NOTE *Pathology*

Three Cases of Canine Gastrointestinal Stromal Tumors with Multiple Differentiations and c-kit-Expression

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ABSTRACT. Three canine gastrointestinal stromal tumors (GISTs) were examined. Histopathologically, the tumor mass in the jejunum (Case 1) consisted of the proliferation of epithelioid cells with abundant eosinophilic or vacuolated cytoplasm. Gangliocyte-like or multinucleated giant cells were scattered. The tumor cells exhibited neural natures mimicking human gastrointestinal autonomic nerve tumors, which were immunopositive for several neuronal markers. Another jejunal mass (Case 2) was composed by a solid proliferation of spindle-shaped cells, arranging in interlacing fascicles and occasional storiform pattern. The tumor seemed to be classified undifferentiated GISTs, that showed no apparent neural or muscular features by ultrastructural and immunohistochemical examinations. In the pyloric mass (Case 3), the spindle cells having eosinophilic processes and elongated nuclei were arranged in sheets. Immunohistochemically, the tumor cells showed muscular natures as regards alpha smooth muscle actin and desmin expression.

KEY WORDS: c-kit, canine, gastrointestinal stromal tumor.

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The non-lymphoid mesenchymal tumors in the gastrointestinal tract commonly derive from myogenous or neurogenous components [7, 15]. However mesenchymal tumors designated as "Gastrointestinal stromal tumors (GISTs)" show neither muscle nor neuronal differentiation, or bi-directional differentiation [15, 16, 19, 22]. It is widely recognized GISTs originate in the interstitial cells of Cajal (ICCs), and currently human GISTs are differentiated from "true" leiomyomas, leiomyosarcomas, schwannomas, and neurofibromas in the abdominal cavity [6, 11, 17, 18, 21]. These ICCs express membrane tyrosine kinase receptor (CD 117) and c-kit protein, encoded by proto-oncogene c-kit [11]. It has been known GISTs also show c-kit-immunoreactivity, while conventional leiomyomas, leiomyosarcomas and shwannomas are negative [6, 11, 17, 18, 21]. Kindblom *et al.* [8] proposed the name "gastrointestinal pacemaker cell tumors", and recently many pathologists diagnosed GISTs based on their c-kit-immunoreactivity [6, 11, 17, 18, 21]. Human GISTs are reported to arise in the esophagus, anus, and even omentum, mesen- and retro-peritoneum besides the gastrointestinal wall [9, 17, 23]. In animals, tumors resembling to human GISTs have been reported in rhesus macaques [1], dogs [10], and horses [2, 3]. The present study describes morphological, immunohistochemical, and ultrastructural features of GISTs with c-kit expression in 3 dogs.

Histopathology: All samples were formalin-fixed and paraffin-embedded. Each paraffin sections of thick 4 μm was stained with hematoxylin-eosin (HE), Masson's trichrome and Watanabe's silver impregnation. A sample from canine jejunum and cerebellum fixed in fresh 4% paraformaldehide was also used as positive control for c-kit antibody. Immunohistochemistry performed using Envision polymer reagent (Dako-Japan, Kyoto, Japan). To block the endogenous peroxidase activity, all sections were immersed into 3% (w/v) H_2O_2, and incubated with primary monoclonal antibodies against vimentin (clone V9, prediluted, Dako-Japan), cytokeratin (AE1/AE3, prediluted, Dako-Japan), desmin (clone D33, prediluted, Dako-Japan), glial fibrillary acidic protein (GFAP, clone 6F-2, prediluted, Dako-Japan), α-smooth muscle actin (SMA, clone 1A-4, prediluted, Dako-Japan), neuron specific enolase (NSE, clone BBS/NC/V1-H14, prediluted, Dako-Japan), and c-kit (clone T595, 1:20, Novo Castra Laboratories Ltd., Newcastle, England), and with rabbit anti-sera against cow S-100 protein (S100, prediluted, Dako-Japan), chromoglanin A (prediluted, Dako-Japan), synaptophysin (1:20, Dako-Japan), and human c-kit (1:25, Dako-Japan). No antigen retrieval method was used for S100 and c-kit. Before immunohistochemistry for other antibodies, sections were treated with autoclave at 121°C for 5 min. The reaction products were visualized with 3,3'-diaminobenzidine tetrahydrochlolide. For electron microscopic study, tissues fixed with 10% formalin (Case 1) or deparaffinized and rehydrated paraffin-embedded blocks (Case 2) were examined.

