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Case Report

Proliferative Potential of a Spinal Nephroblastoma in a Young Dog

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Abstract: The proliferative potential of a spinal nephroblastoma was studied in a young dog. A 4-month-old, female golden retriever showed developing deterioration in her gait and subsequent paralysis of her hind legs. At necropsy, a well-demarcated grayish brown tumor mass was found in the lumbar spinal cord segments between L2 and L3. Histologically, a blastemal cell tumor with a tubule- or glomeruli-like structure was found to be infiltrating intradurally. Proliferating cells at the S-phase, assessed using the bromodeoxyuridine (BrdU) labeling method, were seen occasionally in the tubular cells and glomeruli-like structures and were frequently seen in the blastemal cells. Immunohistochemically, the tubular epithelial cells were positive for cytokeratin, and the blastemal cells were positive for vimentin. The present tumor showed a high potential for growth and invasion, which suggests that it the potential to expand into the adjacent spinal cord. (J Toxicol Pathol 2009; **22**: 79–82)

Key words: nephroblastoma, spinal cord, young dog, BrdU labeling, S-phase cells, immunohistochemistry

Spinal nephroblastoma is unusual and has rarely been reported in juveniles and young dogs of the German shepherd breed, which are predisposed to such tumors[1–5]. Occurrence of this tumor is not well established in animals except for dogs[6,7]. To date, the histogenesis of this neoplasm has been controversial, and this tumor is currently thought to be an extrarenal nephroblastoma based on its histological features as well as immunohistochemistry[3,7]. Spinal nephroblastomas likely develop from the remnants of renal rests trapped between the dura and the developing spinal cord[1,2,5,7]. Regarding the biological behavior of this tumor, little information is available except for a report of an aggressive neoplasm giving rise to a second, less differentiated metastatic focus[5]. We endeavored to assess the proliferative potential of a spinal nephroblastoma in a young dog.

A 4-month-old female golden retriever dog was admitted to a veterinary hospital because of deterioration in her gait and the X-appearance of her hind legs. Physical examination confirmed symmetrical paralysis, and a myelogram showed an intradural mass in the spinal cord from the level of L2 to L3 (Fig. 1). The tumor was resected surgically two months after admission. At the time of surgery through a long midline incision, an encapsulated, intradural grayish brown tumor mass was found in the spinal cord in the L2 to L3 region. Histopathological examination of the operative specimen revealed a suspected ependymoma. Postoperatively, the dog did not recover well; the tumor recurred at the site of the operation, and the dog was euthanized because of poor general condition three months after the operation.

The dog was euthanized by deep anesthesia. A gross examination revealed a subdural lobular mass measuring 6×5×1 mm in the spinal cord at the levels of L2 and L3. The capsular surface was reddish-gray and was covered with thick fibrous connective tissue. The cut surface contained grayish yellow medullary tissue with gelatinous and hemorrhagic areas. It was quite firm and resilient (Fig. 2).

A complete necropsy was performed immediately. Tissue and organ samples were collected and fixed in 10% buffered formalin. After fixation, tissue blocks were dehydrated and embedded in paraffin wax in the usual manner. Sections with a thickness of 3 μm were stained with hematoxylin-eosin (HE).

For immunohistochemistry, the labeled strepto-avidin-biotin (LSAB) method was applied to deparaffinized sections using a commercial kit (DAKO Corp., Santa Barbara, CA, USA). The primary antibodies used were anti-keratin, S-100, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE; polyclonal, DAKO Corp.), anti-cytokeratin AE1/AE3 (monoclonal, Signet labs, Inc., Dedham. MS, USA) and anti-vimentin, (monoclonal, DAKO Corp.). The deparaffinized sections were incubated

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successively in normal goat serum and each primary monoclonal antibody overnight at 4°C and were then incubated in biotinylated anti-mouse immunoglobulin G at room temperature for two hours. The sections were subsequently incubated in PBS containing 0.03% 3.3'-diminobenzidine (DAB; Dojin Chemical Company, Kumamoto, Japan) and 1%H_2O_2 and then counterstained with Mayer's hematoxylin. Negative and substituted serum controls and positive tissue controls were also employed.

To assess the proliferative activity, bromodeoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO, USA) was administered intravenously at a dose of 15 mg/kg one hour prior to euthanasia. BrdU-incorporated cells were identified by immunohistochemical techniques using the LSAB method on deparaffinized sections and a commercial kit with anti-BrdU antibody (monoclonal, Immunotech S.A., Marseilles, France). The deparaffinized sections were incubated successively in a 1:4,000 dilution of a BrdU monoclonal antibody overnight at 4°C and then in biotinylated anti-mouse immunoglobulin G at room temperature for two hours. The sections were then incubated in PBS containing 0.03% DAB and l% H_2O_2 and counterstained with Mayer's hematoxylin. BrdU-positive nuclei exhibited deposits of brown DAB precipitates. The BrdU labeling index (LI) was determined by counting 200 cells. The BrdU LI was expressed as a percentage of the total number of labeled cells scored.

