



Original Research

Mutation analysis of gastrointestinal stromal tumors using RNA obtained via endoscopic ultrasound-guided fine-needle aspiration



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Abbreviations:

GIST

Topic:

gastrointestinal stromal tumor

EUS-FNA

Topic:

endoscopic ultrasound-guided fine-needle aspiration biopsy

PCR

Topic:

polymerase chain reaction

PDGFRA

Topic:

platelet-derived growth factor receptor A

SEL

Topic:

subepithelial lesion

ROSE

Topic:

rapid on-site evaluation

GIMT

Topic:

gastrointestinal mesenchymal tumor

IIT

Topic:

Intention to treat analysis

PP

Topic:

per protocol analysis

ABSTRACT

Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) is useful for pathologically diagnosing gastrointestinal stromal tumor (GIST) before surgery. However, its role in mutation analysis remains unclear. To examine the feasibility of analyzing GIST mutations using mRNA obtained with EUS-FNA, we prospectively enrolled 41 patients with subepithelial lesion from which EUS-FNA was successfully acquired tissue sample. Thirty-two, 5, and 4 subepithelial lesions were diagnosed as GISTs, schwannomas, and leiomyomas, respectively. After RNA was extracted from FNA sample, RNA was converted to cDNA. Full-length sequence of the KIT cDNA amplified via the polymerase chain reaction (PCR) was successful in 31 (96.9%) out of 32 GIST and three out of 9 non-GIST (33.3%). The KIT mutation statuses of 31 GISTs in which KIT cDNA was amplified were successfully determined through directional sequencing. Furthermore, 15 of 16 surgically excised GISTs exhibited the same mutation status in both the EUS-FNA and resected samples. In vitro experiment, the minimum number of cells required to amplify full-length of KIT cDNA from RNA was one-tenth of that required to amplify KIT exon11 gene from DNA. This study clarifies that mutation analysis using RNA obtained with EUS-FNA is feasible and reliable. Moreover, our data would support that RNA-based mutation is superior to DNA-based mutation analysis in GIST.

Abbreviations: GIST, gastrointestinal stromal tumor; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration biopsy; PCR, polymerase chain reaction; PDGFRA, platelet-derived growth factor receptor A; SEL, subepithelial lesion; ROSE, rapid on-site evaluation; GIMT, gastrointestinal mesenchymal tumor; IIT, Intention to treat analysis; PP, per protocol analysis.

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors affecting the gastrointestinal tract [1]. Ninety percent of GIST possess gene mutations, such as mutations in the KIT or platelet-derived growth factor receptor A (PDGFRA) gene. In addition, 95% of GIST are known to overexpress the KIT protein, which is translated from KIT mRNA [1–4]. Gene mutation analysis is considered to provide useful information for both diagnosing and treating GIST, including for predicting drug responses and recurrence after surgery [5–10]. In general, KIT mutations in GIST are analyzed by examining surgically resected tissue samples. Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) is a potentially useful procedure for pathologically diagnosing GIST before surgery [11–13]. However, EUS-FNA samples are generally utilized for pathological examinations because the acquired samples are tiny. A few studies have examined the diagnostic yield of mutation analyses based on examinations of EUS-FNA samples [14–16]. However, the feasibility of performing mutation analyses using EUS-FNA samples have been unclear.

Since the samples obtained with EUS-FNA are usually quite small, the amount of extracted DNA is also extremely low. On the other hand, the fact that almost all GISTs overexpress KIT protein indicates that GISTs contain a large amount of KIT mRNA [17]. Theoretically, there should be a much higher amount of KIT mRNA than KIT DNA in GIST. We hypothesized that analyzing RNA is superior to analyzing DNA for performing mutation analyses of GIST using EUS-FNA samples. The aim of this study was to evaluate the feasibility of mutation analysis of GIST using RNA obtained with EUS-FNA.

Methods

Patients

Between October 2008 and September 2014, Gastrointestinal EUS-FNA was performed to 116 cases of subepithelial lesion (SEL) at Nagoya University Hospital. 85 SELs were suspected of GIST from EUS image. We prospectively got informed consent of this study from 52 patients before EUS-FNA. 41 SELs which were acquired tissue within three passes of EUS-FNA puncture, were registered consecutively in this study (Fig. 1).

Among them, 16 patients with GIST who underwent surgery after EUS-FNA gave their consent for their resected GISTs to be analyzed as well. The

protocols for the collection of the tumor samples and clinical information were approved by the institutional review board at Nagoya University Hospital (IRB approval No. 2008–0611). Written informed consent was obtained from all patients.

