Elevated Expression of T-Antigen in Simian Papovavirus 40-infected Skin Fibroblasts from Individuals with Cytogenetic Defects

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SUMMARY

A number of cytogenetic conditions were examined for expression of simian papovavirus 40 T-antigen in vitro. Skin fibroblasts from patients with Turner's syndrome and trisomy 18 syndrome and most cell lines from Klinefelter's syndrome, trisomy 13 syndrome, chromosomal translocations, chromosome 21 deletions, and single cases of 18q– and 4p– exhibited elevated T-antigen expression, compared to a clinically and cytogenetically normal control population. Thus, T-antigen expression was generally elevated in cells with increased, decreased, or rearranged genetic material involving many different chromosomes.

Variation in T-antigen expression among cell lines may reflect two factors. Individual cell line factors may account for differences within homogeneous clinical groups, whereas population factors appear to account for differences between the various clinical groups and the control population.

The observation of elevated T-antigen expression in diverse cytogenetic conditions suggests that this phenomenon may be a manifestation of chromosomal aberration unrelated to cancer susceptibility.

INTRODUCTION

A number of laboratories have investigated SV40 T-antigen expression and colony formation (transformation) in skin fibroblasts from patients with cytogenetic disorders. These studies have generally shown increased expression of SV40 genetic information in infected cells from cytogenetically abnormal individuals but were usually based on small numbers of cell lines representing a limited spectrum of disorders. Examination of 14 such reports (1, 2, 5, 10, 14, 16, 18, 24) revealed that 52 distinct cell lines from 13 different cytogenetic conditions and 61 distinct normal cell lines were studied. The abnormal cell lines studied were primarily from 17 patients with Fanconi's anemia (1, 2, 5, 14, 17, 18, 22, 24, 23, 27). 19 patients with Down's syndrome (1, 14, 17-19, 22, 24), and 11 patients with other trisomies (14, 16, 18, 24). The remainder included 2 cases of Turner's syndrome (18), 2 cases of ataxia telangectasia (10), and 1 case of triploidy (17). Since these studies were mainly of cell lines with extra or unstable chromosomes, had used various methodologies, and did not have a large or adequately characterized normal control population, a more complete investigation seemed warranted.

Studies from our laboratory have led to modifications in the T-antigen assay system that minimize variability and permit generation of reproducible data (8, 9). A statistically significant increase in T-antigen expression was observed in SV40-infected skin fibroblasts from 10 Fanconi's anemia patients compared to a normal population of 76 individuals (13). For a determination of whether increased expression of SV40 genetic information is a general characteristic of cytogenetic disorders, this assay system was applied to skin fibroblasts from patients with Turner's syndrome, Klinefelter's syndrome, various autosomal trisomy and deletion syndromes, and patients and carriers with chromosome translocations.

MATERIALS AND METHODS

Cell Lines. Most of the human skin fibroblast lines were established from punch biopsy specimens as previously described (18). Cell lines 2278 (GM-5), 2279 (GM-73), 2280 (GM-74), 2281 (GM-85), 2282 (GM-98), 2283 (GM-137), 2284 (GM-143), 2286 (GM-213), 2287 (GM-230), 2290 (GM-284), 2291 (GM-285), 2292 (GM-327), and 2294 (GM-479) were from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, N. J. Cell lines 2267 (CRL-1246), 2375 (CCL-21), 2377 (CCL-65), 2381 (CCL-117), and 2383 (CCL-123) were from the American Type Culture Collection, Rockville, Md. All cells were grown in Dulbecco's modified Eagle's minimal essential medium plus 10% (v/v) fetal bovine serum as previously described (9). The karyotypes of peripheral leukocytes were assessed by the method of Moorehead et al. (15), and fibroblasts were assessed by the method of Sun et al. (21).

Virus. The origin, propagation, and titration of the SV40 small plaque strain used have been described earlier (8).

