ORIGINAL ARTICLE

Deletions of Chromosome 1p and 15q are Associated with Aggressiveness of Gastrointestinal Stromal Tumors

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Background/Purpose: Site-dependent profiles of chromosome imbalances (CIs) have been reported in gastrointestinal stromal tumors (GISTs). However, the role of specific CIs in association with metastasis is not clear.

Methods: Thirteen resected liver metastatic GISTs, including seven from the stomach and six from the small intestine, were analyzed using comparative genomic hybridization (CGH). The CIs associated with metastatic risk were assessed by comparing them with those identified in our previous study of 25 primary GISTs, including 14 from the stomach and 11 from the small intestine.

Results: Synchronous detection of liver metastasis was found more often in patients with intestinal than gastric GIST (5/6 *vs.* 2/7, $p = 0.048$). When compared with the primary tumors, the CI profile of liver metastases was similar in the intestinal group, but became more complex in the gastric group. Deletions of chromosomes 1p and 15q were very common (> 80%) in primary and metastatic tumors of the intestinal group, and exhibited a trend towards increase in the metastatic tumors of the gastric group. Both groups had a doubling in the frequency of 22q deletion in the liver metastases, which was not significantly different. Other CIs, including 9p deletion, increased significantly in the liver metastases of the gastric group, but not in the intestinal group.

Conclusion: Our results, together with clinical findings, indicated a CGH profile associated with the intrinsic aggressiveness of the GISTs. Deletion of 1p and 15q play a critical role in the acquisition of aggressiveness during early GIST development. [*J Formos Med Assoc* 2009;108(1):28–37]

Key Words: chromosomal imbalance, comparative genomic hybridization, gastrointestinal stromal tumor, liver metastasis

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. They are believed to arise from interstitial cells of Cajal (i.e. pacemaker cells).[1] The annual incidence of GIST is estimated at 0.68–1.45 per 100,000. The tumors occur typically in older individuals and arise most often in the stomach, followed by the small intestine, colon, rectum and esophagus.[2,3] Central to the tumorigenesis of GISTs are active mutations in the proto-oncogene *tyrosine-protein kinase* (*KIT*) or *platelet-derived growth factor receptor alpha* (*PDGFRA*) gene, with a detection rate of 60–80% and 3–7%, respectively.[4,5] These mutations have

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been identified as alternative and mutually exclusive genetic events in GIST development, which target seemingly similar downstream pathways.[6,7]

Surgery is the mainstay of treatment, but even after adequate resection, the vast majority of GISTs recur, and in approximately 50% of cases, the liver is the main site of metastasis.[8,9] A small number of patients survive intra-abdominal metastasis for up to 20 years. However, patients with tumors that have metastasized at presentation have a very poor prognosis.[10] The recurrent or advanced tumors are resistant to radiotherapy and chemotherapy, and have had an extremely poor prognosis in the past.[11] Recently, the introduction of imatinib mesylate (trade names Glivec, Gleevec; Novartis, Basel, Switzerland) has greatly altered the clinical course of patients with advanced GIST.[12,13] Its effectiveness depends on the mutational status of *KIT* and *PDGFRA*. Approximately 80% of these patients have a partial response or stable disease. However, acquired resistance is a further clinical challenge, and can develop in half of the patients who initially benefit from the drug.[14,15]

The criteria delineating benign from malignant tumors have not been established reliably. Tumor size, mitotic index, and anatomic site are often used to predict disease-specific survival in patients with primary disease who undergo complete gross resection.[16,17] However, a small subset of small and/or mitotically inactive tumors does metastasize subsequently.[2,18] Many other prognostic markers have also been reported, with variable significance, including patterns of *KIT* and *PDGFRA* mutations.[7] Apparently, the lack of a reliable method of prognostication hampers the selection of patients eligible for imatinib mesylate therapy, a critical step for avoiding waste of resources and possible lack of responsiveness, particularly with the progress of adjuvant and neoadjuvant clinical trials.

