J Toxicol Pathol 2016; 29: 191–194

Case Report

Neuroblastoma at the trigeminal nerve in a cynomolgus monkey (*Macaca fascicularis*)

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Abstract: A male cynomolgus monkey (*Macaca fascicularis*) of 5 years and 11 months of age from the vehicle control group of a 4-week repeated oral dose toxicity study had a spontaneously occurring mass lesion directly attached to the proximal part of the left trigeminal nerve. Histologically, the mass was characterized by a multifocal nodular appearance. Nodular zones showed low to moderate cellularity and were composed of small round cells exhibiting nuclear uniformity. On the other hand, inter-nodular zones were composed of nerve fiber containing septa and closely aggregated highly pleomorphic cells. Immunohistochemically, the small round cells were strongly immunopositive for synaptophysin, neuN, and class III beta-tubulin, while the highly pleomorphic cells were weakly immunopositive for neuN and occasionally immunopositive for class III beta-tubulin and doublecortin, suggesting that the tumor had originated from a neuronal lineage cell. Based on these findings, the mass was diagnosed as a neuroblastoma at the trigeminal nerve.
(DOI: 10.1293/tox.2016-0011; J Toxicol Pathol 2016; 29: 191–194)

Key words: cynomolgus monkey, trigeminal nerve, neuroblastoma

In cynomolgus monkeys, spontaneous neoplastic lesions in the nervous system are rarely encountered in preclinical toxicity studies because animals used in those studies are usually young[1]. To the best of our knowledge, only two cases of a primitive neuroectodermal tumor (PNET) of the cerebellum and an olfactory neuroblastoma in the nasal cavity have been described in cynomolgus monkeys[2, 3]. According to the World Health Organization (WHO) classification of tumors of the human central nervous system (CNS)[4], CNS PNET is defined as a heterogeneous group of embryonal tumors and is composed of undifferentiated or poorly differentiated neuroepithelial cells that have the potential for divergent differentiation along neuronal, astrocytic, or ependymal lines. Among them, PNETs with the potential for only neuronal differentiation are referred to as cerebral neuroblastomas or referred to as ganglioneuroblastomas if ganglion cells are also present[4]. Herein, we report the first case of a spontaneously occurring neuroblastoma at the trigeminal nerve in a cynomolgus monkey.

Received: 9 February 2016, Accepted: 18 March 2016
Published online in J-STAGE: 21 April 2016
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The animal was a male cynomolgus monkey (*Macaca fascicularis*) of 5 years and 11 months of age that had been used in a 4-week repeated oral dose toxicity study as a vehicle control animal. The animal was purpose-bred and kept at Shin Nippon Biomedical Laboratories, Ltd. (SNBL), for laboratory use and was of Cambodian origin. The animal was individually housed in a stainless-steel cage (680 mm × 620 mm × 770 mm) in a conventional facility at a temperature of 23–29°C with a relative humidity of 30–70% and a 12-hour light/dark cycle (lights on from 07:00 to 19:00). Approximately 108 g of pellet food was provided daily. Water was available *ad libitum*. This study was conducted at SNBL and all procedures involving the animal husbandry were approved by the Animal Care and Use Committee of SNBL and performed in accordance with the animal welfare bylaws of SNBL, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

There were no noteworthy findings in terms of clinical observations, body weight, food consumption, hematology, and blood chemistry throughout the quarantine, acclimation, and toxicity study periods. The monkey was anesthetized by an intravenous injection of sodium pentobarbital solution (64.8 mg/ml, 0.4 ml/kg) into the cephalic vein and euthanized by exsanguination at the end of the study period, and a full internal and external macroscopic examination was performed.

A mass that measured 15 × 12 × 10 mm and had an appearance of grey matter was directly attached to the proximal part of the left trigeminal nerve, which was separated from the pontine central nervous tissue (Fig. 1). The mass showed an exophytic growth pattern but did not compress neighboring structures. There were no other gross lesions in this animal. For routine histological examination, most organs and tissues including the present mass were fixed in 10% neutral buffered formalin, and paraffin-embedded sections were prepared and then stained with hematoxylin and eosin.