T-Antigen Assay. This technique has been reported previously (9). Briefly, 3 to 4 replicate cultures of each cell line were infected with 100 plaque-forming units of SV40 virus per cell. After a 3-hr adsorption period, cultures were incubated for 3 days at 37° in the presence of sufficient rabbit
anti-SV40 serum to prevent secondary infection. At this time cultures were fixed and stained by indirect immunofluorescent methods. In each culture, 100 microscopic fields (an average of 3000 cells) were examined for T-antigen expression. The mean proportion of T-antigen-containing cells, weighted according to the number of cells examined per replicate, was calculated ($p_\omega$) and mathematically transformed by the arc sine square root function ($\sin^{-1}\sqrt{p_\omega}$). This quantity was regarded as the estimate of T-antigen expression for a given cell line. The $\sin^{-1}\sqrt{p_\omega}$ transform was used to provide homogeneity of variances required for parametric statistical analysis of these data.

Using these methods we found that T-antigen expression in cells from 76 healthy, karyologically unremarkable individuals was normally distributed and not significantly influenced by age, sex, or ethnic background (13). The probability that $\sin^{-1}\sqrt{p_\omega}$ for a given cell line did not exceed that of the mean value of this representative healthy population ($11.5 \pm 4.0$) was calculated from the cumulative normal probability function (z test).

Assay results do not vary significantly between experiments (9, 13); therefore single determinations of 3 to 4 replicate cultures per cell line were adequate to assess T-antigen expression accurately. Nonetheless, we used internal controls in each experiment. Tumor cell lines A172 and A498 were selected since their levels of T-antigen expression represented the high and low extremes, respectively, and did not change with extensive serial cultivation (9). Skin fibroblast lines were assayed at their lowest available serial passage. This was between passage levels 5 and 10 for lines established in this laboratory or within 3 to 5 passages of receipt from external repositories.

Chart 1. T-antigen expression in SV40-infected skin fibroblasts from individuals with cytogenetic anomalies. T-antigen expression is presented as $p_\omega$ and $\sin^{-1}\sqrt{p_\omega}$. Probabilities were calculated from the cumulative normal frequency distribution with the mean value (11.5 ± 4.0) of $\sin^{-1}\sqrt{p_\omega}$ for a population of 76 normal individuals (13). O, males; O, females. Karyological information is contained within the symbol (i.e., which chromosomes are translocated, deleted, or trisomic). Numbers adjacent to the symbols refer to the cell line designation code.
RESULTS

T-antigen expression in SV40-infected skin fibroblasts from 6 of 8 cases of Turner’s syndrome exceeded the upper 99% confidence limit for the healthy control population (Chart 1A). One of the 6 patients also had acute myelogenous leukemia (Case 0453). The remaining 2 cases exceeded the 95% confidence limit. T-antigen expression was normal in 3 cases (not shown) that phenotypically resembled Turner’s syndrome but had euploid karyotypes. Four of 5 cases of Klinefelter’s syndrome (Chart 1B) showed significantly elevated T-antigen expression, including a case with breast cancer (Case 1654). In 1 case (Case 2280) the extra X chromosome was translocated onto 1 chromosome 14. All 5 cases of trisomy 18 and 2 of 3 cases of trisomy 13 had significantly elevated levels of T-antigen expression (Chart 1C), whereas 1 case of trisomy 7 and 1 of 3 cases of trisomy 13 examined had normal levels. Two of the trisomy 13 cases (Cases 2281 and 2380) contained D;D translocations.

T-antigen expression was significantly elevated in 3 of 6 patients with malformations resulting from translocations (Chart 1D). Four of 5 translocation carriers had significantly elevated levels of T-antigen expression (Chart 1E). None of these cell lines contained extra genetic information in addition to their respective translocations.

Among the chromosome deletion syndromes examined (Chart 1F), marked elevation was observed in single cases with 18q− and 4p− (Wolff’s syndrome) and in 2 of 3 cases with monosomy 21. Results on 1 patient with cri-du-chat syndrome were normal, but the karyotype [80% 45,XY,t(5;22) and 20% 46,XY,5p−] was not typical.

