NOTE *Pathology*

Intestinal Undifferentiated Carcinoma in a Red-Crowned Crane (*Grus japonensis*)

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ABSTRACT. A 33-year-old red-crowned crane (*Grus japonensis*) had a diffuse intestinal thickening from the duodenum to colon. Microscopically, neoplastic cells were arranged in sheets and occasionally nests or cords without gland or squamous differentiation. Metastatic tumor cells were found in the lungs, heart, kidneys and adrenal glands. Immunohistochemically, the neoplastic cells were strongly positive for pan-cytokeratin and cytokeratin 8 and 18 and only partly positive for E-cadherin antibodies. Immunostaining for CD3 was positive in normal lymphocytes, and NSE was also positive in normal nerve fibers. But, the neoplastic cells were not immunoreactive to CD3 and NSE. Based on the histological features and the epithelial characteristics in the immunohistochemical stain, the present case was diagnosed as undifferentiated carcinoma originating from the intestine. Interestingly, the neoplastic cells showed a unique growth pattern; they never invaded the submucosa or muscularis throughout the intestine, whereas they spread lymphogenously or hematogenously to other organs.
KEY WORDS: immunohistochemistry, red-crowned crane, undifferentiated carcinoma.

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The red-crowned crane (*Grus japonensis*) is classified as an endangered species according to the International Union for Conservation of Nature (IUCN) red list categories [3]. Although intestinal adenocarcinomas have been well documented in humans [4], dogs and cats [5, 9, 14], only a few reports have described intestinal undifferentiated carcinoma in humans [4, 11, 12, 15] and there is a little information about it in dogs and cats [5, 9, 14]. Several cases of primary intestinal tumors have been reported in avian species, including adenocarcinoma [2, 8, 18, 19], leiomyosarcoma [16] and lymphosarcoma [17]. To our knowledge, there is no literature on undifferentiated carcinoma in the avian intestinal tract.

A 33-year-old male red-crowned crane (*Grus japonensis*), kept in a crane reserve, exhibited loss of appetite, vomiting and melena. The bird was 6.45 kg in weight and treated with antimicrobial agents, vitamin supplement and transfusion. Three months after the clinical onset, the crane showed a marked weight loss and died (4.55 kg at this time). At necropsy, the intestinal wall from the duodenum to colon was diffusely thickened and firm, especially the ileoceal junction, and the lumens of both ceca were irregularly dilated and contained cloudy and viscoid fluid. On the mucosal surface from the duodenum to jejunum, some milky white or red protruding lesions, up to 2 cm in diameter and with or without a central depression, were found. The mucous membranes from the distal jejunum to the colon were prominently thickened and milky white in color (Fig. 1). The thickened membranes produced stenoses of the lumens. There were a few white foci, 1 to 2 mm in diameter, in the myocardium of the left ventricle and cortex parenchyma of the kidneys. In other organs, a focal ulcer and petechiae of the gastric mucosa and atrophy of muscles in both chests were confirmed.

The gastrointestinal tract, lungs, heart, kidneys, spleen, liver, pancreas, thyroid glands, parathyroid glands, adrenal glands and testes were fixed in 10% neutral buffered formalin, embedded in paraffin-wax and sectioned at 4 μm. After deparaffinization, sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and alcian blue (pH 2.5) and by Grimelius argyrophil methods. Immunohistochemical examination was performed on selected sections by the avidin-biotin peroxidase complex method (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, U.S.A.) with 3'3-diaminobenzidine as chromogen. The slides were counterstained with Mayer's hematoxylin. The primary antibodies used were mouse monoclonal antibodies against human pan-cytokeratin (clone AE1/AE3, Nichirei, Tokyo, Japan), cytokeratin 8 (CK8, clone K_s 8.7, PROGEN, Heidelberg, Germany) and 18 (CK18, clone RGE53, PROGEN), E-cadherin (4A2C7, Invitrogen Corporation, Camarillo, CA, U.S.A.) and neuron-specific enolase (NSE, clone BBS/NC/VI-H14, Dako, Denmark), and a rabbit polyclonal antibody against human CD3 (N1580, Dako, Carpinteria, CA, U.S.A.). For electron microscopic examination, tissue fragments of 1 mm^3 were cut with a sharp razor blade from specific areas of the formalin-fixed paraffin-embedded block, reprocessed, post-fixed in 1% osmium tetraoxide and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

Microscopically, the mucosal thickening and protruding lesions were caused by the growth of neoplastic cells (Fig.

