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NOTE

Pathology

Leydig cell tumor in an Amur tiger (*Panthera tigris altaica*)

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ABSTRACT. A 14-year and 8-month-old intact male Amur tiger presented with an enlarged left testis, measuring 5.7 × 5.5 × 4.5 cm. The cut surface was mottled dark red to reddish brown in color. Microscopically, the enlarged left testis comprised round or polygonal neoplastic cells arranged in a diffuse sheet pattern. These neoplastic cells had a hyperchromatic nucleus and an abundant eosinophilic cytoplasm. Immunohistochemically, these neoplastic cells were positive for vimentin, chromogranin A, synaptophysin, melan-A, inhibin-α, and S100 and negative for desmin and WT-1. Based on these morphological and immunohistochemical findings, the tumor was diagnosed as a Leydig cell tumor.

KEY WORDS: Amur tiger, Leydig cell tumor, testis

The Amur tiger, also known as Siberian tiger, is a population of *Panthera tigris tigris* in the Far east which is critically endangered. Therefore, it is necessary for zoos to develop effective reproduction methods, such as spermatic cryopreservation and artificial insemination, and contribute to conservation [7]. In domestic animals, testicular tumors are categorized into germ cell tumors, such as seminoma and teratoma, and sex cord-stromal tumors, such as Sertoli cell and Leydig cell tumors [1]. These are few reports on cases of testicular tumors in the Felidae family, including the cat, jungle cat, snow leopard and Amur tiger [5, 11, 14, 15, 17]. So far, Sertoli cell tumor is the only testicular tumor reported in Amur tiger [15]. To the best of our knowledge, there are no reports describing Leydig cell tumor in Amur tigers. In this report, we describe the histological and immunohistochemical features of a Leydig cell tumor in an Amur tiger.

A 14-year and 8-month-old intact male Amur tiger that had been kept at the Osaka Municipal Tennoji Zoological Gardens, Osaka, Japan, presented with an enlarged left testis over a 5-month period. Physical examination revealed no other abnormalities. In addition, complete blood count and routine serum biochemical profile were normal. Detailed radiographical and ultrasound examinations revealed no significant lesions in the thoracic and abdominal cavities. The tiger had no previous breeding experience. Subsequently, the bilateral testes and epididymides were surgically excised and submitted to the Nippon Veterinary and Life Science University, Tokyo, in low-temperature condition for histopathological examination and spermatic conservation. Amoxicillin was orally administered for 2 days after the surgical excision to prevent infection. Sperm was collected from the bilateral caudal portion of the epididymides using the flushing method [9]. Total sperm counts in the right and left epididymides were 283.5 × 10^6 and 1.26 × 10^6, respectively. Sperm progressive motility and sperm survival rates were 55.0 and 88.0%, respectively, in the right and 50.0 and 82.0%, respectively, in the left epididymis. No physical abnormality was noted after 9 months.

The testes were fixed in 10% neutral-buffered formalin, routinely processed, and embedded in paraffin wax. Sections (4 μm) were stained using hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) and were subjected to immunohistochemistry using the labeled streptoavidin–biotin method with primary mouse antibodies specific for vimentin (1:1,500; Dako, Glostrup, Denmark), cytokeratin (CK) AE1/AE3 (1:200; Dako), desmin (1:200; Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.), melan-A (1:200;

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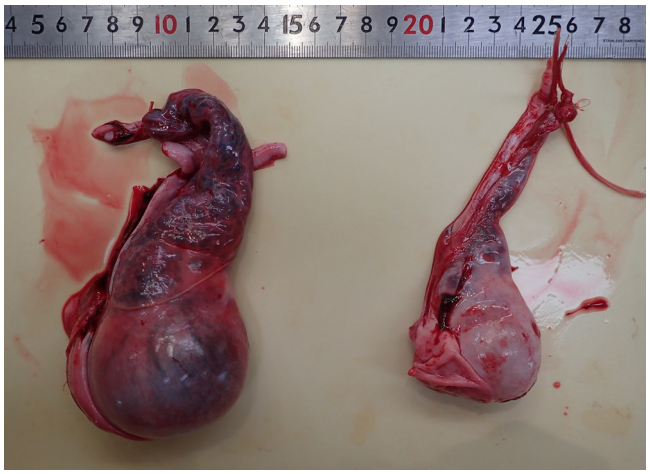

Fig. 1. Bilateral testes. Left, enlarged left testis. Right, right testis.