For electron microscopy, tissue samples from the tumor mass in the spinal cord were fixed with 2% phosphate-buffered glutaraldehyde and 1% osmium tetroxide and routinely processed. Ultrathin section were cut, stained with uranyl acetate and lead citrate and examined with a Hitachi H-8100 electron microscope at 75 kV.

There was infiltrative cellular tumor growth at the level of L2 and L3 in the spinal cord, and this resulted in subdural compression of the adjacent spinal parenchyma (Fig. 3). The tumor was composed of fusiform or round cells with hyperchromatic nuclei and scanty cytoplasm and closely resembled an embryonic metanephrotic blastema. Epithelial tubules of various sizes lined with cuboidal and columnar cells were frequently observed in the clusters or nests of densely packed blastemal cells. Structures resembling immature glomeruli, which are a papilloferous formation of blastemal cells within a cystic space, were observed (Fig. 4). Mitotic figures were prominent among the cells of the blastemal components and were less frequently observed in the tubules with epithelial components. The nests of blastemal cells were separated by various amounts of connective tissue. The spindle and stellated cells accompanying stromal connective tissue had evolved from the round cells of richly cellular blastomous masses, and transitional elements were obvious. The boundary between the tumor and spinal cord tissue was well circumscribed. Fibrous connective tissue was present, and a few foci tumor cells, constituting small tubules, were seen in the parenchyma adjacent to the tumor. A circumscribed area of cartilage, which is normally uncommon, was found in the present case. No teratogenic components were encountered in the tumor.

Immunohistochemical evaluation revealed consistent positive reactions for keratin (Fig. 5) and cytokeratin AE1/AE3 in the epithelial cells and vimentin in the blastemal cells. Other markers (S-100 protein, NSE and GFAP) were negative in both elements.

Ultrastructurally, both the epithelial and blastemal tumor cells had oval or elongated nuclei. Cytoplasmic organelles were poorly developed; however, numerous free ribosomes, a few rough endoplasmic reticula and small round mitochondria were observed. The epithelial cells had abundant junctional complexes and formed conspicuous lumina and microvilli. The basal sides of the epithelial cells were underlined by basement membrane-like materials. Junctional complexes were observed between the epithelial cells on the luminal side.

Immunostaining for BrdU showed only an occasional labeled nucleus in the tubular epithelial cells and frequent positive cells in the nests of blastemal cells (Fig. 6). The BrdU LI was no less than 2% in epithelial cells and was 11.8% in blastemal cells, respectively.

It appears that the present tumor satisfies the criteria for an intradural extramedullary spinal cord tumor in young dogs, as reported by Summers *et al.*[4]. Previously, intradural spinal cord tumors in young dogs have been interpreted as ependymoma[8] or neuroepithelioma[9]. It is now believed that this tumor arises within the embryonic rest of renal tissue in the spinal cord, and it has been suggested that it is an extrarenal nephroblastoma in the spinal cord[6,7]. Because there was no renal neoplasm at a clinical examination, the present spinal cord tumor was obviously the primary tumor. The most striking morphological feature of the present tumor was the mix of blastematous, epithelial and stromal elements, which closely resembled the characteristics of a differentiated nephroblastoma in the kidney[4,7]. In humans, extrarenal nephroblastomas are occasionally seen in various locations such as the pelvis and female reproductive organs[14–16]. The extrarenal location increases the likelihood that these tumors originate over a wide area from remnants of undifferentiated mesodermal tissue before the mesodermic derivatives of myotome, sclerotome and nephrotome develop[14]. Most nephroblastomas originating from the kidney pose few diagnostic difficulties, but those of extrarenal origin are frequently difficult to diagnose and require ultrastructural or immunohistochemical identification of some epithelial feature in order to confirm the diagnosis[4,6,7].

