Heterozygosity loss at 22q and lack of INI1 gene mutation in gastrointestinal stromal tumor

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Abstract

Objectives: Gastrointestinal stromal tumor (GIST) is characterized by *KIT* or *PDGFRA* gene mutation. Although chromosomal losses of 22q are frequent in GIST, it is unclear which tumor suppressor genes might be inactivated in association with such losses. The *INI1* gene, located at 22q11.23, is a tumor suppressor gene that is frequently altered in malignant rhabdoid tumor.

Methods: To elucidate the hypothesis that the *INI1* gene might be altered along with 22q loss in GIST, we examined the loss of heterozygosity (LOH) at 22q11.23, homozygous deletion and mutation of the *INI1* gene, and its gene product expression as well as mutations of *KIT* and *PDGFRA* in 27 cases of GIST.

Results: Among the 27 informative cases, 19 (70.4%) showed LOH of at least one of the microsatellite markers on 22q11.23. None of the cases (0%) showed homozygous deletion or mutation of the *INI1* gene. Immunohistochemically, the INI1 expression was focally reduced in 17/27 (63%) cases, and the INI1 protein level and INI1 mRNA level were each correlated with the presence of 22q11.23 LOH. Although the 22q11.23 LOH was more frequently present in high grade than in low grade tumors, INI1 expression

level was not correlated with tumor grade, tumor size, proliferative activity and the expression levels of cyclin D1 and p16INK4a. *KIT* mutations were found in 18/27 (66.7%) GISTs; however, the *KIT* genotype was not correlated with the status of LOH at 22q11.23.

Conclusions: The results suggest that 22q11.23 LOH is frequently present in GIST irrespective of *KIT* genotype and it might play a role in part of the development of GIST. However, the hemiallelic loss of *INI1* gene causing reduced expression of INI1 protein probably does not have a major impact in the progression of GIST.

Introduction

Gastrointestinal stromal tumor (GIST) is a KIT-positive mesenchymal tumor with a wide spectrum of biological behavior [1]. GISTs demonstrate a gain-of-function mutation of the *KIT* gene or of the *platelet-derived growth factor receptor alpha (PDGFRA)* gene [2-5].

Several studies have revealed the common losses on the chromosome arms 1p, 9p, 14q, and 22q in GIST by using cytogenetic (G-band karyotyping), loss of heterozygosity (LOH), comparative genomic hybridization (CGH), and fluorescence *in situ* hybridization (FISH) analyses [6-12]. There is a possibility that these chromosomal abnormalities are associated with tumorigenesis and stepwise progression of GIST. Several studies have reported that the loss of 9p is associated with malignant behavior of GIST [8,10], and the inactivation of the *p16INK4a* gene located at 9p21 is correlated with higher cell proliferation rate and more aggressive behavior of GIST [13-15]. These data strongly suggest that p16INK4a is a tumor suppressor gene in association with chromosome 9p loss in GIST. Although the allelic losses of 14q and 22q are found in GISTs from low to high-grade, LOH at 22q is more frequently present in high grade than

in low grade GIST, suggesting that LOH at 22q may play a role in earlier step of tumor formation as well as in later progression of GIST [11,12]. However, the tumor suppressor genes linked with chromosomal alterations of 14q and 22q remain unknown.

The *INI1* (also known as *hSNF5* or *SMARCB1)* gene is located at 22q11.23, and its gene product is a member of the ATP-dependent SWI/SNF chromatin-remodeling complex, which regulates chromatin remodeling [16]. In addition, the INI1 protein negatively regulates the G1-S cell cycle via the RB pathway, including upregulation of p16INK4a transcription and downregulation of cyclin D1 [17]. The biallelic inactivation of the *INI1* gene due to homozygous deletion or mutation has been reported in malignant rhabdoid tumor (MRT), a high-grade pediatric sarcoma [18-21]. Given the frequent chromosome 22q loss in GIST, these data encouraged us to elucidate that the *INI1* gene might be altered in GIST.

Materials and Methods

Case materials

A total 27 cases of primary GIST were obtained from the file of Anatomic Pathology, Kyushu University. None of the cases were treated with imatinib prior to the initial surgical operation. All 27 cases are KIT-positive GIST. Each GIST was evaluated for clinicopathologic and histologic features, including tumor size and number of mitoses. Mitoses were counted and summed from 50 high-power fields (HPFs). The grade of each tumor was based on tumor size and mitotic counts, according to the 2001 consensus approach of the National Institutes of Health [1].

