

Original Article

Targeted next-generation sequencing of cancer genes identified frequent *TP53* and *ATRX* mutations in leiomyosarcoma

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Abstract: Leiomyosarcoma is an aggressive soft tissue sarcoma with poor patient survival. The genetic changes of leiomyosarcoma remain to be discovered. In this study, we analyzed the genetic changes of 44 cancer-related genes by using next-generation sequencing in 54 leiomyosarcomas. We identified *TP53* mutations in 19 of the 54 tumors (35%) and *ATRX* mutations in 9 of the 54 tumors (17%). The *TP53*-mutated leiomyosarcomas were limited to female patients ($P = 0.006$). All but 2 of the *TP53*-mutated leiomyosarcomas were located in the uterus ($n = 11$) or retroperitoneum ($n = 6$). The *ATRX* mutations were associated with poorly differentiated leiomyosarcomas ($P = 0.028$) and the presence of tumor necrosis ($P = 0.015$). Kaplan-Meier survival analysis showed that patients with *ATRX*-mutated leiomyosarcomas had worse overall survival than did patients with *ATRX*-wild-type leiomyosarcomas. All of the *ATRX*-mutated leiomyosarcomas showed the alternative lengthening of telomere phenotype. The *ATRX* mutations did not correlate with *ATRX* protein expression, as detected using immunohistochemistry. In conclusion, we identified loss of function of the p53 and *ATRX* pathways being the main mechanisms for leiomyosarcomas. The molecular mechanisms may provide new opportunities to treat these aggressive neoplasms.

Keywords: Leiomyosarcoma, *TP53*, *ATRX*, alternative lengthening of telomeres

Introduction

Soft tissue sarcoma can be broadly categorized as sarcomas with simple karyotypes that frequently involve the fusion or mutation of specific genes and sarcomas with complex karyotypic abnormalities that frequently have numerical chromosomal changes, non-recurrent translocations, and deletions [1]. Sarcomas with complex karyotypes account for approximately two-thirds of sarcoma cases. They frequently harbour genetic alterations in the p53 pathway, such as inactivating *TP53* mutations, homozygous deletions of *CDKN2A*, and *MDM2* amplifications [2, 3]. Other than the p53 pathway, the pathogenesis of sarcomas with complex karyotypes remains largely unknown.

Leiomyosarcoma is a prototypical example of a sarcoma with a complex karyotype. Excluding those of uterine origin, it accounts for 5%-10% of all sarcomas [1]. Leiomyosarcomas are aggressive neoplasms associated with poor patient survival. Leiomyosarcomas are tumors with complex karyotypes without recurrent chromosomal aberrations [4, 5]. The current treatment modalities for leiomyosarcomas include surgical debulking, radiotherapy, and chemotherapy regimens that frequently include gemcitabine and docetaxel [6, 7]. However, no clear survival benefit has been proven for chemotherapy in metastatic tumors, and most patients eventually die from the disease [8, 9]. The outcome is particularly grave for deeply seated, large tumors and tumors associated with the

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Table 1. The 44 cancer-related genes included in this study

<i>AKT1</i>	<i>CDH1</i>	<i>H3F3A</i>	<i>MUTYH</i>	<i>TERT</i> promoter
<i>ALK</i>	<i>CDKN2A</i>	<i>HIST1H3B</i>	<i>NFE2L2</i>	<i>TP53</i>
<i>APC</i>	<i>CHEK2</i>	<i>HRAS</i>	<i>NRAS</i>	<i>TSC2</i>
<i>ARID1A</i>	<i>CTNNB1</i>	<i>IDH1</i>	<i>PALB2</i>	<i>VHL</i>
<i>ARID2</i>	<i>DAXX</i>	<i>IDH2</i>	<i>PIK3CA</i>	
<i>ATM</i>	<i>DICER1</i>	<i>JAK1</i>	<i>PRKACA</i>	
<i>ATRX</i>	<i>EGFR</i>	<i>KIT</i>	<i>PRKAR1A</i>	
<i>BRAF</i>	<i>FGFR3</i>	<i>KRAS</i>	<i>RNF43</i>	
<i>BRCA1</i>	<i>GNAQ</i>	<i>MED12</i>	<i>SMAD4</i>	
<i>BRCA2</i>	<i>GNAS</i>	<i>MEN1</i>	<i>STK11</i>	

