The Journal of Veterinary Medical Science

FULL PAPER

Pathology

Immunohistochemical analysis of beta-catenin, E-cadherin and p53 in canine gastrointestinal epithelial tumors

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ABSTRACT. Wnt/beta-catenin signaling, E-cadherin and p53 reportedly play important roles in the development and/or progression of human gastrointestinal cancer. The present study evaluated the roles of beta-catenin, E-cadherin and p53 in canine gastrointestinal tumors. Endoscopic biopsy or surgically resected samples, a total of 131, including 38 gastric, 13 small intestinal and 80 large intestinal tumors, were obtained from 95 dogs. Those specimens were examined pathologically. Immunohistochemically, nuclear beta-catenin expression was found in 88% (42/48) of polypoid type adenocarcinomas. Most cases of non-polypoid type adenocarcinomas lacked nuclear expression of beta-catenin with the exception of one case (6%, 1/17). Nuclear beta-catenin expression was not observed in signet ring cell carcinomas (0/15), mucinous adenocarcinomas (0/7) and undifferentiated carcinomas (0/4). The findings indicate that nuclear translocation of beta-catenin is closely related to the development of polypoid type adenocarcinomas but not that of non-polypoid type malignant tumors. The immunoreactivity of E-cadherin for tumor cells tended to decline overall in most of cases including benign tumors. Significant immunoreactivity for p53 was not found in 61% of tumors examined (80/131), including malignant tumors (63%, 57/91), while intense p53-immunoreactivity was rarely found in a few cases of malignant tumors (8%, 7/91). We could not conclude clearly significant correlations between histopathological tumor types and immunohistochemical results of E-cadherin or p53. This paper indicates the importance of the nuclear translocation of beta-catenin for the tumorigenesis of canine intestinal polypoid type adenocarcinomas, especially in the colorectum.

KEY WORDS: beta-catenin, carcinogenesis, dog, gastrointestinal neoplasm, immunohistochemistry

J. Vet. Med. Sci.
82(9): 1277–1286, 2020
doi: 10.1292/jvms.20-0297

Received: 19 May 2020
Accepted: 2 July 2020
Advanced Epub:
13 July 2020

The adenoma-carcinoma sequence, the major pathway of colorectal tumorigenesis in humans, is considered to require the accumulation of genetic changes [57]. Inactivation of the *adenomatous polyposis coli* (*APC*) tumor suppressor gene is the first event in this sequence resulting in colorectal adenoma formation [20]. Mutations in *APC* were discovered in patients with human familial adenomatous polyposis (FAP) [13, 26, 40] and were found in more than 80% of sporadic colorectal adenomas and carcinomas [27]. *APC* mutations are associated with an accumulation of intracellular beta-catenin protein, which leads to loss of control of normal beta-catenin signaling [38]. Beta-catenin accumulates in the cytoplasm and nucleus because the mutated *APC* does not bind axin and reduces degradation of beta-catenin [18]. Furthermore, in colorectal tumors, beta-catenin mutations are also detected and activation of beta-catenin-Tcf signaling has been reported [36]. The participation of beta-catenin in intestinal tumorigenesis has been also evaluated in experimental and domestic animals [33, 54]. Consequently, the tumors in the FAP patients or APC^{min} mice are generally formed by polypoid growth and the tumorigenesis is considered to be through the adenoma-carcinoma sequence [41]. We previously showed that Jack Russell Terriers and Miniature Dachshunds were the breeds with the highest predisposition for gastrointestinal tumors, and Jack Russel Terrier was frequently involved in polypoid formed tumors

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

[48]. Recently, a somatic mutation and loss of the wild-type *APC* allele have been reported in the canine breed with gastrointestinal tumors [62]. By contrast, adenocarcinoma shows not only polypoid formation but also occasionally lacked polypoid characteristics and the lesions are flat or depressed in humans and dogs [48, 52]. These carcinomas are considered to be *de novo* because the lesions were small and develop without any intervening precursor lesion [7, 30]. Morphologically, the adenoma-carcinoma sequence or *de novo* tumorigenesis pathway is classified as polypoid growth (PG) type and non-polypoid growth (NPG) type, respectively [21, 49]. NPG carcinomas grow rapidly, resulting in largely advanced carcinoma of the NPG type [31, 35]. In *de novo* carcinogenesis, although little is known about the genetic alterations [16, 37, 61], the involvement of APC and KRAS is considered to be rare [34, 55, 60].

E-cadherin is a calcium-dependent cell-cell adhesion molecule and composed of an adherence junction with alpha-, beta-, and p120-catenin. E-cadherin and these adhesion molecules play an important role in maintaining the epithelial contexture and tumor-suppressive action [22, 43, 53, 59]. Especially, E-cadherin is widely known to play a key role in tumor suppression [39] and/or infiltrative growth, metastasis and poor prognosis [3]. In the case of cancer progression, cancer cells must become detached from neighboring cells, invade through the basement membrane and then migrate into the surrounding tissues. Since mutation of E-cadherin is detected in various malignant tumors [45], cell-cell adhesion molecules may play an important role in cancer invasion and metastasis [11, 56]. Previous studies showed that various tumors [51], including gastric [32, 50] and colorectal [25, 42] often lose E-cadherin expression by immunohistochemistry (IHC). Although there are some previous studies on the role of E-cadherin in canine gastrointestinal tumors [2, 33, 46], little is understood about its role.

