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• *BRIEF REPORTS* •

Analysis of CD117-negative gastrointestinal stromal tumors

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Abstract

AIM: To identify the gastrointestinal stromal tumors (GISTs) that are negative for CD117 expression by immunohistochemistry and to characterize their malignant potential.

METHODS: A total of 108 primary mesenchymal tumors of the gastrointestinal tract were screened to select CD117-negative tumors, from which *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 10, 12, 14, and 18) were sequenced to identify GISTs. Tumor recurrence and distant metastasis were used as the criteria of malignancy.

RESULTS: The result showed that approximately 25% (29/108) of the gastrointestinal mesenchymal tumors were negative for CD117 and approximately 6% (7/108) of the tumors were CD117-negative GISTs. All these CD117-negative tumors had a mutated *KIT* and a wild-type *PDGFRA*. All CD117-negative GISTs with mutations at codons 557/558 of *KIT* had mitotic counts >10/50 high power field, and 75% (3/4) of them showed multiple recurrence or distant metastasis.

CONCLUSION: CD117-negative *KIT* mutated GISTs account for approximately 6% of the gastrointestinal mesenchymal tumors. Tumor recurrence or distant metastasis correlates to both the *KIT* mutations at codons 557/558 and the mitotic counts, but not to the tumor size.

Key words: Gastrointestinal stromal tumor; CD117; KIT mutation; Tumor recurrence; Distant metastasis

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INTRODUCTION

Before our current understanding of the gastrointestinal stromal tumor (GIST), the term stromal tumor was originally introduced to describe mesenchymal tumors of the gastrointestinal (GI) tract that do not have features of Schwann cells or smooth muscle cells[1]. Subsequently, this term refers collectively to all mesencymal tumors regardless of the differentiation phenotype[2]. In 1998, Kindblom *et al*[3] reported that GIST expresses KIT tyrosine-kinase receptor, supporting its origin from a stem cell that differentiates into interstitial cells of Cajal. Since then, GISTs have been defined as KIT-expression mesenchymal tumors of the GI tract[4] regardless of whether there are co-expressed differentiation markers of myogenic phenotype (e.g., α-smooth muscle actin [SMA] or desmin) or neurogenic phenotype (e.g., S-100 and neuron-specific enolase). In this regard, the immunoreaction to a marker for KIT (i.e., CD117 positivity) becomes a requirement for the diagnosis of GISTs with very rare exceptions.

Gain-of-function mutation of *KIT* is believed to be the initial oncogenic step leading to the development of GIST[5]. Several investigators have shown that 52% to 92% of GISTs harbor *KIT* mutation[6-8] and *KIT* mutation is associated with aggressive behavior in GISTs[9,10]. A recent study also showed that approximately 35% of GISTs, lacking mutated *MIT,* have *PDGFRA* mutation[11].

Although the CD117 positivity by immunohistochemistry provides a simple and clear marker for the diagnosis, some authors believe that a few GISTs may not express CD117[12,13]. These tumors, if existed, are likely to be overlooked if the CD117 expression is a requirement for the diagnosis unless mutated *KIT* or *PDGFRA* genes are identified. In the present study, we screened primary mesenchymal tumors of the GI tract that were morphologically indistinguishable from GIST and sequenced the *KIT* and *PDGFRA* exons in all tumors that were negative for CD117. Then, we characterized the CD117-negative GISTs in terms of immunophenotype, mutation pattern, and clinical behavior.

MATERIALS AND METHODS

Specimens and pathology

A total of 108 primary mesenchymal tumors of the GI tract in the pathology archives of Mackay Memorial Hospital were coded as smooth muscle tumors (leiomyoma or leiomyosarcoma), schwannomas, fibromatoses, solitary fibrous tumors, inflammatory myofibroblastic tumors, gastrointestinal autonomic nerve tumors, stromal tumors or GISTs, during the period from 1995 to 2002.

For immunohistochemical staining, 5 μm representative sections of the specimens were deparaffinized with xylene and rehydrated in graded alcohols. Antibody against CD117 (1∶50 dilution; Dako, Carpinteria, CA), S-100 protein (1∶1 500; Dako), desmin (1∶50; Dako), and SMA (1∶100, Dako) were

commercially available. Immunoreaction was detected according to the manufacturer's instructions (Ventana Medical Systems, Tucson, AZ). Staining for CD117 was considered negative if less than 5% of the tumor cells were weakly stained.

