Extraocular orbital tumors induced by human adenovirus type 12 in hamsters

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Intravitreous inoculation of human adenovirus type 12 (Huie strain) in 59 newborn hamsters produced 12 extraocular orbital tumors 27 to 106 days after a single injection. Histopathologic studies suggest that these tumors are an undifferentiated malignant neoplasm that closely resembles a neuroepithelioma that can be induced in the central nervous system by the same virus. Although the neoplastic cells had different architectural patterns, the basic structure showed a uniform perivascular festooning, accompanied by the formation of pseudorosettes. Serial sectioning indicated that the ciliary ganglia and parasympathetic efferent fibers were missing in all 12 eyes. Hypertrophic nerve fibers embedded in tumor mass were occasionally seen. Electron microscopic observations suggest that these tumors are poorly differentiated malignant counterparts of an epithelial tumor with its nidus in neural primordia.

Key words: human adenovirus type 12, intraocular inoculation, neuronal primordia in retrobulbar adnexa, undifferentiated neurogenic tumor.

Since Trentin, Yabe, and Taylor1 discovered that human adenovirus type 12 induced a high incidence of malignant tumors in newborn hamsters, this unique oncogenic DNA virus has been used by a number of scientists to produce neoplasms.2-7, 19 This communication is the first report of virus-induced tumors arising from orbital soft tissues. The nidus of these tumors has not been determined, but their morphology suggests that they should be designated as an undifferentiated neurogenic neoplasm derived from the primordia in the retrobulbar space.

Materials and methods

Preparation of concentrated virus fluid. Human adenovirus type 12 (Huie strain, Flow Laboratories, Baltimore, Md.) was cultured in HeLa cells. Monolayer-cultures were prepared in 250 ml. plastic flasks (Falcon) with Eagle's basal medium supplemented by 15 per cent calf serum. Cultured monolayers of HeLa cells were infected with adenovirus fluid and incubated for 48 hours with Eagle's basal medium supplemented by 2 per cent calf serum. After the medium was decanted, those HeLa cells that yielded the adenovirus-specific cytopathic effect were carefully collected with a policeman (No. 8433). After three cycles of freeze-

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Inoculation of virus fluid. Pregnant hamsters (LVG:LAK strain) were supplied by the Lakeview Hamster Colony, N, J. A single intravitreal inoculation of 0.01 ml. virus fluid, 10^4.5 to 10^5 TCID_{50} HeLa cells per 0.1 ml. was made in the left eyes of 39 hamsters within 24 hours after birth. The injection was made under an operating microscope with a fine hypodermic needle (70M, Metropolitan Supply, Cambridge, Mass.) connected to a microsyringe (No. 710-N, Hamilton).

Twelve control animals were inoculated with supernatant fluid from nonvirus-infected HeLa cells cultured in the same medium as that used for the preparation of virus fluid. All animals were fed a balanced diet of pellets (General Biochemicals).

Immunofluorescence microscopy. Adenovirus-specific T antigen(s) was examined by the direct immunofluorescence microscopic procedure with cryosections of tumors and primary culture cells.

Light microscopy. The tumors were on the temporal side of the eye. There was no tumor cell infiltration in the posterior segment of the eyeball or the optic nerve. Serial sectioning indicated that the ciliary ganglia, parasympathetic efferent fibers, and long ciliary nerves were missing in all 12 eyes. In some cases, enormously hypertrophied nerve fibers and lemmoblastic cells were found within the tumor.

The major part of the tumor was densely packed with small hyperchromatic cells that often formed a uniform perivascular cell-wreath (Fig. 2, white arrow). A perivascular festooning pattern was occasionally seen around larger blood vessels. Rapidly degenerating tumor cells, interspersed with well-preserved cells around blood vessels gave the tumor sections a trabecular or papillary appearance. Tumor cells were mixed with plasma cells and polymorphonuclear leukocytes (Fig. 2, black short arrow) in the retrobulbar soft tissues. High power views of cells that possessed only a scanty cytoplasm consistently showed that the basic cytoarchitectural feature
Fig. 2. Large mass of retrobulbar tumor showing characteristic cell pattern. Numerous small blood vessels with nucleus-free cuffs give tumor its peculiar leopard skin appearance (white arrow). Marked plasma cell infiltration and leukocytes (black arrow) appear in remaining part of retrobulbar tissue. There is no neoplastic encroachment in optic nerve and retina. (Hematoxylin and eosin, ×40).
was perivascular festooning closely associated with pseudorosettes (Fig. 3). Mitotic figures were very common, and giant cells were found occasionally. Collagen or reticulin formation was meager. Silver stains outlined round or elongated ovoid nuclei and nucleoli; debris in the center of pseudorosettes stained intensely with silver (Fig. 4).

