Metachronous Occurrence of Mammary Adenocarcinoma and Another Malignancy in Western European Hedgehogs (Erinaceus europaeus)

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ABSTRACT. Mammary adenocarcinomas showing positive reactivity for estrogen receptor (ER), which were found in two female Western European hedgehogs (Erinaceus europaeus) over 5 years old, were successfully treated with surgical excision, but other malignant tumors occurred subsequently. In case 1 (cribriform carcinoma), the neoplastic tissue was characterized by formation of tubular spaces. In the majority of the tissue, there were large numbers of remaining myoepithelial cells, which were demonstrated distinctly by immunohistochemistry for alpha smooth muscle actin (SMA) and cytokeratin. A forelimb tumor, which occurred 9 months after surgery, was diagnosed as myofibroblastic sarcoma, because the neoplastic spindle-shaped cells showed an SMA-positive and desmin-negative immunophenotype. In case 2 (comedo carcinoma), tumor cell nests showed central comedo necrosis, and lacked SMA-positive myoepithelial cells. Approximately 13 months after surgery, the animal died. Necropsy examination revealed that the animal had renal cell carcinoma, which was judged to be of proximal tubule origin on the basis of immunohistochemistry and electron microscopy.

Key words: hedgehog, mammary carcinoma, estrogen receptor, myofibroblastic sarcoma, renal cell carcinoma.

INTRODUCTION

There have been several reports of neoplasms in African hedgehogs (Atelerix albiventris). Among them, the most common tumors were mammary gland adenocarcinoma, lymphoma and oral squamous cell carcinoma [1]. Histologically, seven mammary gland tumors in African hedgehogs were classified into solid, tubular and papillary types, and tended to be locally infiltrative [2]. Neoplasms in other hedgehog species have been reported rarely [3, 4].

Multiple primaries are common in hedgehogs. Of 35 African hedgehogs affected with tumors, three had more than one type of tumor; mammary adenocarcinoma was included in all three cases [2]. An African hedgehog had mammary adenocarcinoma and a granulosa cell tumor, but was well 10 months postoperatively [5].

In humans, myofibroblastic sarcoma is composed of malignant cells that share ultrastructural features of both fibroblasts and smooth muscle cells [6]. A similar neoplasm was reported in a pig, and consisted chiefly of alpha smooth muscle actin (SMA)-positive, desmin-negative myofibroblastoid cells [7]. In this paper, we report two cases of mammary carcinoma in Western European hedgehogs (Erinaceus europaeus), which were characterized by histology and immunohistochemistry. The diagnosis of other supervening tumors (myofibroblastic sarcoma and renal cell carcinoma) was established on the basis of immunohistochemical and ultrastructural features.
MATERIALS AND METHODS
Two female Western European Hedgehogs (Erinaceus europaeus) over 5 years old developed mammary tumors. In case 1, a large tumor mass, 6 × 5 × 3 cm in size, was formed in the right thoracic region (the second and third glands were involved), and was surgically excised. It was white, with areas of necrosis and hemorrhage. Three months later, ovariohysterectomy was performed because of a 3-cm-diameter endometrial polyp. Nine months after the first surgery, a tumor mass measuring 2.5 × 1 × 1 cm was found on the right forearm. Since amputation of the right forelimb seemed to be unavoidable, the animal was euthanized.

In case 2, ovariohysterectomy was done because of a 3-cm diameter endometrial polyp. A 2-cm-diameter tumor mass in the right axillary region (the first gland was affected) was surgically removed 23 months later. The mass consisted of adipose tissue and a soft white nodule, 5 mm in diameter. One year after the second surgery, the animal showed senile cataract and weight loss. Six weeks later, the liver was enlarged and hard on palpation, and the animal died the next day. At necropsy, the liver was highly enlarged with fragility and was yellowish. There were several white nodules up to 2 mm in diameter in the kidneys.

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE). Selected paraffin sections were labeled by the avidin-biotin-peroxidase complex (ABC) method, using monoclonal antibodies to cytokeratins, pan (KL1) (Biomedica, Foster City, USA), vimentin (Dako, Carpinteria, USA), SMA (Dako, Glostrup, Denmark), desmin (Bio-Science, Emmenbrücke, Switzerland) and estrogen receptor (ER) (Nichirei, Tokyo, Japan), a polyclonal antibody to lysozyme (Biomedica), and an ABC kit (Biogenex, San Ramon, USA). Small pieces taken from the formalin-fixed neoplastic tissues of the forearm (case 1) and the kidneys (case 2) were post-fixed in 1% osmium tetroxide and prepared for electron microscopy (EM).

