NOTE
Pathology

Proliferative changes in the adrenal medulla of aged Chinese native pigs

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ABSTRACT. Four aged retired Chinese native pigs, three females and one male, estimated as over 10-year-old, were subjected to autopsy because of infertility due to aging. Grossly, nodular lesions were found bilaterally in the adrenal medulla of all four pigs. Based on the gross and the histopathological findings, they were diagnosed as either medullary nodular hyperplasia or pheochromocytoma. Immunohistochemically, proliferating cells of all these lesions were immuno-positive for chromogranin-A, indicating adrenal medulla-derived. Ultrastructurally, cytoplasmic neurosecretory granules suggestive of secretion were observed in these proliferating cells. There have been only limited numbers of reports on adrenal medullar proliferative changes including pheochromocytoma in pigs. The present cases will provide a valuable information for the characterization of similar changes in animals and human.

KEY WORDS: adrenal gland, hyperplasia, medulla, pheochromocytoma, pig

Among the neoplasms arising in the adrenal medulla, three types of neuroendocrine tumor are known, those are, pheochromocytoma, neuroblastoma, and ganglioneuroma. These tumors are derived from the neural crest-origin cells (neuroectoderm), which can differentiate into either secretory cells (pheochromocytes) or sympathetic ganglion cells. Pheochromocytomas are tumors of adrenal chromaffin cells, while neuroblastoma and ganglioneuroma are tumors of sympathetic ganglion cells.

Adrenal medullary pheochromocytomas are reported in animals as well as human. In human, they are extremely rare, with an annual incidence of less than 1 per million [1, 19]. In animals, they develop most often in cattle, dogs and rats. Additionally, they are reported in a wolfdog [15], a sea lion [14], a jaguar [12], a cotton-top tamarin [3] and new world primates [8].

In swine, reports of pheochromocytoma are extremely rare. Martinez *et al.* had reported a case of swine malignant pheochromocytoma in 2012 [10]. In addition, there are reports of pheochromocytoma as an incidental finding in a slaughtered pig [16], and a functional pheochromocytoma in an African warthog (*Phacochoerus aethiopicus*) [6].

This paper describes histopathological, immunohistochemical and ultrastructural findings of nodular hyperplasia and pheochromocytoma in the adrenal medulla of four Chinese pigs.

The four Chinese pigs (Short-ear pigs of Taiwan) [11], three females (pig Nos. 1–3) and one male (pig No. 4), that had been kept for breeding, were euthanized through a humane manner and autopsied because of infertility due to aging. The procedures were compiled with the Guidelines for Animal Care and Use of the Nippon Institute for Biological Science. The age of pigs was estimated as over 10 years from their rearing histories, and no apparent clinical symptoms were observed in any pigs at antemortem inspection. Plasma levels of catecholamines and excretion of catecholamine metabolites were not measured. No blood samples were collected from the pigs. Tissue samples of various organs were collected and fixed in 4% phosphate-buffered paraformaldehyde solution and further processed routinely. Four-μm-thick paraffin sections were stained with hematoxylin and eosin (H & E).

Immunohistochemistry was conducted through the labeled streptoavidin biotin [LSAB] method using rabbit polyclonal antibody against chromogranin-A (Dako, Carpinteria, CA, U.S.A.). The secondary antibody used was biotinylated goat anti-rabbit IgG antibody (KPL, Gaithersburg, MD, U.S.A.). The sections were incubated with peroxidase-labeled streptavidin (Dako) and the reactants were visualized with 3, 3′-diaminobenzidine-tetrahydrochloride (Dojindo Laboratory, Kumamoto, Japan) as chromogen. Counterstaining was done with hematoxylin.

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Fig. 1. Gross appearance of a native Chinese pig (Short-ear pig of Taiwan). No noticeable changes are observed. Pig No. 4.
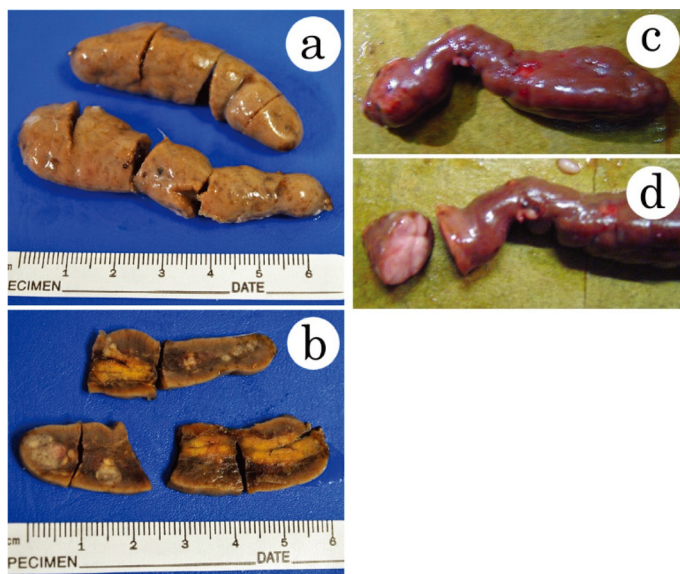

