ONCOLOGY

Effect of γ-Radiation on the Formation of Cell Complexes in HeLa Cell Cultures

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The number of cultured HeLa cells forming cell complexes and the number of cells per complex decrease after repeated irradiation (0.1 and 4.9 Gy at a 3-min interval). The modulating effect of small radiation doses is observed only in confluent cultures.

Key Words: *cell culture; irradiation; small radiation doses; contacting and noncontacting cells; cell complexes*

Cellular interactions are a major factor of tissue organization. Formation of a tissue-like cell population is the first step of cell differentiation.

For instance, at the "subtissue" level the epithelium is organized according to the principle of functional epidermal proliferative unit (EPU) that consist of basal cell and its descendants [1,7,8]. Sometimes these structures (central cell surrounded by peripheral cells) are referred to as "rosettes" [6].

Both prenatal and postnatal development of EPU has been investigated. The existence of subtissue organization was confirmed by analysis of the rat epidermis structure [3].

It was reported that epidermal tumor cells preserve their tissue specificity even in long-term cultures [5].

In the present study we examined the effect of γ-radiation on subtissue organization of the HeLa cell population. Cell monolayer (model of contacting cells) or cell suspension (model of noncontacting cells) was irradiated in two regimes. Two models were employed in an attempt to find out whether the formation of tissue-like structures is determined by the initial state of cell population before irradiation.

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Previously, we demonstrated different responses (assessed by survival of clonogenic cells) of contacting and noncontacting cells to small radiation doses (0.1 Gy) [2].

MATERIALS AND METHODS

HeLa cells were grown in medium 199 supplemented with 10% bovine serum and antibiotics (100 U/ml penicillin and 100 U/ml streptomycin) using the standard procedures [4]. Confluent cultures (logarithmic growth, 3-4 days after seeding 5×10^4 cell/ml) served as a model of contacting cells. Cell suspensions or cells in lag-phase (2 h after seeding in the same concentration) provided a model of noncontacting cells. Cell cultures and suspensions were irradiated once in a dose of 5 Gy or two times: 0.1 and 4.9 Gy at a 3-min interval.

Cell responses was assessed cytomorphologically. Cell complexes and cells in these complexes were counted in a limited field of view (0.5×0.5 mm) per 5000 cells on preparations stained with hematoxylin and eosin; 5-7 preparations were examined in each case.

An Agat-R apparatus (^{60}Co, 0.8 Gy/min) was used as a source of γ-radiation.

Statistical analysis was performed by the methods of variational statistics with the significance level $p<0.05$.

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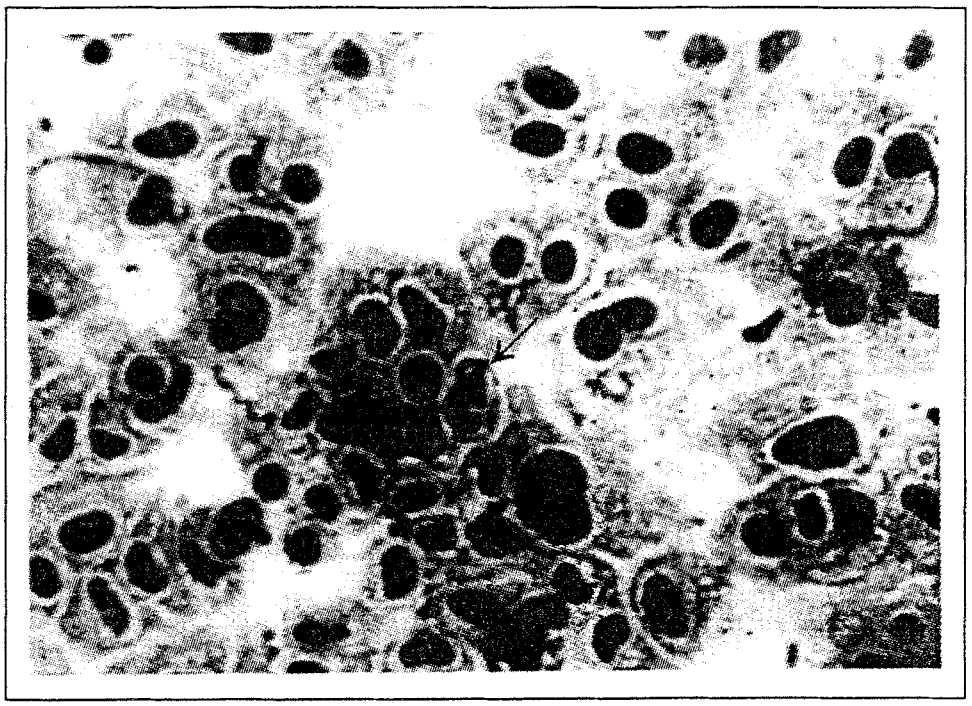