Table 2. Clinicopathological features of 54 leiomyosarcomas

	<i>n</i> = 54
Sex	
Male	11 (20%)
Female	43 (80%)
Age (mean ± SD)	53.7±12.8
Tumor site	
Uterus	24 (44%)
Retroperitoneum	11 (20%)
Others	19 (35%)
Tumor size (cm) (mean ± SD)	10.5±5.0
Cell morphology	
Spindle	36 (67%)
Epithelioid/pleomorphic	18 (33%)
Mitotic activity	
1-9/HPF	8 (17%)
10-19/HPF	26 (55%)
> 19/HPF	13 (40%)
Tumor differentiation	
Well/Moderately	28 (60%)
Poorly	19 (40%)
Necrosis	
Absent	8 (17%)
Present	39 (83%)

great vessels. Further study of the pathogenesis and cell biology of these tumors is necessary for developing more effective treatment modalities.

The genetic changes of leiomyosarcoma remain to be discovered. *TP53* mutation was identified in 16 of 37 extrauterine leiomyosarcomas [10]. A clinical correlation study indicated that tumors with *TP53* mutations have a higher his-

tologic grade or stage at presentation [10]. Ito et al. also reported *TP53* mutations in 39% of leiomyosarcomas [11]. *MED12*, a gene commonly mutated in uterine leiomyoma, were found to be mutated in only 3 of 32 uterine leiomyosarcomas [12]. Whether other genes are mutated remains unknown. In this study, we analyzed the genetic changes of 44 cancer-related genes by using next-generation sequencing and identified frequent mutations of *TP53* and *ATRX* in leiomyosarcomas. The clinicopathological significance of mutations in these 2 genes was also explored.

Materials and methods

Tumor samples

A total of 54 cases of leiomyosarcoma from various sites with available formalin-fixed and paraffin-embedded tissue blocks were retrieved from the archives of the Department of Pathology, National Taiwan University Hospital. Histological and immunohistochemical sections were reviewed to confirm the diagnoses. This study was approved by the Research Ethics Committee of National Taiwan University Hospital, and the specimens were anonymous and analyzed in a blind manner.

Immunohistochemistry and telomere-specific fluorescent in situ hybridization

The detailed methods of the immunohistochemistry and telomere-specific fluorescent in situ hybridization were described previously [13]. The immunohistochemical staining was performed as previously described using an *ATRX* antibody (1:500; Sigma Aldrich, St. Louis, MO, USA). An FITC-labelled PNA probe (Panagene, Daejeon, South Korea) was used for the telomere-specific fluorescent in situ hybridization.

Design of the oligonucleotide probe for capture of cancer-related genes

The capture probe was designed to enrich the open reading frame of 43 cancer-related genes and the *TERT* promoter (-150 to -350), with the sequence being taken from UCSC hg19, NCBI build 37. A list of the genes analyzed is shown in **Table 1**. The NimbleGen Sequence Capture design algorithm was used with 50-105 mer probes (Roche NimbleGen, Madison, WI, USA); a total of 2,100,000 capture probes were used

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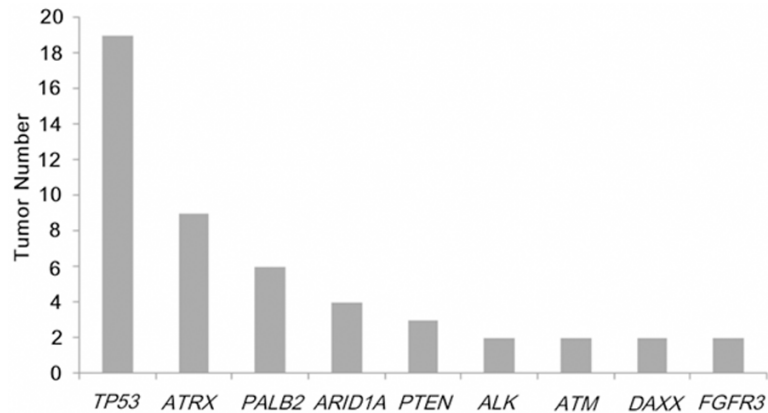


Figure 1. The tumour numbers of each mutated gene. Only genes mutated in 2 or more tumours were included.

for the capture reactions to ensure high-performance captures.

