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Herpesvirus Particles in Prostatic Carcinoma Cells

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Herpesvirus particles were found in cancer cells from a human prostate adenocarcinoma. These particles were identified as herpesvirus on the basis of specific immunofluorescence staining, morphology, and size.

Epidemiological studies on patients with carcinoma of the cervix have implied a link between this malignancy and herpesvirus (HSV) genital infections. This implication comes from the fact that the incidence of carcinoma of the cervix is higher in women with multiple marriages, multiple sex partners, or who start sexual relations early in life; in these groups, antibodies to herpesvirus type 2 are at a higher level than in control normal populations or populations with cancer of other organs (9, 10).

There is suggestive evidence that herpesvirus type 2 which causes genital infections in both men and women is venereally transmitted (6-8) and can persist in the tissue, at least for a limited time, in the absence of overt disease (3) but the primary reservoir for herpesvirus infection was heretofore uncertain. In our previous work, herpesvirus was isolated from 15% of a randomly selected population of 190 males without history of herpesvirus infection, indicating that the male genito-urinary tract can indeed serve as the reservoir for this virus (2). Indirect immunofluorescence staining demonstrated that these isolates were herpesvirus type 2. Since in this investigation herpesvirus was also isolated from patients with diagnosed cancer, we decided to investigate whether or not herpesvirus particles and HSV antigens were present in cells derived from adenocarcinoma of the prostate.

We now report the finding of herpes particles in cancer cells in a patient with adenocarcinoma of the prostate. An irregularly shaped, nodular firm gray prostate tumor weighing 50 grams was surgically removed from a 76 year old male who had not received any previous radiation or chemotherapy. The tumor was densely adherent to the surrounding structures, invading seminal vesicles, nerve vessels, and adjacent soft tissues. Light microscope examination revealed a poorly differentiated adenocarcinoma of the prostate throughout the specimen. The tumor cells as well as cells from normal prostate tissue were tested for the presence of virus by inoculation onto susceptible tissue culture cells, specific immunofluorescence staining, and electron microscopy.

For tissue culture, the tissue was finely minced in Basal minimal media and inoculated onto tube cultures of primary human embryonic kidney cells purchased from HEM Research Inc.; Rockville, Md. The cultures, maintained with Basal minimal media supplemented with 10% calf serum, were incubated at 37 C and examined daily for the presence of viral cytopathic effects. None of the cultures, which were kept for 3 to 4 weeks, exhibited an evident cytopathic effect (CPE). They were considered negative for virus growth.

The presence of herpes viral antigens in tumor cells was determined by indirect immunofluorescence techniques. Small pieces of tumor were grown as tissue explants on methyl cellulose coated cover slips to facilitate adherence of the tissue. The explants were incubated at 37 C and maintained with weekly changes of Basal minimal media containing 10% fetal calf serum, 10% NCTC 109, 1% glutamine, and antibiotics. The tumor explants grew very

poorly and slowly and it was common for them to detach from the cover slip and die, as opposed to the control explants which proliferated more rapidly. Explant cultures in which the cells had proliferated enough to cover at least one-half of the cover slip were fixed in cold acetone for 20 min for specific immunofluorescence staining. The antiserum to herpesvirus used in this test was prepared in three New Zealand white rabbits. The animals were immunized to herpesvirus type 1 (Shealy strain) by weekly intramuscular and intraperitoneal injections of 1 ml of virus stock (10^6 PFU/ml) for a period of 3 weeks. The sera obtained from these animals 2 weeks after the last injection were pooled and the neutralizing activity was tested in human embryonic kidney tube cultures with the Shealy strain (10^3 PFU) as the challenge virus. Under these conditions, this sera inhibited virus CPE at a 1:64 dilution. The fluorescein-conjugated goat anti-rabbit IgG-serum, specific for IgG heavy chain, was obtained from Cappel Laboratories, Inc.; Downing Town, Pa. Rabbit antiserum to herpesvirus at a 1:5 dilution was placed over the coverslip and incubated for 30 min at 37 C in a moist chamber. The cover slip was then washed with an excess of phosphate-buffered saline, and fluorescein-labeled goat anti-rabbit IgG antiserum was added. The cover slip was reincubated as described above, washed again, mounted in 90% glycerol solution, and examined in a Leitz fluorescence microscope. Specific immunofluorescence staining was seen in the nuclear and perinuclear areas and some positive cells were stained throughout the nucleus and cytoplasm (Fig. 1). The stain was intense and evident as compared to the nonspecific fluorescence which was dull and ocurred uniformly in all the cells of the control samples (Fig. 2).

