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 tumors1–3. Occurrence of this tumor is not well established in animals except for dogs4–7. 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 immunohistochemistry3,7. Spinal nephroblastomas likely develop from the remnants of renal rests trapped between the dura and the developing spinal cord1,2,5,7. 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 focus5. 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 antikeratin, 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...
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′-diaminobenzidine (DAB; Dojin Chemical Company, Kumamoto, Japan) and 1% H2O2 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 1% H2O2 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 sections 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 undifferentiated mesodermal tissue before the mesodermal derivatives of myometre, selerotome and nephrotome develop14. Most nephroblastosomas 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 diagnosis4,6,7. 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 BrdU12. The BrdU LI correlates well with the proliferative potential of neoplasms, such as meningioma, astrocytoma and glioblastoma multiformes13. 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\(^1,1\), mammary carcinomas and grade III mastocytomas\(^17\). 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**