INTERACTION BETWEEN NORMAL AND TRANSFORMED CELLS IN INDIVIDUAL AND MIXED CULTURES

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Transformed fibroblast-like cells of lines KhÉTR and KhÉK-40, previously labeled with thymidine-H^3, were seeded on test monolayers of homologous or heterologous cells. The transformed cells were found to multiply on a layer of normal hamster fibroblasts but not to multiply on a layer from cells of the homologous type, although the density of the cell layer in that case was indistinguishable from the density of the normal cells. Specific density-dependent inhibition of growth was thus demonstrated for the transformed cell lines.

KEY WORDS: fibroblast-like cells; transformed cell lines; inhibition of cell division.

A previous investigation [1] showed that some transformed lines of fibroblasts retain their ability to inhibit multiplication, depending on the density of the population (topoinhibition), characteristic of normal embryonic cells. In particular, topoinhibition has been found in two morphological transformed and high-oncogenic lines of hamster fibroblasts KhÉTR and KhÉK-40. The object of this investigation was to study whether the postinhibitory effect of neighboring cells is specific or not: whether the inhibition of cell division persists only in the case of contact between the transformed cells and neighboring cells of the same type or whether it can also be detected after contact with normal fibroblasts.

EXPERIMENTAL METHOD

To investigate the sensitivity of cells to the inhibitory action of population density a method of adding labeled cells to pre-existing layers of homologous or heterologous cells, as suggested by Stoker [6], was used. Three types of cells were chosen: a) embryonic Syrian hamster fibroblast-like cells subcultured once or twice, b) KhÉTR cells – spontaneously transformed Syrian hamster cells after 50-80 passages, c) KhÉK-40 cells (80-100 passages), obtained by treating KhÉTR cells with cytomegaly virus.

Cell cultures were grown in penicillin flasks on coverslips measuring 9 × 18 mm. The seeding density of all types of cells was 100,000 cells/ml medium. To each flask 2 ml of the cell suspension was added. The nutrient medium consisted of equal parts of Eagle's medium and lactalbumin hydrolyzate. The serum concentration was 10%. The medium was changed on the 2nd, 4th, 6th, and 8th days of growth. The final cell density was $6 \cdot 10^5$–$8 \cdot 10^5$ cells/cm^2 for the embryonic hamster cells, $7 \cdot 10^5$–$9 \cdot 10^5$ cells/cm^2 for the KhÉTR cells, and $8 \cdot 10^5$–$11 \cdot 10^5$ cells/cm^2 for the KhÉK-40 cells. To prepare the mixed cultures, stationary cell cultures on the 6th day of growth were used. The coverslips with the cell cultures were subjected to the following operation: with a razor blade areas of the monolayer measuring 4 × 9 mm were taken from the end of the coverslips so that on one coverslip with the cell culture areas of clean glass were formed. Cells previously labeled with thymidine-H^3 (0.1 μCi/ml, 3-4 days, specific activity 1.3 Ci/mmole) were added to these cultures in fresh medium. The newly added cells were thus seeded uniformly on the clean glass and on the existing monolayer. To prevent dilution of the label, cold thymidine was added to the medium in a concentration of 2.5 mg/ml. The specimens were fixed on the 1st, 3rd, and 5th days after addition of the labeled cells in a mixture of acetic acid and alcohol in the ratio of 1:3. The preparations

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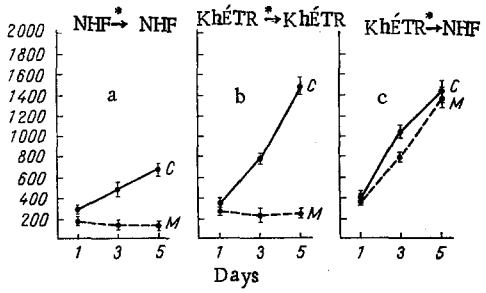

Fig. 1

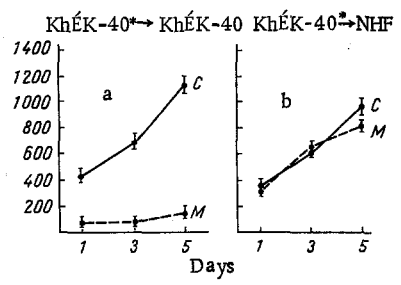

Fig. 2

Fig. 1. Multiplication of labeled KhÉTR cells on a monolayer of KhÉTR cells and on a monolayer of normal hamster fibroblasts (NHF): C) multiplication of added cells on coverslip, M) on monolayer respectively. Abscissa, days after addition of labeled cells; ordinate, density of labeled cells per 100 fields of vision of the microscope (magnification 1.6 × 10 × 100).

