Acta Medica Okayama

Volume 28, *Issue* 1 1974 *Article* 5

FEBRUARY 1974

Brain tumors induced in rats by human adenovirus type 12

Tsuyoshi Murao[*] Hiroyuki Ohmori[†] Hiroshi Sonobe[‡]

Keisuke Matsuo[**] Akira Tsutsumi[††] Katsuo Ogawa[‡‡]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,

Brain tumors induced in rats by human adenovirus type 12*

Tsuyoshi Murao, Hiroyuki Ohmori, Hiroshi Sonobe, Keisuke Matsuo, Akira Tsutsumi, and Katsuo Ogawa

Abstract

Oncogenesis of human adenovirus type 12 in the brain of rats was examined. Newborn rats of Sprague-Dawley and Donryu strains were injected intracranially with human adenovirus type 12. The incidence of intracranial tumors was 91% (30/33) in SpragueDawley and 56% (14/25) in Donryu rats. Except for one tumor nodule located in the parietal cortex of a Sprague.Dawley rat, all tumors developed in the paraventricular areas or in the meninges. Tumors were quite similar histologically to those induced in hamsters and mice resembling the undifferentiated human brain tumors such as medulloblastoma, ependymoblastoma and embryonic gliomas. From the histological features and primary sites of tumor development, it is suggested that the tumors in the brain of rats induced by adenovirus type 12 originate from the embryonic cells in the paraventricular area and also from the undifferentiated supporting cells of the peripheral nerves in the leptomeninges.

---

*PMID: 4275715 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **28**, 47—58 (1974)

BRAIN TUMORS INDUCED IN RATS BY HUMAN ADENOVIRUS TYPE 12

Tsuyoshi MURAO, Hiroyuki OHMORI, Hiroshi SONOBE, Keisuke MATSUO, Akira TSUTSUMI, Katsuo OGAWA

Department of Pathology, Okayama University Medical School, Okayama, Japan (Director: Prof. K. Ogawa)

Received for publication, July 10, 1973

Abstract: Oncogenesis of human adenovirus type 12 in the brain of rats was examined. Newborn rats of Sprague-Dawley and Donryu strains were injected intracranially with human adenovirus type 12. The incidence of intracranial tumors was 91% (30/33) in Sprague-Dawley and 56% (14/25) in Donryu rats. Except for one tumor nodule located in the parietal cortex of a Sprague-Dawley rat, all tumors developed in the paraventricular areas or in the meninges. Tumors were quite similar histologically to those induced in hamsters and mice resembling the undifferentiated human brain tumors such as medulloblastoma, ependymoblastoma and embryonic gliomas. From the histological features and primary sites of tumor development, it is suggested that the tumors in the brain of rats induced by adenovirus type 12 originate from the embryonic cells in the paraventricular area and also from the undifferentiated supporting cells of the peripheral nerves in the leptomeninges.

Efforts to produce neurogenic tumors in experimental animals with chemical carcinogens have been made in mice (1, 2, 3, 4, 5, 6, 7) and also in rats (8, 9, 10, 11, 12, 13). Polyoma virus (14), simian vacuolating virus (15), human adenovirus type 12 (16, 17), Rous sarcoma virus (18), Moloney sarcoma virus (19, 20) and bovine adenovirus type 3 (21, 22) produce neurogenic or intracranial mesenchymal tumors in animals. Of these oncogenic viruses, adenovirus type 12 (AV12) has a high oncogenecity and induces undifferentiated tumors in experimental animals after a short latent period as first reported by TRENTIN, YABE and TAYLOR (23). OGAWA and his associates investigated the oncogenesis of AV12 in hamsters and mice, and attained the conclusion that AV12-induced tumors originate from the subependymal immature cells (16) and the undiffentiated peripheral nerve supporting cells (17, 24, 25, 26, 27). Although AV12 induces intraperitoneal tumors in rats (28), as far as is known, no oncogenic effects in the brains of

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, and a part of which was also supported by The Tokyo Club.

rats have been reported. The following communication is the histomorphological feature and the growth behavior of the intracranial tumors produced in rats by AV12.

MATERIALS AND METHODS

Virus: Human adenovirus type 12, Huie strain, supplied by the courtesy of Prof. Y. YABE of the Institute of Cancer Research, Okayama University Medical School, was propagated in HeLa cells. Details of the method for virus propagation and titration were reported in a previous paper (26).

Animals: Sprague-Dawley and Donryu strain rats, obtained commercially and bred in our laboratory, were used in this experiment.

Virus inoculation: A total of 74 newborn rats, 46 Sprague-Dawley and 28 Donryu, were injected intracerebrally with 0.03 ml of AV12 titering $10^{8.0}$ $TCID_{50}$/0.1 ml in HeLa cells.