In the p53 tumor suppressor gene, gene mutation and protein accumulation are involved in the conversion of adenoma to early carcinoma [24, 44]. Wild-type p53 is an unstable protein with a short half-life for its detection by IHC, but mutant p53 can accumulate within tumor cells creating a stable target for IHC [1, 10, 14, 23]. Indeed, IHC has been widely used as a useful method for mutant p53 detection in humans [8, 9, 28, 47]. Accumulation of the p53 protein was reportedly also detected in various canine tumors by IHC [4, 5, 12, 15]. However, there are some reports that unlike human colorectal tumors malignant progression does not depend on the acquisition of p53 mutations in canine intestinal tumors [33, 58].

Previously, we described the histopathological features, age, sex, and breed predisposition of canine gastrointestinal epithelial tumors [48]. This paper focuses on immunohistochemical features of these tumors. The aim of this study is to investigate the relationship between histopathological features and major makers of oncogenesis in canine gastrointestinal epithelial tumors. The canine gastrointestinal tumors, a total of 131, were immunohistochemically evaluated for beta-catenin, E-cadherin, and p53.

MATERIALS AND METHODS

Cases

Endoscopic biopsy or surgical excision was performed in 95 dogs at the Veterinary Medical Center of the University of Tokyo, the Japan Small Animal Medical Center, and Japan Animal Referral Medical Center between 2013 and 2016. As histopathological cases, 131 specimens of neoplastic lesions from these dogs were used.

Histological examinations

All the samples were fixed in 10% neutral buffered formalin. Paraffin sections of 4 μm, were stained with hematoxylin and eosin (HE). The specimens were diagnosed by two experienced Japanese College of Veterinary Pathology certified veterinary pathologists (J.K.C and K.U) at the Department of Veterinary Pathology of the University of Tokyo. Each lesion was classified according to the "WHO histological classification of tumors of the alimentary system of domestic animals" [17] with modification as follows. Acinar-, papillary- and tubulopapillary adenocarcinoma were classified into PG (Fig. 1) or NPG (Fig. 2) adenocarcinoma, because we focused on histological growth patterns. The PG/NPG classification was based on histological classifications of human intestinal tumors [49]. In brief, PG adenocarcinoma formed a protuberant mass that included pedunculated lesions and sessile or broad-based lesions. NPG adenocarcinoma formed a flat or depressed lesion without intramucosal protuberant growth. Adenomas (n=40), PG adenocarcinomas (n=48), NPG adenocarcinomas (n=17), signet ring cell carcinomas (SRC, n=15), mucinous adenocarcinomas (MUC, n=7) and undifferentiated carcinomas (UND, n=4) were evaluated, and Figs. 1 to 5 show typical histopathological features of these tumors, respectively. These specimens were subjected to IHC for beta-catenin, E-cadherin and p53. The details of the histopathological features, age, sex, and breed predisposition were described in the previous paper [48].

Immunohistochemistry

Paraffin sections were de-paraffinized and treated with 3% hydrogen peroxidase in methanol at room temperature for 5 min and then incubated with 8% skimmed milk in Tris-buffered saline at 37°C for 40 min to block nonspecific reactions. The primary antibodies used were as follows: mouse anti-beta-catenin monoclonal antibody (1:1,000, clone 14/Beta-Catenin, BD Transduction Laboratories, San Jose, CA, USA), mouse anti-E-cadherin monoclonal antibody (1:1,000, 36/E-Cadherin, BD Transduction Laboratories) and rabbit anti-p53 polyclonal antibody (1:50, FL-393, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antigen retrieval was performed by heating the sections using an autoclave at 121°C for 10 min, in pH 6.0 citrate buffer for beta-catenin and E-cadherin and in Dako high pH antigen retrieval solution (Dako, Tokyo, Japan) for p53. The sections were reacted with each primary antibody in a moist chamber at 4°C overnight. After washing, the sections were treated with the Dako Envision Plus Kit (Dako). In order to visualize the immunoreactions, a 3–3′-diaminobenzidine (Dojindo, Kumamoto, Japan) solution containing

