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Synchronous and metachronous multiple gastrointestinal stromal tumors

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Summary. Background & Aims: Sporadic multiple gastrointestinal stromal tumors (GISTs) are rare events especially those developed metachronously. This study aimed to investigate the clinico-pathologic and genetic features defining multiple GISTs. Methods: 624 cases of GISTs were retrieved for retrospective review. 15 cases were identified as multiple GISTs including 13 synchronous and 2 metachronous ones. 32 tumors and 15 normal tissues were obtained from these cases each containing 2-3 tumor nodules and the genomic DNA was extracted for mutational analysis of *KIT* and *PDGFRA* genes. The associated patients were recruited for clinical follow-up studies, including 5 males and 10 females at 49 to 84 years of age. Results: Multiple GISTs comprised of 2.4% of GIST cases in our consecutive series. Twenty-six tumors showed mutations at *KIT* gene in exon 11 and one at *PDGFRA* gene in exon 18. In seven synchronous cases, different tumors from the same patients displayed different genotypes of *KIT* or *PDGFRA*, suggesting their polyclonal origin. In the two multiple GISTs occurring metachronously, the tumors from each patient showed different *KIT* mutations, suggesting that the second tumors were not the relapse or metastasis of the primary GISTs. Conclusions: Based on types of *KIT* or *PDGFRA* mutations and other pathological features, multiple primary GISTs can be differentiated from multiple GISTs resulting from recurrence or metastasis of a single primary tumor. Unlike recurrence or metastasis of GISTs that are malignant, most multiple GISTs are mostly benign and do not require aggressive adjuvant therapy. Therefore, correct diagnosis is critical for proper treatment.

Key words: GIST, Multiple, Sporadic

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract (Miettinen and Lasota, 2006). They exhibit a wide spectrum of clinical features, from indolent and curable to highly malignant diseases that metastasize and become fatal (Miettinen et al., 2002). *KIT* and *PDGFRA* activations are the central tumorigenic events during pathogenesis of the disease (Hirota et al., 1998, 2003; Heinrich et al., 2003). *KIT* mutations occur in 60% to 90% of GISTs (Kindblom et al., 1998; Sircar et al., 1999), frequently in 4 regions of the protein encoded by distinct exons: extracellular domain (exon 9) (Lasota et al., 2000), juxtamembrane domain (exon 11) (Corless et al., 2002; Rubin et al., 2001), tyrosine kinase I domain (exon 13), and tyrosine kinase II domain (exon 17) (Lux et al., 2000). *PDGFRA* gene mutations have been identified in exons 12 and 18 (Hirota et al., 2003; Corless et al., 2005). The discovery that almost all GISTs express KIT/CD117 antigen has led to the development of imatinib mesylate (imatinib, Glivec; Novartis, Switzerland) for the targeted therapy of GISTs (Joensuu et al., 2001), especially for unresectable or metastatic GISTs. Imatinib adjuvant therapy has been shown to dramatically prolong the life expectancy of patients with malignant GISTs (Demetri et al., 2002), and recurrence-free survival after the resection of primary GISTs (Dematteo et al., 2009), but it may not be necessary for nonmalignant GISTs.

Sporadic GISTs are usually present as solitary

lesions. Although relatively rare, they can arise as multiple tumors, often identified as two or more primary tumor lesions synchronously or metachronously present at diagnosis. After Kang et al.(2007) first described 5 sporadic multiple GISTs presented with family history or concurrent neurofibromatosis 1 (NF-1), others have also reported underestimated sporadic multiple GISTs (Haller et al., 2007; Gasparotto et al., 2008; Agaimy et al., 2008). More recently, Agaimy for the first time reported two cases of metachronous presentation of multiple primary GISTs (Agaimy et al., 2009). Therefore, it is necessary to differentiate recurrent or metastasis of primary GISTs from the growth of multiple primary GISTs for the correct staging and therapy.

In the present study, we report 15 cases of sporadic multiple GISTs from the Chinese population including the detailed clinico-pathologic and genetic features, and clinical data collected at a median follow-up period of 33 months (ranging from 3 to 51 months).

Materials and methods

Basic clinical and pathological data

A total of 624 GIST tissues were collected from Dec 2002 to Dec 2009 in Zhongshan Hospital, Fudan University, and stored in the Pathology Archives. The diagnosis of GIST was previously made by two gastrointestinal pathologists (Zhu and Hou) based on the cell type, cellularity, nuclear atypia, invasion and mitosis of the tissue (Hou et al., 2009a,b). There were 427 solitary GISTs, 2 multiple GISTs associated with NF-1, and 195 cases from the consultant file in the same period. The study protocol was approved by the ethics committee of Zhongshan Hospital of Fudan University, and all the patients had given their written consent.

15 sporadic multiple GISTs were retrieved, accounting for 2.4% of all cases. There were 13 synchronous and 2 metachronous multiple GISTs without NF-1. Cases with hyperpigmented lesion, systemic mastocytosis, NF-1, Carney's syndrome, and familial GISTs were excluded. Ten cases presented clinical symptoms typical of GISTs, such as anemia, GI bleeding and abdominal discomfort, and five cases were incidental pathology findings after surgical resection of other tumors including esophageal carcinoma (n=2), gastric cancer (n=1), intestinal diverticular disease (n=1), and omental blood mass (n=1).

