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Gastric Adenocarcinoma in a Cougar (*Felis concolor*)

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ABSTRACT: Diffusely invasive tumors occurred in the stomach of a 9-year-old female cougar (*Felis concolor*) from a zoo in Japan. The tumors consisted of tubular adenocarcinoma cells, and had infiltrative growth to the submucosa and muscularis propria. Tumor cells were positive for carcinoembryonic antigen (CEA), lysozyme, epithelial membrane antigen (EMA), gastrin, alpha-1-fetoprotein (AFP), keratin, and B72.3. Mucin-like materials occurred within cytoplasmic vacuoles.

Key words: Adenocarcinoma, stomach, cougar, *Felis concolor*.

Gastric carcinomas are common malignant neoplasms in humans, but rare in other animal species, including large felids (Head, 1990). Lingeman et al. (1971) reviewed gastric carcinomas in mammals and found 61 cases reported from dogs, four from horses, and one each from a cat, ox, and pig. Sullivan et al. (1987) reported 31 (0.24%) cases of gastric carcinoma among 13,015 dogs. The only known cases in felids involved a squamous cell carcinoma in the cardiac region in a male lion (*Panthera leo*) (von El-Sergany, 1966) and an adenocarcinoma combined with multiple neoplasms including thyroid adenocarcinoma, renal adenoma, and sertoli cell tumor in a captive jungle cat (*Felis chaus*) (Sagartz et al., 1972). The number of reported neoplasms in cougars appears to be limited (Lombard and Witte, 1959; Effron et al., 1977; Hubbard et al., 1983). Kennedy (1976) observed a case of multiple neoplasms including bilateral thyroid adenocarcinoma, bile duct carcinoma, and pulmonary adenocarcinoma in an 18-year-old cougar. Recently, Li et al. (1992) reported a case of functional thyroid follicular adenocarcinoma in a captive cougar.

There are no known reports of gastric carcinoma in the cougar. We describe the features and immunohistochemical findings of a gastric carcinoma in a female cougar (*Felis concolor*).

Originally, the cougar had been sent to the Shizuoka Municipal Zoo at about 1 yr of age in 1984, and had been kept continuously in the zoo. She had been impregnated with chlormadinone acetate (30 mg/kg, Teikoku-Zoki Pharmaceutical Co., Tokyo, Japan) for the purpose of birth control in 1987.

As a 9-year-old, she was examined by the veterinary staff of the Shizuoka Municipal Nihondaira Zoo, Shizuoka, Japan, to evaluate occasional vomiting unassociated with feeding and weight loss in March 1991. The clinical diagnosis was a suspected ulcer, and she was treated orally with 12 mg/kg of a histamine (H_2) receptor blocker (Tagamet; Fujisawa Pharmaceutical Company, Osaka, Japan). By November 1992, she had become quite thin (10.5 kg) and her coat was dull and rough. By December 1992, the animal had become depressed and less active, and could not eat meat. On 6 December 1992 the animal died while in a coma.

A necropsy was performed immediately upon death. The organs were fixed with 10% neutral buffered formalin. Paraffin sections from the liver, spleen, lungs, kidneys, heart, adrenals, thyroids, urinary bladder, ovaries, uterus, mesenteric lymph nodes, pancreas, stomach duodenum, jejunum, ileum, colon and rectum including lesions, were cut at 5 μm, and were stained with hematoxylin and eosin (H&E). Selected sections of the stomach also were stained with periodic acid Schiff (PAS), Alcian blue for acid mucosubstances, and Grimelius for argyrophil cells and Fontana-Masson for argentaffin cells (Vassallo et al., 1971; Vacca, 1985). Deparaffinized sections of the tumor were subjected to