Although *KIT* and *PDGFRA* mutations play a fundamental role in early GIST carcinogenesis, other molecular mechanisms appear to be necessary in tumor progression. Cytogenetic alterations have been proposed to act as a complementary mechanism of GIST development, with accumulation of chromosomal imbalances (CIs) in conjunction with disease progression.[19–31] Some CIs have been suggested to play a prognostic role in this disease, but have revealed a conflicting significance among different studies. For example, some authors have linked losses at 1p and 22q to malignant behavior in GISTs,[21,28–31] whereas others have not identified these aberrations as carrying prognostic value.[22,26]

Although gastric and intestinal GISTs are cytogenetically related, recent studies have revealed consistently that there are substantial site-dependent, genetic differences.[20–23] Evaluation of the prognostic role of the CIs in GISTs thus needs to be examined on a site-specific basis. Moreover, because of the heterogeneous nature of malignant tumors, the CIs of a minor but aggressive tumor component that leads to metastasis can often be overlooked in the analysis of primary tumors using comparative genomic hybridization (CGH). To avoid this pitfall, we performed CGH of 13 cases of surgically resected liver metastatic GISTs, including seven derived from the stomach and six from the small intestine. Assessment of the CIs closely associated with metastatic risk was made by comparing them with the CGH findings of the 25 primary GISTs reported in our previous study.[23]

Methods

Sample selection

In the pathological files at Chi Mei Medical Center, most cases of metastatic GIST to the liver were confirmed pathologically through needle biopsy. The DNA isolated from serial microtome sections of small paraffin-embedded tissue samples theoretically contains a high proportion of truncated nuclei, which may create a certain bias in the CGH analysis. To avoid such a potential pitfall, we collaborated with two other hospitals in Taiwan to collect cases of surgically resected liver metastatic GIST. A total of 13 cases were found consecutively in the archive files of these

hospitals between 1992 and 2004. All tumors included in this study were immunohistochemically positive for CD117. No patients had received chemotherapy or radiotherapy prior to the surgical resection.

Under the guide of the hematoxylin and eosin stained section, the tumor tissue in the paraffin block was selected for DNA preparation. The selected tissue was deparaffinized by treating it twice with xylene at 55°C for 15 minutes each time, followed by washes with absolute ethanol and air-drying. The tissue was then incubated in proteinase K solution (Sigma, St Louis, MO, USA) with 0.5 mg/mL in 10 mM Tris, pH 7.8, 5 mM EDTA, and 0.5% SDS, at 55°C overnight or longer, if needed. DNA in suspension was purified using a phenol/chloroform procedure, and resuspended in 1 × TE buffer.

CGH

The CGH procedure was modified from that described by Kallioniemi et al,[32] and is described in detail elsewhere.[23] Briefly, the metaphase slides from normal females were kept in 95% ethanol at −20°C for at least 48 hours before processing for CGH. DNA from a tumor and genomic DNA from an individual with normal karyotype (reference DNA) were directly labeled with fluorescein-12-dUTP or Texas red-5-dUTP (NEN Life Science, Boston, MA, USA), respectively, using the standard nick-translation procedure. After precipitating the DNA in the presence of Cot1 DNA (Gibco BRL, Gaithersburg, MD, USA), the labeled DNA mixture was hybridized to metaphase spreads on a glass slide for 2–3 days. The slides were washed and then counterstained with 4,6-diamidino-2-phenylindole in an anti-fading solution.

