Activation by 5-Iododeoxyuridine of Shope Papilloma Viral Genome in Cultured VX2 and VX7 Carcinomas

Brief Report

By

T. INOKUCHI, S. IKEJIRI, F. MIZUNO, and T. OSATO

1 2nd Department of Oral Surgery, Kyushu Dental College, Kita-Kyushu
2 Department of Virology, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Japan

With 4 Figures

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OSATO and ITO (5) previously demonstrated the presence of a small amount of immunofluorescent viral antigen in Shope papilloma virus (SPV) (10)-related transplantable carcinomas (7, 9), not only in V x 7 but also in V x 2 which had (1) been stated to have lost the antigen sometime during the period of serial transplantation for over 15 years (9). This paper concerns a remarkable 5-iododeoxyuridine (IUdR)-induction of SPV antigen in such particular tumors.

Transplantable rabbit carcinoma V x 2 and V x 7 (7, 9), originating from SPV-induced papillomas (10), were studied. Cell culture, IUdR treatment, and immunofluorescent staining procedures have been previously described (5, 6, 11). Briefly, finely minced V x 2 and V x 7 tissue fragments were cultured on coverslips and exposed to 25 μg/ml IUdR for 3 days followed by 3 days incubation with drug-free medium. The coverslips were then fixed in acetone and stained with anti-SPV rabbit serum and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit γ-globulin goat antibody (Eiken Kagaku Co.). The rabbit antiserum was a generous gift from Dr. Y. Ito, Kyoto University. The serum was prepared by immunization of rabbits with SPV partially purified by a density gradient centrifugation of cottontail rabbit papilloma tissues. The serum was adsorbed twice with rabbit kidney powder before use and its SPV specificity was determined as described previously (6). Cellular structures were not well retained due to IUdR toxicity. The stained preparations were examined with an Olympus fluorescence microscope with a BG12 exciter filter. The light source was an Osram HBO200 lamp.

Cells grown from V x 2 explants were small and round (Fig. 1) showing a very small immunofluorescence-positive proportion of 0.1 per cent. V x 7 cells were larger and rather polygonal in shape (Fig. 2). About 10 per cent of the cells were reactive with antiviral serum.
When the primary V×2 and V×7 cultures were exposed to IUdR, a remarkable increase of positive cells occurred in both tumors. The immunofluorescence investigations showed that SPV induction became evident in 4.3 and 28 per cent of V×2 and V×7 cells, respectively (Figs. 3 and 4).

The morphological characteristics of cultured V×2 and V×7 tumors noted here were the same as previously described (5). Also, the present immunofluorescence investigations confirmed the previous observations (5), which clearly showed that SPV antigen is demonstrable not only in V×7 carcinoma cells but also in cells of V×2 tumors, though the reactivity of the latter cells occurred in a low frequency.

A remarkable 40 times increase of viral antigen-positive cells was noted in V×2 carcinoma exposed to IUdR, and a 3 times increase was seen in drug-treated V×7. Our findings strongly suggest, on the basis of the recent experience of viral activation by halogenated pyrimidines (1—4, 8, 11), that the whole or parts of the SPV genome has persisted in considerable proportions of V×2 and V×7 carcinoma cells, at least 4 and 30 per cent respectively, for extended periods of 30 years.

Fig. 1. Primary culture of V×2 carcinoma. Small round cells are predominate. Phase contrast. × 200

Fig. 2. Primary culture of V×7 carcinoma. Cells are larger and polygonal. Phase contrast. × 200

Fig. 3. Immunofluorescence of primary V×2 cells exposed to IUdR, stained with anti-Shope viral serum. × 400

Fig. 4. Immunofluorescence of IUdR-treated primary V×7 cells, stained with anti-Shope viral serum. × 400