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Abstract

The oncogenicity of xenotropic pseudotype Kirsten murine sarcoma virus (MSV) was investigated in Sprague-Dawley rats. When fetal or newborn rats were inoculated intracerebrally with xenotropic pseudotype MSV, brain tumors developed after about one month. Tumors were induced both in the cerebrum and the cerebellum. Histologically, the tumors were predominantly glioblastoma multiforme and hemangioendotheliomas. In cerebellar lesions, malignant transformation of vascular endothelial cells, polycystic areas and numerous giant cells were noted. Proliferation of Purkinje cells was also observed in some of the cerebellar tumors. Inoculation of the same virus by other routes, such as s.c., i.p. and i.m., also caused cerebral and cerebellar tumors. Brain tumors thus induced were transplantable subcutaneously into suckling rats.

KEYWORDS: rat, brain tumor, pseudotype MSV, sarcoma virus

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INDUCTION OF RAT BRAIN TUMOR WITH XENOTROPIC PSEUDOTYPE MURINE SARCOMA VIRUS

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Abstract. The oncogenicity of xenotropic pseudotype Kirsten murine sarcoma virus (MSV) was investigated in Sprague-Dawley rats. When fetal or newborn rats were inoculated intracerebrally with xenotropic pseudotype MSV, brain tumors developed after about one month. Tumors were induced both in the cerebrum and the cerebellum. Histologically, the tumors were predominantly glioblastoma multiforme and hemangioendotheliomas. In cerebellar lesions, malignant transformation of vascular endothelial cells, polycystic areas and numerous giant cells were noted. Proliferation of Purkinje cells was also observed in some of the cerebellar tumors. Inoculation of the same virus by other routes, such as s.c., i.p. and i.m., also caused cerebral and cerebellar tumors. Brain tumors thus induced were transplantable subcutaneously into suckling rats.

Key words: rat, brain tumor, pseudotype MSV, sarcoma virus.

Mouse cells contain genetic information of endogenous type-C RNA viruses (1-5). It was recently demonstrated that a pure strain of BALB/c mice had three different sets of information containing codes for type-C RNA virus polypeptides (6). Both BALB virus-1 and BALB virus-2 could be induced from BALB/c mouse cells upon treating them with halogenated pyrimidines, such as iododeoxyuridine (7). BALB virus-1 replicates in cultured cells of NIH Swiss mouse origin. It was shown that BALB virus-2 was not infective to mouse cells, but rather replicated in cells of other animal species (xenotropism) (8, 9). BALB virus-3 was different immunologically from virus-1 and virus-2 and was similar to the endogenous type-C virus isolated from NZB and NIH Swiss mice (10).

Kirsten murine sarcoma virus (Ki-MSV) transformed both BALB/3T3 and NRK cells to non-producer (NP) cell lines, such as K-BALB or K-NRK (11). It was also demonstrated that Ki-MSV were rescued from these NP cells with type-C helper viruses, such as Rauscher MuLV, Kirsten MuLV, BALB virus-1 and BALB virus-2, resulting in the production of pseudotype Ki-MSV. Aaronson and Dunn (12) demonstrated that NRK cells were sensitive not only to BALB virus-2, but also to MSV rescued from NP cells (K-BALB) with BALB virus-2.

From these results it was presumed that rat cells were highly sensitive, not

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only *in vitro* but also *in vivo*, to xenotropic pseudotype MSV, Ki-MSV (BV2), and that tumors could be induced with this virus under certain conditions.

When Ki-MSV (BV2) was inoculated intracerebrally into newborn or fetal Sprague-Dawley rats, brain tumors frequently developed not only in the cerebrum but also in the cerebellum. Brain tumors also developed in the rats inoculated extracerebrally, *i.e.*, subcutaneously, intraperitoneally or intramuscularly, with the same virus at birth.

MATERIALS AND METHODS

Animals. The Sprague-Dawley (SD) rats used were obtained from the Central Laboratory of Experimental Animals, Tokyo.

Viruses and tissue culture. Xenotropic pseudotype Kirsten MSV, Ki-MSV (BV2), in cultured NRK cells was used in this experiment. Virus-infected cells were sent from NIH (Bethesda, Md, U.S.A.) by the courtesy of Dr. Aaronson and Dr. Hino. Eagle's medium with 10 % fetal calf serum was used in most of the cultures. Titration of the xenotropic pseudotype MSV was performed on mink cells cultured in RPMI 1640 medium with 10 % fetal calf serum or NRK cells in Eagle's medium with 10 % fetal calf serum. The titer of Ki-MSV (BV2) was $10^{3.3}$ FFU on mink cells and $10^{4.9}$ FFU on NRK cells.