Sample collection

All EUS-FNA procedures were performed in the course of clinical practice to determine the pathological diagnoses of SEL using an oblique-viewing echoendoscope (GF-UCT240AL-5; Olympus, Japan) with an ultrasound processor with a color Doppler function (EU-ME1; Olympus, Japan). Puncturing was carried out using disposable 22-G puncture needles (EZ Shot2: NA-220H-8022; Olympus, Japan). Endoscopists checked FNA sample visually without rapid on-site evaluation (ROSE) after three puncture passes. When white string tissues were seen, the procedure was finished with registration of this study. If tissue was not obtained within three passes, the procedure was continued until tissue sample was obtained. But such a case was omitted from this study to avoid additional punctures. A part of the obtained sample, which was equivalent to tissue obtained in single session, was assigned for use in RNA extraction.

The sample was immediately immersed in RNAlater™ stabilization solution (Invitrogen, Carlsbad, CA) and was stored at -80°C until just before RNA extraction step.

RNA extraction and cDNA reverse transcription

Each stored sample was deeply frozen with liquid nitrogen and homogenized in the mortar. Total RNA was extracted using 1 ml of TRIzol reagent (Invitrogen, Carlsbad, CA), 0.2 ml of chloroform, 0.5 ml of isopropanol and 1 ml of 75% ethanol according to the manufacturer's instruction. After extracted RNA was diluted by 20 μl of RNA-free water, RNA concentration and absorbance at wavelengths of 260/280 nm were measured with the Nano-Drop 1000™ system (Thermo Fisher Scientific, Waltham, MA) to evaluate the quality and quantity of total RNA. For cDNA synthesis, 3 μl of total RNA was reverse transcribed using PrimeScript™ Reverse Transcriptase (Takara Bio, Inc., Shiga, Japan) according to the manufacturer's protocol.

Amplification of KIT cDNA

Full-length KIT cDNA was amplified with 40 cycles of polymerase chain reaction (PCR) using appropriate primers (Table 1). Template cDNA which was equivalent to 200 ng of RNA was mixed with 2 μl of dNTP, 0.5 μl of each primer, 0.5 μl of KOD FX (TOYOBO CO., LTD, Osaka, Japan), 25 μl of KOD FX buffer and water. A total reaction volume of 50 μl was prepared for PCR. One PCR cycling condition was 1 min of denaturation at 95°C , 0.5 min of annealing at 55°C and 3 min of extension at 72°C . After PCR amplification, agarose gel electrophoresis was used to confirm whether the

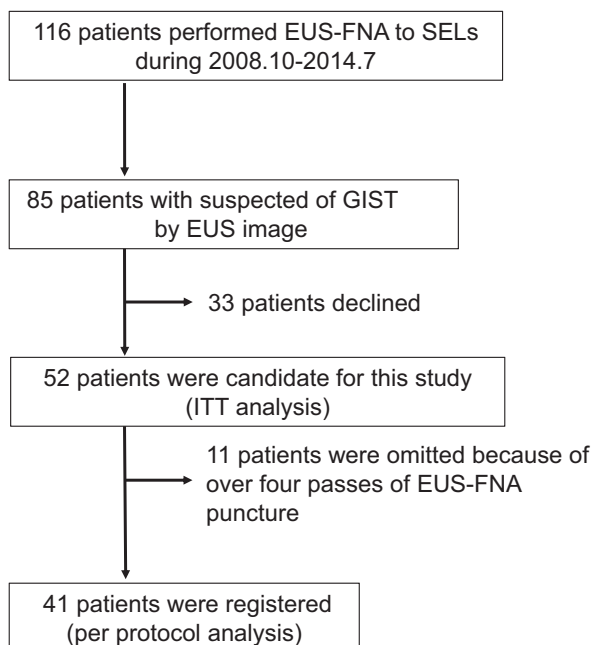


Fig. 1. Flow of patients evaluated in this study.

Table 1
Primer design.

Target cDNA	Forward	Reverse
KIT	AGCTCGGATCCCATCGCTA	CTGCTCAGACATCGTCGTGCAC
nested KIT	AGCTACCGGATGAGAGGCGCT	GTCTGTGCACAAGCAGAGGCTG
PDGFRA	ATCCGGGTTCTGGTCTTAG	CAGGAAGCTGTCTTCCACCAG
Sequencing target		Forward
KIT	exon 9	GTGAATGGCATGCTCCAATGT
	exons 11 & 13	TCACTCCTTTGCTGATTGGT
	exon 17	ATCATGGAGGATGACGAGTGT
PDGFRA	exon 12	ATCTCACTTATTGTCCIGGTT
	exon 14	AGCCGGTCCCAACCTGTCTATG
	exon 18	AGCTTCACTATCAAGITGCC
PCR in vitro experiment		
Target DNA	Forward	Reverse
KIT exon11	CCAGAGTGCTCTAATGACTG	CTGTTATGTGTACCCAAAAGG

amplification had succeeded or failed. In case of failure to KIT amplification by 1st PCR, nested PCR was performed and then checked again by agarose gel electrophoresis. The schematic location where amplified using each primer of KIT was shown in Fig. 2.

