DOG1 Antibody in the Differential Diagnosis of Gastrointestinal Stromal Tumors

A Study of 1840 Cases

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Abstract: Gastrointestinal stromal tumors (GISTs), KIT or platelet derived growth factor receptor α (PDGFRA) signaling driven mesenchymal tumors of the gastrointestinal (GI)-tract and abdomen, require a precise diagnosis so that the patients may benefit from the newly introduced tyrosine kinase inhibitor drugs. The limitations of the current main tools, KIT immunohistochemistry and KIT/PDGFRA mutation analysis, include lack of KIT expression and mutations in some GISTs. In this study we examined 1168 GISTs of different sites and histologic subtypes, and 672 other tumors and normal tissues for discovered on GIST-1 (DOG1) clone K9, a newly introduced immunohistochemical marker, a chloride channel protein. All GISTs and selected non-GISTs were independently evaluated for KIT. In the GI tract, Cajal cells and gastric surface epithelia were DOG1-positive. The overall sensitivity of DOG1 and KIT in GISTs was nearly identical: 94.4% and 94.7%, and results in GISTs were generally concordant. Gastric spindle cell GISTs was nearly uniformly positive for both markers, whereas DOG1 performed slightly better in gastric epithelioid GISTs that included PDGFRA mutant GISTs. In the intestinal GISTs, KIT was slightly more sensitive than DOG1. Negativity for both DOG1 and KIT was observed in 2.6% of GISTs of GI tract. KIT or PDGFRA mutations were detected in 11/24 DOG1-negative GISTs supporting the diagnosis of GIST. DOG1 expression was also generally present in extragastrointestinal and metastatic GISTs. DOG1 was highly specific for GIST, but exceptional DOG1-positive other mesenchymal tumors included uterine type retroperitoneal leiomyomas, peritoneal leiomyomatosis, and synovial sarcomas (positive in 5/42, 4/17, and 6/37 cases). Leiomyomas colonized by DOG1-positive Cajal cells should not be confused with GISTs. DOG1 positivity was relatively common in esophageal squamous cell and gastric carcinomas, whereas it was rare in colorectal carcinomas. DOG1 should be added into the diagnostic panel evaluating GI and other abdominal tumors, but limitations in its sensitivity and specificity should be recognized.

Key Words: DOG1, gastrointestinal stromal tumor, sarcoma, KIT, PDGFRA, mutation, chloride channel protein

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Gastrointestinal stromal tumors (GISTs) are KIT or platelet derived growth factor receptor α (PDGFRA) signaling driven mesenchymal tumors. They can occur anywhere in the gastrointestinal (GI) tract and abdomen and occasionally as metastases at unexpected sites. Proper recognition of GIST has gained increased importance after the availability of KIT and PDGFRA tyrosine kinase inhibitors.[2,5–8,12,15–19,24–26,28–30]

Until recently, KIT immunohistochemistry has been the main tool for the verification of GIST. The problems in the current immunohistochemical identification of GIST include KIT-negative GISTs, a small group especially related to PDGFRA mutant GISTs.[17,18,22] Also, there have been problems of false positivity related to the optimization of KIT antibodies. For example, desmoid tumors have been KIT-positive with certain polyclonal KIT antibodies,[33] although confirmed negative with others,[21] and some authors have also found leiomyosarcomas frequently KIT-positive.[23]

Discovered on GIST-1 (DOG1) transcripts were identified as a typical finding in gene expression profiling studies on GISTs.[31] The corresponding protein with 8 transmembranous passes has been recently identified as a calcium-regulated chloride channel protein.[4,32] The gene, encoded by a locus at chromosome 11q13, is also known as under aliases TMEM16A, FLJ10261, and ORAOV2.[4,14,31]

Polyclonal antibody to DOG1 and monoclonal antibody DOG1.1 have been found to label GISTs apparently independent of KIT/PDGFRA mutation status.[11,20,31] However, a new DOG1 antibody clone K9 has not been tested, and systematic studies of DOG1 expression in different GIST subtypes and sites are not available. Also, the list of epithelial and nonepithelial abdominal tumors in the differential diagnosis of GIST and studied for DOG1 expression remains far from complete.

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In this study, we evaluated the DOG1 expression in 1168 GISTs of different sites, histologic subtypes, and clinical groups, including GISTs presenting at extra-gastrointestinal locations, using a new monoclonal antibody clone K9. We also compared DOG1 to KIT and provided a semiquantitative analysis for their expression in a large series of GISTs. The relationship of DOG1 or KIT-negative GISTs of GI tract to KIT and PDGFRA mutation status was also examined. Data on DOG1-expression of 672 mesenchymal and epithelial tumors that may enter in the differential diagnosis of GIST are also presented, to further elucidate the specificity of DOG1 as a marker for GIST.