Library and capture probe hybridization

Double-stranded DNA extracted from formalin-fixed, paraffin-embedded samples was quantified using a PicoGreen fluorescence assay employing the provided standard (Life Technologies, Carlsbad, CA, USA); 100-1.0 ug of dsDNA in 50 μ L of TE Buffer (10 mM Tris-HCl (pH 7.6), 0.1 mM EDTA) was fragmented to 200-550 bp by using sonication. Shotgun libraries were prepared using the KAPA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA), which contains mixes for end repair and dA addition and ligation, by performing the 'with-bead' protocol to maximise reproducibility and library yield. Indexed (6-bp barcodes) sequencing libraries were amplified using PCR for 9-13 cycles; size selection was performed to remove undesired DNA fragments. A total of 1.0 ug of the shotgun sequencing library, adaptor-specific blocker DNA, and Cot DNA were lyophilised in a 1.5-mL tube and suspended in a hybridization buffer, and heat denatured at 95°C for 10 min before adding the bait-set reagent. The mixture was applied to hybridization-based capture probes for 72 h at 47°C. The hybridised capture probes were then washed using a SeqCap EZ Hybridization and Wash Kits (Roche NimbleGen) at 47°C and at room temperature, respectively. The biotinylated bait was further pulled down using dynabeads M-270 Streptavidin (Life Technologies). The captured DNA was amplified through LM-PCR amplification by using the SeqCap EZ Accessory Kit v2 (Roche NimbleGen), and the PCR prod-

ucts were purified using the QIA Quick Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

Quantification and qualification of captured DNA samples

Purified amplification products were eluted in 50 μ L of molecular grade water, with the quantity and quality of the products being measured using a PicoGreen fluorescence assay and 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 1000/7500 chip. Moreover, real-time quantitative PCR assays with 4 control loci were used to estimate the relative fold enrichment and assess the capture success. The reactions were performed using a LightCycler 480 system (Roche NimbleGen) according to the manufacturer's instructions.

Next-generation sequencing

Post-ligation-mediated PCR products were quantified using PicoGreen dsDNA quantification reagent. The libraries were normalised to 4.0 nM and applied to Illumina MiSeq (Illumina, San Diego, CA, USA), according to the manufacturer's protocols. On completion of the run, the data were base called and demultiplexed on the instrument (Illumina FASTQ 1.9 files with Phred+33 encoding), and the FASTQ format files were used for downstream analysis.

Bioinformatics analysis

The sequence data were mapped to the human genome (hg19) using the BWA aligner 0.77. Local-alignment optimisation was performed using the GATK 1.6. Variant calling was performed only in the genomic regions that had been targeted by the Illumina VariantStudio testing and visualisation.

Confirmatory sanger sequencing

All of the sequence variations of the TP53 and ATRX genes detected using next-generation sequencing were confirmed using Sanger sequencing. In summary, the genomic DNA was extracted using a QIAamp DNA FFPE Tissue Kit