Histologically, the mass lesion was derived from the proximal part of the trigeminal nerve extending from the trigeminal ganglion, and its striking histological feature was its multifocal nodular appearance (Fig. 2a). The mass was composed of multiple nodular zones circumscribed by inter-nodular nerve fibers containing septa in which highly pleomorphic cells were enwrapped. The nodular zones had small round cells exhibiting nuclear uniformity at low to moderate cellularity. These cells were arranged in linear streams and embedded in a neuropil-like stroma-rich background without conspicuous mitotic activity. The inter-nodular zones were composed of nerve fibers containing septa and closely aggregated highly pleomorphic cells. These cells had round to oval or carrot-shaped hyperchromatic nuclei surrounded by scanty cytoplasm with brisk mitotic activity (Fig. 2b). Neoplastic ganglion-like cells were not present within nodular and inter-nodular zones, although matured ganglion cells covered by satellite glial cells were occasionally embedded in inter-nodular zones (Fig. 2c), which were considered to be remnants of normal tissue. A few small reactive astrocytes were admixed within nodular and inter-nodular zones.

Immunohistochemical staining for synaptophysin, neuN, class III beta-tubulin, doublecortin, glial fibrillary acidic protein (GFAP), nestin, and Ki-67 was performed on the present mass. The panels of antibodies used, clones, dilutions, sources, antigen retrieval methods, and positive cells in referenced studies are listed in Table 1. The small round cells in nodular zones were strongly immunopositive for antibodies against synaptophysin, neuN, and class III beta-tubulin. In contrast, the highly pleomorphic cells in inter-nodular zones were weakly immunopositive for neuN and occasionally immunopositive for class III beta-tubulin and doublecortin (Figs. 2d–g). Ki-67 immunoreactivity predominated in the highly pleomorphic cells, not in the small round cells (data not shown). GFAP- and nestin-immunopositive cells, which mostly preseted the typical spider-like appearance of reactive astrocytes, were occasionally found entrapped within nodular and inter-nodular zones (data not shown). These results indicated that the small round cells had a low proliferative neurocytic nature, while the highly pleomorphic cells had a highly proliferative neuroblastic nature.

On histological and immunohistochemical examinations, the present trigeminal nerve mass was characterized by a multifocal nodular appearance with uniform and low proliferative differentiated neurocytic cells resembling the neurocytes of central neurocytomas[5]. The inter-nodular zones were composed of undifferentiated, highly proliferative neuroblastic cells with a characteristic medulloblastoma histology. Thus, the mass consisted of those 2 neoplastic cell populations, both of which exhibited neuronal differentiation.

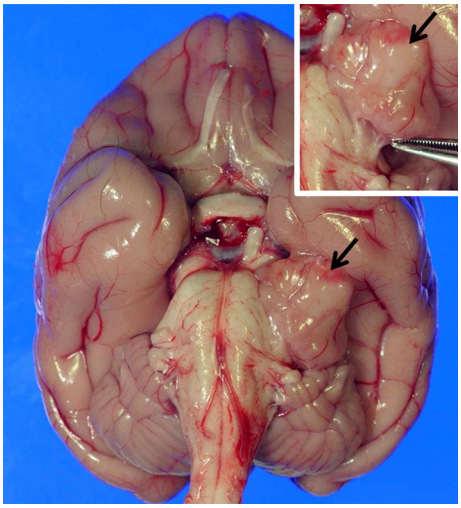

Fig. 1. Macroscopic appearance of the mass (arrows). Inset: The mass was directly attached to the proximal part of the left trigeminal nerve.

Referring to the most current human WHO classification, these biphasic features closely represent the typical appearance of "medulloblastoma with extreme nodularity" (MBEN)[6, 7]. Histologically, MBEN, which was previously termed cerebellar neuroblastoma, is characterized by an extreme degree of nodular appearance within which there are nodules composed of differentiated neurocytic cells exhibiting nuclear uniformity, and these zones alternate with cellular areas composed of highly proliferative undifferentiated cells[6, 7]. Both types of cell predominantly exhibit neuronal differentiation[6–8]. Thus, the histological features of the present case are very similar to those of MBEN.