Immunohistochemistry for INI1, cyclin D1 and p16INK4a

Immunohistochemical staining was performed on the formaline-fixed and paraffin-embedded specimens with the primary antibodies against Ki-67 (MIB-1, dilution: 1/100, Dako Cytomation, Carpinteria, CA, USA), INI1 gene product BAF47 (clone 25, dilution: 1/200, BD Transduction Laboratories, San Diego, CA, USA), cyclin D1 (P2D1F11, dilution: 1/25, Novocastra, Newcastle upon Tyne, UK) and p16INK4a (F12, dilution: 1/50, Santa Cruze Biochemistry, Santa Cruz, CA, USA).

For all antibodies listed above, the proportion of tumor cells with nuclear immunoreactivity was evaluated. As for INI1, reduced nuclear immunoreactivity was considered as abnormal expression (see Result). For cyclin D1, staining of more than 10% of tumor cells was judged as increased expression, as previously reported [22]. For p16INK4a, if less than 20% of tumor cells were stained, the case was considered as decreased expression [13].

Homozygous deletion analysis of *INI1* gene

Homozygous deletion of the *INI1* gene was examined in 27 cases of GISTs, according to our previous study [21]. In brief, genomic DNA was extracted from snap-frozen samples by using standard proteinase K digestion and phenol/chloroform extraction. For a control, we used the MRT cell lines, TM87-16 and TTC549, which are known to have the homozygous deletion of *INI1* exons 1-9. A quantitative real-time polymerase chain reaction (PCR) assay was performed with the primers that specifically amplify exons 1-9 of the *INI1*gene (GenBank accession nos. Y17118-126) and *GAPDH* (GenBank accession no. AY340484) as reference genes. Using the ABI Prism 7700 Sequence

Detection System (PE Applied Biosystems, Foster City, CA, USA), PCR was performed in a total volume of 50 μl in each well, containing 25 μl of SYBR Green PCR Master Mix (PE Applied Biosystems), 50 ng of genomic DNA, and 0.35 μl of 10 μM specific forward and reverse primers. By using ABI Prism 7700 Sequence Detection Software, the gene dosage ratio was calculated as: (average copy number of *INI1* gene)/(average copy number of *GAPDH* gene). Homozygous deletion was judged to have occurred when a sample had a gene dosage ratio of less than or equal to 0.36 in a clinical sample of GIST. This allows for the presence of up to 30% contaminating non-tumor tissue DNA in the template [23]. In MRT cell lines, the gene dosage ratio was less than or equal to 0.001 because there were no contaminating non-tumor cells.

Mutation analysis of *INI1* gene

Mutations in exons 1–9 of the *INI1* gene were examined in 27 cases of GIST, according to the previously described PCR and direct sequencing methods [21]. PCR products for individual exons were analyzed by direct sequencing using the ABI Prism 310 sequence analyzer (PE Applied Biosystems).

LOH analysis of *INI1* gene

LOH was examined by PCR using microsatellite markers on chromosome arms 22q11.23 (D22S303, D22S257, D22S301, D22S345, TOP1P2) in 27 cases of GISTs available for both tumor and non-tumor tissues. The method was essentially the same as our previous study of LOH analysis [24]. In brief, the 5' PCR primers were labeled with 6-carboxyfluorescein (6-FAM). Size markers were labeled with 6-carboxy-X-rhodamine (ROX). PCR of the microsatellite markers was amplified for 35 cycles of denaturing at 95℃ for 1 minute, annealing at 53℃ for 1 minute, and extension at 72℃ for 1 minute. The 6-FAM-labeled PCR products admixed with ROX-labeled size markers were electrophoresed using ABI PRISM 310 (PE Applied Biosystems), and then the data were analyzed by GENESCAN software (PE Applied Biosystems). Allelic ratios were calculated by determining the loss of intensity for each tumor allele compared with that of the normal alleles. In accordance with the manufacturer's protocol, allelic ratios below 0.5 were recognized as LOH.