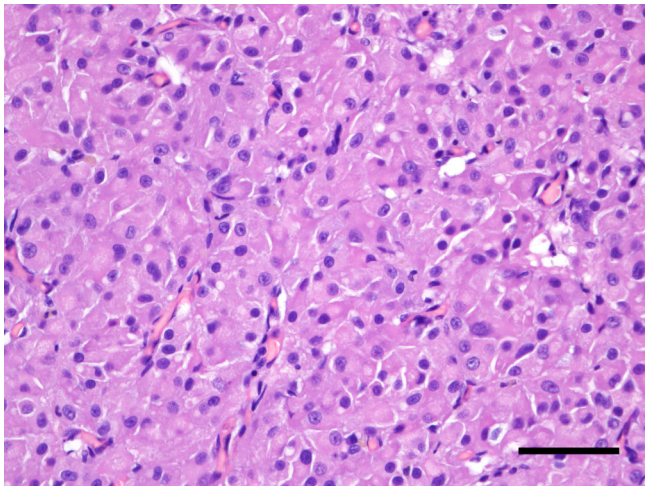

Fig. 2. Leydig cell tumor in an Amur tiger. Neoplastic cells arranged in a diffuse sheet pattern. Neoplastic cells have round nucleus and abundant eosinophilic cytoplasm. HE. Bar=50 μm.

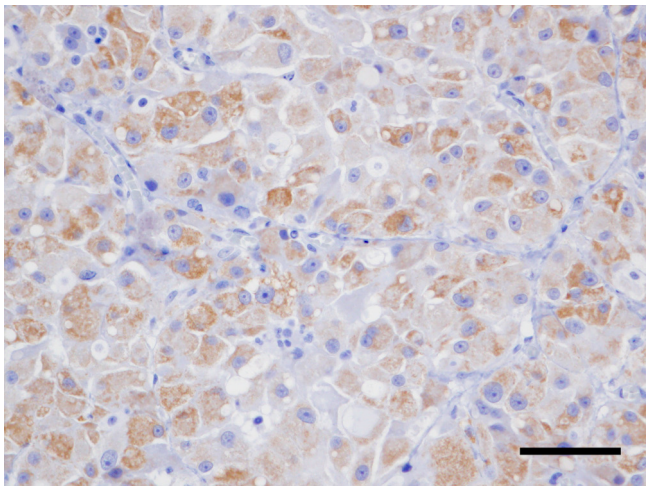

Fig. 3. Leydig cell tumor in an Amur tiger. Neoplastic cells expressing melan-A. Immunohistochemistry. Bar=50 μm.

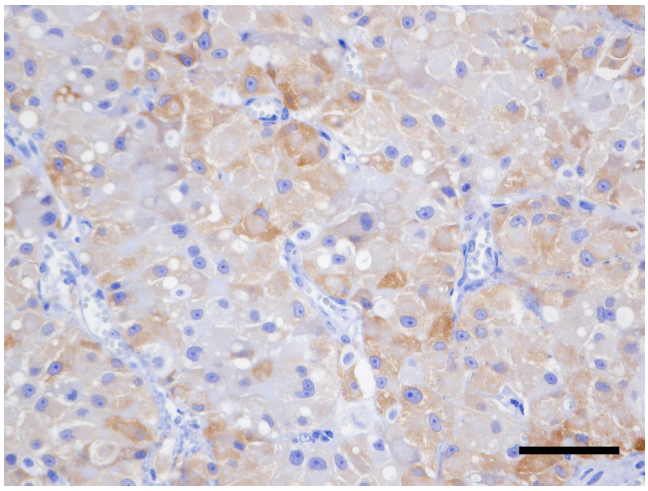

Fig. 4. Leydig cell tumor in an Amur tiger. Neoplastic cells expressing inhibin-α. Immunohistochemistry. Bar=50 μm.

Thermo Fisher Scientific Inc.), Wilm's tumor-1 (WT-1; 1:50; Dako), calretinin (prediluted; Nichirei, Tokyo, Japan), neuron-specific enolase (NSE; 1:200; Dako), and polyclonal antibodies specific for inhibin-α (1:100; AbD Serotec, Oxford, U.K.), S100 (1:1,500; Dako), synaptophysin (1:200; Dako), chromogranin A (1:1,000; Immunostar, Hudson, WI, U.S.A.), and c-kit (1:1,000; Dako). For antigen retrieval, the sections were pretreated at 121°C for 15 min in citrate buffer (pH 6.0) for CK AE1/AE3, vimentin, synaptophysin, S100, chromogranin A, desmin, inhibin-α, NSE, c-kit, calretinin, and melan-A and in Tris-EDTA buffer (pH 9.0) for WT-1. The antibodies used were validated by obtaining a positive reaction with the normal right testis of this case and a negative reaction on replacement of the antibody with normal mouse or rabbit immunoglobulins.

