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DEVELOPMENT OF VIRAL PARTICLES FROM PURIFIED SV 40 DNA IN TRANSFORMING HUMAN CELLS

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Abstract

In vitro cell transformation of human embryonic cells could be induced by DNA extracted from virions of SV 40 purified by density gradient centrifugation. The results shows clearly that cell transformation is induced by incorporation of a part of viral DNA into the genome. On the other hand it has been revealed that viral DNA of SV 40 develop to viral particles in the nucleus of human transforming cells.

INTRODUCTION

Shein, Enders[1] and Koprowski[2] described that SV 40 is infectious to human embryonic kindey cells and induced cell transformation. Although Gerber[3] described a report that deoxyribonucleic acid induced a cytopathic effect identical with the one caused by an intact SV 40 in vitro, the author[4,5] revealed that DNA extracted from SV 40 induced various kinds of tumors, particularly hemangioma of the liver and carcinoma of the intestine in the newborn hamsters.

The present report describes the findings on the oncogenic properties of DNA purified from SV 40 and development to complete viral particles from viral DNA in human transforming cells.

MATERIALS AND METHODS

SV 40 strain, its propagation, purification and biological activities:

SV 40 777 was supplied by Prof. Shimojo, Institute of Medical Science, University of Tokyo. For propagation of the virus, BSC-1 was used. The cells were cultured by MEM supplmented with 10 % calf serum. The virus was propagated on these cells by inoculating 0.5 ml of 10^8 (PFU) per bottle.

For purification 20 ml of concentrated crude virus suspension obtained after one week's propagation was layered gently over 9 ml of saturated KBr solution and centrifuged at 25,000 rpm for 3 hours. The purified virion was obtained from full particles. The virion preparation thus

obtained was further separated into two fractions by CsCl density gradient centrifugation in an SV 39 roter of a Spinco Model L. For the separation the virion particles obtained from the lower band in a KBr cushion, were suspended in CsCl solution (p=1.33) and spun in a rotor No. 40 of a Spinco Model L at 35,000 rpm for 20 hours. Fractions were collected and 1 ml of phosphate buffered saline was added to each fraction. The biological activity of the virion was assayed by the procedures described by Uchida[6].

Viral DNA; its extraction and biological assay

For the extraction of viral DNA the purified virions were dialysed against saline citrae (0.5 M NaCl, 0.015 M sodium citrate) and incubated at 37°C for 2 hours in the presence of 0.1 mg/ml pronase P (Kaken Kagaku), by the method of Yoshiike[7]. From this material DNA was isolated by extracting with phenol three times. Phenol was removed by dialysis against 0.14 M NaCl, 0.01 M phosphate buffer pH 7.3.

The infectivity titration was performed with the cell monolayers cultured in McCoy 5 A medium (10 % bovin serum) by calculating TCID 50.

Cell Used for Transformation

Cells from human embryo (6 weeks old) were used for the observation of cell transformation. The embryonic cells were grown initially in 199 medium (Difco) and McCoy 5 A supplmented with 20 % calf serum respectively. Cell grown on the glass were infected with DNA and after 2 hours' adsorption at 37°C, the medium was discarded and then fresh medium added. Cells grown on coverslips of Leighton tubes were stained with Giemsa and hematoxylin for morphological observation. Immunofluorescent antibody reaction for tumor antigen and surface antigen were also examined by the indirect methods.

For electronmicroscopy, the cells were fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer at 4°C for 30 min and post-fixed with 1 % OsO_4 in phosphate buffer for 30 min. After fixation they were washed with cold water, dehydrated through ethanol siries and embedded in Epon 812. The sections were stained with 5 % uranyl acetate in 7 % aqueous ethanol and observed by electron microscope, Hitachi HU-11 A.