Image acquisition, processing, and evaluation were performed using a fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a Sensys charge-coupled device camera (Kodak KAF 1400 chip; Photometrics, Tucson, AZ, USA), which was controlled using the CytoVision imaging system (Applied Imaging, Santa Clara, CA, USA). CIs were determined based on the calculation of standard reference intervals using CytoVision High-Resolution CGH software, by which we stringently defined DNA losses or gains as significant whenever the tumor profile and the standard reference interval profile at 95% confidence did not overlap.[33] Short chromosomal segments with a test-to-reference fluorescence ratio >1.5 were accepted as having high-level amplification. In each experiment, a negative and positive control with a known chromosomal gain or loss was also included. The negative control DNA was isolated from an individual with a normal karyotype. The positive control DNAs were prepared from Epstein–Barr-virus-transformed lymphoblastoid cell lines with either trisomy 21 (~50 Mb) or an interstitial deletion of 2q23 (~15 Mb).

For mathematical analyses, CIs were expressed as losses, gains or high-level amplifications per chromosomal arm. Assessment of the CIs with high risk of metastasis was made by comparing them with the CGH findings of the 25 primary GISTs reported in our previous study.[23] In the comparison between groups, we used Fisher's exact two-tailed test, the χ^2 test, or the Mann–Whitney *U* test. A value of $p < 0.05$ was considered statistically significant.

Results

As summarized in Table 1, the liver metastatic GISTs were derived from the stomach of seven patients (two female, five male), and from the small intestine of six patients (two female, four male). Patients' age at the diagnosis of primary GIST ranged from 38 to 70 years (female, 59–68 years; male, 38–70 years), with a mean of 58.8 years. Notably, synchronous detection of liver metastasis at the initial diagnosis of the primary GIST was more common in the cases of intestinal origin (5/6, 83.3%) than in those of gastric origin (2/7, 28.6%) ($p = 0.048$). The CGH results disclosed that all liver metastases had a variable number of CIs, involving 2–35 chromosomal arms, as described in detail in Table 1. A detailed description of the CIs of the primary gastric

