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Case Report

A Spontaneous Oligodendroglioma in the Lumbar Portion of the Spinal Cord in a Young BrlHan:WIST@Jcl (GALAS) Rat

Shuji Takeda[1*], Hiroyuki Asano[1], Ryo Ihara[1], Keiko Ogata[1], and Masahiko Kushida[1*]

[1] Toxicology group, Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554-8558, Japan

Abstract: Oligodendroglioma is a rare tumor originating from oligodendrocytes found mainly in the cerebrum in aged rats. Only a few reports have shown spontaneous occurrence of this tumor in the spinal cord, and no report has mentioned its occurrence in young rats. We encountered a case of spontaneous oligodendroglioma in the lumbar portion of the spinal cord in a young (9 weeks old) female BrlHan:WIST@Jcl (GALAS) rat. Here we report the detailed histopathological and immunohistochemical characteristics of this case. No clinical signs, no gross lesions at necropsy, and no specific changes in hematology or blood biochemistry were observed. The tumor was located in the dorsal funiculus in the lumbar portion of the spinal cord and widely spread to the dorsal root nerve. The neoplastic cells showed a sheet-like growth pattern and had small round nuclei, clear cytoplasm and distinct cell borders that resulted in a honeycomb pattern. No mitotic figures or other histological lesions were observed. The neoplastic cells were positively stained for Olig2 and PCNA. The present case was considered to be a low-grade oligodendroglioma based on the histological and immunohistochemical features. To our knowledge, our case is considered to be extremely rare and the first report in a young rat. (DOI: 10.1293/tox.2014-0010; J Toxicol Pathol 2014; 27: 143–146)

Key words: oligodendroglioma, young rat, spinal cord, BrlHan:WIST@Jcl (GALAS)

Spontaneous oligodendrogliomas are found principally in the cerebral hemisphere, basal ganglia and corpus callosum[1, 2]. They are an extremely rare central nervous system tumor in most strains of rat[3]. In Crl:CD(SD), F344/DuCrlCrlj, Wistar Hannover and F344 rats, the incidence of spontaneous oligodendroglioma is reported to be 0.1 to 0.4% in the brain and 0 to 0.1% in the spinal cord after about 32 weeks of age[4–7]. To our knowledge, there have been no reports regarding spontaneous oligodendroglioma in the spinal cord in young rats. Here we report the detailed histopathological and immunohistochemical characteristics of a case of oligodendroglioma in the spinal cord in a 9-week-old BrlHan:WIST@Jcl (GALAS) rat.

Four-week-old BrlHan:WIST@Jcl (GALAS) rats (36 animals of each sex) were purchased from Charles River Laboratories Japan, Inc. (Shiga, Japan), for a toxicity study and housed in suspended aluminum cages (one rat per cage) in a room kept at 24 ± 2 °C and 40–70% RH with a 12-h light/dark cycle. CRF-1 pellet diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were freely available via automatic stainless steel nozzles throughout the study. The animals were observed daily and were used for experiments after a 1-week acclimation period. All experiments were performed in accordance with the Guide for Animal Care and Use of Sumitomo Chemical Co., Ltd. At 9 weeks of age, all animals were euthanized under isoflurane anesthesia, and whole blood samples were withdrawn from the abdominal aorta.

Oligodendroglioma in the spinal cord was found histologically in a female rat in the nontreatment group. This rat showed no clinical signs during acclimation and 4-week test periods, no gross lesions at necropsy, and no specific changes in hematology or blood biochemistry.

Tissue samples including cerebrum, cerebellum, pons, medulla oblongata and cervical and lumbar spinal cord were fixed in 10 vol% neutral buffered formalin and processed by routine methods. The samples were then embedded in paraffin, sectioned in 3-μm slices, stained with hematoxylin-eosin (HE) and examined under a light microscope. For immunohistochemistry, 4-μm-thick sections obtained from paraffin-embedded brain and spinal cord tissues were used. After deparaffinization, the sections were heated in 10 mM citrate buffer (pH 6.0) by microwave for 20 minutes at 98 °C for antigen retrieval and blocked for endogenous peroxidase, and immunohistochemistry using anti-GFAP (glial fibrillary acidic protein) antibody, anti-Iba1 (ionized calcium-binding adapter molecule 1) antibody, anti-PCNA (proliferating cell nuclear antigen) antibody or anti-Olig2 (oligodendrocyte lineage transcription factor 2) antibody

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*Corresponding authors: S Takeda (e-mail: takedas3@sc.sumitomo-chem.co.jp) and M Kushida (e-mail: kushida@sc.sumitomo-chem.co.jp)