Immunohistochemistry

A total of 32 paraffin-embedded tissue blocks were obtained from the 15 multiple GISTs, and subjected to immunohistochemistry staining with a panel of antibodies including CD117 (A4502, KIT polyclonal rabbit, dilution 1:150, Dako, Glostrup, Denmark), CD34 (clone QBEnd 10, mouse, dilution 1:200, Dako), α-smooth muscle actin (α-SMA; 1A4, dilution 1:200, Dako), desmin (D33, dilution 1:200, Dako), and S-100 protein (polyclonal, dilution 1:300, Dako).

Genetic analyses for KIT and PDGFRA

From the above tissue sections, tumor and normal cells were isolated from the same tissue sections by microdissection, and then genomic DNA was extracted from both tumor and normal cells by proteinase K digestion and standard phenol/chloroform extraction, as previously described (Hou et al., 2009c). Exons 9, 11, 13, and 17 of *KIT* gene and exons 12 and 18 of the *PDGFRA* gene were amplified by polymerase chain reaction (PCR), the PCR products were sequenced with both forward and reverse primers, and mutations were identified by comparing the results with known human *KIT* (NM_001093772) and *PDGFRA* (NM_006206) gene sequences in the NCBI GenBank.

Results

Pathological findings

Gross Examinations

Among the 15 cases of multiple GISTs, 11 had 2 tumor nodules (Fig. 1a, case #7) and 2 had three nodules (case #2 and case #6). (Fig. 1b, case #2). In the two metachronous GISTs, the secondary gastric GISTs were identified as new lesions at 7 months after the initial duodenal GIST surgery (Fig. 1c-e, case #14), and 43 months after the initial gastric GIST resection (Fig. 1f-h, case #15), respectively. There were 31 gastric GISTs and 1 duodenal GIST. The tumor size ranged from 0.2 to 12 cm (mean 2.7 cm and median 1.2 cm). All tumors were grossly well circumscribed. The multiple GIST lesions in each case were located in close proximity within 5 cm in 9 patients and over 5 cm in 6 patients. The size ratio of the largest tumor nodule to the small tumor nodule in each patient ranged from 1.2 to 12 (median 3.2 and mean 5.4).

Histologic and immunohistochemical findings

In the 32 GIST specimens, the neoplastic cells were characterized as predominantly spindle shaped in 27 nodules (84.3%), mixed cell types in 3 nodules (9.4%), and predominantly epithelioid cell type in 2 nodules (6.3%). Among multiple GIST nodules from same patients, 12 cases displayed homogenous histopathological and 3 heterogeneous features (Fig. 2a,b, #7). Mitotic figures per 50 high-power fields were 0 in 20 GISTs, 1 in 3 GISTs, 2 in 1 GIST, 3 in 1 GIST, 4 in 3 GISTs, 5 in 1 GIST, 7 in 1 GIST, 9 in 1 GIST, and 25 in 1 GIST. Cellularity was low to mild in 16 cases, moderate in 14 cases, and dense in 2 cases. Nuclear atypia were absent or mild in 19 cases, moderate in 12 cases, and severe in 1 case. Skeinoid fibers were present only in tumors from the duodenal GIST case. Pathological evaluation of aggressive behaviors

(Fletcher et al., 2002) classified 18 very low risk, 6 low risk, 4 intermediate risk, and 4 high risk GISTs. Histopatholgical evaluation of tumor nature (Hou et al., 2009a,b) revealed 26 nonmalignant and 6 low malignant GISTs. Immunohistochemical examination revealed that CD117 was positive in 90.6% cases (29/32, Fig. 2c), and CD34, α-smooth muscle actin (α-SMA), S-100 protein, and desmin were positive in 90.6%, 9.4%, 0%, and 0% cases, respectively.

Molecular Findings

Twenty six GIST masses from the 15 multiple GISTs showed mutations in exon 11 of *KIT* gene and one