Histomorphological examination: The animals manifesting neurological symptoms, such as ataxia, exophthalmus, hyperexcitability, lethargy, etc., were killed under ether anesthesia and a complete necropsy was performed. Brains were fixed in 10% formalin, embedded in paraffin and sectioned serially in the sagittal or the fronal plane. The sections were stained routinely with hematoxylin-eosin and sometimes also with phosphotungstic acid hematoxylin (PTAH), Mallory's azan method, Pap's silver impregnation, Kluver Barrera, and Bodian's nerve fiber stain. Animals dying within twenty days after virus inoculation were discarded, and those surviving more than 270 days after the treatment were sacrificed and sections of the brains were prepared as mentioned above.

Detection of T-antigen: The direct immunofluorescence technique was employed. Fluorescein isothiocyanate-conjugated-globulin was prepared from the tumor-bearing hamster serum and absorbed two times with 100 mg/ml of acetone dried rat brain powder. Details of the method for preparation of the anti-T conjugate were reported previously (26). Three days after virus inoculation, cryostat sections of the brains were prepared from 5 Sprague-Dawley rats, fixed in cooled acetone, and stained with the conjugate. Intracranial tumors induced in 5 Sprague-Dawley and 2 Donryu rats were also examined for the presence of fluorescent T-antigen. The specificity of the fluorescence was examined by the blocking test with the tumor-bearing hamster serum.

RESULTS

Latent period, incidence and distribution of tumors

Thirty-three Sprague-Dawley and 25 Donryu rats survived over twenty days after virus inoculation. In Sprague-Dawley rats, the latent period between virus inoulation and appearance of central nervous symptoms ranged from 55 to 195 days, the average being 120 days. While in Donryu rats, it was from 80 to 191 days, the average being 132 days (Table 1). In all the

TABL 1 TIME OF APPEARANCE OF NEUROLOGICAL SYMPTOMS IN RATS INOCULATED INTRACRANIALLY WITH HUMAN ADENOVIRUS TYPE 12

Days after AV12 inoculation		51 ~ 60	61 ~ 90	91 ~ 120	121 ~ 150	151 ~ 180	181 ~ 210	210 ~ 270
No. of rats manifesting neurological symptoms	Sprague-Dawley strain	1	8	8	6	4	3	0
	Donryu strain	0	1	3	7	2	1	0

rats manifesting neurological disorders, intracranial tumors were found grossly or microscopically. The incidence of tumors was 91% (30/33) in Sprague-Dawley and 56% (14/25) in Donryu rats, showing a definitely higher rate in Sprague-Dawley strain (Table 2). At necropsy, the tumors were very

TABLE 2 INCIDENCE OF INTRACRANIAL TUMORS IN RATS INOCULATED INTRACRANIALLY WITH HUMAN ADENOVIRUS TYPE 12

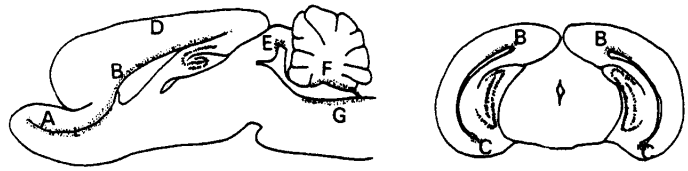
Strain	Effective no. of animals*	Incidence of intracranial tumor (%)
Sprague-Dawley	33	30 (91)
	♀ 18	♀ 17 (94)
	♂ 15	♂ 13 (87)
Donryu	25	14 (56)
	♀ 12	♀ 7 (58)
	♂ 13	♂ 7 (54)

* Effective no. of animals exclude 8 Sprague-Dawley and 3 Donryu rats which died within 20 days after virus inoculation.

soft, greyish-white, and moderately demarcated from the surrounding tissues. The cut surfaces of large tumors showed necrotic and/or hemorrhagic foci in the central area. Seven of 44 tumor-bearing rats developed occlusive hydrocephalus. The changes were confined to the central nervous system and no evidence of tumor was found macroscopically in other organs.

Primary sites of tumor development were shown in Text-fig. 1 and Table 3. In 19 out of 44 tumor-bearing rats, tumors appeared to be multicentric in origin. Of special interest was some connection between the multiplicity and the size of tumors. Although in a few rats only one small tumor nodule was present, each tumorous mass in the case of multiple growths was usually smaller in size than solitary ones. In the case of single

Text-fig. 1. Schematic representation of predilection sites for tumor development in the rat brain.



A: Wall of the olfactory ventricle. B: Wall of the anterior horn and roof of the lateral ventricle. C: Ventral wall of the inferior horn of the lateral ventricle. D: Cortex of the parietal lobe. E: Wall of the recess of the posterior colliculus. F: Roof of the fourth ventricle. G: Floor of the fourth ventricle.