Inoculation of the virus. Newborn and fetal SD rats were inoculated intracerebrally with 0.05 ml and 0.01 ml of Ki-MSV (BV2), respectively. For fetal inoculations, pregnant rats in the 17th to 21st day of gestation were anesthetized with nembutal (20 mg/kg body weight), and a laparotomy was conducted. Exposed fetuses were inoculated intracerebrally with the virus through the uterine wall. Besides cerebral inoculations, newborn rats were inoculated with the same virus, subcutaneously (0.1 ml), intraperitoneally (0.2 ml) or intramuscularly (0.2 ml), and fetuses subcutaneously (0.03 ml) or intraperitoneally (0.03 ml).

Transplantation of tumor tissues. Small pieces of brain tumors were transplanted subcutaneously in the back of newborn rats, using a trocar. Subcutaneous tumors that developed were examined histopathologically.

Autopsy. Organs obtained by autopsy were fixed in a buffered 10 % formalin solution and embedded in paraffin. The sections were stained with hematoxylin-eosin, Mallory PTAH, PAS, and silver stain (Gomori's modified method).

Electron-microscopy. Tissues were fixed in a Millönig buffered glutaraldehyde solution, dehydrated with a series of ethanol, exchanged with QY-1 (n-Buthyl-glycidyl ether) and embedded in Epon-resin. Ultrathin sections were made with a Porter-Blum MT-2 ultra-microtome followed by double staining with uranyl acetate and lead, and were examined under an H-U-500 electron-microscope.

RESULTS

Intracerebral inoculation of Ki-MSV (BV2) into newborn SD rats. As shown in Table 1, among 38 animals, brain tumors were found in 37 (97.4 %) in the cerebrum and in 17 (44.7 %) in the cerebellum. The animals bearing brain tumors showed various symptoms such as enlargement of the skull, lethargy, irritability, static ataxia and rotary motion. In addition to these symptoms, moderate enlargement of the spleen and liver, hemorrhages in various organs such as the lungs, intestinal

tract and lymph nodes were observed. The average latent period of development of brain tumors was 37 days, ranging from 24 to 56 days. Although the viral materials were inoculated into the right hemisphere of the cerebrum, brain tumors were detected not only in both hemispheres of the cerebrum but also in the cerebellum.

TABLE 1. INTRACEREBRAL INOCULATION OF Ki-MSV(BV2) INTO NEWBORN SD-RATS

Ki-MSV (BV2)/NRK culture passage	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlarge- ment of spleen (%)	Enlarge- ment of liver (%)	ALP (day)
13 th	7	6	3	2	1	52
15 th	5	5	5	2	0	26
15 th	2	2	0	0	0	56
30 th	7	7	4	2	1	24
30 th	6	6	5	1	0	25
60 th	8	8	0	0	0	45
60 th	3	3	0	0	0	36
13-60 th	38	37 (97.4)	17 (44.7)	7 (18.4)	2 (5.3)	37

Intracerebral inoculation of Ki-MSV(BV2) into fetuses of SD rats. As shown in Table 2, when the fetuses of SD rats were inoculated intracerebrally with Ki-MSV (BV2), brain tumors were found in the cerebrum in 26 (76.5 %) and in the cerebellum in 31 (91.2 %) among 34 animals, with an average latent period of 24 days. When Ki-MSV (BV2) was diluted 10 times, brain tumors were induced in 8 (61.5 %) out of 13 animals both in the cerebrum and the cerebellum, after an average of 26 days.

TABLE 2. INTRACEREBRAL INOCULATION OF Ki-MSV(BV2) INTO FETUSES OF SD-RATS

Ki-MSV (BV2)/NRK culture passage	Day of gesta- tion	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlarge- ment of spleen (%)	Enlarge- ment of liver (%)	ALP (day)
13 th	18	5	3	5	4	4	28
14 th	18	4	3	4	3	3	21
15 th	21	3	1	1	0	0	17
15 th	18	10	10	9	3	4	26
19 th	18	2	2	2	2	2	18
30 th	19	10	7	10	3	3	24
13-30 th	18-21	34	26 (76.5)	31 (91.2)	15 (44.1)	16 (47.1)	24
20 th*	19	13	8 (61.5)	8 (61.5)	5 (38.5)	4 (30.8)	26

* Inoculum diluted ten times.

Pathological findings of brain tumors. Brain tumors were located in both cerebral hemispheres, frontal lobe, intermediate zone of brain parenchyma, subependymal region and periventricular areas in the cerebrum. Tumors were also found in the fourth ventricle adjacent to the cerebellum and pons, and in the middle and lateral lobes of the cerebellum. The tumors were reddish brown, yellow or yellowish-green (Figs. 1-3).

Microscopic findings. Cerebral tumors were well circumscribed and had necrotic areas and hemorrhages. The lesions showed high cellularity and revealed an area of pseudopalisading with central necrosis and tapering cells (Figs. 4, 5). Proliferation of the perivascular endothelial cells (Fig. 6) and highly anaplastic areas with multinucleated giant cells (Fig. 7) suggested a high grade of malignancy. In other areas, high cellularity, pleomorphism of the cells and vascular obstruction were noted. In addition to these findings, rounded cells of the same size were observed in tumorous lesions, and spindle-shaped cells in mitosis were noted in the corpus callosum. The palisades, pseudo-rosettes, vascularity, pleomorphism, mitotic activity and numerous multinucleated giant cells together are suggestive of glioblastoma multiforme.

