Simian Papovavirus 40 Transformation of Cells from Cancer Patient with XY/XXY Mosaic Klinefelter's Syndrome

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SUMMARY

Transformation frequency by simian papovavirus 40 of fibroblasts from a lung cancer patient with Klinefelter's syndrome and XY/XXY mosaicism was 3 to 10 times higher than that of fibroblasts from individuals with normal karyotypes and no history of cancer. The patient's XXY cell strain also manifested a 3-fold increase in susceptibility to transformation as compared to his XY cell strain. This observation, coupled with the finding of a high frequency of sex chromatin-positive cells in his tumor, could indicate origin of the tumor from the aneuploid cell population.

INTRODUCTION

Oncogenic viruses can produce in vitro neoplastic transformation of susceptible cells accompanied by synthesis of virus-specific tumor antigens and chromosomal aberrations. In addition, loss of certain regulatory properties such as cellular sensitivity to contact inhibition results in the formation of multilayered thick sheets of cells in a disordered, random array. Quantitative assay methods have been developed for estimating the frequency of transformation (11—13, 15, 18). Recent findings (7, 14—16) indicate that fibroblasts from individuals with Down's syndrome and Fanconi's anemia, both of which predispose to leukemia, also have increased susceptibility to in vitro transformation by SV40.2 In fact, susceptibility of fibroblasts to viral transformation has been proposed as a means of identifying individuals at high risk to leukemia and perhaps other neoplasia (7). The value of the transformation frequency test in identifying individuals at high risk to neoplasms other than leukemia has not been fully assessed, nor are reports available to indicate whether individuals whose cells show increased sensitivity to transformation ever develop neoplastic disease. Several workers have proposed that patients with Klinefelter's syndrome are in a high-cancer-risk group (1—4, 6, 17). The present report describes the susceptibility of cells from a cancer patient with Klinefelter's syndrome and XY/XXY sex chromosome mosaicism to in vitro transformation by SV40.

CLINICAL HISTORY

The patient, a Caucasian married man, was first admitted at the age of 38 because of a painful mass, of 4 years' duration, in the right breast. He was 178.4 cm tall and weighed 66.3 kg. He had liver enlargement, spider nevi, “eunuchoid” face with slow-growing and sparse beard, female hair distribution, small prostate, and soft, small testes. He was considered to be borderline mentally retarded. History indicated loss of libido and potency, with probable infertility. Biopsy of the left breast showed focal hyperplasia of the interstitial cells together with sclerosing seminiferous tubules and hyalinization of tubules. The Sertoli cells showed focal proliferation. These findings were consistent with Klinefelter's syndrome. An excisional biopsy of the breast lesion was diagnosed as gynecomastia.

The patient was subsequently admitted at age 45 with undifferentiated bronchogenic carcinoma. He expired shortly after admission from disseminated bronchogenic carcinoma.

MATERIALS AND METHODS

Explant cultures of fibroblasts from skeletal muscle were grown in 60-mm plastic Petri dishes. Cultures were maintained in modified McCoy's medium containing penicillin (50 units/ml), streptomycin (15 mg/ml), phenol red, and 20% fetal calf serum at 37° in a humidified atmosphere continuously flushed with 10% CO2-air mixture. The cells from these cultures were cloned (5) and maintained in McCoy's medium without Ca++ and containing 10% fetal calf serum. After the cultures reached adequate growth, they were vigorously shaken to detach mitotic cells (10). Chromosomes from these cells were analyzed, and cultures with XXY cell strains were identified and separated from those with XY sex chromosome complements.

Fibroblast cultures of skeletal muscle tissue from 2 men and 2 women, i.e., J. B., M. S., B. F., and H. T., who had normal human karyotypes and no history of cancer were used as controls. These cultures were maintained and treated identically to those from the patient.

When the cultures contained approximately 2 to 4 X 10⁴ exponentially growing cells, the medium was removed and the cells were washed with serum-free medium prior to infection with SV40. The virus used was SV40 clone 307L, grown and titrated on monolayer cultures of CV-1 cells, a continuous line of African green monkey cells. The titer of

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2The abbreviation used is: SV40, simian papovavirus 40.

