J Toxicol Pathol 2022; 35: 263–268

Case Report

A case of spontaneous rete testis adenoma in a Sprague–Dawley rat

Masako Imaoka[1*], Tetsuya Osawa[1], Kiyonori Kai[1], and Yoshimi Tsuchiya[1]

[1] Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan

Abstract: A 104-week-old male CD (SD) rat exhibited enlargement of the left testis. Microscopically, this mass was demarcated from the testis by fibrous connective tissue and characterized by cystic dilatation with single-layered columnar cells and papillary proliferation connected to the solid growth area without clear boundaries. In the solid growth area, cells were dissected into irregular alveolar nests by scant fibrous tissue with small blood vessels. The nuclei of proliferating cells were variable in size and round- to oval-shaped, and their cytoplasm was pale or eosinophilic and sometimes contained vacuoles or eosinophilic granules. Immunohistochemically, the tumor cells were positive for vimentin and cytokeratin (CK) 7. Since CK7 was exclusively positive in the rete testis epithelium of the naïve rat, it was valuable to diagnose this tumor as rete testis-originated. Based on these results and the lack of apparent pleomorphism, mitotic figures, and metastasis, the present case was diagnosed as rete testis adenoma. (DOI: 10.1293/tox.2022-0018; J Toxicol Pathol 2022; 35: 263–268)

Key words: Sprague–Dawley rat, rete testis adenoma, cyst formation, spontaneous tumor

Spontaneous rete testis tumors are rarely observed in rodents. Its incidence has been reported to be 1/5,000 (0.02%) or 3/51,230 (0.006%) in Fischer rats[1, 2] and 2/500 in ICR mice[3]. All these spontaneous tumors were diagnosed as rete testis adenocarcinoma, and no cases have been reported in Sprague–Dawley (SD) rats. In this study, we report the first case of rete testis adenoma diagnosed by using histological and immunohistochemical analysis in an aged SD rat.

Initially, 50 male and 50 female CD(SD) rats acquired from Charles River Laboratories Inc. (Tokyo, Japan) were kept for background data collection without treatment with any compounds. Animals were housed in groups of three per wire mesh cage in an air-conditioned room (temperature, 23 ± 2 °C; relative humidity, 55 ± 20%) with a 12-h/12-h light/dark cycle and allowed free access to a commercial standard diet (CRF-1, Charles River Laboratories, Inc.) and chlorinated tap water during the experimental period. One male animal was found dead immediately before the planned autopsy at 104 weeks of age, without abnormal clinical signs before death. At necropsy, the left testis was found to be enlarged and collected. No adhesion was observed with the adjacent tissue and scrotum. Thymus atrophy and pituitary gland enlargement were also observed. The cause of death was not specified.

The testis was fixed in Bouin's solution and trimmed transversely. The section was embedded in paraffin, sectioned into 3-µm slices, and stained with hematoxylin and eosin and periodic acid-Schiff (PAS) reaction. Additionally, immunohistochemistry was performed using a two-step peroxidase 3,3′-diaminobenzidine staining technique with a DAKO EnVision+ system (Dako, Agilent Technologies Inc., Tokyo, Japan) according to the manufacturer's instructions. Staining was performed using antibodies against vimentin, cytokeratin (CK) 7, calretinin, protein gene product 9.5 (PGP9.5), Iba-1, and alpha-fetoprotein (AFP). The sources of the antibodies and the staining results are presented in Table 1. All procedures were performed under the rules for animal experiments according to the Japanese guidelines for animal experiments (Science Council of Japan 2006) and "Regulations for the Use of Animals in Research" approved by the Daiichi Sankyo Institutional Committee of Animal Experiments.

