to multiply on any substrate (in semisolid medium), because this capacity is a characteristic feature of malignant transformation [8]. The aim of this study was to compare the clonogenic activity of DIC on a solid substrate and in a semisolid medium.

MATERIALS AND METHODS

Jungar hamster fibroblasts DEF-4/21 transformed by SV-40 virus were grown in RPMI-1640 medium with 10% cattle serum [1]. Cells in the logarithmic growth phase were irradiated once using an Agat-R γ-device (^{60}Co) at 0.82 Gy/min intensity.

Clonogenic activity of cells was assessed by culturing on solid substrate and in semisolid medium. Cell capacity to form full-value colonies on solid substrate (glass) was assessed by a modified method

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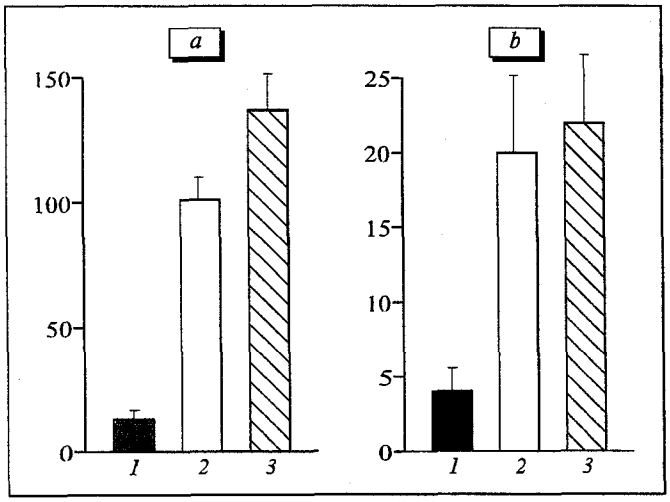

Fig. 1. The efficacy of inoculation of initial cell culture, DIC-10, and DIC-20 on solid substrate (*a*) and in semisolid medium (*b*). Ordinate: inoculation efficacy, %. Here and on Fig. 2: *1*) initial culture; *2*) DIC-10; *3*) DIC-20.

[3,7]. Colonies consisting of less than 50 cells were considered abortive. Half of the dishes from each group was stained and used for morphological analysis of colonies, while in the other half, total count of cells per dish and the mean cell count per colony were determined. Fixed and crystal violet-stained colonies were counted under a Leutz inverted microscope on day 8 of growth. The diameters of colonies were measured with an ocular micrometer.

The efficacy of cell cloning in semisolid medium [2] was assessed in culture medium with 1.2% methyl cellulose (Sigma). Each group included 3-5 dishes. Fourteen days after inoculation, the colonies were counted under microscope and their size measured using an ocular micrometer.

The time course of culture growth was analyzed by growth curves. For this purpose, cell suspension in growth medium (10^4 cells/ml) was inoculated in a dose of 1 ml/well in 24-well plates and incubated in a CO_2 thermostat. Cells grown in wells were counted every day in a Goryaev chamber for 8 days (3-5 wells for each group daily).

RESULTS

In the first series of experiments, we studied the capacity of 3 groups (initial culture, DIC-10, and DIC-20) to form colonies on glass (Fig. 1, *a*). The number of colonies in DIC-10 was 8 times and in DIC-20 10 times higher than in the control; the number of colonies in the DIC groups surpassed the number of inoculated cells. The time of generation apparently decreased and adhesion to the underlayer reduced in DIC. Cells descending from the colonies created additional colonies during a short period.

To verify this hypothesis, we followed up the time course of cell growth in 3 studied groups (Fig. 2, *a*). DIC had a shorter lag period than the control cells, and the growth rate on days 2-8 was 1.4 and 1.6 times higher for DIC-10 and DIC-20, respectively, than cell proliferation in the control culture.

Moreover, the recovery of DIC after exposure to 8 Gy is much more rapid than in the group of initial irradiated cells (Fig. 2, *b*). In the control culture, the count of cells remains very low for 5 days after irradiation, and notable repopulation starts only from days 5-6, while the recovery of DIC starts as early as from day 2 and sharply increases from day 4. The rate of increment in cell count is two times higher in DIC than in the control culture.