RESULTS AND DISCUSSION

The human embryonic cells in primary culture were tested for their abilities to be transformed after being exposed to the viral DNA. The DNA of 3 μg/ml was added to the media and the cells were examined

during definite time intervals. Three months after the addition of the DNA, all the cells were found to contain T-antigen with some morphologic changes showing the malignant transformation. The transformed cells showed the appearance of epitheloid cells, some of which were of multinuclear giant cells (Fig. 1). In the early transforming stage of exposure to the DNA, viral particles were found in the nucleus of some fibroblasts by electron microscopy, suggesting that the complete viral particles developed from the purified viral DNA (Figs. 2 and 3). Cell transformation was also observed on cell lines from hamster embryo and kidney cells, both of which were fibroblasts in native. Although transformant from fibroblastic cell to epithelium-like cell should be characteristic of the SV 40 transformant, viral particles were not discovered in hamster cells. Deoxyribonuclease and anti-SV 40 rabbit serum were used to determine nothing of contamination of SV 40 viral particles in the extracted viral DNA.

Immunological tests revealed that the transformants of cells have the specific tumor antigen, surface antigen, and the biological characteristics of the malignat cell.

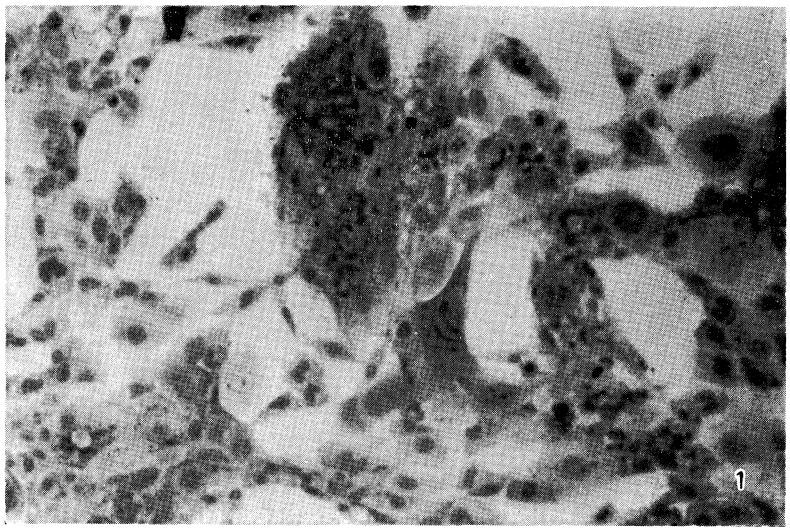
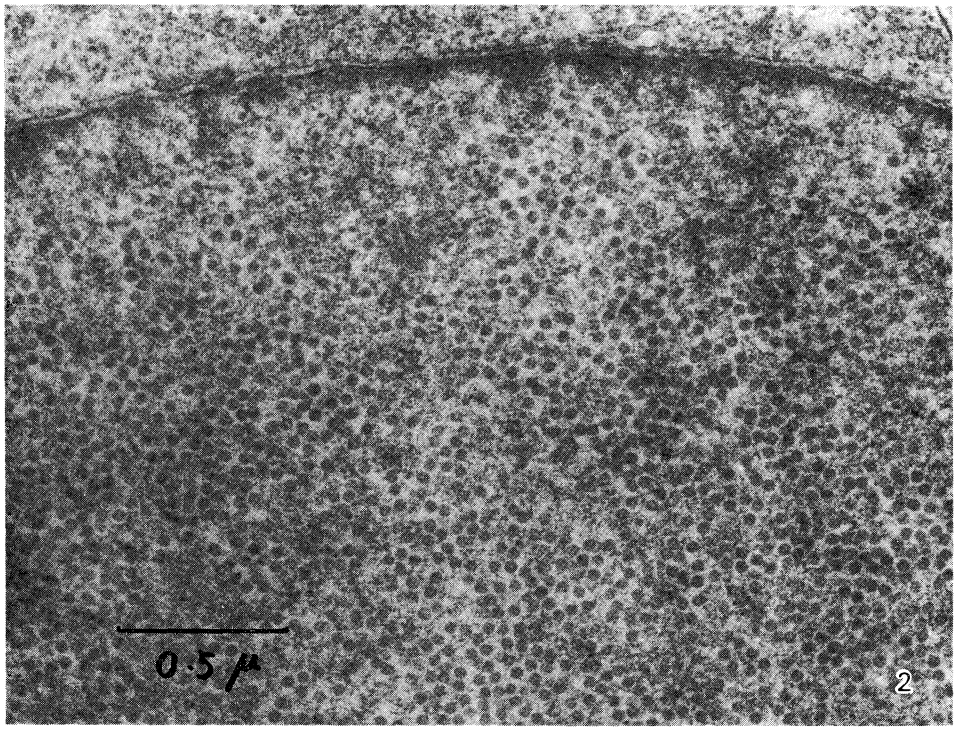


