INDUCTION OF ANGIOGENIC SARCOMAS BY SOME MAMMALIAN ADENOVIRUSES

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The morphogenesis of tumors induced in the soft tissues of hamsters by simian adenovirus Sa7(C8) and the morphology of tumors induced by two other serotypes (SA7-B105 and C626) as well as by type 3 bovine adenovirus were studied. It is postulated that tumors induced by simian adenoviruses and most tumors induced by bovine adenovirus are undifferentiated malignant hemangiopericytomas with a tendency toward differentiation into leiomyosarcoma. On transplantation the structure of the primary tumors persists for a long time.

The oncogenicity of the various groups of adenoviruses of animals has recently been described. The most interesting members of these adenoviruses are the highly oncogenic simian and bovine adenoviruses, not only as models for the study of virus carcinogenesis, but also as possible contaminants of virus vaccines and other biological preparations.

Besides certain differences, there is a basic similarity in the morphology of the tumors induced in the soft tissues of hamsters by the adenoviruses of these species of animals and man [2, 4, 7].

This paper describes the results of a histogenetic analysis of tumors induced in the soft tissues of hamsters by three simian adenoviruses of the SA-7 group and by type 3 bovine adenovirus, strain WBR-1. The virological part of the investigation was carried out by A. D. Al'tshtein, E. M. Tsetlin, N. M. Strizhachenko, and N. A. Graevskaya.

EXPERIMENTAL METHOD

Culture fluid containing the virus was injected subcutaneously and intramuscularly into newborn hamsters. Tumors developed at the site of injection of the virus. The frequency of the tumors induced by these viruses in the hamsters and the times of their appearance are given in Table 1. The animals died from the tumors 1-2 weeks from the time when they became palpable. When the tumors were transplanted, the tumor cells were injected into hamsters of any age, but principally adult. Tumors developed in 100% of the animals after 1-2 weeks.

To study the primary and transplanted tumors induced by simian and bovine adenoviruses, 464 and 70 hamsters respectively were used. The morphogenesis of the tumors was studied in 295 animals. For this purpose the hamsters were sacrificed starting on the 2nd and ending on the 41st day after infection with adenovirus SA7(C8). In addition, cells of a culture of myodermal tissue of a hamster embryo were transformed by the same virus, and after five subcultures in vitro they were injected subcutaneously into adult hamsters. Starting with the 20th day after infection, tumors appeared in all the hamsters at the site of injection of the cells, and these were retransplanted for 24 passages.

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TABLE 1. Frequency of Induction of Tumors in Hamsters and Times of Their Appearance

Virus	Dose per hamster (in PFU)	Method of injection of virus	Incidence of tumors	Latent period (in days)
SA7 (C8)	$10^{5-5,5}$	Subcutaneously	$^{251}/_{257}$	25—79 (35)
SA7 (C8)	$10^{5,2}$	Intramuscularly	$^{10}/_{11}$	25—39 (30)
SA7 (B105)	$10^{3,9}$	Subcutaneously	$^{9}/_{32}$	58—89 (83)
SA7 (C626)	$10^{3,2}$	»	$^{2}/_{11}$	123—138 (131)
WBR-1	$10^{3,7}TCD_{50}$	»	$^{28}/_{61}$	25—287

*Numerator shows number of hamsters with tumors; denominator shows number of animals inoculated which survived as long as the mean latent period.

† Mean latent period shown in parentheses; animals without tumors were observed for 300 days at least.