Pathological findings: A 10-year-old golden retriever male dog (Case 1) showed vomiting and weight loss during the previous 4 months. Clinical examinations revealed a large nodular mass approximately 10 cm in diameter in the jejunum. Surgically removed mass replaced the jejunal wall, and was firm, and white in color. Up to 6 months after surgery, recurrence of the mass has not recognized. Histopathologically, the neoplasm consisted of a solid proliferation of round or polygonal epithelioid cells with

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anisokaryosis and abundant eosinophilic or vacuolated cytoplasm (Fig. 1). The plump spindle cells were partly arranged in sheets. Gangliocyte-like cells with eosinophilic abundant cytoplasm and a large nucleus, and multinucleated giant cells were occasionally observed. The number of mitotic figures of neoplastic cells was 6 per 10 random high-power fields (HPF, 1 HPF=0.216 mm^2). The individual cells were separated by collagenous septa. The tumor cells invaded into the serosa, forming severe coagulation necrosis with moderate inflammatory changes. Ultrastructurally, the aggregated synapse-like dense core granules, approximately150–350 nm in a diameter, were occasionally observed with in the cytoplasm of the neoplastic cells (Fig. 2).

A 9-year-old mongrel female dog (Case 2) showed no specific clinical features, except for mild anemia and anorexia. Palpation and ultrasonography revealed a mass at the jejunum, 3×5 cm in diameter. The cut surface of the surgically removed mass was solid, and white in color. The dog died 2 months after surgery. Histopathologically, the neoplasm of Case 2 proliferated under the submucosa, and partly infiltrated in the mucosa. The neoplastic foci consisted of a solid proliferation of spindle shaped tumor cells with high density. The spindle cells had scant eosinophilic cytoplasm and elongated or round nuclei with high pleomorphism. These cells were predominantly formed interlacing fascicles, and partially storiform nests (Fig. 3). The vacuolated cells were also focally found in scarce infiltration nest. Mitotic rate was greatly high, and counted 80 cells per 10 HPFs. Multifocal coagulation necrosis was also dominant. Individual tumor cells were surrounded by argyrophilic reticulin fibers. Ultrastructurally, the cytoplasm contained numerous mitochondria, cisternae of rough endoplasmic reticulum, polysome, and the bundles of intermediate filaments, average 10 nm in a diameter longitudinally disposed. Cells had occasionally discontinuous basement membranes, and interlocking cytoplasmic processes with adjacent cells.

A 6-year-old, Maltese, female dog (Case 3) had been intermittent vomiting during previous 6 months without improvement by antiemetic drugs. By exploratory celiotomy, a mass, approximately 3 cm in diameter, was found in the pyloric canal. A small neoplastic mass was removed for pathological examination. The dog died a month after surgery. Histopathologically, the neoplasm of Case 3 consisted of a proliferation of spindle cells with eosinophilic cytoplasmic processes arranging fascicles (Fig. 4). The pale round cells were occasionally observed. The nuclei of tumor cells were elongated with blunt end or round. Bizarre nuclei were occasionally observed. Mitotic rate was high and counted 12 cells per 10 HPFs. The extensive necrosis with neutrophilic infiltration was also abundant. Reticulin fibers clearly divided individual tumor cells.

Immunohistochemistry: For immunohistochemical evaluation, antigen-positive cells were counted in 1000 neoplastic cells. The results are summarized in Table 1. In all cases, tumor cells were positive for vimentin in various degrees, and negative for cytokeratin and GFAP. In normal canine jejunum, positive cells with polyclonal c-kit antibody were most located in the external longitudinal muscles, Auerbach's (myenteric) plexus, and partly submucosal plexus (Fig. 5). Purkinje cells and their neurites in the molecular layer were positive with rabbit antiserum against c-kit. In Case 1, immunoreactivity for c-kit was diffusely found in the cytoplasm of neoplastic cells (86.9%, Fig. 6). Spindle-shaped tumor cells in Cases 2 and 3 were weakly to moderately positive with c-kit (12.3 and 27.4%, respectively). All specimens were negative for monoclonal antibody against c-kit. The tumor cells in Case 3 showed marked SMA- (86.9%) and moderate desmin-immunoreactivity (46.7%), suggesting muscular differentiation. A small number of SMA positive neoplastic cells were found in Case 1 (0.2%). All tumor cells in Case 2 were negative for any muscular markers. Epithelioid cells in Case 1 showed scattered intense-positive reaction with S100. All neoplastic cells were weekly positive for NSE. Synaptophysin- and chromoglanin A-positive neoplastic cells were only found in Case 1.