**Histochemical findings.** Only the scanty rim of tumor cell cytoplasm responded to NADH and NADPH tetrazolium oxidoreductases. ATPase was very active in the rim of cytoplasm and in the vascular walls; alkaline phosphatase was positive only in vascular channels. Acid phosphatase was sporadically positive in some degenerating tumor cells. Luxol fast blue stain was moderately positive in the cytoplasm. Lithium carbonate silver did not show differentiated neurofibrillar elements.

Thin sections (2 to 3 μm) from epon-embedded material, stained with toluidine blue, showed the cytologic characteristics of solid tumors. Round or ovoid nuclei usually had several basophilic nucleoli. The nuclear membrane occasionally appeared indented. Tumor cells fused, forming a cytoplasmic syncytium. Intracytoplasmic, basophilic, round inclusions were rather frequent, and mitotic figures were also common.

**Electron microscopy.** The tumor cells varied in size and shape and showed a slightly elongated, ovoid, or cone-shaped cell body with an ovoid nucleus that occupied a large portion of the cell. Each cell appeared to be connected to cytoplasmic processes (Fig. 5). Cytoplasmic organelles were scarce; they showed a few mitochondria and small vesicles. Free ribosomes were frequent, but granular endoplasmic reticulum was rare. Poorly developed Golgi's lamellae and vesicles were observed only occasionally. Irregularly shaped lipid droplets and osmiophilic heterogeneous granules were frequent. Bizarre inclusion bodies (Fig. 6, arrows), consisting of multiple vesicle-like particles, were occasionally found within the cytoplasm as well as in the interstices of the tumor cell syncytium.
Fig. 4. Characteristic argent affinity of tumor cell nuclei. Mitotic and degrading cells, as well as debris in the center of pseudorosettes show intense argent affinity. (Lithium carbonate silver, x250.)

Discussion

The results described are, we believe, the first report of neoplastic transformation of the retrobulbar space caused by adenovirus type 12. The remarkably uniform histopathologic characteristics of these tumors justify their designation as a counterpart of human embryonic neuroepitheliomas for which cytologic criteria are well established.\textsuperscript{9-11} An example of poorly differentiated neuroepithelioma invading the orbit is demonstrated by Hogan and Zimmerman.\textsuperscript{11} The formation of perivascular cell-wreaths and pseudorosettes\textsuperscript{7, 12, 19} is characteristic (Figs. 2, 3, and 4). Cytologically, the poorly differentiated tumor cells resemble an undifferentiated human neuroblastoma.\textsuperscript{12, 15} Furthermore, ultrastructural lesions such as osmiophilic heterogeneous corpuscles and inclusion bodies are suggestive of tumors derived from differentiating neural precursor cells of neural crest origin.\textsuperscript{14} The inclusion bodies described in this paper are frequently encountered in normally developing neuronal precursor cells in the retina of perinatal rodent embryos.

Although there is no direct evidence for asserting a neuroectodermal origin of this tumor, it is significant that the ciliary ganglia were absent in all 12 intraorbital tumors. Occasional appearance of hypertrophic nerve fibers within the neoplastic mass may also imply its neurogenic origin. Indirectly, this suggests that the nidus of the tumors is in neural precursor cells migrating toward the normal location of the ciliary ganglia at the time of inoculation. It seems reasonable to assume that the neuronal precursor populations that are migrating along the ciliary nerve from the brain would be particularly susceptible to adenovirus type 12.

Green and co-workers\textsuperscript{15} suggested that the incorporation and transcription of at least part of the viral genome are obligatory steps in adenovirus oncogenesis. Strohl, Babson, and Rouse\textsuperscript{16} have also stressed that the importance of target cell
determinants in the morphology of tumors produced by DNA viruses may be ordained by their viral genome. However, it is uncertain whether or not the phenotype of the remarkably uniform adenovirus tumor morphology is determined by its viral genome, no matter how different cells in various tissues are involved as a target in adenovirus tumorigenesis. It is puzzling that Strohl, Rabson, and Rouse produced highly uniform adenovirus-typical tumors by using a clonal line of the fibroblastic BHK 21 cells as a target for virus infection. If we insist on a monistic theory to explain the oncogenesis, the tumor they obtained from a purely clonal line of fibroblastic cells should be designated as a sarcoma. However, tissue culture explants of neural retinas tested by Albert, Rabson, and Dalton produced tumors closely resembling human retinoblastomas without rosettes following abortive adenovirus infection. Only a pluralistic theory of adenovirus oncogenesis could explain that the sensory retinas used for tissue culture might be transformed into a sarcoma after an abortive adenovirus infection.

In the present study, three tumors developed in the liver, probably as a result of insinuation of the intraocularly injected virus fluid into the bloodstream. It has been previously documented that intravenous inoculation of adenovirus 12 in newborn hamsters results in tumors in the liver of 100 per cent of the cases. The morphology of these tumors was almost...
identical to that encountered in the retrobulbar tumors. There is no evidence that these hepatic tumors are neurogenic in origin. Nevertheless, ubiquitously distributed neural precursor cells in developing newborn animals may be selectively susceptible to this unique DNA virus, as has been demonstrated in the central nervous system.3 •• ••

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