TABLE 3 DISTRIBUTION OF AV12-INDUCED TUMORS IN THE BRAIN OF RATS

	No. of tumor-bearing rats	Sites of tumors							
		A	B	C	D	E	F	G	H
Sprague-Dawley strain	30	10(3)	26(8)	2(1)	1(1)	2(0)	3(1)	5(1)	4(2)
Donryu strain	14	5(2)	9(3)	0	0	0	3(1)	4(2)	0

a) Alphabets (A~G) refer to corresponding alphabets in Text-fig. 1.
b) H indicates meninges.
c) Numerals mean the total number of tumor-bearing rats.
d) Parentheses enclose the number of rats developing a single tumor in the brain.

large tumors, it was difficult to decide whether such a tumor was of unicentric origin or had been formed by fusion of the true multiple tumors. In one of the Sprague-Dawley rats, multiple tumors showed three different histologic features (Figs. 5, 6, 7). Except for a tumor nodule located in the

Fig. 1. Parasagittal section of the brain of a Sprague-Dawley rat killed 87 days after AV12 inoculation. A pedunculated mass of tumor protruding into ventricular lumen. H-E stain. × about 6.

Fig. 2. Parasagittal section of the brain of a Sprague-Dawley rat killed 69 days after AV12 inoculation. A small tumor produced in parietal cortex. H-E stain. × 100.

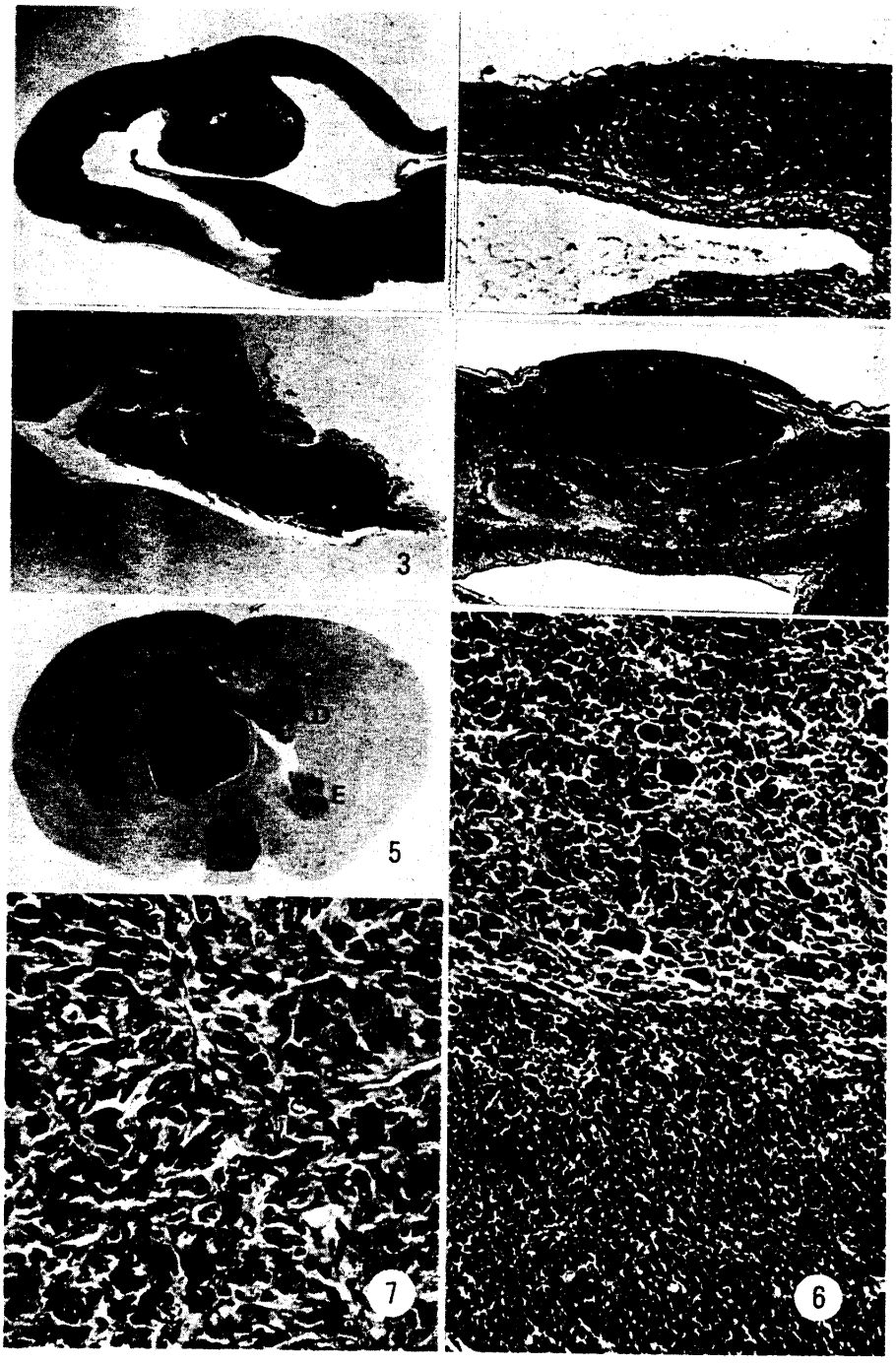
Fig. 3. Parasagittal section of the brain of a Donryu rat killed 104 days after AV12 inoculation. A large mass of tumor occupying the fourth ventricle. H-E stain. × about 10.