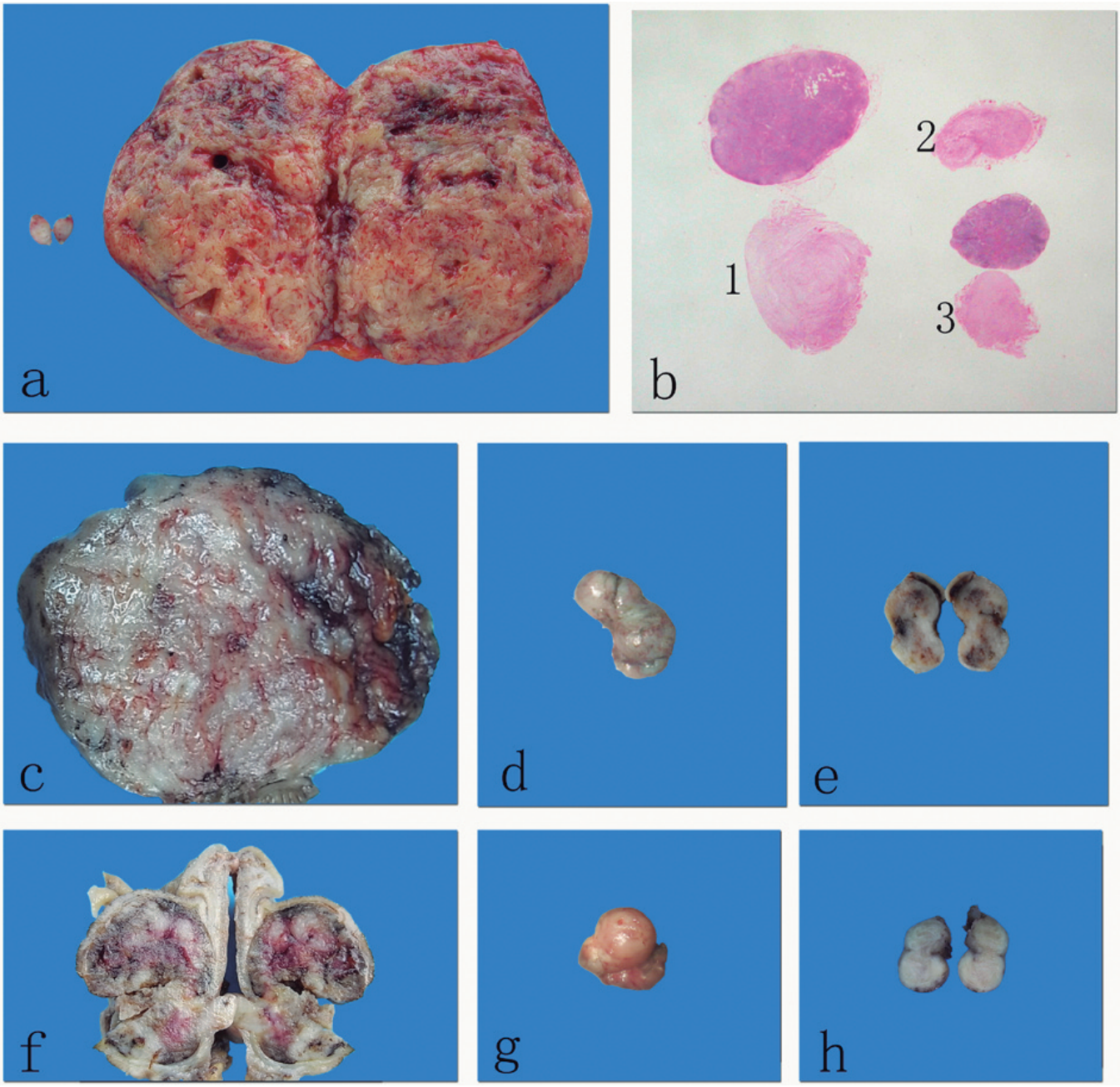

Fig. 1. Gross presentation of sporadic multiple GISTs. **a.** Two GIST masses isolated from stomach synchronously, one was 12 cm and the other was 1 cm. **b.** Three GIST masses from stomach misdiagnosed as lymph nodes in gross examination. The patient had esophageal resection due to esophageal squamous carcinoma, the other two nodules were real lymph nodes. **c.** A duodenal GIST resected from a patient #14. **d.** Gastric GIST was found and endoscopically resected 7 months later in patient #14. **e.** Sectioning of the tumor. **f.** Partial gastrectomy with gastric GIST for patient #15. **g.** Another gastric GIST was resected by endoscopy 43 months after the first operation in patient #15. **h.** Sectioning of the tumor.

Table 1. Demographics and clinical manifestation of 15 multiple GISTs.

Case #	Sex	Age	Clinical manifestation	Resection	Number of tumors isolated	Medical History	Follow up
1	Female	64	Abdominal uncomfortable and melena	Partial gastrectomy	2	No	36 M (alive)
2	Male	84	Dysphagia*	Radical esophageal resection	3	Synchronous esophageal squamous cell carcinoma	Died of esophageal squamous cell carcinoma 48 months later
3	Male	69	Dysphagia*	D2 radical distal gastrorectomy and enucleation of gastric fudus tumors	2	Synchronous gastric adenocarcinoma	Died of gastric adenocarcinoma 23 months later
4	Female	81	GI bleeding	Distal gastrorectomy	2	No	33 M (alive)
5	Male	74	Abdominal emergency*	Wedge gastric resection and intestinal segmental resection	2	Synchronous intestinal diverticular disease	Lost of follow up
6	Female	61	Melena	Partial gastrectory	3	Synchronous gastric minute adenoma	50 M (alive)
7	Female	57	Abdominal uncomfortable	Wedge resection and enucleation	2	No	46 M (alive)
8	Female	69	GI bleeding	Distal gastrorectomy	2	No	36 M (alive)
9	Female	69	Abdominal pain	Wedge resection	2	No	18M (alive)
10	Female	62	Physical examination*	Enucleation	2	Omental blood mass	16 M (alive)
11	Female	66	GI bleeding*	Esophageal resection	2	Esophageal squamous cell carcinoma	15 M (alive)
12	Male	62	Abdominal pain	Wedge resection and enucleation	2	Synchronous leiomyoma of stomach (0.4 cm)	16 M (alive)
13	Female	68	Burn feeling	Partial gastrectomy	2	No	3 M (alive)
14	Female	49	Anemia	Wedge resection	1	History of thyroid papillary carcinoma	23M (alive)
	Female	50	Regular check up	ESD	1	History of thyroid papillary carcinoma and duodeneal stromal tumor	16M (alive)
15	Male	74	Anemia	Partial gastrectomy	1	No	51 M (alive)
	Male	79	Regular check up	ESD	1	Gastric stromal tumor	8 M (alive)

