Increased Susceptibility of Cells from Cancer Patients with XY-Gonadal Dysgenesis to Simian Papovavirus 40 Transformation

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SUMMARY

Genetically susceptible cells in culture can be transformed by oncogenic viruses. Quantitative assay methods have been developed for estimating the frequency of transformation. In an attempt to estimate the susceptibility to transformation of cells from cancer patients with XY-gonadal dysgenesis, fibroblasts from two such patients were infected with simian papovavirus 40, and transformation frequency was determined. Results of the quantitative assay indicate that the cells from these two patients were highly susceptible to transformation. It therefore appears that XY-gonadal dysgenesis resembles in this respect the group of disorders including Fanconi's anemia, Down's syndrome, and Klinefelter's syndrome, in which there is increased transformation frequency of the individual's cells after infection with simian papovavirus 40 in vitro.

INTRODUCTION

SV401 infection of human cells in tissue culture can result in neoplastic transformation of susceptible cells (3, 9). The number of cells undergoing transformation in an infected culture can be assayed quantitatively, and the transformation frequency can be determined for a particular cell strain. The observation that cells from individuals with certain genetic anomalies and at high risk to cancer show increased transformation frequencies after infection with SV40 has generated considerable interest. Fibroblasts from patients with Fanconi's anemia (14, 16), Down's syndrome (15, 17), and Klinefelter's syndrome (5) have been reported to be transformed by SV40 with a significantly higher frequency than fibroblasts from apparently normal individuals tested under identical conditions. In the case of a patient with XY/XXY mosaic Klinefelter's syndrome and bronchogenic carcinoma, we reported extreme susceptibility of aneuploid cell strains that became malignant in vivo to give rise to the tumor. For determination of the range of practical application to transformation assay technique, cells from members of cancer-prone families (13), from patients with colonic cancer (6), and from patients with acute myelogenous leukemia (10) have been tested. Results from these observations indicate that such transformation frequency determination might prove useful for detection of individuals with genetic predisposition to develop cancer and provide a basis for close surveillance and earlier cancer detection.

Clinical and epidemiological studies indicate that the incidence of certain types of tumors is high in patients with XY-gonadal dysgenesis (4, 8, 11). These are phenotypic females with underdeveloped, secondary sex characteristics who present rudimentary or absent gonads, absence of spontaneous menarche, normally developed but somewhat hypoplastic Müllerian duct derivatives, and 46 XY chromosomal constitution. In a series of patients studied, it has been found that individuals with XY-gonadal dysgenesis have an increased potential to develop germ cell tumors at the level of the rudimentary gonads (8). In an attempt to determine oncogenic potentiality of cells from patients with XY-gonadal dysgenesis, skin fibroblast cultures from 2 such patients were exposed to SV40, and susceptibility of cells to transformation was assayed by the technique of Todaro (12). Both patients had a type of germ cell tumor known as dysgerminoma of the rudimentary gonads.

MATERIALS AND METHODS

Fibroblast cultures from skin biopsies from these patients were grown in Ham's F-10 (Colorado Serum Co., Denver, Colo.) supplemented with 20% fetal calf serum. Cells from skin biopsies from 4 apparently normal patients, 2 male and 2 female, with no history of cancer were used as controls. These patients had normal chromosome constitution. Cell cultures between 5th and 8th transfer generations were used for these studies. The virus used was a small plaque variant (SV-S) of SV40, kindly supplied by Dr. George J. Todaro and Dr. Stuart A. Aaronson of the National Cancer Institute, Bethesda, Md. The virus was grown and titrated on primary monolayer cultures of African green monkey kidney cells. The titer of the stock was approximately $5 \times 10^8$ PFU/ml. This stock was further concentrated 10-fold to give a final virus concentration of $5 \times 10^9$ PFU/ml.

Sixty-mm Petri dishes were seeded with approximately 2 X $10^3$ cells. On the following day, the cells were washed with

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1 The abbreviations used are: SV40, simian papovavirus 40; PFU, plaque-forming units.