Mutation analysis

Using the amplified full-length KIT cDNA as a template, exons 9, 11, 13, and 17 were analyzed using the directional sequencing method on an ABI 310 system (Applied Biosystems, Foster City, CA). Sequence primer was shown in Table 1 and Fig. 2). 2 µl of PCR product was mixed with 4 µl of 50 times diluted primer, 1 µl of Big Dye terminator 3.1 Ready Reaction Mix (Applied Biosystems, Foster City, CA), 4 µl of Sequencing buffer and water. A total volume of 20 µl was performed sequencing reaction over 25 cycles of the following step (10 s of denaturation at 96 °C, 5 s of annealing at 50 °C and 1 min of extension at 60 °C). In GISTs which were negative for KIT mutations, exons 12, 14, and 18 of the PDGFRA gene were further analyzed after PCR amplification of full-length PDGFRA cDNA. GIST that did not possess KIT or PDGFRA mutation was defined as wild-type GIST.

Pathological diagnosis

Immunohistochemical staining was performed to distinguish GIST from other mesenchymal tumors in addition to hematoxylin and eosin staining.

For the immunohistochemical staining, commercially available primary antibodies against KIT (polyclonal, 1:500; MBL, Nagoya, Japan), CD34 (clone QBEnd10, 1:600; Dako, Glostrup, Denmark), S100 (polyclonal, 1:1000; Dako), alpha smooth muscle actin (clone 1A4, 1:1000; Dako) and MIB-1 (clone MIB-1, 1:1000; Dako) were utilized.

In vitro experiment

To examine the minimum number of cells required to amplify target KIT gene via PCR, the GIST-T1 cell line (Cosmo Bio, Tokyo, Japan) which is Immunohistochemically positive for c-KIT and has KIT mutation in exon 11 (codon 560–578 deletion), was utilized [18]. After being subjected to trypsin treatment and serum neutralization, the suspended cells were counted using a hemocytometer. The density of the suspended cells was adjusted to 500,000 cells/100 µl, and the suspension was concentrated or diluted to create suspensions with various numbers of cells. Consequently, suspensions with cell counts of 1×10^3 , 5×10^3 , 1×10^4 , 5×10^4 , 1×10^5 , and 2×10^5 were subjected to gene extraction. DNA was extracted using the illustra™ tissue and cells genomicPrep mini spin kit (GE Healthcare, Buckinghamshire, England, UK). RNA extraction was performed as described previously. KIT genomic DNA was amplified over 40 cycles of PCR using appropriate primers which target KIT exon 11 gene (Table 1). Full-length of KIT cDNA was amplified after RNA was reverse transcribed to cDNA. The detailed method was described in previous