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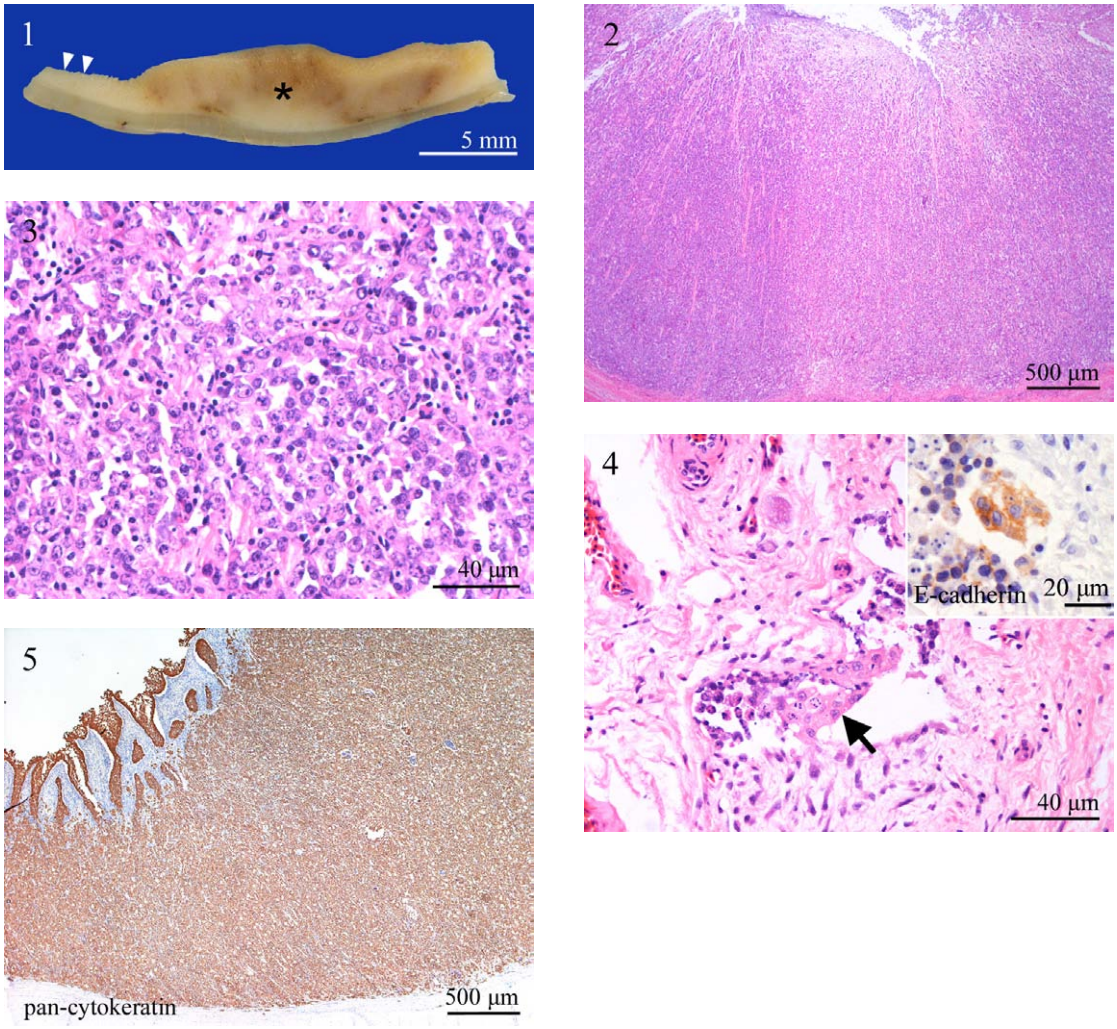

Fig. 1. Jejunum. Mucous membrane is remarkably thickened (*) compared with that of the left side (arrowheads). Photo image after fixation.
Fig. 2. Ileum (ileocecal junction). Neoplastic cells proliferate throughout the lamina propria with the disappearance of all normal intestinal epithelia. The lamina propria is thickened due to broad proliferation of the neoplastic cells. HE.
Fig. 3. Jejunum. Higher magnification of the neoplastic cells. Neoplastic cells grow in nests with thin fibrous stroma. They are round to polygonal, and they have eosinophilic cytoplasm and an atypical round nucleus. HE.
Fig. 4. Ileum (ileocecal junction). Lymphovascular invasion in the serous membrane of the small intestine. Arrow indicates neoplastic cells in lymphovascular spaces. HE. Inset: Ileum. E-cadherin expresses strongly the cytomembrane of the neoplastic cells. IHC for E-cadherin, counterstained with hematoxylin.
Fig. 5. Jejunum. Neoplastic cells have cytoplasmic labeling for cytokeratin. IHC for pan-cytokeratin, counterstained with hematoxylin.