Fig. 2. Gross appearances of adrenal glands. (a, b) Pattern 1. Fixed adrenal glands of pig No.3. Nodular changes are recognized at the cut surface. (c, d) Pattern 2. Left adrenal gland of pig No.1. Nodules are clearly recognized by appearance.

For electron microscopy, paraformaldehyde-fixed tissues from lesion of pattern 2 were refixed in 1% osmium tetraoxide and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (JEM-1400, JEOL, Tokyo, Japan).

At autopsy, no gross abnormalities were found in the body surface of all four pigs (Fig. 1). Adrenal glands of all the pigs showed bilateral proliferative changes, and they were classified into two patterns as follows:

Pattern 1 (P1); The small nodules were recognized by appearance and the boundary was indistinct. On the cut surface, multiple nodules of various sizes were found at the medulla (Fig. 2a and 2b).

Pattern 2 (P2); The multiple nodules larger than P1 were recognized by appearance. On the cut surface, they were clearly recognized as whitish solid nodules at the medulla (Fig. 2c and 2d).

Histopathological characterizations of the adrenal proliferative lesions in each pattern were depicted as follows:

P1: The small nodules consisted of proliferating medullary cells, and they mildly expanded and compressed the surrounding cortex (Fig. 3a and 3b). The proliferating medullary cells had small round nucleus and eosinophilic cytoplasm, being arranged in small lobules. The shapes of the cells were varied from cuboidal or polyhedral to pleomorphic (Fig. 3c).

P2: Some proliferative lesions were expanded to the adrenal surface, displacing the cortex (Fig. 4a and 4b). The nodular lesions consisted of the proliferative medullary cells, and expanded to and remarkably compressed the cortex. The tumor cells had the enriched cytoplasm and atypical nucleus with occasional mitotic figures. The lesions were subdivided into small lobules by fine connective tissue stroma with capillaries (Fig. 4c).

Based on the gross and histological pathology mentioned above, characteristic P1 and P2 lesions were diagnosed as hyperplasia of the adrenal medulla and pheochromocytoma, respectively. Therefore, the proliferative changes in the bilateral adrenal medullae in the four pigs were diagnosed as shown in Table 1.

In order to identify the origin of the proliferative cells in adrenal glands, immunohistochemical staining was performed using anti-chromogranin-A antibody. As a result, both patterns of proliferative changes were positive for chromogranin-A, indicating lesions derived from the adrenal medulla (Fig. 5).

Ultrastructurally, abundant neurosecretory granules were observed in the cytoplasm of the tumor cells in pheochromocytoma (Fig. 6). Unfortunately, discrimination between epinephrine-secreting cells and norepinephrine-secreting cells was not clear, because post mortem autolysis prevented an adequate ultrastructural observation.

There have been only limited numbers of reports on proliferative lesions of the adrenal glands in aged pigs. In this study, we examined four aged retired Chinese native pigs and unexpectedly found proliferative changes in the bilateral adrenal medullae of all four animals. Based on the gross and histophathological findings, the proliferative changes were classified into two patterns, and diagnosed as nodular hyperplasia of the adrenal medulla and pheochromocytoma, respectively.