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the stock preparation was approximately $10^8$ plaque-forming units/ml. The cultures were exposed to 0.05 ml of the 10-fold concentrated virus suspension (approximately 250 plaque-forming units/cell) and were shaken every 15 min for 3 hr at 37° in a humidified CO₂ incubator. The unabsorbed virus was then removed from the cultures and complete medium containing 0.5% of anti-SV40 bovine serum was added to limit further infection by the virus. Twenty-four hr after infection, the cells from cultures of each strain were trypsinized. The patient’s XXY cell strains were pooled from 10 cultures totaling approximately 2 to $4 \times 10^8$ infected cells. An identical procedure was followed for the XY strains. The strains were then subcultured in Petri dishes, and each dish was seeded with 2 to $4 \times 10^6$ cells of the cell suspension. The control cell strains were treated similarly. After 2 weeks, transformed colonies could be seen as small, dense, opaque foci of aggregated cells, growing one above the other without any discernible pattern. Three weeks after seeding, the cultures were fixed in 10% formal 0.76% NaCl solution and stained for 5 min in Jenner stain and for 10 min in Giemsa stain. The darkly stained transformed foci were counted under a low-power dissecting microscope.

RESULTS

Biopsy of the bronchial mucosa and skin revealed several cells with typical Barr bodies. Chromosomal analysis of cells from cultures of leukocytes (8) and from explant cultures (9) of skeletal muscle tissue revealed that 52% of the leukocytes and 53% of the fibroblasts had a modal number of 46 chromosomes with normal male karyotype, whereas 45% of the leukocytes and fibroblasts had 47 chromosomes with an XXY sex chromosome complement (Figs. 1 and 2). A small fraction of hypomodal cells resulted from rupturing of cells during preparation. The diagnosis was Klinefelter’s syndrome with XY/XXY mosaicism.

Results on quantitative assays (Table 1) show that the transformation frequency was 28.5 per $10^4$ cells for XXY and 9.7 per $10^4$ cells for XY cell strains. Cells from the 4 control cultures had transformation frequencies ranging from 1.7 to 3.3 per $10^4$ cells.

Histological examination of tumor sections stained by the Feulgen reaction revealed that 63.5% of tumor cells (a total of 907 tumor cells were scored) were sex chromatin positive. The presence of sex chromatin (Barr bodies) in the tumor cells (Fig. 3) indicates an XXY sex chromosomal make-up.

DISCUSSION

These data indicate that cells from the patient with mosaic Klinefelter’s syndrome were transformed with a 3- to 10-fold higher frequency than were cells from the normal individuals. This suggests that cells from the patient are notably more susceptible to transformation than are cells from normal individuals. Further, the data indicate that the XXY cell strain is more sensitive to transformation than is the XY cell strain from the same patient. Whether the 3-fold increase in susceptibility of the patient’s XY cell strain over cells from the normal individuals was the result of the patient’s terminal disseminated disease or some other cause is not known. The values for transformation frequency of cells from normal individuals are in good agreement with those reported by Todaro et al. (15).

The increased susceptibility to transformation of the XXY cell population from the patient compared to his XY cell population is an interesting finding and may have relevance to origin of the bronchogenic carcinoma from the XXY cell strain. Credence to this possibility is provided by the finding that Barr bodies were observed in 63.5% of the tumor cells. This frequency is significantly ($p < 0.01$) in excess of the frequency which would be expected if the tumor had been a mosaic comprised of 52% cells with XY and 45% with XXY sex chromosome complements, frequencies observed in the patient’s leukocyte and fibroblast cultures.

The results presented here show that a condition characterized by chromosomal abnormality and an existing neoplasm other than leukemia is associated with an increased cellular susceptibility to transformation in vitro by an oncogenic virus. In addition, an increase in transformation sensitivity of aneuploid cells over diploid cells from the same patient has been demonstrated. These observations, together with the finding of a high frequency of sex chromatin-positive cells in the patient’s tumor, pose the intriguing possibility that the tumor may have originated from the aneuploid cell population. The influence of the patient’s active neoplasm on the relative susceptibility of either cell type to transformation is not known. Nonneoplastic patients with this syndrome should therefore be studied. Moreover, it would be of interest to conduct parallel studies on cells from patients with other types of neoplasms, particularly those with chromosomal mosaicism. With improvement and standardization in methodology, quantitative assay of transformation in vitro by oncogenic agents of cells from individuals at high risk to cancer may be a useful tool in cancer detection programs.

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REFERENCES


Figs. 1 and 2. Karyotypes of chromosomes from the patient's 2 cell strains.

Fig. 1. Cell with XY.

Fig. 2. Cell with XXY sex chromosome complement.

Fig. 3. Feulgen-stained histological section of the patient’s bronchogenic carcinoma showing several chromatin-positive cells.
Fig. 1

Fig. 2

Fig. 3
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