Quantitative real-time RT-PCR for *INI1* mRNA expression

The *INI1* gene mRNA expression level was examined by TaqMan assay in 21 cases of GIST. Total RNA was extracted with 1 ml of TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) from snap-frozen tumor samples, and reverse transcription was performed with Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Quantitative PCR was performed by using the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems) and the predeveloped TaqMan assay reagents of *INI1* (Hs00268260-m1) and *GAPDH* (Hs99999905-m1) as endogenous controls. Standard curves were generated using serial dilutions of the cDNA samples of the non-tumor tissue of the gastrointestinal wall. The data obtained for INI1 were standardized by GAPDH, and the ratio of INI1 mRNA to GAPDH mRNA was used as a relative measure of the *INI1* mRNA expression level in each GIST specimen. The MRT cell lines (TM87-16 and TTC549) having homozygous deletion of entire exons of *INI1* were analyzed together as a control.

***KIT* and *PDGFRA* gene mutation analysis**

Mutations in exon 9, 11, 13 and 17 of the *KIT* gene and those in exon 12 and 18 of the *PDGFRA* gene were examined as previously reported [5].

Statistical analysis

The correlations among clinicopathological factors, *KIT/PDGFRA* genotype, INI1 protein level, and the presence of 22q11.23 LOH were analyzed by the chi-squared test. The *INI1* mRNA level according to the INI1 protein level or the presence of 22q11.23 LOH was compared by Mann-Whitney *U*-test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Clinicopathological findings

The clinicopathological findings in GIST are summarized in **Table 1**. The 27 patients included 18 men and 9 women, ranging in age from 38 to 93 years (median: 66 years). The tumors were located in the stomach (n=16), small intestine (n=8), esophagus (n=1), large intestine (n=1), and retroperitoneal soft tissue (n=1). The tumors ranged from 3.5 to 18 cm in size (median: 6.5cm). Mitotic counts varied from 0 to 121 per 50 HPFs (median: 5/50HPFs), and the Ki-67 labeling index varied from 0.3 to 21.2% (median: 5.1%). According to the NIH consensus grading system based on mitotic counts and tumor size, the 27 cases were classified as low (n=6), intermediate (n=6), and high (n=15) grade.

Immunohistochemical staining for INI1, cyclin D1 and p16 proteins

By immunohistochemical staining, most of non-neoplastic cells such as vascular endothelial cells, stromal fibroblastic cells and epithelial cells showed nuclear expression of INI1. Almost all tumor cells showed diffuse and strong nuclear expression of INI1 in 10 of 27 (37%) cases of GISTs. In the remaining 17 cases (63%), the INI1 expression

was focally reduced, showing the mosaic expression pattern of mixed positive and negative nuclei (**Figure 1**). The complete loss of INI1 expression, which was the characteristic staining pattern in malignant rhabdoid tumor, was not observed in any cases of GISTs.

As for cyclin D1, tumor cells showed increased (>10%) nuclear expression in 11/27 (41%) cases. Eight cases (30%) revealed decreased (<20%) nuclear expression of p16INK4a.

Molecular analysis of *INI1* gene

Homozygous deletion and gene mutation of *INI1* were examined in 27 cases of GIST. Only one case (3.7%) showed a TCG(Ser)-to-TCA(Ser) silent mutation at codon 249 of exon 7, resulting in no corresponding amino acid change. None of the 27 cases were considered as homozygous deletions.

As for LOH analysis, among the 27 informative GIST cases, 19 (70.4%) showed LOH with at least 1 of the 5 microsatellite markers on 22q11.23 (**Table 2**).

The mRNA expression of *INI1* was examined in 21 cases of GIST. With respect to the

INI1 mRNA expression index, the median (range) was found to be 5.20 (1.54-20.22). No mRNA expression was observed in MRT cell lines, TM87-16 and TTC549, both of which harbored homozygous deletion of *INI1* exons 1-9.

KIT and PDGFRA gene mutation

Of 27 cases of GIST analyzed, *KIT* gene mutation was found in 18 cases (66.7%), including 17 cases with exon 11 mutation and 1 case with exon 9 mutation (**Table 1**). No *PDGFRA* gene mutation at exon 12 or 18 was observed in any cases. Thus, 9 cases are wild type for *KIT* and *PDGFRA* gene.

