# ONCOLOGY

## CELL LINES PRODUCED FROM TUMORS CAUSED BY VIRUS SV 40

(UDC 616-006+616.988.6]-018-092.4)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 5, pp. 85-88, May, 1965

Original article submitted February 6, 1964

During recent years particular attention of virologists and oncologists has been directed to SV 40 virus, a member of the so-called Papova group [13]. This virus produces cytopathic changes in primary trypsinized cultures of green monkey (Cercopithecus aethiops) kidneys, and in certain transplantable cell lines, consisting of cytoplasmic vacuoles and also leads to formation of specific, oxyphilic intranuclear indusions containing DNA [4, 5, 8, 10]. In addition, SV 40 virus has oncogenic property. Subcutaneous inoculation of this virus into new born Syrian hamsters (Cricetus auratus) leads to formation of sarcoma [1-3, 7, 9]. Intracerebral inoculation of the virus into hamsters brings about formation of ependymous sarcoma [8, 11]. In addition, SV 40 virus transforms hamster and human cells in vitro [4, 6, 12, 14]. Diploid line cells lose their fibroblast-like appearance under the influence of SV 40 virus, epithelioid-like regions as well as giant cells appear in cultures; the culture grows in a 3 dimensional manner as a result of loss of contact inhibition. Because of the increase in metabolism there takes place a rapid change in pH of the culture medium to the acid side. The number of polyploidal cells increases, and significant structural alterations of the chromosomes are observed.

The present study was devoted to investigation of transplantable cell lines obtained from cancers induced by SV 40 virus. This investigation was undertaken to determine certain aspects of inter-relation between the tumorogenic activity of SV 40 virus on cells of new-born hamsters in vivo and the transforming action on cells cultured in vitro.

### METHODS

During 1963 we received several transplantable cell lines derived from cancers of Syrian hamsters, developing after subcutaneous inoculation of SV 40 virus. In the present work we studied cytological characteristics of cell lines CA-SV 40-63-1 and CA-SV 40-63-1 bis. The first cell line was obtained from a subcutaneous cancer, induced by injection of a new-born Syrian hamster with $10^{4.6}$ $CPD_{50}$ (for green monkey kidney cells) of SV 40 virus strain A-426, Rh-2. Line CA-SV 40-63-1 was derived after a lag phase lasting about 5 months. Upon injection of a cell suspension of a cell line CA-SV 40-63-1 (dose $3 \cdot 10^4$ - $2 \cdot 10^5$ cells) into adult Syrian hamsters there appeared on the 8-11th post-injection day cancerous nodules in subcutaneous tissues. One of these cancers (sarcoma) became the source of transplantable cell line CA-SV 40-63-1 bis. The new cell strain appeared without a lag-phase. We subjected the cell line CA-SV 40-63-1 to cytological study beginning with the 3rd passage, and the cell line CA-SV 40-63-1 bis beginning with the first passage. The cell cultures, grown on medium No. 199 with 10% normal bovine serum, were inoculated (cell concentration 150,000/ml of medium) into containers with penicillin, using coverslips. The coverslips with grown cultures were fixed in neutral Shabadash fixative, a mixture of 10% Borim's fluid with 10% of neutral formalin. The following histological and histochemical procedures were used: staining with haematoxylin-eosin, iron hematoxylin, Brashe's methylene green pyronine—to demonstrate RNA, Feulgen's reaction—to demonstrate DNA, Shabadash's method—to demonstrate glycogen, and staining with Sudan III and Sudan black—to demonstrate lipids.

### RESULTS

During the first day of cultivation the predominating part of the cultures of line CA-SV 40-63-1 consisted of growing cells of stellate or spindle-shaped form, resembling mesenchymal elements, reticular cells or fibroblasts at

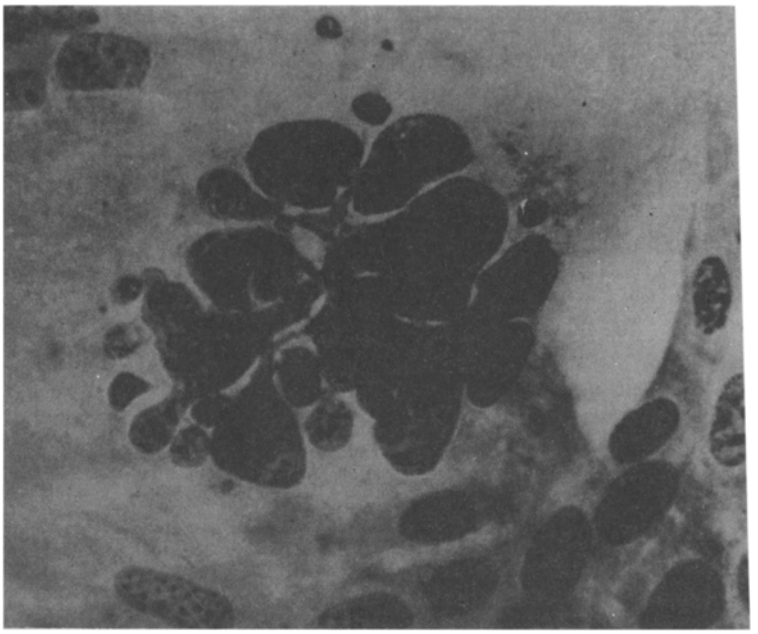

Fig. 1. Cell line CA-SV 40-63-1. Two-day old culture. A giant cell with segmented nucleus. Hematoxylin-eosin stain. × 400.