Cerebellar tumors were observed in 45 % of the animals inoculated at birth and in 91 % of those inoculated during gestation. Hemorrhages, vascularity, proliferation of vascular endothelial cells (Fig. 8), small or medium sized polycystic areas, and multinucleated giant cells (Fig. 9) were observed in these cerebellar tumors. We occasionally observed proliferation of Purkinje cells (Fig. 10) which infiltrated the granular cell layer of the cerebellum.

Subcutaneous transplantation of Ki-MSV(BV2)-induced brain tumors. Minced tissues of Ki-MSV(BV2)-induced rat brain tumors were successfully transplanted subcutaneously into all of 14 newborn rats, as shown in Table 3.

TABLE 3. SUBCUTANEOUS TRANSPLANTATION OF Ki-MSV(BV2)-INDUCED BRAIN TUMOR INTO SD-RATS

Brain tumor used for transplan- tation	Number of animals	Trans- planted tumor "take" (%)	Cerebral tumor (%)	Cerebellar tumor (%)	Enlarge- ment of spleen & liver (%)	ALP (day)
R122377 (6)	6	6	5	5	5	24
R032778 (2)	4	4	0	0	0	8
R032778 (1)	4	4	3	3	4	17
	14	14	8	8	9	17
		(100)	(57.1)	(57.1)	(64.3)	

Tissue culture of Ki-MSV(BV2)-induced brain tumor. Six culture lines from Ki-MSV(BV2)-induced cerebral tumors were obtained. One of these cultures had typical astrocytic cells with numerous processes one month after initiation of the culture (Fig. 11). Supernatants of Ki-MSV(BV2)-induced rat brain tumors did

not transform either NIH/3T3 or BALB/3T3 cells, but transformed NRK cells. The virus found in the culture fluid of the Ki-MSV(BV2)-induced rat brain tumors showed xenotropic characteristics. Numerous C-type viral particles were seen on the surface of the cultured cells (Fig. 12).

Subcutaneous inoculation of Ki-MSV(BV2) into newborn SD rats. When newborn Sprague-Dawley rats were inoculated with Ki-MSV(BV2) subcutaneously, enlargement of the spleen (8/21, 38.1 %) and liver (9/21, 42.9 %), moderate enlargement of the lymph nodes and subcutaneous hemorrhages (9/21, 42.9 %) were detected as shown in Table 4. Cerebral (10/21, 47.6 %) and cerebellar (10/21, 47.6 %) tumors were noted after subcutaneous inoculation of the virus within 18 to 30 days, an average of 22 days.

TABLE 4. SUBCUTANEOUS INOCULATION OF Ki-MSV(BV2) INTO NEWBORN SD-RATS

Ki-MSV (BV2)/NRK culture passage	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlarge- ment of spleen (%)	Enlarge- ment of liver (%)	Hemorrhage in skin (%)	ALP (day)
15 th	6	1	0	4	0	1	18
18 th	6	5	5	3	4	3	30
19 th	9	4	5	1	5	5	20
15-19 th	21	10 (47.6)	10 (47.6)	8 (38.1)	9 (42.9)	9 (42.9)	22

Subcutaneous inoculation of Ki-MSV(BV2) into fetuses of SD rats. When fetuses of SD rats were inoculated subcutaneously with Ki-MSV(BV2), hemorrhages in the subcutaneous tissues (20/39, 51.3 %) and enlargement of the spleen (34/39, 87.2 %) and liver (29/39, 74.4 %) were observed. In addition to this, cerebral tumors (27/39, 69.2 %) and cerebellar tumors (30/39, 76.9 %) were noticed after an average of 24 days as shown in Table 5.

TABLE 5. SUBCUTANEOUS INOCULATION OF Ki-MSV(BV2) INTO FETUSES OF SD-RATS

Ki-MSV (BV2)/NRK culture passage	Day of gesta- tion	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlarge- ment of spleen (%)	Enlarge- ment of liver (%)	Hemor- rhage in skin (%)	ALP (day)
14 th	17	2	0	1	1	1	2	13
15 th	18	3	3	2	3	3	3	13
15 th	20	12	9	10	11	10	10	20
15 th	19	7	7	6	7	6	0	25
15 th	19	9	6	7	6	3	2	33
30 th	18	6	2	4	6	6	3	25
14-30 th		39	27 (69.2)	30 (76.9)	34 (87.2)	29 (74.4)	20 (51.3)	24

Intraperitoneal inoculation of Ki-MSV(BV2) into newborn and fetal SD rats. When newborn SD rats were inoculated intraperitoneally with Ki-MSV(BV2), cerebral and cerebellar tumors developed in 21.1 % (4/19) and 63.2 % (12/19) of the rats, respectively. These rats had enlarged spleens in 15.8 % (3/19), hemorrhagic fluids in the thoracic cavity and hemorrhages in the intestinal tract after an average of 15 days, as shown in Table 6.

