PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 505-507 (1977)

Elevated Expression of T-Antigen in Skin Fibroblasts from Individuals with Cytogenetic Anomalies Infected *in Vitro* with Simian or Human Papovaviruses[1] (39704)

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Clinical groups with elevated cancer risk and increased expression of viral genetic material following *in vitro* infection with simian papovavirus 40 (SV40) include Fanconi anemia (1–5), Down's syndrome (3, 4, 6, 7), trisomy 18 (3, 6), Klinefelter's syndrome (8), and a leukemia-prone family (9). Our laboratory has confirmed and extended this positive correlation between the incidence of SV40-induced T antigen or transformation in infected human skin fibroblasts and some types of increased cancer risk to the cell donor (manuscripts submitted for publication). We have further shown that SV40 T antigen expression by fibroblasts from normal individuals showed no significant differences among various age, sex, or ethnic groups examined (submitted for publication).

Recent reports have shown serological relatedness between the T antigens (10) and partial nucleic acid homology between the genomes of SV40 and the human papovavirus BKV (11). BKV(MM), isolated from a brain tumor and the urine of a patient with Wiskott–Aldrich syndrome (12), has also been shown to complement an SV40 temperature-sensitive mutant defective in an "early" function (13), possibly linked to T-antigen expression (14, 15). In view of these and other general similarities between simian and human papovavirus (10), we questioned whether a human papovavirus might also exhibit elevated T-antigen expression in skin fibroblasts from high cancer risk groups.

Materials and methods. Human skin fibroblast cultures were established from punch biopsies and propagated as reported (16). BKV(MM) was propagated and assayed on human embryonic kidney cells as reported previously (13). The preparation employed contained 1×10^6 tissue culture infectious doses ($TCID_{50}$)/ml assayed on human embryonic kidney cells by limiting dilution methods. SV40 was propagated and assayed on African green monkey kidney (17). The stock contained 2×10^8 PFU/ml.

Human cells were infected with 100 PFU/cell of SV40 or 5 $TCID_{50}$/ml of BKV(MM), as reported previously (18). At 72 hr after infection, indirect immunofluorescent assays for T antigen were performed using hamster sera raised against SV40 T antigen (16). From previous preliminary studies, it was known that these reagents did not stain uninfected human skin cells in over 200 cell strains examined.

The weighted mean proportion (p_w) of T-antigen-containing cells was calculated from three replicate petri plates per determination (18). On each plate, 100 microscopic fields (averaging $3–5 \times 10^3$ cells) were examined for T-antigen expression. Each replicate plate was randomly encoded before staining to prevent operator bias; codes were not broken until the conclusion of the assay.

Results. Figure 1 shows that T-antigen expression by eight human fibroblast strains tended to be similar for SV40 and BKV(MM). For both viruses, the four normal cell strains expressed T antigen less frequently than cell strains from the three clinical groups at high cancer risk. The standard errors were relatively larger for BKV(MM)-infected cells than for SV40-infected cells in every cell strain. This may reflect the less intense fluorescence generally observed in BKV(MM)-infected cells.

[1] This work was supported in part by the National Cancer Institute Contract No. NO1-CP-43216.

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ISSN 0037-9727

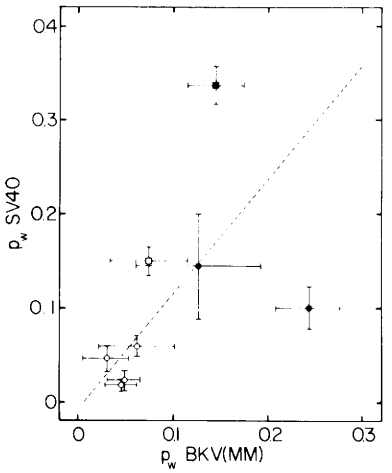

FIG. 1. T-antigen expression by BKV(MM)-infected and SV40-infected human skin fibroblasts. Weighted mean proportions (p_w) for each cell strain and virus examined were calculated as described (18). Each of four normal human fibroblast strains are shown as open circles, each of two strains from Fanconi anemia patients are shown as closed circles, and single strains from patients with Turner's syndrome and Klinefelter's syndrome are shown by open and closed squares, respectively. Bars indicate one standard error about the mean value. The dashed line indicates the nonparametric regression line.

To avoid assumptions about the underlying mathematical distribution of the data, nonparametric statistical analyses were performed. Employing Spearman's rank correlation method (19), data from BKV(MM)-infected cells correlated with results from SV40-infected cells ($r_S = 0.76$; $P_{r_S=0} < 0.05$). Nonparametric linear regression analysis was employed using the methods of Theil (20). Assuming that BKV(MM) and SV40 behaved identically (despite apparent differences in the multiplicity of infection) in all cell strains tested, the expectation is that the regression line of the data in Fig. 1 has a slope of unity and passes through the origin. In fact, the slope estimator possessed a value of 1.27, which was not significantly different from unity ($P > 0.05$). The regression line passes very close to the origin in Fig. 1.

Discussion. The observations of Todaro and his associates that fibroblasts from patients with several diseases associated with increased cancer risk have increased frequency of SV40 transformation and T-antigen expression have been of great interest to oncologists (1, 2, 6). These experiments with SV40, however, require high multiplicities of virus (18), and the significance of increased susceptibility of human cells to infection *in vitro* by a monkey virus has remained obscure. The fact that generally similar susceptibilities are found with a human papovavirus at apparently lower multiplicities provides the previously missing rationale for both of these observations.

Klein (21) has recently suggested that some forms of viral carcinogenesis may require specific genetic changes for development of neoplasia *in vivo* and that chromosomal abnormalities may play a critical role in oncogenesis in man by Epstein-Barr virus. The present findings that T-antigen expression is elevated in human papovavirus-infected fibroblasts from patients with Fanconi anemia, Turner's syndrome, and Klinefelter's syndrome is consistent with this view of viral oncogenesis.

The present results also permit analysis of the relative infectivity of SV40 and BKV(MM) for human cells. It is known that the level of T-antigen expression by SV40-infected cells is multiplicity dependent (18, 22). Since BKV(MM) can be titrated by a limiting dilution T-antigen assay (23), T-antigen expression is probably multiplicity dependent for this virus also. Thus, the level of T-antigen expression is a measure of the number of virus particles infectious for a given cell strain. If it is assumed that 1 $TCID_{50}$ of BKV(MM) consists of a single infectious particle, then BKV(MM) is approximately 20 times more infectious for human cells than SV40. This is derived from the fact that 5 $TCID_{50}$/cell of BKV(MM) (titrated on human cells) induced approximately the same level of T-antigen expression as 100 PFU/cell of SV40 (titrated on monkey cells). This quantitative estimate is consistent with qualitative evidence of the host cell preferences of these viruses (10).

Summary. Human skin fibroblasts were infected with human papovavirus (BK type) or simian papovavirus 40 (SV40) at multiplicities of infection which induced similar frequencies of T-antigen-containing cells at 72 hr after infection. Both viruses exhibited a higher rate of T-antigen expression in cells

from genetically abnormal individuals with Fanconi anemia, Turner's syndrome, or Klinefelter's syndrome than in cells from normal individuals.

The authors wish to express their gratitude to Mark Gunnell, Thomas Archer, Elizabeth Alexander, and Carole Yee for their excellent technical assistance, and Celeste Martin for her patient secretarial assistance.

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Received October 18, 1976. P.S.E.B.M. 1977, Vol. 154.