ESD: endoscopical dissection; *means symptoms not for GIST

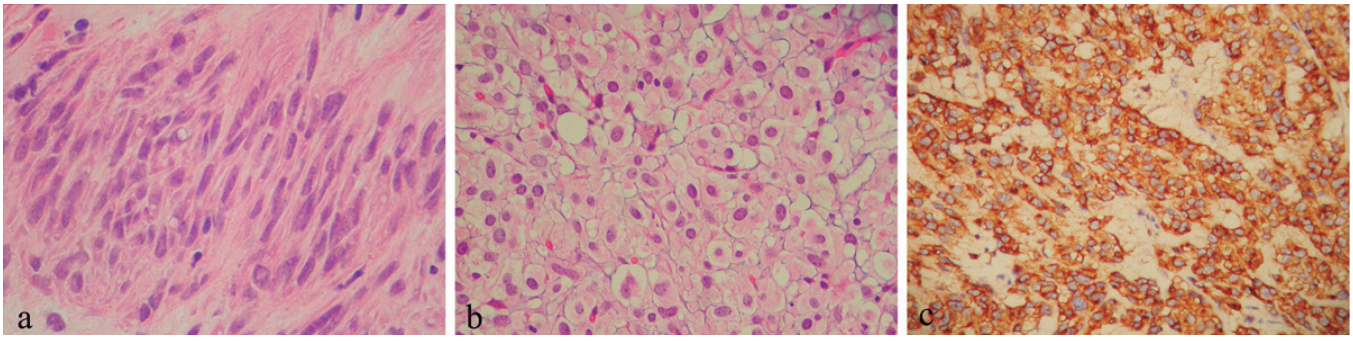

Fig. 2. Different histopathological features of the two lesions in synchronous multiple GISTs shown in Fig. 1a. **a.** The bigger GIST was mostly spindle cell type (H&E). **b.** The smaller lesion consisted of mostly epithelioid cells (H&E). **c.** Tumor cells (in tumor a) expressed CD117 diffusely (Envision). x 400.

showed D842V mutation in exon 18 of *PDGFRA* gene, five had no mutations in any of the examined exons. The overall mutation rate was 84.4% (27/32) while the rate in normal tissues was 0% (Table 2). There were 11 point mutations involving codons 557, 559, 560, and 576; 8 deletions in 5 codons 557-558; 4 duplications involving codons 573-587; and three were a combination of point mutation and deletion. Point mutation and deletion often involved the region between codons 550 to 560, while duplication occurred in the 3' terminal of exon 11. Of the 15 multiple GISTs, 6 had multiple tumor masses displaying similar genotype, with or without gene mutation. However, mutations varied in type and site among multiple GIST lesions in the other 9 cases (60%), especially the one with 3 GIST masses (Fig. 3a-c, case #2) and the two metachronous GISTs (Fig. 4a-d, cases #14 and #15).

Treatment History and Clinical Follow Up

Due to their low or non-malignant nature, all the multiple GISTs in this study were surgically removed and did not require post-surgical adjuvant therapy with imatinib except the two metachronous cases described below. All the patients were followed up in the clinic every 6 months by imaging studies with CT or endoscopy. At the median follow-up period of 33 months (ranging from 3 to 51 months), one patient was

