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Abstract

In the immunofluorescent study it has been revealed that rabbit sera immunized with transformed cells induced by SV-40 DNA, produce circulating antibody capable of re:lcting with intranuclear antigens synthesized by SV-40 complyte virus transforming process, In addition, the result confirmed that SV-40 DNA replicates DNA-containing viruses in the host cell and that also the genome coding for the synthesis of SV-40 tumor antigen is resposible for viral DNA.

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SPECIFIC ANTIGEN OF TUMOR CELL TRANSFORMED BY DNA EXTRACTED FROM SV-40 VIRUS

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It has recently been demonstrated that besides viral antigen the monkey kidney cells in culture infected with SV-40 virus synthesize the specific non-viral antigen as well as in the case of the SV-40 induced hamster tumor cell.

Later on, it has been elucidated that this specific antigen is also present in the virus-affected cells of human (SABIN, SHEIN, KOCH and ENDERS[9]), rabbit, mouse and porcine origin (BLACK and ROWE[10]) in culture that have attained the morphologic transformation by the infection with SV-40 virus. These works have been made mainly by employing the immunofluorescent method by using the antibodies from the serum of the tumor bearing hamster induced by injecting SV-40 virus. The similar hamster tumors have been induced by injecting DNA isolated from the cultured cells with SV-40 virus[11]. To obtain a definite evidence whether or not the tumor induced by the viral DNA is identical with the one induced by the virus itself, we have attempted to reveal the existence of the specific antigen similar to those produced by the viral infection. The cell transformation or the cell dedifferentiation have also been studied morphologically.

MATERIALS AND METHODS

NA-FS cell line : The cell line was originally derived from a hamster tumor (fibrosarcoma) induced by DNA extracted from SV-40 virus[11]. It has undergone 43 passages in tissue culture during 14 months. Attempt to isolate infectious virus from the cells as well as from all transplanted tumors have been unsuccessful. The cells (Photo 1) are grown in 16-ounce bottles in fluid nutrient composed of McCoy's 5 A medium supplemented with 10 percent fetal calf serum. (100 U. penicillin and 100μg streptomycin are added per ml.)

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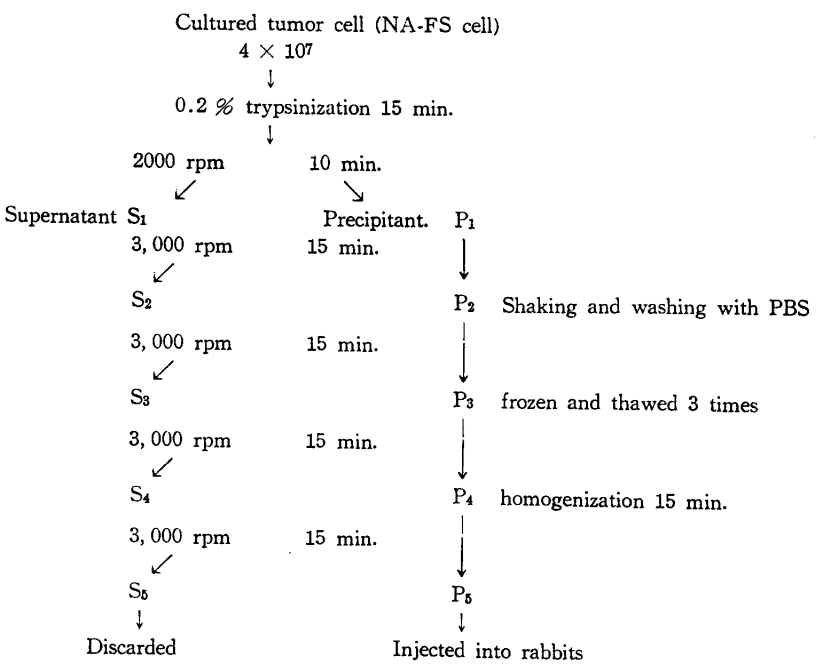
Other cell lines : Normal hamster embryonic kidney cells, Don cells (chinese hamster lung cell : diploid fibroblast), primary human embryo cells (2 months) and intestine cells (2 months), WI-38 (human embryonic lung), Wish cells (human amnion cell) and BS-C-1 cells were used as the control cells.