For electron microscopy the original tumor tissue was fixed with glutaraldehyde with post fixation in osmium tetroxide. Dehydration was performed by increasing concentrations of ethanol prior to embedding in Epon 812. Thin sections were cut with a Porter-Blum MTII microtome and stained with uranyl acetate and lead citrate and examined in a Zeiss 9S-2 electron microscope.

Virus particles are seen only in the nuclei of the cancer cells (Fig. 3). They show a central dense core surrounded by one and sometimes two limiting membranes and their morphology is consistent with that of intranuclear herpesvirus as described by Morgan (7). The size of this particle is 90 to 100 nm as determined by measurements of four to five particles in different electron microscopy fields. That the cells that harbor these particles are indeed cancer cells was confirmed by two independent pathologists who examined not only the tumor but stained thin sections of the area of the Epon block where these sections were cut for electron microscopy. The fact that cells from this tumor were positive for herpesvirus by immunofluorescence tests further supports our belief that this particle is from the herpes group.

The presence of herpes particles in prostatic cancer cells, indicated by both specific immunofluorescence staining and electron microscopy, strengthens the already significant evidence of a direct relationship between herpesvirus and carcinoma of the genital tract. The epidemiological studies linking cervical cancer and herpesvirus infections are strong but circumstantial. They support this relationship but do not determine whether or not herpesvirus is a causative agent in these malignancies.

Duff and Rapp (4) have shown that UV-irradiated herpes simplex virus type 2 can transform cells in vitro and that such cells can cause tumors in animals. A strong implication of the oncogenicity of herpesvirus type 2 in its natural host, man, was provided by Frenkel and co-workers (5) who showed that human cervical cancer cells contained a fragment of herpesvirus DNA in their genome, and by Aurelian, who isolated herpesvirus type 2 from degenerating cervical tumor cells (1).

It is important to stress the fact that the presence of HSV antigens, as determined by specific immunofluorescence was performed in tissue explants and not in repeatedly passed cell cultures. The fact that these explants did not show a lytic infection or viral cytopathic effects indicate the presence of either HSV antigens or noninfectious particles in the cancer cells and is pertinent to the work of Frenkel (5) who found a herpes simpex DNA fragment in cervical cancer cells but no infectious virus. The electron microscopy that revealed viral particles in the nuclear region of the cell was performed in several tissue specimens that were never in tissue culture, but were immediately frozen after surgery, thus precluding the possibility of laboratory contamination.

Our data showing herpesvirus antigens by immunofluorescence and viral particles by electron microscopy indicate a possible relationship between herpesvirus and carcinoma of the prostate. We can not claim that this virus established the state of malignancy in prostatic tissue, but the fact that cells transformed by herpesvirus can cause cancer in another species