MATERIALS AND METHODS

The study was based on 1168 GISTs and 672 other, mostly abdominal mesenchymal and epithelial neoplasms that may enter in the differential diagnosis of GIST. Selected normal tissues were also analyzed. These tissues were accessioned in the Armed Forces Institute of Pathology from 1970 to 2007. All studies were performed on slides derived from multitumor blocks containing 5 to 60 tumors, with the specimen size varying from relatively large to equal or larger than that in thick needle core biopsies. The GISTs included examples from all segments of the GI tract, extragastrointestinal locations, and from all prognostic groups and specific clinical groups, such as children and neurofibromatosis type1 patients. The diagnosis of GIST was based on a combination of histologic features characteristic of GISTs from specific sites and extensive immunohistochemical and molecular evaluation, with studies for exclusion of other specific entities, such as smooth muscle, nerve sheath, and epithelial tumors.[9,24–26] The histologic subtyping of gastric GISTs and assessment whether extragastrointestinal GISTs were compatible for gastric or (small) intestinal type, was based on the experience of the histology of gastric and small intestinal GISTs.[24,26] Parallel data on DOG1 and KIT were obtained on all GISTs and selected non-GISTs. Furthermore, a large number of GISTs were examined for KIT and PDGFRA mutations, as previously described.[17–19] Because previous studies indicate equal DOG1 expression independent of mutation types or wild-type status, the mutation data were presented here only on DOG1 or KIT-negative GISTs of the GI tract.

DOG1 immunostaining was performed using a monoclonal antibody to DOG1, clone K9, Novocastra, NewCastle, UK.[1] The primary antibody was diluted 1:200, and applied after heat-induced epitope retrieval in ethylene diamino-tetra-acetic acid buffer, at pH 8.0, for 1 hour. The results on DOG1 immunostaining were tabulated blindly without knowing the results on KIT. The latter immunostaining was performed using a polyclonal KIT antibody A4502 (Dako Cytomation, Carpinteria, CA), diluted 1:250, with a similar epitope retrieval as used for DOG1. Because CD34, another potential GIST marker, has a low sensitivity for epithelioid and small intestinal GISTs and a limited specificity for GIST, we did not compare our results with those obtained with CD34.[24–26]

RESULTS

Normal Tissues

DOG1 had a narrow distribution. In the adult GI tract, DOG1-positivity was detected in Cajal cells of the esophagus, stomach, small intestine and colon, often concentrated around the myenteric plexus. Increased numbers of positive Cajal cells were seen in carcinomas infiltrating the muscularis propria. Loose fibrous tissue, smooth muscle, and neural elements were negative. DOG1-positivity was also observed on the luminal surface of the gastric body and antral mucosa (variably), salivary gland and pancreatic acini, and intrahepatic bile ducts. Gallbladder glandular elements and transitional epithelium of bladder were positive. Breast and prostatic myoepithelial/basal cells were variably positive. Skin was negative except weak luminal or basal positivity in sweat glands. No DOG1-positive elements were detected in the cerebral gray and white matter, thymus, tonsil, term placenta, testis, and (postmenopausal) ovary. Mast cells were negative in all tissues.

GISTs

A total of 986 of the 1040 GISTs (94.8%) from the different sites of the GI tract and abdomen were positive for DOG1, and 987 of these GISTs (94.9%) were positive for KIT, a nearly identical overall frequency. Positivity for both DOG1 and KIT was present in 960 GISTs of GI tract (92.3%), whereas 27 cases (2.6%) were negative for both DOG1 and KIT. In general, there was a high concordance between the DOG1 and KIT immunostains (Fig. 1). DOG1 and KIT-immunoreactivity was strong in the majority of GISTs, but approximately 25% of cases revealed delicate but distinct cytoplasmic positivity. Approximately half of KIT-negative GISTs were positive for DOG1, and just half of DOG1-negative GISTs were KIT-positive. Most cases showed positivity in $> 30\%$ of tumor cells (Table 1). The staining pattern in both DOG1 and KIT varied from cytoplasmic to membranous.

Some variation between GISTs of different sites and subtypes were noted (Table 2). Although the gastric spindle cell GISTs were nearly always positive for both DOG1 and KIT, the gastric epithelioid GISTs were more consistently positive for DOG1, although often with a delicate pattern of staining similar to one often seen in KIT immunostains. Some gastric epithelioid GISTs were KIT-negative and DOG-positive, but in some cases, the opposite was true (Figs. 2A–C). Small intestinal and duodenal GISTs were typically strongly positive for both markers, but a slightly greater number of cases was positive for KIT than DOG1 (Fig. 2D). All GISTs in neurofibromatosis 1 patient and most GISTs in children, 2 known mutation-negative GIST subsets,[19,29] were positive for both DOG1 and KIT (Table 1).