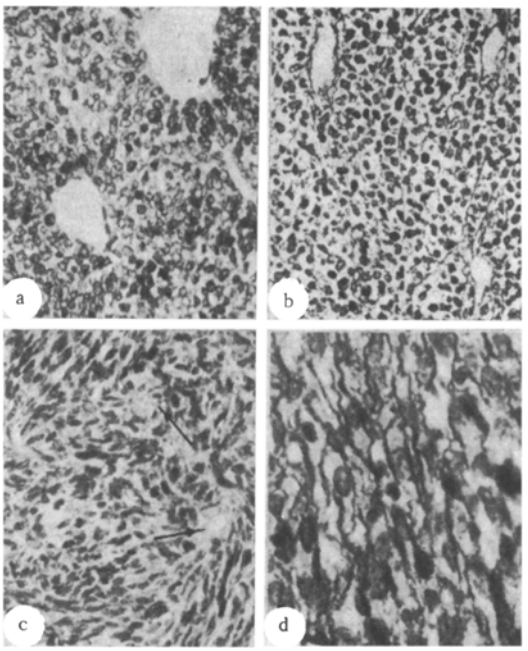

Fig. 1. Morphology of soft-tissue tumors induced in hamsters by SA7(C8) virus: a) tumor resembling hemangiopericytoma: polymorphic cells with large nuclei are arranged around numerous sinusoidal vessels; b) the same tumor: argyrophilic membranes of the vessels can be seen, with tumor cells outside them; c) tumor resembling angioleiomyosarcoma: elongated cells arranged in bundles winding around the vessels (shown by arrows); d) individual argyrophilic "sleeves" around some tumor cells. Staining: a and c) hematoxylin-eosin; b and d) silver impregnation by Gomori's method. Magnification: in a, b, c) 306 × in d) 1000 ×.

EXPERIMENTAL RESULTS

Primary induced tumors usually were multiple and matted together, and the weight of the mass frequently greater than the weight of the animal bearing the tumor; the transplanted tumors, however, were usually single. Tumors induced by simian adenoviruses had clearly defined outlines, and as a rule they did not invade the surrounding tissues, unlike tumors induced by bovine adenovirus, which frequently invaded the surrounding tissues. The tumors were of soft, elastic consistency, pinkish-white on section, with foci of necrosis and hemorrhages. No metastases were observed.

Histological examination showed proliferating tumor cells in the subcutaneous tissue and interstices of the muscles in most animals infected with SA7(C8) virus from the 7th-9th day after infection. Some of them were situated in the capillary wall. At this period the proliferating cells were mostly rather large epithelioid cells. Other foci of proliferation consisted on compactly arranged spindle-cells, frequently with single epithelioid cells. Starting with the 17th day, besides foci of proliferation, larger nodules of tumor tissue could be seen in the subcutaneous areolar tissue with the unaided eye, for they measured about 0.5-1 mm in diameter. Growth of capillaries among the tumor cells could be seen histologically in these nodules, and from the beginning these were lined with typical endothelium. Numerous blood vessels, some of them consisting of sinusoids, surrounded by cells with large nuclei and ill-defined cytoplasm in close contact with them, were present in the tumors after 24 days (Fig. 1a). The tumor cells were outside the argyrophilic membranes of the vessels, and small groups of cells were interwoven with argyrophilic fibers (Fig. 1b). According to Stout [11] and others, these tumors can be regarded as hemangiopericytomas. Later the tumor cells in some places became spindle-shaped and showed a tendency to be arranged in bundles around the vessels, the nuclei of some cells became rod-shaped, and the whole picture resembled that of angioleiomyosarcoma (Fig. 1c). This picture was more clearly defined in the more slowly growing tumors induced by the large-