DNA extraction

To isolate DNA from formalin-fixed tumors, representative paraffin blocks were cut at 8 μm using a clean disposable microtome blade. To ensure representative sampling, excess tissue was trimmed before sectioning. The first and the last sections from each ribbon were subjected to light microscopic examination after routine hematoxylin & eosin staining.

The paraffin sections were transferred directly into the PCR tubes and incubated in 300 μL of xylene at 25 ℃ for 5 min, pelleted at 12 000 *g* for 5 min, re-suspended in 300 μL of absolute alcohol at room temperature, spun down, and lyophilized. The pellets were then processed using the Puregene DNA isolation kit (Gentra, Minneapolis, MN) according to the manufacturer's instructions, which include proteinase K (300 μg/mL) digestion overnight at 55 ℃. The final extracts were dissolved in TE buffer and kept at 4 ℃ for later use.

Polymerase chain reaction (PCR) and DNA sequencing

Four pairs of oligonucleotide primers were used to amplify exons 9, 11, 13, and 17 of *KIT* gene and exons of 10, 12, 14, and 18 of *PDGFRA* gene. The primer pairs to amplify *KIT* were 9R (5'-TGA CAT GGT CAA TGT TGG AA-3') and 9L (5'-AGC CAG GGC TTT TGT TTT CT-3') for exon 9, 11R (5'-TGG AAA GCC CCT GTT TCA TA-3') and 11L (5'-CGT AAT CGT AGC TGG CAT GA-3') for exon 11, 13R (5'-GCA AGA GAG AAC AAC AGT CTG G-3') and 13L (5'-CAT GCG CTT GAC ATC AGT TT-3') for exon 13, and 17R (5'-TGA ACA TCA TTC AAG GGT ACT TTT G-3') and 17L (5'-TTG AAA CTA AAA ATC CTT TGC AGG AC-3') for exon 17. The primer pairs to amplify *PDGFRA* were 10R (5'-AGA TGG TTT GAG AGA TGG TAC TGC-3') and 10L (5'-GGA CAC AGT AGA GTC CAA CAA CGT-3') for exon 10, 12F (5'-TCC AGT CAC TGT CGCT GCT TC-3') and 12R (5'-GCA AGG GAA AAG GGA GTC TT-3') for exon 12, 14R (5'-CTC ACT CTC ATT CAA ACC TAT CAG C-3') and 14L (5'-TC ATA CCC ATC TCC TAA CGG C-3') for exon 14, and 18F (5'-ACC ATG GAT CAG CCA GTC TT-3') and 18R (5'-TGA AGG AGG ATG AGC CTG ACC-3') for exon 18.

PCR was carried out according to previously described procedures[11,14]. The PCR products were sequenced using the ABI PRISM BigDye terminator cycle sequencing ready reaction kit and ABI Prism 377 genetic analyzer (PE Applied Biosystems, Foster City, CA). All PCR products and independent duplicates were sequenced on both strands.

RESULTS

Of the 108 primary mesenchymal tumors of the GI tract examined, 79 tumors were positive for CD117 expression by immunohistochemistry, whereas 29 (Figures 1A and 1B) were negative for CD117, i.e., less than 5% of the tumor cells were weakly stained. All these CD117 negative tumors had positive mast cells in the adjacent areas as an internal control (Figure 1C). Among these CD117-negative tumors, 14 were myogenic (positive for SMA or desmin, negative for S-100), 4 were neurogenic (positive to S-100, negative for SMA and desmin), 9 were null-phenotypic (negative to both S-100 and SMA/desmin), and 2 showed dual differentiation (positive to both S-100 and SMA/desmin).

All these CD117-negative tumors were subjected to *PDGFRA* genomic DNA sequencing for the exons 10, 12, 14, and 18 as well as *KIT* genomic DNA sequencing for the exons 9, 11, 13, and 17. The result showed no detectable mutations in *PDGFRA* gene. However, 7 of them had *KIT* mutations, all of which were located in the exon 11 and consisted of 4 missense mutations and 3 in-frame deletion mutations. Codon 557 (tryptophan) and codon 558 (lysine) were most commonly affected, accounting for 57% (4/7) of the *KIT* mutations.

KIT mutations were identified in 5 of 9 null-phenotypic tumors, 1 of 4 neurogenic tumors (Figure 1D), 1 of 2 dual differentiated tumors and none (0/14) of the myogenic tumors. Fisher's exact test showed a significant heterogeneity in *KIT* mutation frequency according to the tumor type ($P = 0.0051$). A closer look further showed that null-phenotypic tumors and myogenic tumors were more significantly different ($P = 0.0037$).

