Bridges and Multipolar Mitoses in Populations of Rat PA-23 Rhabdomyosarcoma Cells

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Frequencies of abnormal mitoses are estimated in clones (experimental metastases) of two sublines of transplanted rat PA-23 rhabdomyosarcoma selected for increased and decreased frequency of bridges. It is shown that the frequency of cells with bridges is associated with a wide range abnormal mitoses and reflects the incidence of structural chromosomal and genomic mutations in the malignant cell populations.

Key Words: *bridges; multipolar mitoses; abnormal mitoses*

Cytochalasin B induces multipolar mitoses in cultured lymphocytes so that the frequency of bridges in multipolar ana- and telophases becomes significantly higher than in bipolar ones [5]. The induction of multipolar mitoses by cytochalasin B increases the incidence of bridges. A relationship between bridges and multipolar mitoses becomes obvious, postulating that cytochalasin B acts on cytoskeletal proteins [6] and does not cause the emergence of bridge-forming dicentric chromosomes.

For better understanding of this relationship it is necessary to find out whether an increase in the frequency of spontaneously formed bridges leads to an increase in the frequency of multipolar mitoses in cell population. In the present study we used two PA-23 rhabromyosarcoma sublines selected for increased and decreased spontaneous frequency of bridge-containing cells (FBCC). Our goal was to estimate the frequency of multipolar ana- and telophases in these sublines *in vivo*. In addition to multipolar mitoses, we also estimated the frequencies of some other abnormal mitoses.

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MATERIALS AND METHODS

Clones (pulmonary metastases of unicellular origin) of two transplanted rat PA-23 rhabdomyosarcoma sublines selected for increased and decreased FBCC were used.

After five selection cycles, impression smears of 5 clones with low FBCC incidence and 8 clones with high FBCC incidence were prepared. The smears were stained with Hoechst 33258 fluorochrome and analyzed at magnification 400 for the presence of mitoses. Mitotic cells were then examined an immersion microscope at magnification 1000. For each clone, 30-60 anaphases and early telophases and 70-150 metaphases were analyzed.

The frequencies of the following abnormal mitoses were determined and expressed in percent: bridges in ana- and telophases, bridges and lagging chromosomes or their fragments in ana- and telophases, metaphases with lagging chromosomes (metaphases with a chromosome or a group of chromosomes that had lagged from the metaphase plate), metaphases with scattered chromosomes (C-metaphases), and multipolar ana- and telophases.

The data were statistically analyzed by the Wilcoxon—Mann—Whitney *U* test.

RESULTS

After five selection cycles, the mean FBCC was 0.5% in 5 clones with low FBCC and 13.5% in 8 clones

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with high FBCC ($p<0.001$). The percent of FBCC as well as the frequencies of bipolar ana- and telophases and bipolar ana- and telophases with bridges and lagging chromosomes or chromosome fragments in these clones are shown in Fig. 1. Clones with low FBCC had significantly lower frequencies of ana- and telophases with bridges (Fig. 1, *a*) and of ana- and telophases with both bridges and lagging chromosomes or their fragments compared with clones with high FBCC (Fig. 1, *b*) ($p<0.001$). The proportion of bipolar ana- and telophases with bridges in the clones selected for elevated FBCC was 69.8%. The clones with elevated and lowered FBCC did not differ significantly in the incidence of bipolar ana- and telophases with lagging chromosomes or their fragments (Fig. 1, *c*) ($p>0.05$).

As Fig. 2 shows, the frequency of scattered chromosomes and metaphases with lagging chromosomes was markedly decreased in clones with low FBCC compared with clones with elevated FBCC ($p< 0.01$ and $p<0.001$, respectively).

The incidence of multipolar ana- and telophases in the clones with FBCC was significantly higher than in those with lowered FBCC ($p<0.01$) (Fig. 3). It should be noted that out of 183 ana- and telophases only 2 tripolar anaphases and no ana- or telophases with >3 poles were encountered in clones with low FBCC, while the proportion of tripolar ana- or telophases in clones with high FBCC amounted to 61%, and 90.7% of these ana- and telophases had bridges (which occurred in 68 and 75 of the tripolar ana- and telophases, respectively).

The selection of PA-23 clones for increased FBCC led to a significant increases in the incidence of multipolar ana- and telophases, more than 90% of which were with bridges. A positive correlation was established between the frequency of cells with bridges, the frequency of ana- and telophases with bridges, and the frequency of multipolar mitoses. The formation of bridges in ana- and telophases probably results in the emergence of interphase cells with bridges. The mechanisms underlying the mutual influence of bridges and multipolar mitoses remain to be elucidated.