Fig. 1. Population of HeLa cells on day 6 after seeding and irradiation. A typical cell complex is indicated with an arrow. Hematoxylin and eosin, ×200.

RESULTS

In this study, cell complexes are defined as cell clusters formed by the central cell surrounded by at least three cells. The tissue-like organization of intact HeLa cultures with formation of EPU was observed during logarithmic and stationary growth (4-11 days). After irradiation, the dynamics of this process changed. On days 4-6 after seeding and irradiation some cells accumulated around one cell and formed a complex. As Fig. 1 shows, central and peripheral cells are morphologically different: the central cell is round, while peripheral cells have a narrow and wide apices, the narrow apex contacting with the central cell. The wide apex contacts with neighboring cells in the complex or other cells.

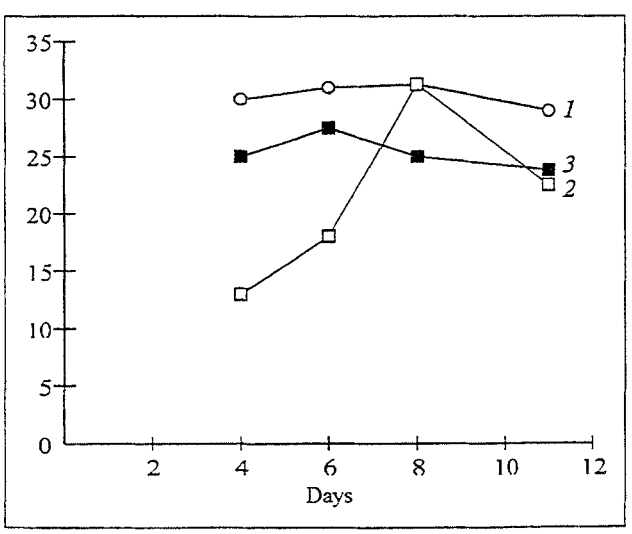

Fig. 2. Relationship between cell complex formation and the initial state of irradiated cells. *1*) control; *2*) confluent culture; *3*) cell suspension. Here and in Figs. 3 and 4: ordinate: number of cell complexes, %.

On day 8 after irradiation, peripheral cells lost their polarity. On day 11, a tendency toward a decrease in the number of contacting cells in the complex was observed. A similar situation developed in the control cultures. The differences consisted in the dynamics of complex formation.

In the control cultures, the number of complex-forming cells throughout the entire experimental period remained at the level of 30% of the total cell number in the field of view (Fig. 2). A graduate activation of complex formation was observed after irradiation of cell cultures with 5 Gy: on day 8 the number of cells in the complexes was twice as that on day 4. In cell suspensions irradiated with the same dose, this parameter remained unchanged during days 4-11. It should be mentioned that on day 8 (transition to the stationary growth phase in intact culture) the number of cells in the complexes reached the maximum in confluent cultures, while in suspensions the complexes contained 1.5-fold less cells compared with complexes formed in monolayers. Thus, the reorganization of the HeLa cell population formed by contacting and noncontacting cells after γ-irradiation in a dose of 5 Gy differ by the dynamics of cell complex formation.

