Radiation-Enhanced Oncogenesis by SV40[1] (34953)

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The induction of primary virus tumors in hamsters by SV40 after an extended period of time after neonatal infection is well documented (1–3). For experimental studies it would be of considerable advantage if the incubation period could be reproducibly shortened. Stoker (4) observed that the frequency of *in vitro* cellular transformation produced by polyoma virus was increased among BHK-21 hamster cells that survived X-irradiation pretreatment. Exposure of mouse fibroblasts to radiomimetic thymidine analogs enhanced the efficiency of SV40 transformation 5-fold (5–7). X-irradiation of the 3T3-mouse cell line markedly sensitized the cells to transformation after infection with SV40 (8). Hamster embryo cells in primary tissue culture were rendered 50-fold more susceptible to SV40 transformation when treated with 150 R of X-irradiation prior to infection than were nonirradiated cells (9). In this study we have asked whether enhancement of virus tumorigenesis occurs *in vivo*, which is similar to that observed *in vitro* after X-ray pretreatment of the SV40 infection site in neonatal hamsters.

Materials and Methods. Virus. Simian virus 40, strain VA45-54 was prepared in green monkey kidney cells (GMK) and contained 10^9 plaque-forming units per ml of virus when assayed in GMK. Full-strength virus and virus diluted 1:1 in Hanks' balanced salt solution were tested. Newborn Syrian golden hamsters were positioned beneath a lead shield disc with tape so that the right scapular region or the left lower flank was exposed tangentially to a 5-mm opening.

Radiation. X-ray from a Maxitron "300" source equipped with 0.5 mm aluminum and 0.42 copper filters was delivered to the center of the disc from a distance of 22 cm. Tissue regions in the openings were 5 cm from target center and were exposed to 150 R of X-irradiation.

Infection. SV40 virus (0.2 ml) was inoculated subcutaneously directly into the irradiated target region immediately after X-ray exposure, and neonates were returned to their mothers and palpated weekly for tumors. Animals were weaned 21 days after birth. Controls included radiation treatment alone, virus infection alone, radiation exposure, and injection of passage fluid control solution containing no virus or radiation exposure at one site and virus injections at another.

Results. Results presented in Fig. 1 show that infection with undiluted virus produced tumors in nonirradiated hamsters beginning after 130 days postinfection, and 80% of the animals developed subscapular tumors by 160 days. However, tumors appeared more than 50 days earlier in hamsters receiving X-ray in the subscapular region prior to neonatal infection, and 88% of the animals had tumors in this group by day 160. Similar results were observed when X-ray exposure and virus were given in the left flank area with the exception that all animals developed tumors.

Using diluted virus suspensions (Fig. 2) in nonirradiated animals no difference could be detected in the time of appearance of the first tumor (130 days) when infected with

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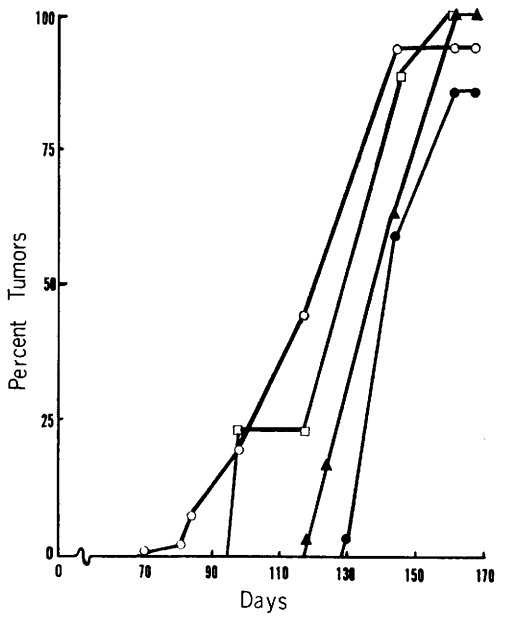

FIG. 1. Appearance of SV40-induced tumors in hamsters after neonatal infection with undiluted virus. Closed circles show data obtained in nonirradiated animals infected in the subscapular space. Open circles depict data obtained when virus is inoculated into the scapular space after X-ray exposure of the area to 150 R, and squares give results for a group irradiated and infected in the left-flank area. Triangles indicate results for animals irradiated in the flank and infected in the scapular space.

diluted virus suspension; however, only 50% of the hamsters developed tumors by day 170. In hamsters receiving irradiation prior to infection in either target area, tumors again appeared earlier (30–45 days) and with 30–50% greater incidence when compared to nonirradiated, infected control. Results were reproducible in three experiments using two to three litters (10–12 animals per litter) per test group. Tumors always occurred at the site of virus injection and were typical SV40 fibrosarcomas.

