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Original Article

Whole exomes sequencing for neurofibromatosis type I patients complicated with gastrointestinal stromal tumors

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Abstract: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract. 85% GISTs carry mutations in the *KIT* or *PDGFRA* and the rest 15% cases which present without *KIT* or *PDGFRA* mutation are called wild type (WT) GISTs. Patients with neurofibromatosis type I (NF1) have higher risk of developing GISTs and all the cases belong to the WT GISTs. Compared with the somatic cases, NF1-associated GISTs are characterized with unique clinical manifestations and are not responding to Imatinib-the first line drug for GISTs. This study was designed to identify the central somatic mutations in NF1-associated GISTs by conducting whole exomes sequencing (WES). We sequenced the tumor and peripheral blood exomes of two patients who developed *KIT/PDGFRA* wild-type GISTs concurrent with NF1. Three novel somatic mutated genes (*PRSS* and *FOLR3,* and *TAS2R43*) were identified in GISTs but not NF1 tumors. And we further test the function of the mutated genes (*PRSS3*, *FOLR3*) in GIST cells and prove the oncogenic roles they play in NF-1 associated GIST. This is the first report, to our knowledge, that WES is applied to study the genomic profile of tumors and peripheral blood exomes in NF1 patients. And it is also the first time that *PRSS3* and *FOLR3* are revealed to be associated with the development of NF1-associated GISTs.

Keywords: Gastrointestinal stromal tumor, neurofibromatosis type I, whole exome sequencing, somatic mutation

Introduction

Neurofibromatosis type 1 (NF1), or von Recklinghausen disease, is the most commonly seen genetic syndrome with an incidence of 1 in 2500 to 3000 and a prevalence of 1 in 4000 to 5000 [1]. As a hereditary disease with an autosomal dominant pattern of inheritance, NF1 is characterized with dermatologic, neurologic and orthopedic manifestations. It is reported that one half of the NF1 cases presented with complete penetrance and the other turned out to be sporadic cases [2, 3]. Diagnosis of NF1 is generally based on the clinical criteria developed by the National Institutes of Health Consensus Development Conference in 1988, where two or more criteria were enough to make the diagnosis [1]. The phenotypes of NF1 patients vary. Some patients were affected severely by the manifestations of the disease while others may live their whole life without any of the complaining. The phenomenon reminds us the necessity of supervising those who were detected with a family history [4]. Though the clinical manifestations of NF1 patients vary, the reasons remain still unknown [5]. As a genetic disease, NF1 patients were predisposed to both benign and malignant tumors of neurogenic and nonneurogenic origin. Of all the tumors, gastrointestinal stromal tumors (GISTs) have attracted great attention for its unique clinical manifestations compared with the common GISTs which harbored *c-KIT* or *PDGFRA* mutation [6].

Firstly described by Mazur and Clark in 1983, GIST is the most common mesenchymal tumor of the gastrointestinal tract, which is mostly

caused by oncogenic mutations in *c-KIT* or *PDGFRA*. Despite that, about 15% of GISTs do not harbor any mutations in the *KIT* or *PDGRA*, and this kind of GIST is called wild type GIST (WT GIST) [7, 8]. According to current studies, GIST is believed to arise from the Cajal cells in the alimentary tract. Cajal cells act like autonomic nerve-related gastrointestinal pacemaker cells that regulate gastrointestinal motility. 70 percent of the sporadic cases occur in the stomach while the rest develop in the intestine or extra gastrointestinal tract. This clinical observation, on the other hand, reflects the reliability of the Cajal cells as the origin of GIST because stomach has been the place where most of the Cajal cells locate [5, 9].

As for GIST associated with NF1, all the cases were proved to be wild type. So far, intestine tract is the only occurring site reported for NF1-associated GIST and most of the cases were reported to be multifocal. Patients who were diagnosed most frequently presented with gastrointestinal bleeding. However, a better prognosis was observed. The unique clinical manifestations indicate that the development mechanism could be different compared to GIST with *c-KIT* or *PDGFRA* mutation. And Imatinib, the first line drug for GIST, was proved to be inefficient for NF1-associated GIST [5, 6, 10]. So far, the mechanism still remains unknown. It no doubt leads to the lack of treatment option for this kind of disease. It is urgent to dig out the central event of NF1-associated GIST. Therefore we try to find out the somatic gene mutation existing in GIST but not in neurofibroma for the same patient. That could provide a hint for our further study.

PRSS3 and FOLR3 proteins have been reported to be associated with tumors. For example, clinical significance and expression of the PRSS3 were revealed to be related to the early development of ovarian cancer [11]. PRSS3 has also been regarded as a potential therapeutic target for metastatic prostate cancer [12]. FOLR3 has also been reported to play a role in lung cancer and uterine leiomyosarcoma [13, 14]. However, no study has indicated the possible link between GIST and the two genes. By conducting a whole exome sequencing for the comparison of expression profile difference in GIST lesion, neurofibroma lesion and peripheral blood in two NF1 patients, we find the possible relationship the two proteins present with the development of NF1-associated GIST. Furthermore, we conduct an experiment in the GIST T1 cells to confirm the vitality of the two proteins.