Table 1. CIs detected in 13 liver metastatic GISTs using comparative genomic hybridization

Case	Sex	Age at Pri	Dx of Mets*	CIs
From stomach				
1	M	38	38	+1q, +2pter-q12, +2q21-qter, −3p13-p24, −3q12-q24, +5 (5q21-q23), −6p24-q25, −9, +12, −14q11-q31, +15, −16q11-q23, +17, +19, +20p, +21
2	F	59	59	−1p31-p36.1, +1q42-qter, −2q21, +3p12, −10p12-p13, 10q21-q22, 15q13-q24, +18q23
3	M	54	55 (1)	−1p33-pter, +1p13-p21, +7q, +8, −9pter-q33, −10p13-q25, −11p15-q13, −11q21-q24, −13, −14, −15, +16p12-p13.2, +20q, +21, −22
4	M	69	70 (1)	−9p13-p23, +11p12-pter (11p15), −15q12-q15, −16q12-q22, +17q21-qter (17q24-q25), −22
5	F	59	61 (2)	−1p, +1q, +2p, +2q33-qter, +3p21, +3p24, +3q21-q23, +3q27-qter, +4p16, +5p13.3, −5q, +6p21-p22, −6q11-q21, +6q24-qter, +7, +8p, +8q21, −9p21-pter, +9q22-qter (9q34), −10, +11p15, +11p11.2-q13, +11q23-qter, +12p13, +12q11-q13, +12q23-qter, +13q32-qter, −14q12-q31, −15q11-q25, +16, +17, +18p11.2, +18q23, +19, +20, +21q22, −22q12
6	M	70	73 (3)	−1p21-pter, +3q27-qter, +4p14-pter, −9p13-p23, +12q24.3, −14q11-q31, +17q24-q25, −20p11.2-12, +21, −22
7	M	51	58 (7)	−1p, +1q21-q24, +1q31-qter, +3p21, +5q31, −4p14-pter, −6p, −7q32-qter, +9q34, +10p11.2, +10q26, +11p11-q13, +11q23, +12p, −12q15-q24.2, −13, −14q21-q22, +16p11.2, +16q21-qter, +17p12-q21, +17q24-qter, +19p (19p13.2-13.3), −19q12, +20, +21, −22
From small intestine				
8	M	48	48	−1p, +1q, −2p12-pter, +2q21-q36, +3p12, +3q24-qter, +5p, −7q11.2, 9, +7q31, +8q, +11p11.2-q13, +11q24-qter, −12p13, −13, +12q24.3, −15q11-q25, +16q11.2, +17q11.2, −18q11.2-q21, +18q22-qter, +19q, +20, −22q13
9	M	54	54	−14q11.2-q24, −15q11.2-q25
10	F	60	60	−1p13-p33, +2q23, +3q26-qter, +6p21-p23, −6q, +7p22, +8p12-p21, +8q242-qter, +9q34, +10p13-pter, +11p15, +11q12-q13, −12p13, +12p11.2-q13, +12q24.1-qter, +14q31-q32, −15q11.2-q25, +16p13.1-pter, +16q21-qter, +17, −18q11.2-q22, +19, −21q21
11	M	65	65	−1p, +1q31, +3p12, +4p, +5p12-p13, −6, +7q, +12q11.2-q23, −13, −14, −15q14-qter, +16p11.2, −18q, +20, −21, −22
12	F	68	68	−1p31-pter, −6q21-q22, −15q15-q25, −18q21, −22
13	M	69	73 (4)	−1p31-p34.2, +3p12, +4p12-p14, +4q21-q31.1, +5p11-p14, −9q21-q22, +10p11.2, −11q13-q22, −15q14-q25, +16p11.2, +21q22