Fig. 2. Multiplication of labeled KhÉK-40 cells on a monolayer of KhÉK-40 cells and on a monolayer of normal hamster fibroblasts (NHF). Legend as in Fig. 1.

were coated with type M emulsion, developed after 7-10 days, and stained with Mayer's hematoxylin. From 3 to 5 slides were taken at each point. The number of labeled nuclei in the cell culture and on the clean glass was determined and in each case 100 fields of vision of the microscope were examined (magnification 1.6 × 10 × 100). In each experiment 2000-3000 fields of vision were thus counted. The percentage of labeled cells at the site of injury to the monolayer was 87-94% on the 5th day after addition, i.e., practically all the cells remained labeled after this period.

EXPERIMENTAL RESULTS

It will be clear from Figs. 1c and 2b that the transformed cells adhered well and multiplied both on the coverslip and on the pre-existing monolayers of embryonic fibroblast-like cells. The density of these cells was $6.5 \cdot 10^5$-$8.2 \cdot 10^5$ cells/cm^2. The monolayer formed from embryonic Syrian hamster fibroblast-like cells thus did not inhibit the multiplication of the transformed cells. Labeled embryonic hamster cells did not proliferate on the monolayer consisting of the same cells (Fig. 1a).

The ability of the transformed cells to adhere to and proliferate on the monolayer of cells of the homologous type is demonstrated in Fig. 1a, b and Fig. 2a. It will be clear from these figures that the transformed cells adhered both to the clean glass and to a monolayer of cells of the same type. Cells attached to this monolayer, unlike cells on the glass, virtually did not multiply. The cell density of the monolayer was $7 \cdot 10^5$-$9 \cdot 10^5$ cells/cm^2. Multiplication of transformed KhÉTR and KhÉK-40 cells, as well as their individual cultures, is thus inhibited by the presence of neighboring cells of the same type.

Experiments have shown that the division of some transformed cell lines can be inhibited by normal cells [4, 7], whereas the division of other transformed lines was not inhibited under these circumstances [2, 3, 8]. The authors cited do not give data on the patterns of proliferation of the transformed cells when seeded on a monolayer of homologous cells; for that reason the specificity of inhibition of multiplication observed in a heterologous system remains undetermined for these lines.

The data described above show that cells of transformed lines KhÉTR and KhÉK-40 adhere to a monolayer of the same type of cells but do not multiply on it. These experiments confirmed the results obtained by the writer previously [1] by a different method: transformed KhÉTR and KhÉK-40 cells, like embryonic hamster fibroblasts, if grown in isolated culture remain sensitive to density-dependent growth inhibition. Meanwhile contact with embryonic fibroblasts, by contrast with contact with homologous cells, does not inhibit the multiplication of transformed fibroblasts: cells of lines KhÉTR and KhÉK-40 multiply on a thick monolayer of embryonic hamster cells just as well as on clean glass. In the present experiment cell cultures of embryonic and transformed fibroblasts with similar densities were specially used. Consequently, the sensitivity of the transformed cells to density-dependent growth inhibition depends on the character of the contacting cells and not on their density.

It is not yet clear whether this specificity of growth inhibition is characteristic of normal cells. Normal fibroblasts are more adhesive to glass than transformed and, for that reason, when normal cells are added to a monolayer of transformed fibroblasts they can pass through it, adhere to the glass, and multiply on it: the conditions of growth on the glass and on the cell monolayer are different and this makes analysis of results of this type difficult.

The mechanism of topoinhibition remains unexplained: it is not known whether contact interaction between cell surfaces or local changes in the medium induced by an increase in the cell population density, such as changes in the local pH [5], play a role in this case. The problem of which changes in transformed cells determine the differences in their sensitivity to the inhibitory action of cells of different types requires further study.

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