Materials and methods

Patients

This study was approved by the Institutional Review Board of the Zhongshan Hospital, Fudan University.

Case #1 (174568): A 57-year-old Chinese woman was admitted to the hospital for treatment of a submucosal tumor in duodenum, revealed by gastroscopy. There were no special symptoms relating to the tumor. The history of the patient, however, was complex, beginning with the diagnosis of NF1 at the age of 15 years for the presence of multiple neurofibromas and café-au-lait spots covering the whole body. No close predecessor presented any of the classical cutaneous characteristics, but her only son presented the cutaneous characteristics of NF1. She received a partial small bowel resection for perforation of the small intestine caused by a tumor; the pathologic diagnosis was small-bowel GIST. After resection, no adjuvant therapy was performed. At the time of the recent hospitalization, the patient underwent distal gastrectomy, resection of part of descending duodenum, and Billroth II style reconstruction. The main tumor, 2.0 cm in diameter, was on the wall of the duodenal bulb; three additional submucosal tumors with diameters of 0.3-0.5 cm were found in the duodenum. The histological diagnosis of these tumors was GIST. The tumors were composed of spindle cells, but there were ≤5 mitoses in 50 high-power fields. Immunohistochemical staining showed that the tumor cells expressed CD117, CD34, and DOG-1. As determined by PCR and by direct sequencing of the PCR products, there were no mutations in exons 9, 11, 13, and 17 in the KIT gene or in exons 12 and 18 in the PDGFRA gene. For this case, the GIST, NF1, and the blood samples were obtained after written consent was signed by the patient prior to adjuvant therapy.

Case #2 (171685): A 58-year-old Chinese woman was admitted to the hospital for a giant abdominal tumor. The clinical manifestation

was dull pain in upper abdomen over the previous two weeks. Blood analysis showed moderate anemia (hemoglobin level, 8.0 g/dL). An abdominal CT scan revealed a lesion, 20 cm in diameter, in the upper abdominal cavity. The lesion had a cystic center and a solid marginal area. The diagnosis, GIST, was proved by needle core biopsy and pathology assay. The case history of the patient was not complex, beginning with the diagnosis of NF1 at the age of 20 years for the presence of multiple neurofibromas and café-au-lait spots covering the whole body. No close relatives presented any of the classical cutaneous characteristics. Physical examination confirmed the presence of multiple neurofibromas, café-au-lait spots, and skin fold freckling. Based on these findings, it was diagnosed as a GIST associated with NF1. The patient first received imatinib (400 mg/d) as neoadjuvant therapy. But one month later, she developed a severe adverse reaction, and a CT image showed that the tumor had enlarged. Subsequently, an operation was performed, and the tumor, along with part of the small intestine, was resected. The postoperative course was favorable, without any major complications. The main tumor, on the wall of the intestine, was 20 cm in diameter; multiple submucosal tumors with diameters of 1-2 cm were present in the intestine. The histological diagnosis of these tumors was GISTs. The tumors were composed of spindle cells, with nuclear palisading. Mitoses were rare, as determined in 50 high-power fields. Immunohistochemical assays revealed that the tumor cells expressed CD117, CD34, and DOG-1. As determined by PCR and direct sequencing of the PCR products, there were no mutations in exons 9, 11, 13, and 17 in the KIT gene or in exons 12 and 18 of the PDGFRA gene. The blood sample and the GIST tissue samples were obtained during the operation, which was performed after the first round of imatinib therapy. Written consent was obtained from the patient.

Exome sequencing and copy number analyses

Genome DNA was extracted with the QIAamp DNA Mini Kit (Qiagen), and genomic DNA libraries were constructed following the protocols recommended by Illumina (Illumina, San Diego, CA). Whole-exome enrichment was conducted using a TruSeq Exome Enrichment Kit (Illumina). Captured DNA libraries were sequenced using the Illumina HiSeq2000 Genome Analyzer, which yielded 200 (2 X 100) base pairs from the final library fragments. Genomic DNA isolated from tumors was amplified and fragmented using a core SNP 6 reagent kit and DNase I (Affymetrix). Then the sample is hybridized onto Affymetrix GeneChip® genome-wide human SNP array 6.0 arrays and the arrays were scanned on an Affymetrix GeneChip® scanner 3000 7G. Data was initially analyzed on the Affymetrix Genotyping Console™ and subject to further in-house analysis. Raw CEL data of SNP 6.0 arrays was processed on the Genotyping Console (version 4.1.1.834, Affymetrix). Segment summary of each sample was generated by the Genotyping Console with default configuration. Then all summaries were processed by a proprietary algorithm to retrieve HUGO gene symbols of genes that are located within the segments regions, with copy number status and gene annotations. The resulting reads were aligned to the hg19 reference genome using Burrows-Wheeler Aligner (BWA). The Genome Analysis Toolkit (GATK) was applied for base quality score recalibration, insertion and/or deletions (indels) realignment, and removal of duplicates. Somatic single-nucleotide variants (SNVs) were detected using MuTect, and somatic indels were detected using Pindel. Results for SNVs and indels were combined and compared to the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) to identify novel mutations. Potential functional effects for the mutations were predicted using SnpEff, PolyPhen and PROVEAN.