Statistical analysis

Tables 3 and 4 summarize the correlations among the presence of 22q11 LOH, INI1 protein expression level, KIT genotype and other clinicopathological parameters. The presence of 22q11 LOH was correlated with decreased expression of INI1 protein ($p=0.008$) and a higher risk grade ($p=0.038$) (**Table 3**). The presence of 22q11 LOH tended to be correlated with greater numbers of mitoses and higher Ki-67 labeling

indexes (p=0.070, 0.070, respectively), but these differences did not reach the statistical significance. In addition, the decreased INI1 protein expression also tended to be correlated with higher mitoses and a higher Ki-67 labeling index (p=0.081, 0.081, respectively), but these differences did not reach the statistical significance (**Table 4**). The primary site, tumor size and KIT genotype were not each correlated with LOH status or with INI1 protein level.

We further checked the possible correlation between INI1 alteration (LOH or protein loss) and cyclin D1 or p16INK4a. The presence of LOH at 22q11 was not correlated with the protein expression levels of cyclin D1 and p16INK4a (**Table 3**). The decrease of INI1 protein expression tended to be correlated with lower level of p16INK4a expression (p=0.087), but it was not correlated with cyclin D1 expression level (**Table 4)**.

We then compared the mRNA levels of *INI1* (**Figure 2**). The expression level of *INI1* mRNA was significantly lower in GISTs with decreased INI1 protein than in those with preserved INI1 protein (median 4.02 [range: 1.53-5.51] vs 8.94 [3.09-20.22]) (p=0.0049). Likewise, the presence of 22q11 LOH was significantly associated with a lower level of

INI1 mRNA expression (median 4.03[range 1.53-6.97] vs 13.93[2.99-20.22])

(p=0.0046).

Discussion

Despite the central role of *KIT* or *PDGFRA* mutations, the additional molecular events leading to the development of GISTs remain unclear. Frequent losses in the chromosome arms 1p, 9p, 14q, and 22q in sporadic GISTs suggest that some kinds of tumor suppressor genes may be altered in association with such chromosomal abnormalities [6-12]. According to the previous studies, about 50-70% of GISTs show LOH with at least one of microsatellite markers on 22q [11,12]. In particular, LOH is frequently found in the chromosome region between 22q11 and 22q13.

INI1 is known as a tumor suppressor gene located at 22q11.23. INI1 gene product negatively regulates the cell cycle progression from G0/G1 into the S phase via the upregulation of *p16INK4a* transcription and the downregulation of *cyclin D1* transcription, resulting in the inhibition of hyperphosphorylation of RB and E2F1-dependent transcription of target genes [17,25,26]. In malignant rhabdoid tumor (MRT), both alleles of the *INI1* gene are inactivated by homozygous deletion or by a combination of heterozygous deletion (LOH) and mutation, resulting in cell cycle progression [16,18]. Biallelic alterations of the *INI1* gene in MRT fit the “two-hit” theory

of the tumor suppressor gene.

In the current study, we focused on the *INI1* gene located at 22q11.23, and found frequent (70.4%) LOH at 22q11.23 in GIST. Moreover, the LOH was more frequently present in high grade than in low grade tumors. The result is consistent with a previous large study showing that the accumulation of LOH at 22q11-22q13 was more frequently present in malignant than benign GISTs [11]. However, neither point mutation nor homozygous deletion of *INI1* gene was observed, suggesting the hemiallelic but not biallelic manner of *INI1* alteration in GIST. In addition, although the presence of LOH at 22q11.23 was significantly associated with decreased expression of INI1 protein (BAF47), the expression level of INI1 was not correlated with tumor grade, tumor size and mitotic activity in our series of GISTs. The results suggest that INI1 is less likely to play a major role in the progression of GIST. Rather, other as-yet-unidentified genes mapped 22q may play more significant role.

The role of hemiallelic loss of the *INI1* has been controversial. A recent study has revealed that the RB-dependent cell growth was caused by the loss of one allele of the *INI1* gene in a fibroblast cell line [27]. In the present study of GIST, there was a

significant correlation between the LOH at *INI1* locus and the lower levels of INI1 gene products (mRNA or protein), but biallelic alteration of *INI1* was not found. The result suggests that hemiallelic loss of *INI1* gene resulted in decreased expression of INI1 gene products. The decreased expression of INI1 protein was observed in GISTs from low to high grade, and was not correlated with tumor size, mitotic activity and Ki-67 labeling index. Given these results, along with the above-mentioned function of INI1, the haploinsufficiency of INI1 might help tumor cells proliferate in relatively early step of the pathogenesis of GIST.