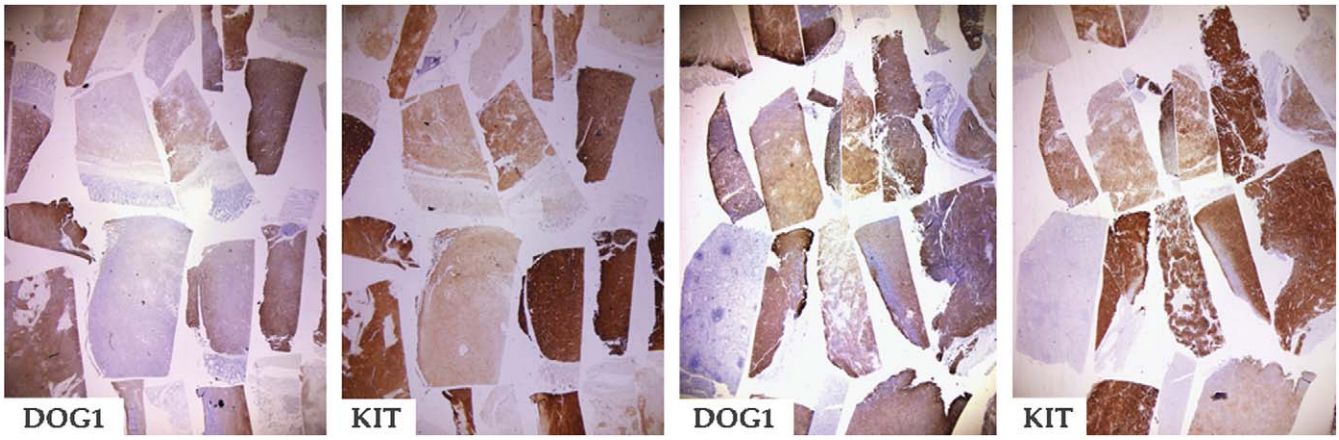

FIGURE 1. Examples of 2 multitumor blocks immunostained for DOG1 and KIT. The blocks contain gastrointestinal stromal tumors and a smaller number of smooth muscle tumors, seen as negative for both markers. Note the high concordance for both positive and negative results between the 2 antibodies.

In extragastrointestinal GISTs from the omentum, DOG1 was detected in 117/128 cases (91.4%) and KIT in 119/128 cases (93.0%), only marginally lower numbers than those obtained from primary GI GISTs (Table 1). GISTs in the abdomen were DOG1-positive in 83/90 cases and KIT-positive in 82/90 cases. Omental GISTs constituted of a mixture of gastric and small intestinal type GISTs, whereas small intestinal type GISTs dominated among the abdominal GISTs. Both groups contained large numbers (from 30% to > 50%) of abdominal metastases of GISTs.

KIT and PDGFRA Mutation Status of Cases Negative for DOG1, KIT, or Both

Analysis of the mutation status of GISTs of the GI tract negative for DOG1, KIT, or both has been summarized in Table 3. The double-negative cases most often showed wild-type sequences, with 1 KIT exon 11 mutation and 3 PDGFRA mutations. DOG1-negative and KIT-positive GISTs contained 5 PDGFRA mutations and 2 KIT exon 11 mutations, and 5 wild-type sequences, whereas DOG1-positive and KIT-negative GISTs showed PDGFRA mutations in 10 cases, no KIT mutations, and wild-type sequences in 3 cases. All PDGFRA mutations occurred in gastric GISTs.

DOG1 in Tumors Other Than GISTs

A wide variety of GI (and some other) mesenchymal neoplasms were immunohistochemically DOG1 negative. These included typically or variably KIT-positive tumors such as mastocytoma, blastic extramedullary myeloid tumor, metastatic melanoma to the GI tract, seminoma, and numerous KIT-negative entities representing the spectrum of mesenchymal tumors of the abdomen and GI tract that can be potentially confused with GIST, for example GI leiomyosarcoma, schwannoma, dedifferentiated liposarcoma, and glomus tumor (Table 4).

Exceptional non-GISTs that revealed DOG1 positivity included half of GI intramural leiomyomas (esophagus, stomach, and intestines). Although the tumor cells proper were negative, numerous DOG1-positive

TABLE 1. Discovered on Gastrointestinal Stromal Tumor 1 and KIT-positivity in 1168 Gastrointestinal Stromal Tumors of Different Sites and Clinical Groups

	DOG1-positivity				KIT-positivity			
Site or clinical group	3+	2+	1+	0	3+	2+	1+	0
Stomach (n = 537)	455	35	17	30	413	60	24	40
Small intestine (n = 372)	352	6	-	14	360	3	-	9
Duodenum (n = 57)	53	-	-	4	56	1	-	-
Esophagus (n = 10)	8	1	-	1	10	-	-	-
Colon and rectum (n = 64)	59	-	-	5	55	4	1	4
Omentum (n = 38)	34	-	-	4	33	2	2	1
Abdomen, NOS (n = 90)	79	3	1	7	71	8	3	8
Gastric GISTs in children (n = 12)	11	-	-	1	8	-	-	4
Duodenal/small intestinal GISTs in neurofibromatosis 1 patient (n = 16)	16	-	-	-	16	-	-	-
Total for all sites (n = 1168)	1040	45	18	65	998	78	30	62

Hyphen indicates no cases in the group. GISTs in children and neurofibromatosis in 1 patient are included in totals of the numbers for specific sites.