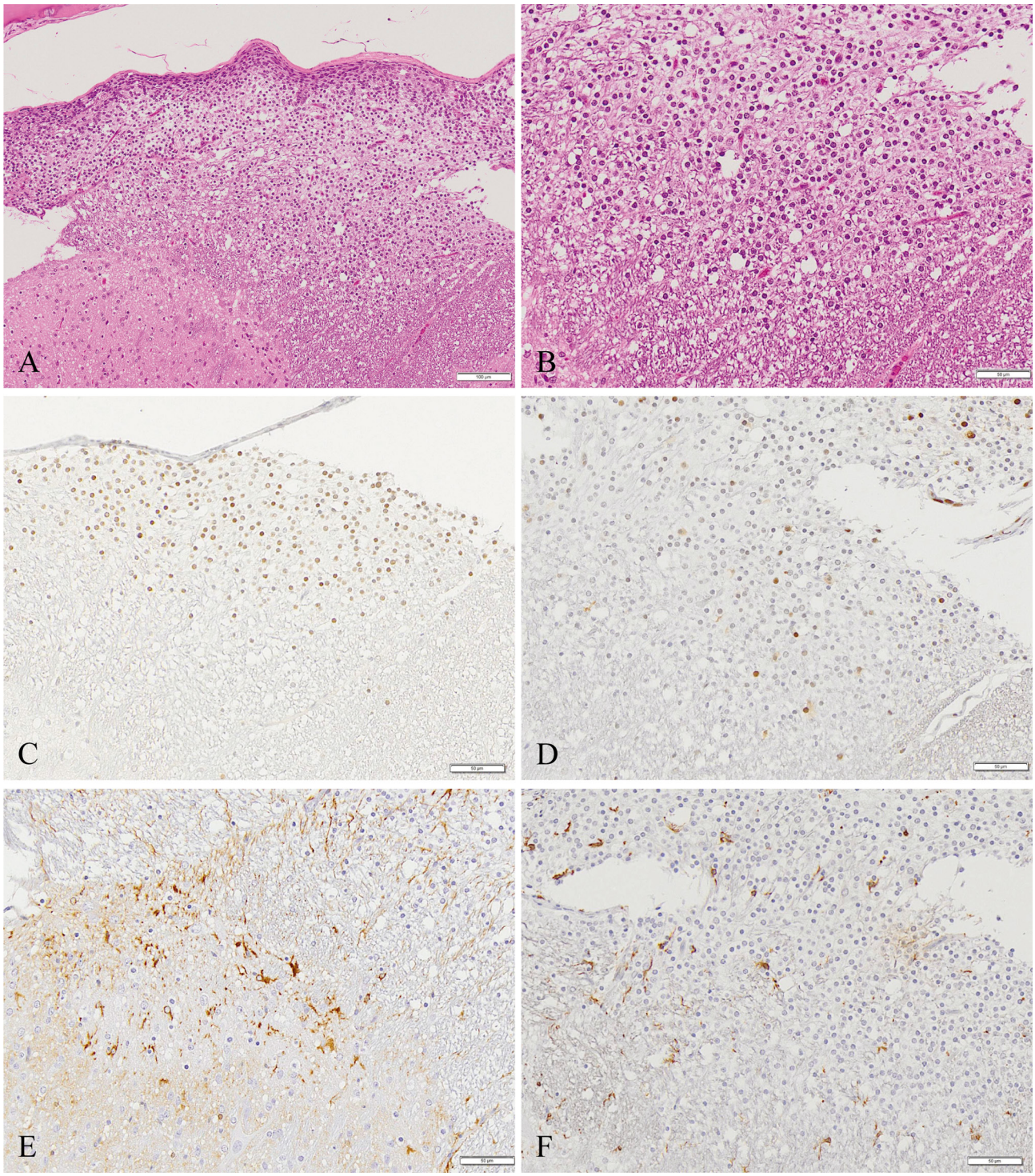

Fig. 1. HE staining and immunohistochemistry of the tumor in the spinal cord. A: The tumor was located in the dorsal funiculus in the lumbar portion of the spinal cord and widely infiltrated the dorsal-root nerve tissue. B: The neoplastic cells showed a sheet-like growth pattern and had small round nuclei, clear cytoplasm and distinct cell borders that resulted in a honeycomb pattern. C: The nuclei of neoplastic cells were stained positive for Olig2. D: The nuclei of neoplastic cells were stained positive for PCNA. E: Nonneoplastic spindle or polygonal cells with round to oval nuclei observed in this tumor were positively stained for GFAP. F: Some nonneoplastic spindle cells in the tumor were positively stained for Iba1.

was performed. The sections were incubated with anti-PCNA mouse monoclonal antibody (1:100 dilution, Dako Japan Inc., Kyoto, Japan), anti-GFAP rabbit polyclonal antibody (1:2000 dilution, Dako Japan Inc.), anti-Olig2 rabbit poly-

clonal antibody (1:500 dilution, Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) or anti-Iba1 rabbit polyclonal antibody (1:1000 dilution, Wako, Osaka, Japan) for 1 hour at room temperature. Immunoreactivity was detected and visualized using Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) and a DAB Map kit (Ventana Medical Systems, Inc., Tucson, AZ, USA) before the sections were counterstained using hematoxylin.