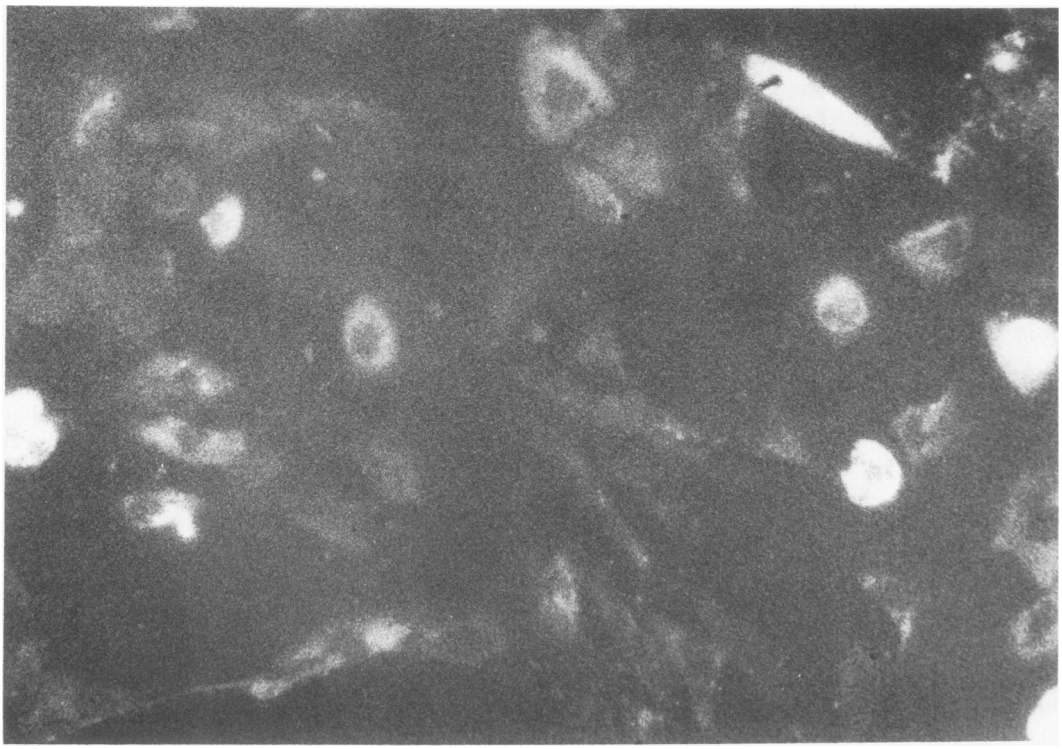

FIG. 1. *Fluorescent staining of human prostatic cancer cells. Specific immunofluorescence for herpesvirus is seen in both nuclear and perinuclear areas and some positive cells are stained throughout.*