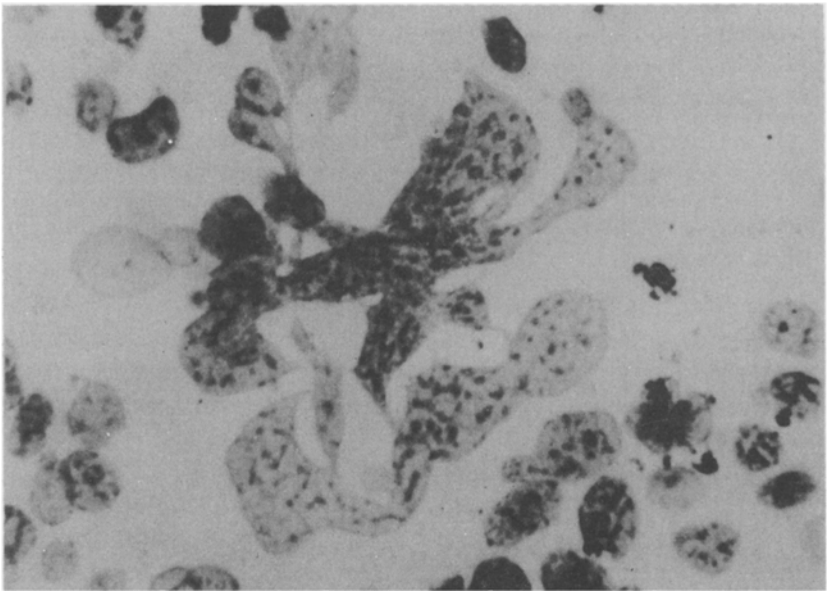

Fig. 2. Cell line CA-SV 40-63-1. Two-day old culture. Irregular nucleus of a giant cell. Uneven deposition of Feulgen positive material in different cell segments. Feulgen stain. × 400.

different stages of differentiation. The cells formed a syncytium of different density. Along with syncytium connecting cells in cultures of CA-SV 40-63-1 there were observed elements resembling hystiocytes of loose connective tissue.

Among the cells joined in a syncytium it was possible to differentiate elements with the regular distribution of chromatin in nuclei, as well as with the vacuolated nuclei and with sharply hyperplastic nuclei.

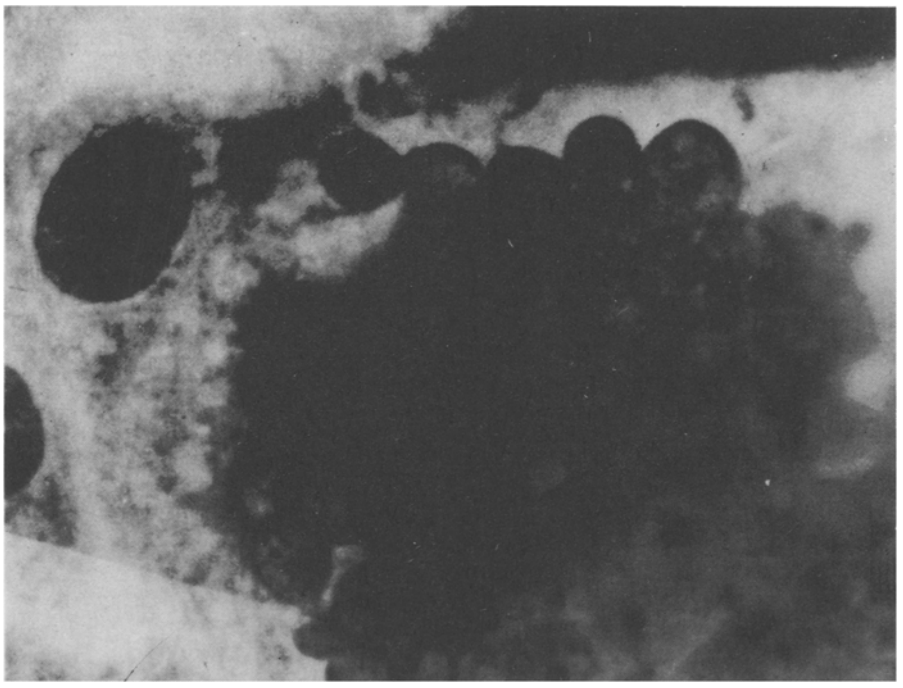

Fig. 3. Cell line CA-SV 40-63-1 bis. A giant multinuclear cell. Stained with iron hematoxylin. × 700.