RESULTS
Case 1
Histologically, in the mammary tumor, there were widespread areas of necrosis, which were encircled by abundant collagenous fibrous tissue. The most predominant growth pattern was cribriform or solid (Fig. 1), but small intracellular or intracytoplasmic lumina were detected even in solid areas (Fig. 2). In parts, there were tubulopapillary structures and tumor cell nests showing central necrosis. A few invasive neoplastic cells were in the stroma, but there were no tumor cells in the neighboring lymph nodes.

The tissue of the forearm tumor, which was not encapsulated and was invasive into surrounding muscular and adipose tissues, was considerably cellular, but in parts, less cellular areas with abundant collagenous stroma were seen. The neoplastic cells were spindled with fusiform to elongated nuclei, finely clumped chromatin and medium-sized nucleoli. The mitotic rate ranged from 2 to 4 per high-power field (HPF).

The tissue of the mammary tumor, which was not encapsulated and was invasive into surrounding muscular and adipose tissues, was considerably cellular, but in parts, less cellular areas with abundant collagenous stroma were seen. The neoplastic cells were spindled with fusiform to elongated nuclei, finely clumped chromatin and inconspicuous nucleoli (Fig. 3). Occasional mitoses were seen (1-3/HPF).

Immunohistochemically, ER-positive neoplastic cells were present in large numbers in the mammary tumor. Many myoepithelial cells, which were positive for cytokeratin and SMA and negative for desmin, remained in more than 50% of the neoplastic cell nests (Fig. 4), and a few to several in the other nests. In the encapsulating and stromal tissues, there were great numbers of stromal cells showing positivity for SMA and vimentin and negativity for desmin and cytokeratin (Fig.

Fig. 1 Case 1, mammary tumor. Malignant cells are arranged in a cribriform pattern, produced by the existence of many tubular spaces. HE, ×200.

Fig. 2 Case 1, mammary tumor. The tumor tissue consists of solid sheets of cells, but a fair number of intercellular or intracytoplasmic lumina (arrows) are visible. HE, ×630.
Mammary Adenocarcinoma in Hedgehogs

Fig. 3 Case 1, forearm tumor. Spindle-shaped neoplastic cells are arranged in bundles in a cellular area. Mitotic figures are present (arrows). HE. × 200.

Fig. 4 Case 1, mammary tumor. Immunostain for SMA shows strong immunoreactivity in the myoepithelial component and in the stromal myofibroblastic component (top right). ABC. × 200.

4). The same immunophenotype was observed in the forearm tumor (Fig. 5).

Ultrastructurally, in the forearm tumor, the neoplastic cells were characterized by moderate quantities of rough endoplasmic reticulum (RER) and thin filaments with focal densities (Fig. 6). Frequently, glycogen particles were present in the cytoplasm, and pinocytotic vesicles, subplasmalemmal densities, dense bodies or lipid droplets were seen in occasional cells.

Case 2

Histologically, the neoplastic tissue of the mammary gland was composed of solid masses of neoplastic cells, many of which had necrotic centers (Fig. 7). In places, tubular spaces were present. The neoplastic cells were cuboidal or polyhedral in shape. The nuclei were round to oval, with medium-sized to small nucleoli and finely dispersed chromatin. A high nucleus-cytoplasm ratio and considerable mitotic figures (3-8/HPF) were seen. Ductal hyperplasia and mast cell infiltration were detected in the surrounding tissue.

The unencapsulated neoplastic tissues, which existed mainly in the renal cortex, consisted of solid islands of neoplastic cells, but in places glandular and cystic formations were present. Some neoplastic cell foci were necrotic with cholesterol crystals. The neoplastic cells were oval, cuboidal or polyhedral with round to oval nuclei, inconspicuous nucleoli and finely

Fig. 5 Case 1, forearm tumor. Residual striated muscle cells (top left) and vascular smooth muscle cells (arrows) stain positively for desmin, but surrounding neoplastic cells are unstained. ABC. × 400.

Fig. 6 Case 1, forearm tumor. A myofibroblastoid cell is characterized by moderately developed organelles and bundles of fine filaments with focal densities (arrows). EM. × 7,500.
clumped chromatin. Some cells had abundant clear cytoplasm, while others had eosinophilic cytoplasm, and transitional forms were seen (Fig. 8). Mitoses were present (1-3/HPF). Frequently, foci of dysplastic tubules were formed in the cortex, and were accompanied by stromal fibrosis and ciliated epithelial cells in a few tubules (Fig. 9). There was severe fatty degeneration in the liver.