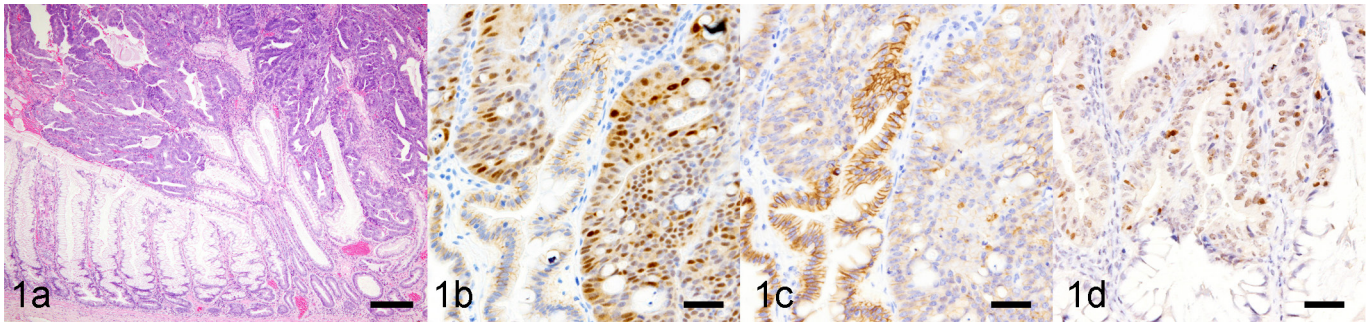

Fig. 1. Polypoid growth type of adenocarcinoma, sample number 119. Dog, large intestine. (a) The neoplastic lesions protrude into the lumen. Hematoxylin and eosin stain (HE). Bar, 200 μm. (b) Nuclei of neoplastic cells are positive for beta-catenin. Score 3. Immunohistochemistry (IHC). Bar, 200 μm. (c) Cell membrane of neoplastic cells is weakly positive for E-cadherin. Score 3. IHC. Bar, 40 μm. (d) Nuclei of neoplastic cells are sporadically positive for p53. Score 1. IHC. Bar, 40 μm.

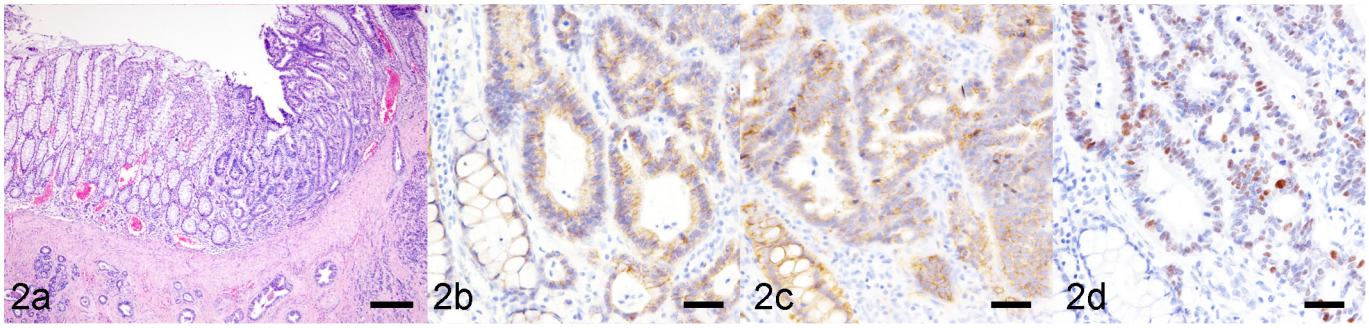

Fig. 2. Non-polypoid growth type of adenocarcinoma, sample number 122. Dog, large intestine. (a) The surface of the mucosa is flat, and the tumor cells invade into the submucosal layer. Hematoxylin and eosin stain (HE). Bar, 200 μm. (b) Nuclei of neoplastic cells are negative for beta-catenin. Score 0. Immunohistochemistry (IHC). (c) Cell membrane of neoplastic cells is heterogeneously positive for E-cadherin. Score 1. IHC. (d) Nuclei of neoplastic cells are sporadically positive for p53. Score 2. IHC. (b–d) Bar, 40 μm.

0.03% H_2O_2 was used. All slides were counterstained with Mayer's hematoxylin. For negative controls, the primary antibody was omitted from the reaction. Beta-catenin and p53 were assessed using nuclear-positive ratios and E-cadherin was assessed by the area of reduction for membranous-positive-intensity. The slides were scored based on the proportion of cells affected as follows: score 0, <1% tumor cells nuclei showing immunoreactivity; score 1, 1–25% showing immunoreactivity; score 2, 26–50% showing immunoreactivity; score 3, >50% showing immunoreactivity for nuclear beta-catenin and p53, and on the contrary the ratio of cells showing a reduced area of immunoreactivity for E-cadherin.

Statistical analysis

Statistical analysis was performed using the SAS Release 9.1.3 (SAS Institute Inc., Cary, NC, USA). Comparisons of the means of different groups were performed with a one-way analysis of variance, followed by Tukey's honestly significant difference test. *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Immunoreactivity of beta-catenin

Immunohistochemically, beta-catenin was located on the cell membrane of normal epithelial cells. In the case of tumors, immunoreactivity representing nuclear beta-catenin expression was evaluated as the significant finding. The results of beta-catenin immunoreactivity for each tumor are represented in Table 1 and Supplementary Table 1. Nuclear expression of beta-catenin in tumor cells was observed in 20% (1/5) of gastric- and 94% (33/35) of colorectal adenomas. In more than half (55%, 22/40) of adenomas, the results were evaluated as score 1, and there were no cases evaluated as score 2 or 3 in gastric tumors. In PG adenocarcinoma, nuclear expression of beta-catenin was observed in 45% (5/11) of gastric, 100% (3/3) of small intestine and 100% (34/34) of colorectal tumors (Fig. 1b). Although the gastric (Fig. 6a, 6b) and intestinal (Fig. 7a, 7b) PG tumors were similar morphological structures the immune-response was different (Figs. 6c, 7c, Table 1). Especially in the colorectal tumors, 47% (16/34) of PG adenocarcinomas were evaluated as score 3, the score was higher than the gastric tumors. In contrast, the nuclear expression of beta-catenin was not found in any NPG/diffuse type of tumors including NPG (1/17, Fig. 2b), SRC (0/15, Fig. 3b),

