MITOTIC BEHAVIOR OF TRANSPLANTABLE CELL CULTURES OF LINE CA-SV-40-63-1

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The mitotic behavior of transplantable cell cultures obtained from subcutaneous tumors of the Syrian hamster induced by injection of SV-40 virus was studied. A similarity was found between the mitotic behavior (delay of division in metaphase, numerous pathological mitoses) and the changes observed during transformation of human lung diploid cells under the influence of SV-40 virus, suggesting that the disturbances of mitotic behavior during transformation are possibly neoplastic in character.

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Oncogenic virus SV-40 causes transformation of cells of certain mammals and man in vitro [6-12, 14, 15]. A previous investigation [2] showed that transformation of diploid cells of human embryonic lung by SV-40 virus leads to essential changes in mitotic behavior consisting of an increase in mitotic activity, metaphase delay, and a sharp increase in the number of pathological mitoses. It was decided to study the mitotic behavior of transplantable cell cultures of line CA-SV-40-63-1 obtained from subcutaneous tumors of the Syrian hamster. The cytology of cultures of line CA-SV-40-63-1 has already been studied to some extent [3-5].

In this investigation the mitotic behavior of cells of CA-SV-40-63-1 cultures was studied at two passages (46th and 65th) in order to determine for how long the transformed cells retain their special qualities during passage of the culture.

EXPERIMENTAL METHOD

A suspension of CA-SV-40-63-1 cells in medium No. 199 with 10% bovine serum (concentration of suspension 10^5 cells/ml) was poured in volumes of 1.5-2 ml into penicillin flasks with bands of cover slips. The 3-day cultures were fixed in Shabadash's neutral mixture. The preparations were stained with Carazzi's hematoxylin, iron hematoxylin, and by the Feulgen reaction. The mitotic activity was expressed as the number of dividing cells per thousand counted. The percentage of pathological mitoses was determined. Pathological forms of mitoses were classified by Alov's method [1]. The numerical results were analyzed statistically by the Fisher–Student method.

EXPERIMENTAL RESULTS

The mitotic activity of the cells at the 46th passage was 37.4±2.6%, and at the 65th passage 61.5±2.6%. In the same preparation areas were found with widely different mitotic activity. In some foci of proliferation at the 46th passage the mitotic activity varied from 18 to 49%, and at the 65th passage from 37 to 78%.

Cultures of both passages showed marked delay of dividing cells in metaphase (Fig. 1): the proportion of dividing cells in metaphase was 74 and 84.5% respectively ($P < 0.05$). Pathological mitoses in the cultures studied amounted to 63.5±5.1% and 79±4.9% ($P < 0.05$). Different forms of pathological mitoses were observed: pathological prophase (early separation of chromatids, irregular spiralization of chromosomes, peripheral location of chromosomes beneath the nuclear membrane, very irregular loosening of the prophase coil); pathological metaphases (K-metaphases–colchicine-like metaphases with dispersal and deletion of chromosomes, triple and multipolar metaphases); pathological ana- and telophases (asymmetrical figures, single and multiple bridges, multipolar ana- and telophases). The predominant form of pathological

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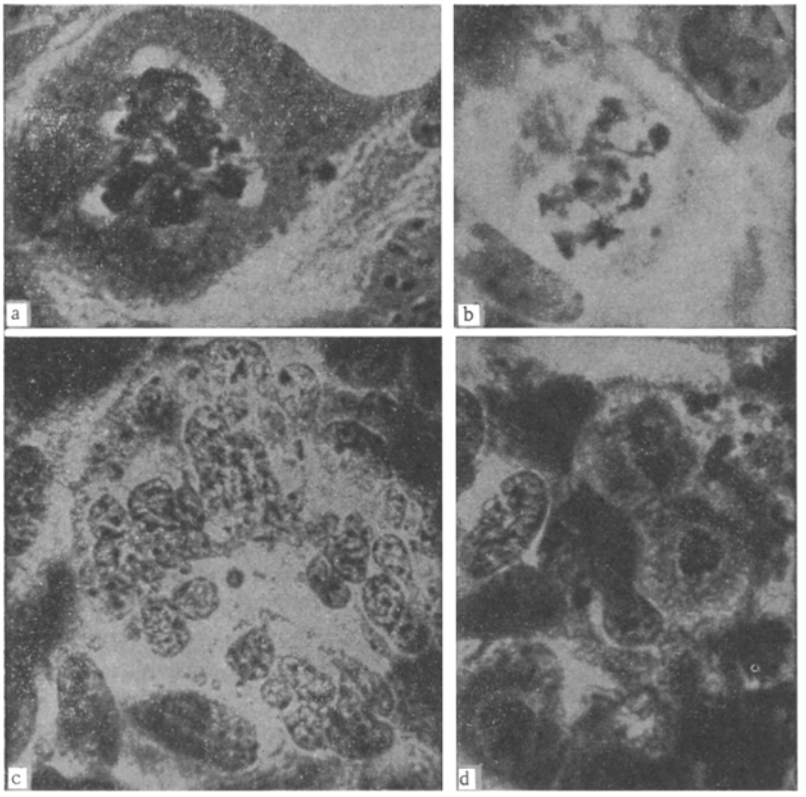

Fig. 1. Cells of line CA-SV-40-63-1. a) Multipolar metaphase; b) telophase with bridges; c) cell with numerous micronuclei and segmented nuclei; d) delay of cell division at the metaphase stage. Carazzi's hematoxylin, 600×.

mitoses (77.6 and 86.4%) were the K-metaphases, with very short and thick chromosomes. In most cases this type of pathological mitoses was associated with other forms of mitotic pathology, such as dispersal and deletion of chromosomes in metaphase, and multipolar figures.

In the subsequent stages of mitosis various combinations of pathological forms likewise were observed, such as the formation of bridges with asymmetrical arrangement of chromosomes. Many dying cells in K-metaphase were present. Multiple mitoses were particularly numerous in dividing giant cells: the chromosomes were collected into separate groups, sometimes joined by bridges. Figures of this type were seen in metaphase and also in ana- and telophase. This process sometimes terminated with the formation of multinuclear cells with nuclei of different sizes, determined by the size of the preceding group of chromosomes. If this process did not reach completion, the micronuclei remained connected with each other and with the larger nuclei by thin isthmuses. The giant cells as a rule were polyploid, the chromosome number sometimes reaching several hundreds, and impossible to count accurately. Polyploidy of these cells is probably the result of endomitosis. Endomitosis was evidently combined with the pathological mitoses mentioned above.

CA-SV-40-63-1 cultures are thus characterized by a well marked mitotic imbalance. Heteroploidy of this line was maintained both by a continuous polyploidization and by an irregular distribution of chromosomes (pathological mitoses) between daughter nuclei. In this way populations may arise consisting of cells differing in their karyologic characteristics and viability.

It has now been shown that a new tumor T-antigen is synthesized in the nuclei of all cells of cultures transformed by SV-40 virus. It may be postulated that the disturbances of mitotic behavior described above also represent a manifestation of the radical disorganization of the nuclear structures connected with the constant influence of foreign genetic information on all the living processes of the transformed cells. The features distinguishing the mitotic behavior of cell cultures of line CA-SV-40-63-1 discovered in these experiments are similar to changes in mitotic behavior observed during transformation of diploid cells from the human lung.

This suggests that the changes during transformation of human diploid cells by SV-40 virus are possibly neoplastic in character.

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