When virus solutions were diluted ten times and inoculated into fetuses as described previously, cerebral tumors in developed 28.6 % (4/14) of the animals and cerebellar tumors in 21.4 % (3/14), with enlargement of the spleen in 21.4 % (3/14) and of the liver in 7.1 % (1/14), after an average of 34 days.

TABLE 6. INTRAPERITONEAL INOCULATION OF Ki-MSV(BV2) INTO NEWBORN OR FETAL SD-RATS

Recipients	Ki-MSV (BV2)/NRK culture passage	Day of gestation	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen (%)	Enlargement of liver (%)	Hemorrhage in skin (%)	ALP (day)
Newborns	19 th		9	2	8	3	0	4	15
			10	2	4	0	0	1	15
			19	4 (21.1)	12 (63.2)	3 (15.8)	0 (0)	5 (26.3)	15
Fetuses	20 th*	20	6	2	1	2	1	1	26
	20 th	19	4	1	0	0	0	0	53
	20 th	17	4	1	2	1	0	0	28
	20 th		14	4 (28.6)	3 (21.4)	3 (21.4)	1 (7.1)	1 (7.1)	34

* Inoculum diluted ten times.

Intramuscular inoculation of Ki-MSV(BV2) into newborn SD rats. When Ki-MSV (BV2) was inoculated intramuscularly into newborn SD rats, thigh tumors developed in 88.2 % (15/17) at the site of inoculation after an average of 76 days. Cerebral and cerebellar tumors (5.9 %, 1/17) and an enlargement of the spleen (58.8 %, 10/17) and liver (23.5 %, 4/17) were also observed as shown in Table 7.

TABLE 7. INTRAMUSCULAR INOCULATION OF Ki-MSV(BV2) INTO NEWBORN SD-RATS

Ki-MSV (BV2)/NRK culture passage	Number of animals	Thigh tumor (%)	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen (%)	Enlargement of liver (%)	ALP (day)
25 th	7	5	0	0	2	3	32
30 th	10	10	1	1	8	1	106
25-30 th	17	15 (88.2)	1 (5.9)	1 (5.9)	10 (58.8)	4 (23.5)	76

DISCUSSION

Gliomas, meningiomas, hemangiomas, hemangioendotheliomas have been found in Moloney MSV-induced rat brain tumors (13, 14). These brain tumors were mainly composed of large round cells suggestive of gliomas (15-17). Similar findings were observed in Rous sarcoma virus-induced mouse brain tumors (15, 16).

We investigated the induction of rat brain tumors with xenotropic pseudotype Ki-MSV. As was reported by Aaronson (12), NRK cells showed sensitivity of xenotropic pseudotype MSV. It was presumed that rat cells were sensitive to these viruses '*in vivo*' and tumors would be induced in rats under the appropriate conditions.

Yung and his associates (18) reported that 'gliomas' were induced in Wistar/Furth rats on the twenty-sixth postnatal day when they were inoculated intracerebrally with Ki-MSV at 10 days of age.

As was reported in this paper, brain tumors were induced at a high incidence when xenotropic pseudotype Ki-MSV was inoculated intracerebrally into either newborn or fetal SD rats. Histologically the brain tumors thus induced were glioblastoma multiforme with proliferation of perivascular endothelial cells, multinucleated giant cells, necrotic areas and pseudopalisades.

Although we detected cerebellar tumors in rats, tumors were rarely found in the cerebellum of rats inoculated with ecotropic Moloney MSV (17).

When brain tumors induced with Ki-MSV (BV2) were transplanted subcutaneously into SD rats, tumorous changes were detected in the brain, in addition to tumor development at the site of transplantation. It was presumed that the virus produced from the transplanted subcutaneous tumor induced *de novo* tumorous lesions in the brain.

Kirsten and his coworkers (19) reported that intraperitoneal inoculation of Kirsten mouse erythroblastosis virus (MEV) into newborn Wistar/Furth rats caused malignant lymphomas, erythroblastosis, multiple sarcomas, and polyostotic osteolytic lesions, associated with cerebral or cerebellar petechial hemorrhages. The findings that Kirsten observed may be related to the results which we obtained by inoculation of xenotropic pseudotype Kirsten MSV, Ki-MSV (BV2). It seems to be of interest that Ki-MSV (BV2) readily induced cerebellar tumors in rats.

The occasional observation of the proliferation of Purkinje cells in rats inoculated intracerebrally with xenotropic pseudotype Kirsten MSV was particularly interesting. These cells infiltrated the granular layer and appeared to cluster on the loose stroma in which few granular cells were seen. In some cases, partial or complete disappearance of the granular layer was observed. These findings bring to mind "dysplastic gangliocytoma of the cerebellum" in humans (20), also referred to as gangliomatosis of the cerebellum (21), diffuse hypertrophy of the cerebellum cortex (22), Purkinjeoma or Lehrmitte-Duclos disease (23). Further study must be done to clarify the possible interaction of Purkinje cells with Ki-

MSV(BV2).