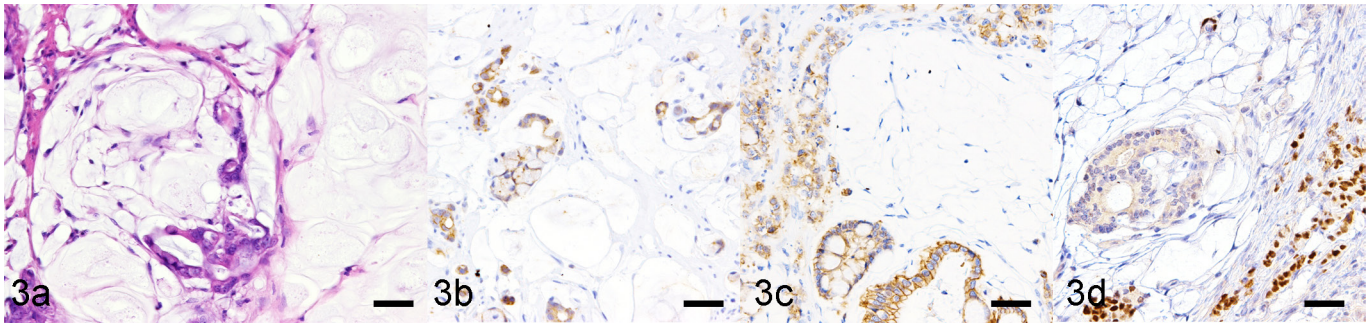

Fig. 3. Mucinous adenocarcinoma, sample number 46. Dog, small intestine. (a) Neoplastic cells proliferate with the outer cellular mucin into the submucosa, and neoplastic cells form solitary or gland-like structures. Hematoxylin and eosin stain (HE). (b) Nuclei of neoplastic cells are negative for beta-catenin. Score 0. Immunohistochemistry (IHC). (c) The immunoreactivity for E-cadherin is reduced or lost in the cell membrane of neoplastic cells forming solitary neoplastic foci, while neoplastic cells with glandular or tubular structures are intensely immunopositive for E-cadherin. Score 2. IHC. (d) The nuclei of the neoplastic cells forming solitary neoplastic foci show positive immunoreactivity for p53. The nuclei of the neoplastic cells with glandular or tubular structures are negative for p53. Score 1. IHC. (a–d) Bar, 40 μm.

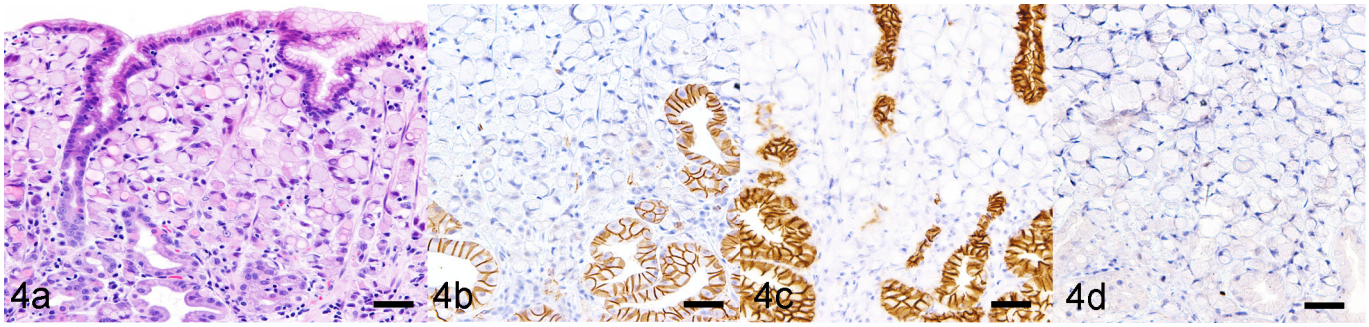

Fig. 4. Signet ring cell carcinoma, Sample number 32. Dog, stomach. (a) "Signet ring" formed neoplastic cells are diffusely observed in the lamina propria. Hematoxylin and eosin stain (HE). (b) Nuclei of neoplastic cells are negative for beta-catenin. Score 0. Immunohistochemistry (IHC). (c) Cell membrane of neoplastic cells is completely negative for E-cadherin. Score 3. IHC. (d) Nuclei of neoplastic cells are negative for p53. Score 0. IHC. (a–d) Bar, 40 μm.

