T-Antigen Expression in Human Skin Fibroblasts Is Not Regulated by an Endogenous Interferon Response to SV40 Infection

Brief Report

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Summary

Exogenous interferon may affect SV40 T-antigen expression, depending on the chromosomal complement, time of treatment, and biological factors in human cells. However, no evidence was found for endogenous interferon response to SV40 infection in the regulation of T-antigen expression.

Increased expression of T-antigen or transformation has been reported in simian papovavirus 40 (SV40)-infected skin fibroblasts from patients with various cytogenetic anomalies, some of which are associated with high cancer risk (8—10, 19, 20). Interferon (IF) may be a factor in regulating the level of T-antigen expression in a given cell line, since the frequency of T-antigen expression in non-transformed human cells is sensitive to depression by IF treatment before SV40 infection (12). Moreover, some cytogenetic defects associated with elevated T-antigen expression (8—10, 19, 20) also have altered IF production or sensitivity (17, 18). If IF is involved in regulating T-antigen expression, then normal cell lines may simply produce more IF in response to SV40 infection, and/or be more sensitive to IF action, than cell lines with a high frequency of T-antigen expression. Also, IF may be a factor explaining biological variation of T-antigen expression among individuals with particular cytogenetic and clinical states (9). To evaluate these issues, human skin fibroblasts from diploid individuals and patients with monosomy 21 and trisomy 21 were examined.

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It must be emphasized that this study is concerned with the role of IF in the 72 hour T-antigen assay (5, 6), and not necessarily with other aspects of the biological interaction of SV40 and IF under all possible experimental conditions. This is not to deny the importance of these other aspects, but to focus attention on the possible role of IF in an assay previously shown to have some efficacy as an in vitro indicator of cancer risk (8, 9).

Human skin fibroblast lines were established from punch biopsy materials as previously described (5). All cultures were propagated and maintained in Dulbecco-modified Eagle’s medium (DMEM) supplemented with 10 per cent fetal calf serum (GIBCO, Grand Island, N.Y.) in a humidified atmosphere containing 10 per cent CO2 at 37° C. All cell lines were free of detectable mycoplasma infection. The cytogenetic status of all cell lines was verified by the method of SUN et al. (15). FS-4 cells were obtained from Drs. Vilček and Havell, and propagated as described by them (21).

SV40, small plaque isolate, was propagated and titrated in African green monkey kidney cells as described by TAKEMOTO and coworkers (16). Human cells were infected with 100 PFU/cell (6). After adsorption of the virus for 3 hours, cultures were washed with DMEM and incubated for 72 hours in the presence of goat anti-SV40 serum to prevent secondary infection. T-antigen expression (weighted mean proportion of T-antigen containing cells in 2—4 replicate cultures) was determined by indirect immunofluorescent methods (5). Uninfected cells contained no detectable T-antigen.

Newcastle disease virus (NDV, strain CG) was propagated in 9—11 day old embryonated eggs (13). Vesicular stomatitis virus (VSV), Indiana strain, was propagated in primary chick embryo fibroblast cultures (4) and harvested at the first sign of cytopathology, about 14 hours post infection.

Laboratory reference stocks of human IF were produced by superinduction of FS-4 cells exposed to poly I. poly C as described by VILSEK and HAVELL (21). Mock interferon was produced identically, except that the poly I. poly C was omitted. IF activity was assayed by reduction of dye-binding due to VSV cytopathic effect in FS-4 cells using the microwell technique (1). IF activity is expressed in terms of IF units (IU) standardized against Reference Human Standard (69/19) prepared by the Medical Research Council, Mill Hill, London, England.

IF production in skin fibroblast lines was examined by seeding 7.5 × 10³ cells/cm² in T-75 flasks (Falcon Plastics, Oxnard, Calif.), the same cell density as employed in petri dishes for T-antigen assays. After 24 hours, cell cultures were infected with 100 PFU/cell of NDV or SV40. Fluids from NDV-infected cultures were harvested at 24 hours after infection, adjusted to pH 2.0—2.5 for 6 days at 4° C (to inactivate virus), neutralized, and examined for IF activity. Fluids from SV40-infected cultures were harvested 72 hours after infection and concentrated 10-fold by ultrafiltration employing an Amicon stirred cell with a PM10 membrane (Amicon Corp., Lexington, Mass.). Preliminary experiments had shown that the method of concentration employed caused no detectable loss of interferon activity. Since these samples contained no detectable infectious SV40 (<5 PFU/ml) by plaque assays, they were not subjected to low pH treatment for virus inactivation. Sham-infected cultures were also employed as controls for each cell line.