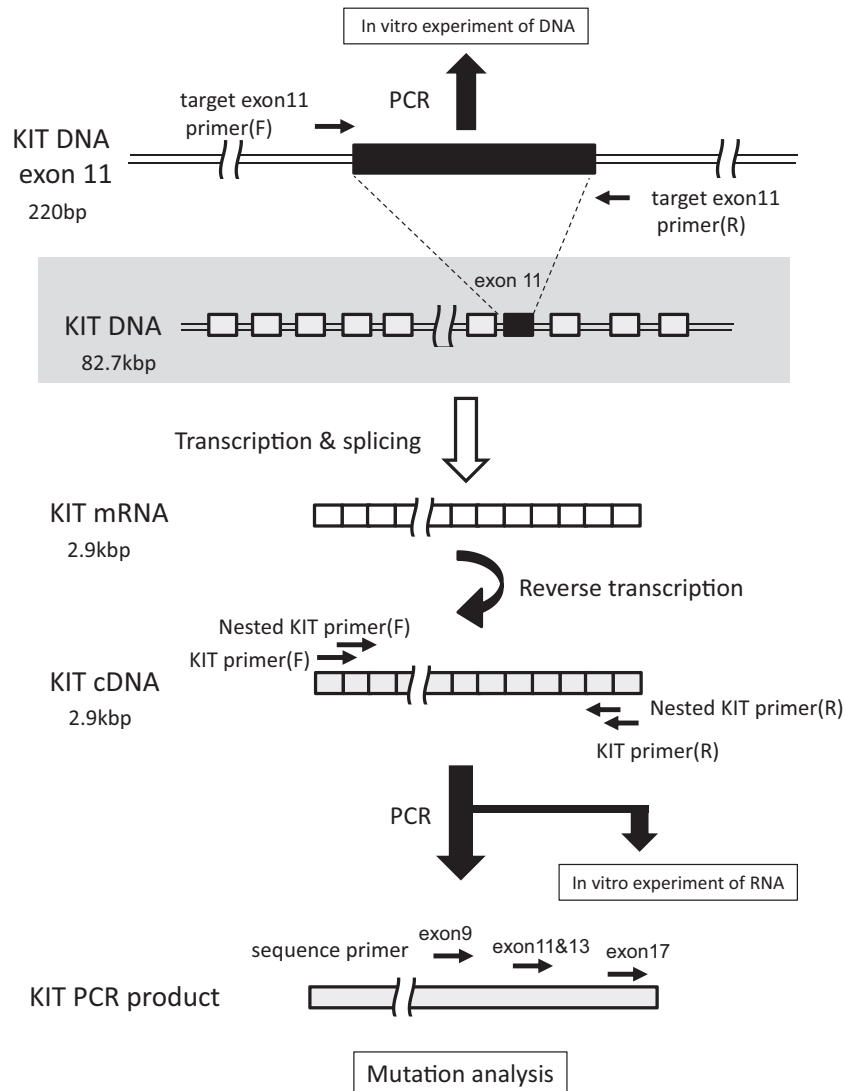


Fig. 2. The location of each primers on schematic images of DNA, mRNA, cDNA and PCR product related with KIT gene.

paragraphs. The schematic locations of cDNA or DNA where amplified were shown in Fig. 2. For each PCR product, agarose gel electrophoresis was used to confirm whether the PCR amplification had succeeded or failed. All experimental procedures were repeated three times with same condition.

Statistical analysis

The correlation between the quantity and quality of the extracted RNA was evaluated using Spearman's rank correlation coefficient. The success rates of KIT amplification during the first PCR and/or nested PCR was described with both per protocol analysis (PP) and Intention to treat analysis (ITT). The success rate of KIT amplification was compared between GIST and non-GIST using Fisher's exact test in per protocol analysis. Statistical significance was defined as $P < .05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24 (IBM, Armonk, NY, USA).

Results

Patient characteristics

Although 52 patients were candidate for this study, 11 patients were omitted because of over four passes of EUS-FNA puncture (Fig. 1). The characteristics of 41 patients enrolled in this study are shown in Table 2. Thirty-two of 41 tumors were pathologically diagnosed with GISTs. Five and four tumors were diagnosed with schwannomas and leiomyomas, respectively.

Quantity and quality of RNA

RNA extraction was successfully achieved from all samples. The median RNA concentration was 213.2 ng/ul (range: 28–1498). The median 260 nm/280 nm absorbance ratio, which indicates RNA quality, was 1.71 (range: 1.30–1.95). The correlation between RNA concentration and 260 nm/280 nm absorbance ratio is shown in Fig. 3a. There is a strong correlation between the RNA concentration and the 260 nm/280 nm absorbance ratio ($R = 0.748, P < .0001$).

Success rate of KIT amplification

All samples were subjected to cDNA reverse transcription and PCR amplification. The success rate of the first PCR was 70.7% (PP), 55.8% (ITT), and the overall success rate of both the 1st PCR and nested PCR was 83.0% (PP), 65.4% (ITT).

Among 32 GISTs, the success rate of the first PCR was 87.5%, and the overall success rate of both the 1st PCR and nested PCR was 96.9%. On the other hand, the success rate of the 1st PCR was 11.1%, and the overall success rate of both the 1st PCR and nested PCR was 33.3% among non-

Table 2
Patients' characteristics.

	All patients	GIST	Non-GIST
No.	41	32	9
Gender			
Male	22	18	4
Female	19	14	5
Age, y, mean (SD)	65.7 ± 11.5	65.5 ± 11.1	66.3 ± 13.2
Tumor size (mm), median, range	25 (16–240)	27.5 (16–240)	25 (20–43)
Tumor location			
Esophagus	3	1	2
Stomach	37	30	7
Duodenum	1	1	0
Pathological diagnosis			
GIST	32	32	–
Schwannoma	5	–	5
Leiomyoma	4	–	4