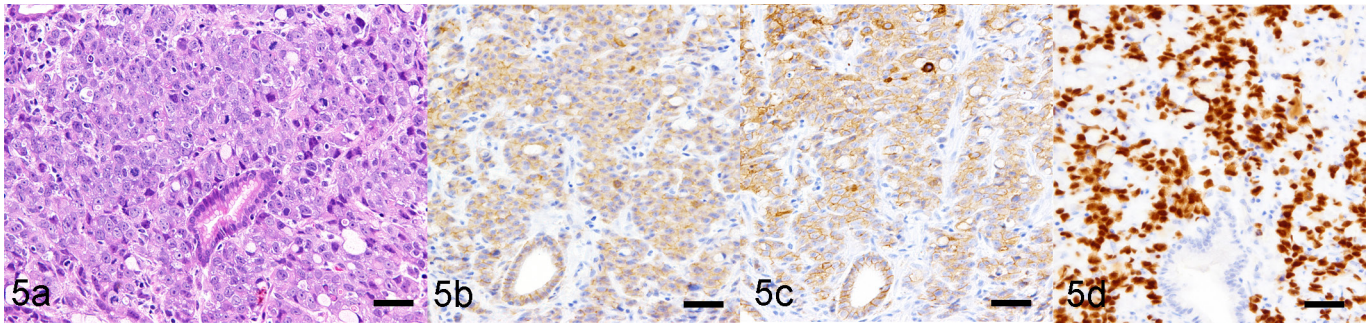

Fig. 5. Undifferentiated carcinoma, sample number 37. Dog, stomach. (a) Neoplastic epithelial cells that show no specific differentiation diffusely proliferate in the lamina propria. Hematoxylin and eosin stain (HE). (b) Nuclei of neoplastic cells are negative for Beta-catenin. Score 0. Immunohistochemistry (IHC). (c) Cell membrane of neoplastic cells is feebly and occasionally positive for E-cadherin. Score 2. IHC. (d) Nuclei of neoplastic cells are strongly and diffusely positive for p53 throughout the lesion. Score 3. IHC. (a–d) Bar, 40 μm.

MUC (0/7, Fig. 4b) and UND (0/4, Fig. 5b), except for one case in a colorectal NPG tumor. There were statistically significant differences between PG adenocarcinoma and NPG/diffuse type tumors in large intestine.

Immunoreactivity of E-cadherin

Immunohistochemically, the cell membrane of normal epithelial cells was immunopositive for E-cadherin. The results of E-cadherin immunoreactivity for each tumor are represented in Table 2 and Supplementary Table 1. The immunoreactivity of

Table 1. Comparison of immunohistochemical profiles of beta-catenin in gastrointestinal tumors

Diagnosis:	Adenoma	Adenocarcinoma		SRC	MUC	UND
Growth pattern:	PG	PG	NPG			
Beta-catenin						
Stomach (n=38):	5	11 [3]	7 [7]	12 [12] b*	0	3 [3]
Score 0	4 80%	6 [1] 55%	7 [7] 100%	12 [12] 100%	0	3 [3] 100%
Score 1	1 20%	5 [2] 45%	0	0	0	0
Score 2	0	0	0	0	0	0
Score 3	0	0	0	0	0	0
Small intestines (n=13):	0	3 [1]	3 [3]	1 [1]	5 [5]	1 [1]
Score 0	0	0	3 [3] 100%	1 [1] 100%	5 [5] 100%	1 [1] 100%
Score 1	0	1 33%	0	0	0	0
Score 2	0	1 33%	0	0	0	0
Score 3	0	1 [1] 33%	0	0	0	0
Large intestines (n=80):	35	34 [6] a*	7 [7] a*, b**	2 [2] b**	2 [2] b**	0
Score 0	2 6%	0	6 [6] 86%	2 [2] 100%	2 [2] 100%	0
Score 1	21 60%	9 26%	0	0	0	0
Score 2	4 11%	9 [1] 26%	0	0	0	0
Score 3	8 23%	16 [5] 47%	1 [1] 14%	0	0	0
All sites (n=131):	40	48 [10]	17 [7] a**, b**	15 [15] a**, b**	7 [7] a**, b**	4 [4] b**
Score 0	6 15%	6 [1] 13%	16 [16] 94%	15 [15] 100%	7 [7] 100%	4 [4] 100%
Score 1	22 55%	15 [2] 31%	0	0	0	0
Score 2	4 10%	10 [1] 21%	0	0	0	0
Score 3	8 20%	17 [6] 35%	1 [1] 6%	0	0	0

PG, polypoid growth type; NPG, non-polypoid growth type; SRC, Signet-ring cell carcinoma; MUC, Mucinous adenocarcinoma; UND, Undifferenciated carcinoma. The Number of cases showing invasion or metastasis is enclosed in bracket. Statistically significant differences are shown as follows: a**, $P<0.01$, vs PG adenoma; b*, $P<0.05$, vs PG adenocarcinoma; b**, $P<0.01$, vs PG adenocarcinoma.