In a second series of experiments we examined the modifying effect of small radiation dose (0.1 Gy)

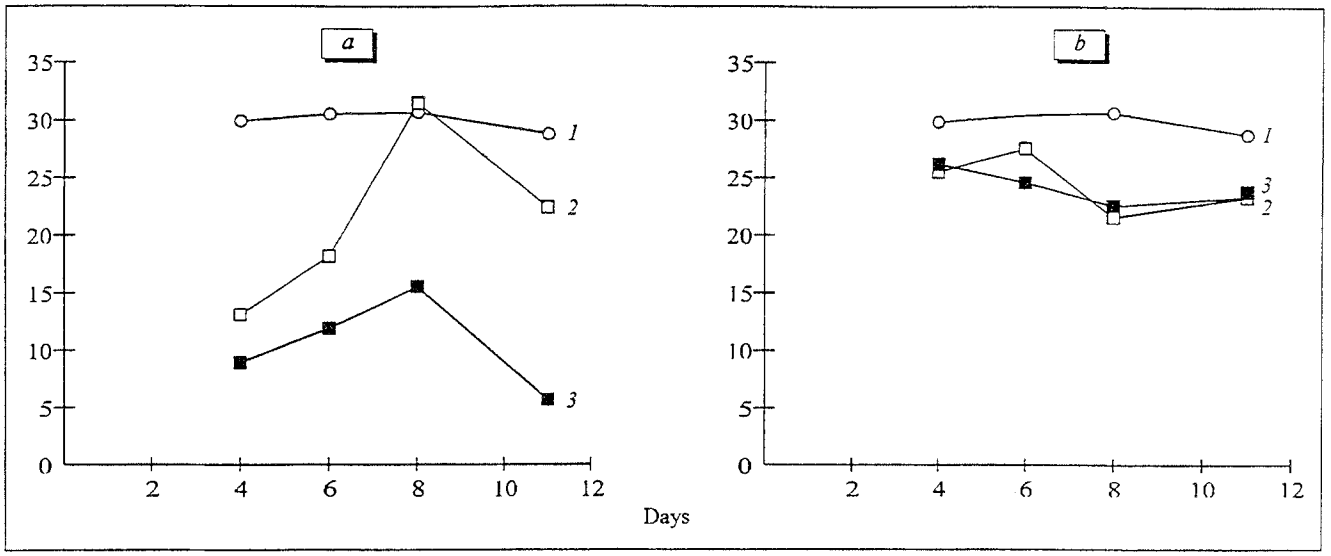

Fig. 3. Total number of complex-forming cells in cell monolayer (*a*) and suspension (*b*). *1*) control; *2*) 5 Gy; *3*) 0.1 Gy+3 min+4.9 Gy.

on the formation of cell complexes in a confluent culture or cell suspension. Figure 3 shows that irradiation of confluent cultures with 0.1 Gy 3 min before irradiation with 4.9 Gy markedly suppresses complex formation (Fig. 3, *a*); in cell suspension this effect was not observed (Fig. 3, *b*). Generally, 20-

