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A paraganglioma in the posterior wall of the left atrium originating from the aortic body in a Wistar Hannover rat

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ABSTRACT

A small cardiac tumor was detected in the posterior wall of the left atrium of a 110-week-old female Wistar Hannover rat (Slc: Wistar Hannover/Rcc) during a carcinogenicity historical control study. Tumor was consisted of 2 different cells. Most of the tumor cells were polygonal to oval in shape and had slightly basophilic and granular cytoplasm. These cells were arranged in distinctive cell nests, called 'Zellballen', and were separated by reticulin fibers. The nuclei were round to slightly oval. A few mitotic figures were found. Cytoplasmic granules of tumor cells were negative for Fontana–Masson and Periodic acid Schiff (PAS) staining. Immunohistochemical staining revealed that the chief cells in the tumor were positive for the neuroendocrine markers synaptophysin and chromogranin A but were negative for S-100 protein, vimentin, cytokeratin, α-smooth muscle actin, and calcitonin. In contrast, the surrounding sustentacular cells, other type of tumor cells, were positive for only S-100 protein. The immunohistochemical properties of the tumor cells were quite similar to those of the aortic body. The tumor cells had infiltrated the myocardium of the left atrium and were also noted within vessels. Based on these findings, the tumor was diagnosed as a paraganglioma originating from the aortic body.

1. Introduction

Paragangliomas are rare neoplasms of neural crest origin. These tumors are most frequently reported in dogs (Richards and Mawdesley-Thomas, 1969; van Zwieten et al., 1979) and are extremely rare in rats. One exception is female rats of the WAG/Rij strain, in which paragangliomas are found in nearly 6% of aging animals (van Zwieten et al., 1979). The occurrence of these neoplasms in the aortic body is relatively uncommon in humans and animals. One study described a paraganglioma that arose from the aortic body and attached to the aorta of a 3-year-old male white rat (Trevino and Nessmith, 1972). The occurrence of paragangliomas in the hearts of 3 Fischer 344 rats has also been reported (Hall et al., 1987). Furthermore, the paraganglioma originating from the aortic body are very uncommon in the Wistar Hannover rat (Weber et al., 2011).

The histopathological pattern of paragangliomas is similar in tumors from different species and in different locations. Individual tumor cells are polygonal to oval and are arranged in distinctive cell nests, called 'Zellballen'. These nests are separated by reticulin fibers and are surrounded by sustentacular cells. These pathologic features have been well documented as typical of paragangliomas in WAG/Rij rats (van Zwieten et al., 1979).

To our knowledge, although a few cases of paragangliomas associated with the aortic body have been reported in Wistar Hannover rats (Weber et al., 2011), a detailed immunohistochemical (IHC) analysis of these tumors have not been previously reported in this strain of rats. To determine the origin of this tumor, we compared the morphological and IHC features of the tumor to those of the normal aortic body.

2. Materials and methods

The neoplasm was found by histological examination of a female Wistar Hannover rat (Slc: Wistar Hannover/Rcc) that was sacrificed during a 2-year carcinogenicity historical control study. The

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Table 1
Antibodies used in IHC staining of the tumor and aortic bodies.

Specificity	Antibody	MW (kDa)	Unmasking	Dilution
Synaptophysin (SY 38)	mAb (DAKO) Mouse	34	Microwave/TB	1:100
Chromogranin A	pAb (ABCAM) Rabbit	68	Microwave/CB	1:250
S-100	pAb (DAKO) Rabbit	40–68	Microwave/CB	1:800
Vimentin	mAb (DAKO) Mouse	57	Microwave/CB	1:1500
α-Smooth muscle actin	mAb (DAKO) Mouse	42	Microwave/TB	1:800
Cytokeratin	mAb (DAKO) Mouse	40–68	Microwave/TB	1:800
Calcitonin	pAb (DAKO) Rabbit	15	Microwave/CB	1:800

pAb: polyclonal antibody; mAb: monoclonal antibody; MW: molecular weight; CB: citrate buffer, pH 6.0; TB: tris buffer, pH 9.0.

animal was housed individually in stainless steel wire cage under barrier conditions of 23 ± 3 °C, 55 ± 20% relative humidity, and a 24-h light–dark cycle. The animal had free access to a Teklad-certified, irradiated, global 18% protein rodent diet 2918C (Harlan Laboratories, Inc., USA) and tap water via an automatic water supply system. The procedure for animal care was approved by the Institutional Animal Care and Use of Committees (IACUC) of Biotoxtech Co. Ltd. based on the Animal Protection Act. The animal's clinical signs were observed once a day. No clinical signs were evident until necropsy. The animal was euthanized by exsanguination via the abdominal aorta under isoflurane anesthesia and necropsied under the supervision of a pathologist. The heart was fixed in 10% neutral buffered formalin, routinely dissected longitudinally, and both halves were embedded. After routine tissue processing, the aortic root was serially sectioned at 4 μm and stained with H&E. These sections were also used for histochemical and IHC studies.