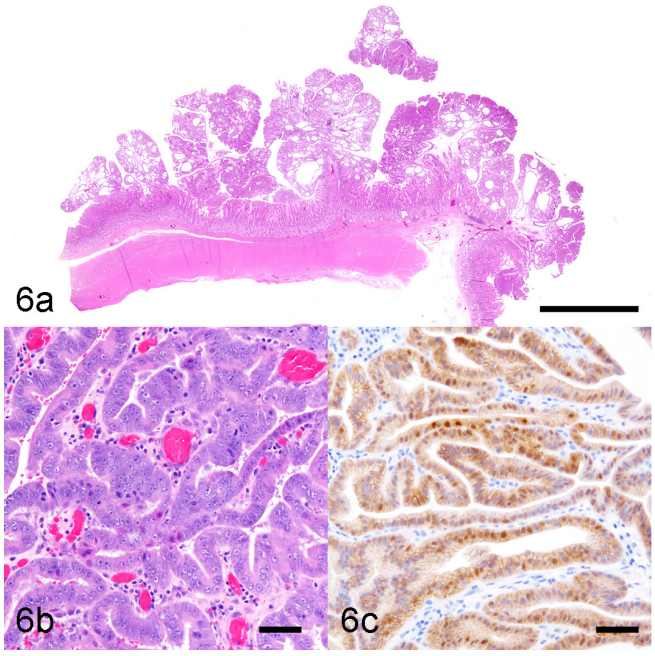

Fig. 6. Polypoid growth type adenocarcinoma, sample number 13. Dog, stomach. (a) Neoplastic epithelial cells prelude into the lumen. Hematoxylin and eosin stain (HE). Bar, 500 μm. (b) Higher magnification of Fig. 6a. Bar, 60 μm. (c) Nuclear beta-catenin positive cells are sparsely observed. Immunohistochemistry. Bar, 60 μm.

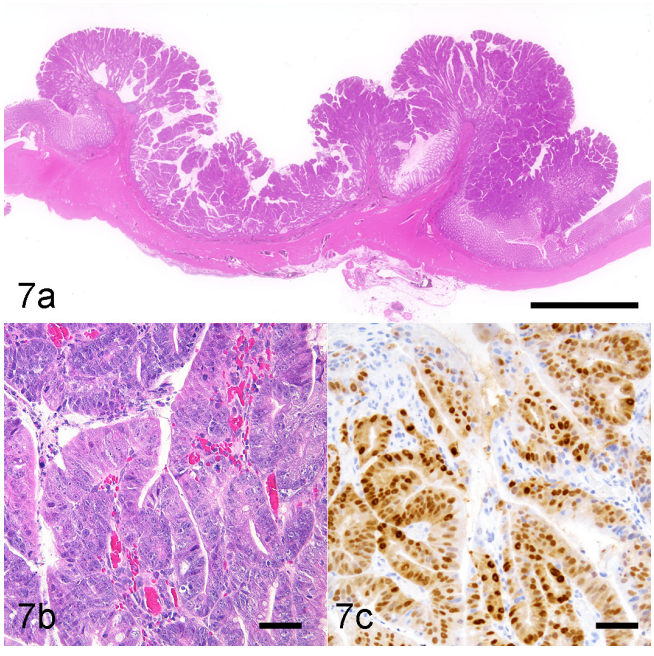

Fig. 7. Polypoid growth type adenocarcinoma, sample number 91. Dog, intestine. (a) Neoplastic epithelial cells prelude into the lumen. Hematoxylin and eosin stain (HE). Bar, 500 μm. (b) Higher magnification of Fig. 7a. Bar, 60 μm. (c) Most of the neoplastic cells show positive for nuclear beta-catenin and high intensity. Immunohistochemistry. Bar, 60 μm.

Table 2. Comparison of immunohistochemical profiles of E-cadherin in gastrointestinal tumors

Diagnosis:	Adenoma	Adenocarcinoma		SRC	MUC	UND
Growth pattern:	PG	PG	NPG			
E-cadherin						
Stomach (n=38):	5	11 [3]	7 [7]	12 [12]	0	3 [3]
Score 0	1 20%	0	0	0	0	0
Score 1	1 20%	6 [2] 55%	1 [1] 14%	0	0	0
Score 2	2 40%	2 18%	4 [4] 57%	5 [5] 42%	0	2 [2] 67%
Score 3	1 20%	3 [2] 27%	2 [2] 29%	7 [7] 58%	0	1 [1] 33%
Small intestines (n=13):	0	3 [1]	3 [3]	1 [1]	5 [5]	1 [1]
Score 0	0	0	1 [1] 33%	0	0	0
Score 1	0	0	0	0	1 [1] 20%	0
Score 2	0	2 [1] 67%	1 [1] 33%	0	1 [1] 20%	0
Score 3	0	1 33%	1 [1] 33%	1 [1] 100%	3 [3] 60%	1 [1] 100%
Large intestines (n=80):	35	34 [6]	7 [7] a**, b**	2 [2]	2 [2] b*	0
Score 0	0	0	0	0	0	0
Score 1	1 3%	2 6%	2 [2] 29%	0	1 [1] 50%	0
Score 2	10 29%	5 [1] 15%	4 [4] 57%	1 [1] 50%	1 [1] 50%	0
Score 3	24 69%	27 [5] 79%	1 [1] 14%	1 [1] 50%	0	0
All sites (n=131):	40	48 [10]	17 [7]	15 [15]	7 [7]	4 [4]
Score 0	1 3%	0	1 [1] 6%	0	0	0
Score 1	2 5%	8 [2] 17%	3 [3] 18%	0	2 [2] 29%	0
Score 2	12 30%	9 [2] 19%	9 [9] 53%	6 [6] 40%	2 [2] 29%	2 [2] 50%
Score 3	25 63%	31 [5] 65%	4 [4] 24%	9 [9] 60%	3 [3] 43%	2 [2] 50%

PG, polypoid growth type; NPG, non-polypoid growth type; SRC, Signet-ring cell carcinoma; MUC, Mucinous adenocarcinoma; UND, Undifferenciated carcinoma. The Number of casess showing invasion or metastasis is enclosed in bracket. Statistically significant differences are shown as follows: a**, $P<0.01$, vs PG adenoma; b*, $P<0.05$, vs PG adenocarcinoma; b**, $P<0.01$, vs PG adenocarcinoma.