0 indicates negative; 1+, < 10% tumor cells positive; 2+, from 10% to 30% of tumor cells positive; 3+, > 30% of tumor cells positive; DOG1, discovered on GIST-1; GISTs, gastrointestinal stromal tumors; NOS, not otherwise specified.

TABLE 2. Discovered on Gastrointestinal Stromal Tumor 1 and KIT-immunoreactivity in Different Histologic Subtypes of Gastric Gastrointestinal Stromal tumors

	DOG1				KIT			
Subtype	3+	2+	1+	0	3+	2+	1+	0
Spindle cell sclerosing (n = 36)	33	-	1	2	32	3	-	1
Spindle cell, palisading vacuolated (n = 116)	113	2	1	-	112	4	-	-
Spindle cell, hypercellular (n = 47)	43	1	-	3	43	1	1	2
Spindle cell, sarcomatous (n = 68)	62	2	1	3	57	6	2	3
Epithelioid, sclerosing (n = 115)	90	8	6	11	70	22	10	13
Epithelioid, discohesive (n = 44)	21	14	5	4	17	9	7	11
Epithelioid, hypercellular (n = 31)	26	2	1	2	23	3	1	4
Epithelioid, sarcomatous (n = 9)	8	1	-	-	8	-	1	-
Epithelioid/spindle and mixed types (n = 71)	59	5	2	5	51	12	2	6
Total for column (n = 537)	455	35	17	30	413	60	24	40

Hyphen indicates no cases in the group.
0 indicates negative; 1+, < 10% tumor cells positive; 2+, from 10% to 30% of tumor cells positive; 3+, > 30% of tumor cells positive; DOG1, discovered on GIST-1.

spindle cell elements between smooth muscle cells were often present consistent with the colonization by Cajal cells. These cells were also KIT-positive, in addition to mast cells (Fig. 3A). Uterine type leiomyomas in the retroperitoneum and peritoneal leiomyomatosis lesions (smooth muscle actin, desmin, and estrogen receptor positive tumors) showed focal DOG1-immunoreactivity in the tumor cells in 5/42 and 4/17 cases, respectively. However, KIT positivity in these tumors was limited to mast cells only (Fig. 3B). Synovial sarcomas (6/37)

