

<Case Report>

Embryonal rhabdomyosarcoma in the abdominal cavity of an aged Sprague-Dawley rat

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Abstract : We report a spontaneous embryonal rhabdomyosarcoma in the abdominal cavity of an aged (88-week-old) Sprague-Dawley rat. The animal had a firm lobulated 5 × 5 × 4.5 cm mass in the abdominal cavity that was whitish to tan with necrotic and hemorrhagic plaques. Microscopically, the mass contained nodules with spindle or globoid shaped neoplastic cells with abundant eosinophilic cytoplasm and round or elongated nuclei mixed with other spindle cells with a filamentous appearance and scanty cytoplasm. Multinucleated cells and cross-striations were also observed. The neoplastic cells were positive for vimentin, desmin, and alpha-smooth muscle actin, especially the small spindle cells.

Keywords : abdominal cavity, embryonal rhabdomyosarcoma, rat

Rhabdomyosarcoma (RMS) is described as a highly invasive and metastatic malignant tumor arising in the skeletal muscle of animals [5]. The incidence of RMS is very rare in both animals and humans [5, 13]. In laboratory animals, naturally occurring RMS is < 0.2% in Fischer 344 and Wistar rats [7, 14]. In the Sprague-Dawley rat, which is one of the most commonly used laboratory rat strains, Merck Research Laboratories (USA) reported a 0.02% incidence as unpublished historical control data [4].

RMS occurs mainly in the subcutaneous tissue within the head, neck, and thoracic areas in humans and animals, including laboratory rats [2], but it is very rare in other areas. Thus far, spontaneous RMS has been reported in the urinary bladder, kidney, heart, lung, and female reproductive system of domestic animals [5]. RMS also rarely develops in skeletal muscle of the body cavity in laboratory animals [4].

RMS is further classified into embryonal, botryoid, alveolar, and pleomorphic types based on the histological features. Among them, the embryonal type is particularly rare in both animals and humans. In laboratory animals, to the best of our knowledge, only one spontaneous embryonal RMS has been reported: near the right genu in a 7-month-old Sprague-Dawley rat. This type of tumor usually occurs in young animals [5].

We herein report a case of embryonal type RMS that spontaneously occurred in the abdominal muscle of a relatively old (88 weeks of age) Sprague-Dawley rat.

The animal was purchased from the Central Lab Animal, Seoul, Korea. It had been in a control group of a chronic tox-

icity study (ChemOn, Korea). The rat was emaciated, and a firm, solitary mass of 5 × 5 × 4.5 cm was found in the abdominal cavity. The mass was mainly colored yellow with a few fluid-filled cystic areas of necrosis, hemorrhage, and gelatinous degeneration. On cut section, the tumor was multilobulated, firm, and generally yellowish with hemorrhagic foci (Fig. 1A). No other masses were found in any other organs.

The mass was fixed in 10% neutral phosphate-buffered formalin and embedded in paraffin. Four-micrometer-thick sections were prepared on glass slides and stained with hematoxylin and eosin for light microscopic examination. Histochemical Masson's trichrome stain was utilized according to the protocols provided by the manufacturer. Immunohistochemical staining was performed on 4-μm-thick sections of formalin-fixed, paraffin-embedded tissue using the ABC method. Briefly, after deparaffinization and hydration, the tissue sections were treated with 0.03% H₂O₂ in methyl alcohol for 30 min at room temperature to inactivate endogenous peroxidase. Sections were then incubated with primary antibodies for vimentin (V9; Cell Marque, USA), desmin (Clone D33; Dako, USA), and alpha-smooth muscle actin (Clone 1A4; Dako) overnight at 4°C. The slides were then incubated in the appropriate biotinylated secondary antibodies for 40 min, followed by incubation with ABC reagent for 30 min at room temperature (VECTASTAIN ABC Kit; Vector Laboratories, USA). The positive reaction was visualized by DAB substrate for 2 min, and the sections were then counterstained with Mayer's hematoxylin.