E-cadherin for tumor cells tended to decline overall in most of the cases including benign tumors. The immunoreactivity for E-cadherin did not depend on the histological type of the tumors. In PG adenocarcinomas, the surface of the neoplastic foci showed intense immunoreactivity for E-cadherin, whereas the immunoreactivity was reduced in the central area of the neoplastic foci (Fig. 1c). There were no cases that showed the complete loss of immunoreactivity in tumors with glandular or tubular structures of both PG and NPG types (Fig. 2c), whereas NPG types showed statistically lower scores than PG types in the intestine (Table 2). By contrast, the diffuse type carcinomas namely MUC (Fig. 3c), SRC (Fig. 4c) and UND (Fig. 5c) showed severe reduction or complete loss of immunoreactivity for E-cadherin in almost all of the neoplastic lesions (Fig. 4c). In MUC, individual neoplastic cells with signet ring cell-morphology showed obviously reduced reactivity, whereas the cell membrane of the neoplastic tubular structures was intensely immunopositive for E-cadherin (Fig. 3c).

Immunoreactivity of p53

The normal epithelial cells were completely immunonegative for p53. The results of p53 immunoreactivity for each tumor are represented in Table 3 and Supplementary Table 1. A total of 61% (80/131) of the gastrointestinal epithelial tumors, including malignant tumors (63%, 57/91) were evaluated as score 0 (Fig. 4d). Although about half of gastrointestinal adenomas (43%, 17/40) had weak and occasional nuclear immunoreactivity for p53, all of these cases were evaluated as score 1. By contrast, 8% of the malignant tumors (7/91) exhibited intense immunoreactivity for p53. These tumors including 2% of PG adenocarcinomas (1/48, Fig. 1d), 6% of NPG adenocarcinomas (1/17, Fig. 2d), 20% of SRC (3/15) and 50% of UND (2/4, Fig. 5d) were evaluated as score 3. In MUC, a small number of neoplastic cells within a limited area of the neoplastic foci showed intense immunoreactivity for p53 (Fig. 3d). We could not definitely conclude significant correlations between histopathological tumor types and immunohistochemical results of p53, although all cases evaluated as score 3 had evidence of interstitial/submucosal invasion or metastasis (Table 3). There was no significant difference between histological growth patterns.

DISCUSSION

The evaluation of IHC using beta-catenin indicated different results between the gastric and intestinal tumors. All of the PG type adenocarcinomas (100%, 37/37) in the small and large intestines showed significant nuclear expression of beta-catenin. By contrast, the nuclear expression of beta catenin was obviously rare in PG type adenocarcinomas in the stomach as compared to that in the intestines. The observations suggest that dysregulation of beta-catenin plays an important role in canine intestinal PG type adenocarcinomas, especially in the colorectum. In addition, the different degree of nuclear expression of beta-catenin between gastric and intestinal tumors may indicate the different roles of beta-catenin in the tumorigenesis of these tumors between the

Table 3. Comparison of immunohistochemical profiles of p53 in gastrointestinal tumors

Diagnosis:	Adenoma	Adenocarcinoma				SRC			MUC			UND		
Growth pattern:	PG	PG		NPG										
p53														
Stomach (n=38):	5	11	[3]	7	[7]	12	[12]		0			3	[3]	
Score 0	5 100%	9	[2] 82%	4	[4] 57%	9	[9] 75%		0			2	[2] 67%	
Score 1	0	2	[1] 18%	2	[2] 29%	1	[1] 8%		0			0		
Score 2	0	0		0		0			0			0		
Score 3	0	0		1	[1] 14%	2	[2] 17%		0			1	[1] 33%	
Small intestines (n=13):	0	3	[1]	3	[3]	1	[1]		5	[5]		1	[1]	
Score 0	0	2	67%	1	[1] 33%	0			2	[2] 40%		0		
Score 1	0	1	[1] 33%	2	[2] 67%	0			3	[3] 60%		0		
Score 2	0	0		0		0			0			0		
Score 3	0	0		0		1	[1] 100%		0			1	[1] 100%	
Large intestines (n=80):	35	34	[6]	7	[7]	2	[2]		2	[2]		0		
Score 0	18 51%	19	[2] 56%	5	[5] 71%	2	[2] 100%		2	[2] 100%		0		
Score 1	17 49%	9	[2] 26%	1	[1] 14%	0			0			0		
Score 2	0	5	[1] 15%	1	[1] 14%	0			0			0		
Score 3	0	1	[1] 3%	0		0			0			0		
All sites (n=131):	40	48	[10]	17	[7]	15	[15]		7	[7]		4	[2]	
Score 0	23 58%	30	[4] 63%	10	[10] 59%	11	[11] 73%		4	[4] 57%		2	[2] 50%	
Score 1	17 43%	12	[4] 25%	5	[5] 29%	1	[1] 7%		3	[3] 43%		0		
Score 2	0	5	[1] 10%	1	[1] 6%	0			0			0		
Score 3	0	1	[1] 2%	1	[1] 6%	3	[3] 20%		0			2	[2] 50%	

PG, polypoid growth type; NPG, non-polypoid growth type; SRC, Signet-ring cell carcinoma; MUC, Mucinous adenocarcinoma; UND, Undifferenciated carcinoma. The Number of casess showing invasion or metastasis is enclosed in bracket. There is no significant difference between growth patterns.

stomach and intestines.