Histologically, the tumor was located in the dorsal funiculus in the white matter at lumbar portion of the spinal cord, and it has spread extensively into the dorsal root nerve tissue. The neoplastic cells showed a sheet-like growth pattern and had small round nuclei, clear cytoplasm and distinct cell borders that resulted in a honeycomb pattern (Fig. 1A, B). No mitotic figures and no other histological lesions were observed.

Immunohistochemically, the nuclei of neoplastic cells were stained positively for Olig2 and PCNA, indicating that these neoplastic cells were derived from oligodendrocytes and had increasing cell proliferation activity, but were negative for GFAP or Iba1 (Fig. 1C–F). On the other hand, nonneoplasitic spindle or polygonal cells with round to oval nuclei observed around this tumor were positively stained for GFAP and Iba1, indicating that these cells were reactive astrocytes and microglia (Fig. 1C–F). These histopathological and immunohistochemical results suggested that the tumor was an oligodendroglioma.

Light microscopically, a typical oligodendroglioma in rat brain is characterized by 1) uniform cells with small, round, darkly stained nuclei, clear cytoplasm and delicate cell membranes resulting in honeycomb structures; 2) vascular endothelial hypertrophy and hyperplasia; and 3) hemorrhage and necrosis[8]. Oligodendroglioma is broadly classified into two categories, low grade and high grade. Low-grade oligodendroglioma is composed of neoplastic cells having chromatin-rich nuclei, and few mitotic figures are present[9]. In contrast, high-grade oligodendroglioma may extend over multiple regions of the brain or spinal cord, causes extensive damage, has foci of necrosis and hemorrhage, exhibits pronounced cellular atypia and pleomorphism and demonstrates invasive growth[1]. The neoplastic cells are slightly smaller than in low-grade tumors but also contain chromatin-rich nuclei, and some mitotic figures are present[9]. In our study, the neoplastic cells showed a typical honeycomb pattern, no mitotic figures and no necrosis or hemorrhage, suggesting that the tumor was a low-grade oligodendroglioma.

Immunohistochemically, in spontaneous oligodendroglioma in rats, neoplastic oligodendrocytes are positive for Olig2 but negative for GFAP[10][REMOVED HYPERLINK FIELD]. In some cases, oligodendrogliomas show mixtures of different cell types and are diagnosed as mixed gliomas[8]. In such cases, GFAP is a useful marker to determine whether the astrocytes are reactive or neoplastic because reactive astrocytes are positive for GFAP[8, 11]. In our study, the neoplastic cells were positively stained for Olig2 but negative for GFAP, indicating these cells were derived from oligodendrocytes, and nonneoplastic spindle or polygonal cells in this tumor were positively stained for GFAP, indicating that these cells were reactive astrocytes, not neoplastic cells. Some nonneoplastic spindle cells in the tumor were positively stained for Iba1, indicating that they were reactive microglia.

Neoplasms of the nervous system, whether spontaneous or induced, are infrequent in laboratory rodents and usually observed in the brain rather than the spinal cord[9]. In neoplasms of the rat central nervous system, spontaneous astrocytoma occurs most commonly, followed by oligodendroglioma and granular cell tumor[12]. Spontaneous oligodendrogliomas are found principally in the cerebral hemisphere, and in a few cases, they have been reported in the rat spinal cord[1–4]. Oligodendroglioma generally occurs at a relatively advanced age: the youngest previously reported case was in the brain of a Crl:CD(SD) rat at 32 weeks of age[12].

In BrlHan:WIST@Jcl(GALAS) rats, the most common spontaneous central nervous system tumor was reported to be granular cell tumor, followed by astrocytoma, oligodendroglioma and mixed glioma[5]. The incidence of spontaneous oligodendroglioma in the brain of BrlHan:WIST@Jcl(GALAS) rats was reported as approximately 0.13% in both sexes, but it was not reported in the spiral cord or in young rats[5].

On the other hand, the selective induction of brain tumors including glioma, oligodendroglioma and mixed glioma in SD rats at 14–52 weeks after birth by transplacental administration of ethylnitrosourea (ENU) on the 18th day of gestation has been reported[13], and the histological features of the reported oligodendroglioma resemble those of our case.

In conclusion, the tumor was diagnosed as an oligodendroglioma (low grade) based on its histological and immunohistochemical features. To our knowledge, there have been only a few reports on spontaneous oligodendroglioma in the spinal cord in Han:SPRD and Crl:CD(SD) rats[2, 4], and none have been reported in young rats or other rat strains. Therefore, our case is considered to be extremely rare and to be the first report in a young (9 weeks old) BrlHan:WIST@Jcl (GALAS) rat.

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