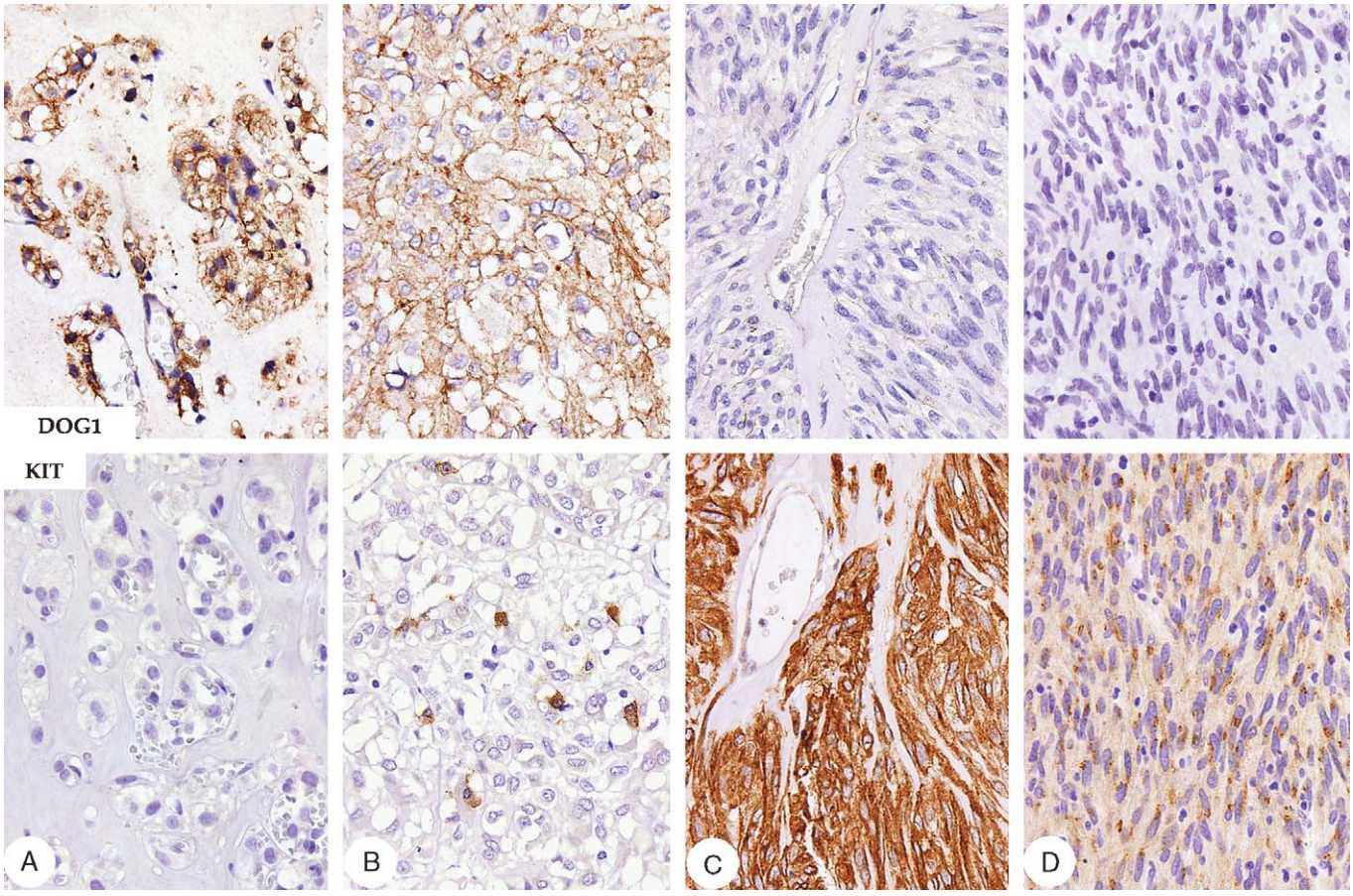

FIGURE 2. Paired examples of DOG1 (upper row) and KIT immunostaining (lower row) in 4 GIST. A, Gastric sclerosing epithelioid GIST and another gastric epithelioid GIST are positive for DOG1 and negative for KIT. Note positive mast cells in lower panel of B. C, Gastric hypercellular spindle cell GIST negative for DOG1 and positive for KIT. D, Small intestinal GIST negative for DOG1 and positive for KIT. Note the perinuclear dot-like KIT immunostaining in this case. GIST indicates gastrointestinal stromal tumors.

TABLE 3. KIT and Platelet Derived Growth Factor Receptor α Mutation Status in Those Gastrointestinal Stromal Tumors That Were Negative for Discovered on Gastrointestinal Stromal Tumor 1, KIT, or Both

	Cases Analyzed	Wild-type	KIT Exon 11	PDGFRA Exon 12	PDGFRA Exon 14	PDGFRA Exon 18
DOG1 negative, KIT negative	12	8 cases 3 gastric 5 small intestinal	1-V559D colonic		1-N659K gastric	2-D842V both gastric
DOG1 negative, KIT-positive	12	5 cases 2 gastric 2 duodenal 1 small intestinal	1-557-558 del gastric 1-W557R small intestinal			4-D842V 1-D842Y all gastric
DOG1-positive, KIT negative	14	3 cases all gastric		1 V561D gastric	1-N659K gastric	6-D842V 2-843-846del 1-842-844del All gastric
Total	38	16	3	1	2	16

The predicted amino acid sequence is shown for each mutation.
DOG1 indicates discovered on GIST 1; PDGFRA, platelet derived growth factor receptor α.

showed focal DOG1-positivity in the epithelial or spindle cell components (Fig. 3C).

GI carcinomas (Table 5) showed focal to extensive DOG1 expression, especially in squamous cell carcinomas of the esophagus (Fig. 3D) and intestinal carcinomas of the stomach, whereas diffuse signet ring cell carcinomas of the stomach and colorectal carcinomas were usually negative.

TABLE 4. Discovered on Gastrointestinal Stromal Tumor 1-immunoreactivity in Mesenchymal Tumors Other Than Gastrointestinal Stromal Tumors, and Other Nonepithelial Tumors