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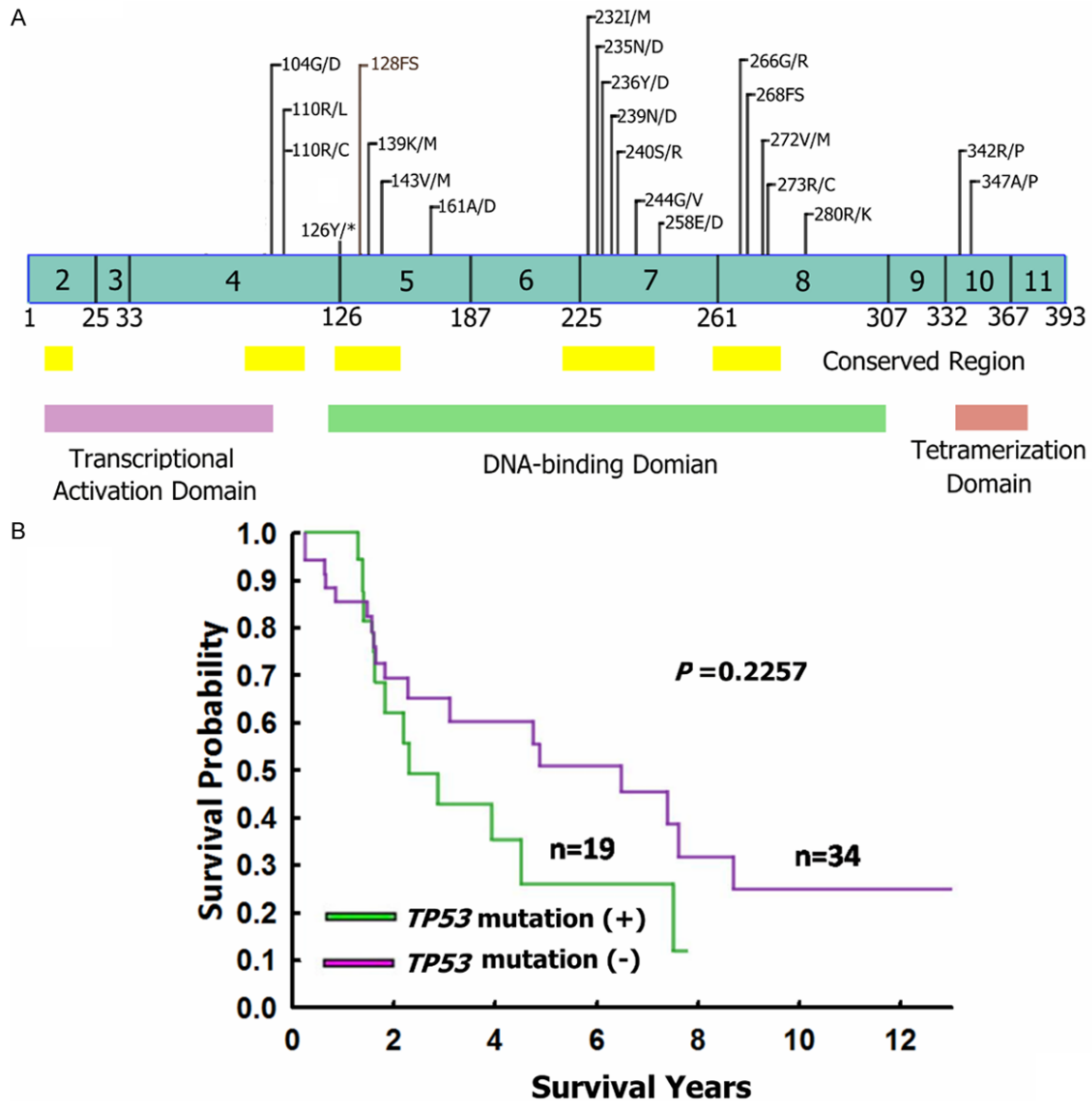


Figure 2. Mutations of *TP53* in leiomyosarcomas. A. The distribution of *TP53* mutations in leiomyosarcomas. *: stop codon; FS: Frameshift mutation. B. Kaplan-Meier survival analyses of patients with *TP53*-mutated and *TP53*-wild-type leiomyosarcomas.

(Qiagen). The target sequences were PCR-amplified. After purification, direct sequencing was performed using an automated ABI 3770 sequencer (Applied BioSystems, Foster City, CA, USA).

Statistical analysis

The data analyses were conducted using Epi Info Version 3.3.2 software (Centres for Disease Control and Prevention) and SPSS Version 19.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test. Con-

tinuous variables were analyzed using the Student *t* test. Survival curves were generated using the Kaplan-Meier method, and differences were calculated using the log-rank test. All statistical results were considered significant at a 2-tailed *P* value of < 0.05.

Results

Patients and tumor samples

A total of 54 cases of leiomyosarcoma were examined. **Table 2** summarises the clinical information. Forty-three of the patients were

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Table 3. Relation of clinicopathological features with TP53 and ATRX mutations in leiomyosarcoma

