Transformation of Human Embryonic Fibroblasts by Photodynamically Inactivated Herpes Simplex Virus, Type 2 at Supra-optimal Temperature

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SUMMARY

Infection of human embryonic fibroblast cell monolayers with neutral red and light-inactivated herpes simplex virus, type 2 (HSV-2) at supra-optimal temperature (42 °C) resulted in persistence of viable cells in suspension culture at 37 °C which have properties in common with virus transformed cells: formation of cell aggregates, HSV-2-specific antigens and colony formation in soft methyl cellulose medium. These data are consistent with the idea that photodynamic inactivated HSV-2 has potential oncogenic activity.

There is published evidence that ultraviolet irradiation (Duff & Rapp, 1971) or photodynamic inactivation (Rapp, Jui-Lien & Jerkofsky, 1973) of herpes simplex virus, type 1 (HSV-1) or HSV-2 results in virus which can transform hamster cells. HSV transformed cells are oncogenic when inoculated into syngeneic animals (Rapp & Duff, 1974; Jui-Lien, Jerkofsky & Rapp, 1975). Photodynamic inactivation of HSV occurs following treatment of virus with a heterocyclic dye (e.g. neutral red, proflavin or toluidine blue) and subsequent exposure of the treated virus to visible light.

Other investigators showed that temperature sensitive (ts) mutants of HSV can transform rat cells (Macnab, 1974), hamster cells or human cells (Takahashi & Yamanishi, 1974). Darai & Munk (1973) reported that human cells showed transformed-like morphology at 37 °C following HSV-2 infection at 42 °C, a temperature non-permissive for HSV-2-specific DNA synthesis (Marcon & Kucera, 1975). Recently, we (Melvin & Kucera, 1975) found that HSV-2 can stimulate human cell DNA synthesis during incubation at 42 °C or following shift-down from 42 °C to 37 °C. The capacity to stimulate host cell DNA synthesis is a property shared with other oncogenic DNA viruses (Dulbecco, Hartwell & Vogt, 1965; Fox & Levine, 1971; St Jeor et al. 1974; Gerber & Hoyer, 1971). Since photoinactivation is used in the treatment of recurrent HSV infections of the skin and mucous membranes in humans (Felber, 1971; Friedrick, 1973; Kaufman et al. 1973) experiments were designed to determine whether photoinactivated HSV-2 can induce cell transformation in human embryonic fibroblasts (HEF). Results indicated that HEF cells exposed to neutral red and light-inactivated HSV-2 at 42 °C possessed some properties of transformed cells during subsequent incubation at 37 °C. Preliminary results of these experiments have been published (Kucera & Gusdon, 1975).

Preparation of secondary HEF cell cultures and HSV-2 stocks was previously described (Melvin & Kucera, 1975). Tests for possible mycoplasma contamination in virus stocks were negative. The method used for preparing neutral red and light-inactivated HSV-2 was reported by Wallis & Melnick (1964). The ANG strain of HSV-2 used was obtained from L. Falk, University of Chicago, Chicago, Illinois. This strain of HSV-2 was previously used by Darai & Munk (1973) to abortively infect human cells which subsequently acquired some properties associated with cell transformation. Our method for establishing cell
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transformation was as follows. Monolayer cultures of HEF cells in 75 cm² Falcon flasks (BioQuest, Oxnard, California) were washed once with 0.01 M-tris-HCl buffered saline pH 7.0, and infected with neutral red and light-inactivated HSV-2 (approx. 90% inactivation of p.f.u.) at an input multiplicity of 3 inactivated p.f.u. of HSV-2/cell. After a virus attachment period of 1 h at 37 °C, the virus inoculum was decanted and the cell monolayers were overlaid with 10 ml of growth medium (minimal essential medium supplemented with 20% heat-inactivated foetal calf serum, 10% tryptose phosphate broth, 100 units penicillin/ml, 100 μg streptomycin/ml, 0.075% NaHCO₃ and 2 mM-L-glutamine). All cultures were incubated at 42 °C to abort the lytic functions of partially inactivated HSV-2 (Darai & Munk, 1973). After 8 days at 42 °C to block residual infectious virus multiplication (Marcon & Kucera, 1975) the infected cultures were shifted-down to 37 °C and maintained at this temperature. Results showed that uninfected control cells did not survive during incubation at 42 °C. In contrast, about 50% of the infected cells were floating in the culture medium. These cells were viable by trypan blue dye exclusion tests. They were subcultured by low speed centrifuging (450 g for 5 min) and resuspension to a density of 5 x 10⁶ cells/ml of fresh growth medium.