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REFERENCES

1. Aaronson, S.A., Hartley, J.W. and Todaro, G.J.: Mouse leukemia virus: "Spontaneous" release by mouse embryo cells after long-term in vitro cultivation. *Proc. Natl. Acad. Sci. USA.* **64**, 87-94, 1969.
2. Huebner, R.J., Kelloff, G.J., Sarma, P.S., Lane, W.T. and Turner, H.C.: Group-specific antigen expression during embryogenesis of the genome of the C-type RNA tumor virus: Implications for ontogenesis and oncogenesis. *Proc. Natl. Acad. Sci. USA.* **67**, 366-376, 1970.
3. Lowy, D.R., Rowe, W.P., Teich, N.M. and Hartley, J.W.: Murine leukemia virus: High-frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Science* **174**, 155-156, 1971.
4. Gelb, L.D., Milstien, J.B., Martin, M.A. and Aaronson, S.A.: Characterization of murine leukemia virus-specific DNA present in normal mouse cells. *Nature New Biol.* **244**, 76-79, 1973.
5. Chattopadhyay, S.K., Lowy, D.R., Teich, N.M., Levine, A.S. and Rowe, W.P.: Evidence that the AKR murine-leukemia-virus genome is complete in DNA of the high-virus AKR mouse and incomplete in the DNA of the "virus-negative" NIH mouse. *Proc. Natl. Acad. Sci. USA.* **71**, 161-171, 1974.
6. Stephenson, J.R., Reynolds, R.K., Tronick, S.R. and Aaronson, S.A.: Distribution of three classes of endogenous Type-C RNA viruses among inbred strains of mice. *Virology.* **67**, 404-414, 1975.
7. Stephenson, J.R. and Aaronson, S.A.: A genetic locus for inducibility of C-type virus in BALB/c cells: the effect of a nonlinked regulatory gene on detection of virus after chemical activation. *Proc. Natl. Acad. Sci. USA.* **69**, 2798-2801, 1972.
8. Aaronson, S.A. and Stephenson, J.R.: Independent segregation of loci for activation of biologically distinguishable RNA C-type viruses in mouse cells. *Proc. Natl. Acad. Sci. USA.* **70**, 2055-2058, 1973.
9. Stephenson, J.R. and Aaronson, S.A.: Demonstration of a genetic factor influencing spontaneous release of a xenotropic virus of mouse cells. *Proc. Natl. Acad. Sci. USA.* **71**, 4925-4929, 1974.
10. Stephenson, J.R., Tronick, S.R. and Aaronson, S.A.: Isolation from BALB/c mouse cells of a structural polypeptide of a third endogenous type-C virus. *Cell* **3**, 347-353, 1974.
11. Aaronson, S.A. and Weaver, C.A.: Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. *J. Gen. Virol.* **13**, 245-252, 1971.
12. Aaronson, S.A. and Dunn, C.Y.: Endogenous C-type viruses of BALB/c cells: Frequencies of spontaneous and chemical induction. *J. Virol.* **13**, 181-185, 1974.
13. Ribacchi, R. and Giraldo, G.: Intracranial tumors in rats injected intracerebrally with murine sarcoma virus (MSV), Moloney's strain. Preliminary report. *Lav. 1st. Anat. Istol. Pathol.*

- Perugia* **26**, 141-148, 1966.
14. Ida, N., Ikawa, Y., Ogawa, K., Takada, M. and Sugano, H.: Cell culture from a rat brain tumor induced by intracerebral inoculation with murine sarcoma virus. *J. Natl. Cancer Inst.* **53**, 431-447, 1974.
 15. Kumanishi, T., Ikuta, F. and Yamamoto, T.: Brain tumors induced by Rous sarcoma virus, Schmidt-Ruppin strain. III. Morphology of brain tumors induced in adult mice. *J. Natl. Cancer Inst.* **50**, 95-109, 1973.
 16. Ikuta, F. and Kumanishi, T.: Experimental virus-induced brain tumors. In *Progress in Neuropathology* 2, ed. H.M. Zimmerman, Grune & Stratton, Inc. New York and London, pp. 253-334, 1973.
 17. Ida, N., Ikawa, Y., Ogawa, K., Takada, M. and Yokogi, E.: Intracranial tumors in rats and mice produced by MSV-CS-Moloney substrain. *Proc. Jpn. Cancer Assoc. 29th Ann. Meeting*, p. 97, 1970.
 18. Yung, W.K., Blank, N.K. and Vick, N.A.: 'Glioblastoma': Induction of a reproducible autochthonous tumor in rats with murine sarcoma virus. *Neurology* **26**, 76-83, 1976.
 19. Kirsten, W.H. and Mayer, L.A.: Morphologic responses to a murine erythroblastosis virus. *J. Natl. Cancer Inst* **39**, 311-335, 1967.
 20. Rubinstein, L.J.: Atlas of tumor pathology. Tumors of the central nervous system. Armed Forces Institute of Pathology, Washington, D.C. 1972.
 21. Russell, D.S. and Rubinstein, L.J.: Ganglioglioma: A case with a long history and malignant evolution. *J. Neuropathol. Exp. Neurol.* **21**, 185-193, 1962.
 22. Oppenheimer, D.R.: A benign "tumor" of the cerebellum. Report on two cases of diffuse hypertrophy of the cerebellar cortex with a review of nine previously reported cases. *J. Neurol. Neurosurg. Psychiat.* **18**, 199-213, 1955.
 23. Ambler, M., Pogacar, S. and Sidman, R.: Lhermitte-Duclos disease (granule cell hypertrophy of the cerebellum). Pathological analysis of the first familial cases. *J. Neuropathol. Exp. Neurol.* **28**, 622-647, 1969.