	TP53 mutation		P value	ATRX mutation		P value
	(+)	(-)		(+)	(-)	
	N (%)	N (%)		N (%)	N (%)	
Sex			0.006			0.44
Male	0 (0%)	11 (31%)		1 (11%)	10 (22%)	
Female	19 (100%)	24 (69%)		8 (88%)	35 (78%)	
Tumor site			0.018			0.085
Uterus	11 (58%)	13 (37%)		7 (78%)	17 (38%)	
Retroperitoneum	6 (32%)	5 (14%)		1 (11%)	10 (22%)	
Others	2 (11%)	17 (49%)		1 (11%)	18 (40%)	
Age (mean ± SD)	54.8±10.8	53.1±13.9	0.651	54.46±12.9	53.6±12.9	0.651
Tumor size (cm) (mean ± SD)	12.8±5.6	9.1±4.0	0.012	13.4±4.1	10.7±5.0	0.097
Cell morphology			0.840			1.000
Spindle	13 (68%)	23 (66%)		6 (67%)	30 (67%)	
Epithelioid/pleomorphic	6 (32%)	12 (34%)		3 (33%)	15 (30%)	
Mitotic activity			0.302			0.180
1-9/10 HPF	1 (6%)	7 (12%)		0 (0%)	8 (21%)	
10-19/10 HPF	11 (65%)	15 (52%)		4 (50%)	22 (56%)	
>19/10 HPF	5 (29%)	8 (36%)		4 (50%)	9 (23%)	
Tumor differentiation			0.937			0.028
Well/Moderately	10 (59%)	18 (60%)		2 (25%)	26 (67%)	
Poorly	7 (41%)	12 (40%)		6 (75%)	13 (33%)	
Necrosis			0.126			0.015
Absent	1 (19%)	7 (0%)		0 (0%)	8 (21%)	
Present	16 (81%)	23 (100%)		8 (100%)	31 (79%)	
ATRX expression			0.167			0.207
Lost	9 (47%)	10 (29%)		5 (55%)	15 (33%)	
Intact	10 (53%)	25 (71%)		4 (44%)	30 (67%)	
ALT			0.163			0.008
Negative	5 (26%)	16 (46%)		0 (0%)	21 (47%)	
Positive	14 (74%)	19 (54%)		9 (100%)	24 (53%)	

women; 24 of the cases originated in the uterus, and 30 of the cases had extrauterine origins. The most common extrauterine site was the retroperitoneum ($n = 11$). The remaining 19 cases originated in various other sites (intra-abdominal: $n = 7$; lower extremities: $n = 5$; upper extremity, skin, chest wall, thyroid, back, paraspinal region, thoracic cavity: each $n = 1$). The patients' overall mean and median ages were 53.7 and 53.8 years, respectively (range: 28-88 years); the mean ages of the patients with tumors in the uterus and in extrauterine sites were 51 and 56 years, respectively. The tumor sizes were known in 44 cases and ranged from 1.5 to 23 cm. The overall mean and median tumor sizes in the cases were 10.5 and 10 cm, respectively.

A summary of the histological features is shown in **Table 2**. Of the tumors, 36, 12, and 6 were composed predominantly of spindle, epithelioid, and pleomorphic cells, respectively. To simplify statistical analysis, the epithelioid and pleomorphic cell morphologies were combined as a single feature. Because none of the tumors had $\geq 50\%$ tumor necrosis and because there were relatively few well-differentiated tumors in our series, these parameters were also simplified to 'absent' or 'present' and 'well/moderately differentiated' or 'poorly differentiated', respectively. The tumors exhibiting predominantly epithelioid or pleomorphic cell morphologies occurred mostly in the uterus (8 and 3 cases, respectively). In 7 cases, only sections of metastatic tumors or small biopsy samples