**Years between diagnosis of primary and metastatic GISTs in parentheses. Pri = primary GIST; Dx = diagnosis; Mets = metastatic GIST.*

($n = 14$) and intestinal ($n = 11$) GISTs reported in our previous study[23] can be found in the Progenetix database at http://130.60.44.174/progenetix/P14730211/.

For the gastric group, the liver metastatic tumors ($n = 7$) had CIs that involved 6–39 chromosomal arms, with gains more prevalent than deletions. The average number of chromosomal arms with gain and deletions were 11.1 and 7.3, respectively. However, the primary tumors ($n = 14$) reported in our previous study[23] had more deletions than gains, with gains and deletions involving an

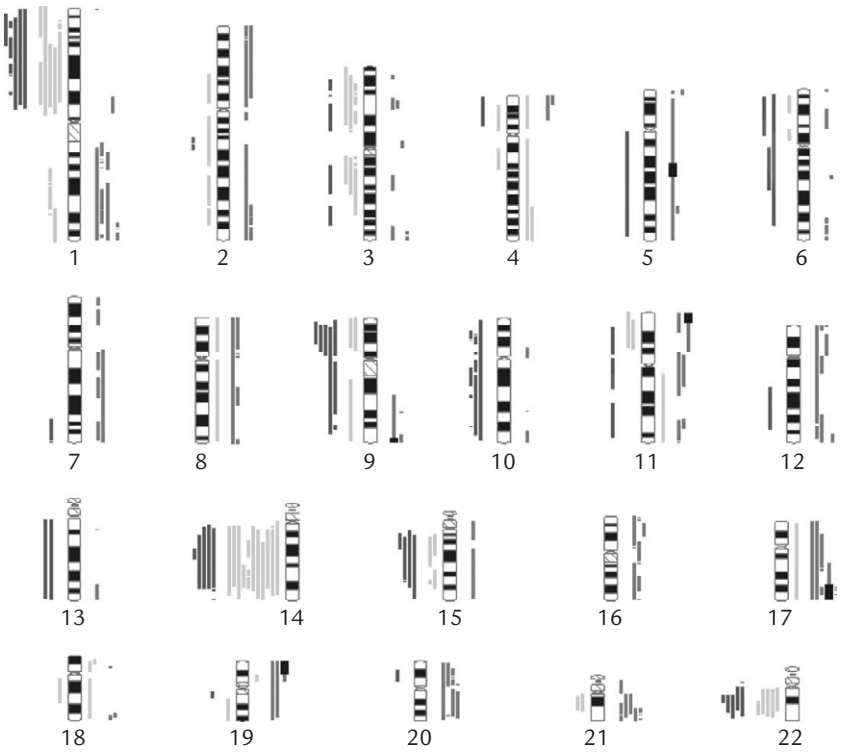

Figure 1. CGH profile of gastric GISTs, including 14 primary tumors (gray bars) and seven liver metastases (dark bars). Each bar represents one tumor, with gains on the right side and losses on the left side of the ideogram of each chromosome. The broad dark boxes represent high-level amplification.

average of 0.9 and 3.1 chromosomal arms, respectively. The CIs detected in primary and liver metastatic tumors are compared in Figure 1. High-level amplification was not found in any of the 14 primary tumors, but was detected in five different sites in four samples of liver metastasis (as underlined in Table 1 and indicated by broad dark boxes in Figure 1).

With respect to the intestinal group, the liver metastatic tumors ($n = 6$) had CIs that involved 2–26 chromosomal arms, with gains slightly more common than deletions. The average number of chromosomal arms with gains and deletions were 8.3 and 6.3, respectively. Similarly, for the primary tumors ($n = 11$) reported in our previous study,[23] gains were more common than deletions, with gains and deletions involving an average of 6.5 and 5.3 chromosomal arms, respectively. The CIs detected in both primary and liver metastatic tumors are compared in Figure 2. No sample of primary or metastatic tumor showed a chromosomal region with high-level amplification.

Compared with the primary GISTs, the cumulative CI profile of the liver metastases was very similar to that of the intestinal group but not the gastric group (Figure 3). As indicated by the dotted rectangle, 9p deletion seemed to be particularly more prevalent in the metastatic tumors than in the primary intestinal GISTs. However, from the detailed comparison of each group of primary and metastatic GISTs (Table 2), it was evident that 9p deletion was significantly more prevalent in the metastatic tumor of the gastric group than the intestinal group. Deletion of 14q was the most common alteration in the primary gastric GISTs, but did not become more prevalent in the metastatic lesions of either group. Deletion of 22q, the second most common CI in the primary gastric GISTs, doubled in frequency in the liver metastases of both groups, with no significant difference. Deletions of 1p and 15q were very common (> 80%) in both primary and metastatic tumors of the intestinal group, and also exhibited a trend towards increase in the metastatic tumors of the gastric group. Most of the other common CIs listed in Table 2 increased significantly in the liver metastases of the gastric group but not the intestinal group.