Considering the normal function of the INI1 protein that negatively regulates the G1-S cell cycle via the up-regulation of *p16INK4a* transcription and the down-regulation of *cyclin D1* transcription, the *INI1* gene alteration in GIST cells might cause the decrease of p16INK4a expression and increase of cyclin D1 expression, enabling the cell cycle progression. However, in the current study, we failed to find the significant correlation between INI1 alteration (LOH or protein loss) and the protein expression level of cyclin D1 or p16INK4a, although the decrease of INI1 protein expression tended to be correlated with lower level of p16INK4a expression. One possible explanation for this

result is that the expression of cyclin D1 and p16INK4a is regulated by another molecular mechanism; for example, as previously reported, the chromosome 9p loss decreases the p16INK4a protein expression level, particularly in high-grade GISTs [13,15]. In addition, the *cyclin D1* mRNA can be up-regulated in GIST by activated KIT-derived intracellular signaling such as RAS-RAF-ERK and JAK-STAT pathways [28]. Further investigation is expected to elucidate the interactions and significant roles of INI1 and G1-related factors such as RB, cyclin D1 and p16 in the cell-cycle progression of GIST cells by using functional studies such as the silencing of *INI1* gene.

The reduced nuclear INI1 expression by immunohistochemical stain was seen in 63 % of GISTs in this study. Recently, reduced expression of INI1 protein has been reported not only in MRT but also in sarcomas such as epithelioid sarcoma and extraskeletal myxoid chondrosarcoma [29,30]. To the best of our knowledge, this is the first report to show the decreased immunohistochemical expression of INI1 protein in GIST. In contrast to the staining pattern (diffuse and complete loss) of INI1 in MRT [31,32], GISTs showed a mosaic pattern of INI1 expression, consistent with the loss of expression in a subset of tumor cells. Recently, similar mosaic immunostaing pattern (focal loss of

expression) of INI1 has been reported in schwannomatosis [33]. Interestingly, some cases of schwannomatosis are found to have the biallelic *INI1* gene alterations [34], which contradicts the relationship between protein expression pattern and the underlying molecular alteration of INI1 in MRT and GIST.

As mentioned above, there is a possibility that the alteration of tumor suppressor genes mapping to 22q, other than INI1, might play a role in the pathogenesis and progression of GIST. Pylkkänen et al. have detected the frequent LOH of *NF2* gene locus at 22q12.2, but failed to find *NF2* gene mutation and reduced expression of its gene product, suggesting that *NF2* gene was unlikely to be a tumor suppressor gene involved by the tumorigenesis of GIST [12]. According to a large study of LOH in GISTs, 22q13.33(D22S922) and 22q11.22(D22S425) were the “hot-spot” of deleted regions [11]. Further investigation of other tumor suppressor genes mapping to 22q would be expected.

In conclusion, the results of current study suggest that LOH at 22q11.23 is frequently present in GIST irrespective of *KIT* genotype and it may play a role in the development of GIST. However, it is less likely that INI1 plays a major role in the progression of GIST. Rather, other as-yet-unidentified genes mapped 22q may play more significant role in the

tumorigenesis and progression of GIST.

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Figure legends

Figure 1. Histological finding and immunohistochemical staining for INI1.

a. Representative morphology of GIST (HE staining). The spindle-shaped cells proliferate in fascicular pattern.

b. Immunohistochemical staining for INI1. INI1 expression is focally reduced with mixture of nuclear-positive and -negative tumor cells, showing mosaic pattern.

c. Immunohistochemical staining for cyclin D1. A representative case of GIST shows increased nuclear expression of cyclin D1.

d. Immunohistochemical staining for p16INK4a. A representative case of GIST shows decreased nuclear expression of p16INK4a. Arrow indicates vascular endothelial cells as internal positive control.

Figure 2. INI1 mRNA expression level.

a. Decreased expression of INI1 protein is correlated with lower INI1 mRNA expression levels (p=0.0049).

b. The presence of LOH at 22q is correlated with lower INI1 mRNA expression level

(p=0.0046).

