MITOTIC BEHAVIOR OF A CULTURE OF HUMAN DIPLOID CELLS AT VARIOUS STAGES OF TRANSFORMATION BY VIRUS SV40

I. A. Alov, M. E. Aspiz,
V. N. Blyumkin, V. I. Gavrilov,
and A. I. Solov'eva

UDC 616.988-018.1-092:612.014.483

The cytologic mechanisms of transformation of normal cells have received little study. The view has long been held that one of the mechanisms of transformation of normal into tumor cells is the appearance of pathological mitoses, which lead to the formation of a mutant cell population [1, 7]. However, the possibility cannot yet be ruled out that neoplastic transformation of cells and the appearance of pathological mitoses are simply parallel phenomena. One argument in support of a causal relationship between these processes would be the appearance of pathological mitoses during various forms of malignant change and of abnormal mitoses in the period of cell transformation.

Tissue culture experiments have shown that spontaneous malignant transformation of cells during prolonged cultivation is accompanied by the appearance of numerous pathological mitoses [7]. An increase in the number of pathological mitoses has been observed in the early stages of chemical carcinogenesis [2].

In the present investigation the changes in mitotic behavior were studied during exposure of cells to the action of oncogenic virus SV40, which has the property of inducing transformation of human cells in vitro [6, 12]. This transforming action of virus SV40 has led several workers to study changes in mitotic activity [11, 13] and the appearance of chromosomal aberrations [5, 9, 10, 14] in cells infected with this virus.

EXPERIMENTAL METHOD

Experiments were carried out on diploid cells from the lungs of a human embryo (DCHL, strain No. 1)*. The cells were grown on Earle's medium. At the 7th passage the culture was infected with virus SV40 (strain No. 426)†. Virus-containing fluid was added at the rate of 1 ml to 100 ml medium. After adsorption for 3 h at 37°, the liquid was aspirated from the flask and the layer of cells carefully washed off with Hanks's solution. Fresh medium (30% Earle's medium with 10% calf serum and 70% "conditioned" medium from the flask with DCHL-1 cells of the 5th passage) was then poured into the flask. The DCHL-1 cultures, chronically infected with virus SV40 and grown on cover slips, were fixed with Shabadash's neutral mixture at various stages of transformation of the cells: 23, 46, 81, 97, and 109 days after infection of the culture. The same culture not infected with virus served as original control. The preparation were stained with Carazzi's hematoxylin or iron hematoxylin. The mitotic behavior and pathological forms of mitosis were studied in accordance with the scheme suggested by I. A. Alov [1]. The mitotic activity was counted in 1000 cells, and at the same time the relative percentages of the individual phases of mitosis were determined. The number of pathological mitoses was expressed as a percentage of the total number of dividing cells. Statistical analysis of the numerical results was carried out by the Fisher–Student method.

*Strain DCHL-1 was obtained from the Tissue Culture Laboratory of the D. I. Ivanovskii Institute of Virology.
†Strain No. 426 of virus SV40 was obtained from the L. A. Tarasevich Control Institute.

Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR; Laboratory of Tissue Culture, Institute of Virology, Academy of Medical Sciences of the USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences of the USSR, A. P. Avtsyn). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 122-125, November, 1967. Original article submitted February 13, 1967.

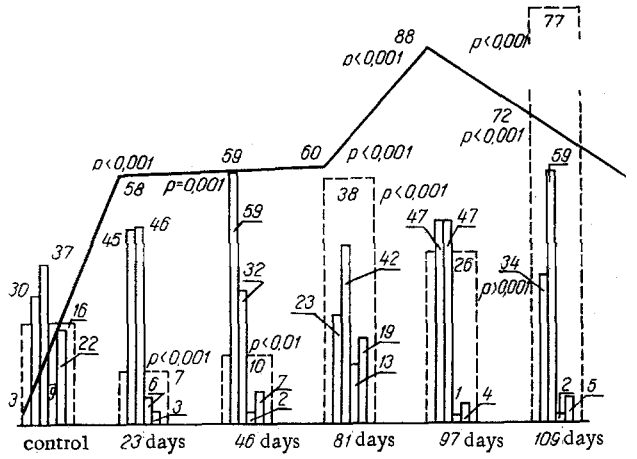

Fig. 1. Changes in mitotic behavior of DCHL-1 cells in the period of transformation induced by virus SV40. Wide broken columns—mitotic activity; narrow columns—percentages of individual phases of mitosis; the curve denotes changes in the number of pathological mitoses.

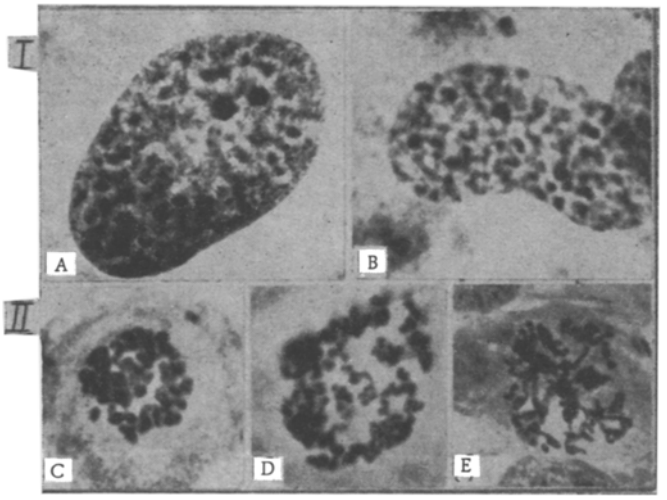

Fig. 2. Changes in DCHL-1 cells in the transformation period. I: A) Virus inclusions in nucleus; B) heterochromatinization; II—pathological mitoses; C) K-metaphase of "plant type"; D) scattering of shortened chromosomes in metaphase; E) scattering of relatively unchanged chromosomes in metakinesis.