Diagnosis	
Desmoid, including 7 from the abdominal cavity	0/20
Desmoplastic small round cell tumor	0/7
Endometrial stromal sarcoma, abdominal metastases	0/8
Extramedullary myeloid tumor, soft tissues, and skin	0/5
Ewing sarcoma, extraskeletal	0/11
Glomus tumor, stomach, including 1 malignant	0/14
Inflammatory fibroid polyp, small intestine	0/65
Inflammatory myofibroblastic tumor, GI-tract	0/15
Kaposi sarcoma, soft tissue, and skin	0/19
Leiomyoma, intramural, in the GI-tract	0/25*
Leiomyoma, of muscularis mucosae of colon/rectum	0/9
Leiomyoma, uterine type, abdomen and retroperitoneum	5/42
Leiomyomatosis peritonealis disseminata	4/17
Leiomyosarcoma, small intestine and abdomen	0/14†
Leiomyosarcoma, retroperitoneum	0/54
Liposarcoma, dedifferentiated, abdomen	0/18
Malignant peripheral nerve sheath tumor	0/8
Mastocytoma, skin	0/3
Melanoma, metastatic to GI-tract	0/67
Neuroblastoma	0/7
PEComa, abdominal	0/3
Schwannoma, stomach or colon	0/33
Seminoma, testicular	0/16
Solitary fibrous tumor, including 7 from the abdomen	0/19
Synovial sarcoma, including 2 from the stomach	6/37
Undifferentiated sarcoma, small intestine, colon/rectum	0/26
Total	15/562

*Positive Cajal cells detected in half of the cases.
†Includes 3 metastases of uterine leiomyosarcoma.
GI indicates gastrointestinal.

DISCUSSION

The precise diagnosis of GIST has become the utmost important because of the availability of first and second-generation tyrosine kinase inhibitor drugs (imatinib, sunitib, and others) specifically targeting the constitutional KIT activation by tumor-specific oncogenic mutations.[2,7,8] In this regard, specific diagnosis enables delivery of potentially life-saving treatment to the right patients. In contrast, selection of the appropriate patient population for this very expensive treatment is part of heath care resource optimization.

In this study we extensively evaluated DOG1 antibody, clone K9, in the diagnosis of GIST. There were 2 components in the study: (1) analysis of large numbers of GISTs from different sites to test the sensitivity of this marker, and (2) to test the specificity of DOG1 on a wide variety of GI, and abdominal mesenchymal and epithelial tumors, and KIT-positive tumors relevant in the differential diagnosis of GIST.

DOG1 gene was found and named as a transcript and protein that is highly expressed in GISTs.[31] *DOG1* gene has also been independently discovered in other contexts, and is known under aliases TMEM16A, FLJ10261, and ORAOV2. In humans, it is localized in chromosome 11q13, and is overexpressed in head and neck squamous cell carcinomas. [13,14] Recently, the same gene product, a transmembrane protein with 8 transmembranous passes, has been identified as a calcium-dependent, receptor-activated chloride channel protein, also named as anoctamin 1.[4,32] Therefore, DOG1 does not seem to be directly related to oncogenic activation of KIT (and PDGFRA) in the GIST.

It is of interest that DOG1 labels GI Cajal cells, similar to KIT, as noted in previous studies.[11,31] However, DOG1 has a narrower distribution than KIT being absent in mast cells, melanocytes, and germ cells. However, earlier studies on polyclonal DOG1 antibody found reactivity in mast cells, although monoclonal antibody DOG1.1 did not detect the mast cells.[11,31] Our findings in DOG1-positivity in GI and some other