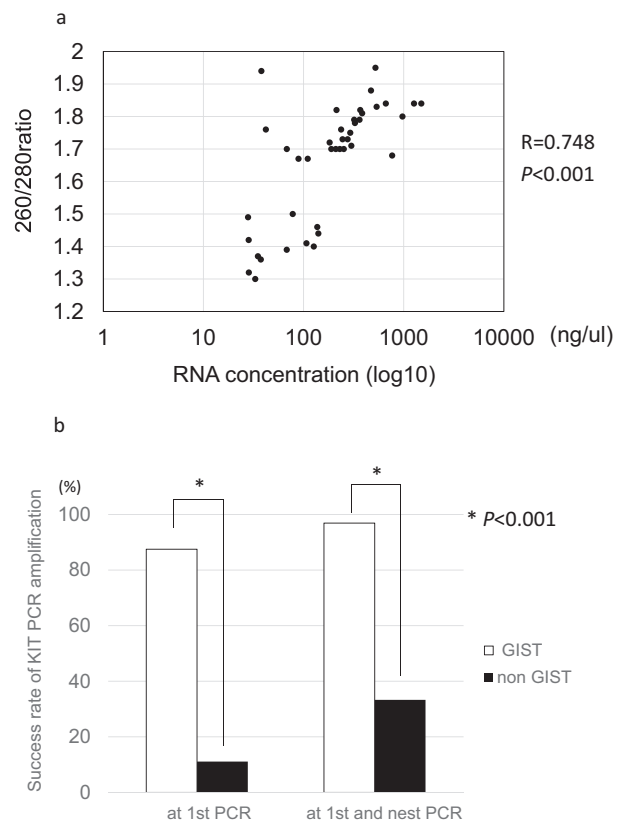


Fig. 3. The correlation between the RNA concentration and 260 nm/280 nm absorbance ratio of the RNA extracted from EUS-FNA samples obtained from 41 SEL (Fig. 3a). A comparison of the success rate of KIT amplification between GIST and non-GIST (Fig. 3b).

GISTs. The success rate of KIT amplification was significantly higher among GISTs than among non-GISTs ($P < .001$) (Fig. 3b).

The KIT amplification flowcharts for all SELs, GISTs, and non-GISTs are shown in Fig. 4a, b, and c, respectively. c-kit cDNA was successfully amplified by 1st PCR in 29 out of 41 SELs. c-kit cDNA was successfully amplified from 5 of the remaining 12 SELs via nested PCR. In 7 SELs, KIT amplification was failed. Three non-GISTs from which the KIT gene was amplified were all schwannomas. We compared RNA concentration and 260 nm/280 nm absorbance ratios between the samples which were succeed by 1st PCR and those which were failed by 1st PCR. However, neither of these parameters differed significantly between two groups (Supplementary Table 1).

Gene mutations in GIST

The results of GIST mutation analysis are shown in Table 3. All 31 GISTs in which full-length KIT cDNA was amplified were revealed their KIT mutation statuses completely. Twenty-seven GISTs carried KIT mutations. KIT exon 11 mutations were detected in 25 of the 27 GISTs with KIT mutations (92.6%). Regarding the types of mutations found in exon 11, insertions, point mutations, and deletions were detected in 6, 12, and 7 GISTs, respectively. Of the remaining two GISTs, one duodenal GIST exhibited an insertion in exon 9, and the other had a point mutation in exon 13. Two GISTs carried point mutations in exon 18 of PDGFRA. The remaining two GISTs that have neither KIT nor PDGFRA mutations were wild type.

Sixteen GISTs were surgically resected after EUS-FNA. The surgical specimens from these cases were subjected to gene mutation analysis using the same method as was employed for the EUS-FNA samples. The gene mutation analysis was successful in all 16 cases. One GIST in which KIT amplification from the EUS-FNA sample failed was confirmed to be a wild-type GIST through the surgical sample. Complete correspondences