Among the proliferative lesions, nodular hyperplasia of adrenal medulla was diagnosed according to the following critical points; 1) histopathological finding of nodular proliferation of medullar cells, 2) mild compression to the cortex by the proliferative medullary lesion and 3) without atypism of the proliferating cells. By contrast, pheochromocytoma was diagnosed according to the following points; 1) noticeable gross mass formation at the surface of adrenal gland, 2) severe compression to the cortex
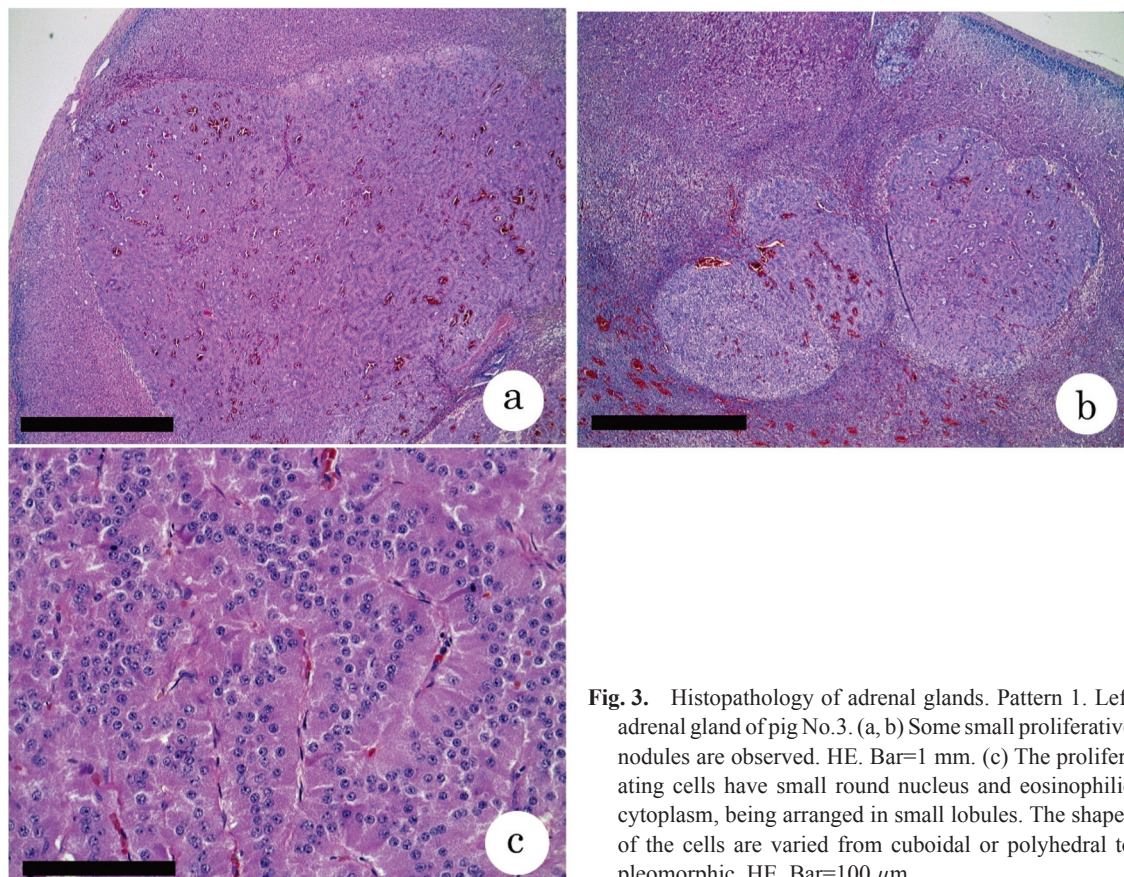
Fig. 3. Histopathology of adrenal glands. Pattern 1. Left adrenal gland of pig No.3. (a, b) Some small proliferative nodules are observed. HE. Bar=1 mm. (c) The proliferating cells have small round nucleus and eosinophilic cytoplasm, being arranged in small lobules. The shapes of the cells are varied from cuboidal or polyhedral to pleomorphic. HE. Bar=100 μm.
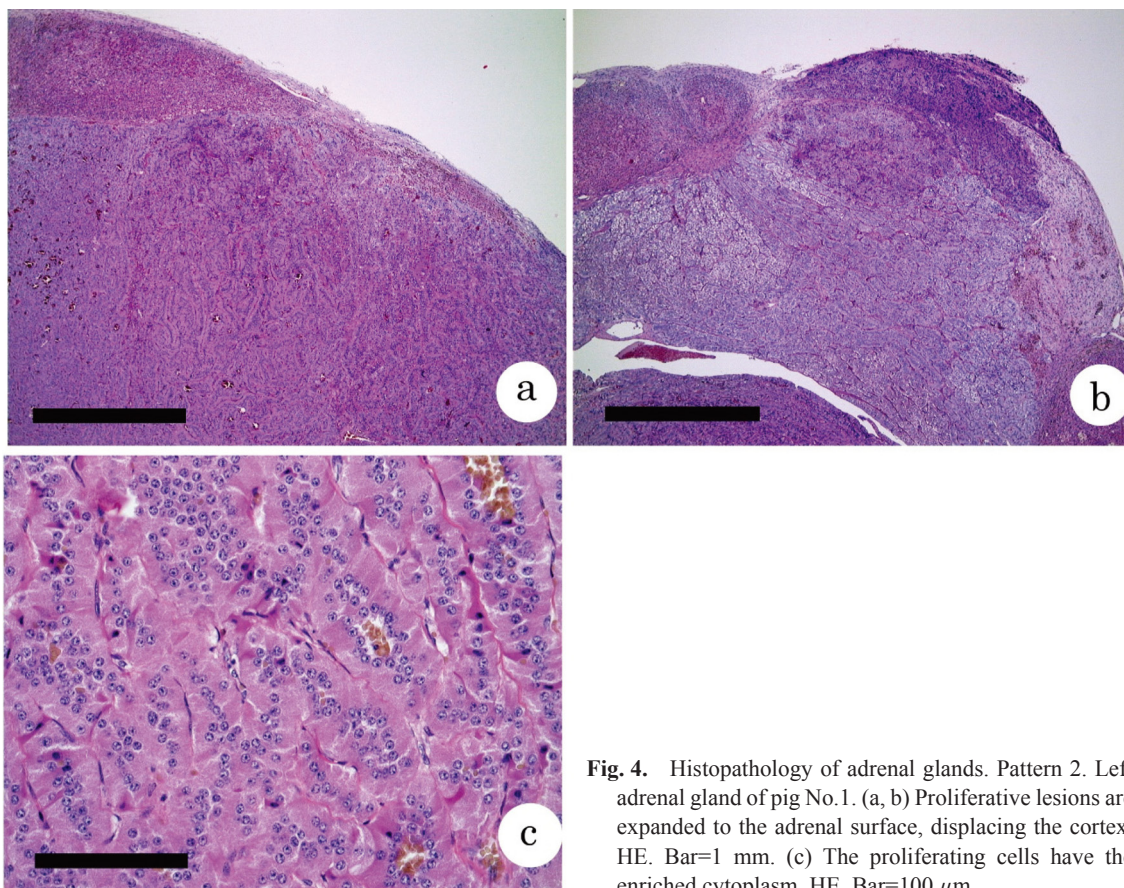