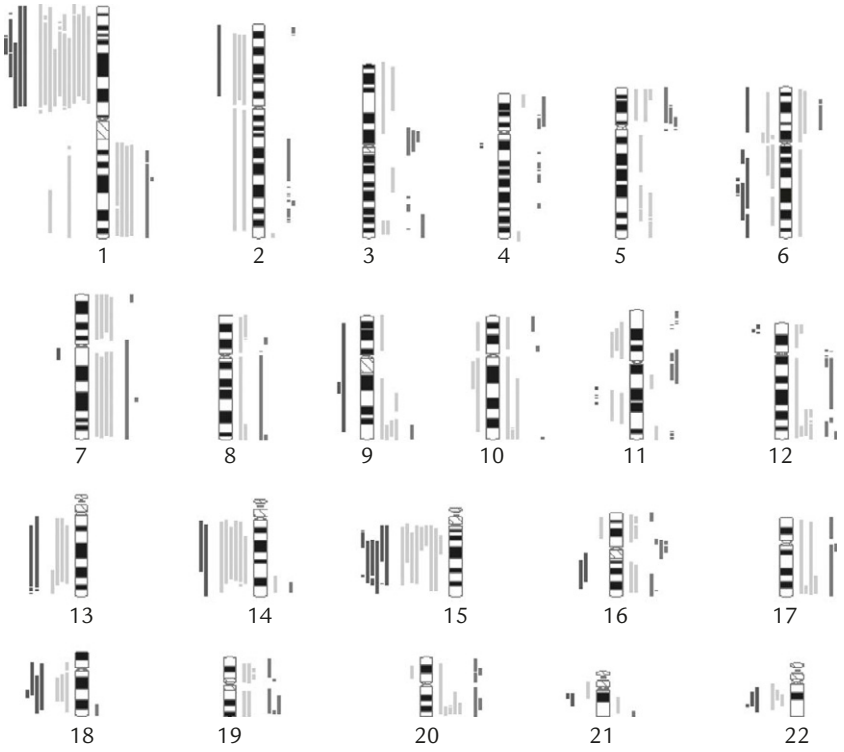

Figure 2. CGH profile of intestinal GISTs, including 11 primary tumors (gray bars) and six liver metastases (dark bars). Each bar represents one tumor, with gains on the right side and losses on the left side of the ideogram of each chromosome.

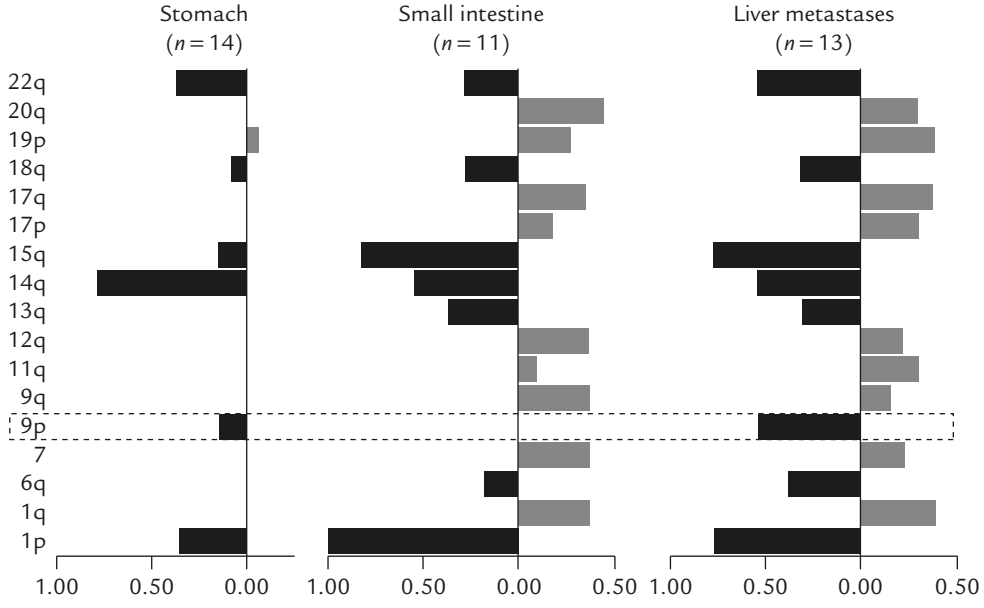

Figure 3. Comparison of the CGH profile of primary GISTs (including 14 from the stomach and 11 from the small intestine) and liver metastases. The dark bars on the left side of each group indicate the relative frequency of deletion of the corresponding chromosome arm, and the gray bars on the right side are frequencies of chromosomal gains.