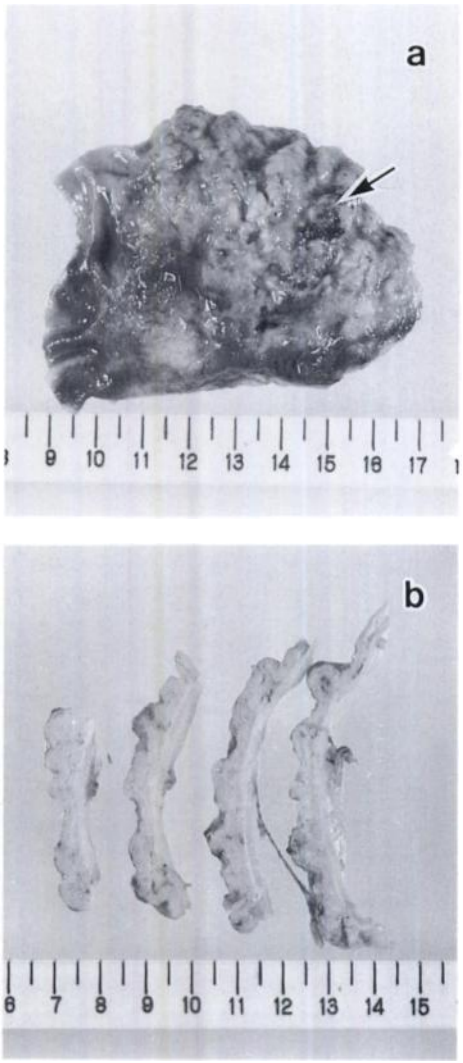

FIGURE 1. (a) Gross appearance of formalin-fixed stomach. The tumor is present in the antrum to body region of the stomach, and shows diffuse invasion of tumors, partially hemorrhagic. (b) The sections of the tumor is firm and grayish-white. Invasion under muscularis mucosa is evident. Scale is in centimeters.

FIGURE 2. Gastric adenocarcinoma in the cougar. The stomach shows diffuse involvement of adenocarcinoma cells into submucosa (sm). mm = muscularis mucosa, mp = muscularis propria. H&E stain. Bar = 400 μm.

immunohistochemistry using the labeled streptavidin-biotin (LSAB) method (Dako Corporation, Santa Barbara, California, USA), according to the manufacturer's instructions. The peroxidase reaction was initiated by 0.06% diaminobenzidine (Doujin Chemicals, Kumamoto, Japan) and 0.01% H_2O_2. The sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany). The antibodies used were anti-carcinoembryonic antigen (CEA), anti-lysozyme, anti-epithelial membrane antigen (EMA), anti-gastrin, anti-alpha-1-fetoprotein (AFP), anti-keratin (Dako Corporation), and monoclonal antibody (MAb) B72.3 (Japan Tanner Corporation, Kobe, Japan). Samples of formalin-fixed tumor tissue were washed in phosphate buffer, fixed with 2% phosphate buffered glutaraldehyde and 1% osmium tetroxide (Polysciences, Inc., Warrington, Pennsylvania, USA), dehydrated in a series of graded ethanols and embedded in Epon 812 (Polyscience Inc.). Ultrathin sections were cut and double-stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with an electron microscope (H-800, Hitachi Limited, Tokyo, Japan).

At necropsy, the animal was cathectic, with minimal subcutaneous fat storage. A diffusely invasive carcinoma was located in the antrum to body region, slightly anterior to the lesser curvature of the stomach (Fig. 1a). The stomach wall affected by the tumor was rigid and markedly thickened. The mucosal surface was covered with gelatinous mucus, and partially hemorrhagic. The section of the tumor was firm and grayish-white (Fig. 1b); invasion of the tumor under the muscularis mucosa was evident. No evidence of metastasis was

FIGURE 3. Tumor cells of the cougar form irregular tubular structures in the submucosa. Some resemble those of stomach gland and others resemble those of intestinal epithelium. mp = muscularis propria. H&E stain. Bar = 250 μm.

found in the regional lymph nodes. Additional gross findings included severe fibrinous pleuritis, pulmonary edema and moderate atrophy of both ovaries.

Microscopically, the tumor was a well-differentiated tubular adenocarcinoma with infiltrative growth to the upper layer of the muscularis propria (Fig. 2). Mucosal atrophy with disappearance of the gastric glands was prominent, and a small number of signet ring carcinoma cells grew diffusely in the lamina propria, often with desquamation into the lumen. In the middle and deep areas of the tumor, some tumor cells contained irregular tubular structures, some of which were very similar to gastric glands; other tumor cells resembled the intestinal epithelium with mucin within the cytoplasm (Fig. 3). The

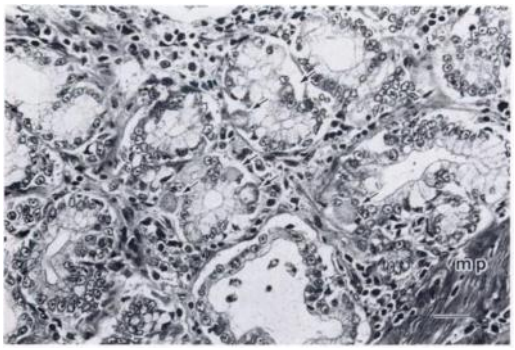

FIGURE 4. The tumor often contained cells resembling parietal cells with central spherical nuclei and intensely eosinophilic cytoplasms (arrows). mp = muscularis propria. H&E stain. Bar = 26 μm.