Fig. 4. Parasagittal section of the brain of a Sprague-Dawley rat killed 84 days after AV12 inoculation. Tumor occupying subarachnoid space. H-E stain. × 40.

Fig. 5. Coronal section of the brain of a Sprague-Dawley rat killed 113 days after AV12 inoculation. Five tumor nodules are shown. A, D and E nodules have the identical histological appearance. H-E stain. × about 6.

Fig. 6. Higher magnification of A and B nodules shown in Fig. 5. The A nodule (upper part) has the histological characteristics of glioblastoma multiforme, while the B nodule (lower part) shows the histological features of medulloblastoma. H-E stain. × 150.

Fig. 7. Higher magnification of C nodule shown in Fig. 5. Tumor has the histological resembrance to spongioblastoma polare. H-E stain. × 345.


3
5
7
6

parietal cortex of one Sprague-Dawley rat (Fig. 2), all tumors were situated in the paraventricular area or leptomeninges. Although small tumors arising from ventricular wall were covered by a layer of ependyma, large ones had occassionally invaded the choroid plexuses and filled the ventricular cavities. Some of them were pedunculated (Fig. 1). All the tumors located in the roof of the fourth ventricle were so large that it was impossible to decide from which region they had originated. In such cases, the tumor might have originated in the medullary velum or the vermis of cerebellum (Fig. 3). Of 4 tumors involving the meninges, two were located in the subarachnoid tissue with compression of cerebral cortex (Fig. 4).

Histomorphological observations

Tumors both of Sprague-Dawley and Donryu rats showed the identical histologial features. Although many different shapes and arrangements of cells were seen in most of the tumors, the predominant feature tended to be one of the following histological characteristics.

(1) The predominant cell was round and had a round nucleus with a heavily stained nuclear membrane enclosing fine chromatin granules (Figs. 6, 8). The cytoplasm was scant and most of the cells formed no protoplasmic process. However, a few of them had short protoplasmic processes and round nuclei with a single prominent nucleolus (Fig. 9). The cytoplasm was stained light blue with Kluver-Barrera method and no Nissl bodies were noted. A few multinucleated giant cells and numerous mitotic figures were seen. The cells were closely packed and showed no characteristic arrangement. A PTAH stain disclosed no intercellular fibrils. With Bodian method no argyrophilic fibers were noted in the tumor cells. The sections stained with Mallory's azan method and Pap's silver impregnation revealed a small

Fig. 8. Section of tumor arising in a Donryu rat at the roof of fourth ventricle. Tumor has no characteristic architecture and most of the tumor cells have a small amount of cytoplasm and a round nucleus with a heavily stained nuclear membrane. G: Granular layer of cerebellum. H-E stain. × 345.

