J. Comp. Path. 2016, Vol. ■, 1–5

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Malignant Oestrogen-producing Teratoma in a Cat

Y. Machida*, M. Michishita*, M. Wada†,§, T. Hori†, E. Kawakami†, H. Yoshimura‡, K. Ohkusu-Tsukada*, K. Taya¶ and K. Takahashi*

** Laboratory of Veterinary Pathology, † Laboratory of Reproduction and, ‡ Laboratory of Physiological Pathology, Faculty of Veterinary Medicine, Nippon Veterinary and Life Science University, Tokyo, § Family Animal Clinic, Nagareyama City, Chiba Prefecture and ¶ Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan*

Summary

A 5-year-old female domestic shorthair cat was presented with abdominal distension and serum biochemical evaluation indicated a high concentration of oestradiol (32.81 pg/ml). Exploratory laparotomy revealed a large cystic mass in the right ovary with cystic fluid containing a high level of oestradiol (18.80 pg/ml). The tumour was composed of immature neuroectodermal tissue, mature cartilage, smooth muscle, adipose tissue and aggregated, poorly differentiated mesenchymal cells. It contained cysts of various sizes that were lined by epithelium of different types. The basal layer of the lining epithelium was shown to express aromatase by immunohistochemistry. The findings suggest that this was a novel, malignant, oestrogen-secreting teratoma and that the aromatase-positive, neoplastic cells may have been the source of elevated levels of serum oestrogen.

Keywords: cat; oestrogen; ovary; teratoma

Teratomas are rare tumours composed of cells derived from two or three germ layers showing various stages of maturation. They develop multiple tissue types that are foreign to the part of the body in which they are located. Teratomas are categorized as germ-cell tumours, which include germinoma, embryonal carcinoma, endodermal sinus tumour and choriocarcinoma, each of which represents the neoplastic transformation of embryonic tissue (Kennedy *et al.*, 1998). The most common sites for teratoma are the ovary and testis, but the tumour also occurs less commonly in intracranial or retrobulbar locations. Descriptions of hormone-secreting teratomas are limited to a human case of a malignant oestrogen-secreting tumour arising from a mature sacrococcygeal teratoma (Yoshida *et al.*, 2011).

The aromatase complex, which is also known as aromatase cytochrome P450 protein, is a key enzyme in steroidogenesis that catalyses the biosynthesis of oestrogens from androgens. It also plays important roles in sexual differentiation, fertility and carcinogenesis (Bulun *et al.*, 1994; Conley and Hinshelwood, 2001; Subramanian *et al.*, 2008). Aromatase is expressed in a variety of human cells and tissues, including the fetal brain, testicular Leydig cells, placental syncytiotrophoblast, adipose stromal cells in both males and females, and ovarian granulosa and luteal cells (Conley and Hinshelwood, 2001). In women, oestrogens are produced by aromatase-mediated catalysis of circulating inactive steroids, which contributes to tumour cell proliferation and malignancy in hormone-dependent breast cancer (Subramanian *et al.*, 2008) and endometrial tumours (Bulun *et al.*, 1994).

Correspondence to: K. Takahashi (e-mail: kimimasa@nvlu.ac.jp).

0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2016.11.273

A 5-year-old female domestic shorthair cat was admitted to the Family Animal Clinic, Nagareyama City, Chiba Prefecture, Japan, with abdominal distension. On exploratory laparotomy, a large mass was identified in the right ovary (13.5 × 10.5 × 8.0 cm). The left ovary was reduced in size. There were no abnormalities of other abdominal viscera and the mass was removed as part of a total ovariohysterectomy. Samples of the mass, uterus and left ovary were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE).