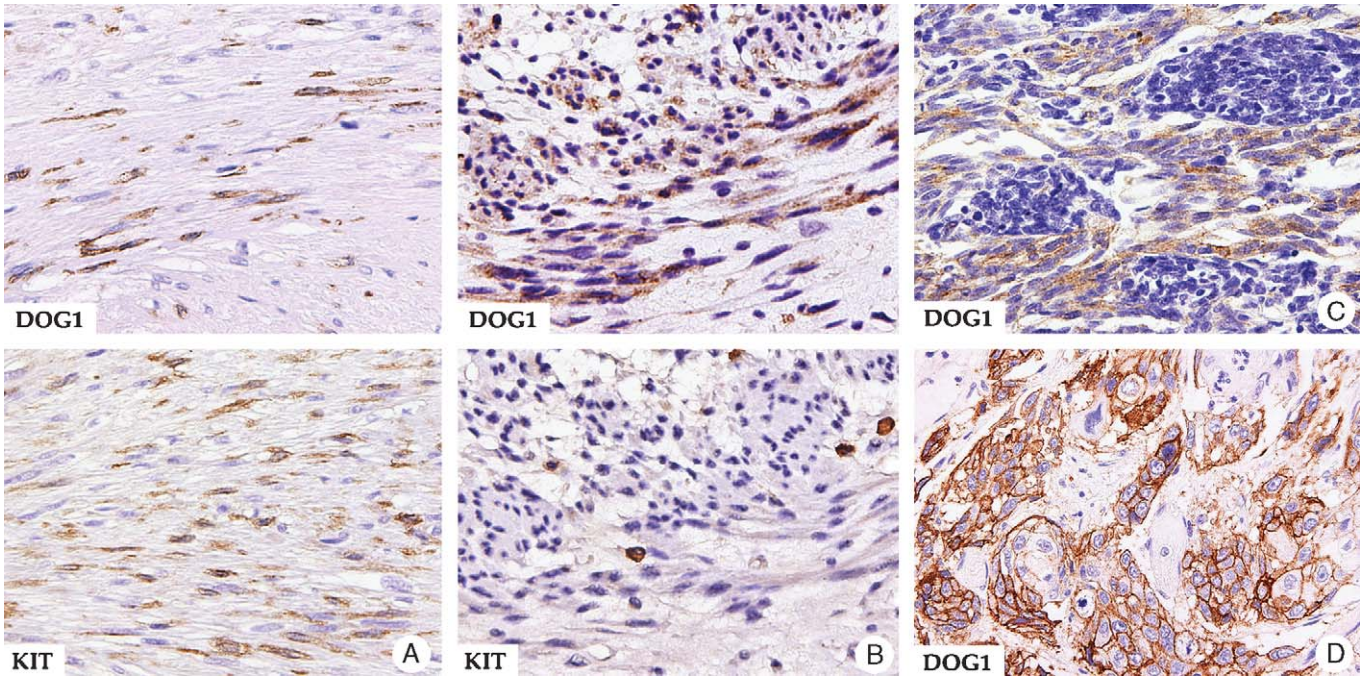

FIGURE 3. Exceptional examples of DOG1 immunoreactivity in tumors other than gastrointestinal stromal tumors. A, DOG1 and KIT immunoreactive Cajal cell-like elements are often detected in intramural leiomyomas of the gastro intestinal tract. Note the additional positive mast cells in the KIT immunostaining. B, Uterine type leiomyoma of the retroperitoneum shows DOG1-positivity in 10% of cases. KIT immunostaining of the same case reveals mast cells as the only positive elements. C, Rarely, synovial sarcoma contains DOG-positive tumor cells, as seen here in the epithelial component of a biphasic example. D, Esophageal squamous cell carcinoma is DOG1-positive with a membrane staining pattern.

epithelia are consistent with the findings provided by the manufacturer's specification sheet.[1]

In this study we found DOG1-positivity with the K9 monoclonal antibody in a great majority of GISTs of all sites, (1103/1168 cases, 94.4%). In comparison, 87% of GISTs in a large series were positive with the DOG1.1 monoclonal antibody[11] indicating that the new K9 clone may be more sensitive. In general, our study showed a great concordance between the results of DOG1 and KIT: 92.3% of cases were positive for both. In a majority of cases, the positivity was strong, but the gastric epithelioid GISTs were less intensely positive than the gastric spindle cell and intestinal GISTs. Compared with KIT, DOG1 had a nearly identical overall performance, but was slightly more sensitive in gastric epithelioid GISTs, whereas a greater number of small intestinal and duodenal GISTs were negative for DOG1 than KIT. Approximately half of KIT-negative GISTs were positive for DOG1, and just half of DOG1-negative GISTs were KIT-positive. These results indicate that use of both markers together is beneficial in problem cases, such as tumors with unexpectedly KIT-negative or positive results. It is possible that even greater detection sensitivity could be reached by analyzing the larger specimens in clinical practice, given sometimes the focal immunoreactivity.

DOG1 and KIT-negative (double-negative) cases are a special dilemma. This small group comprised only 27/1040 GISTs of the GI tract (2.6%) in this study. Mutation analysis revealed that many of these cases had PDGFRA and occasionally KIT mutations, verifying such cases as GISTs. However, more commonly these tumors were wild-type for all "hot-spot" exons. This raises the possibility that some DOG1 and KIT-negative tumors, despite histologic appearances compatible with GIST, still represent other biologic entities. Nevertheless, mutation negativity in itself is not evidence against GIST, as several mutation-negative subgroups are known, such as pediatric GISTs, those in neurofibromatosis 1 patient, and sporadic wild-type GISTs.[2,5,19,29]

Additional studies, such as analysis of phosphorylation status of KIT, would be of interest for DOG1 and KIT-negative GISTs.[5] However, these studies are not widely available for clinical setting and are not feasible in formalin-fixed and paraffin-embedded tissues, and could

TABLE 5. Discovered on Gastrointestinal Stromal Tumor 11-immunoreactivity in Epithelial Tumors of the Gastrointestinal Tract

Diagnosis	
Carcinoid, small intestine	0/6
Carcinomas	
Gastric adenocarcinoma, intestinal type	8/29
Gastric adenocarcinoma, signet ring cell-type/diffuse	3/13
Colonic adenocarcinoma	1/20
Cholangiocarcinoma of liver	1/6
Squamous cell carcinoma of esophagus	9/15
Undifferentiated sarcomatoid carcinoma of small intestine	0/21
Total	22/110

therefore not be performed in this retrospective series. Antibody to protein kinase C θ, a downstream effector in the KIT-signaling pathway, has been suggested as a supplementary GIST marker.[3,10,27] However, our experience (Miettinen et al, unpublished) has shown that immunohistochemical staining for protein kinase θ is often indistinct and difficult to interpret. Therefore, a small gray area remains, where the histologic diagnosis of GIST cannot be supported by objective parameters. In clinical cases in which KIT/PDGFRA mutation testing and KIT phosphorylation analysis remain negative in KIT/DOG1-negative cases, a trial with KIT tyrosine kinase inhibitors might still be prudent until a better understanding of these tumors is developed. However, clinical response to imatinib in KIT-negative wild-type GISTs is known to be poorer than in typical GISTs.[2] Additional biologic and genetic studies for this subgroup are warranted.