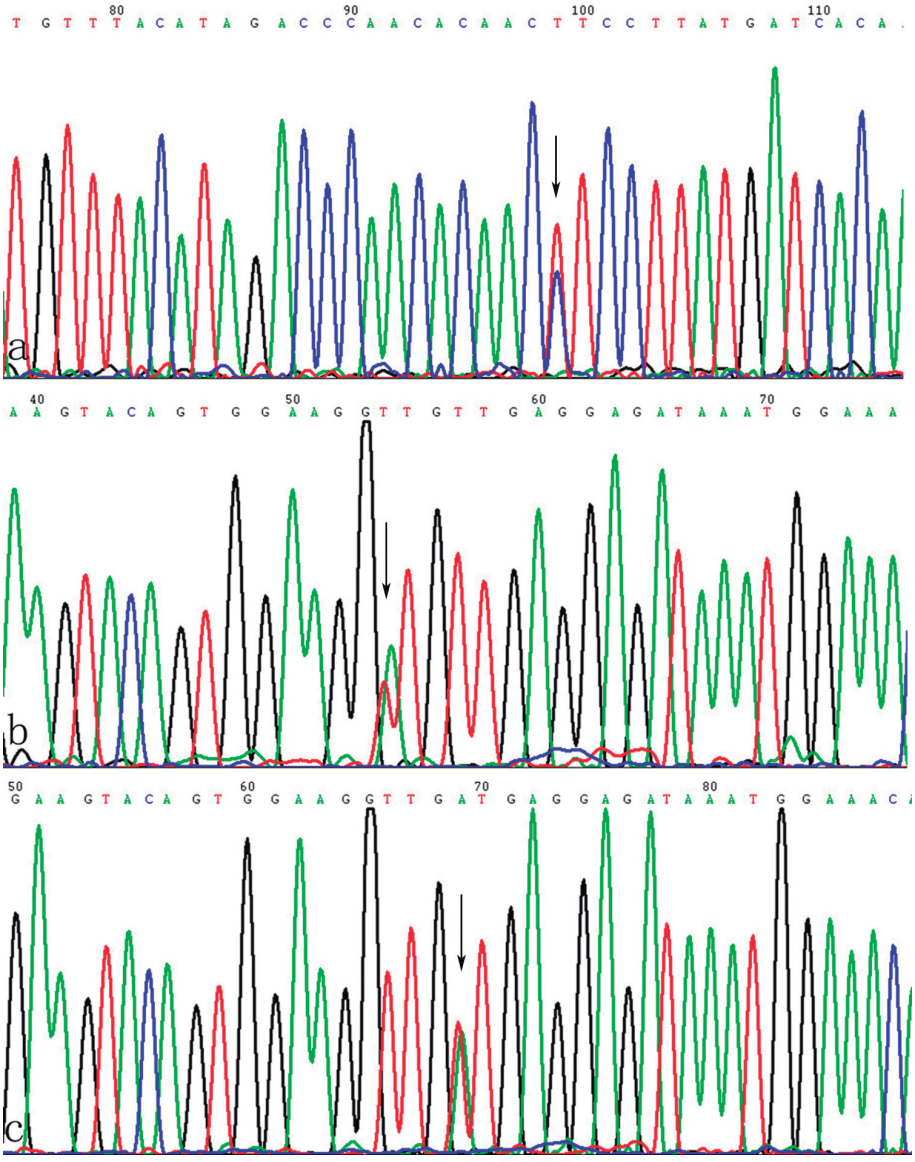

Fig. 3. Three synchronous GISTs from Fig 1b were identified with different mutations (arrows indicated) in exon 11 of KIT gene. **a.** 576 CTT to CCT point mutation. **b.** 559 GTT to GAT point mutation. **c.** 560 GTT to GAT point mutation.

lost to follow up, one patient died of esophageal carcinoma at 48 months, and one patient died of gastric adenocarcinoma at 23 months. Twelve patients were still alive disease-free at 3 to 51 months after the tumor resection (Table 1).

Case analysis on metachronous GISTs

In the two metachronous gastric GIST cases, one patient (case #14) was diagnosed to have thyroid papillary carcinoma, and a huge abdominal mass, a uterus leiomyoma, and a smaller thymoma were identified by regular radiology examination. After a laparotomy, a duodenal GIST was identified as low malignancy based on histopathological parameters (Hou et al., 2009a). She did not receive any additional therapy after this surgery and was closely followed up. Seven months later, a small gastric nodule was found by

Table 2. The clinicopathological features and genetypes of 15 multiple GISTs.