The question of bridges in tripolar ana- and telophases deserves a separate discussion. The following relationships between bridges and multipolar ana- and telophases can be suggested. The normal arrangement of chromosomes in the metaphase plate is orderly [3,4], but dicentric chromosomes from which bridges are formed [2] appear to influence the spatial orientation of chromosomes in the metaphase plate in such a way that metaphases with scattered chromosomes emerge (Fig. 2, *a*), with subsequent formation of tripolar mitoses [1]. On the other hand, the formation of bridges may be influenced by tripolar mitoses.

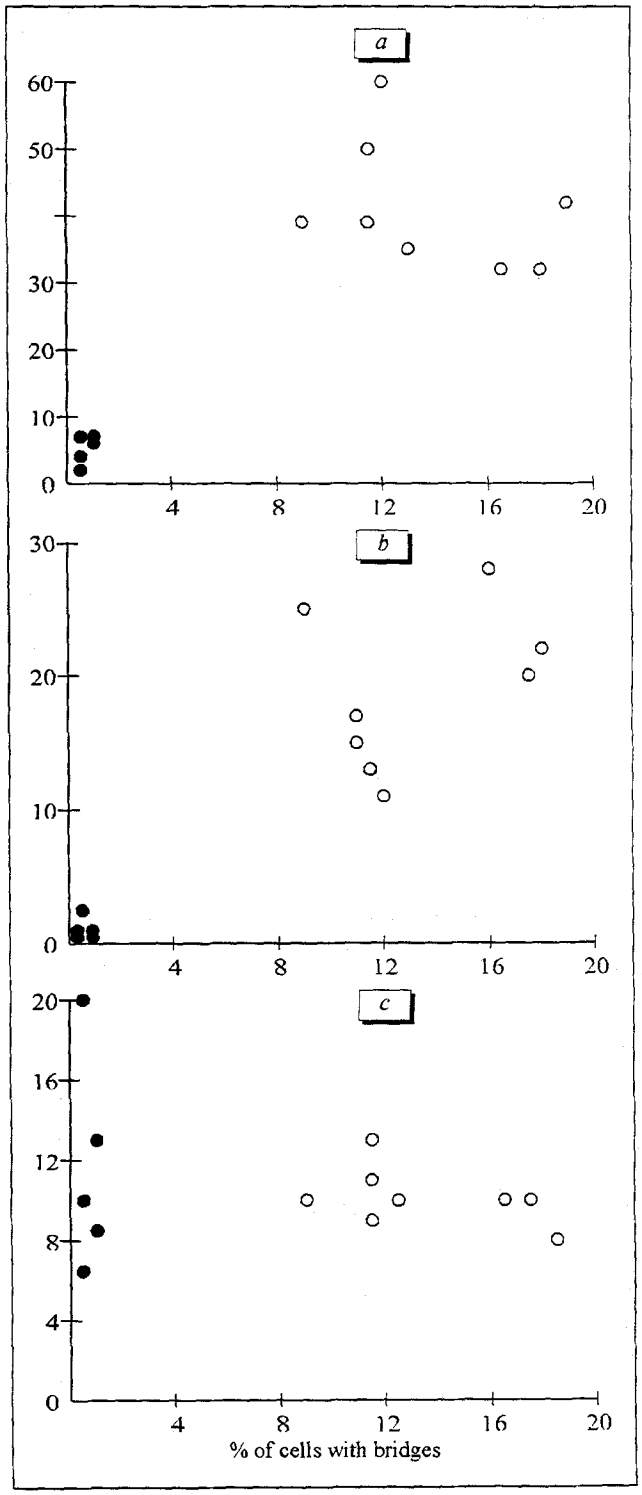

Fig. 1. Percent of bipolar ana- and telophases (ordinate) with bridges (*a*), with bridges and lags (*b*), and with bridges *and* lagging chromosomes or chromosome fragments (*c*) in clones of rat PA-23 rhabdomyosarcoma sublines with elevated (white circles) and lowered (black circles) frequencies of cells with bridges.