Although DOG1 seems highly specific for GISTs, especially among GI mesenchymal tumors, there are rare exceptions of positive non-GISTs. Uterine type retroperitoneal leiomyomas and biologically related retroperitoneal leiomyomatosis lesions were DOG1-positive in > 10% of cases, which is a potential pitfall in the use of DOG1. However, the smooth muscle nature of these tumors can be usually histologically easily identified by their tinctorial quality in hematoxylin and eosin staining and cellular morphology with blunt-ended nuclei. If the problem still remains unresolved, a wider panel of markers including smooth muscle actin, desmin, and estrogen/progesterone receptor easily identifies uterine type leiomyomas and leiomyomatosis lesions that are almost always positive. Furthermore, these tumors are KIT-negative with mast cells only positive. Previous studies using a polyclonal and monoclonal DOG1 antibody (DOG1.1) did not specifically evaluate the estrogen-dependent smooth muscle tumors.[11,31] One should not confuse the intramural GI leiomyomas with DOG1 and KIT-positive dendritic-shaped Cajal cells with GISTs. These cells seem to colonize the tumors from the surrounding muscularis propria or myenteric plexus, perhaps as a peculiar reaction incited by the smooth muscle proliferation, and the same phenomenon can be seen in carcinomas infiltrating in the muscularis propria.

Previous studies with different DOG1 antibodies have reported sporadic positivity in rare examples of non-GISTs. One study with a polyclonal antibody showed positivity in 1/20 synovial sarcomas, 1/40 leiomyosarcomas, 1/4 fibrosarcomas, and 1/9 Ewing sarcomas.[29] Monoclonal antibody DOG1.1 showed rare positivity in leiomyosarcoma (1/326), synovial sarcoma (1/39), and desmoplastic melanoma (1/10).[10] In our study, examples of most these entities did not reveal DOG1-positivity indicating a high, comparable specificity of the K9 clone, as seen for the DOG1.1 monoclonal antibody. However, synovial sarcomas revealed infrequent positivity also in our study.

GI carcinomas may be DOG1 positive. In this study, the highest frequency of DOG1 positivity was seen esophageal squamous cell carcinomas and gastric carcinomas of intestinal type, whereas gastric carcinomas of diffuse type and colorectal carcinomas were less commonly positive. DOG1-immunoreactivity is not unexpected in carcinomas, considering that an alternative gene name ORAOV2 refers to "overexpressed in oral carcinoma".[12] A previous study on DOG1.1. monoclonal antibody found positivity in hepatocellular, adenoid cystic, and pulmonary carcinomas, 2/3 colon adenomas, and 1/4 duodenal carcinomas, but GI carcinomas were not extensively studied.[11] Although the differentiated carcinomas can hardly be confused with GIST in larger specimens, limited biopsies may be more problematic. Therefore, application of epithelial markers in DOG1-positive GI-biopsies may be warranted. Our findings on GI sarcomatoid carcinomas, the GI carcinomas that could be easily confused with GIST, were consistently DOG1(and KIT-negative) further aiding their distinction from GIST.

Consistent DOG1-negativity of many regularly or potentially KIT-positive tumors, such as metastatic melanoma, seminoma, Ewing sarcoma, and extramedullary myeloid tumor, makes this marker as a valuable adjunct tool in the differential diagnosis of GIST.

In conclusion, we analyzed over 1840 GI and other tumors for DOG1, a new marker originally identified in expression array studies of GISTs, using a new monoclonal antibody clone K9. DOG1 and KIT-immunoreactivity is present in 94% of GISTs, indicating equal sensitivity for these markers. DOG1 is especially useful in KIT-negative gastric epithelioid GISTs. However, 2.6% of KIT-positive GISTs of the GI tract (especially small intestinal ones) are negative for DOG1, indicating the need to use both KIT and DOG1 antibodies in problem cases, as well as KIT and PDGFRA mutation analysis to support the diagnosis of GIST. DOG1 should be added into the immunohistochemical panel evaluating GI, abdominal, and selected other KIT-negative and positive tumors suspected of GISTs. The nature of both DOG1 and KIT-negative GISTs needs further study, and DOG1 expression in GI carcinomas and some smooth muscle tumors should be considered in the differential diagnosis of GIST.

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