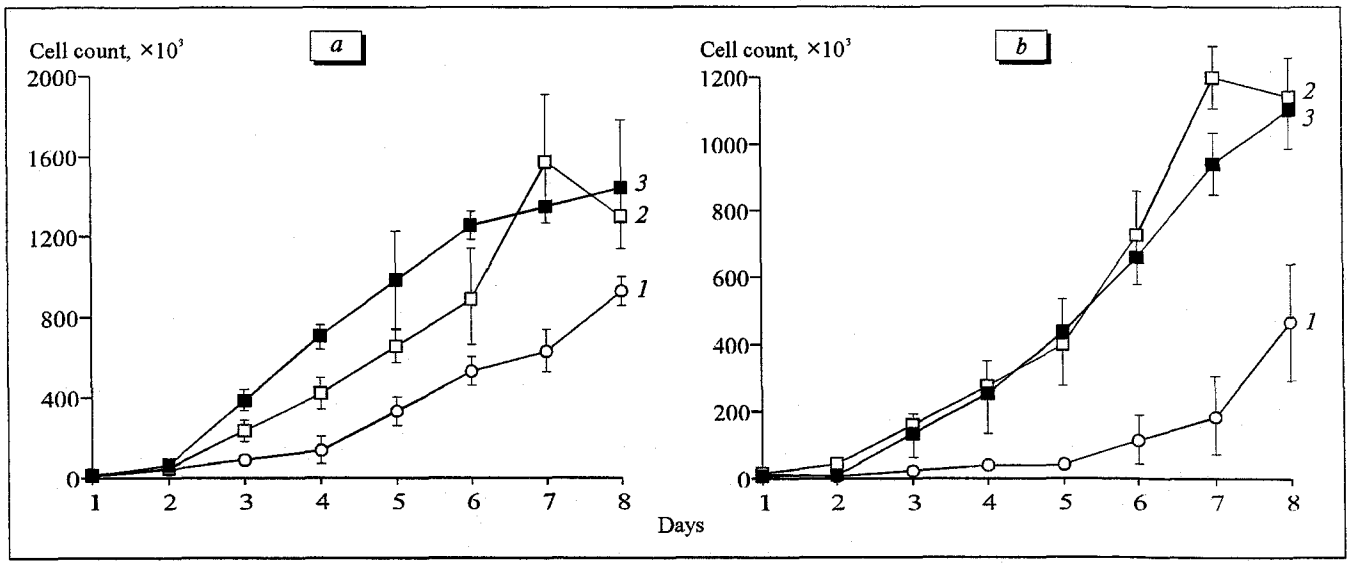

Fig. 2. Time course of fibroblast growth under normal conditions (*a*) and after irradiation in a dose of 8 Gy (*b*). Radiation power 0.82 Gy/min.