Fig. 4. Histopathology of adrenal glands. Pattern 2. Left adrenal gland of pig No.1. (a, b) Proliferative lesions are expanded to the adrenal surface, displacing the cortex. HE. Bar=1 mm. (c) The proliferating cells have the enriched cytoplasm. HE. Bar=100 μm.

Table 1. Diagnosis of proliferative changes in four pigs

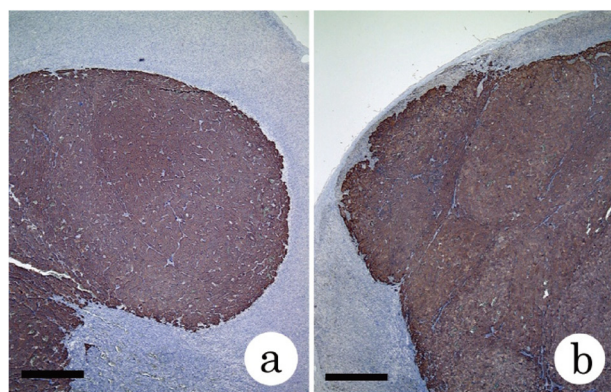| Pig No.	Left	Right
1	Pheochromocytoma	Hyperplasia of medulla
2	Pheochromocytoma	Pheochromocytoma
3	Hyperplasia of medulla	Hyperplasia of medulla
4	Hyperplasia of medulla	Pheochromocytoma

Fig. 5. Left adrenal gland. (a) Pattern 1. Pig No. 3. (b) Pattern 2a. Pig No.1. Cells in the proliferative lesions are positive for chromogranin-A. Immunohistochemistry. Bar=500 μm.
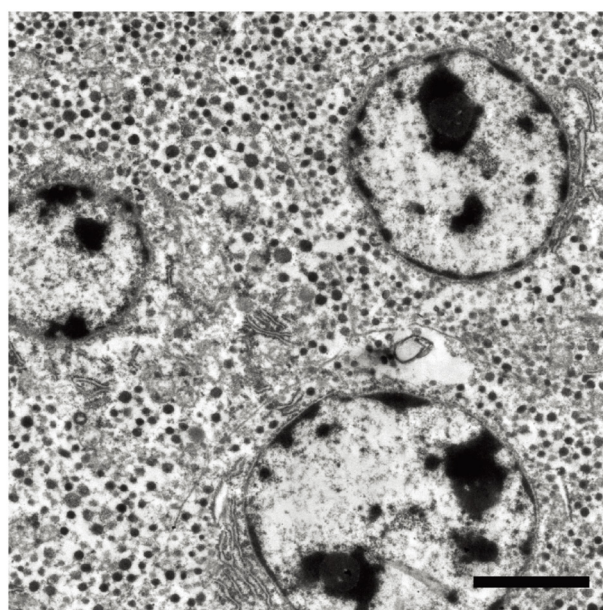