LEGENDS FOR FIGURES

Fig. 1. *Ki-MSV(BV2)-induced rat brain tumor* (ID No. R020778-1) Both cerebral and cerebellar tumors developed in SD rats 25 days after newborn rats were inoculated intracerebrally with xenotropic pseudotype Ki-MSV. Diffuse hemorrhages are noted in the whole cerebellum and sporadic petechial lesions are observed in the cerebral hemispheres.

Fig. 2. *Ki-MSV(BV2)-induced rat brain tumor* (ID No. R020778-1) An area of the pons is involved on the left side. A cerebropontine angle tumor, which proved to be a glioblastoma, is also seen.

Fig. 3. *Ki-MSV(BV2)-induced rat brain tumor* (ID No. R122377-1) A sagittal section through the brain stem and cerebellum. Brain tumors are located in the cerebral hemispheres, frontal lobe, intermediate zone of the brain parenchyma, subependymal region and periventricular areas in the cerebrum.

Fig. 4. *Ki-MSV(BV2)-induced rat brain tumor* (cerebrum, $\times 100$, H & E stain, ID No. R122777-3) This highly cellular glial tumor shows pseudopalisades in the middle of the photomicrograph. Two multinucleated giant cells are seen in the upper portion of the pseudopalisades.

Fig. 5. *Ki-MSV(BV2)-induced rat brain tumor* (cerebrum, $\times 400$, H & E stain, ID No. R121977) A higher magnification of the pseudopalisades. Anaplastic cells of astrocytic lineage tend to cluster around the central areas of necrosis, resulting in the classic glioblastoma multiforme appearance. Numerous tapering cells are seen.

Fig. 6. *Ki-MSV(BV2)-induced rat brain tumor* (cerebellum, $\times 100$, H & E stain, ID No. R062978-7) Vascular endothelial proliferation is prominent, and hyperchromatic endothelial cells form new capillary blood vessels, which are both features of the vascular patterns of glioblastoma multiforme.

Fig. 7. *The Ki-MSV(BV2)-induced rat brain tumor* (cerebrum, $\times 100$ H & E stain, ID No. R020678-1) Numerous giant cells in the microcellular area in a glioblastoma are observed.

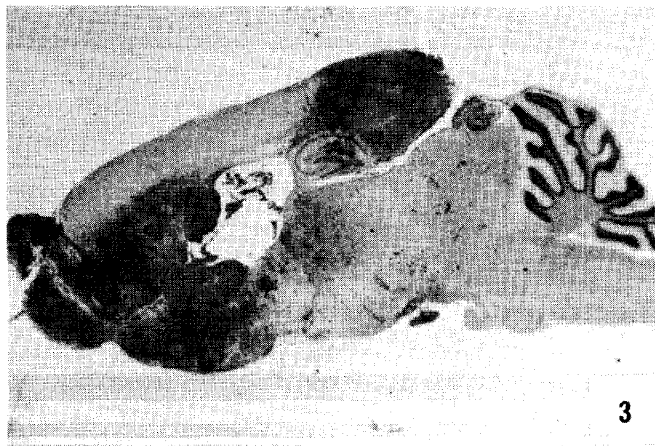
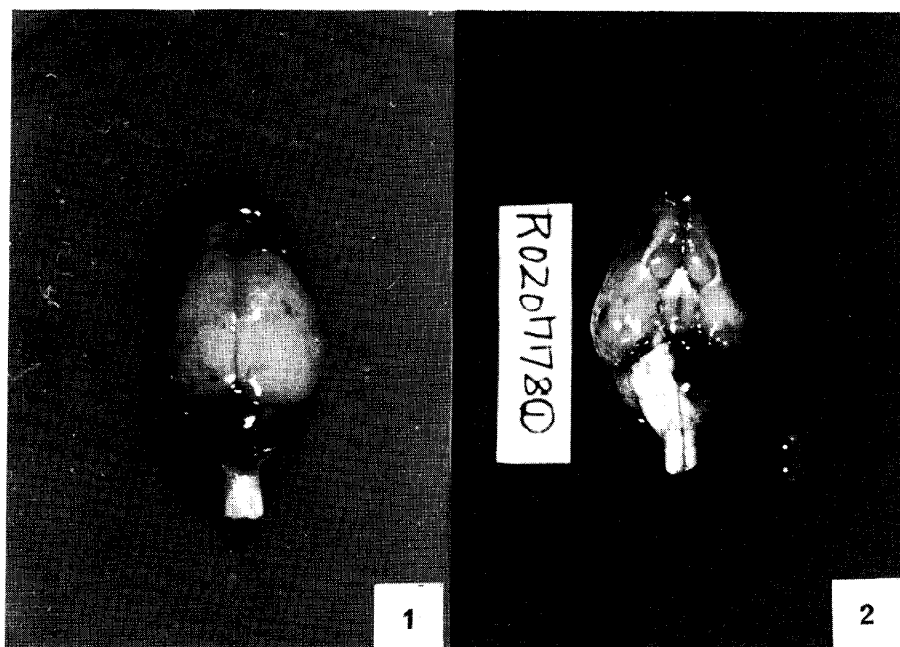
Fig. 8. *Ki-MSV(BV2)-induced rat brain tumor* (cerebellum, $\times 400$, H & E stain, ID No. R041078-1) Vascular endothelial proliferation is present.