The cut surface of the testis after fixation was white to milky white in color, and no distinct structure was observed, excluding cyst formation in the macroscopic mass. However, under a light microscope, the testicular tissue was found to be atrophied and crescent-shaped, and the mass was located between the testis and epididymis. The mass was demarcated from the surrounding tissue by fibrous connective tissue, but in the region close to the epididymis, the mass was still surrounded by fibrous tissue like tunica albuginea (Fig. 1a and 1b). The tumor was speculated to be located in the tunica albuginea, not in the testicular parenchyma or interstitium. The cystic dilated area was lined by a single layer of columnar epithelium, and the cells forming the pap-

Received: 9 February 2022, Accepted: 18 April 2022
Published online in J-STAGE: 7 May 2022
*Corresponding author: M Imaoka
(e-mail: imaoka.masako.hf@daiichisankyo.co.jp)

Table 1. Antibodies Used for Immunohistochemistry and Summarized Staining Results

Antibodies	Species and clonality (clone)	Source	Positively reacted components		
			Naïve SD rat	The present case	
			Testis	Testis	Tumor
Vimentin	Mouse mAb (V9)	Agilent (Dako)	Rete testis epithelium Sertoli cells Leydig cells Mesothelium Vascular endothelium Others	Sertoli cells Leydig cells Mesothelium Vascular endothelium Others	Tumor cells
Cytokeratin 7	Rabbit mAb (EPR17078)	Abcam	Rete testis epithelium Mesothelium	Mesothelium	Tumor cells
Calretinin	Mouse mAb (6B8.2)	Millipore	Leydig cells	Leydig cells	−
PGP9.5	Mouse mAb (13C4/I3C4)	Abcam	Sertoli cells	Sertoli cells	−
Iba-1	Rabbit pAb	Wako	Macrophages Sperm	Macrophages	−
AFP*	Mouse mAb (189502)	R&D Systems	−	−	−

PGP9.5: protein gene product 9.5; AFP: alpha-fetoprotein; mAb, monoclonal antibody; pAb: polyclonal antibody; −: negative.
Antigen retrieval using citric acid buffer solution (pH 9.0, 98 °C, 20 min) was performed for all stains. The secondary antibody was EnVision+ System-HRP Labelled Polymer (Dako, Agilent Technologies Inc.).
* A rat yolk sac specimen was used as the positive control.

illary stalk protruded into the inside of the cyst (Fig. 1c). In the center of the mass, cells proliferated with a solid growth pattern, and the boundaries between this area and the papillary proliferation area were unclear. In the solid growth area, cells were dissected into irregular alveolar nests by scant fibrous tissue with small blood vessels. Some duct-like structures were dilated and filled with blood or eosinophilic fluid. The nuclei of proliferating cells were variable in size and round to oval in shape, and their cytoplasm was pale or eosinophilic (Fig. 1d and 1e). In the solid area, some cells possessed cytoplasmic vacuoles or eosinophilic granules that were positively stained by PAS reaction (Fig. 1f). Pigment-laden macrophages and hemorrhage were found in the solid area, but the proliferating cells displayed few mitotic figures and no invasive growth toward the surrounding tissue. The displaced testis exhibited Sertoli-seminiferous tubules, cystic dilatation of the tubules, and mineralization.

Based on the aforementioned histopathological features, immunohistochemistry was performed using the antibodies listed in Table 1. First, the staining characteristics of each antibody in the testicular component of the naïve animal were tested because the present animal's paraffin block was stored for approximately 20 years and it was unclear whether the "correct" reaction was obtained. The section prepared from a 7-week-old male SD rat in the control group of the toxicology study was fixed with formalin–sucrose–acetic acid solution for 2 days. Positive reactions in the neoplastic cells were determined by comparing the naïve testis and atrophied testicular tissue in the present case. The results are presented in Table 1.

As shown in Fig. 2a and 2b, vimentin was positively stained in the rete testis, Sertoli cells, and Leydig cells in the 7-week-old testis. As a similar positive reaction was obtained in the testicular tissue of the present animal, the present staining condition was considered reliable. In tumor cells, vimentin showed a positive reaction in both papillary and solid growth areas (Fig. 2c and 2d). As for CK7, a specific reaction in the rete testis of the 7-week-old rat was observed (Fig. 3a). CK7 immunostaining was strongly positive in the cells lining the cyst and in most of the papillary areas. In the solid area, most tumor cells were negative or weakly positive, but strongly positive cells were sometimes observed (Fig. 3b and 3c). Staining for antibodies including calretinin, PGP9.5, and Iba-1 was negative in the neoplastic cells based on the corresponding results obtained in the 7-week-old testis and testicular tissue of the present case (Table 1 and Fig. 4). AFP was judged to be negative with reference to the positive control specimen, namely, the yolk sac from the placental tissue obtained from a pregnant SD rat (data not shown).