Antisera : NA-FS cell lines were used for production of hamster anti-tumor serum and NA-FS cell line was transplanted in weaning hamsters by subcutaneous inoculation (1×10^6/ml). Serum was collected as late as possible after transplantation. On the other hand, antisera were collected from SV-40 induced tumor bearing hamsters.

Rabbit anti-NA-FS cell serum : Rabbit anti-NA-FS serum was obtained for the purpose to determine the presence and the localization of cellular antigens of fibrosarcoma cells induced by SV-40 DNA. After washing with phosphate buffer saline solution (PBS, pH. 7.2) and treating with 0.2 percent trypsin the cell suspension of NA-FS cultured cells (8×10^6), was prepared as shown in Table 1.

Table 1

Preparation of NA-FS cell for immunization to rabbit



The NA-FS cells were injected into rabbit subcutaneously with Freund adjuvant (Microbiological Ass. Inc.) twice a week for the first two weeks (total 10

times). Rabbit blood was collected by heart puncture after the injection of 4×10^8 cells in total into rabbits. The serum was diluted in PBS (1 : 3 v/v) and adsorbed by a mouse liver powder (Microbiological Ass. Inc.). Control sera were pooled from normal hamsters and rabbits.

Conjugates : For the indirect test, fluorescein-labeled rabbit serum globulin and hamster globulin were used. The conjugate was dissolved in distilled water (1 : 5 v/v) according to the instruction indicated and then diluted in PBS (1 : 3 v/v) and kept in −70°C refrigerator.

Immunofluorescent technic : NA-FS cells grown on coverslip of Leighton tubes (Bellco Co.) were washed twice in PBS, fixed for 5 minutes in acetone at room temperature and air-dried. In the indirect test, a group of NA-FS cells was treated with a 3-fold dilution of rabbit anti-NA-FS cell serum (inactivated 56°C, 30 minutes) at 37°C for 40 minutes in humid atmosphere, washed twice in PBS for 5 minutes, and rinsed in distilled water. The NA-FS cells were then added with fluorescein-labeled goat antiserum to rabbit globulin for 40 minutes at 37°C, the washing process was repeated, and the coverslip mounted in buffered glycerol (pH. 8.5). The other NA-FS cell group was treated with a 3-fold dilution of tumor bearing hamster serum (unheated serum) and added with a 3-fold dilution of fluorescein-labeled rabbit antiserum to hamster globulin in the same manner. The specificity of reactions in the indirect test was checked repeatedly by testing control cells treated with the sera of SV-40 tumor bearing hamster and rabbit antiserum against NA-FS cells (Table 2). NA-FS cells were treated also with normal sera of rabbits and hamsters.

Table 2 Specific Tumor Antigen of Cultured Cells Treated with Rabbit Antiserum Against NA-FS Cell (indirect method)

Cell Lines	Rabbit Anti-NA-FS Serum
NA-FS cell	Positive
Fibroblastic transformed cell of intestine 407 infected with SV-40 [15)]	10 percent positive
Hamster kidney cell	negative
Human embryo cell	negative
Human intestine cell	negative
Intestine 407	negative
WI-38	negative
Wish cell	negative
Don	negative
BS-C-1	negative

RESULTS

The antigen of the NA-FS cell transformed by SV-40 DNA *in vivo* and cultured *in vitro* reacted with anti-sera from rabbits immunized with NA-FS cells (Photos 2 and 3). Specific fluorescence was seen in the nucleus where it was particulate (Photo 2) and in the nuclear membranes apparently in equal amounts contrary to the behavior of normal hamster fibroblasts. Particularly nuclei reacted at the site of the nuclear membrane of the NA-FS cell and this reaction seemed to be more pronounced with the NA-FS cells reacted with anti-NA-FS cell-rabbit serum (Photos 2 and 3). The nucleolei did not react and these findings were similar to the results shown in the SV-40 transformed cells by RAPP[5,6] and POPE *et al.*[7] On the same basis the results reported here suggest the presence of a similar cellular antigen in the tumor cells induced by SV-40 DNA.