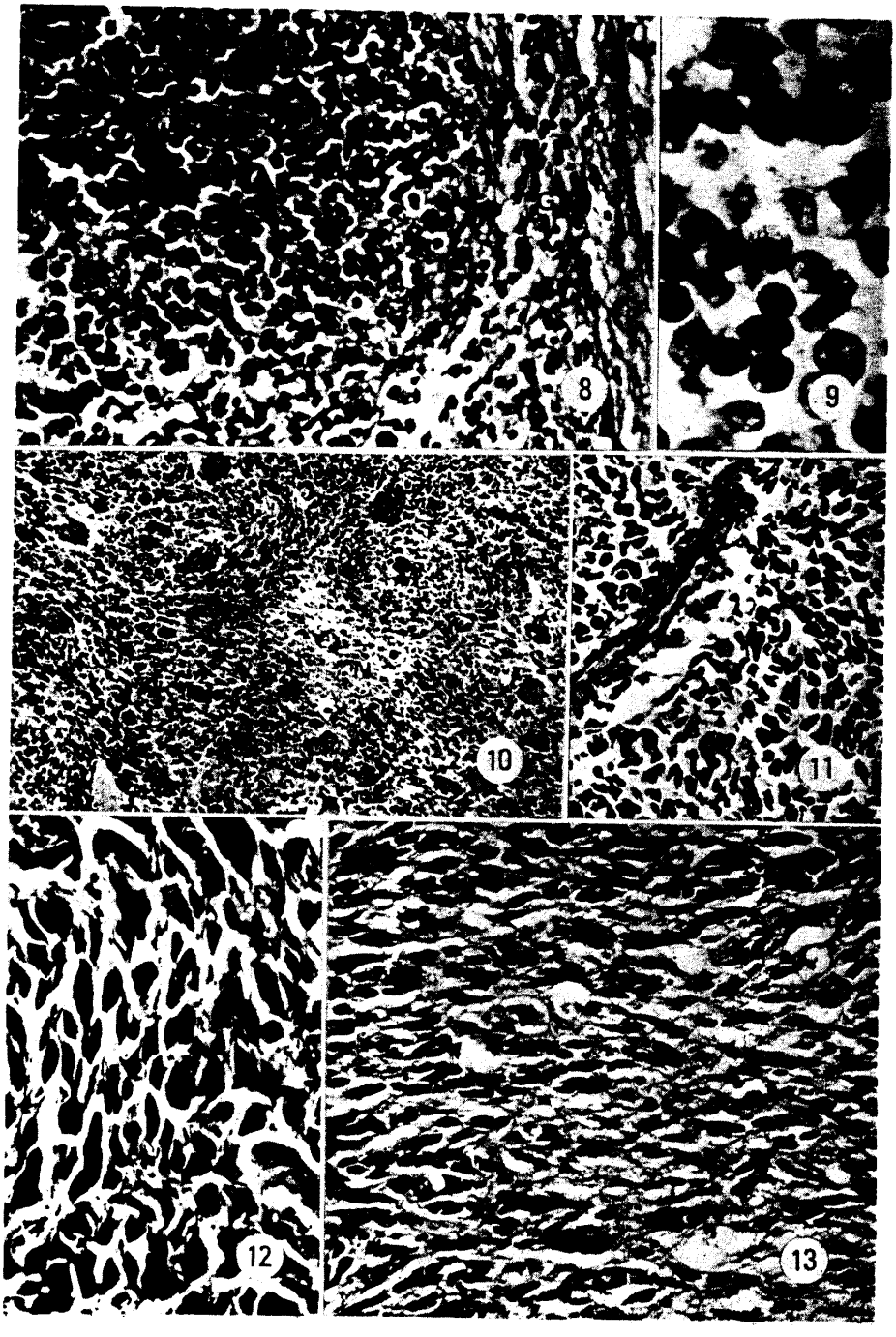
Fig. 9. Higher magnification of Fig. 8 specimen. A few tumor cells have a round nucleus with a large nucleolus. H-E stain. × 1000.

Fig. 10. Section of tumor arising in a Donryu rat at the paraventricular area of the left lateral ventricle. Tumor cells are pleomorphic, and arranged in pseudopalisades around focus of necrosis (N). H-E stain. × 150.

Fig. 11. Pap's silver impregnation of the same tumor in Fig. 10. Reticulin fibers are restricted around a capillary. × 400.

Fig. 12. Section of tumor arising in a Donryu rat at the paraventricular area of the left lateral ventricle. Fine protoplasmic processes are shown. PTAH stain. × 1000.

Fig. 13. Section of tumor arising in a Donryu rat at the paraventricular area of the left lateral ventricle. Tumor cells are mainly spindle in shape and have fine protoplasmic processes. Some of them show the features of immature astrocytes. H-E stain. × 345.


8
9
10
11
12
13

amount of collagen and reticulin in the region of the blood vessels, respectively. The tumor of this type resembled human medulloblastoma.

(2) The tumor was mainly composed of tadpole shaped cells attaching to the connective tissue around the small blood vessels with tapering protoplasmic processes, forming pseudorosettes (Fig. 14). With PTAH stain the tumor cells showed blue-colored fibrillary processes being oriented to the perivascular connective tissue. However, neither true rosettes nor blephaloplasts were noted. Multinucleated giant cells were occasioally seen. The histological findings of the tumor corresponded to those of human ependymoblastoma.

(3) The tumor was composed of cells of various size and shape ; polyhedral, spindle or carrot-shaped with round, oval or elongated nuclei. The cytoplasm formed usually one or more fibrillary processes. Throughout the tumor there were many small foci of hemorrhage and necrosis (Fig. 10). Around the foci of necrosis the tumor cells were arranged in palisades. Mitotic figures and bizarre multinucleated giant cells were numerous (Figs. 6, 10). The tumor tissue was interspersed with dilated and capillary-like blood vessels with a single layer of endothelial cells. There was no proliferation of endothelial cells. A small number of collagenic and reticular fibers were demonstrated in the stroma around blood vessels from which they extended outward for a short distance between the tumor cells (Fig. 11). The infiltration of lymphocytes and plasma cells was occasionally noted. Although there was no endothelial hyperplasia of the blood vessels, these histologic features corresponded to embryonic glioma with some resemblance to human glioblastoma multiforme.

