原　著

Functional Malignant Sertoli Cell Tumor with Massive Metastasis in the Retroperitoneum in a Dog

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Abstract : A male, mixed breed, over 8 years old dog had a mass palpated in the right cryptochidism located at the inguinal subcutaneous region. At necropsy another large mass was observed in the retroperitoneum. The masses in the right testis and in the retroperitoneum were approximately 7.5 cm and 10.0 cm in diameter, respectively. Histopathological examination revealed that both masses were composed of polygonal cells with moderate to high grade atypia. The tumor cells formed alveolar or cord structures separated by fibrous stroma, and showed a tendency to orient perpendicularly to the basement membrane. Immunohistochemically, the tumor cells were positive for vimentin, inhibin-α and Wilms' tumor-1. The testis was diagnosed with malignant Sertoli cell tumor, and the mass in the retroperitoneum was the metastasis from primary testicular tumor. In addition, marked hypoplasia of hematopoietic cells in the bone marrow, squamous metaplasia in the prostate and the gynecomastia were observed. It was suggested that the toxicity of hormones, such as estrogen and others, produced from the functional malignant Sertoli cell tumors was responsible for these lesions.

Key words : functional sertoli cell tumor, metastasis, dog

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Introduction

According to the World Health Organization (WHO) classification of tumors of domestic animals, there are three major types of testicular tumors in dogs: sex cord stromal tumors, germ cell tumors, and mixed germ cell-sex cord stromal tumors[6)].

The most common types of testicular tumors in dogs are Sertoli cell tumor and interstitial (Leydig) cell tumor in sex cord stromal tumors, as well as seminoma in germ cell tumors[3)], which have been reported to occur with approximately same frequency[4)]. The majority of these tumors are benign, and metastatic tumors are described rarely and in most cases are seminomas[6,13)].

Immunohistochemistry is currently included in the routine diagnostic protocol for testicular tumors in order to differentiate cases in human medicine[14)]. It has been also suggested that immunohistochemistry could be an important tool for the evaluation of canine testicular tumors[5,8)].

In this case, the dog with remarkable cytopenia and right testicular tumor had a large mass in the retroperitoneum which was detected for the first time at necropsy. We examined histologically and immunohistologically about the dog in order to confirm the origin of two masses.

Materials and Methods

In an intact male Japanese Shiba mixed dog, over 8 years old (exact age was unknown because it was the sheltered), a mass was found at the first medical examination for castration. It existed in the right cryptorchidism located at the inguinal subcutaneous region. Size of the mass was about 4 cm (accurate size was not available). The castration and surgical treatment for mass were not performed, because it had showed hematologic abnormalities including

thrombocytopenia and leukopenia (Table 1). Estrogen and testosterone level in blood was examined 11 months before the death, and they were within the normal range (Table 1). At that time, the abnormality associated with tumor was not recognized other than the cytopenia. The dog died unexpectedly 14 months after the first examination, and necropsy was performed at the Nippon Veterinary and Life Science University.

Tissues were fixed in 10 % neutral-buffered formalin and routinely embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin (HE). Serial sections were subjected to immunohistochemistry (IHC) using the Envision system™ (Dako, Glostrup, Denmark), with mouse monoclonal antibodies specific for human pan-cytokeratin (clone AE1/AE3, 1 in 300 dilution; Dako), human vimentin (clone V9, 1 in 500 dilution; Dako), human Melan-A (clone A103, 1 in 50 dilution; Dako), human inhibin-α (clone R1, 1 in 50 dilution; AbD Serotec, Oxford, UK.), humanWilms' tumor-1 (WT1) (clone 6F-H2, 1 in 50 dilution; Dako)[5,8]. For antigen retrieval, the sections were pre-treated at 121 ℃ for 10 min in citrate buffer (pH6.0) for pan-cytokeratin, vimentin, Melan-A, inhibin-α, or in Tris-EDTA buffer (pH9.0) for WT1. For negative control, normal mouse IgG (Dako) was used instead of the each antibodies.