A laparotomy was conducted on three occasions over the following 12 months, until the death of the cat. Blood samples were collected prior to each laparotomy and at 14 days after the second laparotomy. The concentrations of oestrogen and progesterone in the blood, cystic fluid within the tumour and ascitic fluid were measured by using the VIDAS Oestradiol II and VIDAS Progesterone enzyme-linked fluorescence assay kits (Arkray, Kyoto, Japan) in a SPOTCHEM VIDAS SV-5020 automated fluorescence immunochemistry analyser (Arkray). Biochemical assay prior to the first laparotomy revealed a high concentration of serum oestradiol (32.81 pg/ml, normal <5.0 pg/ml) despite a lack of oestrus (Shille *et al*., 1979) (Supplementary Fig. 1). In contrast, the concentration of serum progesterone (1.83 ng/ml, normal <1 ng/ml) was considered close to normal (Shille *et al*., 1979) (Supplementary Fig. 1). The concentration of oestradiol in the cystic fluid from the tumour was 18.80 pg/ml and that of progesterone was 0.92 ng/ml. Seventy-seven days after the first laparotomy, the cat again displayed abdominal distension and poor appetite. Serum biochemistry indicated a high concentration of oestradiol (41.73 pg/ml) and an almost normal concentration of progesterone (1.57 ng/ml) (Supplementary Fig. 1). The second laparotomy revealed a large intra-abdominal mass (17.0 × 8.0 × 4.0 cm) that was widely adherent to the greater omentum. The mass was removed. Fourteen days later, the concentration of serum oestradiol reduced to 19.17 pg/ml. However, 40 days after the second laparotomy, the cat exhibited anorexia and was found to have high concentrations of oestradiol in the serum (51.01 pg/ml) and ascitic fluid (15.66 pg/ml), and a third laparotomy was conducted. Numerous masses were found in the liver, greater omentum and abdominal wall, and it was difficult to remove all of them surgically. Bleomycin (10 mg/m^2) was administered at weekly intervals, but the cat died 45 days later.

The masses removed during the second and third laparotomies were fixed and processed as described previously. Sections were subjected to immunohistochemistry (IHC) using primary antibodies specific for pancytokeratin (clone AE1/AE3, 1 in 200 dilution; Dako, Glostrup, Denmark), cytochrome P450 aromatase (aromatase; rabbit polyclonal, 1 in 4,000 dilution; Yoshida and Osawa, 1991), inhibin-α (rabbit polyclonal, 1 in 50 dilution; AbD SeroTec, Kidlington, UK), S100 protein (S100; rabbit polyclonal, 1 in 1,500 dilution; Dako), glial fibrillary acidic protein (GFAP; rabbit polyclonal, 1 in 500 dilution; Dako), nestin (rabbit polyclonal, 1 in 1,000 dilution; Merck Millipore, Darmstadt, Germany) and α-smooth muscle actin (αSMA; clone 1A4, 1 in 400 dilution; Dako). After reaction with the specific primary antibodies at 4°C overnight, the sections were incubated with biotinylated goat anti-mouse IgG or anti-rabbit IgG antibodies (1 in 500 dilution; Dako), followed by incubation with a 1 in 500 dilution of peroxidase-conjugated streptavidin (Dako) at room temperature for 30 min. Antibody binding was 'visualized' using 3,3′-diaminobenzidine tetrahydrochloride and sections were counterstained with haematoxylin. For antigen retrieval, the sections were autoclaved at 121°C for 10 min in citrate buffer (pH 6.0) for sections labelled for pancytokeratin, inhibin-α, S100, GFAP, nestin and αSMA and in Target Retrieval Solution pH 9.0 (Dako) for sections labelled for aromatase. As a negative control, primary antibodies were substituted with phosphate buffered saline. The ovaries from a 4-year-old normal cat undergoing routine ovariohysterectomy were used as a positive control.

The ovarian mass was encapsulated with a smooth glistening outer surface. On cross section it was composed predominantly of grey to brown soft tissue, and contained clear serous or mucinous small cysts of varying size (2−15 mm diameter) with some haemorrhagic and necrotic foci.

The ovarian mass and the subsequent masses removed at the second and third laparotomies were identical histologically. They were composed predominantly of immature neuroectodermal tissue that commonly exhibited primitive neuroepithelial rosettes, accompanied by foci of mitotically-active glial cells (Fig. 1). Neoplastic mesodermal tissue, mature cartilage (Fig. 1), smooth muscle, adipose tissue and aggregated, poorly differentiated, mesenchymal cells were present. Cysts of various sizes were identified, lined by stratified squamous epithelium (Fig. 2), ciliated pseudostratified epithelium (Fig. 3) or vacuolated epithelium (Fig. 4). Although the stratified squamous and ciliated pseudostratified epithelial tissues resembled the epidermis and lining of the respiratory tract, respectively, the vacuolated epithelium was histologically unique. Mitotic figures were noted (2−3 per ×400 field) in the

Please cite this article in press as: Machida Y, et al., Malignant Oestrogen-producing Teratoma in a Cat, Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.11.273