(4) The tumor was mainly composed of spindle-shaped cells with elongated nuclei and bipolar protoplasmic processes (Figs. 7, 13), forming interlacing bands. A few of protoplasmic processes were stained blue with PTAH (Fig. 12). The stroma was scant, and with Mallory's azan stain no collagenic fibers were demonstrated between the tumor cells. A few giant cells were

Fig. 14. Ependymoblastoma in the olfactory bulb of a Sprague-Dawley rat. H-E stain. × 400.

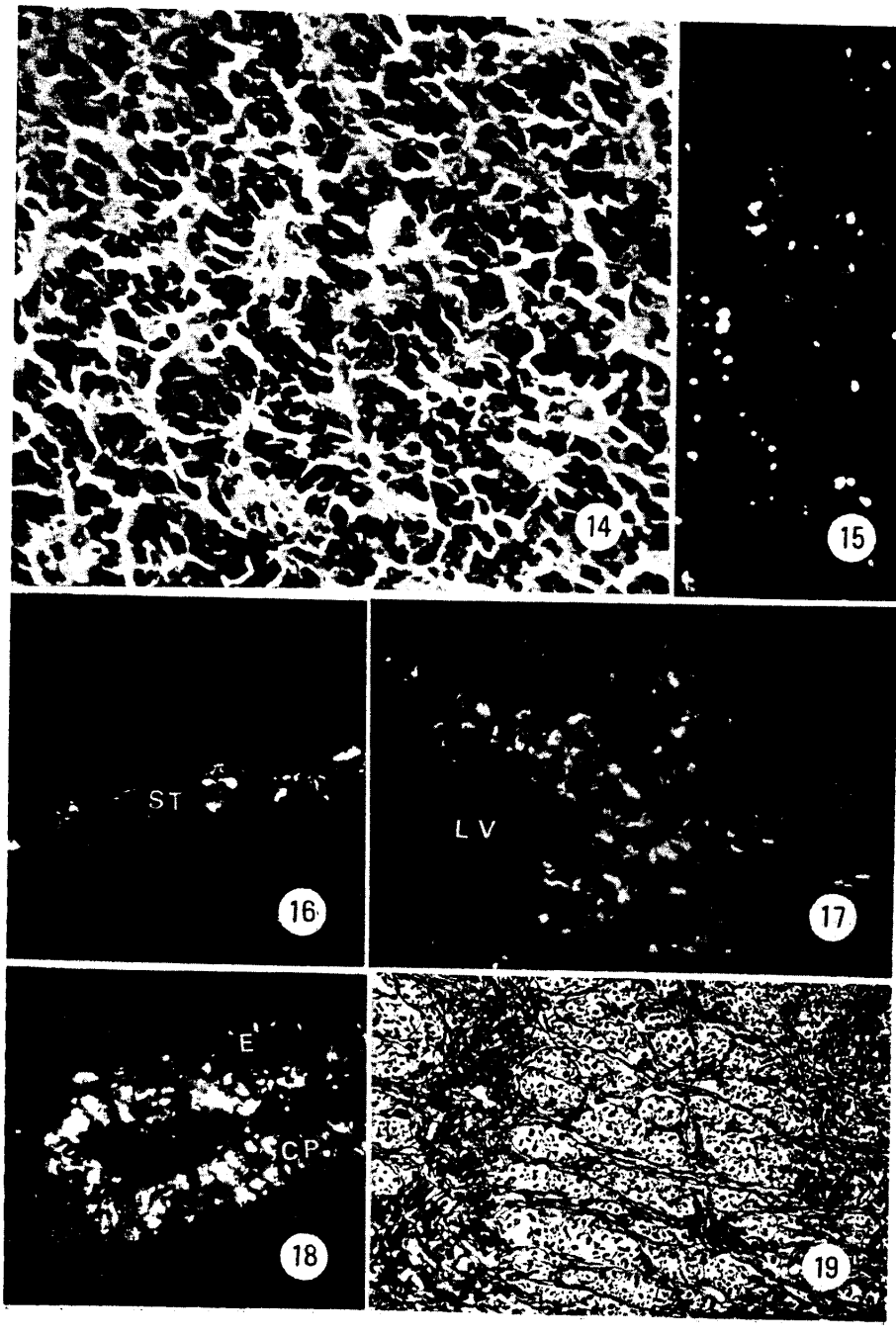
Fig. 15. Fluorescent T-antigens in the tumor arising in a Sprague-Dawley rat. Stained with anti-T conjugate. × 200.

Fig. 16. Cells with fluorescent T-antigens in the subarachnoid tissue (ST), stained on the 3rd day after AV12 inoculation. × 200.

Fig. 17. Cells with fluorescent T-antigens in the paraventricular area of lateral ventricle (LV), stained on the 3rd day after AV12 inoculation. × 200.