In this study, the diagnoses of GIST were based on their morphological features and c-kit immunoreactivity. In positive controls, canine Purkinje cells was intensely positive for c-kit antibody as previously mentioned [14]. Rabbit antiserum against c-kit that recognizes C' terminus of the c-kit intracellular domains, labeled GISTs neoplastic cells and the specific regions in canine non-neoplastic intestine, together with canine Purkinje cells and molecular layer [14]. These observations supported that the immunohistochemical results in 3 GISTs were specific for c-kit expression. Previously, c-kit expression was reported in the tissue for dogs and equine using same polyclonal antibody [3]. In contrast, the monoclonal antibody for c-kit that binds N-terminal C2-like extracellular domains, labeled no canine specimens, suggesting no cross-reaction for canine tissues. The antigenic sites of c-kit C'-terminus may be quite conservation though interspecies.

Human GISTs have revealed remarkable variability in their morphologic appearances and differentiations. In microscopic observations, neoplastic cells compose of spindle, epithelioid, nuclear palisading, myxoid, anaplastic pattern, and occasionally ganglioma-like morphology [17, 24]. Immunohistochemical evaluations indicate GISTs differentiate toward 4 categories as following, (1) smooth muscle type, (2) neural type, (3) bi-directional type, and (4) undifferentiated type [20]. Ultrastructurally, neural differentiation is based on synapse-like structures [4, 9, 12]. Herrera *et al.* [5] sub-classified GISTs showed neural pattern by electron microscopy as "Gastrointestinal autonomic nerve tumors (GANTs)". Subsequently, many GANTs have been reported as a subtype of human GISTs [4, 8, 9, 12, 13, 20], and in equine GISTs [3]. Muscular differentiation is evidenced by focal accumulation of intracytoplasmic thin filaments with occasional dense patch, subplasmalemmal attachment plaques, micropinocytotic vesicles, and discontinuous basal lamina [24].