Cell culture and siRNA transfection

The human gastrointestinal stromal tumor cell line, GIST-T1, were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin and maintained in an incubator with a humidified atmosphere of 5% CO_2 at 37°C. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Shanghai, China). The siRNA (GenePharma, Shanghai, China) was transfected using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The sequences of siRNA were shown as follows: NC: UUC UCC GAA CGU GUC ACG UTT, ACG UGA CAC GUU CGG AGA ATT; PRSS3-

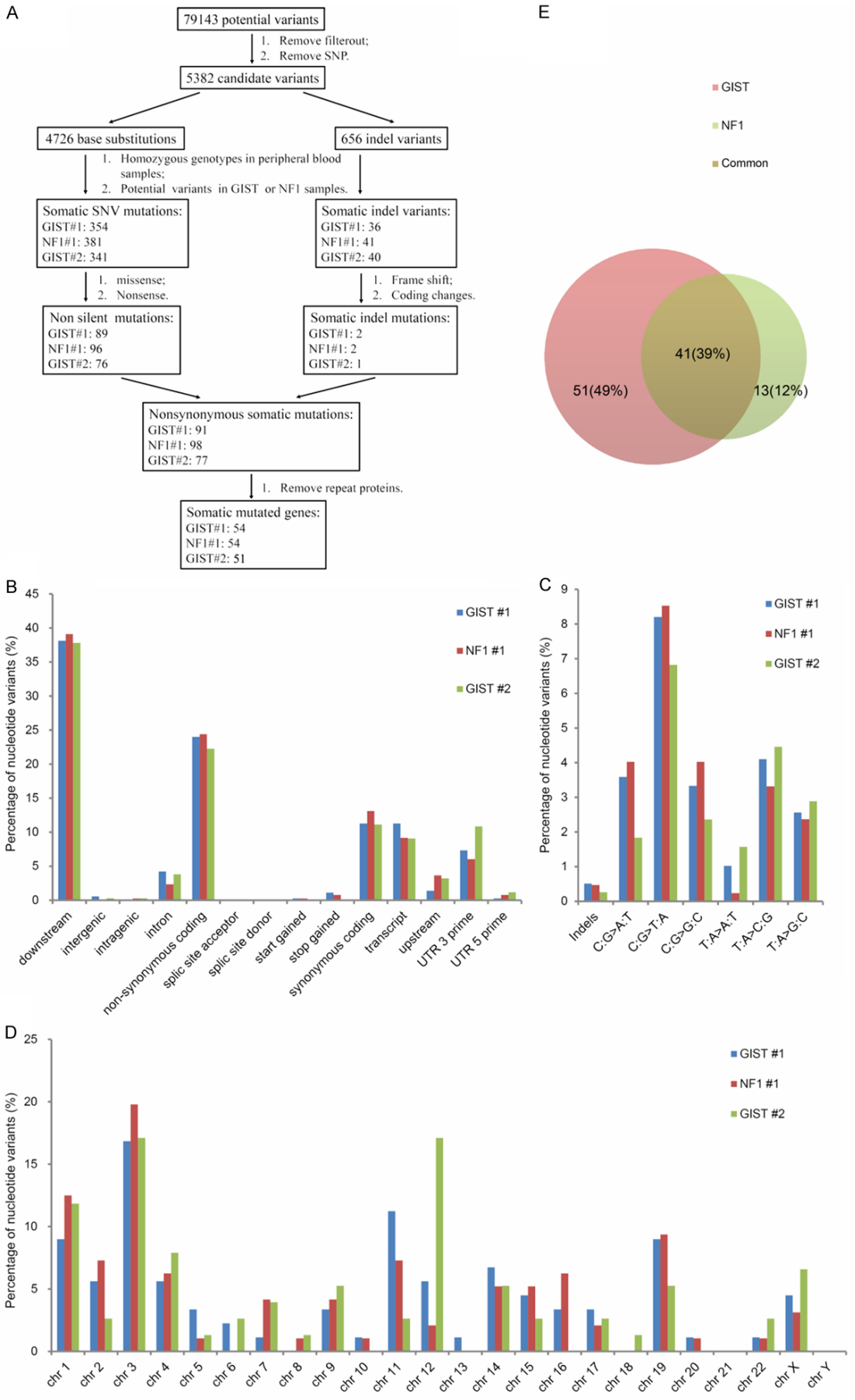
A
79143 potential variants
1. Remove filterout;
2. Remove SNP.
5382 candidate variants
4726 base substitutions
656 indel variants
1. Homozygous genotypes in peripheral blood samples;
2. Potential variants in GIST or NF1 samples.
Somatic SNV mutations:
GIST#1: 354
NF1#1: 381
GIST#2: 341
Somatic indel variants:
GIST#1: 36
NF1#1: 41
GIST#2: 40
1. missense;
2. Nonsense.
1. Frame shift;
2. Coding changes.
Non silent mutations:
GIST#1: 89
NF1#1: 96
GIST#2: 76
Somatic indel mutations:
GIST#1: 2
NF1#1: 2
GIST#2: 1
Nonsynonymous somatic mutations:
GIST#1: 91
NF1#1: 98
GIST#2: 77
1. Remove repeat proteins.
Somatic mutated genes:
GIST#1: 54
NF1#1: 54
GIST#2: 51
E
GIST
NF1
Common
41(39%)
51(49%)
13(12%)
B
Percentage of nucleotide variants (%)
GIST #1
NF1 #1
GIST #2
downstream
intergenic
intragenic
intron
non-synonymous coding
splic site acceptor
splic site donor
start gained
stop gained
synonymous coding
transcript
upstream
UTR 3 prime
UTR 5 prime
C
Percentage of nucleotide variants (%)
GIST #1
NF1 #1
GIST #2
Indels
C:G>A:T
C:G>T:A
C:G>G:C
T:A>A:T
T:A>C:G
T:A>G:C
D
Percentage of nucleotide variants (%)
GIST #1
NF1 #1
GIST #2
chr 1
chr 2
chr 3
chr 4
chr 5
chr 6
chr 7
chr 8
chr 9
chr 10
chr 11
chr 12
chr 13
chr 14
chr 15
chr 16
chr 17
chr 18
chr 19
chr 20
chr 21
chr 22
chr X
chr Y