Approx. 4 weeks after HSV-2 infection and subculture of the ‘floating’ cells a morphological transformation, characterized by formation of cell aggregates, occurred in the cultures (Fig. 1). The cells increased in quantity and were subcultured at 3 to 5 week intervals as described above. We interpret these data to indicate the possible presence of HSV-2 genetic information in the infected cells. Assays for infectious virus production in the transformed cells were all negative when tissue culture fluids from the infected cells were inoculated on to HEF cell monolayers in a standard plaque assay.

The continued presence of the HSV-2 genomes in the infected HEF cells was examined by specific immunofluorescence tests. Rabbit antiserum raised against HSV-2 antigens was obtained by subcutaneous inoculation of 2 ml of disrupted HSV-2 infected rabbit kidney cells (antigen) mixed with an equal volume of complete Freund’s adjuvant. Beginning 4 days

Fig. 1. Human embryonic fibroblast cells in suspension culture showing formation of cell aggregates following infection with photodynamic inactivated HSV-2.
Fig. 2. Immunofluorescence in human embryonic fibroblast cells infected with photodynamic inactivated HSV-2.

later, the immunized rabbit was given a total of 7 semiweekly subcutaneous injections of 2 ml of the same antigen mixed with an equal vol. of incomplete Freund's adjuvant at 4 different sites. One week after the final injection, 0.75 ml of antigen was injected intravenously. After 14 days the rabbit was exsanguinated and the antiserum was heat inactivated (56 °C, 30 min), adsorbed against foetal calf serum and uninfected rabbit kidney cells prior to use in the indirect immunofluorescence test. The results of these experiments (Fig. 2) showed distinct nuclear and cytoplasmic immunofluorescence in a small proportion (5 to 10 %) of the transformed cells. Fluorescence was not detected in uninfected HEF cell controls or with pre-immune rabbit serum which was used in place of post-immune serum (data not shown).

The HSV-2-transformed HEF cells have the capacity to grow and form colonies in soft methyl cellulose overlay medium (Kucera & Simonson, 1974) a property shared with other virus-transformed cells (Macpherson & Montagnier, 1964). Controls consisted of HEF cells continuously maintained as stock cultures at 37 °C. Cell colonies were not observed in the mock-infected control HEF cells after 24 days' incubation at 37 °C (data not shown).

In summary, the infected HEF cells possess the following characteristics of transformed cells: (1) formation of cell aggregates (Fig. 1) and detection of HSV-2-specific antigen(s) (Fig. 2) in abortively infected cells; (2) colony formation in soft methyl cellulose overlay medium, and (3) persistence of viable cells in serial subculture as compared to mock-infected HEF cell controls. We interpret these data to indicate that cell aggregation and production of HSV-2-specific antigens are consequences of an inherited active virus gene function(s) in these cells.

Photoinactivation experiments with other oncogenic DNA viruses (e.g. SV40, HSV-1, HSV-2) showed that inactivated viruses have the potential to transform hamster embryo fibroblasts (Rapp et al. 1973). Our results confirmed published work by Rapp et al. (1973)
that photoinactivation of HSV-2 does not destroy the cell transformation potential of the virus. The HSV-2-transformed HEF cells are maintained in suspension cultures in contrast to monolayer cultures as previously described for HSV-2-transformed human embryonic lung cells (Darai & Munk, 1973). From results of transformation of mammalian cells by inactivated HSV-2 reported here and by others (Rapp & Duff, 1974) an oncogenic potential of neutral red and light treatment of HSV lesions in humans should be recognized.

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REFERENCES


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