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Case Report

Adenocarcinoma of Barrett's esophagus in a dog

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Abstract. An endoscopic examination revealed a mass in the distal esophagus of a 9-year-old intact male bulldog. Histopathologically, the mass was composed of cuboidal to columnar neoplastic epithelial cells and extended from the squamous epithelium of the esophageal mucosa, indicating that the tumor was derived from Barrett's esophagus. Moreover, highly atypical foci that exhibited a cribriform pattern and high mitotic indices were also observed. The epithelial cells on the surface of the lesion often produced mucus that was positive for Alcian blue and immunohistochemically positive for MUC5AC. The neoplastic epithelial cells were diffusely positive for cytokeratin 7 and p53, and occasionally positive for cytokeratin 20. Based on these findings, the tumor was diagnosed as an adenocarcinoma. This report describes the clinical and pathological features of a spontaneous case of adenocarcinoma of Barrett's esophagus in a dog. (DOI: 10.1293/tox.2017-0009; J Toxicol Pathol 2017; 30: 239–243)

Key words: adenocarcinoma, Barrett's esophagus, dog

Barrett's esophagus is a condition of the lower esophagus in which the cells of the normal squamous mucosa transform into columnar mucosal cells[1]. It is considered to be an adaptation to chronic injuries, such as chronic inflammation due to gastroesophageal reflux[2]. It has been reported that it is more likely that the metaplastic cells in tumors of Barrett's esophagus originate from migrating gastric cardia progenitors with metaplastic potential rather than from squamous progenitor cells in the esophageal mucosa[3]. Clinical and genetic studies have strongly suggested that Barrett's esophagus is a precancerous condition that is related to the development of esophageal adenocarcinoma[4]. However, whether esophageal adenocarcinoma emerges from metaplastic cells or directly arises from migrating gastric cardia progenitors remains unknown.

Experimental models of Barrett's esophagus have been produced in several animal species[5]. The rat models have been studied most extensively, but the structural and physiological differences between the human and rodent esophagus affect the applicability of the results of rodent studies to humans. Subjecting dogs to pharmacological or surgical interventions can produce ideal animal models of Barrett's esophagus. Kawaura et al. performed cardiectomy or gastrectomy in 50 dogs as acid or alkaline reflux models and endoscopically biopsied the esophageal mucosa every 3 months[6]. Among the 50 dogs, 24 developed Barrett's esophagus, and 9 of them progressed to Barrett's esophagus with mild dysplasia, including 2 dogs that progressed further to adenocarcinoma after 60 months post operation.

Spontaneous Barrett's esophagus is rare in animals, so it has not been studied in detail. There has been one case report about a dog that spontaneously developed adenomatous polyps in Barrett's esophagus[7]. The present study describes the clinical, histopathological, and immunohistochemical features of spontaneous adenocarcinoma of Barrett's esophagus in a dog.

A 9-year-old intact male bulldog exhibited hypersalivation, an increasing incidence of vomiting, and weight loss. The animal was referred to the Veterinary Medical Center, the University of Tokyo, for examination. Blood biochemistry tests revealed a reduced plasma potassium concentration

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(2.9 mmol/l, reference range: 4.37–5.35 mmol/l) and an elevated plasma C-reactive protein level (6.5 mg/dl, reference range: <1 mg/dl)[8]. Computed tomographic image revealed a mass with a diameter of 19 mm in the lower esophagus. An endoscopic examination detected a mass in the mucosa of the distal esophagus (Fig. 1A). The mass as well as the surrounding mucosa were slightly dark red compared with the proximal esophageal mucosa. No significant lesions were observed in the stomach or duodenum during the endoscopic examination. Mucosal biopsy specimens were collected from the esophagus, stomach, and duodenum using endoscopic biopsy forceps, and fixed in 10% neutral buffered formalin. After a histopathological examination, no further treatment was performed, and the dog died 4 months later. We did not have consent to perform a necropsy.