The clinical and histologic features of these CD117-negative GISTs are summarized in Table 1. Among these patients with CD117-negative GISTs, there were five men and two women, at an average age of 52.7 years (range, 44 to 69 years). Three tumors occurred in the stomach, two in small intestine, and two in rectum. Nearly all CD117-negative GISTs were composed of spindle tumor cells. The mitotic counts ranged from 2 to 367 per 50 HPF. Four of these tumors (#106, #133, #142, and #146) had mitotic count greater than 10/50 HPF. The tumor sizes ranged from 4.5 cm to 40 cm (range, 4.5 to 40 cm). Four tumors (#112, #142, #146, and #151) measured larger than 10 cm in greatest dimension, two (#106 and #133) measured 5-10 cm, and one (#23) smaller than 5cm. If tumor recurrence or metastasis was used as the criterion for malignancy, three cases (#133, #142, and #146) belonged to this category.

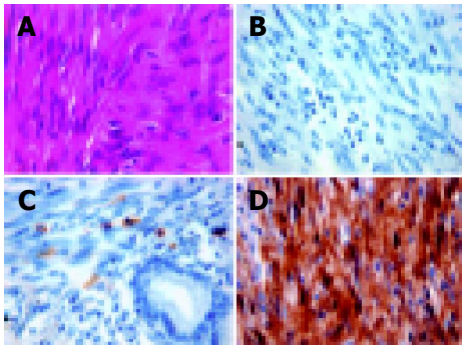

Figure 1 Light microscopic examination of case #23 (A) shows interlacing spindle tumor cells (hematoxylin & eosin stain). The tumor cells were negative for CD117 by immunohistochemistry (B), while the adjacent mast cells were immunoreative to CD117 (C). This tumor was strongly positive for S-100 (D). (magnification ×400).

Table 1 Clinical data of CD117-negative GISTs harboring *KIT* mutations

Case Number	Age (yr) Sex Location	Tumor size[1] Cell type MI[2]	S-100	SMA[3] or desmin	Clinical behavior (follow-up months)
#23	52	4.5 cm	+	-	No evidence of disease after excision (24 mo)
	Female	Spindle			
	Stomach	MI = 2			
#106	69	5.5 cm	-	-	No evidence of disease after excision (15 mo)
	Female	Spindle			
	Stomach	MI = 30			
#112	44	13 cm	-	-	No evidence of disease after excision (12 mo)
	Male	Spindle			
	Rectum	MI = 7			
#133	56	6 cm	-	-	Alive with local recurrence for 5 times (35 mo)
	Male	Spindle			
	Rectum	MI = 13			
#142	45	15 cm	-	-	Died of disease (48 mo); failed to Grivec treatment
	Female	Spindle			
	Small intestine	MI = 367			
#146	53	17 cm	-	-	Alive with multiple metastasis (36 mo); remission after Glivec treatment
	Female	Mixed			
	Small intestine	MI = 100			
#151	50	40 cm	+	+	No evidence of disease after excision (17 mo)
	Female	Spindle			
	Stomach	MI = 3			

[1]Size represents the single greatest dimension. [2]MI, mitotic index (mitoses per 50 high power fields). [3]SMA, α-smooth muscle actin.

DISCUSSION

Acknowledging the fact that further studies are needed to fully understand the molecular basis of GIST, it would seem that gain-of-function mutation of *KIT* is one of the critical oncogenic steps in the tumor development. CD117 positivity may not correlate well to *KIT* mutation[12,13] because mutated *KIT* in some tumors has a low expression at protein level or the epitope of receptor has defects. Alternatively, fixation of the specimen and sources of the antibody (C-19 of Santa Cruz *vs* A4502 of DAKO) may influence the reaction, resulting in false negative staining by immunohistochemistry. In this study, all CD117-negative tumors had immunoreactive mast cells in their adjacent tissues, thus excluding the possibility of technical failure.

In this study, we screened a total of 108 mesenchymal tumors of the GI tract. The result showed that 29 (25%) of these tumors were negative for CD117 expression. Among them, seven harbored mutated *KIT* and none had mutated *PDGFRA*. About 57% *KIT* mutations in CD117-negative GISTs were missense mutations, causing a higher ratio of missense to deletion/insertion mutation than that of the CD117-positive GISTs, which was 23:77 in our series (unpublished observation). We could not explain the reason for this phenomenon.