DISCUSSION

This study examined T-antigen expression in skin fibroblasts from patients with a spectrum of trisomy, deletion, and translocation disorders involving both autosomal and sex chromosomes. Significantly increased expression (P < 0.05) was found in 30 of 39 cell lines from such patients, and only 1 case failed to exceed the normal mean. These findings extend our previous reports of elevated T-antigen expression (Chart 1E). None of these cell lines contained extra genetic information in addition to their respective translocations.

Among the chromosome deletion syndromes examined (Chart 1F), marked elevation was observed in single cases with 18q− and 4p− (Wolff’s syndrome) and in 2 of 3 cases with monosomy 21. Results on 1 patient with cri-du-chat syndrome were normal, but the karyotype [80% 45,XY,t(5;22) and 20% 46,XY,5p−] was not typical.

A major concern here is the variation of T-antigen expression within groups of patients with a particular cytogenetic disorder. Among individuals with translocations differences in T-antigen expression may reflect the specific chromosomes involved, but the variation within relatively homogeneous categories (e.g., Turner’s syndrome, Klinefelter’s syndrome, trisomy 18, and monosomy 21) suggests that noncytogenetic factors within individual cell lines also regulate the level of T-antigen expressed. This is supported by the observations that different aliquots of the same cell line gave relatively homogeneous results in the same and in different experiments and that the level of T-antigen expression generally remains constant over several cell passages (9). Since differences between cell lines are greater than those accounted for by experimental variation, it appears that at least 2 biological factors control the expression of T-antigen in cells. Individual cell line factors seem to account for differences within homogeneous clinical groups, whereas population factors account for differences between normal individuals and those with cytogenetic abnormalities. Individual variation may explain the elevation in all 8 patients with Turner’s syndrome in this study, whereas 2 patients previously reported from another laboratory had normal values (18).

These observations suggest that cell lines differ in their regulation of T-antigen expression. Experiments utilizing radioactively labeled SV40 virus do not support the hypothesis that cells containing cytogenetic anomalies absorb virus more efficiently than do normal cells (Ref. 1; J. M. Kelley and A. S. Lubiniecki, unpublished observations). Therefore, it is possible that as yet undefined biological factors in cell lines with cytogenetic abnormalities explain these differences. These biochemical changes could be of fundamental significance in considering the mechanism by which cytogenetic abnormalities predispose to cancer.

Another question arising from these data concerns the relationship of elevated T-antigen expression to increased cancer risk. Patients with Fanconi’s anemia and Down’s syndrome are prone to acute leukemia (6, 11). Turner’s syndrome is associated with various tumors derived from neural crest tissue (25); Klinefelter’s syndrome is associated with male breast cancer (7); and trisomy 18 is associated with premalignant lesions of Wilms’ tumor (4). All 5 syndromes exhibit elevated SV40 T-antigen expression but so do cytogenetic disorders that are not presently associated with high cancer risk. Although some of these disorders are too rare or lethal to permit an analysis of cancer risk, the finding of elevated T-antigen expression in diverse cytogenetic syndromes suggests that it may be a manifestation of chromosomal aberration that is unrelated to cancer susceptibility.

It remains possible, however, that elevated T-antigen expression in cytogenetic syndromes is a predisposing factor for certain neoplasms in combination with other risk factors such as the estrogen hormonal imbalance that leads to breast cancer in Klinefelter’s syndrome. This notion is consistent with the finding of increased colony formation and T-antigen expression in karyologically normal families prone to leukemia and certain other cancers (3, 12, 20). Perhaps the SV40 assay measures a variety of molecular abnormalities analogous to but not necessarily identical with the different DNA repair defects seen in patients with xeroderma pigmentosum. Further experiments are presently underway in this laboratory that should lead to clarification of the relationship between T-antigen expression and susceptibility to cancer.

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