The biopsy samples were processed for routine histology, embedded in paraffin, cut into 4 µm-thick sections, and stained with hematoxylin and eosin (HE) and Alcian blue (pH 2.5). Immunohistochemistry was performed using the primary antibodies listed in Table 1. A horseradish peroxidase-labeled polymer was used as a secondary antibody (Dako EnVision + System, Dako, Tokyo, Japan). The resultant antigen-antibody complexes were visualized with 3,3-diaminobenzidine, and then the sections were counterstained with hematoxylin. Normal esophageal, gastric, and ileal tissue samples from another dog, which were collected during a routine necropsy, were used as positive controls. Negative controls were obtained by omitting the primary antibodies.

A histopathological examination of the esophageal mass revealed that the normal esophageal mucosa was replaced by an exophytic nodular mass composed of papillary projections, tubules, and glandular acini lined by a simple columnar to cuboidal epithelium separated by fine fibrous connective tissue, which was mildly expanded by scattered small mature lymphocytes and plasma cells (Fig. 1B). However, in the deeper regions of the tumor mass, the neoplastic cells showed significant atypia and were arranged in irregular glandular structures or cribriform patterns (Fig. 1C). The mitotic count of the atypical area was high (29 mitotic figures per 10 high-power fields). Goblet cells positive for Alcian blue were often found on the apical surface of the mass (Fig. 2A). Immunohistochemistry revealed that these surface cells were positive for mucin 5AC (MUC5AC) (Fig. 2b). The neoplastic cells were also positive for cytokeratin (CK) 7 and CK20 but negative for CK5/6 (Figs. 2C–E). CK7 positivity was observed throughout the mass; however, CK20 positivity was mainly observed on the surface of the mass and occasionally observed in deeper areas. The nuclei of the neoplastic cells were positive for p53 (Fig. 2F), whereas the cells in the normal esophageal mucosa as well as the esophageal glands of another dog were negative for p53. In contrast to the neoplastic cells, the squamous epithelial cells were negative for CK7, CK20, and p53, although they were positive for CK5/6. The nuclei of the neoplastic cells were frequently and randomly positive for Ki-67. In the present case, no significant histological lesions were seen