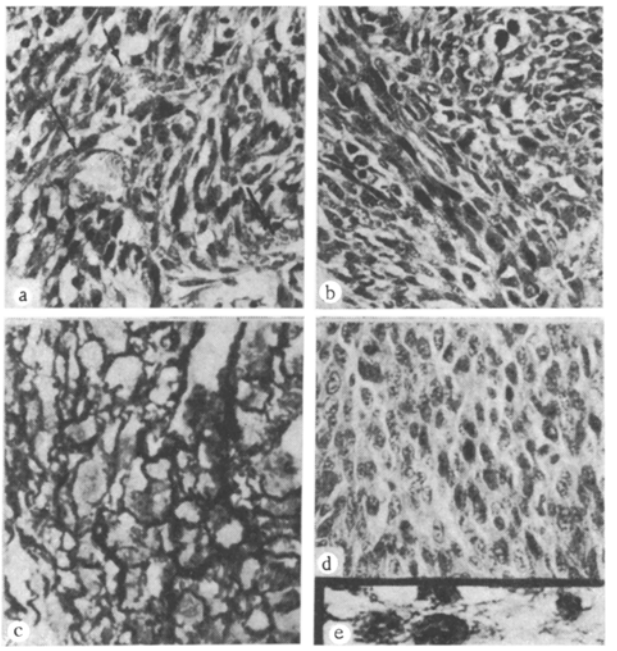

Fig. 2. Tumors of soft tissues of hamsters induced by type 3 bovine adenovirus: a) area of tumor resembling angioleiomyosarcoma (vessels indicated by arrows); b) first passage of tumor in adult hamster, tumor has appearance of leiomyosarcoma; c) same area with individual argyrophilic "sleeves" around cells; d) leiomyosarcoma of hamster which developed from cells of a culture of a tumor with the structure of an undifferentiated hemangiopericytoma injected into it; e) irregularly arranged longitudinal fibrils in tumor cell. Staining: in a, b, and d) hematoxylin-eosin; c) silver impregnation by Gomori's method; e) Heidenhain's iron hematoxylin.

plaque variant of SA7(C8) virus [1]. Similarity to leiomyosarcoma was also observed in tumors which developed in the soft tissues of the hamsters after inoculation with a culture of hamster embryonic cells transformed by SA7(C8) virus. In the more highly differentiated tumors, together with the characteristic cyto- and histotypical picture there were other signs to indicate that these tumors were of the smooth-muscle type: the cells were surrounded by individual argyrophilic "sleeves" (Fig. 1d), the staining properties of the cytoplasm of some cells were similar to those of the sarcoplasm (it stained red with azan and glycogen began to appear in it), while staining with hematoxylin phosphotungstate revealed numerous ill-defined fibrils. Although the tumors grew by infiltration, their growth was chiefly expansive in character.

The transplanted tumors, which were studied until the 20th passage, retained the structure of the primary tumors, but with each successive passage the features of anaplasia increased in degree.

Tumors induced by stains B105 and C626 in the soft tissues of hamsters were similar to tumors induced by strain C8 but differed from them in the somewhat higher degree of anaplasia and polymorphism of the cells and by the presence of many giant cells.

All tumors induced in the soft tissues of the hamsters by the simian adenoviruses were of the same type. Bovine adenovirus induced tumors of a different structure [7]. Meanwhile, among the tumors induced by this adenovirus, the chief group consisted of tumors histologically similar to those described above. They also contained areas resembling angioleiomyosarcomas (Fig. 2a). The leiomyosarcoma structure was particularly clearly seen in tumors after the first passage in adult hamsters (Fig. 2b, c). The picture of leiomyosarcoma was observed in tumors which developed from a line of tumor cells taken through more than 150 passages in vitro (Fig. 2d, e).

Despite the signs of increasing anaplasia and polymorphism of the tumor cells, the structure of the hemangiopericytoma persisted after transplantation until the 50th passage, but later the tumors could be defined only as undifferentiated.

Hull et al. [5] describe tumors induced in hamsters by various simian adenoviruses as undifferentiated with some characteristics of lymphomas, while Berman [2] describes them as small-cell sarcomas of embryonic type. Merkow et al. [8], who studied subcutaneous tumors in hamsters induced by simian adenovirus SV-30, consider that the tumors arise from the mesenchyme. It is concluded from the results of the present investigation that, despite the low level of differentiation of these tumors, they can be regarded as malignant hemangiopericytomas with a tendency toward differentiation into leiomyosarcoma. Stout [12] and others considered that the cells of the hemangiopericytoma, like the epithelioid cells of glomus tumors, are derivatives of pericytes. According to Zimmermann [14] and others (3, etc.), these cells are transitional to smooth-muscle cells, and this view is supported by electron-microscopic evidence [6, 9, 10]. The view expressed above that pericytes can be regarded as "targets" for the oncogenic action of adenoviruses used in this investigation could be confirmed the early formation of tumor nodules by proliferation of the epithelioid cells found in the capillaries were present after inoculation of SA7(C8) virus. However, no connection was found between the foci of proliferation and the blood vessels. This apparently contradicts the fact that the definitive tumors all have the same type of structure and are hemangiopericytomas. A possible explanation is that the targets are not only the pericytes, or indeed not pericytes at all, but some other (evidently cambial) cells which, as a result of integration of the virus genome into the cell genome, obtain a specific direction of differentiation toward the smooth-muscle cell. Results described in the literature indicate the leading role of the virus in the morphology of cloned cells transformed by it in vitro, just as in the tumors induced by viruses [13].

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