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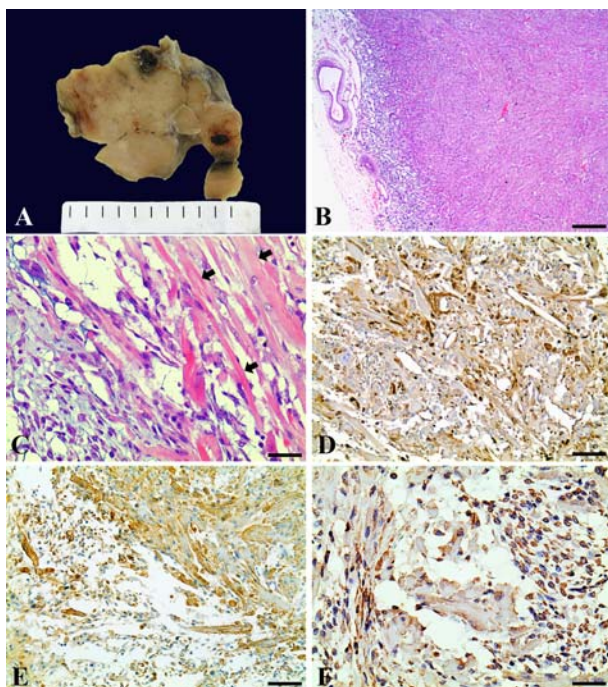


Fig. 1. Gross morphology (A), Histopathology (B and C), and Immunohistochemistry for vimentin (D), desmin (E) and α -smooth muscle actin (F). H&E staining (B and C), and ABC method (D-F). Scale bars = 250 μ m (B), 50 μ m (D and E), 25 μ m (C and F).

Histologically, the tumor comprised multiple nodules of spindle-shaped neoplastic cells that were incompletely encapsulated within fibrous connective tissue (Fig. 1B). At the interface with the abdominal musculature, the border of the tumor was indistinct because the tumor cells invaded and were admixed with normal skeletal fibers. There were extensive areas of neoplastic cells that varied in shape from spindle to globoid with abundant eosinophilic cytoplasm, admixed with other spindle-shaped neoplastic cells with filamentous appearance of basophilic scanty cytoplasm arranged in a fascicular pattern (Fig. 1C). In several areas, the small spindle shaped neoplastic cells were predominant with a loose myxoid arrangement (left low part of Fig. 1C). These neoplastic cells had variously sized single or multiple, round or elongated nuclei with one or two prominent nucleoli. With both Masson's trichrome staining and hematoxylin and eosin staining, cross-striations were evident in the cytoplasm of the well-differentiated neoplastic cells (arrow in Fig. 1C), representing rhabdomyocytic differentiation. In the immunohistochemical staining, the neoplastic cells were positive for vimentin and desmin (Fig. 1D and E). On the other hand, alpha-smooth muscle actin, known as the actin isoform of smooth muscle, was expressed in the small cells with filamentous feature of cytoplasm, in general, and often in some neoplastic cells among the more differentiated tumor cells (Fig. 1F).

The neoplastic cells were negative with Masson's trichrome

stain in contrast to the blue dyed collagen fibers between the tumor cells (data not shown). The mitotic index, calculated in 10 high-power fields (HPF; $\times 400$ fields), was 0.1/HPF.

According to the histological, histochemical, and immunohistochemical results, this case was diagnosed as an embryonal type of RMS.