Table 1. Clinicopathological and immunohistochemical findings in 27 cases of GIST

Case	Age (y)	Sex	Site	Size (cm)	Mitosis (/50HPF)	Grade	Ki-67 (%)	KIT Mutation	INI1 IHC
1	74	F	Stomach	17.0	28	High	15.2	KIT exon11	preserved
2	65	M	Stomach	14.0	50	High	16.0	KIT exon11	decreased
3	60	M	Stomach	13.0	8	High	16.4	KIT exon11	decreased
4	60	M	Stomach	5.0	112	High	9.0	KIT exon11	decreased
5	40	M	Stomach	6.5	121	High	8.9	KIT exon11	decreased
6	62	F	Stomach	4.0	12	High	8.1	KIT exon11	decreased
7	71	M	Small intestine	8.5	6	High	8.0	-	decreased
8	76	M	Small intestine	7.2	25	High	8.9	KIT exon11	decreased
9	38	M	Small intestine	13.0	2	High	4.1	KIT exon11	preserved
10	47	M	Small intestine	5.5	18	High	5.1	-	decreased
11	46	F	Small intestine	6.0	10	High	8.9	KIT exon9	preserved
12	91	M	Small intestine	10.3	5	high	10.8	-	decreased
13	88	M	Colon	18.0	25	High	9.0	-	decreased
14	70	F	Esophagus	5.5	20	High	21.2	KIT exon11	preserved
15	88	M	Pelvic cavity	15.0	5	High	7.0	KIT exon11	decreased
16	79	F	Stomach	5.3	1	Intermediate	1.5	KIT exon11	preserved
17	93	F	Stomach	7.0	0	Intermediate	4.6	KIT exon11	decreased
18	49	M	Stomach	6.5	0	Intermediate	1.1	-	decreased
19	74	M	Stomach	6.5	4	Intermediate	3.6	KIT exon11	preserved
20	68	M	Stomach	6.8	2	Intermediate	2.9	-	decreased
21	66	M	Small intestine	5.5	1	Intermediate	2.0	-	decreased
22	53	F	Stomach	4.0	2	Low	3.0	-	preserved
23	73	F	Stomach	3.8	2	Low	1.0	KIT exon11	preserved
24	63	M	Stomach	4.3	1	Low	0.3	KIT exon11	decreased
25	60	M	Stomach	4.0	4	Low	4.6	-	preserved
26	70	F	Stomach	3.5	0	Low	1.3	KIT exon11	preserved
27	72	M	Small intestine	4.0	1	Low	3.0	KIT exon11	decreased

IHC, immunohistochemistry

HPF, high-power-field

Table 2. The reuslt of loss of heterozygosity at 22q11.23 analysis in GIST

Case	D22S303	D22S257	D22S301	D22S345	TOP1P2
1	□	□	□	○	●
2	□	□	□	□	●
3	●	□	□	●	●
4	●	●	●	□	●
5	●	●	●	□	●
6	●	□	●	●	○
7	●	□	□	□	●
8	□	□	□	□	●
9	□	●	●	●	□
10	●	□	●	●	●
11	□	○	□	□	□
12	□	●	●	□	□
13	□	□	○	□	□
14	●	□	●	●	●
15	□	○	□	□	●
16	□	□	□	○	□
17	□	□	○	○	□
18	□	□	●	□	●
19	○	○	○	○	□
20	●	□	●	●	●
21	□	●	□	●	□
22	○	○	□	□	○
23	○	□	□	○	○
24	□	●	●	□	●
25	□	□	□	○	●
26	□	□	○	□	○
27	□	●	●	●	●

Black circles (●) indicate loss of heterozygosity (LOH), white circles (○) indicate retain of heterozygosity, and white squares (□) indicate noninformative markers.