Result

Macroscopically, the right cryptorchidism showed enlarged (7.5 × 5.0 × 4.2cm) bulging sphere and the cut surface revealed white color and multiple nodules. The left testis showed moderately atrophy (2.5 × 1.5 × 1.0cm) (Fig. 1a, b). A large mass (10.0 × 10.0 × 7.0cm) in the retroperitoneum widely covered the right kidney over the caudal to the abdominal cavity side (Fig. 1c). The right kidney and both sides of adrenal gland were attached to the surface of the mass, and these border were clear. The cut surface revealed solid and spongiform with muddy hemoid liquid exuded partly (Fig. 1d).

Histologically, the testicular tumor was composed of elongate, polygonal to spindle cells with small, round to elongate nuclei which revealed moderate to high grade atypia. They were arranged in alveolar or cord

Table 1. Hematological data

Parameter	Reference values	14 months[1] (first examination)	11 months[1]	1 week[1]
Erythrocytes ($10^6/\mu l$)	5.5 ～ 8.5	5.8	1.4	5.9
Hemoglobin (g/dl)	12 ～ 18	11.2	3.2	11.7
Leucocytes (/μl)	6,000 ～ 17,000	4,300	1,800	2,500
Platelets ($10^3/\mu l$)	175 ～ 400	1.0	0.5	0.1
Estradiol Ⅱ (pg/ml)	＜15.0 (male)	NA[2]	9.71	NA
Testosterone (ng/ml)	＜4.47 (intact male)	NA	1.76	NA

[1] Period before the dog death
[2] NA: Not available

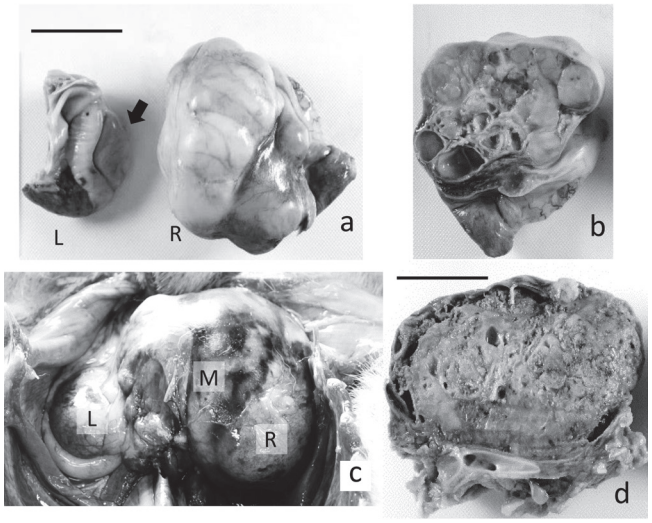


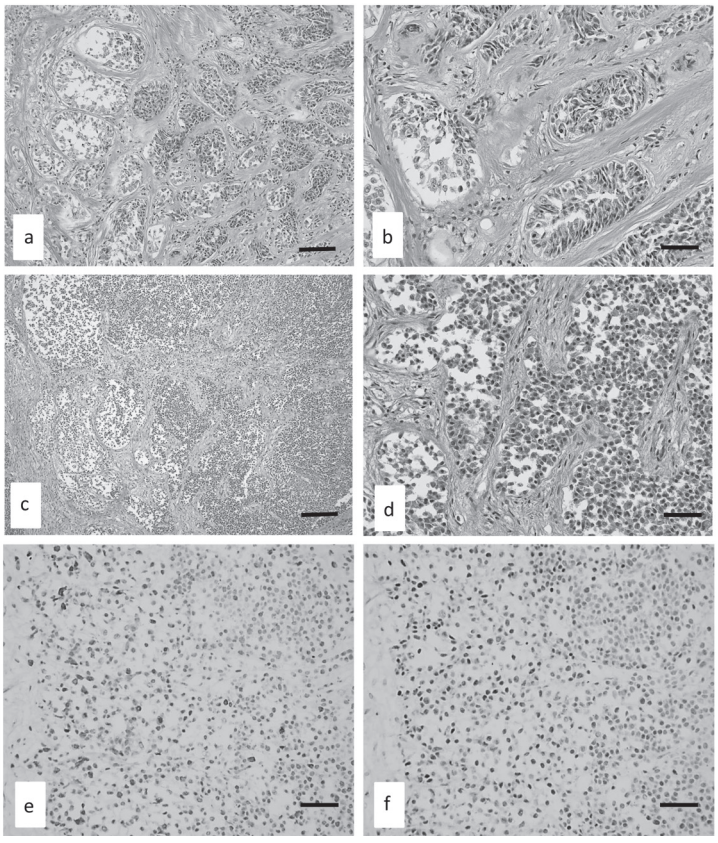
Fig. 1 Macroscopic findings. (a) Left (L) testis (arrow) is moderately atrophic. Right (R) inguinal cryptochidism is enlarged. (b) The cut surface of the right testis reveales white and lobulation by stroma. (c) A large mass (M) and left (L) and right (R) kidney in the retroperitoneum at necropsy. The mass widely covers the right kidney. (d) The cut surface of the mass showed in (c). The surface reveales solid and spongiform with muddy hemoid liquid exuded partly. Bar, 5.0 cm.