Fig. 6. Right adrenal gland. Pheochromocytoma. The ultrastructure of proliferating tumor cells. Pig No. 4. Abundant neurosecretory granules are observed in the cytoplasm. Bar=2 μm.

by the proliferating medullary lesion and 3) with atypism of the proliferating cells. Therefore, patterns 2 was considered as pheochromocytoma. "Malignant" pheochromocytoma is used to designate adrenal medullary tumors that invade through the adrenal capsule and into adjacent tissues (e.g., posterior vena cava or periadrenal fat) and/or metastasize to distant sites (e.g., liver, regional lymph nodes or lungs) [13]. Such findings to suggest malignancy were not observed in pattern 2.

In the case of functional pheochromocytoma, some clinical signs such as tachycardia, edema and cardiac hypertrophy are observed because of the excessive chatecholamine production [13]. In human, clinical diagnosis of adrenomedullary tumors is based on the observation of paroxysmal or sustained hypertension, increased plasma levels of catecholamines as well as enhanced excretion of various catecholamine metabolites in urine [2]. In the present case, no animal showed any of such noticeable clinical symptoms, but it is unknown whether the present cases are functional pheochromocytoma or not because blood and/or urine were not tested. By the ultrastructural observation, abundant secretory granules were found in the cytoplasm of tumor cells in the present case, while it is not clear that the cytoplasmic granules reflect the function of the tumors.

Pheochromocytomas develop most often in cattle, dogs and rats. They also occur infrequently in other species, and the occurrence in swine is extremely rare. In a 1968 abattoir survey of 3.7 million swine in Great Britain, only one 2.5-year-old female was diagnosed with an adrenal pheochromocytoma [16]. This extremely low prevalence was suggested to be due to slaughter before reaching maturity in most animals [10]. In addition to this, finding the neoplastic changes in the adrenal gland is extremely difficult in slaughter inspection except cases of a considerable size of tumor [10]. The present four aged pigs examined were autopsied because of infertility due to aging. The age of all pigs were estimated over 10-years-old from their rearing histories, and proliferative changes were unexpectedly found in the adrenal medulla of all four animals. Under these circumstances, there might be two possibilities in the finding of rare tumors. One is that the occurrence of pheochromocytoma might increases with age and common even in swine. It means that the extremely low prevalence of pheochromocytoma in swine would be due to the slaughter before reaching maturity. The other is that the high frequency of pheochromocytoma might be specific in the native Chinese pig strain.

Pheochromocytomas are derived from the adrenal medulla. Besides pheochromocytomas, neuroblastoma and ganglioneuromas are also derived from the adrenal medulla. Neuroblastomas are differentiated from pheochromocytomas by being composed of small tumor cells with hyperchromatic nucleus and scant amount of cytoplasm that often resemble lymphocytes and tend to form pseudorosettes [4]. Ganglioneuromas are composed of multipolar ganglion cells and neurofibrils with a prominent fibrous connective tissue stroma [4]. In addition, immunohistostaining for chromogranin-A is also helpful for the differential diagnosis. Antibodies to chromogranin-A can be used for the demonstration of this unique protein in chromaffin cells [8]. These histopathological and immunohistochemical findings were helpful for the differential diagnosis in present case.

Diffuse or nodular adrenal medullary hyperplasia appears to precede pheochromocytoma in bulls, humans with thyroid C-cell tumors, and laboratory rats [4]. In human, the syndrome of familial medullary thyroid carcinoma (MTC), pheochromocytoma, and parathyroid hyperplasia (also known as multiple endocrine neoplasm (MEN) type 2) is inherited as an autosomal dominant trait [17, 18], and the pheochromocytomas occurring in patients with familial MTC may represent extreme degrees

of nodular hyperplasia in the medulla [7]. And it was suggested that the adrenal medullar hyperplasia may be the precursor of pheochromocytoma [5, 9]. Progression of hyperplasia to pheochromocytoma was also described in old bulls with thyroid C-cell tumors [20]. In the present cases, there was no persuasive evidence of the relationship between medullar nodular hyperplasia and pheochromocytoma.

Conclusively, the proliferative lesions in the adrenal medulla that are considered to be extremely rare in swine were found bilaterally in four aged native Chinese pigs. According to gross and histopathological findings, they were diagnosed as either medullary nodular hyperplasia or pheochromocytoma.

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