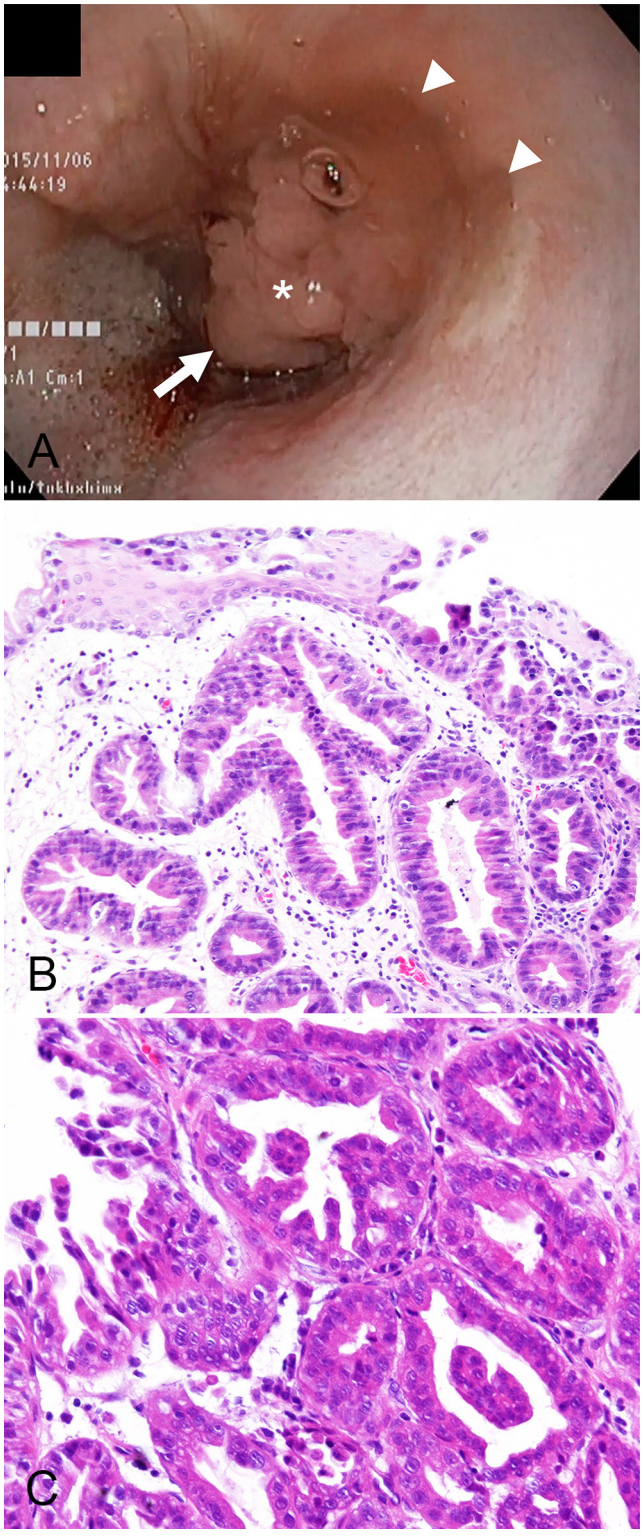

Fig. 1. (A) An endoscopic image of a mass protruding from the mucosa of the distal esophagus is shown. The mass (arrow) as well as the surrounding mucosa (arrowheads) were slightly dark red in color. An asterisk indicates the area that was collected for histopathological examination. (B) Cuboidal to columnar epithelial cells extended from the squamous epithelium of the esophageal mucosa (upper left). Lymphocytes and plasma cells infiltrated the stroma. (C) The atypical area exhibited a cribriform pattern and a loss of cellular polarity. (B) HE stain, ×100. (C) HE stain, ×200.

Table 1. Primary Antibodies for Immunohistochemistry

Antibody	Host (clone)	Dilution	Antigen retrieval	Manufacturer
Cytokeratin 5/6	Mouse (D5/16B4)	1:50	Heat, pH 9.0	Dako, Tokyo, Japan
Cytokeratin 7	Mouse (OV-TL 12/30)	1:50	Proteinase K	Dako, Tokyo, Japan
Cytokeratin 20	Mouse (Ks20.8)	1:50	Proteinase K	Dako, Tokyo, Japan
p53	Rabbit	1:50	Heat, pH 9.0	Santa Cruz Biotechnology, Dallas, TX, USA
MUC5AC	Mouse (45M1)	1:100	Heat, pH 6.0	Abcam, Cambridge, UK
Ki-67	Mouse (MIB-1)	1:100	Heat, pH 6.0	Dako, Tokyo, Japan

