Case Report

Spontaneous Glioblastoma Multiforme in an AKR/J Sea Mouse

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Abstract : A spontaneous brain tumor was found in a 46-week-old male AKR/J Sea mouse. The tumor occupied the forebrain, and invaded the nasal mucosa of nose along the nerve. The tumor cells showed a wide variety of sizes and shapes ; round, polygonal, oval, and fusiform with eosinophilic cytoplasm. Bizarre giant cells with multiple nuclei, foci of necrosis and hemorrhage, and mitotic figures were common findings. According to these findings, the tumor was diagnosed as glioblastoma multiforme, a highly malignant astrocytic neoplasm. The tumor cells reacted negatively against the antibody for glial fibrillary acidic protein (GFAP), and had no glial filaments under ultrastructural examination. It is presumed that those immunocytochemical and ultrastructural findings show poor differentiation in this case. (*J Toxicol Pathol* 1999 ; 12 : 151~153)

Key words : Glioblastoma multiforme, Glial fibrillary acidic protein (GFAP), Mouse.

Spontaneous tumors in the central nervous system (CNS) have been reported in many animal species, especially in dogs¹. In mice, however, these tumors are rare² except for VM and BRVR strains which have a high incidence of astrocytomas^{3,4}. Experimentally, CNS tumors are induced by several procedures, for example intracerebral injection of chemicals using polycyclic hydrocarbons as methylcholanthrene⁵ and certain viral agent such as avian sarcoma virus⁶, murine sarcoma virus⁷, and human adenovirus 12⁸ in mice. Recently, we encountered a spontaneous tumor in the brain of an AKR mouse. This paper describes the histopathological, immunohistochemical and ultrastructural features of the tumor.

A 46-week-old male AKR/J Sea mouse (Clea Japan, Inc., Tokyo), which was used in the non-treated group of a toxicity study, was necropsied following manifestation of marked weakness with decrease in locomotor activity, and an increase in respiration rate. At necropsy, a soft mass was observed from the cerebral frontal part to the olfactory bulb. The lesion was whitish-gray with an ill-defined border. Rough surface of the cranial bone facing the olfactory bulb was also observed. All organs and tissues were fixed in 10% neutral buffered formalin, processed by conventional method, embedded in paraffin, cut into 4 μ m-thick sections, and stained with hematoxylin and eosin (H.E.). The nasal tissue was decalcified with 10% formic acid formalin for ten days before processing, and examined at level I, II, and III according to the method of Nagano⁹. For immunohistochemical examination, the paraffin-embedded sections of the brain and the nasal cavity were stained for glial fibrillary acid protein (GFAP), S-100 protein, vimentin, and lysozyme by the labeled streptavidin biotin (LSAB) method. The source and

working dilution of these antibodies are shown in **Table 1**. A part of formalin-fixed tumor tissue in the brain was processed for ultrastructural examination.

Histopathologically, the tumor had high cellularity and occupied the cerebral frontal part and the olfactory bulb. The tumor cells showed a wide variety of sizes and shapes, which were round, polygonal, oval, and fusiform with eosinophilic cytoplasm (Fig. 1a, b). Most of them had pleomorphic and hyperchromatic nuclei with conspicuous nucleoli. Bizarre giant cells with irregular-shaped multiple nuclei were conspicuous in the tumor. The cellular borders were indistinct and mitotic figures were common. Necrosis, hemorrhage, and small foci of aggregated neutrophils were observed in some area of the tumor. The tumor cells had invaded the cranial bone and submucosa in the posterior nasal cavity, and were present in the bundle of nerve fiber beneath the olfactory epithelium at level II (Fig. 2). No tumor cells were observed in the other organs or tissue. Immunohistochemically, the tumor cells stained positively for vimentin (Fig. 3). Less than 10% of them reacted positively for S-100 protein. For GFAP and lysozyme, they reacted negatively (**Table 1**). Some histiocytic giant cells around necrosis and neutrophils reacted positively for lysozyme. Ultrastructurally, the tumor cells had rugged nuclei with large nucleoli. Numerous mitochondria and rough endoplasmic reticulum were observed, but glial filaments were not (Fig. 4).

In this case, the tumor occupied the forebrain and invaded the nasal mucosa along the nerve. No neoplastic growth was observed in the other organs or tissues. The findings suggest that this tumor originated from the brain. The CNS tumors in mice are classified according to the criteria used in human neoplasm as classification for mice has not yet been established, although it is unclear whether rodent brain tumors have a similar biologic behavior as their human equivalents². Human glioblastoma multiforme is histologically characterized by prominent cellular pleomorphism with occasional multinucleated giant cells, and prominent vascular proliferation and necrosis¹⁰. This case was diagnosed as glioblastoma multiforme because of a resem-

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Fig. 1. Photomicrograph of glioblastoma multiforme in the forebrain. (a) The tumor cells show a wide variety of sizes and shapes. Bizarre giant cells (arrows) and necrosis (asterisk). H.E. $\times 200$ (b) Mitotic figure of bizarre giant cell and small aggregation of neutrophils. H.E. $\times 400$

Table 1. Immunoreactivity of the Present Case

Antibodies	Source	Dilution	Reactivity	
			Tumor Cells	Astrocytes
Vimentin (RAb*)	MEDAC	1 : 50	+	+
GFAP (RAb)	DAKO	1 : 600	—	+
S-100 (RAb)	DAKO	1 : 1000	—(+)	+
Lysozyme (RAb)	DAKO	1 : 500	—	—

*Rabbit polyclonal antibody.

+ ; Positive, — ; Negative

(+) ; Less than 10% of tumor cells react positively.

blance to human glioblastoma multiforme except for prominent vascular proliferation. Glioblastoma multiforme is defined as a highly malignant astrocytic neoplasm, but the dedifferentiation is so great that the astrocytic origin can not be established by H.E. stain. Evidence of astrocytic differentiation is shown by immunohistochemistry and electron microscopy¹⁰. The immunoreactivity is positive for vimentin and S-100 protein, while reactivity for GFAP is highly variable. Generally, well-differentiated cells are positive and undifferentiated cells are often negative¹¹. In this case, the tumor cells did not stain for GFAP although normal and non-neoplastic reactive astrocytes did. It is known that most astrocytomas in rodents react negatively for GFAP, whereas normal and reactive astrocytes show positive reaction. It is speculated that in rats, GFAP could have undergone a conformational change affecting the epitope(s) to GFAP antibody in the tumor formation¹². Ultrastructurally, however, tumor cells had no glial filaments in this case. Thus, it is presumed that those immunocytochemical and ultrastructural findings show poor differentiation in the present case.

Fig. 2. Photomicrograph of the olfactory mucosa at level II. The tumor cells in the bundle of nerve fiber in the lamina propria (arrows). $\times 280$

Histiocytic sarcoma was considered as a differential diagnosis, but was not adopted because the ultrastructural findings showed that the tumor cells were without developed lysosome or phagosomes.

AKR mouse is the strain with a large incidence of virus induced T-cell lymphoma¹³. The astrocytic tumor, however, appeared in one of eighty mice in this study, and to our

Fig. 3. Photomicrograph of the tumor with positive immunoreactivity for vimentin. Methylgreen counterstain. $\times 200$

Fig. 4. Electron micrograph. The tumor cells have rugged nuclei with large nucleoli. Numerous mitochondria and rough endoplasmic reticulum. Bar = $1\ \mu\text{m}$. $\times 7,600$

knowledge, no astrocytic tumor has been reported in AKR mice. Thus, the present tumor may not be common to the AKR strain.

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