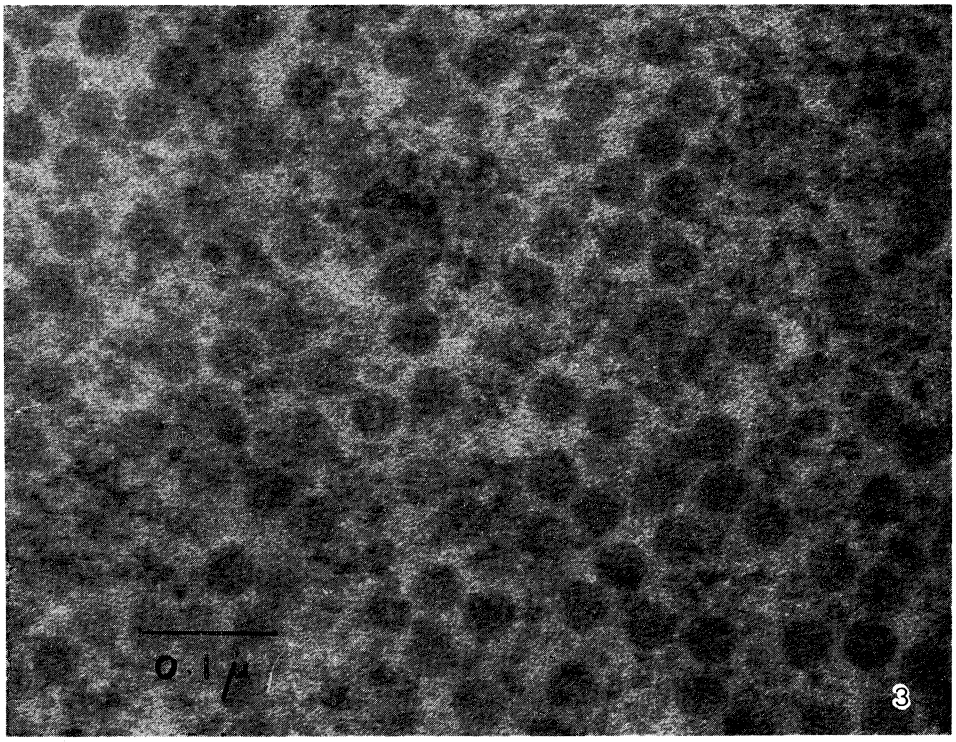
Fig. 1.: In vitro human transformed cells induced by DNA purified from SV 40 (3 months cultivation). 10×20. Giemsa stain.

The present investigation demonstrated that human cells were transformed into malignant cell in vitro by SV 40 DNA just as in the viral particle itself. Presumably the viral DNA is incorporated into the genome of the host cell and induced the genetical change of nucleic acids in the target cells. On the other hand viral DNA developed to viral particles which might be infectious source continuously in the human cell lines and it is significant thing to distinguish the oncogenic differences in the viral particles developed from nucleic acids in the different cells.



Figs. 2 and 3.: Electron microscopic findings of SV 40 viral particles developing from extracted viral DNA in the nucleus of human transforming cell

NM: Nuclear membrane. N: Nucleus.



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