Figure 1. A. Flowchart for the process of screening and identifying somatic mutated genes in GIST and NF1 tumors after exome sequencing; B. The distribution of somatic SNVs in gene regions; C. The spectra of somatic non-synonymous mutations in different samples; D. The distribution of somatic non-synonymous SNVs in chromosomes; E. The distribution of somatic mutated genes in GIST and NF1 tumors. The numbers of mutations are indicated in the corresponding circles.

Table 1. Somatically mutated genes in the exome sequences of two *KIT* and *PDGFRA* wild-type, NF1-associated GISTs

Gene name	Gene ID	Genomic coordinate	Patient	Allele mutation	Amino acid mutation
NF1	4763	chr17: 29585454	GIST#1	T>A	Y1067*
		chr17: 29657440	GIST#2	T>A	S1557R
PRSS3	5646	chr9: 33798574	GIST#1	G>A	S175N
			GIST#2		
FOLR3	2352	chr11: 71847122	GIST#1	C>T	H40Y
			GIST#2		
TAS2R43	259289	chr12: 11244261	GIST#1	G>C	L190V
			GIST#2		
		chr12: 11244321	GIST#1	T>G	S170R
			GIST#2		
		chr12: 11244323	GIST#1	T>C	K169R
			GIST#2		

240: CCU CCU ACC CUG GAA AGA UTT, AUC UUU CCA GGG UAG GAG GTT; PRSS3-825: GAC AGC UCC AAG GAG UUG UTT, ACA ACU CCU UGG AGC UGU CTT; FOLR3-244: GGA CUG AUC UCC UCA AUG UTT, ACA UUG AGG AGA UCA GUC CTT; FOLR3-547: GGA UGU GCC CUU AUG CAA ATT, UUU GCA UAA GGG CAC AUC CTT; FOLR3-684: GCA CCU UUG AGU CCU ACU UTT, AAG UAG GAC UCA AAG GUG CTT.

Result

To identify the genetic lesions in *KIT/PDGFRA* wild-type, NF1-assocated GISTs, genome DNA was extracted from GIST tissue, NF1 tissue, and peripheral blood from one patient (case #1). The DNA preparations were used to perform whole exome, next-generation sequencing, which allows identification of the genetic alterations in genes. For another patient (case #2), with NF1 and GIST, only GIST tissue and peripheral blood were obtained. From the three tumor samples and two peripheral blood samples, 79,143 potential variants were obtained when aligned with the hg19 reference genome sequence. 5,382 candidate variants, including 4,726 base substitutions and 656 small somatic insertion and/or deletions (indel) variants were identified (**Figure 1A**). Through a comparison of the sequencing results for tumor samples and peripheral blood samples, we proposed 354 (GIST#1), 381 (NF1#1), 341 (GIST#2) candidate somatic singlenucleotide variant (SNV) mutations and 36 (GIST#1), 41 (NF1#1), 40 (GIST#2) somatic indel variants (**Figure 1A**). In the GIST and NF1 tumor samples, the "downstream region" was the most frequent location, followed by the "non-synonymous coding region", among the somatic SNVs (**Figure 1B**). We focused on the mutations located in the gene coding exome regions. In addition, 1-2 somatic indel mutations were identified in each sample, according to sequence changes in gene coding regions. Together, there were 91 (GIST#1), 98 (NF1#1), and 77 (GIST#2) non-synonymous somatic gene mutations in DNA of the tumors (**Figure 1A**).