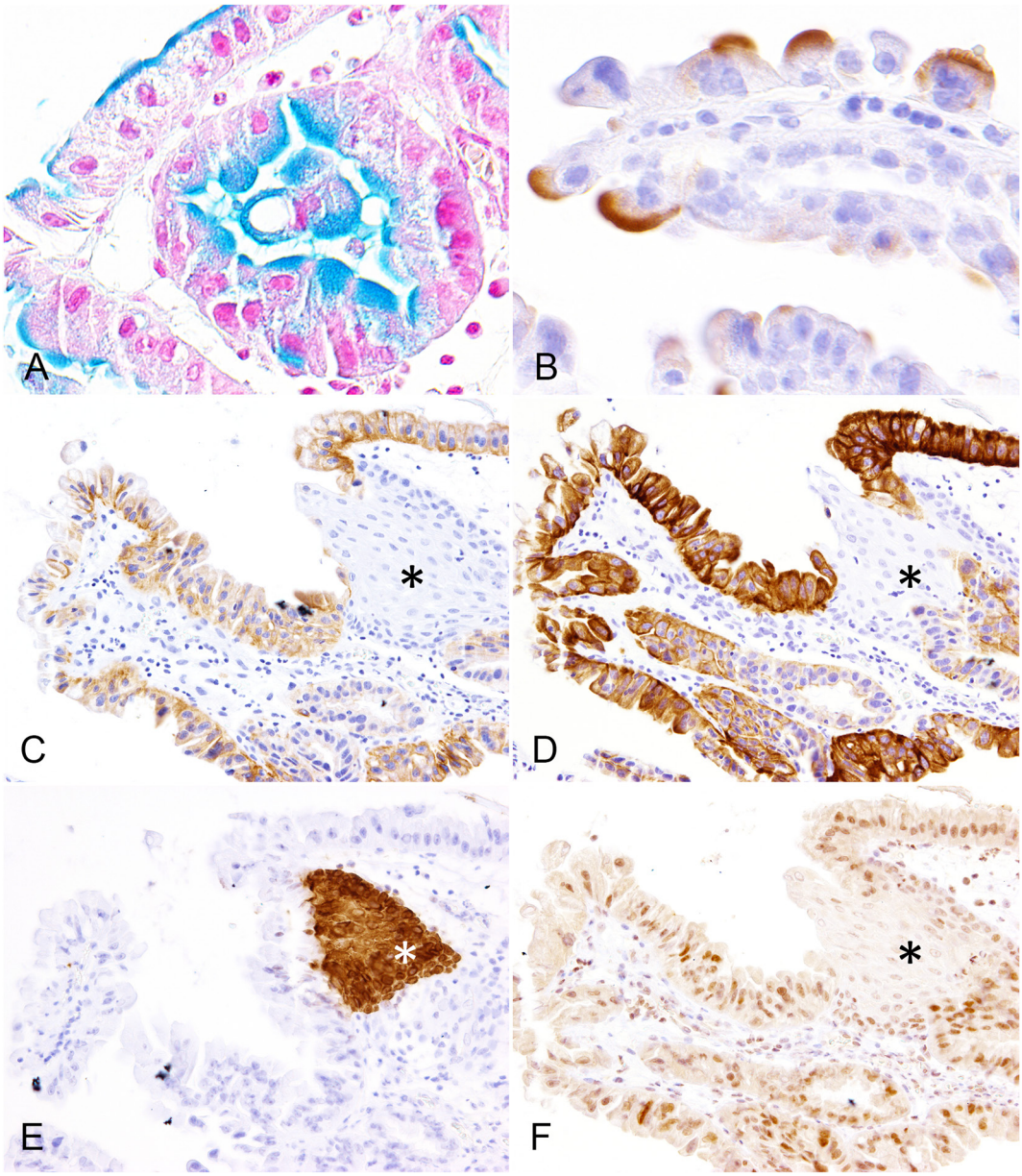

Fig. 2. The surface columnar cells in the lesion were positive for Alcian blue (A) and immunohistochemically positive for MUC5AC (B). The cuboidal to columnar neoplastic cells were positive for CK7 (C) and CK20 (D), and negative for CK5/6 (E). The squamous epithelium of the remaining esophageal mucosa (asterisk) was negative for CK7 (C) and CK20 (D) and positive for CK5/6 (E). The nuclei of the cuboidal to columnar neoplastic cells were positive for p53, whereas those of the squamous epithelial cells were negative for p53 (F). (A) Alcian blue (pH 2.5) stain, ×400. (B–F) Immunohistochemistry; (B) ×400, (C–F) ×200.

in the dog's stomach or duodenum, except for a mild *Helicobacter* spp. infection in the gastric mucosa. *Helicobacter* spp. infection was not found in the neoplastic tissue of the esophagus.