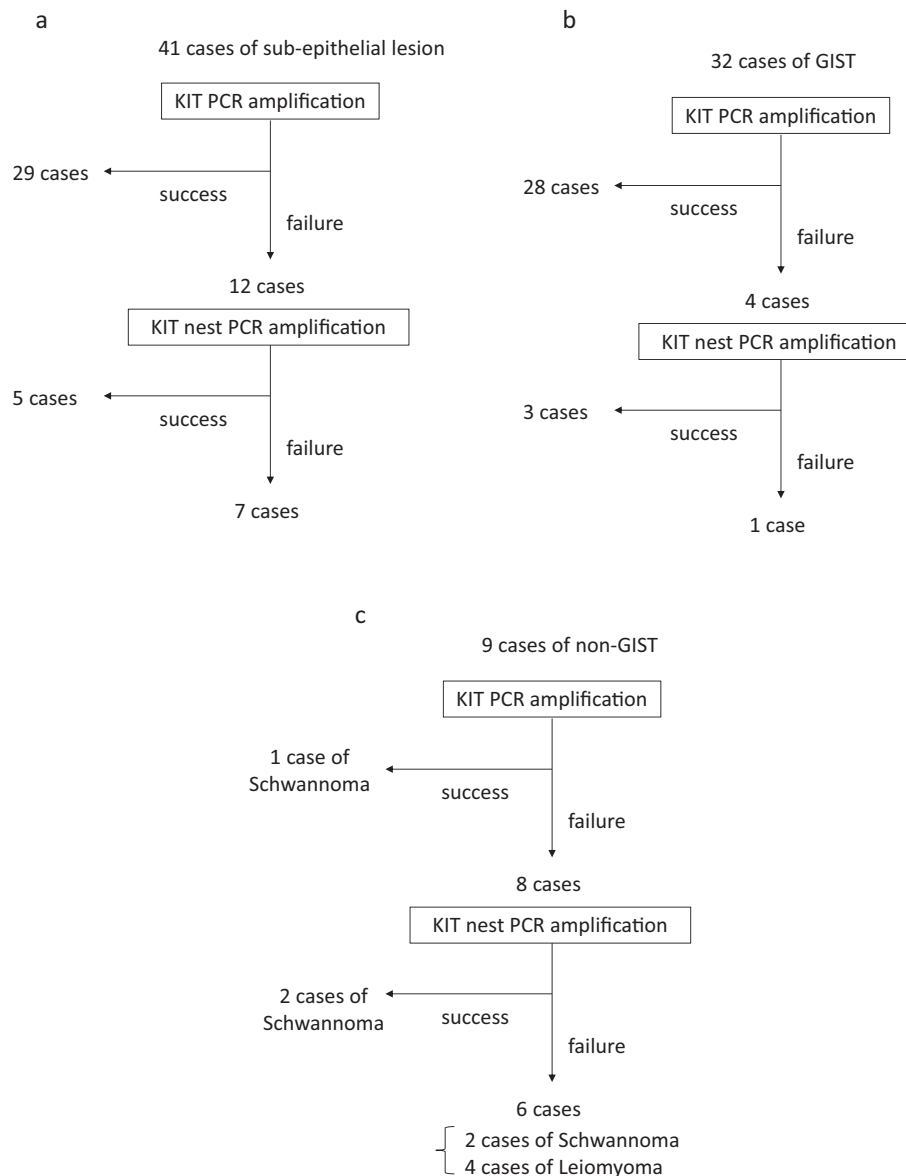


Fig. 4. Flowcharts of KIT amplification from SEL (Fig. 4a) After each SEL had been pathologically diagnosed as a GIST or non-GIST, the flowchart for GIST (Fig. 4b) or non-GIST (Fig. 4c) was followed.

between the mutation statuses of the surgical and EUS-FNA samples were seen in the remaining 15 GISTs.

Comparison of the minimum number of cells required for PCR-based amplification of the KIT gene between DNA and RNA samples

The minimum number of cells required for KIT amplification from DNA was 5×10^4 [4] (Fig. 5). In the case of RNA, which was converted to cDNA, the minimum number of cells required was 5×10^3 [3], which was one-tenth of the value for DNA samples. The results of this experiment indicate that using RNA is superior to the conventional DNA method for mutation analyses of small samples, such as those obtained with EUS-FNA.

Discussion

Although EUS-FNA is used to pathologically diagnose SEL, gene mutation analysis using EUS-FNA samples has been quite limited. Because the FNA sample which is tiny tissue has limitations regarding tumor purity and DNA quantity, such gene analyses have the concerns that extracted DNA is

insufficient or is not from a tumor. Here, we clarified the feasibility of mutation analysis using RNA extracted from EUS-FNA samples. First, we confirmed that RNA could be extracted from all EUS-FNA samples collected from SEL and that there was a positive correlation between the quantity and quality of extracted RNA. Secondly, we found that the success rate of KIT amplification was significantly higher among GIST than among non-GIST (96% vs. 33%). However, there was no relationship between the success rate and the quality or quantity of extracted RNA, even among GIST. In addition, 15 surgical samples out of 16 GISTs showed that the mutation status was consistent with EUS-FNA sample. Finally, an in vitro experiment using GIST-T1 cell line indicated that the minimum number of cells required to amplify KIT gene from RNA was one tenth of that required to amplify it from DNA.

Mutation analysis is useful not only for diagnosing GIST but also for predicting the effects of molecular targeting medicines and the risk of recurrence after surgery [4–10,19,20]. However, such analyses have been exclusively performed using DNA extracted from surgically resected samples. In unresected GIST due to metastasis, there is no opportunity to get mutation information although it contributes to assume the effectiveness of tyrosine kinase inhibitors.

Table 3Characteristics of mutation status in patients with GIST ($n = 32$).