For non-synonymous somatic SNVs, the C: G>T: A substitution was most frequent, and the T: A>A: T transversion was most rare, in both GIST and NF1 tumors (**Figure 1C**). However, the prevalence of base-pair mutations in the two types of tumors showed different patterns. T: A mutations were more likely to be found in the GIST tumor genome, whereas NF1 tumors showed more C: G mutations (**Figure 1C**). Our results suggest that, in NF1-associated GIST tumors, predominant C: G>T: A substitution, accompanied by T: A mutations, is a characteristic of the mutation process. The distributions of non-synonymous somatic SNVs in chromosomes are shown in **Figure 1D**. Of the non-synonymous somatic mutations found in three paired tumor samples from two patients, 105 genes were identical (**Figure 1E**), whereas 51 genes (49%) were detected only in GIST samples (**Table 1** and Supplementary Table 1), 13 genes (12%) were detected only in NF1 tumors (Supplementary Table 2), and 41 genes (39%) were detected in both tumor types (Supplementary

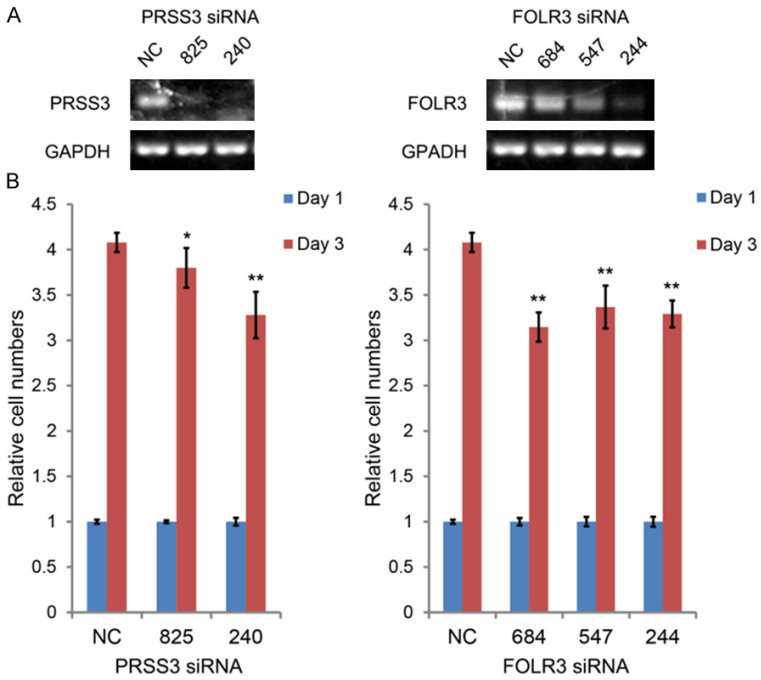

Figure 2. A. After transfected with different siRNA (50 nM) for 72 h, GIST-T1 cells were harvested for mRNA extraction; and the expression of PRSS3 and FOLR3 was determined by RT-PCR; B. GIST-T1 cells were transfected with PRSS3 or FOLR3 siRNA, and cell growth was evaluated by the CCK-8 assay. **P*<0.05; ***P*<0.01 compared with NC group (mean ± SD, Student *t* test).

Table 3), suggesting that the two types of tumors shared most mutations but the tumor-specificity also existed.

Of the 51 GIST-specific, non-silent mutated genes, in addition to the *NF1* gene, *PRSS3*, *FOLR3*, and *TAS2R43* genes were found in GIST tissues of both patients (**Table 1**). To further investigate the influence of these proteins on the progression of GISTs, specific siRNAs were used to inhibit their expressions in GIST-T1 cells (**Figure 2A**). As shown in **Figure 2B**, after endogenous PRSS3 or FOLR3 was knocked down, the growth of GIST-T1 cell was significantly reduced. However, when TAS2R43 was knocked down, the growth of GIST-T1 cell was not significantly affected. So the result was not listed. These results suggest that PRSS3 and FOLR3 play oncogenic roles in GIST tumorigenesis.

Discussion

As a tumor suppressor gene located on chromosome 17q11.2, NF1 is responsible for a protein called neurofibromin. Neurofibromin is involved in the downregulation of the rat sarcoma viral oncogene homologue (RAS)-mitogen activated protein kinase (MAPK) pathway [15]. Though the most frequent detected disease in NF1 is generally derived from nervous system, NF1 patients could sometimes be accepted into clinic for non-nervous system diseases such as GIST, phaeochromocytoma and even breast cancer [5]. Of all the non-nervous system diseases, GIST has attracted the most attention for doctors majored in general surgery.

Though defined as a non-nervous system disease in NF1, GIST may as well not be classified as simple as that in our opinion. The reason we put forward that opinion is that GIST has long been regarded as the tumor originated from Cajal cells. As is said previously, Cajal cells play a role as autonomic nerve-related gastrointestinal pacemaker cells that regulate gastrointestinal motility. On the other hand, its function is nerve-related. That prompts us to think about whether the aberrant expression of neurofibromin in NF1 patients contributes to the development of GIST. After reviewing the previous studies, we found no direct evidences were available. However, some indirect evidences were detected. Johanna analyzed the 50 GISTs derived from 15 NF1 patients microscopically and find hyperplastic foci (diffuse and focal) of the interstitial cells of Cajal that were often associated with microscopic GIST in the surrounding intestinal muscle wall [16]. Study based on autopsy indicated a higher frequency of GIST occurrence in NF1 patients compared with somatic GIST. 3 of 12 patients proved to develop GIST according to the pathology test [17]. More involving studies are urgently needed.

