Potential application of genomic profiling for the diagnosis and treatment of patients with sarcoma

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Received March 31, 2020; Accepted December 14, 2020

DOI: 10.3892/ol.2021.12614

Abstract. Sarcomas represent a heterogeneous group of mesenchymal malignancies arising at various locations in the soft tissue and bone. Though a rare disease, sarcoma affects ~200,000 patients worldwide every year. The prognosis of patients with sarcoma is poor, and targeted therapy options are limited; therefore, accurate diagnosis and classification are essential for effective treatment. Sarcoma samples were acquired from 199 patients, in which *TP53* (39.70%, 79/199), *CDKN2A* (19.10%, 38/199), *CDKN2B* (15.08%, 30/199), *KIT* (14.07%, 28/199), *ATRX* (10.05%, 20/199) and *RB1* (10.05%, 20/199) were identified as the most commonly mutated genes (>10% incidence). Among 64 soft-tissue sarcomas that were unclassified by immunohistochemistry, 15 (23.44%, 15/64) were subsequently classified using next-generation sequencing (NGS). For the most part, the sarcoma subtypes were evenly distributed between male and female patients, while a significant association with sex was detected in leiomyosarcomas. Statistical analysis showed that osteosarcoma, Ewing's sarcoma, gastrointestinal stromal tumors and liposarcoma were all significantly associated with the patient age, and that angiosarcoma was significantly associated with high tumor mutational burden. Furthermore, serially mutated genes associated with myxofibrosarcoma, gastrointestinal stromal tumor, osteosarcoma, liposarcoma, leiomyosarcoma, synovial sarcoma and Ewing's sarcoma were identified, as well as neurotrophic tropomyosin-related kinase (*NTRK)* fusions of *IRF2BP2-NTRK1*, *MEF2A-NTRK3* and *ITFG1-NTRK3*. Collectively, the results of the present study suggest that NGS-targeting provides potential new biomarkers for sarcoma diagnosis, and may guide more precise therapeutic strategies for patients with bone and soft-tissue sarcomas.

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Abbreviations: NGS, next-generation sequencing; FISH, fluorescence *in situ* hybridization; GA, genomic alteration; GIST, gastrointestinal stromal tumor; SNV, single nucleotide variant; Indel, insertion-deletion polymorphism; CNV, copy number variation; TMB, tumor mutational burden; DFSP, dermatofibrosarcoma protuberans; TMB-H, high tumor mutational burden; ICPI, immune checkpoint inhibitor

Key words: sarcoma, diagnosis, next-generation sequencing, genomic profiling, tumor mutational burden, biomarker

Introduction

Sarcoma is a rare malignant tumor that frequently occurs in, or originates from, the bone, cartilage or connective tissue (1). Globally, almost 200,000 patients are affected by sarcoma each year (2). The prognosis of patients is poor, and the choice of approved targeted drugs is somewhat limited (3,4). Surgery is currently the primary treatment option for most sarcomas, but local recurrence does occur (5). Targeted molecular therapies have yielded improved clinical outcomes (6-8). However, due to diagnostic difficulties, bone and soft-tissue sarcomas are often only diagnosed at the advanced stage, resulting in a 50-60% 5-year survival rate (9,10). Therefore, a more accurate system for sarcoma diagnosis and classification is urgently required.

Sarcoma includes soft-tissue sarcomas and primary bone sarcomas (11). Soft-tissue sarcomas comprise >50 subtypes (12), and the most frequently observed subtypes include liposarcoma, leiomyosarcoma, undifferentiated soft-tissue sarcoma, fibrosarcoma and synovial sarcoma (13). Primary bone sarcoma subtypes include Ewing's sarcoma and osteosarcoma (14). Histopathological examination, such as the analysis of histological sections and fluorescence *in situ* hybridization (FISH), are still the only available methods for the accurate diagnosis of sarcomas. However, due to their

rarity and diversity, the classification of sarcomas remains a challenge. The identification and application of potential biomarkers is a convenient, rapid and accurate strategy for identifying sarcoma subtypes, and is conducive to improving diagnosis and prognostic prediction (15-18).

