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Immunohistochemical Study of Pancreatic Neuroendocrine Tumor in *Panthera tigris tigris*

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ABSTRACT: The histological and immunohistochemical characteristics of a case of pancreatic neuroendocrine tumor are described in a 14-yr-old female Bengal tiger (*Panthera tigris tigris*) housed at the New Biblical Zoo of Jerusalem, Jerusalem, Israel, 1994. The neoplastic cells were immunohistochemically negative for insulin and glucagon, slightly positive for neuron-specific enolase, moderately positive for serotonin and somatostatin, and markedly positive for chromogranine A and gastrin. This is the first documentation of a pancreatic neuroendocrine tumor in the tiger.

Key words: Pancreas; Neuroendocrine tumor; *Panthera tigris tigris*; Immunohistochemistry.

Pancreatic neuroendocrine tumors have not been described in the tiger (*Panthera tigris*): These neoplasms are also relatively rare in the domestic cat (Dill-Macky, 1993). There are only a few reported cases of pancreatic islet neoplasia in cats. Gastrinomas, insulinomas and tumors of unknown endocrine activity have been reported (Middleton et al., 1983; McMillan et al., 1985; Peterson and Randolph, 1989; Van der Gaag et al., 1988; O'Brien et al., 1990). Our objective was to document the histological and immunohistochemical features of a pancreatic neuroendocrine tumor in a Bengal tiger (*Panthera tigris tigris*), an endangered species according to the IUCN classification.

A 14-yr-old female tiger, housed at the Biblical Zoo of Jerusalem, Jerusalem, Israel, was unwilling to leave her house. By evening of the same day she was unconscious with tonic twitching and finally became comatous. The only remarkable sign, noticed in the last weeks of her life, was her abundant body fat. Her usual diet had included about 13 kg of beef meat twice a week during the summer and three times that quantity during the cold season. Euthanasia was elected and was performed via intramuscular injection of 10 mg/kg ketamine hydrochloride (Ketaset, Fort Dodge, Iowa, USA) with 2 mg/kg Rompun sedation (Bayer, Wupertal, Germany) and 100 mg/kg of Nembutal (Ceva, Abbott, Brussells, Belgium).

A post mortem examination was performed at the zoo. Tissues (pancreas with its attached mass, kidneys and ovaries), preserved in 10% buffered formalin, were evaluated at the Pathology Department, Kimron Veterinary Institute, Bet Dagan, Israel (Reference No. 59025). For histology, 5 μm paraffin sections were prepared and stained with hematoxylin and eosin (HE), Periodic Acid Schiff (PAS), Masson's trichrome and Gomori's method for reticulin (Bancroft and Stevens, 1977). At necropsy, the animal had a swollen and soft abdomen. Internally, extremely large amounts of fat pad deposits were noted in the abdomen, around the pericardium, kidneys, and in the subcutis.

A 7 to 8 cm diameter multinodular, gray-white, moderately firm mass was attached to and compressing the pancreas. The gastric mucosa had several ecchymotic hemorrhagic areas. All other organs were unremarkable.

Immunohistochemical staining for neuron-specific enolase (NSE), insulin, glucagon, chromogranin A, gastrin, serotonin and somatostatin (Dako Corp., Carpinteria, California, USA and Biogenex Laboratories, San Ramon, California, USA) were performed using the avidin-biotin peroxidase complex (ABC) technique (Hsu et al., 1981). Appropriate positive control normal endocrine tissues were used. For

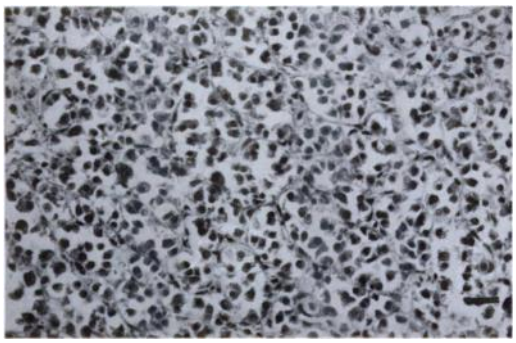

FIGURE 1. Pancreatic neuroendocrine tumor of a *Panthera tigris tigris*, Jerusalem, Israel, 1994. The neoplastic cells are organized in small lobules, subdivided by a delicate network of connective tissue fibers. H&E. Bar = 50 μm.

negative controls, the primary antiserum was omitted. The intensity of immunohistochemical reactions were graded subjectively as negative, slight, moderate, and marked. Electron microscopy examination was not done due to unsuitable fixation of tissues in formalin and advanced postmortem deterioration.