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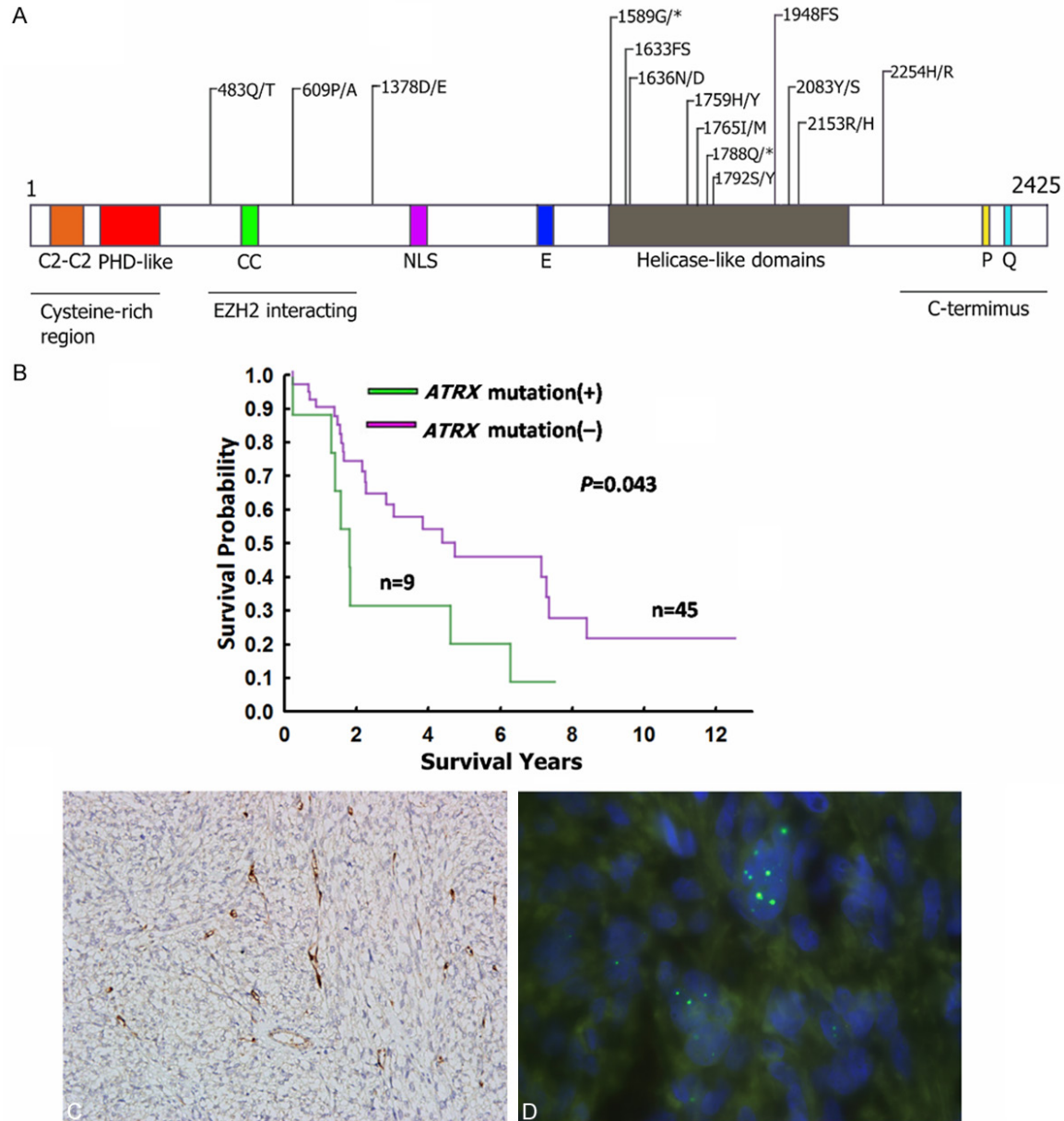


Figure 3. Mutations of *ATRX* in leiomyosarcomas. (A) The distribution of *ATRX* mutations in leiomyosarcomas. Two patients had the p.G1589* mutation. *: stop codon; FS: Frameshift mutation; CC: Coiled-coil domain; NLS: Nuclear localization signal; E: Negative tract; P: P domain; Q: Q domain. (B) Kaplan-Meier survival analyses of patients with *ATRX*-mutated and *ATRX*-wild-type leiomyosarcomas. (C) Immunostaining demonstrated the loss of *ATRX* expression in a leiomyosarcoma. (D) Telomere-specific fluorescent in situ hybridization revealed the presence of large and bright signals. (C) and (D) were taken from a case with *ATRX* mutation.

were available for histological review and were not graded.

Prevalence of mutations among the 44 genes analyzed

An adequate library was obtained from 54 formalin-fixed, paraffin-embedded tumor samples

for subsequent sequencing. The mean coverage of each target region was 425×. False positive sites were removed under the following filter conditions, as determined using Integrative Genomics Viewer software: a mean coverage > 100×, each variant coverage > 20×, a variant frequency of each sample > 5%, and $P < 0.01$; in the coding region (except for the *TERT* pro-

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moter), not a synonymous mutation and not strand-biased variants, by using the Integrative Genomics Viewer (IGV) software. Subsequently, these variants were searched in the 1000 Genome database was searched for these variants to distinguish between SNPs and mutations.