With continuous developments in molecular biology, next-generation sequencing (NGS) technology has enabled more accurate and efficient molecular characterization. The Cancer Genome Atlas and the International Cancer Genome Consortium have characterized the genome and genomic alterations (GAs) of most types of cancer (19,20), and a number of recent studies have focused on the molecular profiling of sarcomas (21,22). Sarcomas can be divided into the following subgroups according to genetic heterogeneity: i) Gene fusions; ii) genomic amplifications; and iii) extensive combinations of genomic imbalances and point mutations (23-25). There is also evidence to suggest that some sarcomas possess unique molecular characteristics, such as the *SYT-SSX* fusion in synovial sarcoma, the *EWS-ATF1* fusion in clear cell sarcoma, and the *EWSR1-FLI1* fusion in Ewing's sarcoma (26-28). Specific molecular characterizations not only assistant in the classification of sarcoma, but can also guide treatment programs. For example, imatinib has demonstrated good efficacy in gastrointestinal stromal tumor (GIST) patients with *KIT*, *PDGFRA*, *CSF1* and *ABL* mutations (29-31); since 2013, palbociclib has undergone phase II clinical trials in liposarcoma patients with *CDK4* mutations (32). Therefore, a clear classification system and a precise molecular description of sarcoma subtypes are necessary for subsequent diagnosis and treatment.

The present study aimed to identify GAs for the mutational profiling of 199 patients with sarcoma (both soft-tissue and primary bone sarcoma). By comparing molecular-based classification with traditional immunohistochemical categorization, the accuracy and necessity of NGS technology for sarcomas classification was confirmed. The results provide comprehensive and accurate information of GAs, which suggest novel biomarkers for sarcoma diagnosis that may guide precise therapeutic strategies for patients with bone and soft-tissue sarcomas.

Materials and methods

Patient enrollment and sample collection. The present study was approved by the Ethics Committees of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China) and the First Affiliated Hospital of Sun Yat Sen University (Guangzhou, China). A total of 199 patients with sarcoma were enrolled between January 1, 2008 and December 31, 2018. Tumor tissues were collected from patients, fixed in formalin and embedded in paraffin. Matched blood samples were collected as controls for GA detection.

Identification of GAs and measurement of tumor mutational burden (TMB). Total DNA was obtained from both formalin-fixed paraffin-embedded (FFPE) tumor tissues and matched blood samples of each patient using the QIAamp DNA FFPE Tissue Kit and QIAamp DNA Blood Midi Kit (both Qiagen GmbH), respectively. The DNA samples were sequenced using the next-generation sequencing-based YuanSu450™ gene panel (OrigiMed®), in a laboratory certified by the College of American Pathologists (CAP) and the Clinical Laboratory Improvement Amendments (CLIA). The genes were captured and sequenced with a mean depth of 800x using the NextSeq 500 system (Illumina, Inc.). Single nucleotide variants (SNVs) were identified using MuTect (v1.7, www.broadinstitute.org/cancer/CGA), and insertion-deletion polymorphisms (indels) were identified using PINDEL (V0.2.5, https://www.pindel.com). The functional impact of these mutations was annotated using SnpEff3.0 (http://snpeff.sourceforge.net). Copy number variation (CNV) regions were identified by Control-FREEC (v9.7, http://boevalab.inf.ethz.ch/FREEC/index.html.) with the following parameters: Window=50,000, and step=10,000. Gene fusions/rearrangements were detected using the following in-house pipeline: Paired-end reads with an abnormal insert size of >2,000 bp (aligned to the same or different chromosomes) were collected; discordant read pairs were clustered according to the pairing relationship, and consistent breakpoints from the paired-end discordant reads (within a single cluster) were identified to establish potential fusion/rearrangement breakpoints. Gene fusion/rearrangements were assessed using the Integrative Genomics Viewer (v2.4, http://software.broadinstitute.org/software/igv/ReleaseNotes/2.4.x). The TMB of each patient was calculated by counting the number of somatic mutations (including SNVs and indels) per megabase (Mb) of the sequence examined.

Statistical analysis. Statistical analyses were performed using SPSS version 22.0 (IBM Corp) and significant differences were detected using Fisher's exact test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of patients with sarcoma. A total of 199 patients with soft-tissue or osteogenic sarcomas were enrolled in the present study. This included 105 male and 94 female patients, with a median age of 50 years (range, 1-86 years). The TMB values of all patients were identified, from which 197 valid values were obtained with a median of 1.5 muts/Mb (range, 0.7-24.5 muts/Mb) (Table I).