Fig. 18. Fluorescent T-antigens in choroid plexus epithelium (CP) and ependyma (E), stained on the 3rd day after AV12 inoculation. × 200.

Fig. 19. Coronal section of medulla oblongata of a Donryu rat. Tumor cells occupying the space around and also within the nerve bundles. Bodian stain. × 250.


14
15
ST
16
LV
17
E
CP
18
19

noted. The tumor had histological resemblance to human spongioblastoma polare.

In most of the tumors 4 types of the histological characteristics mentioned above were mixed, although a few tumors exhibited one histologic type. There was no close relationship between the site of tumor and its histological type. However, the amount of stroma and the inflammatory reaction was influenced by the site of invasion of the tumor. There were numerous original and newly formed blood vessels, hemorrhage, and leukocytes infiltration in the tumors invading the choroid plexuses. In all cases, however, no endothelial hyperplasia was noted. The tumors growing in the meninges were histologically quite similar to those in the brain.

The growth of the tumor was infiltrative as well as expansive. Most of the large tumors showed "secondary structures" described by SCHERER (29), such as perivascular, perifascicular (Fig. 19), intrafascicular and subependymal growth. The tumor cells were found disseminated by the cerebrospinal fluid to the ventricular wall, choroid plexuses and leptomeninges. However, it was impossible to find tumor cells within the lumen of the blood vessels, and no metastatic foci were observed in the remote parts of the body.

Fluorecsent microscopic examination

On the third day after AV12 inoculation, fluorescent cells were observed in the subararachnoid tissue (Fig. 16), paraventricular area (Fig. 17) and epithelium of the choroid plexus (Fig. 18). In all 5 rats tested, no cells with fluorescent T-antigens were found in other areas. All the 7 intracranial tumors tested showed the presence of fluorescent T-antigens. Most of the T-antigens were present in the tumor cells as fluorescent rods and granules (Fig. 15). There was no relationship between the shape of fluorescent T-antigens and the histological feature of tumors.

DISCUSSION

Results of the present experiment indicate that AV12 produces intracranial tumors of rats in a high incidence, and that the histogenesis of these intracranial tumors of rats may be quite similar to that previously described in hamsters and mice (17, 26). Although most of the carcinogen-induced tumors exhibit the histologic characteristics of differentiated gliomas, AV12-induced tumors are composed of immature cells with a poor resemblance to the differentiated glial or nerve cells. From this fact, the origin of the AV12-induced tumor was misinterpreted as mesenchymal cells (16, 30, 31). However, MUKAI *et al.* (32) investigated the intraperitoneal tumors induced by AV12 and supported the theory of neuroectodermal origin proposed by OGAWA *et al.* (24).

In the present experiment, the cells with fluorescent T-antigen were distributed in the paraventricular area, choroid epithelium and subarachnoid tissue. Except for choroid epithelium, these areas corresponded to the primary sites of tumor development. In the brain of rats within 24 hr after birth, many embryonic cells remain in the subependymal area. Similarly, undifferentiated peripheral nerve supporting cells might be distributed in the meninges. From these reasons as well as from histological features of the tumors, it is evident that AV12 causes the neoplastic transformation of undifferentiated neuroectodermal cells located in the subependymal area and menings. As for a small tumor nodule produced in the parietal cortex of a Sprague-Dawley rat, it may be assumed that a few number of embryonic calls can migrate from subependymal area to cortex in an undifferntiated state. Although the question as to the kinds of embryonic cells constituting the normal matrix zone, has not been clearly elucidated, we can draw a conclusion that AV12-induced tumors in the brain of rats might originate from the matrix cell with a tendency to glial differentiation.