Radiation exposure, even to this very local region (2–5% of the hamster trunk area) may have resulted in modest immunologic suppression. Tumors were generally palpable 10 days earlier in control animals receiving 150 R of X-ray in the left flank and undiluted virus in the scapular space when compared with nonirradiated, infected hamsters (Fig. 1). This group also developed 20% more late tumors than infected, nonirradiated controls using undiluted virus only. It seems unlikely that the small degree of enhancement of SV40 oncogenesis produced by radiation-induced depression in immunologic reactivity could account for the marked enhancement of tumor appearance when infection occurred in the irradiated tissue zone. Tumors did not occur in control groups receiving no virus.

Virus tumors were examined for the presence of SV40 tumor or T antigen using the indirect fluorescent-labeling procedure (10) and were uniformly observed to contain the virus neoantigen.

Discussion. Observations from this (9) and other laboratories (4–8) have clearly established that a variety of rodent cell types are greatly sensitized to transformation *in vitro* when exposed to X-irradiation prior to or shortly after SV40, adenovirus 31, or polyoma virus infection. Additionally, we re-

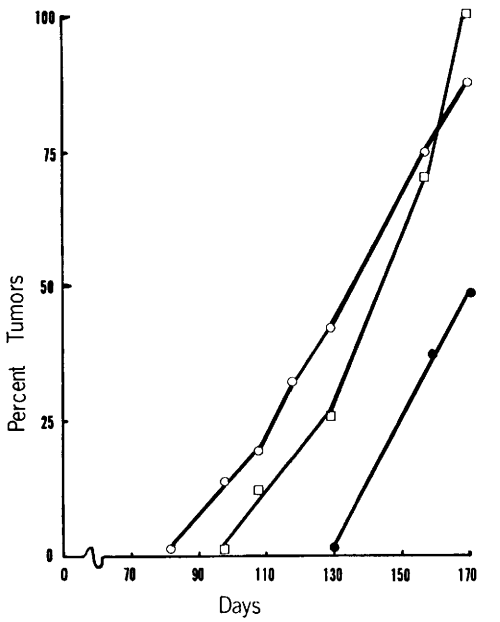

FIG. 2. Appearance of SV40-induced tumors in hamsters after neonatal infection with diluted virus. Symbols are for groups treated as shown in Fig. 1. No difference could be detected between animals receiving flank irradiation and dilute virus inoculation and virus alone.

cently reported that thymidine analogs and aging of primary cells *in vitro* rendered the cells increasingly sensitive to SV40 transformation (9). Physical damage to the DNA of the irradiated cells (11), recycling the cells to a partcular mitotic phase postirradiation, or induced DNA synthesis (8) or repair (9) may be responsible for the observed enhancement *in vitro*.

The purpose of this report is to relate the observation that a similar sensitization of target cells can be achieved in an animal model system. It appears practicable to use the system described here to ascertain whether a number of nononcogenic, teratogenic viruses can now be inoculated into irradiated neonatal hamsters with resultant tumor production. The increased sensitivity to oncogenesis observed may also be useful in more precisely evaluating the safety of a number of viruses currently used in live agent, human vaccines. In addition, chemotherapeutic studies may be more conveniently carried out using the shorter induction period provided by preirradiation. Since relatively low doses of X-ray were used in this work, the possibility must be considered that exposure of human fetuses to irradiation may activate latent virus to produce tumors later in life. Preliminary findings indicate that X-ray pretreatment of neonates markedly increases the tumorigenicity of weakly oncogenic viruses such as adenovirus 3.

Summary. Exposure of hamster and mouse embryo cells to X-irradiation prior to infection with Simian virus 40 (SV40) significantly increased the frequency of transformation *in vitro*. Neonatal hamsters were tested for increased sensitivity to SV40 tumorigenicity after exposure to localized, low-level X-ray at the site of virus inoculation *in vivo*. Results showed that irradiation of a target area prior to infection markedly decreased the time to tumor appearance and increased the frequency of tumor occurrence. No tumors occurred in irradiated animals that did not receive virus. Although low doses of localized X-ray may have resulted in modest immunologic suppression, this was not a primary factor in tumor enhancement. Tumors appearing in SV40-infected animals uniformly possessed viral neoantigens.

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