tumor often contained cells resembling parietal cells; these were rounded or pyramidal cells with central spherical nuclei and intensely eosinophilic cytoplasms (Fig. 4). Mitotic figures occasionally were observed in the tumor. A moderate fibrous stromal reaction and a moderate inflammatory reaction were observed around the tumor growth. Some argyrophilic cells were found in sections stained by the Grimelius method, but no argentaffin cells were found in Fontana-Masson stained sections in the tumor. Based on the periodic acid Schiff (PAS) reaction and Alcian blue staining, there was acid or neutral mucus within the cytoplasm of the adenocarcinoma cells and signet ring cells. The eosinophilic cytoplasm of the parietal-like cells were PAS and Alcian blue negative.

The tumor had a strong immunostaining response with antibody to CEA, lysozyme, EMA, gastrin, AFP, keratin and MAb B72.3 (Fig. 5a to d).

Ultrastructurally, the tumor cells contained mucin-like materials within cytoplasmic vacuoles (Fig. 6a). The tumor cells bore microvilli and formed lumina, and were jointed by desmosomes (Fig. 6b). Some tumor cells contained abundant mitochondria in the cytoplasm.

Based on gross, histologic, and ultrastructural characteristics, we classified this case as a well-differentiated tubular ade-

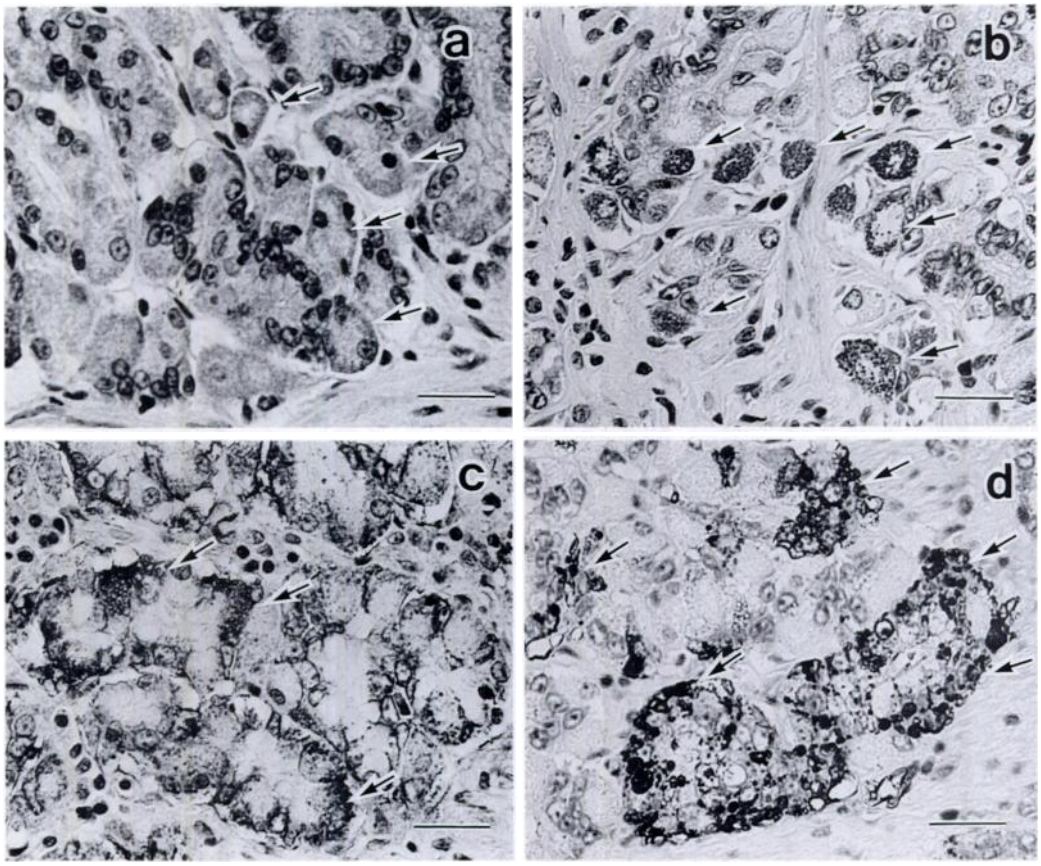

FIGURE 5. Binding of antisera to (a) carcinoembryonic antigen (CEA), (b) lysozyme, (c) epithelial membrane antigen (EMA) and (d) B72.3 is positive in some tumor cells of the cougar (arrows). Labeled streptavidin-biotin (LSAB) method with hematoxylin counterstaining. Each bar = 22 μm.