In humans, the pathogenesis of gastric cancer was divided into two categories namely diffuse type and intestinal type, and these are believed to result from distinct pathogenetic pathways [6, 19]. The intestinal type carcinomas develop after stepwise progression from chronic gastritis to atrophic gastritis, intestinal metaplasia, dysplasia to intestinal type carcinoma. This inflammatory sequence progressing to gastric cancer is particularly prevalent in the gastric tumors caused by *Helicobacter pylori* (*H. pylori*) infection [6, 19]. On the other hand, diffuse type tumor is believed to arise as a *de novo* cancer [19]. Although there are various interpretations of the involvement of dysregulation of the Wnt/beta-catenin pathway in human gastric cancer, Wnt/beta-catenin is reportedly involved in more than 30% of gastric cancers [6].

In the intestinal carcinoma, the adenoma-carcinoma sequence that is associated with multistep gene mutations including APC and beta-catenin dysregulation is the major pathway of the colorectal tumorigenesis in humans [27]. The literature indicated that the intestine is more susceptible to beta-catenin than the stomach, and these classical theories in humans are consistent with the present findings in canine intestinal tumors. In this study, we could not clarify the molecular mechanism of nuclear accumulation of beta-catenin and the roles of genetic mutations including beta-catenin gene or *APC*. The nuclear accumulation of beta-catenin and *APC* mutation have been reported in dogs including, Jack Russell Terrier [62, 63]. Since in the present study the high score of nuclear beta-catenin was also detected in the colorectal tumor of Jack Russell Terrier, mutation of *APC* gene of the canine breed might be associated with the phenomena.

In the present study, no nuclear reactivity of beta-catenin was noted in 94% (16/17) of NPG type carcinomas. Likewise, diffuse type carcinomas including SRC, MUC and UND, the nuclear expression of beta-catenin was not observed. The genetic and molecular factors contributing to NPG type carcinomas remain controversial, and the relationship between beta-catenin and NPG carcinomas also has not been verified. Although APC plays an important role in the degradation of beta-catenin, the molecule is not always involved in NPG carcinomas [60]. The present results in canine tumors indicate that NPG carcinomas including SRC, MUC and UND developed *via* a pathway that may not require APC/beta-catenin mutation such as the *de novo* pathway. A previous report describes that beta-catenin played an essential role in canine intestinal tumors [33], however our results indicate that NPG type carcinomas in canine intestines may not require beta-catenin dysregulation for their tumorigenesis.

The immunoreactivity for E-cadherin in epithelial tumors of canine GI tracts was depended on the growth patterns and the location of the neoplastic cells. Namely, in PG type tumors the neoplastic cells on the superficial area facing the luminal space showed intense membrane expression of E-cadherin and the immunoreactivity was markedly reduced or lost in the deeper areas. In addition, some cases of diffuse type carcinomas including SRC, MUC or UND showed complete loss of immunoreactivity for E-cadherin. However, a relationship between the histological pattern and the immunoreactivity for E-cadherin was not clear, because various tumors including benign tumors showed downregulation of E-cadherin. Similar downregulation of E-cadherin

expression has also been reported in several types of intestinal tumors in both humans [29] and dogs [33]. E-cadherin is essential for the formation and maintenance of normal epithelial structures [45], and most tumors with decreased reactivity of E-cadherin have abnormal cellular architecture and loss of tissue integrity. Hence, our results suggest that downregulation of E-cadherin is involved in the tumor formation of abnormal tissue architecture rather than malignant alteration or invasion in canine gastrointestinal tumors.

The intense immunoreactivity of nuclear p53 was found in a few cases (9%, 7/91) of gastrointestinal tumors examined in dogs. All these 7 cases were evaluated as score 3 with evidence of malignancy such as invasion or metastasis, while no specific histological patterns were noted in these tumors. Even in some benign tumors, significant immunoreactivity for p53-positive was also observed, while the reactivity score was low and had no evidence of malignancy. Unlike human colorectal tumors, canine malignant behavior was reportedly not accompanied by acquisition of p53 mutations [33, 58]. Our results also suggest that abnormal p53 is not absolutely required in all of the tumors for malignant progression, however, when most of the tumor cells show a high-level of nuclear p53 expression, the tumor may have a high malignant potential.

In conclusion, this experiment provides new insight into the relationship between specific histological patterns and beta-catenin dysregulation in canine intestinal tumors. The nuclear localization of beta-catenin is involved in the development of PG tumors, but not diffuse-types of carcinoma including NPG carcinomas, SRC, MUC and UND. The results suggest that PG adenocarcinomas mainly developed from polypoid adenomas through the conventional adenoma-carcinoma sequence pathway. By contrast, NPG carcinomas may arise thorough different mechanisms, such as the *de novo* pathway. To conclude the role of E-cadherin and p53 on the tumorigenesis in canine gastrointestinal tumors, further molecular biological studies will be needed.

ACKNOWLEDGMENTS. The authors would like to thank Mr. Pete Aughton, BSc, D.A.B.T., Dip.R.C.Path., ITR Laboratories Canada Inc., for language editing of this paper.

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