The most distinctive feature of the present case was that the mass was demarcated from the testis by fibrous connective tissue. Papillary proliferation by single-layered columnar cells was considered to originate from the cyst-lining epithelium and was connected to the solid growth area. Based on this positional information, we speculated that the origin of this tumor was the rete testis or seminiferous tubules connected to the rete testis. Immunohistochemical results for CK7 indicated that the tumor was of rete testis origin. Additionally, the tumor was not considered malignant because of the lack of apparent pleomorphism, mitotic figures, and invasive growth. The tumor was diagnosed as a rete testis adenoma.

The differential diagnoses may include mesothelioma, Sertoli cell tumor, and interstitial cell (Leydig cell) tumor. The papillary and solid growth of the tumor cells gave the impression of mesothelioma, and the irregular alveolar nests or vacuolated cytoplasm of neoplastic cells in the solid

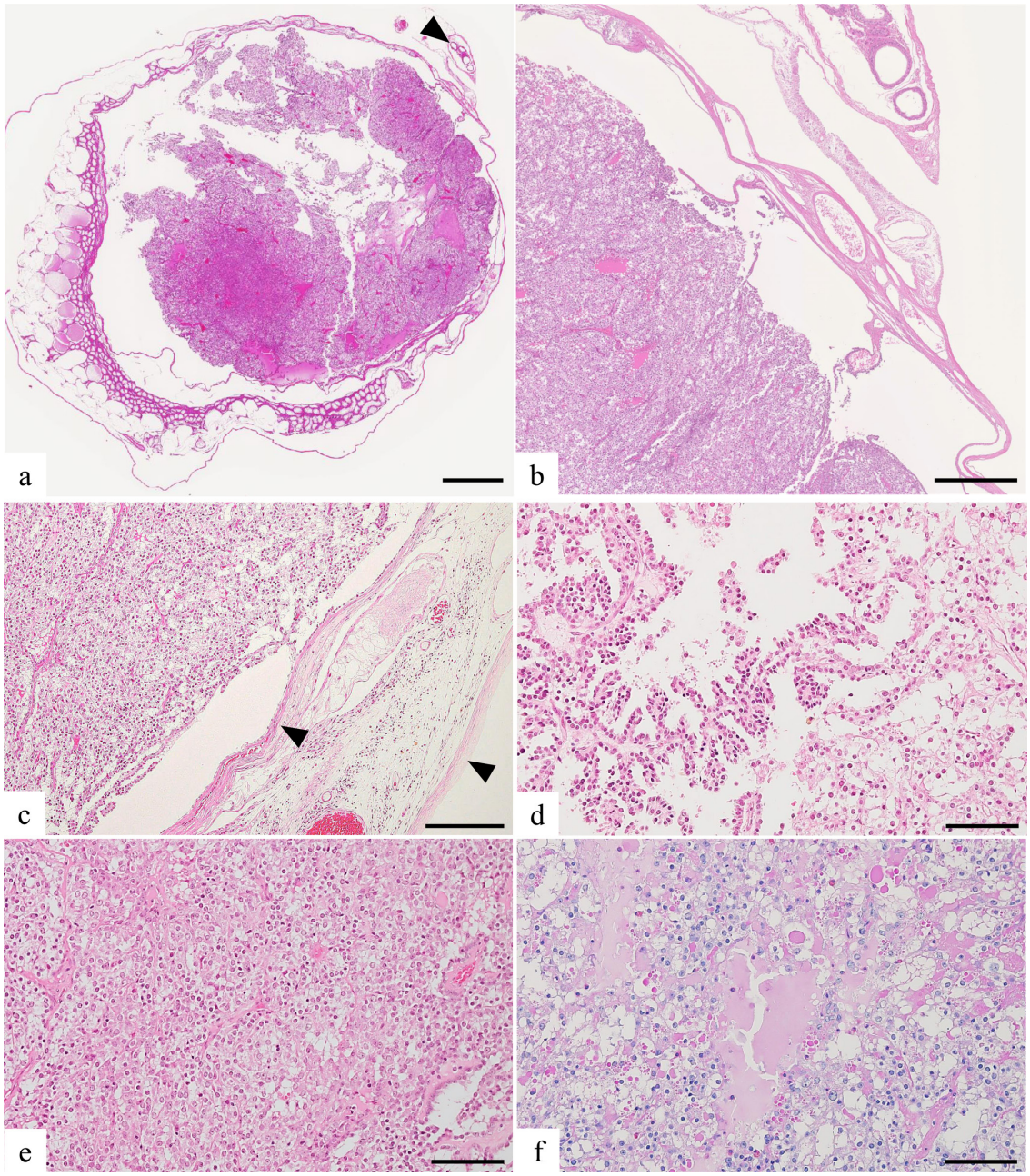

Fig. 1. Microscopic characteristics of the tumor. a) At lower magnification, the mass was demarcated from the testis by fibrous connective tissue. The atrophied testis was crescent-shaped and was observed around the mass. The epididymis was also identified (arrowhead). Hematoxylin and eosin (H&E) staining, bar=2 mm. b) In the region close to the epididymis, the mass was surrounded by fibrous tissue like tunica albuginea. H&E staining, bar = 500 µm. c) The cyst in the mass was lined by a single layer of columnar epithelium, and the cells forming the papillary stalk protruded into the inside of the lesion. The tumor was clearly demarcated with testicular tissue by the tunica albuginea located on both sides of the atrophied seminiferous tubules (arrowheads). H&E staining, bar=200 µm. d) Representative features of the papillary proliferation area. The boundaries of this area and the solid growth area were unclear. H&E staining, bar=100 µm. e) In the solid growth area, cells were dissected into irregular alveolar nests by scant fibrous tissue with small blood vessels. H&E staining, bar=100 µm. f) In the solid growth area, vacuoles or eosinophilic granules were sometimes present in the cytoplasm. The granules were positively stained by the periodic acid-Schiff reaction. Bar=100 µm.

growth area implied a Sertoli cell-originated tumor or Leydig cell tumor. Of these, mesothelioma was the most likely diagnosis according to the immunohistochemistry results. In rodents with mesothelioma, it is well known that the tumor cells express both vimentin and CKs (including CK7) when using a pan-CK antibody such as clone AE1/AE3[4, 5]. Moreover, specific expression of CK7 has been reported in human mesothelioma[6]. However, these immunohistochemi-