Based on the immunoreactivity to S-100 and SMA/desmin, these 29 CD117-negative tumors could be divided into four groups. It is the current concept that GISTs are derived from stem cells that differentiate into interstitial cells of Cajal, which are positive for CD117 by immunohistochemistry. Therefore, GISTs without CD117 expression are probably better regarded as "null phenotypic" when immunostaining for SMA/S-100 is also negative, as opposed to the "prototypic" GISTs that are immunopositive for CD117. In this study, we also found that one of four CD117-negative and S-100-positive tumors harbored mutated *KIT* (Figure 1). Because patients with *KIT*-mutant tumors (regardless of their immunoreactivity to S-100) can benefit from imatinib treatment just like other GIST patients, it is better to classify this kind of tumor as GIST instead of *KIT*-mutant "nerve sheath tumor" at least for the therapeutic purpose. The myogenic group is distinct because none of the tumors in this group harbored *KIT* mutation, suggesting that true myogenic differentiation and *KIT* mutation are mutually exclusive. However, when the true myogenic tumors were excluded, the heterogeneity in *KIT* mutation became insignificant ($P = 0.765$, Fisher's exact test). This finding supports the argument[10] for differentiation of true smooth muscle tumors from GIST, and argues against differentiating other tumors from GIST.

So far, all the grading systems for GIST are derived from the observation on the CD117-positive tumors. Most of these systems, such as the NIH Consensus Guidelines for grading[4], give weight to both mitotic count and tumor size. According to this system, there were one low-risk tumor (#23) and six high-risk tumors in our CD117-negative GISTs. In contrast, some grading systems such as the three-tiered grading scheme[15] emphasize mitotic count and cytologic feature but not tumor size. Based on this scheme, there were two low-risk tumors (#23 and #151), one intermediate-risk tumor (#112), and four high-risk tumors (#106, #133, #142, and #146). Because three of the four high-risk tumors in this grading system (#133, #142, and #146) showed either multiple recurrence or distant metastasis, the three-tiered grading scheme correlates better with the clinical behavior of CD117-negative GISTs. This finding suggests that tumor size might not be an important prognostic factor in CD117-negative GISTs, as opposed to CD117-positive tumors. In fact, only two of four tumors with their sizes >10 cm in this study were malignant and the largest tumor

(case #151) measuring 40 cm was microscopically of low risk and clinically benign.

Mutations at certain positions may be associated with aggressive tumor behavior. All CD117-negative GISTs with mutations at codons 557/558 of *KIT* were high-risk in the three-tiered grading scheme[15], and vice versa. Therefore, 75% (3/4) of them showed multiple recurrence or distant metastasis. A similar observation was also made in CD117-positive GISTs with mutations at codons 557/558 occurred in 79% (11/14) of malignant tumors[10]. In contrast, mutations at codons 557/558 only accounted for 34% (15/34) of all GISTs with exon 11 genomic mutations[6].

In conclusion, CD117-negative GISTs do exist (Figure 2), accounting for approximately 6% of the gastrointestinal mesenchymal tumors. They are immunohistochemically different from smooth muscle tumors, which are positive for SMA/desmin but negative for S-100 and CD117. A final diagnosis of CD117-negative GIST depends on the presence of mutated *KIT* or *PDGFRA*. Although generalizations about the malignant potential of CD117-negative GISTs cannot be made, our limited cases show a correlation to *KIT* mutations at codons 557/558 and mitotic counts, but not to tumor size.

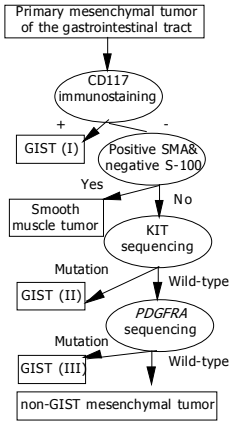

Figure 2 Proposed algorithm for the diagnostic approach to the primary mesenchymal tumor of the gastrointestinal tract. GIST (I) represents the prototypical GISTs, which were diagnosed based on positive CD117 by immunohistochemistry. If tumors were negative for both CD117 and S-100 but positive for α-smooth muscle actin or desmin, they were true smooth muscle tumors. The rest of the tumors should be examined for PDGFRA and KIT genes. Those harboring mutated PDGFRA, labeled GIST (III), belonged to the recently described entity[11], whereas those containing mutated KIT, labeled GIST (II), were described in this study. The remaining tumors were not GISTs.

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