DISCUSSION

BIRIULIKA and his associates[12] were the first to demonstrate an abnormal antigen in a virus-induced tumor when they worked with Rous sarcoma. More detailed studies of the relationship between the inducing virus and the new cellular antigen in the transformed cells were later made by SJÖGREN and his co-workers[13], and by HABEL[14], using polyoma virus-induced tumors in the mouse and hamster.

Detection of new complement-fixing antigens following transformation of cells by SV-40 virus has not resolved the problem of the proportion of the cells

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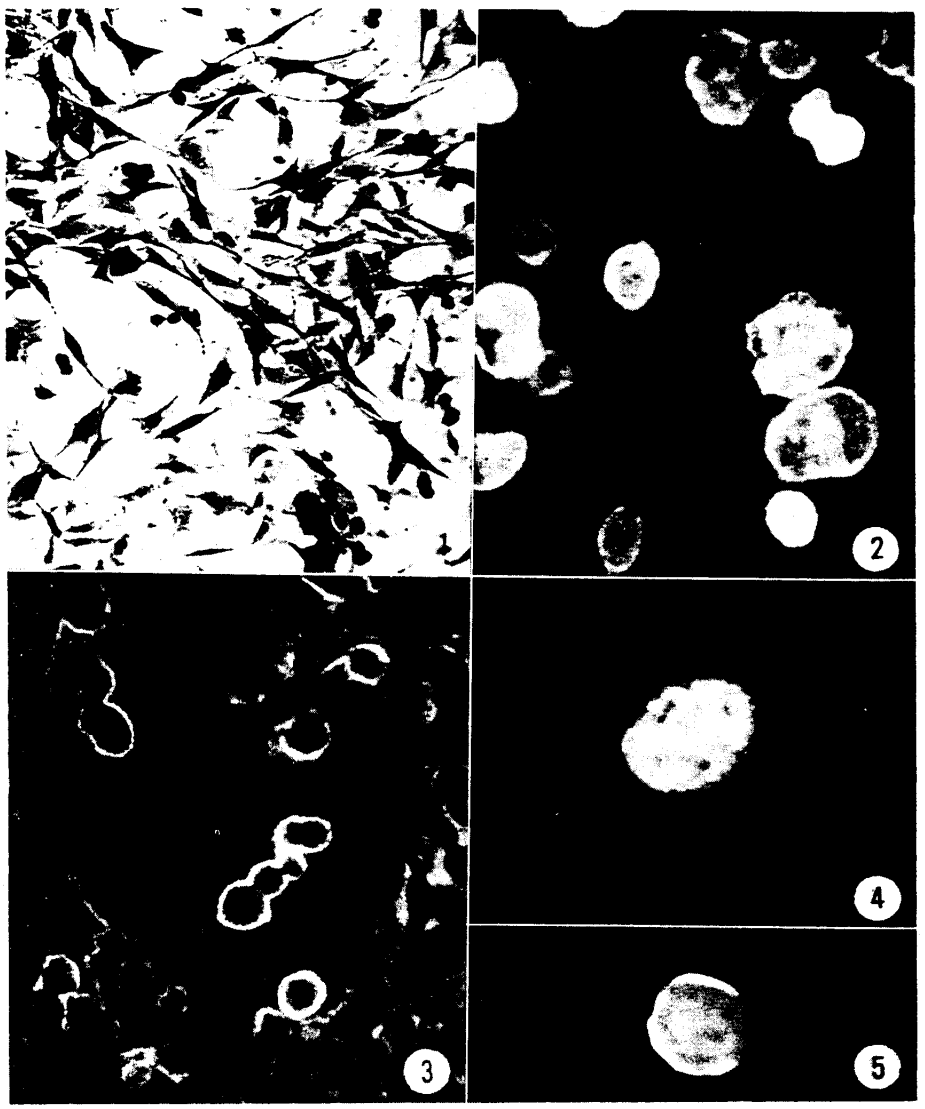
Photo 1 Cultured cells of the subcutaneous hamster fibrosarcoma induced by SV-40 DNA (NA-FS cell) 26 passages (360 days) × 100, H-E staining.