RMS is classified into four types in domestic animals and human based on histological features: embryonal, botryoid, alveolar and pleomorphic. Embryonal RMS is characterized by loose and dense cellularity involving irregularly shaped, primitive mesenchymal-like cells with a round euchromatic nucleus and cytoplasmic processes. In this type myogenesis of serial stages of differentiation is observed from undifferentiated mesenchymal cells to elongated myoblasts and fully differentiated muscle fibers [9, 12]. It is another characteristics of embryonal RMS that high percentage of the cases express alpha-smooth muscle actin in an association with differentiation stage [3, 8], as also evidenced in this case. Botryoid RMS has a grape-like myxoid and lobulated gross morphology, and the neoplastic cells proliferate into the lumen of tubular organs, such as the bladder, bile duct, vagina, or conjunctiva, as multinodular excrescences of various size [12]. On the other hand, alveolar RMS consists of indistinguishable and undifferentiated small round to oval cells surrounded by fibrovascular septae, resembling floating cells in alveolar structures [12]. Pleomorphic RMS, also called anaplastic RMS, is characterized by high cellular nuclear pleomorphism. In this type, proliferating anaplastic cells are haphazardly arranged to form clusters or are interlacing [1, 5]. Among the subtypes of RMS, the present case was diagnosed as embryonal type based on the histological features. This case was unusual in that the tumor developed in the intra-abdominal region of an aged rat. Embryonal RMS usually occurs on the neck and head at a young age in domestic animals and humans [5, 11], and there has been only one reported case in laboratory animals [6]. The cellular morphology and arrangement patterns of neoplastic cells were similar to those in the reported domestic animal and human cases [9, 10]. This is a rare case of embryonal RMS spontaneously developed in the abdominal muscle of a laboratory rat.

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References

1. Bisby TM, Pratt SM, Fenton RK, Baird AN, Thompson CA, Lin TL. What is your diagnosis? Perifemoral mass in a cow. *Vet Clin Pathol* 2009, **38**, 343-347.
2. Chang SC, Inui K, Lee WC, Hsuan SL, Chien MS, Chen CH, Chang SJ, Liao JW. Spontaneous rhabdomyosarcoma in a young Sprague-Dawley rat. *Toxicol Pathol* 2008, **36**, 866-870.
3. Clément S, Orlandi A, Bocchi L, Pizzolato G, Foschini

- MP, Eusebi V, Gabbiani G.** Actin isoform pattern expression: a tool for the diagnosis and biological characterization of human rhabdomyosarcoma. *Virchows Arch* 2003, **442**, 31-38.
4. **Conner MW.** Spontaneous rhabdomyosarcoma in a young Sprague-Dawley rat. *Vet Pathol* 1994, **31**, 252-254.
 5. **Cooper BJ, Valentine BA.** Tumors of muscle. In: Meuten DJ (ed.). *Tumors in Domestic Animals*. 4th ed. pp. 319-363, Iowa State University Press, Ames, 2002.
 6. **Germann PG, Werner M, Schäfer HJ, Hübel U.** Embryonal rhabdomyosarcoma in a 7-month-old Sprague-Dawley rat. *Vet Pathol* 1994, **31**, 272-274.
 7. **Haseman JK, Hailey JR, Morris RW.** Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. *Toxicol Pathol* 1998, **26**, 428-441.
 8. **Hicks J, Flaitz C.** Rhabdomyosarcoma of the head and neck in children. *Oral Oncol* 2002, **38**, 450-459.
 9. **Kaplan AM, Creager AJ, Livasy CA, Dent GA, Boggess JF.** Intra-abdominal embryonal rhabdomyosarcoma in an adult. *Gynecol Oncol* 1999, **74**, 282-285.
 10. **Kato Y, Notake H, Kimura J, Murakami M, Hirata A, Sakai H, Yanai T.** Orbital embryonal rhabdomyosarcoma with metastasis in a young dog. *J Comp Pathol* 2012, **147**, 191-194.
 11. **Murakami M, Sakai H, Iwatani N, Asakura A, Hoshino Y, Mori T, Yanai T, Maruo K, Masegi T.** Cytologic, histologic, and immunohistochemical features of maxillofacial alveolar rhabdomyosarcoma in a juvenile dog. *Vet Clin Pathol* 2010, **39**, 113-118.
 12. **Parham DM.** Pathologic classification of rhabdomyosarcomas and correlations with molecular studies. *Mod Pathol* 2001, **14**, 506-514.
 13. **Stewart H.** Tumours of the soft tissue. In: Turusov VS (ed.). *Pathology of Tumours in Laboratory Animals: Tumours of the Mouse*. Vol. 2. pp. 487-526, IARC Scientific Publications, Lyon, 1979.
 14. **Walsh KM, Poteracki J.** Spontaneous neoplasms in control Wistar rats. *Fundam Appl Toxicol*. 1994, **22**, 65-72.