The first case associated with gastrointestinal neoplasms resembling leiomyomas in NF-1 patients could be dated back to 1969, when GIST has not yet been well elucidated and defined as one kind of tumor [18]. As is known to us, GIST is the most commonly seen mesen-

chymal tumor. The most important index for diagnosing GIST is the positive immunohistochemistry staining for CD117 and DOG-1. *C-KIT* or *PDGFRA* mutation has been recognized as the central event for the development of GIST. Of all the detected mutation, exon 11 leads as the most frequently affected site (60%) and exon 9 follow (15%) [2]. Besides the mutation analysis, GIST was also characterized as solitary tumor classified into three types: spindle cell-type (70%), epithelioid-type (20%) and mixed-type (10%) [2, 8]. Most of the primary tumors were solitary lesion distributing along the alimentary tract, with the stomach as the most common site (60%), followed by the small intestine (25%) [2, 15]. Though demonstrated CD117 and DOG-1 expression, NF1-associated GIST were different from the sporadic cases in many aspects [19, 20]. Based on the existing studies, all of the NF1-associated cases were detected without *c-KIT* or *PDGFRA* mutation, indicating that the molecular pathogenesis for NF1-associated cases were different from the sporadic cases [21]. NF1-associated cases could only been found in intestine. No gastric origin cases have been reported yet [20]. Primary cases tended to be multifocal and mixed-type cases were more frequently seen [20, 21]. The deep cause of the differences still remains unknown.

As a member of serine protease family, trypsin is encoded by PRSS1, PRSS2 and PRSS3. The relationship between PRSS3 and cancers has been highlight recently, especially in the study of lung cancer and ovarian cancer [12, 22, 23]. Though mainly expressed by pancreas, PRSS3 could be found in some epithelial cancers and cancer cell lines [23]. Besides, overexpression of PRSS3 has been reported to be associated with progression of cancers [22, 23]. Some studies have even put forward the opinion that over expression of PRSS3 can not only promote the cell proliferation and invasion in ovarian cancer but also act as a potential marker for predicting the metastasis. FOLR3 belongs to the folate receptor gene family and participate in the transport and binding of folate and the naturally occurring form of folic acid [24]. It is a secretory molecular expressed predominantly in hematopoietic cells [25]. Most of the studies before have focused on its function on metabolic pathway. Seldom has a study relieved how the aberrant FOLR3 could affect the occurrence of cancer, letting along the NF1-associated GIST. Our study has selected PRSS3 and FOLR3 as the keys in the development of NF1-associated GIST for the statistical analysis results derived from WES for the two NF1 patients. The cell experiment proved that those two genes played oncogenic roles in the GIST T1 cells. Our results give a primary impression that PRSS3 and FOLR3 are potential treatment targets for NF1-associated GIST. That evidence is still not enough. Our group has successfully established the PDTX models, relevant studies are underwent now in our lab [26]. More telling evidence is urgently needed.

Conclusion

In summary, through next-generation sequencing of the exome of DNA from GIST and NF1 tissue of the patients with *KIT/PDGFRA* wild-type GISTs concurrent with NF1, we identified three potential novel driver mutation genes in GISTs, which may reveal the underlying etiology of the *KIT/PDGFRA* wild-type GISTs developing in NF1 patients. The genes could be targets for intervention and/or therapy for GISTs that develop from NF1.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Somatically mutated genes in the exome sequences of GIST