Photo 2 Tumor antigen detected in NA-FS cells by the treatment with rabbit anti-NA-FS cell serum. Intranuclear ántigens are observed in tumor cells, particularly in mitotic cells. 22 passages × 200

Photo 3 Tumor antigen detected in NA-FS cells by the treatment with rabbit anti-NA-FS cell serum. Antigens are localized in site of the nuclear membrane of tumor cells. 24 passages × 200

Photo 4 Tumor antigen in the NA-FS cells by the treatment with SV-40 tumor bearing hamster serum. Note nuclear antigen by the treatment with SV-40 induced tumor bearing hamster serum (tumor size 30 mm × 30 mm. 227 days post-inoculation of SV-40) 35 passage. × 430

Photo 5 Tumor antigen in the NA-FS cell by the treatment with SV-40-tumor bearing hamster serum. Note accumulation of antigen in the site of the nuclear membrane. 35 passages × 430



in transformed cultures involved in production of such antigens. A new immunofluorescent method to detect antigens in the nuclei of SV-40 transformed cells (RAPP and MELNICK[5,6], POPE and ROWE *et al.*[7]) resolves the question of the intracellular site of the antigen production as well as that of cells involved.

The antigen produced in the cells transformed *in vivo* by SV-40 DNA appears to be similar in all the SV-40 transformed cells to what previous investigators have detected in the cells transformed by the SV-40 virus *in vivo*, or *in vitro*

as well as in those human cells. The localization and the appearance of the SV-40 DNA-induced tumor antigen are similar to those seen when cells react with sera from SV-40 virus induced tumor bearing hamsters. On the other hand, it has been demonstrated that cells react strikingly with sera from rabbits immunized with hamster-transformed cells induced by DNA, at the site of the nuclear membrane as well as the nucleus. The nature of the new antigen has not been determined but the results obtained from immunofluorescent study using the rabbit anti-hamster tumor cell show that it appears to be cellular in origin rather that viral and is present only as the result of cell transformation induced by DNA. In addition, since it has been confirmed that all of the cells in transformed cultures, regardless of whether they are of the hamster or of the human origin, synthesize the antigen, it was expected that such a technique would prove effective in the detection of the transformed cells induced by SV-40 virus.

Recently, other works have established that the specific, SV-40 tumor CF antigens are not a consequence of malignant transformation because serologically identical antigens, which are not a part of the CF antigens of the SV-40 viral particles, are shown to be produced early after SV-40 virus infection of normal cells, and which quickly react with their antigens following transformation of cells by SV-40 virus. This fact is considered to be indicative of the integration of a part of the viral genome with that of the host cells. It was found that the specific tumor antigens consist of at least two different components and present in different concentrations in tumor cells and in the SV-40 infected normal cells[9].

In the immunofluorescent study it has been revealed that the rabbit sera immunized with transformed cells induced by SV-40 DNA *in vivo* and cultured *in vitro*, produce circulating antibodies capable of reacting with intranuclear antigens synthesized by SV-40 transforming process. In addition, it was noticed that the cells infected with infectious DNA produce viral antigens in the host cell (BS-C-1) as shown in the previous studies[5] and also synthesize the intranuclear antigen in the host cell. The results indicate that infectious DNA can be developed into complete viral antigen, replicated DNA-containing virus in host cell and that also the genome coding for the synthesis of the SV-40 tumor antigen is responsible for viral DNA.

SUMMARY

In the immunofluorescent study it has been revealed that rabbit sera immunized with transformed cells induced by SV-40 DNA, produce circulating antibody capable of reacting with intranuclear antigens synthesized by SV-40 complete virus transforming process.

In addition, the result confirmed that SV-40 DNA replicates DNA-containing viruses in the host cell and that also the genome coding for the synthesis of SV-40 tumor antigen is resposible for viral DNA.

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