Fig. 9. *Ki-MSV(BV2)-induced rat brain tumor* (cerebellum, $\times 400$, H & E stain, ID No. R041078-1) Numerous anaplastic and irregular giant cells are noted.

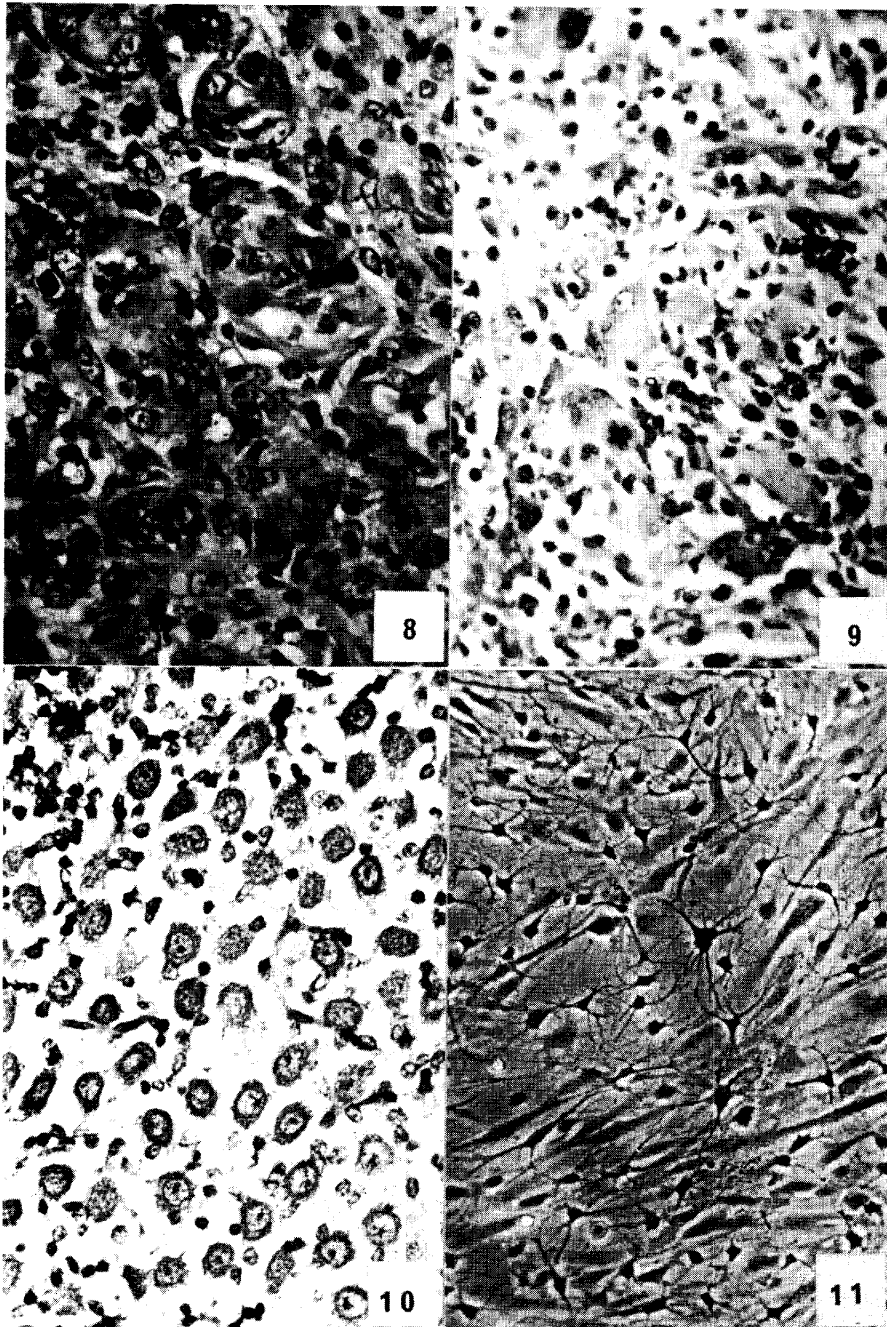
Fig. 10. *Ki-MSV(BV2)-induced rat brain tumor* (cerebellum, $\times 400$, H & E stain, ID No. R062978-7) A cluster of Purkinje cells replaces the granular layer.