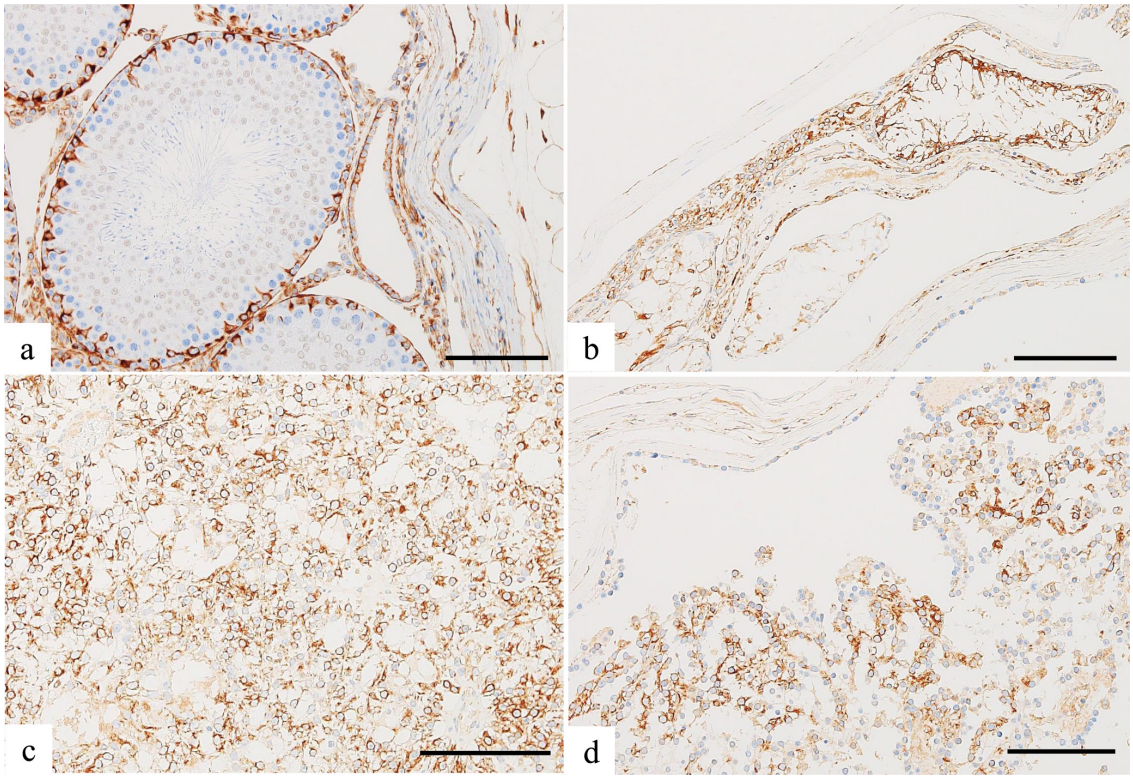

Fig. 2. Immunohistochemistry for vimentin in an intact testis and in the present case. a) The rete testis and seminiferous tubule of a 7-week-old rat. The cytoplasm of the rete testis, Sertoli cells, and Leydig cells was positive for vimentin. Bar=100 µm. b) The atrophied seminiferous tubule in a section of the present lesion. Similar staining specificity was observed in a 7-week-old rat. Bar=100 µm. c) Tumor cells showed positive reaction in the solid growth area in the present case. Bar=100 µm. d) In the papillary growth area, positive reaction was obtained diffusely. Bar=100 µm.