nocarcinoma derived from the antrum to body region. The commonest site of involvement in gastric carcinoma is the antrum region in both humans (Fine and Mo, 1990) and dogs (Patnaik et al., 1977). It now is common to adopt the gastric carcinoma classifications used with humans in all animals; carcinomas are divided into tubular or intestinal, diffuse and intermediated types (Head, 1990). Most canine gastric adenocarcinoma are of the diffuse type (Lingeman et al., 1971). The histologic features in the cougar case closely resembled those of the tubular adenocarcinoma in humans. The tumor was positive for PAS or Alcian blue, and PAS positive neutral mucus containing cells might represent gastric type cells, while Alcian blue positive acid mucus containing cells might represent metaplastic intestinal cells. In the present cougar case, some argyrophilic cells were found in the tumor. Scanziani et al. (1993) found argyrophilic cells in 13 of 22 gastric carcinomas in dogs and demonstrated that a significant proportion of gastrointestinal mucosal tumors of the dog contain a mixture of epithelial and endocrine cells.

Expression of an epithelial antigen, such as EMA and keratin, has been noticed in gastrointestinal cancer in humans. Expressions of CEA and AFP also have been demonstrated in human gastric cancers (Hockey et al., 1984; Ooi et al., 1990). Lysozyme also is believed to be a useful marker for gastrointestinal tumors, and it has been con-

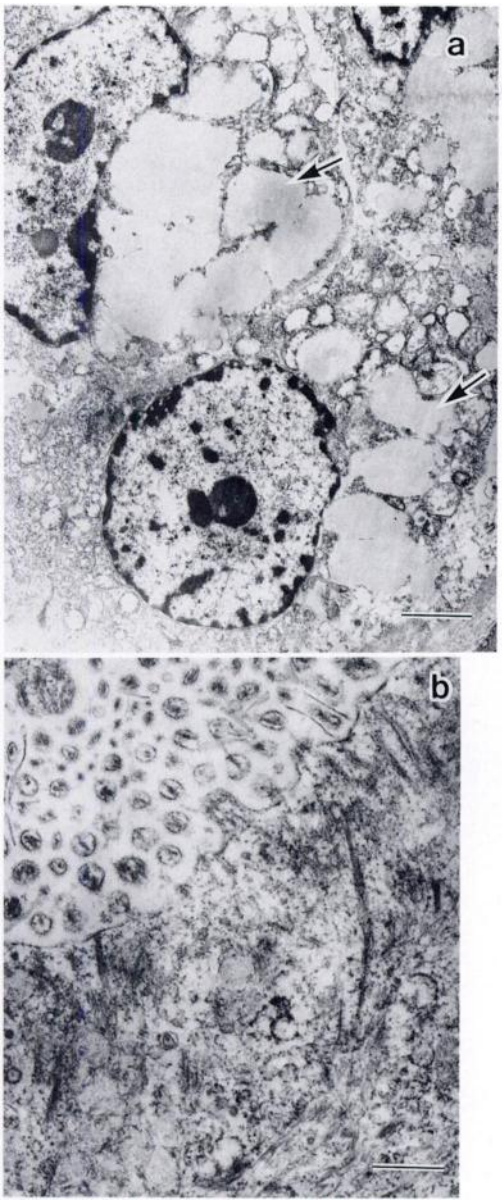

FIGURE 6. (a) Electron micrograph of tumor cells with mucin-like vacuoles. Bar = 2 μm. (b) The tumor cells bear microvilli and form lumen. Bar = 0.6 μm.

sidered that gastric cancer showing strong lysozyme immunoreactivity commonly takes a malignant course (Tahara et al., 1982). Monoclonal antibody (MAb) B72.3, which reacts with a tumor-associated glycoprotein designated mucin-like glycoprotein (TAG-72), has been identified in both human and canine carcinomas, but generally is not found in normal human or canine tissues (Stramignoni et al., 1983; Lyubsky et al., 1988; Clemo et al., 1993). Mitochondria-rich tumor cells by electron microscopy might correspond to parietal-like cells by light microscopy.

Among humans, gastric cancer can develop in young adults and is associated with such factors as carcinogenic activity of the polycyclic hydrocarbons present in smoked meat and fish, and genotype (Wynder et al., 1963; Davis, 1989). Recently, human gastric carcinomas have been associated with infection by the gastric *Helicobacter pylori* (Parsonnet et al., 1991). More recently, cheetahs (*Acinonyx jubatus*) were reported to have gastritis due to an organism that is closely related to a human pathogen (Eaton et al., 1993). Further investigation is needed to determine any correlation between *Helicobacter pylori* infection and gastrointestinal cancer in the large felidae.

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