REFERENCES

1. SELIGMAN, A.M. and SHEAR, M.J. : Studies in carcinognesis. VIII. Experimental production of brain tumors in mice with methylcholanthrene. *Am. J. Cancer* **37**, 364-395, 1939
2. PEERS, J.H.: The response of the central nervous system to the application of carcinogenic hydrocarbons. I. Dibenzanthracene. *Am. J. Path.* **15**, 261-272, 1939
3. PEERS, J.H.: The response of the central nervous system to the application of carcinogenic hydrocarbons. II. Methylcholanthrene. *Am. J. Path.* **16** 799-816, 1940
4. ZIMMERMAN, H.M. and ARNOLD, H.: Experimental brain tumors. I. Tumors produced with methylcholanthrene. *Cancer Res.* **1**, 919-938, 1941
5. ZIMMERMAN, H.M. and ARNOLD, H.: Experimental brain tumors. II. Tumors produced with benzpyrene. *Am. J. Path.* **19**, 939-955, 1943
6. ZIMMERMAN, H.M. and ARNOLD, H.: Experimental brain tumors. III. Tumors produced with dibenzanthracene. *Cancer Res.* **3**, 682-685, 1943
7. KAWAI, S. *et al.*: Experimental studies on brain tumors in the mouse. *Tr. Soc. Path. Jap.* **48**. 1150-1151, 1959 (in Japanese)
8. SWEET, W.H. and BAILEY, P.: Experimental production of intracranial tumors in the white rat. *Arch. Neurol. & Psychiat.* **45**, 1047-1049, 1941
9. WEIL, A.: Experimental spinal cord tumors. Exhibition at the *Amer. Neurol. Assoc.*, Chicago, U.S.A., 1942
10. RUSSEL, W.O.: The response of the central nervous system of the rat to methylcholanthrene. *Cancer Res.* **5**, 140-156, 1954
11. NAKAMURA, Y.: Histopathological studies on experimental brain tumor. *Med. J. Osaka Univ.* **6**, 919-946, 1956
12. ITO, T. and IKUTA, F.: The nature of gliomas, with special reference to the experimental brain tumor of rats. *Shinkei Shinpo* **5**, 118-153, 1961 (summary in English)
13. DRUCKREY, H. *et al.*: Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an Bd-ratten. *Z. Krebsforsch.* **69**, 103-201, 1967
14. RABSON, A.S. and KIRSCHSTEIN, R.L.: Intracranial sarcomas produced by polyoma virus in Syrian hamsters. *Arch. Path.* **69**, 663-671, 1960

15. KIRSCHSTEIN, R. L. and GERBER, P.: Ependymomas produced after intracerebral inoculation of SV 40 into new-born hamsters. *Nature* **195**, 299-300, 1962
16. HUEBNER, R. J. *et al.*: Oncogenic effects in hamsters of human adenovirus types 12 and 18. *Proc. Natl. Acad. Sci.* **48**, 2051-2058, 1962
17. OGAWA, K. *et al.*: Tumor induction by adenovirus type 12 and its target cells in the central nervous system. *Gann* **60**, 383-392, 1969
18. RABOTTI, G. F. and RAINE, W. A.: Brain tumors induced in hamsters inoculated intracerebrally at birth with Rous sarcoma virus. *Nature* **204**, 898-899, 1964
19. RIBACCHI, R. and GIRALDO, G.: Intracranial tumours in rats injected intracranially with murine sarcoma virus (MSV), Moloney's strain. Preliminary report. *Lav. Anat. Pat. Perugia* **26**, 141-155, 1966
20. IDA, N. *et al.*: Intracranial tumors in rats and mice produced by MSV-CS-Moloney substrain. *Proc. Jap. Cancer Ass.*, p97, 1970 (in Japanese)
21. LEVENBOOK, I. S. and STRIZHACHENKO, N. M.: Morphology of tumors induced in hamster soft tissues by bovine adenovirus type 3. *Int. J. Cancer* **8**, 531-540, 1971
22. MOTOI, M. and OGAWA, K.: Oncogenesis of bovine adenovirus type 3 in the brain of hamsters. *Tr. Soc. Path. Jap.* **61**, 113, 1972 (in Japanese)
23. TRENTIN, J. J., YABE, Y. and TAYLOR, G.: The quest for human cancer viruses. *Science* **137**, 835-841, 1962
24. OGAWA, K. *et al.*: Histogenesis of malignant neoplasia induced by adenovirus type 12. *Gann* **57**, 43-52, 1966
25. OHMORI, M.: Electron microscopic studies on the tumor induced by adenovirus type 12. *Acta Med. Okayama* **19**, 199-208, 1965
26. MURAO, T.: Induction of intracranial tumors in mice by human adenovirus type 12. I. Immunofluorescent studies on T antigen and the predilection sites for tumor development in the brain. *Acta Path. Japonica* **22**, 45-51, 1972
27. MURAO, T.: Induction of intracranial tumors in mice by human adenovirus type 12. II. Enhancement by N, N'-dimethylnitrosourea. *Acta Med. Okayama* **25**, 261-268, 1971
28. HUEBNER, R. J. *et al.*: Specific adenovirus complement-fixing antigens in virus-free hamster and rat tumors. *Proc. Natl. Acad. Sci.* **50**, 379-389, 1963
29. SCHERER, H. J.: Structural development in gliomas. *Am. J. Cancer* **34**, 333-351, 1938
30. PEREIRA, M. S. *et al.*: Infection with adenovirus type 12. *Lancet* **1**, 198-199, 1964
31. CHINO, F. *et al.*: Pathological studies on the oncogenesis of adenovirus type 12 in hamsters. *Japan. J. Med. Sci. Biol.* **20**, 483-500, 1967
32. MUKAI, N. and KOBAYASHI, S.: Undifferentiated intraperitoneal tumors induced by human adenovirus type 12 in hamsters. *Am. J. Path.* **69**, 331-340, 1972