The following 3 stains were used: the Fontana–Masson staining for argentaffin granules, PAS staining for glycogen granules, and Gomori's reticulum stain for reticular fibers.

IHC staining of the tumor was performed using an indirect avidin–biotin peroxidase method, using the primary antibodies listed in Table 1. A biotin–streptavidin–horseradish peroxidase commercial detection kit (Dako, CA, USA) was used for IHC evaluation. The protocol used in this study was based on that previously described by Brown et al. (2003), with modifications. Positive control cells were as follows: pancreatic islet cells for synaptophysin and chromogranin A staining, the peripheral nerve of the colon for S-100 protein staining, the smooth muscle of the colon for vimentin and α-smooth muscle actin staining, the mammary gland for cytokeratin staining, and the thyroid gland for calcitonin staining. Primary antibody diluents without primary antibody were used as a negative control. Nonspecific immunoreactivity was not detected, and background staining was minimal. Stained samples were examined qualitatively and graded as negative (−), mildly positive (+), and strongly positive (++) based on the stain intensity and cell quantity.

In addition, in order to confirm similarity of cellular nature between the neoplastic lesion and the normal aortic bodies, we investigated 10 normal Wistar Hannover rats from this study in same manner.

3. Results

Necropsy revealed no remarkable findings in any organ. Microscopic examination revealed a small cardiac tumor in the posterior wall of the left atrium (Fig. 1A). Histopathologically, the predominant tumor cells were arranged into 'Zellballen' (Fig. 1B), which were separated by delicate reticulin fibers. The pattern was clearly demonstrated using Gomori's method for reticulin fibers (Fig. 2). The tumor cells were polygonal to oval in shape and had slightly basophilic and granular cytoplasm with indistinct cell boundaries. The nuclei were oval, with stippled chromatin and a single central nucleolus. A few mitotic figures were found (Fig. 1B). There was no obvious or apparent pleomorphism or necrosis. These cells correspond to chief or type I cells. In certain sections, a second cell type could be seen at the periphery of the cellular nests. These cells had scanty, eosinophilic cytoplasm with smaller hyperchromatic oval or lenticular nuclei, consistent with sustentacular or type 2 cells (Fig. 1B). Characteristically, the neoplastic cells forming nests had invaded between the cardiac muscle fibers. The muscle fibers adjacent to the tumor showed degenerative changes, such as vacuolation, loss of myofiber, and atrophy (Fig. 1B). Tumor emboli were present in the vascular lumen (Fig. 3A), as confirmed by vimentin staining in the vascular wall (Fig. 3B).

The aortic bodies in the normal animals were located in adipose tissue around the base of the aorta or pulmonary artery (Fig. 4A and B). Frequently, the smallest aortic body was part of the perineural tissues of the ganglion. Histopathologically, the organization of

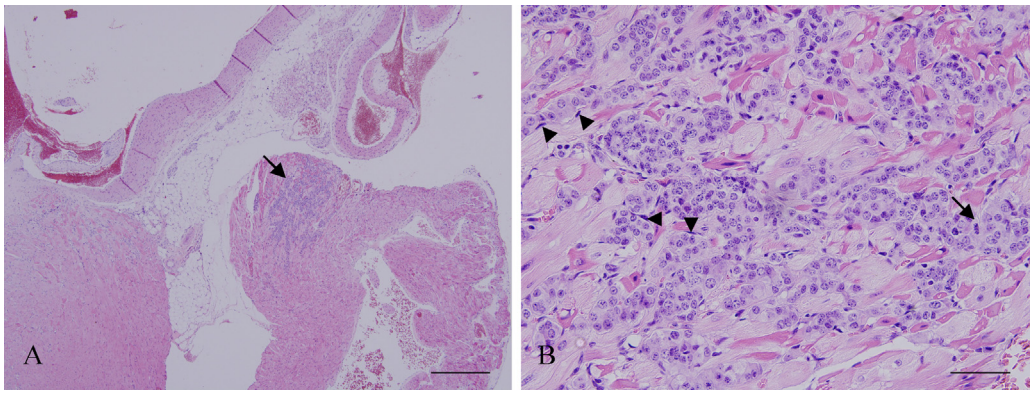

Fig. 1. Location and pattern of tumor. A: The tumor (arrow) was located in the left atrial wall. The aorta is located in the upper left, the left ventricular wall occupies the lower left corner, and the nerve fiber and pulmonary artery are in the upper right. H&E; scale bar = 500 μm. B: The tumor cells are arranged in distinctive cell nests, surrounded by sustentacular cells (arrow head). The nuclei are round to slightly oval, and a few mitotic figures were found (arrow). The tumor cell nests infiltrated between myocardial fibers. Note vacuolation and increased acidophilia of some fibers in the region of tumor cell invasion. H&E; scale bar = 50 μm.