Discussion

The development and progression of cancer is believed to involve a multistep genetic process, with changes both at the molecular and cytogenetic level. While primary changes are important for cellular transformation and tumor initiation, secondary non-random changes accumulate at later stages and are responsible for biological tumor progression and dissemination.[34] For GISTs, mutually exclusive gain-of-function *KIT* or *PDGFRA* mutations have been identified as

Table 2. Comparison of the frequencies of common CIs between primary and liver metastatic GISTs of gastric and intestinal origin*

	Stomach			Small intestine		
	Primary ($n=14$)	Liver metastasis ($n=7$)	p	Primary ($n=11$)	Liver metastasis ($n=6$)	p
Gains						
1q42-q44	0	4 (57)	0.002	4 (36)	1 (17)	NS
12q24.3	0	3 (43)	0.008	4 (36)	2 (33)	NS
17p11.2-p12	1 (7)	3 (43)	MS	2 (18)	1 (17)	NS
17q24-q25	1 (7)	5 (71)	0.002	4 (36)	1 (17)	NS
19p13	1 (7)	3 (43)	MS	3 (27)	1 (17)	NS
20p12	0	3 (43)	0.008	1 (9)	2 (33)	NS
20q13.1	0	3 (43)	0.008	5 (45)	2 (33)	NS
21q22	0	5 (71)	<0.001	1 (9)	1 (17)	NS
Deletions						
1p32-p34	5 (36)	5 (71)	NS	11 (100)	5 (83)	NS
9p21-p23	2 (14)	5 (71)	0.009	0	1 (17)	NS
10p12-p13	0	3 (43)	0.008	1 (9)	0	NS
10q21-q22	0	3 (43)	0.008	2 (18)	0	NS
13q	0	2 (29)	MS	4 (36)	2 (33)	NS
14q	11 (78)	5 (71)	NS	6 (55)	2 (33)	NS
15q	2 (14)	4 (57)	MS	9 (82)	6 (100)	NS
22q	5 (36)	5 (71)	NS	3 (27)	3 (50)	NS

**Data presented as n (%). NS = no statistical significance; MS = marginal statistical significance, with p ranging from 0.035 to 0.049 (Fisher's exact test).*

primary steps in tumorigenesis. Rare variant *KIT* or *PDGFRA* mutations have also been found in association with anatomic site and distinct clinical phenotype in selected GIST subsets.[7] However, the mutational status alone cannot fully explain the diverse and apparently site-dependent biology of these tumors. Other non-random genetic changes identified at the cytogenetic level may eventually complete the picture.

Gunawan et al[22] and the authors of the present study[23] have described a site-dependent heterogeneous CI profile in GISTs, which has been confirmed in a recent CGH analysis of 203 samples.[20] Overall, deletion of 14q was more frequent in gastric GISTs, while deletions of 1p, 6q, 13q, 15q and 22q and gain of 5p occurred more often in intestinal tumors. Such site-dependent heterogeneity of the CGH pattern does not relate to tumor genotypes of *KIT* and *PDGFRA* genes.[21] In the present analysis of 13 cases, we noticed that liver metastases present at the initial diagnosis of the primary tumor was more common for the intestinal group (83.3%, 5/6) than for the gastric group (28.6%, 2/7) ($p=0.048$). Moreover, as depicted in Figure 3, we noticed that the CI profiles between primary intestinal and liver metastatic tumors were very similar. Such an association suggests that the CIs that are particularly prevalent in the intestinal GISTs might be closely associated with the intrinsic aggressive characteristics of the intestinal GISTs.[7,17]

As shown in Table 2, the comparative results disclosed that deletions 1p and 15q were very common in primary and metastatic GISTs of intestinal origin, with detection frequencies ranging from 82% to 100%. For the gastric group, these alterations also showed a similar trend towards an increase in the metastatic tumors. The lack of statistical significance for the gastric group was likely the result of the small sample size in our study. However, when combined with the CGH findings of another large study of primary gastric GISTs ($n=116$) reported by Gunawan et al,[20] we noticed that these two alterations were

significantly more prevalent in the liver metastases than in the primary tumors of gastric origin, with $p = 0.003$ for 1p deletion (5/7 *vs.* 25/116) and $p < 0.001$ for 15q deletion (4/7 *vs.* 9/116). These findings indicate that deletions of 1p and 15q play an important role in the acquisition of aggressiveness in the early stage of tumorigenesis of intestinal GISTs. To the best of our knowledge, the genes residing on 1p and 15q have not yet been fully elucidated as potential candidates. An interesting tumor-associated gene that maps to 1p36 is ENO1, also known as MYC promoter-binding protein 1. The binding of ENO1 represses MYC expression and prevents the stimulation of cell proliferation.[35]