In the mammary lesion, almost all neoplastic cells showed positive immunostaining for ER (Fig. 10) and myoepithelial cells were absent. In the kidneys, the neoplastic cells reacted intensely with the anti-lysozyme antibody (Fig. 11). In contrast to intensely stained proximal tubules, most tubules in the distal nephron showed less intense staining for lysozyme.

In the kidneys, two types of neoplastic cells were confirmed by electron microscopy; they contained abundant glycogen (Fig. 12) or many mitochondria in the cytoplasm. There were intermediate forms with a fair amount of glycogen and several mitochondria. Pinocytotic vesicles and lipid droplets were detected in all types of cells, sometimes in large numbers.

**DISCUSSION**

It is conventional to subdivide carcinoma of the human breast into two main pathologic categories, in situ carcinoma and invasive carcinoma [8]. In case 1, invasive neoplastic cells were confirmed, and the most predominant growth pattern was cribriform. This tumor could be regarded as an invasive cribriform carcinoma [9], but had an extensive in situ...
component, which was demonstrated distinctly by the presence of SMA-positive myoepithelial cells at the periphery of tumor cell nests [10]. If human invasive carcinomas are accompanied by such a component, it conveys a better prognosis and a decreased frequency of nodal metastases [8, 10]. In addition, the presence of ER in many tumor cells in case 1 is a finding supporting the view that this tumor was a low grade carcinoma [11]. As for this mammary carcinoma, neither metastasis nor recurrence was detected 9 months after mastectomy.

Among human ductal carcinomas in situ, the comedo type is characterized by solid nests with central necrosis, and the neoplastic cells are usually negative for hormone receptors [9]. Similar lesions were predominant in case 2, and a diagnosis of comedo carcinoma was made [9]. As in human counterpart neoplasms [9], myoepithelial cells were absent, but ER was observed in almost all tumor cells. Such a tumor is considered to be relatively low in malignancy [11], and actually the animal was in good condition for a long time after surgery.

In humans, low-grade myofibroblastic sarcoma is a recently discerned entity, and is composed entirely of cells that have immunohistochemical and ultrastructural features of myofibroblasts [6]. In case 1, the tumor in the forearm was considered to be malignant on the basis of relatively high mitotic activity and cellularity. This tumor showed immunoreactivity for SMA but not for desmin was interpreted to show myofibroblastic differentiation, and a diagnosis of myofibroblastic sarcoma was made. The diagnosis was supported by ultrastructural findings [7], though there were frequent cells with abundant glycogen granules, suggestive of a histogenetic relation to smooth muscle.

Human clear cell renal cell carcinomas are composed of cells with abundant clear cytoplasm, but many of them have extensive areas in which the cytoplasm is eosinophilic [12]. These carcinomas are thought to show differentiation into the direction of proximal tubules [13]. Two similar cell types were seen in the present renal cell carcinoma. In addition, the neoplastic cells were strongly positive for lysozyme, and most neoplastic lesions and foci of dysplastic tubules were localized in the cortex. These findings suggested the proximal tubule origin of the carcinoma [13].

In the present cases, two types of malignant neoplasms were observed. Multiple neoplasms in the same hedgehog are a frequent occurrence [2]. In three hedgehogs with mammary gland carcinomas, concurrent neoplasms were detected, including uterine malignancies in two cases [2]. In a case report, a hedgehog had mammary adenocarcinoma and a granulosa cell tumor [5]. In addition to mammary tumors, there were uterine polyps in the present cases. Thus, hedgehogs with mammary adenocarcinoma are prone to have proliferative disorders in the uterus and ovary, synchronously or metachronously. In addition to mastectomy, ovariohysterectomy would be effective for survival of hedgehogs with mammary carcinoma. Moreover, ovariectomy may be of value early in the course of estrogen-positive mammary carcinomas [5].

要約

5歳を超える年齢の雌のナミハリネズミ2頭において、エストロジェンレセプター陽性の乳腺癌を手術により治療することに成功したが、その後、別種の悪性腫瘍が発生した。症例1では、乳腺の腫瘍組織は腺腔の形成を特徴として、豊富な組織像を呈していた。腫瘍組織の大部分は多形性膿皮細胞が混在していたが、α平滑筋アクチノシンとサイトゲラシンが陽性であった。術後9か月で前腕部に線維形細胞の増殖を特徴とした腫瘍ができ、線維形の腫瘍細胞は平滑筋アクチノシンで陽性に染まりオストミンで染まらなかったことから、筋線維芽細胞肉腫と診断した。

症例2の乳腺癌は免疫染色の形態学的特徴を示し、腫瘍中心部には新しい組織があり、平滑筋アクチノシン陽性の膿皮細胞は残存していた。約13か月後このハリネズミは死亡した。
REFERENCES