structures dissected and surrounded by abundant fibrous stroma. They showed partly a tendency to orient perpendicularly to the basement membrane (Fig. 2a, b). Focal necrosis was observed in the tumor. The vascular invasion was found around the tumor. The tumor cells of the retroperitoneum were similar to the testicular tumor cells, but they revealed higher malignancy and lower differentiation than the testicular ones ; they were smaller and polygonal form and had lower tendencies to orient perpendicularly to the basement membrane (Fig. 2c, d). The frequency of mitotic figures per high-power (×400) field was 2-3 in the testicular tumor and 4-5 in the retroperitoneal tumor. Except retroperitoneal mass, the metastases were not observed.

IHC revealed that the tumor cells were strong positively for vimentin, partly positive for inhibin-α and WT1 (Fig.2e, f). The testicular tumor cells were partly weak positive, and the tumor cells of the retroperitoneum were negative for Melan-A. The tumor cells were negative for pan-cytokeratin.

The marked hypoplasia of hematopoietic cells was observed in bone marrow of the sternum. Especially, myelocytes and megakaryocytes were hardly found. The invasion of adipose cells was significantly found in the bone marrow cavity (Fig. 3). The low formation of the white pulp was observed in spleen. In addition, the characteristic features of the paraneoplastic syndrome were confirmed in other organs. In prostate, the prominent squamous metaplasia of the columnar epithelium in acinus with the inflammatory cells, neutrophils and plasma cells, and the stromal hyperplasia were found. In mammary glands, large acini lined with enlarged columnar epithelium were observed. The acini were often expanded, and lactescent secretion and foamy macrophages were found in the lumen.

In the organ except the above, significant pathological changes were not confirmed. In the right and left adrenal gland and right kidney adhered to retroperitoneal tumor, histological abnormalities were not observed. The cause of sudden death of the dog was considered that



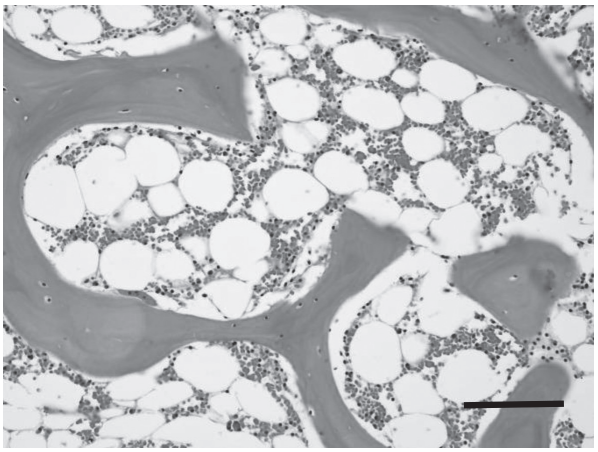


Fig. 3 Hypoplasia of hematopoietic cells in the sternum bone marrow. Myelocytes and megakaryocytes are hardly observed. Bar, 100 μm.