If two kinetochores function in a dicentric chromosome (sometimes only one kinetochore functions in polycentric chromosomes [7,8]) and their distribution

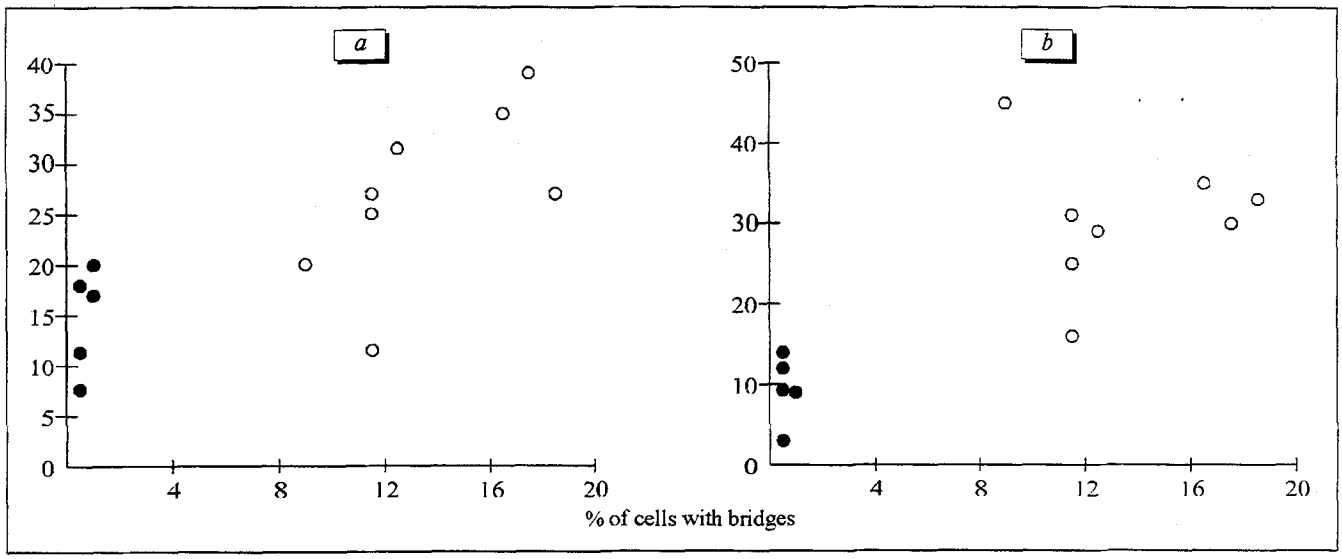

Fig. 2. Percent of the frequencies of metaphases (ordinate) with scattered chromosomes (with C-metaphases) (*a*) and lagging chromosome (*b*) in clones of rat PA-23 rhabdomyosarcoma sublines with high (white circles) and low (black circles) frequencies of cells with bridges.

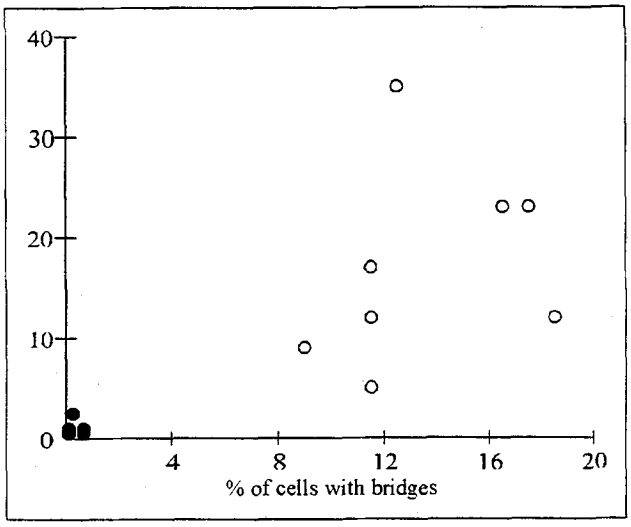

Fig. 3. Percent of the frequencies of multipolar ana- and telophases (ordinate) in clones of rat PA-23 rhabdomyosarcoma sublines with elevated (white circles) and lowered (black circles) frequencies of cells with bridges.

between the poles is random, then the probability of bridge formation by dicentric chromosome in a bipolar mitosis is lower than in tripolar mitosis under the same conditions. Multipolar mitoses provoke the formation of bridges from dicentrics rather than the formation of dicentric chromosomes.

From our results it can be concluded that the incidence of cells with bridges is related to the incidence of multipolar mitoses, and that this relation holds true for a variety of abnormal mitoses in populations of malignant cells. Further research into the mechanisms by which these parameters are related is necessary for a better understanding of the relationship between chromosomal aberrations and genomic mutations in cell populations.

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