In the analyzed leiomyosarcoma samples, mutations were identified in 22 of 44 genes. The genes with mutations in more than 2 tumors are shown in **Figure 1**. Only 3 genes-*TP53*, *ATRX*, and *PALB2*-had mutation frequencies higher than 10%.

In the 54 tumors, *TP53* was mutated in 19 (35%), *ATRX* in 9 (17%), and *PALB2* in 6 (11%). The 6 *PALB2* mutations were p.C1127R (c.3379 A>G), p.E1018D (c.3054 C>G), p.T1012/I (c.3035 G>A), p.R825T (c.2674 C>G), p.I309V (c.925T>C), and p.D498Y (c.1492 C>A). Because only 6 tumors harboured *PALB2* mutations, the following sections focus on the clinicopathological significance of the *TP53* and *ATRX* mutations.

Clinicopathological significance of the TP53 mutation in leiomyosarcoma

Figure 2A shows the locations of the *TP53* mutations in the leiomyosarcomas. All of the mutations were located in exons 4-10, mainly in the DNA-binding domains and mostly in the evolutionary-conserved regions [14]. Three leiomyosarcomas had 2 mutations. Most of the mutations were missense mutations, but one nonsense mutation and 2 frameshift mutations were also detected.

The correlations of the *TP53* mutation with clinicopathological features are shown in **Table 3**. The *TP53*-mutated leiomyosarcomas were limited to female patients ($P = 0.006$). All but 2 of the *TP53*-mutated leiomyosarcomas were located in the uterus ($n = 11$) or retroperitoneum ($n = 6$), whereas 49% of the *TP53*-wild-type leiomyosarcomas were located in body sites other than the uterus or retroperitoneum ($P = 0.018$). *TP53* mutations were also associated with larger tumor size (12.8 ± 5.6 versus 9.1 ± 4.0 , $P = 0.012$). The *TP53* mutation did not correlate with patient age or survival, cell morphology, mitotic activity, tumor differentiation, or tumor necrosis (**Table 3** and **Figure 2B**).

Clinicopathological significance of ATRX mutation in leiomyosarcoma

Figure 3A shows the locations of the *ATRX* mutations in the leiomyosarcomas. Most of the mutations were located in the helicase-like domain. Most of the mutations were missense mutations, but 2 nonsense mutations and 2 frameshift mutations were also detected. Two tumors harboured 2 mutations. One tumor harboured 5 mutations (p.S1792Y, p.I1765M, p.H1759Y, p.Q1788*, and p.P609A).

The correlations of the *ATRX* mutation with clinicopathological features are shown in **Table 3**. *ATRX* mutations were associated with poor differentiation ($P = 0.028$) and the presence of tumor necrosis ($P = 0.015$). The *ATRX* mutation did not correlate with patient sex or age, tumor site or size, cell morphology, or mitotic activity. The Kaplan-Meier survival analysis showed that the patients with *ATRX*-mutated leiomyosarcomas had worse overall survival than did patients with *ATRX*-wild-type leiomyosarcomas (**Figure 3B**).

Correlation with ATRX mutation and alternative lengthening of telomeres (ALT)

Previous studies have shown that *ATRX* mutations are highly correlated with the loss of *ATRX* expression and the ALT phenotypes in pancreatic neuroendocrine tumor and glioma [15, 16]. Therefore, we performed immunostaining for *ATRX* protein and telomere-specific fluorescent in situ hybridization for all of the tumors. *ATRX* expression was lost in 19 leiomyosarcomas (35%). ALT was detected in 33 leiomyosarcomas (61%). Although the *ATRX* mutation did not correlate with the loss of *ATRX* expression, all 4 tumors with nonsense and frameshift mutations were immunonegative for *ATRX*. All of the *ATRX*-mutated leiomyosarcomas showed the ALT phenotypes ($P = 0.008$). **Figure 3C** and **3D** show the results of the immunostaining and telomere-specific fluorescent in situ hybridization of an *ATRX*-mutated leiomyosarcoma.

Discussion

In this paper, we present a parallel sequencing analysis of 44 cancer-related genes from a collection of 54 leiomyosarcomas. Our work represents the first systematic, large-scale sequencing analysis of leiomyosarcomas. We found that

TP53 and *ATRX* mutations in leiomyosarcoma

TP53 and *ATRX* were frequently mutated in the leiomyosarcomas.