In the GISTs of gastric origin, the most common CI is 14q deletion, which showed a similar frequency in the primary tumors (78%) and liver metastases (71%). However, as depicted in Table 2, the alteration was present in only 55% (6/11) and 33% (2/6), respectively, of the primary and metastatic GISTs of intestinal origin. Hence, it is unlikely to be associated with metastatic risk of this disease. The next most prevalent CIs of primary gastric GISTs are deletions of 1p (36%), which had doubled to 71% in liver metastases. Compared with tumors of intestinal origin, in contrast to 1p deletion with high prevalence, 22q deletion also doubled from 27% to 50% in the liver metastases at a relatively low rate. These findings seemingly imply that 22q deletion also plays a role, with less critical significance, in the acquisition of aggressiveness by GISTs. This alteration has been reported in association with high-risk GISTs previously, including by our own group,[20,23,28,30] but was not seen in another previous study.[26] Recently, Gunawan et al,[20] based on CGH analysis and long-term follow-up, proposed three major cytogenetic pathways in GISTs, one initiated by 14q deletion, one by 1p deletion, and another by 22q deletion. They indicated that 22q deletion appears to initiate the critical transition to an unfavorable cytogenetic sub-pathway, by accumulating gain of 8q and deletions of 9p and 9q. However, in the current study of 13 liver metastases, eight samples had 22q deletion, including five and three of gastric and intestinal origin, respectively. Only three samples had gain of 8q and/or deletions of 9p and 9q.

Another alteration worthy of note is 9p deletion, which is one of the CIs commonly reported in association with high-risk GISTs.[26,30,36,37] In the study of El-Rifai et al[26] of clinically malignant GISTs, the authors found that three of five liver metastatic GISTs (of unknown primary origin) had 9p deletion. In a microsatellite analysis of GISTs, Sabah et al[37] demonstrated that the loss of heterozygosity at 9p21 was a common finding in high-risk (malignant or recurrent) tumors, but was absent in those of low malignant potential. However, as shown in Table 2, we found that the association between 9p deletion and liver metastasis is significant only in the gastric group but not in the intestinal group. Similarly, Schneider-Stock et al[38] found that a high predictive value for p16INK4 (mapped to 9p21) alterations is only significant in the group of benign and borderline GISTs with regard to the clinical outcome. Recently, in another much larger series of 284 primary GISTs,[39] they further pointed out that loss of p16INK4 seemed to identify a subgroup of gastric GISTs with a worse prognosis ($p = 0.037$), whereas it had no additional value for predicting prognosis in intestinal GISTs.

As depicted in Table 2, some other CIs, including deletions of 10p, 10q and 13q and gains of 1q, 12q, 17p + q, 19p, 20p + q and 21, were significantly more prevalent in liver metastases than in primary tumors of gastric origin. Some of these, including 10q deletion and gains of 17q and 20q, have been found more frequently in high-risk and/or clinically malignant GISTs in previous studies.[23,26] However, we did not discern a trend towards similar increases of these CIs in the liver metastases that arose from intestinal GISTs. Therefore, it indicates that a role for these alterations for the acquisition of aggressiveness during the early stage of GIST progression is unlikely to be as critical as that of deletions of 1p, 15q, 22q and 9p.

In conclusion, among the common CIs, deletions of 1q and 15q play an important role in

the acquisition of aggressiveness during the early stage of GIST development. Deletion of 22q plays a similar role with less critical significance. Finally, 9p deletion is significant for the gastric GISTs, but not in those arising from the small intestine.

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