However, the term medulloblastoma including MBEN is unavailable for a tumor arising from the trigeminal nerve, since it is not generally used for tumors that occur in any extracerebellar regions[6]. In the literatures, medulloblastomas are commonly referred to as PNETs of the cerebellum capable of differentiating along a wide range of histogenetic lines[7–9] According to the WHO classification, CNS PNETs with the potential for only neuronal differentiation are termed cerebral neuroblastomas or ganglioneuroblastomas[6]. In the present case, the tumor occurred in the trigeminal nerve, and neoplastic ganglion-like cells were not present. Therefore, the diagnosis of neuroblastoma at the trigeminal nerve was ultimately made.

With respect to the nature and origin of this tumor, it might have originated from neural crest cells, which represent a transient, multipotent, migratory cell population that gives rise to a diverse cell lineage including peripheral

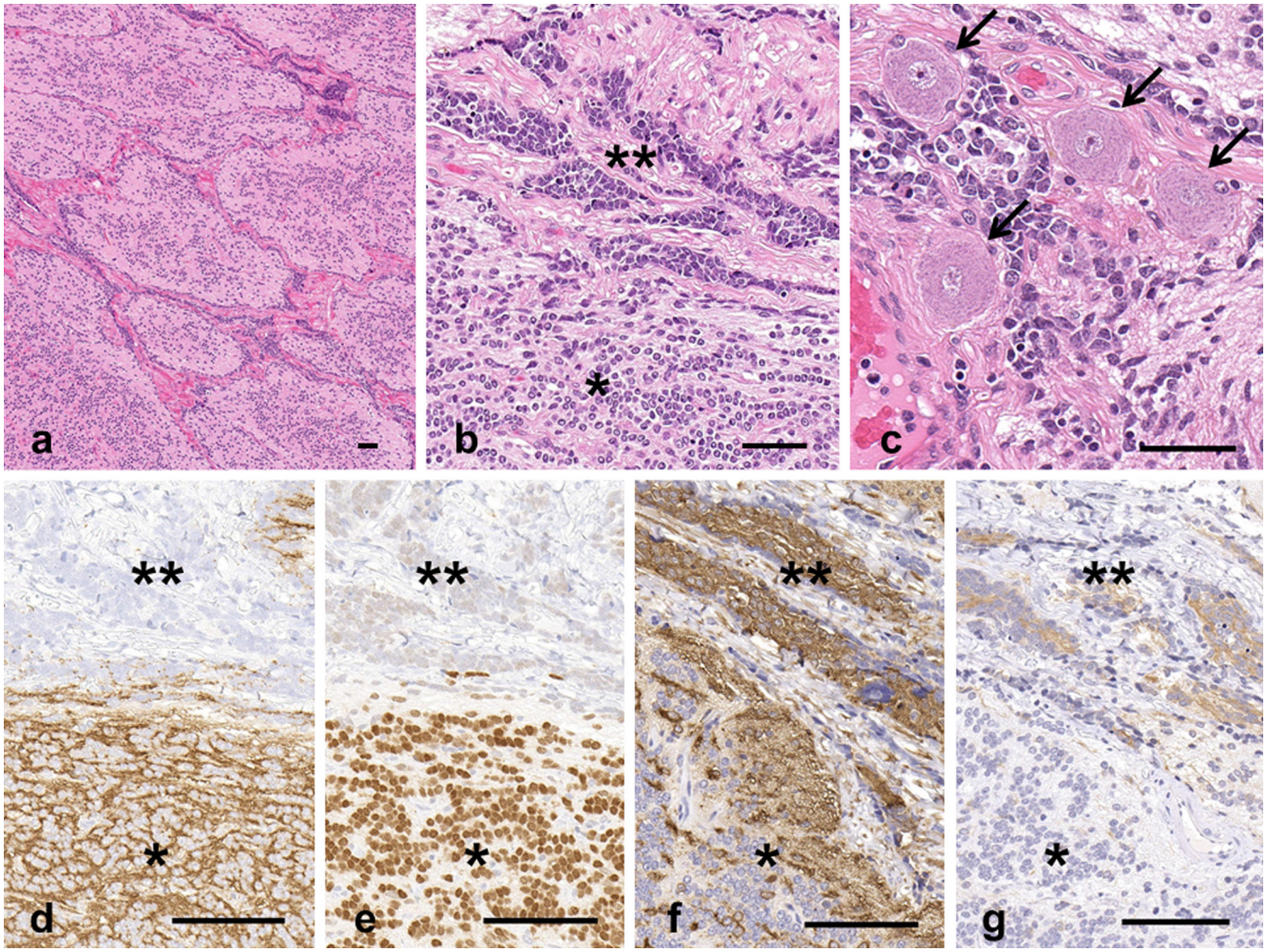

Fig. 2. Histological features (a–c) and immunohistochemical findings for synaptophysin (d), neuN (e), class III beta-tubulin (f), and doublecortin (g) of the mass. (a) The mass was characterized by multifocal nodular zones circumscribed by inter-nodular nerve fibers containing septa in which highly pleomorphic cells were enwrapped. (b) Nodular zones showed low to moderate cellularity and were composed of small round cells exhibiting nuclear uniformity (asterisk). On the other hand, inter-nodular zones were composed of nerve fibers containing septa and closely aggregated highly pleomorphic cells (double asterisks). (c) Matured ganglion cells covered by satellite glial cells (allows) were occasionally embedded in inter-nodular zones, which were considered to be remnants of normal tissue. (d–g) The small round cells were strongly immunopositive for antibodies against synaptophysin, neuN, and class III