The columnar epithelial cells that extended from the squamous mucosa were considered to be indicative of metaplasia of the esophageal mucosa. Based on the presence of this finding together with the inflammatory changes and mucous production exhibited by the epithelial cells, the dog was diagnosed with Barrett's esophagus. Moreover, the areas that displayed significant atypia (i.e., irregular tissue structure, high mitotic count) were diagnosed as adenocarcinoma of Barrett's esophagus.

Elevated C-reactive protein indicates an inflammatory response, and in the present case, it likely reflects the inflammatory changes that were grossly and microscopically observed in the esophagus. Persistent inflammation of the esophagus is considered an important factor in the pathogenesis of Barrett's esophagus in humans. In regard to *Helicobacter* spp. infection, an inverse relation of gastric *Helicobacter pylori* infection and Barrett's esophagus has been demonstrated in human studies[9, 10]. In dogs, gastric infection with *Helicobacter* spp. is common; however it has not been associated with any kind of neoplastic change[11]. Due to the small number of cases of Barrett's esophagus, it is difficult to evaluate the relation of gastric *Helicobacter* spp. infection and Barrett's esophagus in the dog.

Immunohistochemically, Barrett's esophagus is characterized by the presence of gastric-type mucin, i.e., MUC5AC, on the surface epithelium[12]. In the current study, the surface neoplastic cells of the esophagus and the normal gastric mucosa were positive for MUC5AC, indicating that the affected esophagus was producing gastric-type mucin. However, MUC5AC staining was decreased in the areas that displayed significant atypia in the present case, which agrees with the findings of adenocarcinoma of Barrett's esophagus in humans[13]. The CK expression of Barrett's esophagus is characterized by diffuse CK7 staining and surface staining of CK20[14], which were also observed in the present canine case. CK20 is a marker of differentiated intestinal epithelial cells, and its expression is decreased in the esophageal and intestinal adenocarcinoma cells of humans and dogs[15, 16].

In human patients with Barrett's esophagus, nuclear accumulation of p53 is related to the progression of dysplasia and tumor development, and this finding is absent in Barrett's esophagus without dysplasia[17]. Moreover, it is strongly associated with a p53 gene mutation in adenocarcinoma of Barrett's esophagus. In an experimental study involving canine models of Barrett's esophagus, nuclear p53 positivity was only detected in dogs that had developed adenocarcinoma[6]. In the present study, nuclei of the neoplastic cells were diffusely positive for p53. Moreover, Ki-67-positive neoplastic cells were distributed randomly, indicating aberrant cell proliferation.

To the best of our knowledge, this is the first report about spontaneous esophageal adenocarcinoma in a dog. The immunohistochemical characteristics of the present canine case will aid in the detection and diagnosis of spontaneous esophageal neoplastic lesions derived from Barrett's esophagus.