Tumor lesion	Site	Size (cm)	Cell type	Cellularity	Nuclear atypia	Mitosis (/50HPFs)	Risk*	Malignancy**/parameter	KIT or PDGFRA gene mutation
1a	Cardia	3.5	S	Moderate	Moderate	3	Low	Nonmalignant	Exon 11, WK557-558 deletion
1b	Greater curvature	0.3	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, WK557-558 deletion
2a	Fundus	0.3	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, L576 P point mutation
2b	Fundus	0.4	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, V559D point mutation
2c	Fundus	0.8	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, V560D point mutation
3a	Greater curvature	1.5	S	Moderate	Moderate	1	Very low	Nonmalignant	Exon 11, WK557-558 deletion
3b	Greater curvature	1.3	S	Moderate	Moderate	1	Very low	Nonmalignant	Exon 11, WK557-558 deletion
4a	Anterior wall of gastric body	4.0	E	Moderate	Moderate	2	Low	Malignant/muscle invasion	Exon 18 of PDGFRA gene, D842V
4b	Anterior wall of gastric body	1.0	S	Mild	Mild	0	Very low	Nonmalignant	No mutation
5a	Greater curvature	2.5	M	Moderate	Moderate	0	Low	Malignant/muscle invasion	No mutation
5b	Lesser curvature	1.0	M	Mild	Mild	0	Very low	Nonmalignant	No mutation
6a	Greater curvature of gastric body	6	S	Moderate	Moderate	4	Intermediate	Nonmalignant	Exon 11, 551-558 PMYEVQWK deletion
6b	Greater curvature of gastric body	0.5	S	Mild	Mild	0	Very low	Nonmalignant	Exon 11, Q556H and WK557-558deletion
6c	Greater curvature of gastric body	0.5	S	Mild	Mild	0	Very low	Nonmalignant	Exon 11, V559D point mutation
7a	Lesser curve of stomach	12	S	Moderate	Moderate	4	High	Nonmalignant	Exon 11, V559D point mutation
7b	Back wall of stomach	1	E	Mild	Mild	0	Very low	Nonmalignant	No mutation
8a	Antrum of anterior wall	4.5	S	Moderate	Moderate	9	Intermediate	Mlignant/muscle invasion	Exon 11, 576-587LPYDHKWEFPR duplication
8b	Cardia	0.5	S	Mild	Mild	0	Very low	Nonmalignant	No mutation
9a	Lesser curvature	11	S	Moderate	Moderate	0	High	Nonmalignant	Exon 11, 574-576TQL duplication
9b	Lesser curvature	6	S	Dense	Moderate	25	High	Mlignant/active mitoses	Exon 11, 574-576TOL duplication
10a	Anterior wall	0.3	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, V559D point mutation
10b	Posterior wall	0.4	S	Sparse	No	0	Very low	Nonmalignant	Exon 11, W557R point mutation
11a	Left of fundus	0.3	S	Mild	Mild	0	Very low	Nonmalignant	Exon 11, V559D point mutation
11b	Left of fundus	0.2	S	Mild	Mild	0	Very low	Nonmalignant	Exon 11, V559D point mutation
12a	Anterior wall	3	S	Moderate	Moderate	5	Intermediate	Nonmalignant	Exon 11, 560V deletion
12b	Posterior wall	0.3	S	Mild	No	0	Very low	Nonmalignant	Exon 11, 560V deletion
13a	Fundus	3	S	Moderate	Mild	0	Low	Nonmalignant	Exon 11,V559D point mutation
13b	Fundus	0.6	S	Mild	Mild	0	Very low	Nonmalignant	Exon 11, T553F and 554-559EVQWKV deletion
14a	Dudeoneum	9	S	Moderate	Moderate	4	Intermediate	Malignant/muscle invasion	Exon 11, 551P deletion and M552L
14b	Lesser curvature	2.8	S	Moderate	Mild	0	Low	Nonmalignant	Exon 11, 557-558WK deletion
15a	Antrum of posterior wall	5.5	M	Dense	Severe	7	High	Malignant/muscle invasion	Exon 11, I571L and 572-579DPTQLPYD duplication
15b	Cardia	2	S	Moderate	Mild	1	Low	Nonmalignant	Exon 11, V559D point mutation

endoscopy and removed by endoscopical dissection. Although it was pathologically confirmed to be a nonmalignant gastric GIST (Joensuu et al., 2001), and no adjuvant therapy was required, the patient then requested aggressive therapy in the hope to prevent the recurrence of the first tumor. Therefore, she received imatinib therapy for one year after the second resection, and is still alive disease-free.

The other patient (case #15) was diagnosed of a low grade malignant GIST at age 74. After tumor resection, he also rejected adjuvant therapy and was followed up clinically. However, 43 months later, a second gastric mass was found by imaging study and then was removed with endoscopy. When the first and second tumors were reviewed together for comparison (Table 2), the first GIST was identified as malignant with mixed cell types (Fig. 4a), immonohistochemically positive for CD117 and CD34, with ATA to CTA mutation in codon 571 and 572-579 (GAC CCA ACA CAA CTT CCT TAT GAT) duplication in exon 11 of *KIT* gene (Fig. 3b), while the second GIST was identified as nonmalignant, presented as pure spindle cell type (Fig. 4c), positive for CD117 but negative for CD34, and with V559D point mutation (Fig. 4d). The results indicated that the two GIST masses were polycolonal GISTs rather than a recurrence of the single primary tumor. Also, due to the malignancy of the first tumor, additional imatinib therapy was recommended but was rejected by the patient due to financial issues. The patient has been clinically well and is still in regular long-term follow-up.

Discussion

Multiple GISTs can be divided into 4 subtypes: (1) familial multiple GISTs with germline mutations of *KIT* or *PDGFRA* gene (Nishida et al., 1998; Isozaki et al., 2000; Beghini et al., 2001; Hirota et al., 2002; Chompret et al., 2004; Robson et al., 2004; Carballo et al., 2005; Hartmann et al., 2005), (2) multiple GISTs associated with NF-1 without mutations of *KIT* or *PDGFRA* (Kinoshita et al., 2004; Joo et al., 2004; Takazawa et al., 2005; Andersson et al., 2005; Miettinen et al., 2006), (3)