beta-tubulin, while they were immunonegative for doublecortin (asterisks). In contrast, the highly pleomorphic cells were weakly immunopositive for antibodies against neuN and occasionally immunopositive for class III beta-tubulin and doublecortin, while they were immunonegative for synaptophysin (double asterisks). Bars = 50 μm.

Table 1. Primary Antibodies Used for Immunohistochemical Evaluation

Antibody	Clone	Dilution	Source	Antigen retrieval	Positive cells in referenced studies
Class III beta-tubulin	5G8	1:2000	Thermo[a]	Autoclave, citrate buffer, pH 6.0	Neuronal lineage cells[12]
Doublecortin	Polyclonal	1:200	SCB[b]	Autoclave, citrate buffer, pH 6.0	Neuroblasts[13]
Glial fibrillary acidic protein (GFAP)	Polyclonal	1:700	Dako[c]	None	Resting and reactive astrocytes[14]
Ki-67	MIB-1	1:50	Dako	Autoclave, citrate buffer, pH 6.0	Proliferating cells[15]
Nestin	Polyclonal	1:20	IBL[d]	None	Neural stem cells, astrocyte progenitors and reactive astrocytes[14, 16]
NeuN	A60	1:400	Millipore[e]	Autoclave, citrate buffer, pH 6.0	Neuronal lineage cells[17]
Synaptophysin	SY38	1:40	Dako	Autoclave, citrate buffer, pH 6.0	Neuronal lineage cells[17]

[a]Thermo Fisher Scientific, Inc., Waltham, MA, USA; [b]Santa Cruz Biotechnology Inc., Dallas, TX, USA; [c]Dako, Glostrup, Denmark; [d]Immuno-Biological Laboratories, Gunma, Japan; [e]Merck Millipore Corporation, Darmstadt, Germany

neurons and glia and migrate toward the periphery to form the trigeminal ganglion during the development of the trigeminal nerve[10, 11]. In this context, migration defects and/or entrapment of these cells might be considered to be involved in the pathogenesis of the present case.

To the best of our knowledge, this is the first report of a neuroblastoma spontaneously occurring at the trigeminal nerve in a cynomolgus monkey.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

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