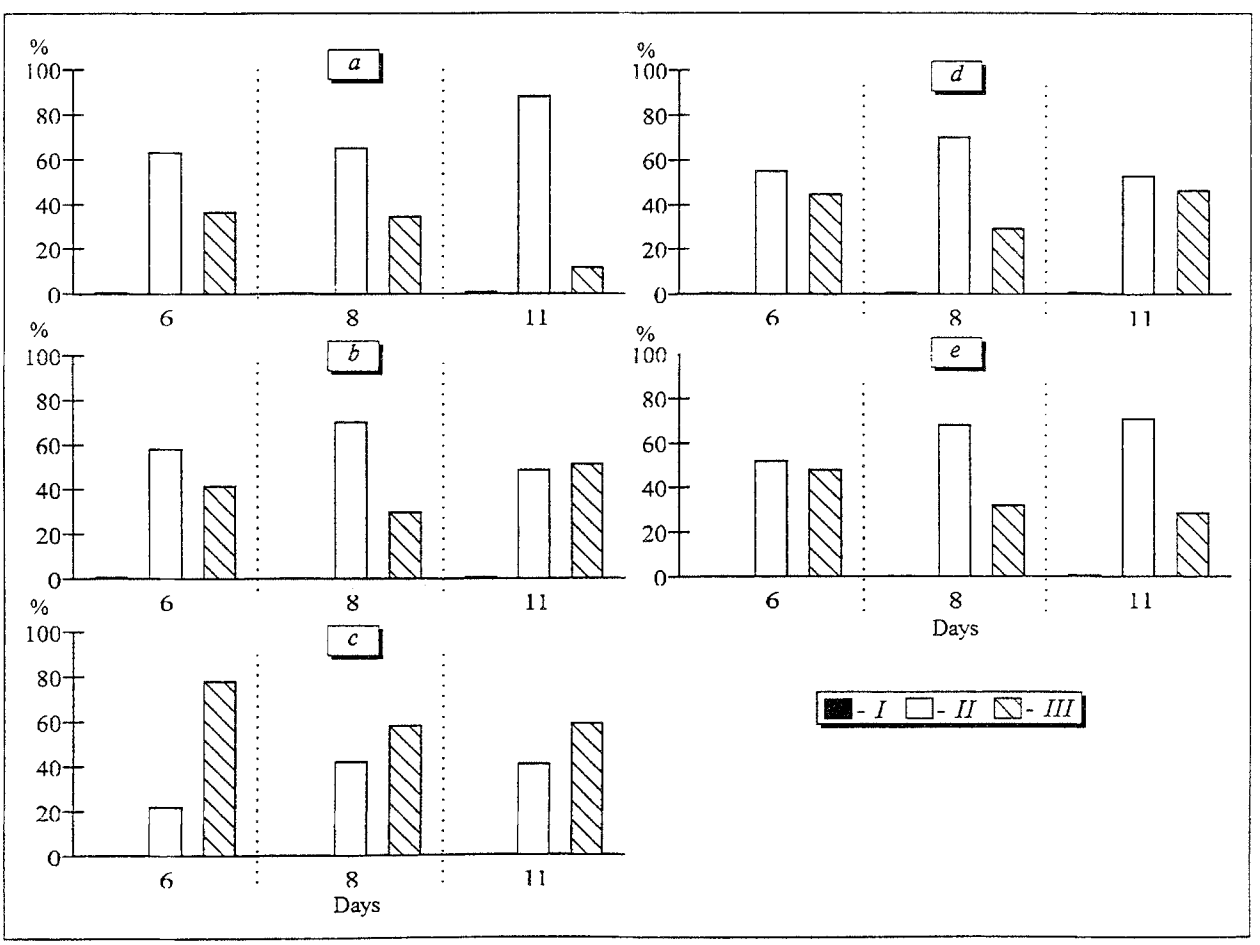

Fig. 4. Cell complexes containing different numbers of cells. *a*) control (intact cells); *b*) confluent culture, 5 Gy; *c*) confluent culture, 0.1 Gy+3 min+4.9 Gy; *d*) cell suspension, 5 Gy; *e*) cell suspension, 0.1 Gy+3 min+4.9 Gy. *I*) complex containing 9 and more cells; *II*) complex containing 5-8 cells; *III*) complex containing 3-4 cells.

30% of cells formed complexes. These results indicate that small radiation doses modify the formation of cell complexes only in a confluent culture.

Figure 4 illustrates the effect of γ-irradiation on the number of cells in the complex formed in cell cultures and suspensions. Complexes formed by 5-8 cells predominated in the control throughout the observation period (Fig. 4, *a*). On days 6-8 of culturing, the numbers of small (3-4 cells) and large (>9 cells) complexes were almost equal. On day 11, the number of complexes consisting of 3-4 cells decreased. After irradiation in a dose of 5 Gy, the numbers of cells in the complexes were practically the same in cell monolayer and suspension. It should be mentioned that on day 11 (similar to the control), the number of complexes consisting of 5-8 cells slightly decreased in cell monolayer, while in cell suspension it remained practically unchanged. The situation was different after repeated irradiation: although complexes formed by 5-8 cells predominated (as after a single-dose irradiation) and small complexes predominated on day 6, large complexes were not observed throughout the experiment. The effect of 0.1 Gy irradiation was observed in confluent cultures but not in cell suspensions.

Our results indicate that the time course of reorganization of the HeLa cell population after γ-irradiation is different in confluent culture and cell suspension. Irradiation in a dose of 0.1 Gy markedly decreases both the number of complex-forming cells and the number of cells in the complex. This effect is observed in cell monolayer but not in cell suspension.

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