Mutations of *TP53* in leiomyosarcomas have been reported previously [10, 11]. Our study showed that *TP53*-mutated leiomyosarcomas were limited to female patients and that all but 2 of the *TP53*-mutated leiomyosarcomas were located in the uterus or retroperitoneum. By contrast, Konomoto et al. showed *TP53* mutations in 8 of 15 superficial-type extrauterine leiomyosarcomas and 8 of 22 deep-type extrauterine leiomyosarcomas. The reason for this discrepancy is unknown. Advances in sarcoma classification since the 1998 study of Konomoto et al. may change the diagnosis of some of their cases. The *TP53* mutation rate of Ito's study (39%) was similar to that of our study. Pérot et al. showed mutations of *TP53* coding regions in 15 of 35 leiomyosarcomas (42%) [17]. However, the sex distribution and tumor sites were not mentioned in these 2 studies. Our finding that *TP53* mutations were highly correlated with the uterine or retroperitoneal leiomyosarcomas in female patients supports the recent hypothesis that uterine and retroperitoneal leiomyosarcomas in female patients have similar histogenesis. A cluster analysis of immunohistochemical markers showed that uterine and retroperitoneal leiomyosarcomas in female patients shared similar expression features for the estrogen and progesterone receptors and WT1 [18]. Uterine and retroperitoneal leiomyosarcomas are typically larger than their trunk and extremity counterparts [19], which may account for the size difference between *TP53*-mutated and *TP53*-wild-type leiomyosarcomas.

ALT is a major telomere-maintaining mechanism for sarcomas with complex karyotypic abnormalities [20]. *ATRX* and *DAXX* form a dimer that acts as a histone chaperone to deposit histone variant H3.3 to GC-rich regions of the genome, including the telomeres, and plays a critical role in telomere stability [21-23]. It is hypothesized that dysfunction of the dimer leads to telomere instability, increased telomere homologous recombination, and ultimately alternative lengthening of telomeres. Consistent with previous reports on pancreatic neuroendocrine tumors and glioma, all of the leiomyosarcomas with *ATRX* mutations in the present study were positive for the ALT phenotypes.

However, 15 leiomyosarcomas in our study showed a loss of *ATRX* expression without somatic mutations being identified in those cases, indicating that other mechanisms may be responsible for the loss of *ATRX* function in these tumors. According to provisional data from The Cancer Genome Atlas (<http://cancergenome.nih.gov/>), 7.4% (7/56) of the sarcomas harboured homozygous *ATRX* deletions, which might partly account for the loss of *ATRX* expression in some of the cases.

In most tumors, only one mutation in the *TP53* or *ATRX* gene was identified. The dominant negative effect of the mutant p53 over the wild-type p53 may cause the inactivation of the p53 tetramer complex [24]; consequently, monoallelic mutation may abolish the tumor suppressor activity of p53. The *ATRX* gene is located in the X chromosome. In female cells, one X chromosome is inactivated through methylation [25]. This Lyonization effect may have inactivated the wild-type allele of the *ATRX* genes in the tumors.

We detected 6 *PALB2* mutations in our cohorts. *PALB2* has a critical function in DNA damage repair [26]. Germline *PALB2* mutations predispose individuals to a high risk of developing familial breast cancer [27]. In a nation-wide epidemiological study in Sweden, leiomyosarcoma was associated with maternal breast cancer [28]. Additional large-scale studies should be performed to determine whether the *PALB2* mutation underlies the genetic mechanism for these familial breast cancer leiomyosarcoma cases.

In conclusion, we found frequent *TP53* and *ATRX* mutations in leiomyosarcomas. The *TP53* mutations were predominantly identified in the uterine and retroperitoneal leiomyosarcomas in female patients, indicating that leiomyosarcomas in those 2 sites share similar pathogenesis. The *ATRX* mutations were highly correlated with the ALT phenotypes. Recently, ataxia telangiectasia and Rad3-related kinase have been shown to play crucial roles in homologous recombination and ALT, with inhibitions of this kinase causing apoptosis of cancer cells with alternative telomere lengthening [29]. Identifying *ATRX* mutations in leiomyosarcomas may provide new opportunities for treating these aggressive neoplasms.

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Abbreviations

ALT, alternative lengthening of telomeres.

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