The proliferative potential of the present tumor was assessed by BrdU immunohistochemistry. BrdU is a thymidine analogue that is incorporated into DNA-synthesizing nuclei (S-phase), and the incorporated BrdU in the nuclei can be detected using antibodies against BrdU[12]. The BrdU LI correlates well with the proliferative potential of neoplasms, such as meningioma, astrocytoma and glioblastoma multiforms[13]. Nephroblastoma is a highly malignant type of tumor found in human juveniles and

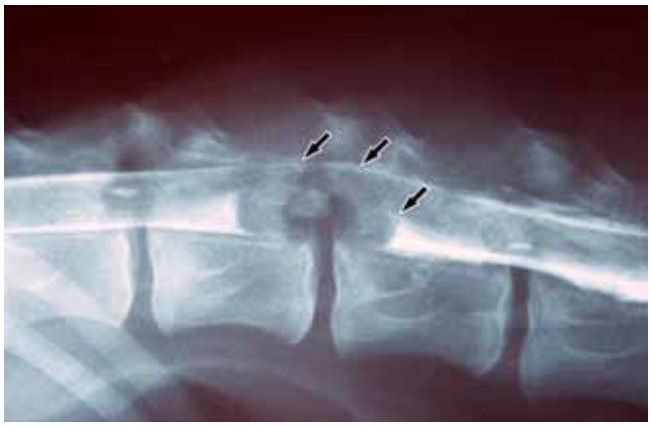

Fig. 1. Lumbar spinal cord myelogram. Arrows delineate an area of widening of the spinal cord extending from the second to third lumbar vertebrae.

Fig. 2. Cut surface of spinal mass between the spinal cord at the second to third lumbar vertebrae. Neoplastic tissues compressed the spinal parenchyma (arrows). Bar=5 mm.

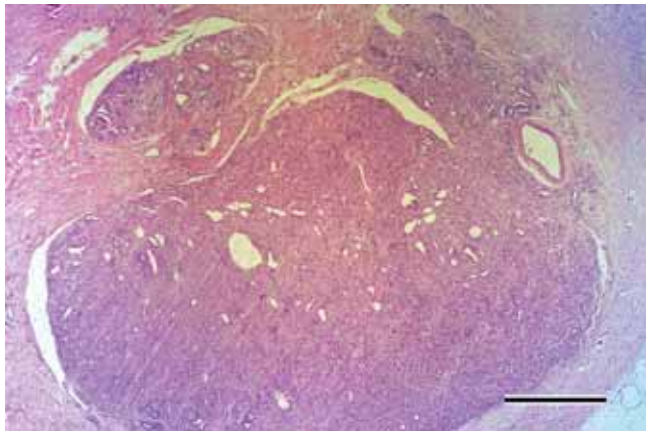

Fig. 3. Expansive tumor growth compressing the spinal parenchyma. Note the multiple nests of blastemal cells surrounded by stellated neoplastic stromal cells. HE stain. Bar=200 μm.

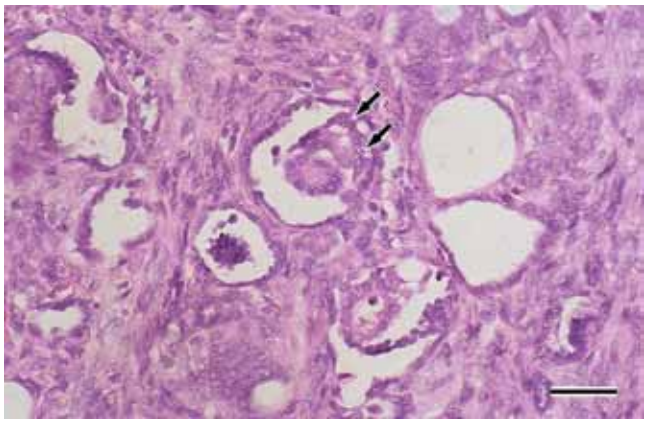

Fig. 4. Frequent tubular epithelial cells were seen in the blastemal component. The mass is composed of three distinct elements, dense sheets of polygonal blastemal cells, a delicate fibrous supporting stroma and an epitheloid component forming tubules and structures resembling fetal glomeruli (arrows). HE stain. Bar=50 μm.

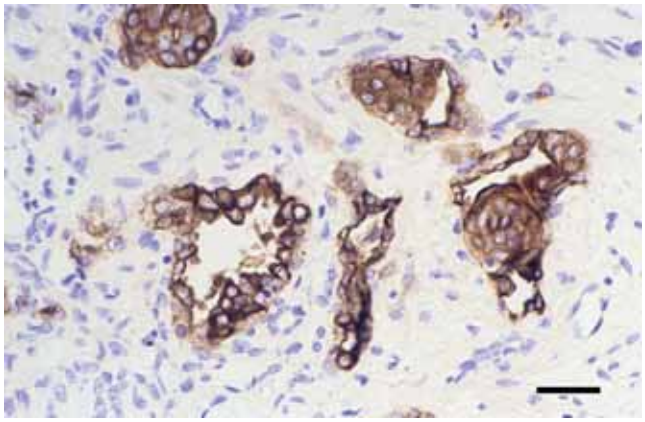

Fig. 5. Tubular epithelial cells are positive for keratin by immunohistochemical staining. Bar=50 μm.