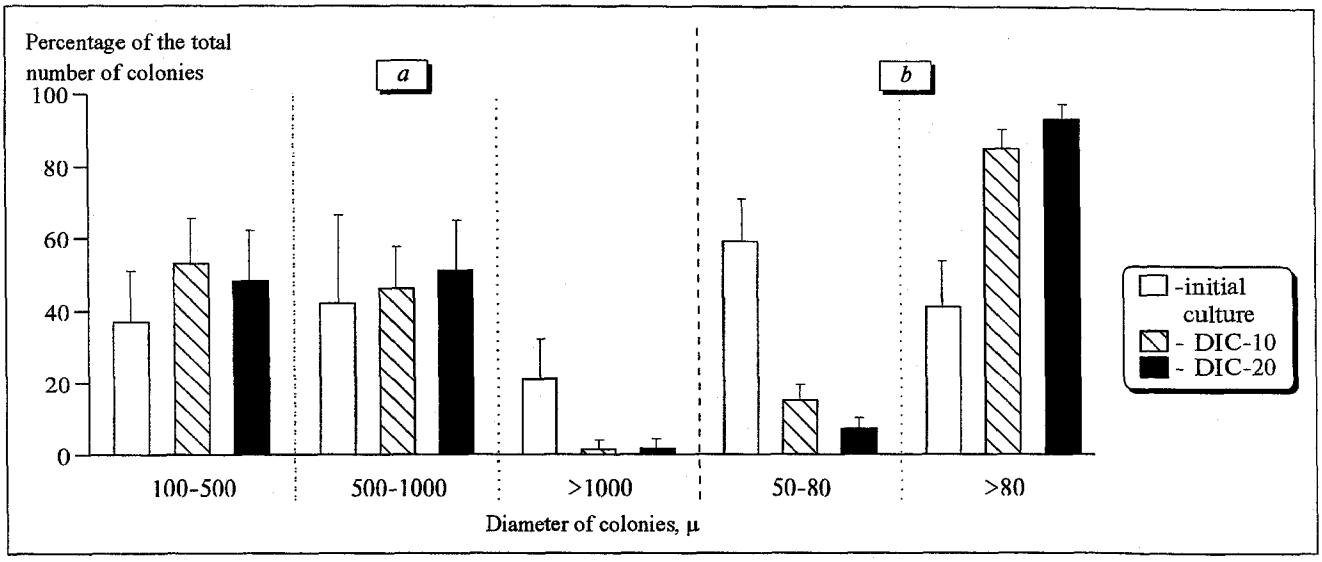

Fig. 3. Distribution of colonies by size for the initial cells, DIC-10 and DIC-20 on solid substrate (*a*) and in semisolid medium (*b*).

From our results it can be concluded that increased rate of cell growth in DIC groups is due to increased clonogenic activity and to a shorter generation of proliferating cells. Thus, the proliferative potential of DIC is essentially higher than of the initial cell line.

Distribution of colonies by size characterizes the state of cell population [6]. Usually, high irradiation dose leads to an increase in the proportion of small colonies [9]. Comparison of the distribution of colonies by size in 3 tested cultures showed that the proportion of large colonies (>1 mm) in the initial cells is 5 times greater than in the descendants (Fig. 3, *a*). Morphological analysis showed that colonies formed by DIC were more compact and dense, and at least 3 more variants of colonies, differing by morphology, could be distinguished among them; this indicates a greater heterogeneity of DIC clonogenic cells in comparison with the initial cell strain. The mean number of cells per colony was 552 for the initial culture and 1715 and 817 cells for DIC-10 and DIC-20, respectively. Although DIC colonies were small, they were 1.5 (DIC-20) and 3 (DIC-10) times larger than colonies of descendants from the initial culture.

These results indicate that descendants from cells survived after high-dose irradiation differ from the initial cell culture functionally and morphologically and can be regarded as an individual substrain.

We investigated a possible relationship between high proliferative activity of DIC on solid substrate and its capacity to form colonies in semisolid medium. Figure 1, *b*, shows that EI of initial cells is 4% in medium with methyl cellulose and 5 times higher in the DIC groups. Comparison of efficacy of inoculation on solid substrate (Fig. 1, *a*) and in semisolid medium (Fig. 1, *b*) shows that EI of initial cells in semisolid medium is 3 times lower and of DIC groups 5 times lower than on solid substrate. Therefore, not all clonogenic DIC which form colonies on solid substrate can form them in semisolid medium. Close values of EI in semisolid medium (20-22%) and of the resistant cell fraction in DIC (17-20%), detected in previous studies, are worthy of note. Presumably, only the most viable radioresistant cells form colonies in semisolid medium.

The morphology of colonies grown in semisolid medium from the initial culture and from descendants of cells survived after high-dose irradiation is similar. In order to characterize the efficacy of cell growth in semisolid medium, we compared the sizes of colonies formed by DIC and initial cells (Fig. 3, *b*). In semisolid medium, DIC formed mainly large colonies: the number of colonies larger than 80 μ was twice as large as that after inoculations of the initial cells. This fact confirms a high proliferative potential of DIC cultures. In semisolid medium, DIC form mainly large morphologically homogenous colonies, while on the glass they form small compact colonies differing morphologically. Comparison of DIC groups growth on two substrates permits us to conclude that inoculation of DIC in medium with methyl cellulose is a sort of a "filter" selecting truly clonogenic cells and discarding common clonogenic cells generating colonies only on solid substrate.

These data indicate that descendants from cells survived after high-dose irradiation acquire some properties distinguishing them from the initial cell strain. The most important of them is a high pro-

liferative potential observed both on a solid substrate and in a semisolid medium. Combination of new properties of DIC, such as high radioresistance and clonogenic activity, ensure their capacity to recover tumor growth rapidly and effectively.

Some scientists [4,5] believe that variants more likely to generate colonies in semisolid medium than the initial cells emerge as a result of mutations. In addition, tumor cell capacity to multiply on any substrate (in semisolid medium) is a characteristic feature of malignancy of the isolated substrains. In our case, further malignant degeneration of DIC survived after high-dose exposure could be caused by mutations induced by high-dose γ-irradiation.

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