Fig. 2 (a) Tumor cells of the right inguinal cryptochidism. HE. Bar, 200 μm. (b) High-power image of the testicular tumor. HE. Bar, 50 μm. (c) Tumor cells of the mass in retroperitoneum. Tumor cells resemble to testicular tumor cells with higher malignancy. HE. Bar, 200 μm. (d) High-power image of the retroperitoneal tumor. Cells of the right area have higher malignancy. HE. Bar, 50 μm. (e) Tumor cells are partly positive for inhibin-α. The higher malignant tumor cells are not labeled. IHC. Bar, 50 μm. (f) Tumor cells are partly positive for WT1. IHC. Bar, 50 μm.

circulatory disturbance due to long-term anemia.

Discussion

In this case, large tumor mass in the retroperitoneum which was found in the first time at necropsy. The different diagnosis for this tumor included primary testicular tumor arising from the kidney, adrenal glands, or lymph nodes and metastasis from the right testicular tumor. So, the histological and immunohistochemical characteristics were examined and compared between the right testicular and retroperitoneal tumor.

Histologically, the morphological characteristic of the retroperitoneal tumor cells were similar to testicular ones. Immunohistochemically, both tumor cells were positive for vimentin, and partly positive for inhibin-α and WT1. Inhibin-α, a peptide hormone that suppresses follicle-stimulating hormone, is immunohistochemically detected only in Sertoli and Leydig cells in canine testis[1,8]. WT1 has been identified as a tumor suppressor gene responsible for pediatric renal tumors, and recently known as a transcriptional factor in various kinds of tumors. In human, WT1 is detected in Sertoli-Leydig cell tumors[11]. Considering histological and immunohistochemical features, it was strongly suggested that the mass in the retroperitoneum was metastatic lesion from the testicular primary tumor; malignant Sertoli cell tumor in intratubular to diffuse form[6].

Excessive estrogen from testicular tumors is reported to cause the paraneoplastic syndrome, the feminizing syndrome and cytopenia in dogs[3,7,9]. Estrogen myelotoxicosis is descrived to occur in 15 % of dogs with Sertoli cell tumor presenting with male feminizing syndrome[10] and hormone production is generally proportional to tumor size[10,12]. Interestingly, at 11months before of the death when the dog had already shown the cytopenia, but the serum estrogen was within the normal level. The mechanism of the paraneoplastic syndrome by Sertoli cell tumor is not fully understood. Moreover, it is not proven that estrogen solely is responsible for all sign of the syndrome including feminization and bone marrow hypoplasia. It has been suggested that other secretory products from the tumor cause this syndrome[3,10,12]. In this case, although the measurement of the inhibin level was not performed, testicular tumors and the metastatic lesion expressed inhibin-α immunohistochemically. Inhibin or other hormones produced from the two tumors might have effect on the paraneoplastic syndrome, at least cylopenia.

The metastasis of malignant Sertoli cell tumors, including in previously neutered dogs, have been reported[2,15]. However, the size of almost all metastasis descrived was about less than 5cm in diameter. This dog might be rare case of functional malignant Sertoli cell tumor with a massive metastasis in the retroperitoneum.

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後腹膜腔への巨大な転移腫瘤を伴った機能性悪性セルトリ細胞腫の犬の１例

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要　　約

著しい血球減少症を示し，右鼠径部に腫大した潜在精巣を有していた雄雑種犬に，剖検時に右精巣のみならず後腹膜腔にも巨大な腫瘤が認められた。右精巣腫瘤は直径 7.5 cm，後腹膜腫瘤は直径 10.0 cm の大きさであった。両腫瘤では中等度から高度の異型を示す多角形腫瘍細胞が，胞巣ないし索状構造を形成しながら増殖し，腫瘍細胞は胞巣の壁に柵状に配列する傾向を示していた。免疫組織化学的検索において，腫瘍細胞は vimentin，inhibin-α，WT1 に陽性を示した。以上より，右精巣腫瘍は悪性セルトリ細胞腫であり，後腹膜腫瘤は精巣腫瘍の転移と考えられた。また，骨髄における顕著な造血細胞低形成，乳腺の雌性化，前立腺における扁平上皮化生が認められたことから，本症例はホルモン産生性の機能性悪性セルトリ細胞腫と考えられた。

キーワード：イヌ，骨髄低形成、セルトリ細胞腫

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