No.	Amplification		Mutation status				Type
	1st PCR	Nested PCR	Gene	Exon	Codon ^{b,c}		
1	Failure	○	None	–	–	–	–
2	○		KIT	11	V559D		PM
3	○		KIT	9	503AY504		I
4	○		KIT	13	K642E		PM
5	○		PDGFRA	18	D842V		PM
6	○		KIT	11	550–558		D
7	○		KIT	11	555		D
8	○		KIT	11	W557R		PM
9	○		KIT	11	579DPTQLPYD580		I
10	○		KIT	11	W557R		PM
11	○		PDGFRA	18	D842V		PM
12	○		KIT	11	580DH581		I
13	○		KIT	11	V560E		PM
14	○		KIT	11	573DP574		I
15	○		KIT	11	585YDHWKWEFP586		I
16	○		KIT	11	V560D		PM
17	○		KIT	11	L577P		PM
18	○		KIT	11	V561D		PM
19	○		KIT	11	L577P		PM
20	○		KIT	11	V560D		PM
21	○		KIT	11	L576P		PM
22	○		KIT	11	561–562		D
23	Failure	○	KIT	11	560		D
24	○		KIT	11	V559D		PM
25	○		KIT	11	558		D
26	○		KIT	11	580DPTQLPYDH581		I
27	○		KIT	11	560		D
28	Failure	○	None	–	–		–
29 ^a	Failure	Failure	None	–	–		–
30	○		KIT	11	V559D		PM
31	○		KIT	11	585LPYDHWKWEFP586		I
32	○		KIT	11	557–558		D

^a Mutation status of No.29 was analyzed using surgical tissue sample because of failure to amplification by FNA sample.

^b Each letter indicates the amino acid that was inserted or substituted due to the gene mutation (e.g., V559D indicates that valine was substituted for aspartic acid at codon 559 due to a point mutation, and 503AY504 indicates that alanine and tyrosine were inserted between codons 503 and 504).

^c The numbers in the codon column indicate deleted codons (e.g., 557–558 indicates that codons 557 and 558 were deleted). Abbreviations. D, deletion; PM, point mutation; I, insertion.

In this study, we proved that mutation analyses can be conducted using RNA extracted from EUS-FNA specimens collected from GIST. Clinically, RNA-based mutation analysis in unresectable GIST would offer informative data that can aid drug selection.

The feasibility of KIT analysis using RNA was shown in almost all GIST but a few in non-GIST. We speculate that it was resulted due to the abundant KIT mRNA in GIST. However, it is interesting that one-third of non-GIST showed KIT amplification by PCR. There are two possibilities why KIT was amplified even though non-GIST generally does not express KIT protein. One is that gastrointestinal mesenchymal tumors (GIMTs) including leiomyoma and schwannoma expressed small amount of KIT mRNA which cannot detect by protein level such as immunohistochemical staining. The other is that intestinal cells of Cajal was contained in GIMTs by chance. Anyway, we think these does not disturb clinical management because all cases which were diagnosed of leiomyoma or schwannoma did not possess KIT mutation. Regarding PDGFRA mutation, 4 GISTs without KIT mutation were analyzed after amplification of full-length PDGFRA cDNA. All 4 succeeded amplification and read sequence although PDGFRA expression has not examined pathologically to diagnose of GIST. It may be also advantage of RNA analysis that both sequences are performed using EUS-FNA sample.

There have been a few reports about mutation analysis using EUS-FNA specimens [14–16,21]. These mutation analyses were performed using DNA, rather than RNA. Gomes et al. reported that they were able to perform molecular analysis using DNA from 51 out of 85 cell samples. Thus, the