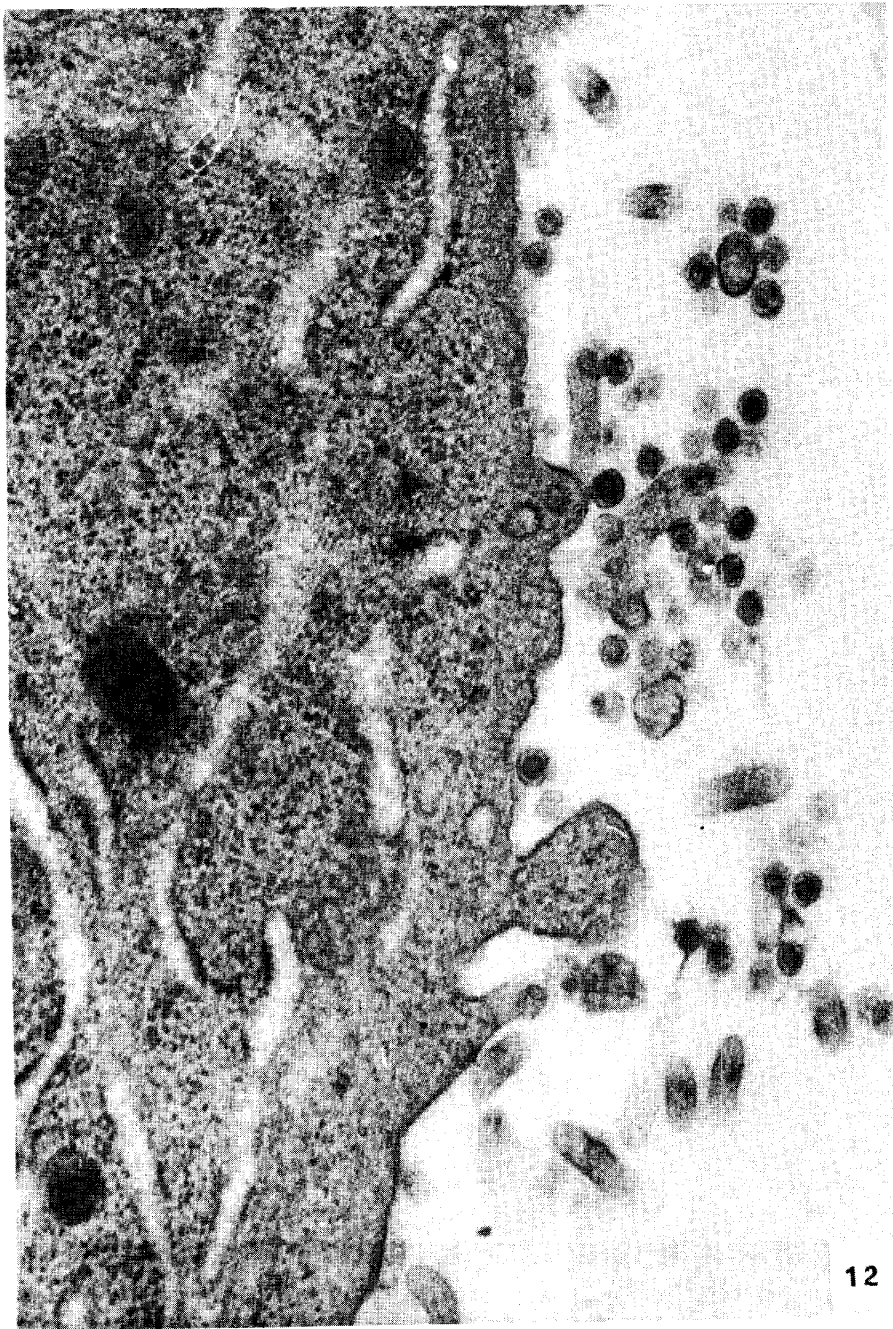
Fig. 11. *Cultured cells from a Ki-MSV(BV2)-induced rat brain tumor* (Phase microscope, ID No. R-122777-2) Many astrocytic cells with numerous thin processes are present. The supernatants of the culture fluids contained viruses.

Fig. 12. *Electron-microscopic findings of the cultured cells of a Ki-MSV(BV2)-induced rat brain tumor* ($\times 36000$) Numerous C-type viral particles are seen on the surface of the cultured cells.









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