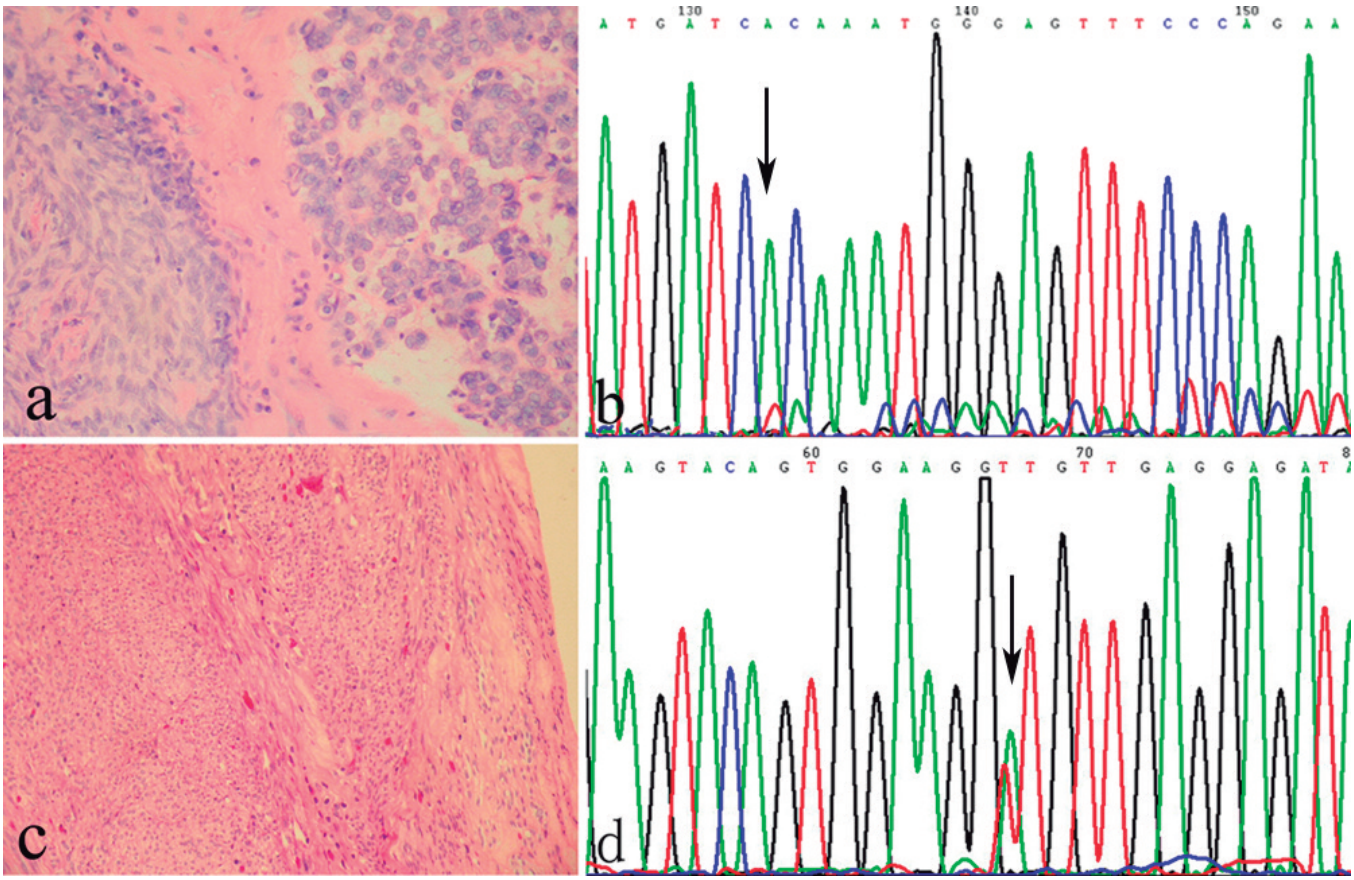

Fig. 4. Metachronous gastric GISTs in patient #14 (Fig. 1f and g). **a.** H&E staining of the first gastric GIST showing mixed cell type in which spindle and epithelioid cells had distinct demarcation. **b.** Sequencing results of exon 11 of KIT gene for the first GIST showing point mutation with ATA to CTA in 571 and 572-579 (GAC CCA ACA CAA CTT CCT TAT GAT) duplication(arrow indicated). **c.** The second GIST was pure spindle cell type. **d.** Sequencing result of exon 11 of KIT gene for the second GIST showing point mutation from 559 GTT to GAT (arrow indicated).

multiple GISTs associated with Carney's triad without mutations of *KIT* and *PDGFRA* (Carney, 1999; Zhang et al., 2010), and (4) sporadic multiple GISTs (Kang et al., 2007; Haller et al., 2007; Gasparotto et al., 2008; Agaimy et al., 2008, 2009). The first 3 subtypes have been investigated extensively but the last subtype was only described recently. From prior reports, most multiple GISTs were diagnosed synchronously, among which there were cases of multiple microscopic lesions less than 10 mm or identified from incidental findings without symptoms. These cases may not be clinically significant (Agaimy et al., 2008; Chetty, 2008). However, some multiple lesions of larger size associated with clinical symptoms might have been mis-diagnosed as metastasis (Gasparotto et al., 2008). Rarely, patients could develop metachronous GISTs and those multiple lesions may be easily mis-diagnosed as recurrence (Agaimy et al., 2009).