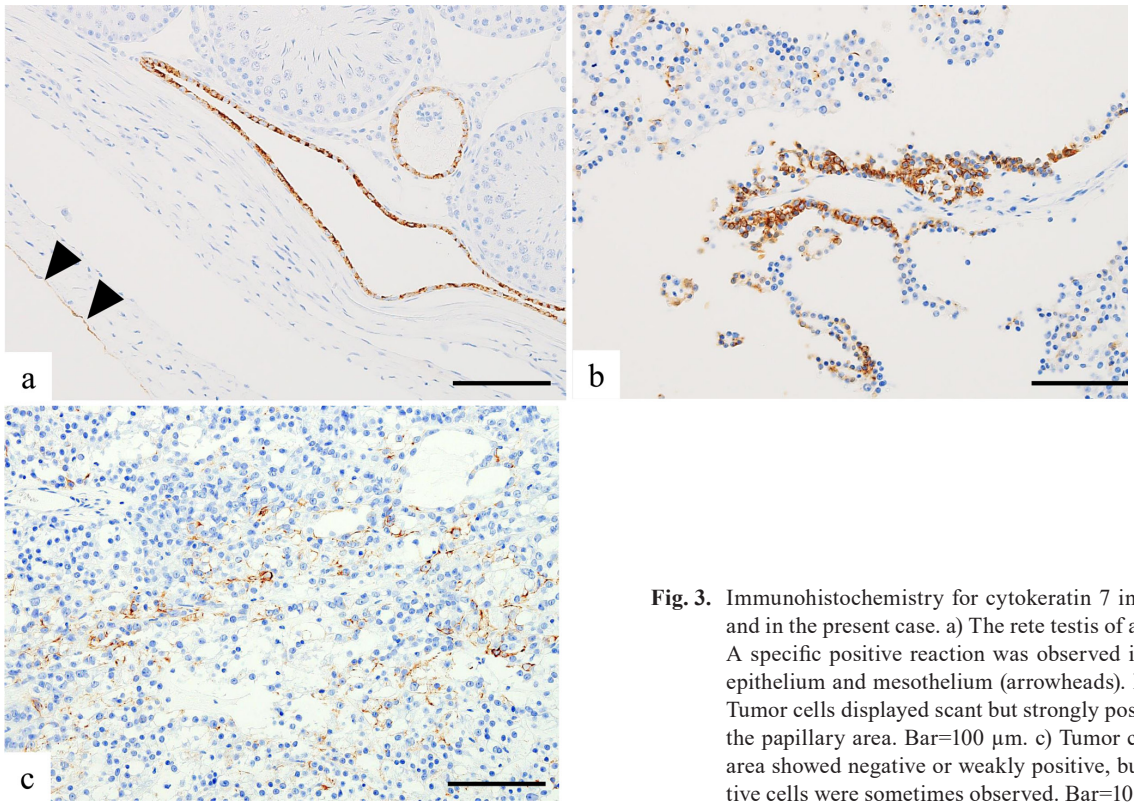

Fig. 3. Immunohistochemistry for cytokeratin 7 in an intact testis and in the present case. a) The rete testis of a 7-week-old rat. A specific positive reaction was observed in the rete testis epithelium and mesothelium (arrowheads). Bar=100 µm. b) Tumor cells displayed scant but strongly positive staining in the papillary area. Bar=100 µm. c) Tumor cells in the solid area showed negative or weakly positive, but strongly positive cells were sometimes observed. Bar=100 µm.