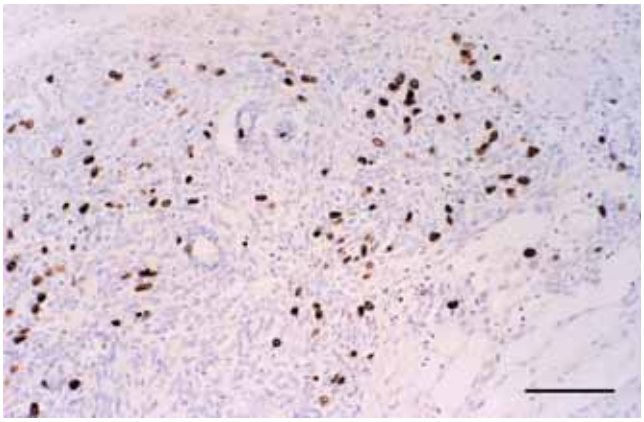

Fig. 6. Immunostaining for BrdU. Many labeled nuclei are seen in the blastemal cells, and there are also occasional labeled cells in the tubular epithelial cells. Bar=100 μm.

animals and tends to recur rapidly after surgical removal[7,10]. The present tumor showed a higher BrdU incorporated index (11.8%) in the blastemal components, and this is roughly equal to those of malignant tumors such as squamous cell carcinomas[11], mammary carcinomas and grade III masctocytomas[17]. As the present tumor contained many BrdU labeled cells with a higher BrdU LI, the present tumor was considered to be malignant and to have the potential for recurrence.

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References

1. Macri NP, Alstine WV, and Coolman RA. Canine spinal nephroblastoma. J Am Anim Hos Assoc. **33**: 302–306. 1997.
2. Neel J and Dean GA. What is your diagnosis? A mass in the spinal column of a dog. Vet Clin Pathol. **29**: 87–89. 2000.
3. Pearson GR, Gregory SP, and Charles AK. Immunohistochemical demonstration of Wilms' tumor gene product WTI in canine "neuroepithelioma" providing evidence for its classification as an extrarenal nephroblastoma. J Comp Pathol. **116**: 321–327. 1997.
4. Summers BA, deLahunta A, McEntee M, and Kuhajda FP. A novel intradural extramedullary spinal cord tumor in young dogs. Acta Neuropathol. **75**: 402–410. 1988.
5. Terrell SF, Platt SR, Chrisman CL, Homer BL, deLahunta A, and Summers BA. Possible intraspinal metastasis of a canine spinal cord nephroblastoma. Vet Pathol. **37**: 94–97.2000.
6. Koestner A and Higgins RJ. Tumors of the nervous system. In: Tumors in Domestic Animals, 4th ed. DJ Meuten (ed.) Iowa State Press, Ames, 697–738. 2002.
7. Meuten DJ. Tumors of the urinary system. In: Tumors in Domestic Animals, 4th ed. DJ Meuten (ed.) Iowa State Press, Ames, 509–546. 2002.
8. Zachary JF, O'Brien DP, and Ely RW. Intramedullary spinal ependymoma in a dog. Vet Pathol. **18**: 697–700. 1981.
9. Tamke PG and Foley GL. Neuroepithelioma in a dog. Canadian Vet J. **28**: 606–608. 1987.
10. Hardwick DF and Stowenes D. Wilms tumors. J Urol. **85**: 903–910. 1961.
11. Yoshida K, Yanai T, Iwasaki H, Sakai H, Ohta J, Kati S, Ishikawa K, Lackner AA, and Masegi T. Proliferative potential of canine oral epulides and malignant neoplasms assessed by bromodeoxyuridine labeling. Vet Pathol. **36**: 35–41. 1999.
12. Grazner HG. Monoclonal antibody to 5 -bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science. **218**: 474–476. 1982.
13. Nagashima T, DeArmond SJ, Murovic J, and Hoshino T. Immunohistochemical demonstration of S-phase cells by anti-bromdeoxyuridine monoclonal antibody in human brain tissues. Acta Neuropathol. **67**: 155–159. 1985.
14. Aterman K. Extrarenal nephroblastomas. J Cancer Res Clin Oncol. **115**: 409–412. 1989.
15. Johnson F, Luttenton C, and Limbert D. Extrarenal and urothelial Wilms tumor. Urology. **15**: 370–373. 1980.
16. Mirkin LD, Azzarelli B, and Seo IS. Extrarenal Wilms' tumor with cerebellar metastasis in a four-year-old girl with spina bifida. Am J Clin Pathol. **93**: 805–809. 1990.
17. Ishikawa K, Sakai H, Hosoi M, Ohta G, Yanai, T, and Masegi T. Immunohistochemical demonstration of S-phase cells in canine and feline spontaneous tumors by anti-bromodeoxyuridine monoclonal antibody. J Toxicol Pathol. **18**: 135–140. 2005.