In this study, we introduced a series of 15 sporadic multiple GISTs from a cohort of Chinese patients. They were comprised of 2.4% of 624 consecutive GIST cases over 7 years deposited in our GIST registry, excluding 2 multiple GISTs associated with NF1. This frequency is in line with the recently reported rate of 1.1% to 1.6% (Gasparotto et al., 2008), but much lower than the rate of 10% in a separate report by Agaimy, especially in gastric body (Agaimy et al., 2008). These results indicate that sporadic multiple GISTs, especially those incidentally discovered, do not occur frequently, therefore the correct diagnosis requires meticulous inspection (Agaimy et al., 2007) or the use of serially sectioned tissues (Kawanowa et al., 2006). For example, in our series, multiple GIST nodules presented in case #2 (Fig. 1b), #3, and #11 were initially mis-diagnosed as lymph nodes in gross examination for synchronous carcinoma, therefore the reported rate of multiple GISTs in our cases was lower than the actual rate. In addition, multiple lesions of GIST can be found in different sizes from each patient, and the distance between lesions also varies among patients. The size ratio of GISTs in one patient can be as high as 12 (case #7), and tumor masses can be found on both the anterior and posterior walls at operation (case #10). These observations indicate that careful examination is necessary to avoid missing multiple tumors, especially during the gastric surgical procedure, since most multiple GISTs originate in the stomach.

Among the 15 multiple GISTs, 31 were in the stomach and only one in the duodenum, consistant with previous observations that most GISTs arise from the GI tract. Rarely, the nodules can also grow outside of the GI tract and reside in the peritoneum (Gasparotto et al., 2008), but they are not truly metastasis of a primary GIST and in fact are multiple primaries. For example, Gasparotto et al (2008) confirmed that 5 cases of such multifocal GISTs were initially mis-diagnosed as a higher grade disease, several patients were treated unnecessarily with imatinib after surgical resection of the primary GISTs.

We examined the 32 tumors for mutations in 4 exons of *KIT* and 2 exons of *PDGFRA*. Mutations in exon 11 of the *KIT* gene were found in 26 tumor masses, including point mutation and deletion, as well as the combination of point mutation, deletion and duplication. Three previous known mutations mainly involve juxtamembrane domain deletions of codons 557 and 558, and duplication at the 3' terminal. One tumor mass with epithelioid cell type displayed D842V point mutation in exon 18 of *PDGFRA* gene, which has been shown to be the hot spot mutation in GISTs of epithelioid cell type (Medeiros et al., 2004, Wasag et al., 2004). The overall mutation of the study cohort was 84.4%, similar to what has been reported previously in solitary and multiple GIST (Gasparotto et al., 2008; Agaimy et al., 2008, 2009; Wardelmann et al., 2002).

It is interesting that multiple tumor nodules in the same patient can have different genotypes, as shown in 60% (6/15) of our cases (Fig. 3a-c). This phenomenon has also been demonstrated in recent reports. The discordance of genotypes was 100% (4/4) in Haller's (Haller et al., 2007), 90% (10/11) in Agaimy's (Agaimy et al., 2009), and 70% (7/10) in Gasparotto's (2008) reports. The independent genotype is instrumental for the recognition of the polyclonal origin of multiple tumors, especially in peritoneum nodules as indicated by Gasparotto (Gasparotto et al., 2008), and metachronous GISTs by Agaimy et al. (2009). Correct staging of GISTs is the key to personalized therapy in the imatinib era.

For coexistence or metachronous development of multiple tumors, determining whether these tumors are recurrent of a single primary or multiple primaries is helpful to predict the malignancy of the tumors, and select the proper treatment. In our case #14, genetic tests identified different mutations of *KIT* gene in the two tumors developed metachronously, the second tumor was then considered as a new primary tumor rather than the metastasis of the first duodenal GIST. In case #15, the longer lag phase between the two GISTs made it harder to decide whether they were metastasis or multiple primaries. However, the heterogeneity in histopathological, immnological and genotypic features revealed their polyclonal origins (Fig. 4a-d). After extensive discussion in our multidisplicinary team meeting at the hospital, both cases were diagnosed as sporadic primaries based on the molecular biology findings. Due to the malignant nature of the first tumor, to prevent possible tumor recurrence, both patients were recommended to receive imatinib adjuvant therapy after the first surgery, but were rejected by the patients due to financial considerations. Patient #14 then accepted imatinib therapy for a year after the second resection, but patient #15 never did. Although post-surgical adjuvant therapy with imatinib has been shown to dramatically prolong life expectancy (Demetri et al., 2002) and disease-free survival (Dematteo et al., 2009) of patients with malignant GISTs, patient #15 had been doing well at 51 months after the first tumor resection or 8 months after the second resection at his last visit.

In summary, in this study, we reported a series of

sporadic multiple GISTs, both synchronous and metachronous. Most of them could be characterized by heterogeneous *KIT* or *PDGFRA* gene mutations, immunohistochemical spectrum or histopathological pattern, suggesting their polyclonal origins. Although imatinib is generally recommended for recurrent or metastatic GISTs, it is prudent to differentiate metastases or recurrence from real multiple co-existing or metachronous primaries. For less malignant multiple GISTs, surgical resection and post-operational follow up might be sufficient. Therefore, for a targeted treatment, it is critical to make correct diagnosis of sporadic multiple GISTs by meticulous clincopathological, immunohistochemical and genetic evaluations.

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