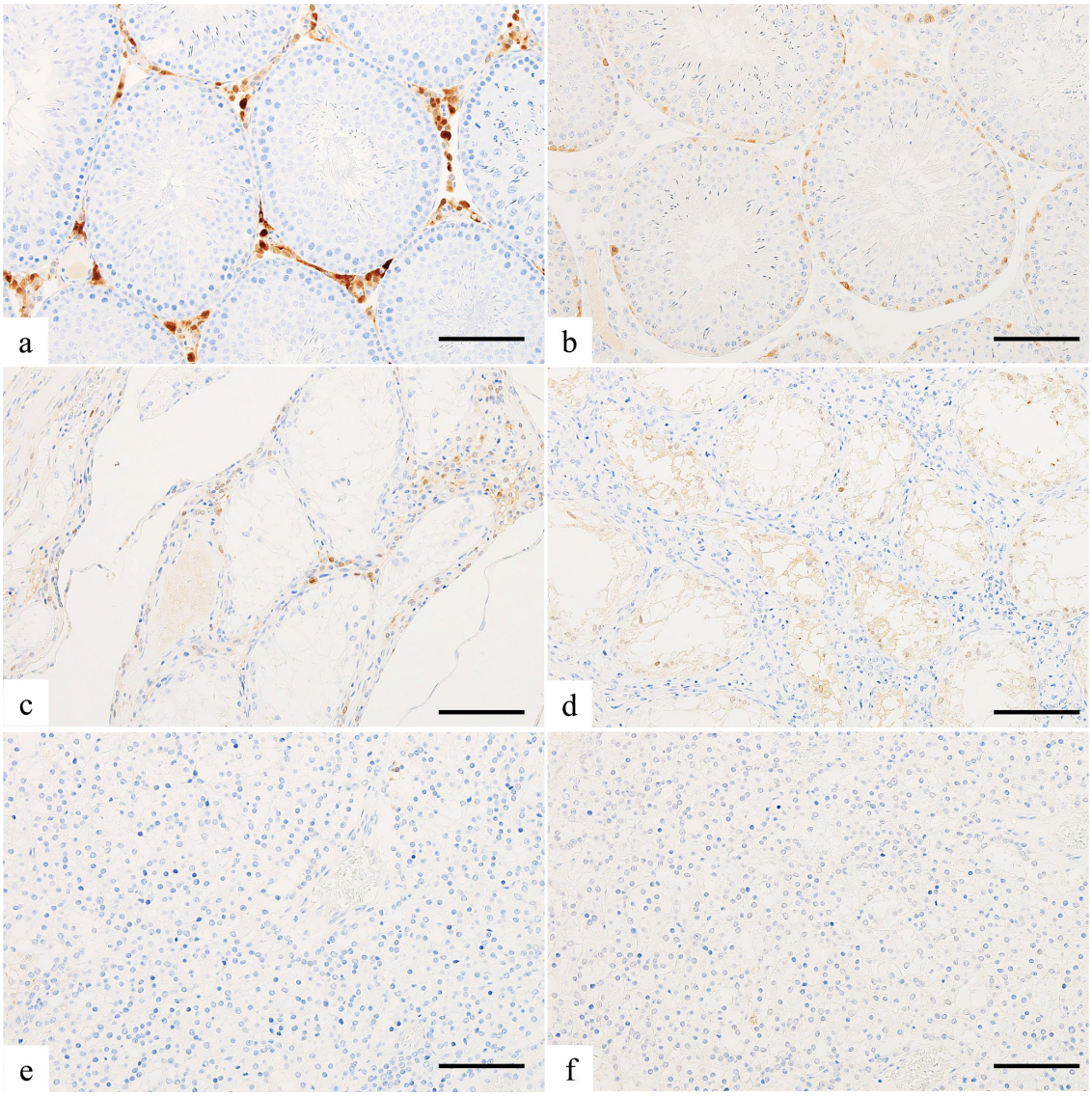

Fig. 4. Immunohistochemistry for calretinin (a, c, and e) and PGP9.5 (b, d, and f) in an intact testis and in the present case. a) and c) Calretinin-positive Leydig cells were observed in the testis of a 7-week-old rat and testicular tissue in the present case, respectively. Bar=100 µm. e) Tumor cells were negative. Bar=100 µm. b) and d) PGP9.5-positive Sertoli cells were seen in the testis of a 7-week-old rat and testicular tissue in the present case, respectively. Bar=100 µm. f) Tumor cells showed negative reaction. Bar=100 µm.

cal characteristics were not considered a strong basis for diagnosis, as the present tumor tissue was demarcated by testicular tissue, as shown in Fig. 1a and 1b, differing from mesothelioma, which arises from outside the testis. Other diagnoses, such as sex-stromal-originated tumors, could be dismissed based on positive result for CK7 and negative results for PGP9.5 or calretinin.

The rete testis is a convoluted sac- or duct-like structure lined by flattened cuboidal epithelium located in the cranial pole of the testis. The seminiferous tubule connects to the rectus tubules and intratesticular rete. The intratesticular portion of the rete testis is under the tunica albuginea, passes through the tunica, and connects the extratesticular rete and efferent ducts of the epididymis[7, 8]. Rete testis-derived proliferative lesions including tumors are extremely rare in rodents, and all previously reported cases were adenocarcinoma. According to Mitsumori *et al.*, two of the three adenocarcinomas in F344 rats were considered to have originated within the intratesticular portion of the rete, and histologically, tubular/glandular structures lined by cuboidal epithelium were prominent in combination with marked fibrosis, hemorrhage, and necrosis[2, 7]. In contrast, little is known about benign proliferating lesions in the rete testis, including adenoma. Although one report mentioned adenomatous hyperplasia in F344 rats, the histological features resembled adenocarcinoma rather than that in the present case[9]. In mice, rete testis-derived cystadenoma and cystadenocarcinoma have been reported, which exhibit papillary or solid growth of tumor cells in cysts lined with a single-layered cuboidal epithelium[3]. Although immunohistochemistry was not performed in this previous report, the present case exhibits some histological resemblance to this