Grossly, the sizes of the left and right testes were 5.7 × 5.5 × 4.5 cm and 4.0 × 3.3 × 2.2 cm, respectively (Fig. 1). The cut surface of the enlarged left testis was mottled dark red to reddish brown in color, whereas that of the normal right testis was uniformly yellowish brown in color. Microscopically, the enlarged left testis was found to comprise a large nodule, measuring approximately 4 cm in diameter, encapsulated by a delicate fibrovascular tissue. The large nodule was composed of round or polygonal neoplastic cells arranged in a diffuse sheet pattern. These neoplastic cells had a hyperchromatic nucleus and an abundant eosinophilic cytoplasm (Fig. 2). The number of mitotic figures was 2 per 10 high-power (400×) fields. Moreover, multifocal areas of hemorrhage in the neoplastic tissue and moderate atrophy of seminiferous tubules surrounding the neoplastic tissue were observed. In the right testis, Leydig cell hyperplasia and mild atrophy of seminiferous tubules were observed.

Immunohistochemically, the neoplastic cells were positive for vimentin, chromogranin A, synaptophysin, melan-A (Fig. 3), inhibin-α (Fig. 4), and S100 and negative for CK AE1/AE3, desmin, and WT-1. Normal Leydig cells were positive for vimentin,

chromogranin A, melan-A, and S100 and negative for inhibin-α, synaptophysin, desmin WT-1, and CK AE1/AE3, whereas normal Sertoli cells were positive for vimentin and WT-1 and negative for inhibin-α, synaptophysin, melan-A, desmin, and S100.

Based on the morphological and immunohistochemical findings, a diagnosis of Leydig cell tumor was finally established. According to the WHO classification of tumors in domestic animals, Leydig cell tumors are histologically categorized into three types: solid-diffuse, cystic-vascular, and pseudoadenomatous [10]. This case corresponded to the solid-diffuse type. In humans, Leydig cell tumors are often characterized by cytoplasmic inclusions (Reinke crystals), which are strongly PAS-positive and are significant for diagnosing Leydig cell tumors. However, no Reinke crystals were observed in this case.

As testicular tumors of animals can be morphologically diagnosed without using immunohistochemistry, the immunohistochemical features of such tumors are not well characterized. However, immunohistochemistry is potentially a useful tool for characterizing testicular tumors [1]. Therefore, it is crucial to immunohistochemically characterize testicular tumors, including Sertoli cell tumors, Leydig cell tumors, seminoma, and mixed germ cell sex cord-stromal tumors, and normal tissues of Felidae animals to understand the development of testicles and the immunostaining properties of different tumors. In domestic animals, sex cord-stromal tumors exhibit positivity not only for endocrine markers, such as chromogranin A, NSE, and inhibin-α, but also for vimentin, melan-A, and S100 [1, 15]. However, immunohistochemical characterization of Leydig cells of normal and neoplastic tissues in Amur tigers remains unclear. In dogs, a variety of markers are useful to well characterize these cells in normal and neoplastic tissues. Neoplastic Leydig cells express vimentin, S100, melan-A, inhibin-α, calretinin, and c-kit but not cytokeratin, desmin, and PGP9.5, similar to normal Leydig cells [4, 8, 12, 13]. Neoplastic Sertoli cells express c-kit, E-cadherin, and desmin in addition to vimentin, calretinin, inhibin-α, and melan-A, which are also expressed in neoplastic Leydig cells [2, 6, 12]. In cats, sex cord-stromal tumors, both Sertoli cell and Leydig cell tumors, express vimentin and NSE [3, 11]. Melan-A, S100, and PGP9.5 are variably expressed in normal and neoplastic Leydig cells, whereas calretinin and cytokeratin AE1/AE3 are not [11, 17]. In a previous case report of feline Sertoli cell tumor, neoplastic cells were negative for melan-A [11]. Together with the results of the present study, melan-A may be a useful diagnostic marker for identifying Leydig cell tumors not only in the cat but also in other Felidae species including the tiger. NSE, calretinin, and c-kit have been used in diagnosing canine and feline testicular tumors, although the expression pattern of calretinin differs among feline and canine sex cord-stromal tumors, in particular, Leydig cell tumors [8, 11]. Unfortunately, in this study, the antibodies, such as NSE, calretinin, and c-kit, did not cross-react in the normal and tumor tissues (data not shown). Inhibin-α, a gonadal protein synthesized by Sertoli and Leydig cells, is involved in suppressing the secretion of follicular-stimulating hormone from the pituitary gland [4, 16]. In addition to normal Sertoli and Leydig cells, this protein is also expressed in neoplastic sex cord-stromal tumor cells [4]. In this case, although diffuse expression of inhibin-α was observed in neoplastic Leydig cells, no expression of inhibin-α was observed in normal Leydig and Sertoli cells, and the mechanism underlying this phenomenon remains unclear.

In conclusion, to the best of our knowledge, this is the first report of a Leydig cell tumor in an Amur tiger. It provides insights into the morphological and immunohistochemical features of Leydig cell tumor of Amur tigers.

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