Gene name	Gene ID	Genomic coordinate	Patient	Allele mutation	Amino acid mutation
PRAMEF22	653606	1:13036736	GIST#1	T>A	Y270N
RGPD3	653489	2:107049425	GIST#1	A>C	L570R
DSPP	1834	4:88537088	GIST#1	A>G	N1092D
		4:88537261	GIST#1	A>T	E1149D
ZDHHC11	79844	5:848742	GIST#1	C>T	V86I
FAM153A	285596	5:177163580	GIST#1	A>G	F145L
HLA-DRB1	3123	6:32549582	GIST#1	G>T	T135N
RAET1L	154064	6:150343211	GIST#1	A>G	M85T
TAS2R30	259293	12:11286214	GIST#1	C>G	Q210H
PARP4	143	13:25021201	GIST#1	G>A	L1080F
MUC16	94025	19:9002587	GIST#1	C>T	R13410Q
		19:9002597	GIST#1	C>T	D13407N
FRG1B	284802	20:29628251	GIST#1	A>G	N85D
RBMXL3	139804	X:114425136	GIST#1	A>G	S378G
PRAMEF16	654348	1:13497572	GIST#2	G>A	C290Y
		1:13497584	GIST#2	C>T	P294L
MST1P9	11223	1:17085006	GIST#2	C>T	G459E
RBMXL1	494115	1:89449237	GIST#2	T>A	R91S
OR2T29	343563	1:248722611	GIST#2	C>G	S61T
FER1L5	90342	2:97361378	GIST#2	T>C	S1319P
SNED1	25992	2:242011113	GIST#2	G>C	E1238Q
SORCS2	57537	4:7735143	GIST#2	G>A	G1068D
ALB	213	4:74279140	GIST#2	G>T	D133Y
FHDC1	85462	4:153896049	GIST#2	A>C	T536P
BDP1	55814	5:70848945	GIST#2	T>A	F407I
OR12D3	81797	6:29342440	GIST#2	A>G	F209L
SASH1	23328	6:148664232	GIST#2	G>A	G10E
PRSS1	5644	7:142460339	GIST#2	G>A	C121Y
MLL3	58508	7:151970840	GIST#2	C>T	S321N
PABPC1	26986	8:101727750	GIST#2	T>C	I150V
CBWD3	445571	9:70912519	GIST#2	A>C	E232D
TAS2R31	259290	12:11183475	GIST#2	G>A	R154W
		12:11183484	GIST#2	C>G	E151Q
		12:11183485	GIST#2	T>A	K150N
PTPRO	5800	12:15669792	GIST#2	T>A	Y561N
GXYLT1	283464	12:42512971	GIST#2	T>C	Y106C
FAM186A	121006	12:50745822	GIST#2	T>G	E1598A
EP400	57634	12:132445256	GIST#2	A>C	H31P
		12:132445273	GIST#2	T>C	S37P
POTEM	641455	14:20007593	GIST#2	T>C	I365M
GALC	2581	14:88452853	GIST#2	T>C	N115S
GOLGA6B	55889	15:72954595	GIST#2	A>G	K284E
FURIN	5045	15:91424716	GIST#2	C>T	H665Y
CCDC165	23255	18:8786031	GIST#2	G>A	R610H
COL5A3	50509	19:10078030	GIST#2	C>T	G1484D
ANGPTL6	83854	19:10206822	GIST#2	C>A	G140W
SIN3B	23309	19:16987383	GIST#2	G>T	E951D
ZNF285	26974	19:44901381	GIST#2	C>T	G16S
NEFH	4744	22:29885644	GIST#2	C>A	A672E
WNT7B	7477	22:46318965	GIST#2	G>A	S258L
HNRNPH2	3188	X:100667122	GIST#2	G>A	R49K
MAGEC1	9947	X:140993640	GIST#2	T>A	S150R

Supplementary Table 2. Somatically mutated genes in the exome sequences of NF1

Gene name	Gene ID	Genomic coordinate	Patient	Allele mutation	Amino acid mutation
FLG	2312	1:152280685	NF1#1	C>A	G2226V
KCNN3	3782	1:154842250	NF1#1	G>T	P159Q
		1:154842253	NF1#1	G>T	P158H
POTEF	728378	2:130832185	NF1#1	G>A	R954W
LRP1B	53353	2:141625828	NF1#1	G>A	L1330F
HSPD1	3329	2:198363406	NF1#1	C>T	G56E
ADAM29	11086	4:175899129	NF1#1	C>T	T818M
RP1L1	94137	8:10467448	NF1#1	C>T	G1387E
HOMEZ	57594	14:23744820	NF1#1	A>T	D539E
GOLGA6L10	647042	15:82637166	NF1#1	T>C	E307G
TPSAB1	7177	16:1291545	NF1#1	C>T	T115I
		16:1291598	NF1#1	G>A	V133I
		16:1291608	NF1#1	A>G	H136R
CYP2A7	1549	19:41381671	NF1#1	A>G	I420T
ZSCAN18	65982	19:58600105	NF1#1	C>G	S168T
FAM182A	284800	20:26061945	NF1#1	A>G	G40
		20:26061948	NF1#1	G>T	G100
		20:26061956	NF1#1	C>A	A103E

Supplementary Table 3. Somatically mutated genes in the exome sequences in both GISTs and NF1