cystadenoma.

Rete testis tumors in rodents are believed to be negative for vimentin, according to textbooks[7, 8, 10, 11], but little published information is available. On the contrary, the rete testes of rabbits and dogs are known to express vimentin and CKs. In dogs, the rete testis expresses vimentin, CAM5.2 (CK10 and CK18), and PKK 1 (CK8, CK9, and CK19)[12], and a case of adenocarcinoma also represents the expression of vimentin and CK[13]. Normal rete testis in rabbits have been reported to express vimentin, CK10, and CK18[14]. In the present case, we compared the expression of several antibodies in the rete testis from a naïve 7-week-old SD rat to that in our case and observed positivity for vimentin and CK7 in the intact epithelium. Additionally, CK7 showed a positive reaction exclusively in naïve rete testes. These careful but basic observations have enabled the present diagnosis, highlighting their importance in tumor diagnosis.

Tumors derived from rete testes are also rare in humans. According to a textbook[15], typical adenoma in rete testes is recognized as polypoid nodules composed of tubules that project into the dilated lumen of the rete testis. The tubules resemble those observed in benign Sertoli cell tumors[15]. Regarding benign tumors of the rete testis, cystic adenoma and sertoliform cystadenoma have been reported to have papillary and solid growth patterns, in which immunoreactivity for cytokeratin is positive and negative, respectively[16, 17]. This information partly matches the findings of the CK7 positivity in the present case.

In conclusion, based on histopathological and immunohistochemical characteristics, the tumor was diagnosed as rete testis adenoma. To the best of our knowledge, this is the first report of a benign rete testis tumor in rats.

Disclosure of Potential Conflicts of Interest: The authors have no conflicts of interest.

Acknowledgment: We are grateful to Dr. Satoshi Furukawa, Nissan Chemical Co., for providing histological specimens of rat placenta for AFP immunohistochemistry.

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