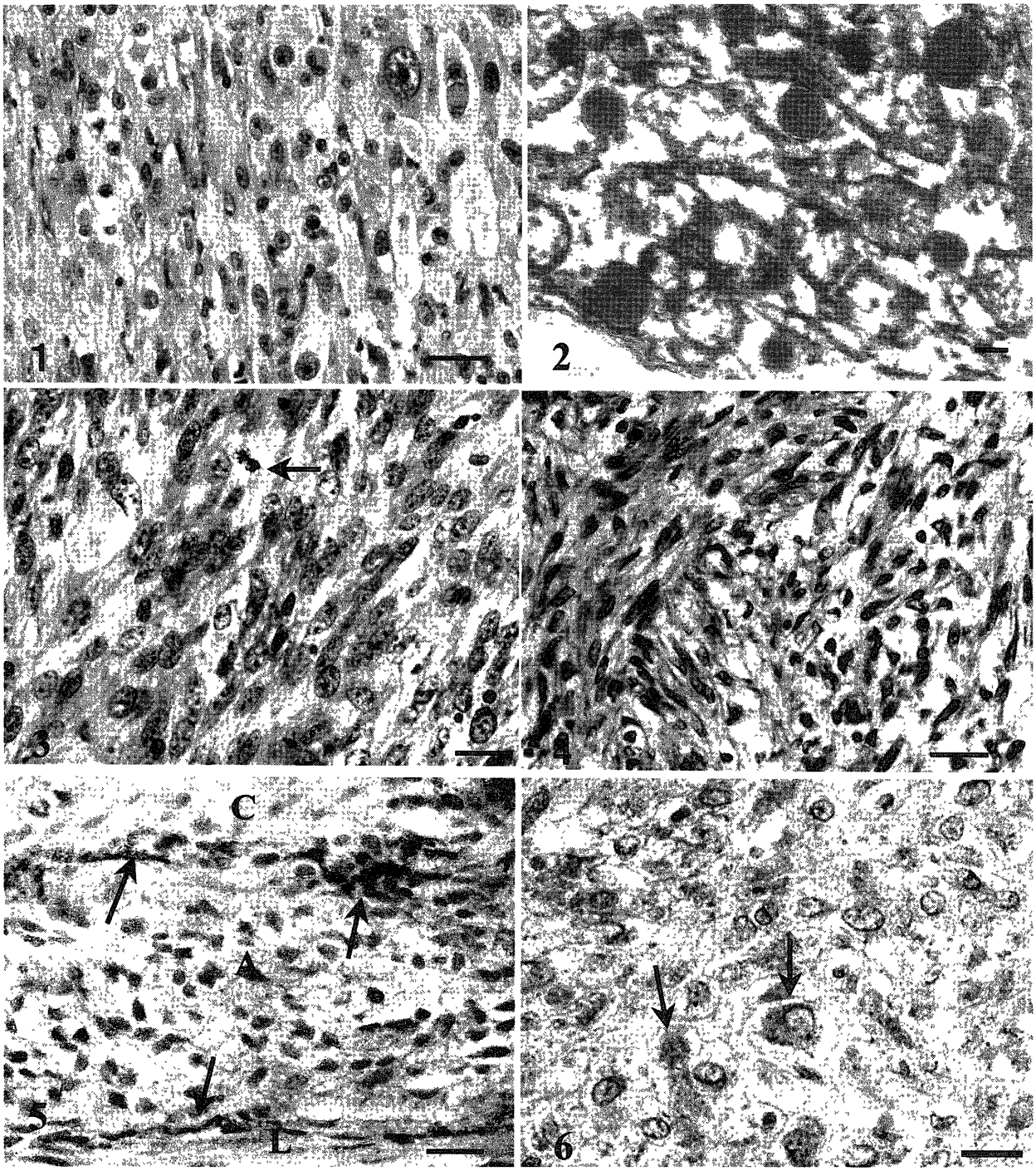

Fig. 1. Case 1. The tumor mass consists of solid proliferation of epithelioid cells with vacuolated or abundant cytoplasms with anisokaryosis. HE. Bar = 25 μm.

Fig. 2. Case 1. Tumor cells contain numerous aggregated electron-dense synapse-like granules. Electron microscope. Bar=200 nm.

Fig. 3. Case 2. The tumor consists of the proliferation of spindle shaped cells having scant cytoplasm and elongated nuclei with high mitotic activity (arrow: mitotic figure). HE. Bar=25 μm.

Fig. 4. Case 3. Spindle tumor cells arranging in interlacing-bundles. The tumor cells have cytoplasmic processes and blund-end or oval nuclei. HE. Bar=25 μm.

Fig. 5. Normal jejunum. Interstitinal cells surrounding Auerbach's plexus and between longitudinal muscular layers (arrows) are immunoreactive for polyclonal c-kit antibody. L: longituginal layer, A: Auerbach's plexus, and C: circular layer. Immunostaining for c-kit. Bar=50 μm.

Fig. 6. Case 1. Epithelioid cells showing moderate to intense immunoreactivity for polyclonal c-kit antibody (arrows). Immunostaining for c-kit. Bar=20 μm.

Table 1. The results of immunoreactivity of canine GISTs for antibodies examined

Case No.	Keratin	Vimentin	GFAP	Desmin	SMA	S100	NSE	Syn	ChrA	c-kit
1	0	81.1	0	0	0.2	36.3	14.8	14.8	11.2	86.9
2	0	57.5	0	0	0	14.1	10.1	0	0	12.3
3	0	46.9	0	46.7	83.7	14.1	14.2	0	0	27.4

Value is percentage: immunoreactive cells per a thousand neoplastic cells Keratin; cytokeratin AE1/AE3, Syn, synaptophysin, ChrA; chromoglanin A, c-kit; rabbit antiserum for c-kit.

Case 1 showed epithelioid pattern with expression for several neuronal markers. The expression for S100 was stronger as compared to those in the other cases, and the immunoreactivity for SMA very limited. Ultrastructurally, neoplastic cells had occasionally neurosecretory granules. These findings indicate the neoplasm might differentiate toward neural type, but is not fully consistent with human GANTs. Generally, neoplasmic cells in human GANTs interconnect to adjacent cells without basal membranes, and no muscular natures are unusual in these tumors [4, 9, 12, 13]. The mild SMA immunoreactivity may indicate that the neoplasm in Case 1 refrained the pluripotential differentiation. On the other hands, neoplastic cells consisted of the proliferation spindle cells with mild S100- and NSE-immunoreactivity. No muscular natures including immunoreactivity for SMA and desmin were recognized. Immunohistochemically, the neoplasm seemed to have the natures of neural type, but no specific neural features were confirmed ultrastructurally. These findings indicate that the neoplasm in Case 2 should be classified undiffentiated type. The c-kit-immunoreactivity in Case 2 was less than those in the other cases. The low level of c-kit expressions was reported in human undifferentiated stromal tumors with aggressive biological behavior [22]. The tumor of Case 3 was composed of a proliferation of spindle cells with marked SMA- and desmin-expressions, and mild S100- and NSE-immunoreactivity. These results seemed to indicate the bi-directional differentiation of this tumor. However, the tumor could be considered as muscular type because of the wide range specificity of the neural markers. As previously mentioned, S100-immunoreactivity doesn't always mean the neuronal natures [10]. In addition, the specificity of NSE is also questionable [19]. On the other hand, muscular differentiation in GISTs is thought the most popular pattern [16, 20]. Considering the poor clinical prognosis and malignant morphological features of the tumor in Case 3, the neoplasm might be a low grade GIST with muscular differentiation.

In conclusion, the present study showed the morphological features of 3 canine GISTs with various differentiations, such as muscular, neural, and undifferentiaied patterns. For the diagnosis of GIST, c-kit expression and ultrastructural examinations might be needed to discriminate from another mesenchymal tumors in the gastrointestinal tract.

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