2). The most marked proliferation of tumor cells was seen in the ileum, where the normal epithelia had completely disappeared. The neoplastic cells were round to polygonal with eosinophilic cytoplasm and had an atypical round nucleus with conspicuous nucleoli. The neoplastic cells were arranged in sheets and occasionally nests or cords with thin fibrous stroma, and most tumor cohesion was poor (Fig. 3). The mean mitotic index was approximately 3 under a high-power field (×400). The neoplastic cells located in the lamina propria, but they did not invade the submucosa or muscularis throughout the thickened intestine. Metastases to the lungs, heart, kidneys and adrenal glands were observed, and the histological findings of the metastatic cells were similar to those of the neoplastic cells in the intestinal tract. Lymphovascular invasion was observed in the serous membrane of the small intestine and adrenal glands (Fig. 4). The gastric mucosa was focally ulcerated, but no neoplastic cells were found.

Positive PAS reaction was confirmed in the mucus with the goblet cells in the normal intestinal glands, but not in the

neoplastic cells. Alcian blue stain also detected no production of mucus. Argyrophilic granules were detected in the non-neoplastic intestinal endocrine cells by Grimelius stain, but not in the neoplastic cells.

Immunohistochemically, the intestinal and the metastatic tumor cells were strongly and diffusely positive for pan-cytokeratin and CK 8 and 18 antibodies as well as the normal epithelial cells in the small and large intestines (Fig. 5). Although all tumor cells were weakly stained for E-cadherin in the cytoplasm, strong positive reactions were seen in the cytomembrane of tumor cells, infiltrating the lymphovascular spaces, as well as the normal epithelial cells (Fig. 4, inset). Immunolabeling for CD3 was positive in the normal lymphocytes, and NSE was also positive in the normal nerve fibers. But, the neoplastic cells were not immunoreactive to CD3 and NSE.

Ultrastructually, cytoplasmic secretory granules were not observed in the neoplastic cells.

In humans and domestic animals, undifferentiated carcinoma is defined by the epithelial component of neoplastic cells with no evidence of squamous or glandular differentiation, and immunohistochemical stain for cytokeratin is useful in the diagnosis of this tumor; it is difficult to distinguish with neuroendocrine carcinoma or non-epithelial tumors in the intestinal tracts [4, 9, 11, 12, 15].

Immunohistochemistry with several antibodies, including anti-cytokeratin, NSE and CD3, has been performed for tumor diagnosis in birds [10, 13, 17]. In humans and domestic animals, the antibodies to chromogranin A, synaptophysin and NSE are helpful to distinguish with neuroendocrine carcinoma [4, 9]. In the present case, the positive control tissue from this crane showed immunoreactive for NSE, but all of the tumor cells were negative. However, polyclonal rabbit anti-human Chromogranin A (A0430, Dako, Glostrup, Denmark) and monoclonal mouse anti-synaptophysin (clone SP15, Millipore, Billerica, MA, U.S.A.) did not specifically react with any tissue sections in this crane. Previously, diffuse intestinal T-cell lymphoma in a yellow-naped Amazon parroto has been reported. It showed diffusely and markedly distending mucosal membrane, similar to the present case, and immunohistochemistry with CD3 in the control tissue and neoplastic cells were successful [17]. This finding supports our results since the normal lymphocytes also stained with CD3, but the tumor cells were negative in our case. Additionally, in the present case, tumor cells were diffusely and strongly stained for cytokeratins, and the tumor cells infiltrating the lymphovascular spaces specifically expressed E-cadherin. This indicates the present tumor cells had epithelial characteristics. Based on the histological features and the epithelial characteristics of the immunohistochemical stains, the present case was diagnosed as undifferentiated carcinoma originating from the intestine.

The diffuse infiltration of the neoplastic cells throughout the intestinal tract in our case strongly suggested that the intestinal tract was the primary site. We suspected the ileocecal junction as a primary tumor location, where the most marked tumor growth was seen with the disappearance of all normal intestinal epithelia, and the neoplastic cells horizontally spread in the jejunum, ceca and colon. Among avian species, primary intestinal carcinomas are rare, and all reported cases have been diagnosed as adenocarcinomas [2, 8, 18, 19]. On the other hand, ovarian carcinomas, which are the most common tumor in hens, are likely to metastasize to the intestinal tract and may be confused with intestinal carcinoma [7]. However, that was not the case in the present report involving a male crane.

In humans, primary undifferentiated carcinoma of the intestine is extremely rare, and patients with this tumor have a poorer prognosis than patients with adenocarcinoma [4, 11, 12, 15]. This tumor of the small intestine was found in the jejunum and the ileum, but not in the duodenum [11, 12, 15]. Additionally, the tumor in all reported cases was a nodulated mass lesion, and many cases extended to the deeper layer, such as muscularis and serosa, and metastasized to lymph node [11, 12, 15]. Thus, the growth pattern of human undifferentiated carcinoma of the small intestine is widely different from the present case.

This is, to our knowledge, the first description of avian intestinal undifferentiated carcinoma in a red-crowned crane. There have been a few reports associated with tumor in cranes [1, 6, 10]. It is unclear if the unique growth pattern in the present case is specific to this species. Further investigations are needed to reveal the ecology of the red-crowned crane, as it is an endangered species.

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