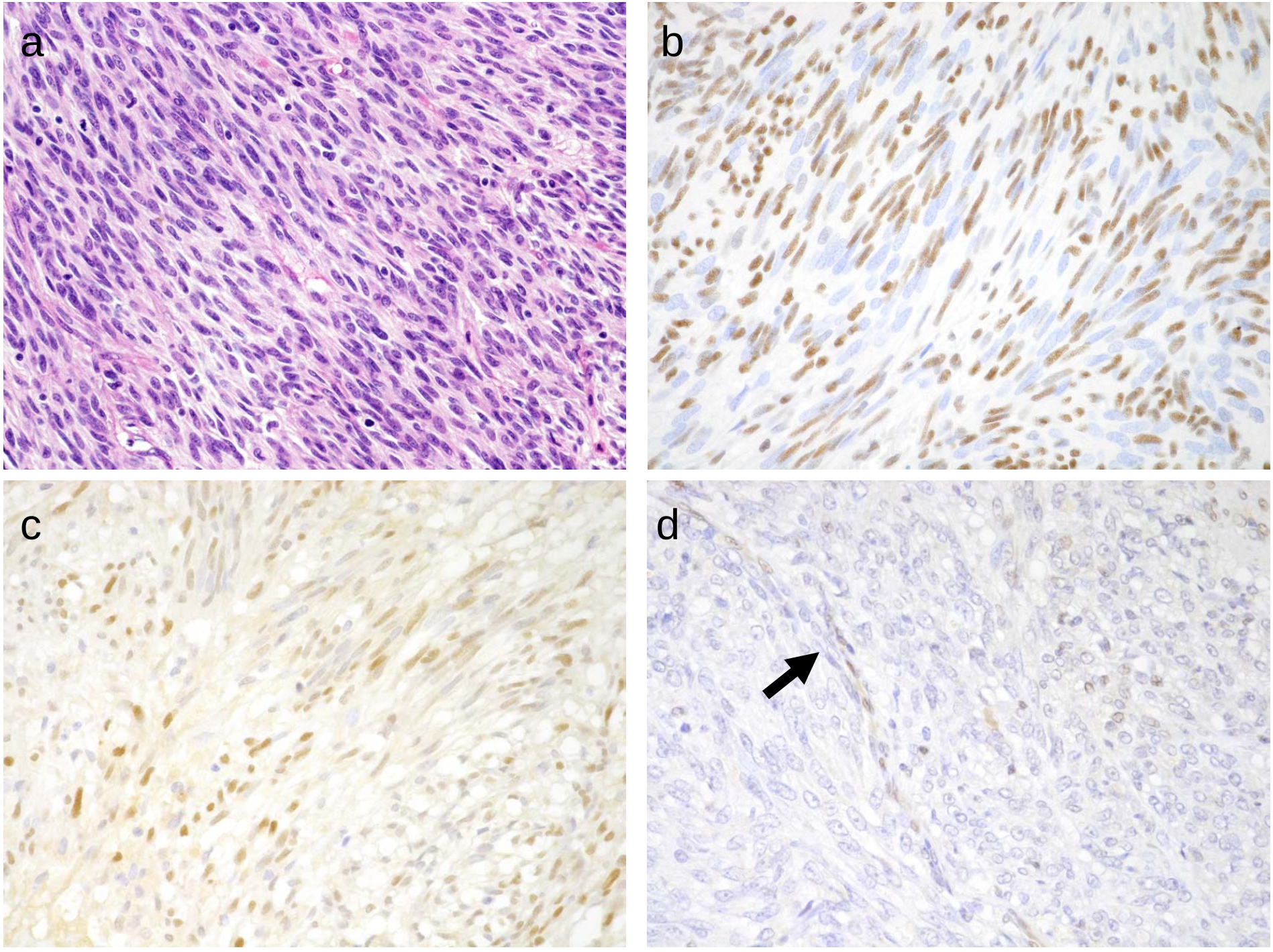
Table 3. Summary of the correlation between the 22q11.23 LOH and clinicopathological factors

Factors		22q11.23 LOH		
		+	-	*p*-value
		(n=19)	(n=8)	
INI1 protein				
	decreased (n=17)	15	2	0.008*
	preserved (n=10)	4	6	
Risk grade				
	high (n=15)	13	2	0.038*
	low, intermediate (n=12)	6	6	
Size				
	<5cm (n=7)	4	3	0.373
	≥5cm (n=20)	15	5	
Mitoses				
	<5/50HPF (n=13)	7	6	0.070
	≥5/50HPF (n=14)	12	2	
Ki-67 labeling index				
	<5% (n=13)	7	6	0.070
	≥5% (n=14)	12	2	
Site				
	stomach (n=16)	10	6	0.280
	others (n=11)	9	2	
KIT mutation				
	positive (n=18)	12	6	0.676
	negative (n=9)	7	2	
CyclinD1				
	<10% (n=16)	11	5	0.824
	≥10% (n=11)	8	3	
p16INK4a				
	<20% (n=8)	6	2	0.733
	≥20% (n=19)	13	6	