By far the more characteristic appearance of line CA-SV 40-63-1 in all passages was the presence of many large mononuclear and polynuclear giant cells. Among the giant mononuclear cells there were elements with ball-shaped or ovoidal flask-shaped nuclei as well as cells with irregular multilobular nuclei, resembling nuclei of megakaryocytes of red bone marrow or markedly hypertrophic nuclei of neutrophils (Fig. 1, 2). Extensive nuclear budding was characteristic of such nuclei, which led to the formation of numerous daughter hyperchromic nuclear segments, gradually decreasing in size towards periphery of the cell. Among the multinuclear cells one finds particles several hundred microns in diameter. The nuclei in symplasts may vary in shape and dimensions (the result of budding, mentioned above). One also encounters symplasts containing a large number (up to 100 and above) of nuclei of different sizes (Fig. 3). The chromatin in the nuclei of giant cells frequently occupies marginal position, some nuclei display structure, and large vacuoli are formed in some other nuclei. One can also observe strongly expressed nuclear hyperplasia.

Line CA-SV 40-63-1 has considerable mitotic activity. The frequency of mutation in 1000 cells in a 24 h culture is about 50, in a 2 day culture—80-90; in a 3 day—80-100. Atypical mitotic figures are predominant, particularly polycentric division figures and mitosis with chromosomal retention. The number of chromosomes in giant cells surpasses that in diploidal cells; there is a sharp variation in the number of chromosomes in different cells (sharply expressed anaploidy of the cell line).

A significant number of giant mononuclear and polynuclear cells is observed already in a 24 h culture (12-25%). In a 2 day culture of line CA-SV 40-63-1 20-35% of the monolayer are giant cells. In individual passages cultures contained up to 50% of giant cells.

Cells of CA-SV 40-63-1 contained an insignificant amount of glycogen and lipids. The RNA concentration varied in different cells. DNA is distributed unevenly in segmented nuclei.

One of the more important peculiarities of CA-SV 40-63-1 is a rapid transition from stormy growth to "growth" destruction. Because of this, it was necessary to pass the culture frequently (not less than once every 4 days).

The cells of CA-SV 40-63-1 had a high tumorogenic activity in relation to the tissues of Syrian hamsters. Tumors brought about by subcutaneous inoculation of a suspension of CA-SV 40-63-1 cells developed very rapidly. Usually, the animals succumbed towards about the middle of the 2nd month after injection. The tumor nodules

reached up to 9 • 7 cm in size. Upon microscopic examination, it was possible to show that these nodules are polymorphic sarcomas with a large number of giant mononuclear and polynuclear cells. The giant cells of the studied tumors did not differ by their cytological and cytochemical markers from similar cells observed in cultures in vitro, although somewhat smaller in size, which is probably related to the treatment of the material (condensation upon imbedding in paraffin) and because the cells in a culture monolayer are spread on glass surface. The nuclei of most of the tumor cells were similar in appearance to the nuclei of cells of a CA-SV 40-63-1 culture; they exhibit marginal location of chromatin, nuclear hyperplasia, and some vacuolated or honeycomb nuclei. Sarcomas, produced by injection of hamsters with CA-SV 40-63-1, were poor in connective tissue. They were highly vascularized with sinusoidal-like vessels. The early appearance of many hemorrhages and necrotic nodules of different size was characteristic of the above described tumors.

Sarcomas produced by subcutaneous inoculation of hamsters with SV 40-63-1 cells constantly metastasized to lungs. The metastatic nodules localized under the pleura and in peribronchial tissue. We also observed metastasis of tumors to kidney. In one case the cells metastasized to spleen and in another to liver. In one hamster the tumor grew through the posterior peritoneal wall and the pancreas, destroying most of its acini.

One of the tumors, resulting from injection of a hamster with SV 40-63-1, was trypsinized. The primary cell culture thus obtained was used for establishing a new transplantable cell line which became known as CA-SV 40-63-1 bis. The new cell stain differed from the above described line CA-SV 40-63-1 in that it developed without a lag phase.

The cultures of CA-SV 40-63-1 bis consisted of syncytially connected cells, resembling mesenchymal reticular cells and fibroblasts and also from a large number of giant mononuclear and multinuclear elements, similar to the giant cells of the first line. Giant cells comprise at least 50% of the cultures of CA-SV 40-63-1 bis line. In all other respects (mitotic activity, presence of atypical mitosis, sharply expressed anaploidy, histochemical determinants) the culture differs only slightly from strain CA-SV 40-63-1.