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Table 1
Transformation frequency of cell strains by SV40

<table>
<thead>
<tr>
<th>Subject</th>
<th>Karyotype</th>
<th>$T_f/10^4$ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient I</td>
<td>44 + XY</td>
<td>70.4 ± 1.9</td>
</tr>
<tr>
<td>Patient II</td>
<td>44 + XY</td>
<td>73.5 ± 0.7</td>
</tr>
<tr>
<td>Control I</td>
<td>44 + XY</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Control II</td>
<td>44 + XY</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>Control III</td>
<td>44 + XY</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Control IV</td>
<td>44 + XY</td>
<td>2.1 ± 1.5</td>
</tr>
</tbody>
</table>

$T_f = (\text{No. of transformed foci/total no. of infected cells plated}) \times 10^4$.

phosphate-buffered saline solution, infected with 0.5 ml of the 10-fold concentrated virus suspension (2.5 X 10⁹ PFU/dish), and shaken every 15 min for 3 hr at 37°C in a humidified CO₂ incubator. At the end of the infection period, the unabsorbed virus was completely removed from the cells by washing the cultures 5 times with phosphate-buffered saline solution followed by addition of complete medium containing 0.5% anti-SV40 bovine serum (Flow Labs, Inc., Rockville, Md.), added primarily to limit further infection of cells by the virus. On the next day, the cultures were trypsinized and subcultured, with each subculture containing approximately $2 \times 10^4$ cells. Within 2 to 3 weeks, transformed colonies could be seen as small, dense foci of aggregated cells growing one above the other in a random formation. Eighteen days after seeding, the cultures were fixed in 10% buffered formalin and stained with hematoxylin. The control cultures were treated identically. For determination of colony and monolayer morphologies of all the cell cultures, mock-infected cultures were stained and examined as described.

Chromosomes were studied following short-term leukocyte cultures and fibroblast cultures of all these individuals.

RESULTS

Results of the quantitative assays are shown in Table 1. The mean transformation frequency of cells from Patient I was $70.4 \pm 1.9/10^4$ cells, and that from Patient II was $73.5 \pm 0.7/10^4$ cells. Cells from 4 normal patients showed transformation frequencies ranging from 2.1 to 4.2/10^4 cells.

DISCUSSION

Several reports have so far indicated that cells from patients with chromosomal abnormalities and belonging to a high-cancer-risk group display increased susceptibility to transformation by SV40. These reports have specifically demonstrated that cells from Fanconi’s aplastic anemia (14, 16), Down’s syndrome (15, 17), and Klinefelter’s syndrome (5) are highly susceptible to transformation in vitro by SV40, in comparison to normal individuals. These 3 syndromes are associated with inborn chromosomal aberrations, which for some reason predispose to neoplastic disease. Recent reports also indicate that normal diploid cells from certain cancer patients show increased susceptibility to transformation by SV40 (6, 13). Observations by our group have indicated that cells from colonic cancer patients belonging to cancer-prone families show higher sensitivity to transformation than those from patients with cancer of the colon but without any family history of cancer. Recently, it has been shown that skin fibroblasts from patients with xeroderma pigmentosum, a recessively inherited autosomal disorder that predisposes to cancer of the skin, appear not to show increased susceptibility to transformation by SV40 (1). The basis for this apparent lack of increased susceptibility is not clear and warrants further investigation. It has been demonstrated that increased susceptibility to transformation by SV40 is proportional to the increase in the number of SV40 T-antigen-positive cells (2). In Paget’s disease of the vulva, a condition which is indicative of an underlying carcinoma, it has been shown by this technique that there is an increase of SV40 T-antigen-positive cells, in comparison to the amount of normal human cells (7). As previously pointed out, XY-gonadal dysgenesis is not due to any gross abnormalities of the chromosomes, but the available evidence seems to implicate either an X-linked recessive gene or a male-limited, autosomal dominant gene. It seems that this defective gene results in the development of abnormal sex characteristics and an increased potentiality to develop cancer of the rudimentary gonads. This predisposition to cancer, together with the findings of increased susceptibility of cells from these patients to viral transformation, is an interesting observation that may be relevant to the origin of germ cell tumor in patients with XY-gonadal dysgenesis. Furthermore, for evaluation of the applicability of this technique, it will be important to test patients with XY-gonadal dysgenesis who have not yet developed cancer in order to determine whether these individuals can be detected in the general population.

The data reported here, together with previous observations from other laboratories on the correlation between increased susceptibility of cells to transformation in vitro by SV40 and high incidence of neoplastic diseases in patients with certain types of genetic disorders, strongly encourage use of such in vitro assays to detect individuals with increased potentiality to develop cancer.

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REFERENCES


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