EXPERIMENTAL RESULTS

Three weeks after infection of the cultures with virus SV40, residual manifestations of the cytopathic action of the virus and the first signs of commencing transformation were observed. Signs of degeneration were noted in some cells, and intranuclear virus inclusions in others. Only a few resistant cells had begun to proliferate. Heterochromatinization of the nuclei was observed in many interphase cells, and it could also be traced in the subsequent stages of transformation. The mitotic activity at this period was reduced by more than half in comparison with the uninfected cells (Fig. 1). However, the relative increase in the number of first phases of mitosis indicates that a new wave of mitoses had now begun, leading to an increase in mitotic activity in the subsequent stages of transformation of the culture. Even during this initial stage of transformation, the number of pathological mitoses increased sharply—from 2.9% in the control to 58% of all the dividing cells.

In the course of the next stages of transformation (46-109 days after infection of the cultures), a gradual increase in mitotic activity took place, reaching a maximum after 109 days, i.e., in the final stage of transformation of the cells. The increase in mitotic activity was accompanied by a change in the relative proportions of the individual phases of mitosis. In the first half of the transformation period the relative number of prophases and metaphases increased, while in the second half metaphases were predominant. Comparison of the changes in the phases of mitosis with the changes in mitotic activity shows that the increase in the relative number of prophases usually coincided with the beginning of an increase in mitotic activity, and reflected the beginning of a new wave of mitoses (an increase in the number of cells commencing mitosis). The relative increase in the number of metaphases, coinciding with the maximum of mitotic activity, was probably connected with some delay in the course of this phase of mitosis. This is characteristic of the transformation of normal into tumor cells [1, 3, 8].

With the beginning of transformation, the number of pathological mitoses increased sharply (Fig. 1), rising steadily and reaching a maximum 97 days after infection with the virus. It is interesting to note that the increase in the number of pathological mitoses and their maximum preceded the stimulation of mitotic activity and its maximum. Cells with an unbalanced karyotype, which arise as a result of pathological mitoses, possibly become capable of increased proliferation. This hypothesis merits special study.

The predominant form of abnormal mitosis (36-54%) during transformation is that of K-metaphases (Fig. 2). This form of pathological mitosis is characterized either by scattering of greatly shortened and thickened chromosomes (K-metaphases of "plant type"), or by agglutination of the chromosomes into coarse, lumpy, metaphase plates (K-metaphases of "animal type").

Heterochromatinization of the interphase nuclei and the appearance of K-metaphases with shortened chromosomes are probably the result of a single chain of processes, associated with excessive spiralization of the chromosomes in the transforming cells.

About half of all the pathological mitoses consisted of other forms of disturbances of the normal course of mitosis: scattering of chromosomes in metaphase, three-group metaphases, deletion of chromosomes and their fragments in metakinesis and during divergence toward the poles, and asymmetrical mitoses.

The study of the period of transformation of cells infected with virus SV40 thus shows that the changes in mitotic behavior at this period are characterized by an increase of mitotic activity, a sharp increase in the number of pathological mitoses, and a relatively slow course of metaphase.

LITERATURE CITED

1. I. A. Alov, Vestn. Akad. Med. Nauk SSSR, No. 11, 58 (1965).
2. A. P. Vasil'eva, Byull. Éksp. Biol., No. 10, 83 (1966).
3. I. A. Kazantseva, Arkh. Pat., No. 11, 31 (1966).
4. A. N. Mustafina, Tsitologiya, No. 2, 249 (1966).
5. H. Cooper and P. Black, J. Nat. Cancer Inst., 30, 1015 (1963).
6. H. Koprowski, J. Ponten, F. Jensen, et al., J. Cell. Comp. Physiol., 59, 281 (1962).
7. A. Levan and J. J. Beisele, The Genetics of Cancer [Russian translation], 273, Moscow (1961).
8. W. H. Lewis, Ann. New York, Acad. Sci., 51, Art. 8, 1287 (1951).
9. P. Moorhead and E. Saksela, J. Cell. Comp. Physiol., 62, 57 (1963).
10. Idem, Hereditas, Lund., 52, 271 (1965).
11. G. Petersson, J. Fogh, E. Harven, et al., Virology, 28, 303 (1966).
12. H. M. Shein and J. Enders, Proc. Nat. Acad. Sci., Washington, 48, 1164 (1962).
13. H. Shein, J. Enders, L. Palmer, et al., Proc. Soc. Exp. Biol., New York, 115, 618 (1964).
14. S. Wolman, K. Hirschhorn, and G. Todaro, Cytogenetics, 3, 45 (1964).