The transplantable cell line CA-SV 40-63-1 was obtained from subcutaneous tumor (sarcoma) produced by introduction of SV-40 virus into a Syrian hamster. In contrast to the majority of anaploidal cell strains (SOTS, HeLa, Hep-2, KB and other), having the tendency for individual cytological leveling, line CA-SV 40-63-1 retained the cytotypical differentiation typical of the initiating tumor (sharply defined cellular polymorphisms and a tendency to formation of symplasts, as well as predominance of giant cells with irregular nuclei). It may be assumed that the above described cytological peculiarities of cultures of CA-SV 40-63-1 are primarily the results of tumorogenic transformation of cells in vivo under the influence of oncogenic virus. At the same time, the manner of establishing of this cell line confirms that by far not all the tumorogenic cells have been genetically useful in developing a transmissable cell line. An extensive number of cells, growing under newly encountered in vitro conditions, died and only an insignificant part survived. Expressed qualitative genetic changes (transformation of tumor cells in vitro) became apparent in the surviving cells only toward the end of the 5th month. As a result of this process, the cells acquired the property of continuous in vitro passage. These transformed in vitro elements had high oncogenic activity. Subcutaneous injections of these cells into hamsters led to development of rapidly growing metastatic tumor.

At the same time the cells from these tumors, regardless of the prolonged presence in the inoculated animal, after becoming adapted to serial passage in vitro retained this cytological characteristic in the future. The last statement is based on the ease of obtaining (without a lag phase) transplantable line CA-SV 40-63-1 bis. (Strains CA-3 and CA-4 have been obtained in a similar manner—without a lag phase.)

Preparations used in studying cell lines CA-SV 40-63-1 and CA-SV 40-63-1 bis confirm that carcinogenicity in vivo brought about by virus SV 40 is not identical with transformation in vitro, leading to the development of a transplantable cell line. At the same time the cytological similarity of the original tumor, produced by SV 40 virus and the derived cell line, confirm that cells of CA-SV 40-63-1 retain most of genetic markers, obtained in the course of tumorogenesis in vivo.

## SUMMARY

A transplantable cellular strain, CA-SV 40-63-1, was produced from a sarcoma in a Syrian hamster induced by injection of SV 40 virus. The cellular strain formed after a prolonged lag-phase. The CA-SV 40-63-1 cultures were characterized by a marked cellular polymorphism, high mitotic activity, a tremendous number of giant mono- and polynuclear cells. The administration of cells of the CA-SV 40-63-1 strain to Syrian hamsters causes the formation

of sarcomas of which the cellular composition bears a striking resemblance to that of the CA-SV 40-63-1 strain. One of such tumors gave rise to a new cellular strain, CA-SV 40-63-1 bis, which formed without the lag-phase. The cytological characteristics of the 2nd cellular strain are close to those of the CA-SV 40-63-1 strain. Certain aspects of the problem of relationships between the processes of malignization of cells in vivo and transformation of cells in vitro are also discussed.

## LITERATURE CITED


1. N. N. Vasil'eva, A. T. Kravchenko, and V. I. Gavrilov et al., Problems in Virology, No. 2 (1964), p. 222.
2. V. I. Gavrilov, N. N. Vasil'eva, and N. N. Dodonova et al., ibid, No. 5 (1963), p. 583.
3. G. I. Deichman and E. L. Prigozhina, ibid, No. 3 (1962), p. 277.
4. V. Ya. Karmisheva, A. N. Mustafina, and G. I. Kolyaskina et al., In: The Morphology of Cytopathic Activity of Viruses. Proceedings of a Symposium, Moscow (1963), p. 69.
5. V. Ya. Karmisheva, N. M. Shestopalova, and V. N. Reingol'd et al., Problems in Virology, No. 4 (1963), p. 460.
6. H. Cooper and P. Black, J. nat. Cancer Inst., 30 (1963), p. 1015.
7. B. Eddy, G. Bormar, and W. Berkeley et al., Proc. Soc. exp. Biol. (N. Y.), 107 (1961), p. 191.
8. P. Gerber and R. Kirschstein, Virology, 18 (1962), p. 582.
9. A. Girardi, B. Sweet, and V. Slotnick et al., Proc. Soc. exp. Biol. (N. Y.), 109 (1962), p. 649.
10. H. Hsiung and W. Gaylord, Jr., J. exp. Med., 114 (1961), p. 975.
11. R. Kirschstein and P. Gerber, Nature, 195 (1962), p. 299.
12. H. Koprowski, J. Ponten, and F. Jensen et al., J. cell. comp. Physiol., 59 (1962), p. 281.
13. J. L. Melnick, Science 135 (1962), p. 1128.
14. H. Shein and I. Enders, Proc. nat. Acad. Sci. (Washington), 48 (1962), p. 1164.


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