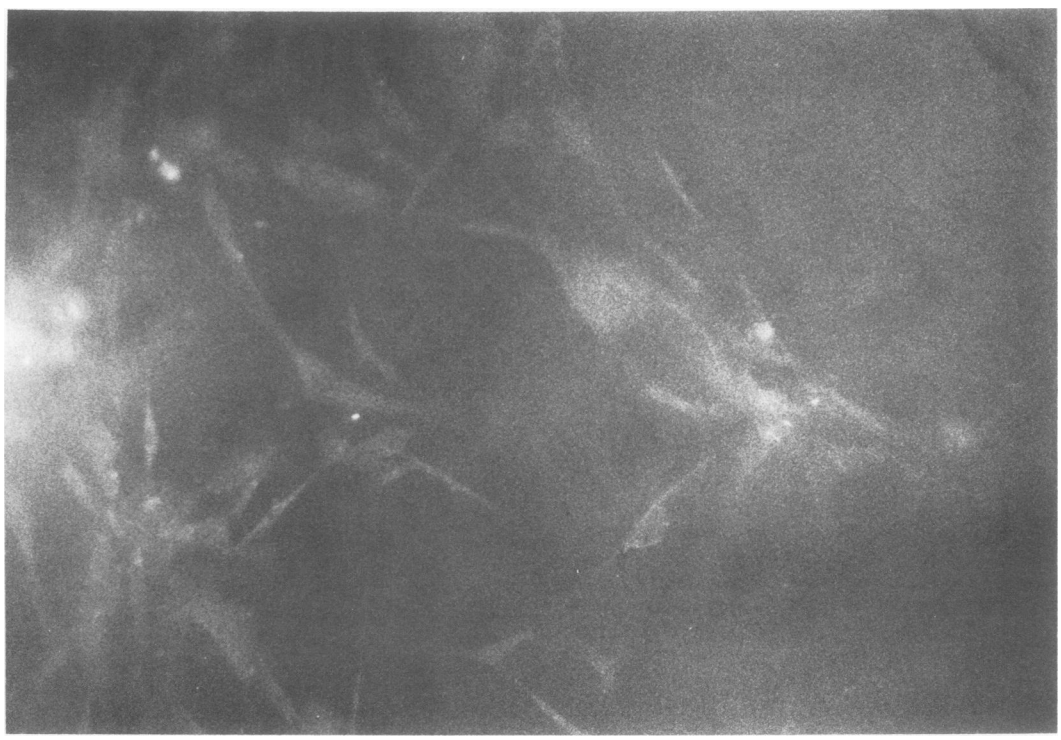

FIG. 2. *Control tissue from a normal prostatic tissue exhibiting background fluorescence only.*

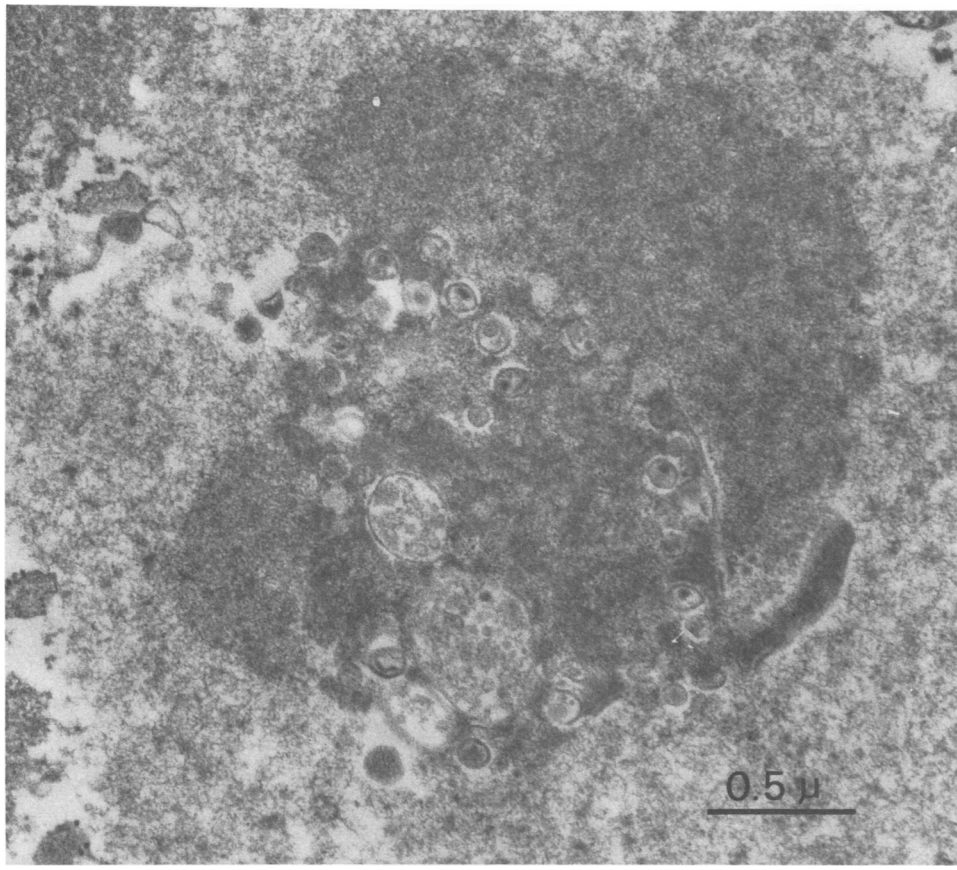

FIG. 3. *Virus particles in the original tumor specimen. Particles are seen in the nuclear region of the cancer cells and have an average size of 90 to 100 nm; magnification ×38,000.*

(4) demonstrates the oncogenic potential of this virus and suggests the importance of further investigation. Additional study of the conditions required for herpesvirus oncogenesis will be necessary.

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Takashi Sakimoto did the electron microscopy.

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