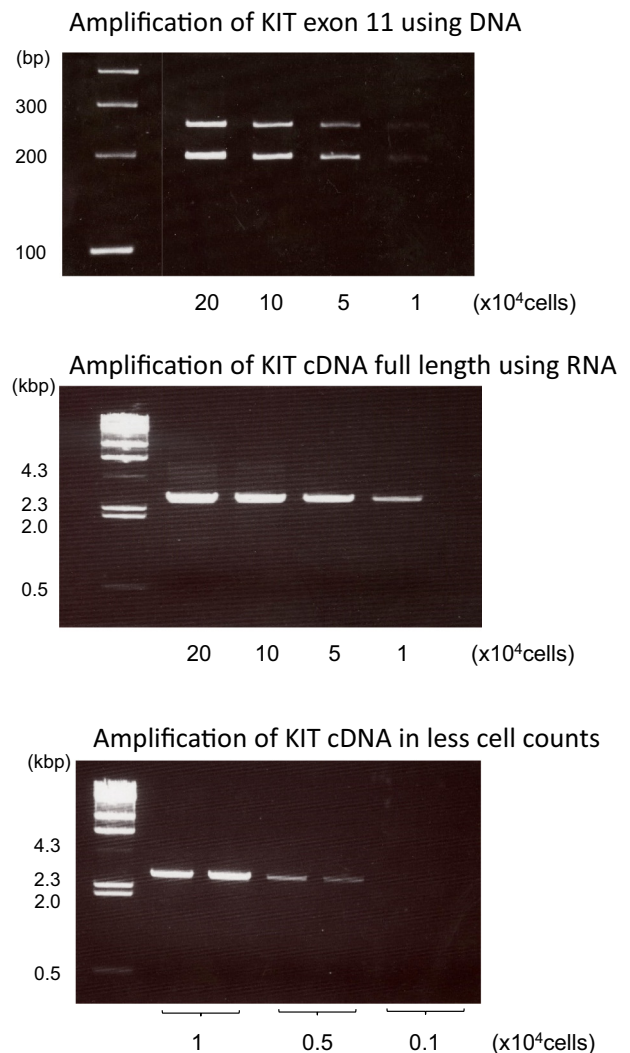


Fig. 5. The PCR products amplified using the KIT primers at each cell density. The upper panel shows the KIT exon 11 products amplified from DNA. Because of the deletion of 57 bases in exon 11, two bands appeared. The middle panel shows those amplified from full-length KIT cDNA, which is an alternative to RNA. The minimum number of cells required to amplify cDNA was investigated, and the PCR products amplified from less than 10,000 cells are shown in the lower panel.

success rate of this approach was only 60% [14]. On the other hand, a previous study described mutation analysis of GIST using EUS-guided biopsy samples. In the latter study, in which the analyses were performed using extracted DNA, it was shown that the success rate of sequencing was 98%. Interestingly, no EUS-FNA samples were subjected to sequencing because of their poor quality and small size [16]. Recently, Gleeson et al. examined the gene mutations in EUS-FNA samples using targeted next-generation sequencing. Sequencing results were successfully obtained in 19 out of 20 (95%) subjects. However, the 20 subjects were limited among 32 subjects which had fulfilled the cell number criteria after cytologic smears [21].

To the best of our knowledge, there have not been any reports about mutation analysis using RNA, even with regard to analyses of surgical samples. The present study is the first to demonstrate that gene mutation analysis using RNA obtained with EUS-FNA is feasible. In addition, it indicated that RNA analysis, rather than DNA analysis, has a great advantage in GIST, which contains lots of KIT mRNA in each cell. In other words, the tumors in which RNA sequencing is less likely to be successful are what KIT is no or less expressed, so less of a problem if the assay fails. Utilizing RNA for gene analysis would be of benefit, especially when a sample is a small volume or a high percentage of admixed non-tumor cells. In the present study,

the success rate of the mutation analysis was 96%. When EUS-FNA samples are not suitable for immunostaining or pathological evaluation in SEL, RNA based mutation analysis would aid the diagnosis of GIST. Furthermore, our result would support the utility of RNA based genetic testing using the clinical samples in other tumors that overexpress some mRNA with a mutation.

This study had a few limitations. Firstly, it was conducted at a single center and involved a relatively small sample size. Secondly, this study contains a selection bias because the registration was done after confirming EUS-FNA sample was obtained within 3 passes. Thirdly, EUS-FNA has been less common for SELs in the latest clinical practice. Due to the development of puncture needles, the name of the examination has been changing to Endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB), which offers more volume of specimens than EUS-FNA. However, the needle we used in this study belongs to the conventional type for EUS-FNA. We believe that mutation analysis using RNA is also applicable to EUS-FNB. Fourthly, the comparison of RNA- and DNA-based gene analyses was not performed simultaneously because the EUS-FNA samples were too small to allow two analyses to be performed. To directly prove the superiority of RNA analysis over DNA analysis, comparisons of mutation analysis success rates should be conducted simultaneously using DNA and RNA from the same samples in surgically resected tissue. Even consideration with these limitations, this study has revealed a novel efficient approach to mutation analyses in GIST using a combination with RNA and EUS-FNA.

In conclusion, mutation analysis using RNA obtained with EUS-FNA was found to be highly feasible and provided reliable results. This approach is suggested to be superior to DNA-based mutation analysis of GIST using EUS-FNA samples.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tranon.2020.100848>.

CRediT authorship contribution statement

Kohei Funasaka: Conceptualization, Methodology, Data curation, Writing - original draft, Formal analysis. **Ryoji Miyahara:** Investigation. **Kazuhiro Furukawa:** Investigation. **Tsunaki Sawada:** Resources. **Keiko Maeda:** Resources. **Takeshi Yamamura:** Resources. **Takuya Ishikawa:** Resources. **Eizaburo Ohno:** Resources. **Masanao Nakamura:** Resources. **Hiroki Kawashima:** Resources. **Yoshiki Hirooka:** Supervision. **Naoki Ohmiya:** Supervision. **Mitsuhiro Fujishiro:** Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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