Gene name	Gene ID	Genomic coordinate	Patient	Allele mutation	Amino acid mutation
FTH1	2495	11:61735080	GIST#1, NF1#1	G>T	S1*
GOLGA6L6	727832	15:20740252	GIST#1, NF1#1	C>A	E500*
		15:20740292	GIST#1, NF1#1	G>C	H486Q
		15:20740293	GIST#1, NF1#1	T>C	H486R
		15:20740294	GIST#1, NF1#1	G>T	H486N
OTOA	146183	16:21747639	GIST#1, NF1#1	G>T	E196*
GRIK3	2899	1:37319270	GIST#1, NF1#1	C>A	L386F
HRNR	388697	1:152188569	GIST#1, NF1#1	C>T	G1846S
IGFN1	91156	1:201178667	GIST#2, NF1#1	C>G	T1549R
		1:201178849	GIST#2, NF1#1	G>A	E1610K
		1:201179068	GIST#1, GIST#2, NF1#1	G>A	G1683R
		1:201180208	GIST#1, NF1#1	G>C	G2063R
		1:201180214	GIST#1, NF1#1	G>A	V2065M
		1:201180217	GIST#1, NF1#1	A>G	N2066D
HLX	3142	1:221053600	GIST#1, NF1#1	A>C	Q134P
REG3A	5068	2:79385823	GIST#1, NF1#1	T>G	H50P
ANKRD36	375248	2:97820417	GIST#1, NF1#1	T>G	L400R
		2:97820434	GIST#1, NF1#1	T>G	F406V
PRR21	643905	2:240982172	GIST#1, NF1#1	C>T	M76I
MUC4	4585	3:195505816	NF1#1	G>C	T4212S
		3:195505829	GIST#1, NF1#1	G>A	P4208S
		3:195505855	GIST#1, NF1#1	G>T	S4199Y
		3:195507323	NF#1	T>C	T3710A
		3:195507433	NF#1	G>A	A3673V
		3:195507982	NF#1	T>G	H3490P
		3:195508451	NF#1	G>T	P3334T
		3:195508835	GIST#1, GIST#2, NF1#1	C>T	A3206T
		3:195510457	NF#1	A>G	V2665A
		3:195510731	GIST#1, NF1#1	C>T	A2574T
		3:195510755	GIST#1, NF1#1	T>C	T2566A
		3:195510779	GIST#1, NF1#1	C>T	A2558T
		3:195511283	NF#1	C>G	A2390P
		3:195511285	NF#1	T>C	D2389G
		3:195511286	NF#1	C>T	D2389N
		3:195511465	GIST#1, NF1#1	G>A	A2329V
		3:195513446	NF#1	T>C	S1669G
		3:195513515	NF#1	C>T	A1646T
		3:195515008	NF#1	C>G	G1148A
CRIPAK	285464	4:1388656	GIST#1, NF1#1	C>T	R103C
		4:1388817	GIST#1, NF1#1	C>G	P173R
FRG1	2483	4:190876293	GIST#1, GIST#2, NF1#1	C>A	P140Q
		4:190878569	GIST#2, NF1#1	T>C	L150S
		4:190878571	GIST#2, NF1#1	G>A	A151T
PCDHA13	56136	5:140263173	GIST#1, NF1#1	C>A	S440R
MUC12	10071	7:100636551	NF#1	C>G	P1046A
		7:100636552	NF#1	C>T	P1046L

		7:100645731	NF#1	G>A	E3963K
LRRC4	64101	7:127670327	GIST#1, NF1#1	C>G	A123P
C9orf150	286343	9:12775880	GIST#2, NF1#1	T>G	C56G
		9:12775883	GIST#2, NF1#1	A>G	S57G
CYLC2	1539	9:105767469	GIST#1, NF1#1	A>G	K186E
PAPPA-AS1	493913	9:119160776	GIST#1, NF1#1	G>A	S97F
AGAP7	653268	10:51465138	GIST#1, NF1#1	C>T	E440K
MUC6	4588	11:1017169	GIST#1, NF1#1	G>A	P1878S
		11:1017591	GIST#1, NF1#1	C>G	R1737P
		11:1018182	GIST#1, NF1#1	G>T	T1540N
		11:1018186	GIST#1, NF1#1	G>T	P1539T
		11:1018192	GIST#1, NF1#1	C>T	V1537I
PRDM10	56980	11:129801109	GIST#1, NF1#1	T>G	E161D
KRT75	9119	12:52822136	GIST#1, NF1#1	C>G	R429P
KRT8	3856	12:53298675	GIST#2, NF1#1	A>C	S109A
CR383656.1		14:19378189	GIST#1, NF1#1	G>T	R199L
POTEG	404785	14:19563409	GIST#1, NF1#1	C>T	A308V
TEP1	7011	14:20876580	GIST#1, GIST#2, NF1#1	G>A	H7Y
		14:20876588	GIST#1, GIST#2, NF1#1	A>G	L4P
TPSB2	64499	16:1279438	GIST#1, NF1#1	C>T	A85T
ZFPM1	161882	16:88599698	GIST#1, NF1#1	G>C	E444D
NCOR1	9611	17:16068377	GIST#1, NF1#1	C>G	K178N
CDC27	996	17:45234432	GIST#1, NF1#1	G>T	S169Y
MAP1S	55201	19:17837593	GIST#1, NF1#1	C>G	P441R
ZNF208	7757	19:22155725	GIST#1, NF1#1	C>G	W604S
		19:22156826	NF#1	C>A	G337V
ZNF676	163223	19:22363701	GIST#1, NF1#1	G>A	A273V
		19:22363702	GIST#1, NF1#1	C>T	A273T
ZNF492	57615	19:22836805	GIST#1, NF1#1	G>A	A40T
ZNF579	163033	19:56089870	GIST#1, NF1#1	G>C	A379G
POM121L1P	25812	22:22985728	GIST#1, NF1#1	C>T	V277I
ZXDB	158586	X:57618845	GIST#1, GIST#2, NF1#1	G>A	E122K
		X:57618849	GIST#1, GIST#2, NF1#1	A>C	E123A
FMR1	2332	X:147010263	GIST#1, GIST#2, NF1#1	A>C	K119N
RBFOX3	146713	17:77111776	GIST#2	C>G	A8P