Microscopically, the mass was multilobated, with thick, vascularized fibrous trabeculae subdividing the tumor lobules. The tumor invaded and replaced the pancreas. Only a few remnants of normal pancreatic tissue were noted in the periphery. No defined capsule was present. The neoplastic cells were arranged in typical endocrine-like clusters, forming solid alveoli or nests surrounded by delicate connective tissue fibers (Fig. 1). The fibers were silver positive with Gomori's stain. The closely packed, fairly uniform, round to polygonal cells had prominent granular acidophilic cytoplasm and small spherical nuclei with a single prominent nucleolus. Only a few neoplastic cells, which were dispersed at random, had atypical and pleomorphic nuclei. Up to two mitotic figures were present for every 10 high power field; the radius of each field was 500 μm. Small aggregates of lymphocytes were occasionally noted, infiltrating among neoplastic clusters located at the periphery of the mass. A prominent fibrovascular stroma was distributed among the tumoral cell aggregates. The tumor tissue was immunohistochemically negative for insulin and glucagon, slightly positive for NSE, moderately positive for serotonin and somatostatin, and markedly positive for chromogranin A and gastrin.

No serum secretory products were evaluated in the tiger; consequently, the functional activity of the tumor was not determined. The referring veterinarian concluded that the suspected cause of death was an endocrine pancreatic tumor.

The histological and immunohistochemical features were evidence for a pancreatic neuroendocrine tumor strongly positive for gastrin. Due to lack of evidence for invasion of lymphatics or blood vessels, absence of metastases, and the minimal cellular atypia, the tumor was considered benign.

Neuroendocrine cells are multimessenger systems; they are able to change their pattern of hormone expression, their morphology or both, in response to micro-environmental signals (Tischler, 1989). This plasticity might help to explain many of the apparently abberant characteristics of neoplastic neuroendocrine cells (Tischler, 1989).

Immunohistochemically, neuroendocrine tumor cells can be identified by means of hormonal and nonhormonal markers. The latter includes the chromogranins, synaptophysin, and NSE antigens (Capen and Martin, 1969). Neoplasms originating from the pancreatic islets may be classified according to their predominant secretory product and the resulting functionality, such as gastrinoma, insulinoma or glucagonoma (Ashley, 1990).

Neoplasms derived from neuroendocrine cells may produce two or more peptide hormones which are usually synthesized by their normal counterparts or secreted by endocrine or non-endocrine cells. The secreted product may be mature or immature, with varying degrees of peptide hormonal processing (Betton, 1992). Although hormonal content positivity oc-

curred within the mass of the present case, apparently no clinical signs characteristic of excess hormone secretion in the blood was evident. In fact, the tiger behaved normally until the day of death. Jubb (1993) states that there is a poor correlation between the immunohistochemical profile of islet cell tumors and clinical disease.

Active gastrin-secreting neoplasms in domestic cats are associated with vomiting, weight loss, listlessness and alternating diarrhea and obstipation (Middleton et al., 1983; Peterson and Randolph, 1989). In one case of insulinoma in a cat, seizure episodes were noted, concomitant with low blood glucose concentration (McMillan et al., 1985).

As no other pathological changes were found in the tiger, taking into consideration the limited number of organs checked histologically, we believe that the pancreatic tumor, leading to some metabolic abnormalities, was the cause of death. Although evidence for hormonal synthesis was present in the neoplastic cells, the lack of serum assays for hormones contents prevented conclusions regarding it's clinical hormonal functionality.

The physiological control mechanisms which regulate the secretion of neuroendocrine cells may lead, upon their continuous and prolonged stimulation, to secretion of peptide hormone, to compensated feedback hypertrophy, followed by hyperplasia and neoplasia of the neuroendocrine cell concerned (Betton, 1992). Perhaps the obese state resulting from prolonged overfeeding contributed to the development of the pancreatic tumor in the tiger. In this regard, it is interesting to note that a food-restricted diet significantly decreased the incidence of pancreatic islet carcinoma in laboratory rats (Keenan et al., 1995).

The nomenclature of pancreatic endocrine tumors is supplemented by the functional activity, depending on which hormones are formed and secreted by the tumor (Kloeppel and Heitz, 1988). The pancreatic tumor in this tiger was classified as a multihormonal (serotonin, somatostatin and gastrin producing) pancreatic neuroendocrine tumor.

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