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References

1. DeMeester SR, and DeMeester TR. Columnar mucosa and intestinal metaplasia of the esophagus: fifty years of controversy. Ann Surg. **231**: 303–321. 2000. [Medline] [CrossRef]
2. Naini BV, Souza RF, and Odze RD. Barrett's Esophagus: a comprehensive and contemporary review for pathologists. Am J Surg Pathol. **40**: e45–e66. 2016. [Medline] [CrossRef]
3. Hayakawa Y, Sethi N, Sepulveda AR, Bass AJ, and Wang TC. Oesophageal adenocarcinoma and gastric cancer: should we mind the gap? Nat Rev Cancer. **16**: 305–318. 2016. [Medline] [CrossRef]
4. Ross-Innes CS, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M, Malhotra S, di Pietro M, Ivakhno S, He M, Weaver JM, Lynch AG, Kingsbury Z, Ross M, Humphray S, Bentley D, Fitzgerald RC. Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Study Group. Oesophageal Cancer Clinical and Molecular Stratification OCCAMS Study Group. Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. Nat Genet. **47**: 1038–1046. 2015. [Medline] [CrossRef]
5. Kapoor H, Lohani KR, Lee TH, Agrawal DK, and Mittal SK. Animal models of barrett's esophagus and esophageal adenocarcinoma-past, present, and future. Clin Transl Sci. **8**: 841–847. 2015. [Medline] [CrossRef]
6. Kawaura Y, Tatsuzawa Y, Wakabayashi T, Ikeda N, Matsuda M, and Nishihara S. Immunohistochemical study of p53, c-erbB-2, and PCNA in barrett's esophagus with dysplasia and adenocarcinoma arising from experimental acid or alkaline reflux model. J Gastroenterol. **36**: 595–600. 2001. [Medline] [CrossRef]
7. Gibson CJ, Parry NM, Jakowski RM, and Cooper J. Adenomatous polyp with intestinal metaplasia of the esophagus (*Barrett esophagus*) in a dog. Vet Pathol. **47**: 116–119. 2010. [Medline] [CrossRef]
8. Nakamura M, Takahashi M, Ohno K, Koshino A, Nakashima K, Setoguchi A, Fujino Y, and Tsujimoto H. C-reactive protein concentration in dogs with various diseases. J Vet Med Sci. **70**: 127–131. 2008. [Medline] [CrossRef]
9. Fischbach LA, Graham DY, Kramer JR, Rugge M, Verstovsek G, Parente P, Alsarraj A, Fitzgerald S, Shaib Y, Abraham NS, Kolpachi A, Gupta S, Vela MF, Velez M, Cole R, Anand B, and El Serag HB. Association between Helicobacter pylori and Barrett's esophagus: a case-control study. Am J Gastroenterol. **109**: 357–368. 2014. [Medline] [CrossRef]

10. Wang C, Yuan Y, and Hunt RH. Helicobacter pylori infection and Barrett's esophagus: a systematic review and meta-analysis. Am J Gastroenterol. **104**: 492–500, quiz 491, 501. 2009. [Medline] [CrossRef]
11. Neiger R, and Simpson KW. Helicobacter infection in dogs and cats: facts and fiction. J Vet Intern Med. **14**: 125–133. 2000. [Medline] [CrossRef]
12. Warson C, Van De Bovenkamp JH, Korteland-Van Male AM, Büller HA, Einerhand AW, Ectors NL, and Dekker J. Barrett's esophagus is characterized by expression of gastric-type mucins (MUC5AC, MUC6) and TFF peptides (TFF1 and TFF2), but the risk of carcinoma development may be indicated by the intestinal-type mucin, MUC2. Hum Pathol. **33**: 660–668. 2002. [Medline] [CrossRef]
13. Glickman JN, Blount PL, Sanchez CA, Cowan DS, Wongsurawat VJ, Reid BJ, and Odze RD. Mucin core polypeptide expression in the progression of neoplasia in Barrett's esophagus. Hum Pathol. **37**: 1304–1315. 2006. [Medline] [CrossRef]
14. Wong HH, and Chu P. Immunohistochemical features of the gastrointestinal tract tumors. J Gastrointest Oncol. **3**: 262–284. 2012. [Medline]
15. Driessen A, Nafteux P, Lerut T, Van Raemdonck D, De Leyn P, Filez L, Penninckx F, Geboes K, and Ectors N. Identical cytokeratin expression pattern CK7+/CK20- in esophageal and cardiac cancer: etiopathological and clinical implications. Mod Pathol. **17**: 49–55. 2004. [Medline] [CrossRef]
16. Uchida E, Chambers JK, Nakashima K, Saito T, Ohno K, Tsujimoto H, Nakayama H, and Uchida K. Pathologic features of colorectal inflammatory polyps in miniature dachshunds. Vet Pathol. **53**: 833–839. 2016. [Medline] [CrossRef]
17. Bian YS, Osterheld MC, Bosman FT, Benhattar J, and Fontolliet C. p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. Mod Pathol. **14**: 397–403. 2001. [Medline] [CrossRef]