FAILURE OF HERPES SIMPLEX VIRUS TYPE 2 TO SUBSTITUTE FOR DIMETHYL-BENZANTHRACENE IN TWO-STAGE SKIN CARCINOGENESIS

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There are some indications that herpes simplex virus (HSV) may be mutagenic. Specific chromosomal changes have also been demonstrated in cultured cells infected with HSV. To further investigate the mutagenic activity of HSV type 2 (HSV-2) we used mouse skin as a model system for carcinogenesis. Inoculation of the back skin of 4-week-old Senac mice with live virus twice per week for one week or with inactivated virus twice per week for two weeks was used to initiate the mouse skin. After initiation with HSV-2, 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied twice weekly for 50 weeks as a promoter. During a period of 52 weeks, no skin carcinoma was found in the experimental groups, whereas 55% of control mice treated with 9, 10-dimethyl-1, 2-benzanthracene (DMBA) and then with TPA-developed skin carcinoma. The results demonstrate that HSV-2 could not substitute for DMBA in this animal model of two-stage skin carcinogenesis.

HSV-2 has been suspected for many years of being a contributing cause of squamous cell cervical cancer. Duff and Rapp and others have clearly demonstrated that transformed cells arise at low frequencies from both primary and established rodent or human cells cultured after abortive infection with HSV under various nonpermissive protocols, including ultraviolet (UV) inactivation of the virion. Transformed cell lines have been established from most experiments with rodent cells, and some lines produce tumors in experimental animals. It has been reported that repeated vaginal inoculation of HSV-2 inactivated with either formalin or UV irradiation can induce cervical and/or vaginal cancers in mice and that the incidence of these cancers can be decreased by prior vaccination or increased by local application of croton oil after inoculation of inactivated HSV-2. In addition, mouse lip cancer can be induced with HSV inoculation followed by treatment with croton oil or TPA. More recently, induction of cervical cancer in mice by long-term frequent vaginal...
exposure to live or inactivated HSV could not be repeated.\textsuperscript{18} There also are contradictory reports with regard to detection of HSV-specific DNA, RNA and virus antigens in cervical cancer tissues.\textsuperscript{19} Several groups have concentrated on identifying the viral genes responsible for morphologic transformation and the gene products that persist in transformed cells; however, results have not been consistent and no single gene product has been associated with transformation.\textsuperscript{19,20}

It is well established that all the human herpesviruses induce chromosomal aberrations.\textsuperscript{21-24} However, due to the failure to consistently demonstrate HSV-2 sequences in cervical cancer biopsies and the suspected mutagenicity of HSV, zur Hausen\textsuperscript{25} has proposed that HSV may act as an initiator. By measuring the rate of mutation at the HGPRT locus in cells exposed to UV- or neutral red-inactivated HSV-1, Schlehofer and zur Hausen\textsuperscript{26} later found that HSV is as potent a mutagen as the chemical carcinogen 4-nitroquinoline-1-oxide. Although the mutagenic activity of HSV has been suggested, its possible role as an initiator that mutagenizes susceptible target cells for transformation has not been studied further.

Carcinogenesis is assumed to be a multi-stage disease process in which a single normal cell progresses to malignancy in discrete sequential stages. This was shown by Rous and Kidd\textsuperscript{27} who reported that skin carcinogenesis was a two-stage process that could be enhanced by irradiation of the skin. The two stage concept of initiation-promotion was first formulated and described by Berenblum.\textsuperscript{28} Subsequently, Boutwell\textsuperscript{29} and Slaga\textsuperscript{30,31} defined many important aspects of the initiation and promotion of two-stage carcinogenesis, specifically that skin tumors can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation stage) followed by repetitive treatment with a noncarcinogenic promoter (promotion stage). The also dissolved in acetone (50 μg/ml).

**Experimental Animals**

Four-week-old female Sencar mice were obtained from Harlan Sprague Dawley. Mice were housed at a maximum of 5 animals per cage and were supplied with tap water and Purina Lab Rodent Chow \textit{ad libitum}. To enhance the contact of agents with skin, the hair on the back hind quarters of mice was clipped once per week.

**Viral Inoculation of Mice**

The right side of the skin of the hind back quarters of each mouse in groups I and II was slightly abraded with a razor blade. Then 0.1 ml of live virus suspension (group I) or inactivated virus suspension (group II) was dropped onto the abraded area (2 × 2 mm\textsuperscript{2}) twice weekly for one week (group I) or 2 weeks (group II). One week after virus infection, we applied 5 μg of TPA in 0.1 ml of acetone twice weekly. This treatment continued for 50 weeks.

**Application of DMBA**

Instead of viral inoculation, a single application of 10 nM of DMBA in 0.1 ml of acetone was made to the hind back skin of mice (without abrading) in group V. After one week, twice weekly applications of TPA (5 μg/0.1 ml acetone) were begun and continued for 50 weeks. Several groups and different treatments of both sides of the mouse back skin served as initiation phase which requires only a single application of carcinogen is essentially an irreversible step, whereas the promotion phase is initially reversible.