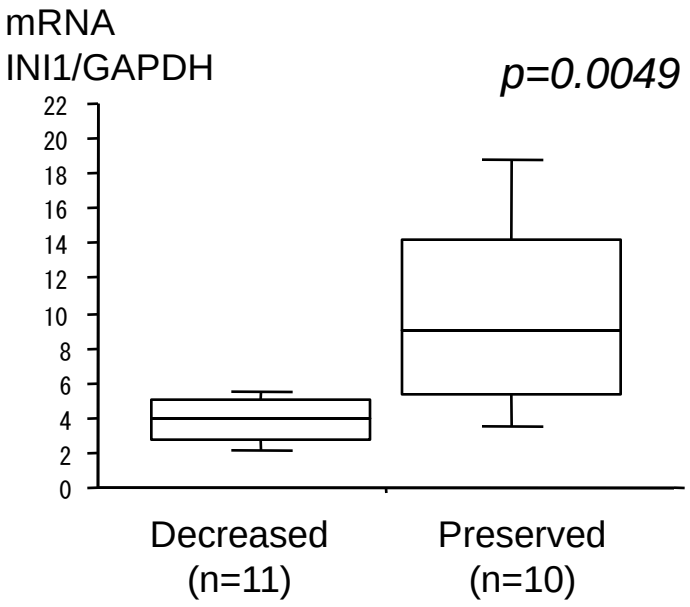
* statistically significant

Table 4. Summary of the correlation between the INI1 protein expression and clinicopathological factors

Factors		INI1 protein expression		
		decreased (n=17)	preserved (n=10)	*p*-value
Risk grade				
	high (n=15)	11	4	0.212
	low, intermediate (n=12)	6	6	
Size				
	<5cm (n=7)	3	4	0.200
	≥5cm (n=20)	14	6	
Mitoses				
	<5/50HPF (n=13)	6	7	0.081
	≥5/50HPF (n=14)	11	3	
Ki-67 labeling index				
	<5% (n=13)	6	7	0.081
	≥5% (n=14)	11	3	
Site				
	stomach (n=16)	9	7	0.383
	others (n=11)	8	3	
KIT mutation				
	positive (n=18)	10	8	0.406
	negative (n=9)	7	2	
CyclinD1				
	<10% (n=16)	12	4	0.118
	≥10% (n=11)	5	6	
p16INK4a				
	<20% (n=8)	7	1	0.087
	≥20% (n=19)	10	9	

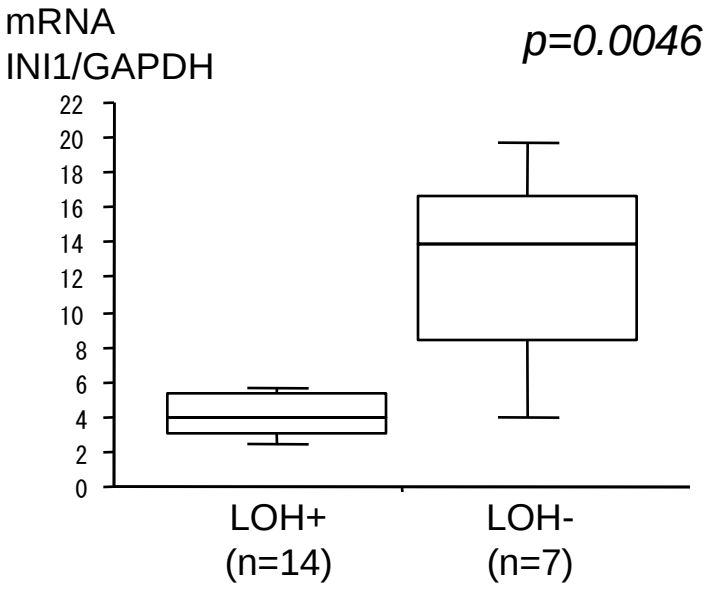

a
b
c
d

(a)



INI1 protein expression

(b)



LOH at 22q11