GAs in 199 patients with sarcoma. Based on NGS targeting of 450 cancer-associated genes, a total of 1,077 clinically relevant GAs were identified in 288 genes (Fig. 1), with an average of 5.41 alterations per sample (range, 0-21). Among these GAs, CNV was the most frequent mutation type (49.21%, 530/1,077), followed by SNV/short indel (39.83%, 429/1077), gene fusion (7.99%, 86/1,077) and long indel (2.97%, 32/1,077) (Fig. 1 and Table SI). The most commonly mutated genes with a mutation frequency of >10% were *TP53* (39.70%, 79/199), *CDKN2A* (19.10%, 38/199), *CDKN2B* (15.08%, 30/199), *KIT* (14.07%, 28/199), *ATRX* (10.05%, 20/199) and *RB1* (10.05%, 20/199). Notably, most mutations in *TP53*, *KIT* and *ATRX* were SNVs, while those in *CDKN2A*, *CDKN2B* and *RB1* were CNVs (Fig. 2).

NGS aids the diagnosis of sarcoma. All sarcomas were pathologically diagnosed before sample collection, after

Table I. Clinicopathological features of 199 patients in the sarcoma cohort.

Variable	Value
Sex, n (%)	
Male	105 (52.76)
Female	94 (47.24)
Median age (range), years	50 (1-86)
Median TMB (range), muts/Mb	1.5 (0.7-42.5)

TMB, Tumor mutational burden.

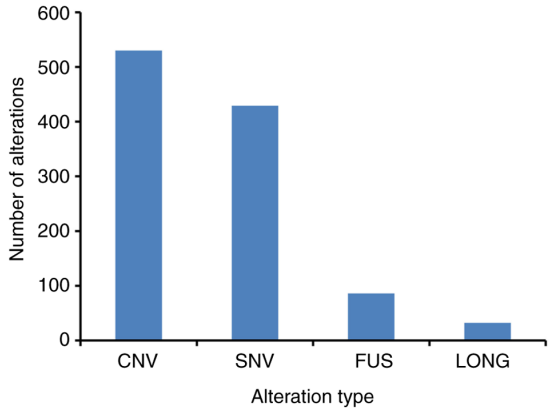

Figure 1. Statistical distribution map of variation types. CNV, copy number variations; SNV, single nucleotide variants; FUS, gene fusion; LONG, long insertion-deletion polymorphism.

which an experienced pathologist was invited to make a second diagnosis. Only those with the same diagnostic results were considered to be classified, while those with inconsistent or undetermined diagnostic results were considered as unclassified. As a result, 23 GISTs, 22 osteosarcomas, 12 myxofibrosarcoma, 11 liposarcomas, 11 leiomyosarcomas, 9 synovial sarcomas, 5 chondrosarcomas, 5 aggressive fibromatosis, 5 rhabdomyosarcomas, 4 Ewing's sarcomas, 4 angiosarcomas, 4 undefferentiated pleomorphic sarcomas, 3 mesotheliomas, 2 epithelioid hemangioendotheliomas, 2 myofibroblastic sarcomas, 2 myxoid sarcomas, 1 dermatofibrosarcoma protuberan, 1 solitary fibrous tumor, 1 embryonic undifferentiated sarcoma, 1 alveolar soft part sarcoma, 1 clear cell sarcoma, 1 malignant granular cell tumor, 1 myolipoma, 64 unclassified soft-tissue sarcomas and 4 unclassified osteogenic sarcomas were identified (Table II). Therefore, further classification was carried out according to the results of NGS.

According to the NGS detection results, 15 additional sarcoma cases were identified and classified, including 3 liposarcomas with amplifications in *MDM2* and *CDK4*, 3 Ewing's sarcomas with *EWSR1* fusions, 3 dermatofibrosarcoma protuberans (DFSP) cases with fusions of *PDGFB* (*COL1A1-PDGFB*), 2 leiomyosarcomas with mutations of *RB1* and *TP53*, 2 infantile fibrosarcomas with the fusion of *ETV6-NTRK3*, 1 GIST with a mutation in *PDGFRA*, and 1 *NTRK* rearranged spindle cell mesenchymal tumor. Among them, 1 leiomyosarcoma was misdiagnosed as a GIST before NGS auxiliary diagnosis. However, 54 cases remained unclassifiable. The primary characteristic mutations of these 15 sarcomas are listed in Table III.

Association of GAs with sarcoma subtypes. The mutational landscapes of sarcoma subtypes including GIST, osteosarcoma, liposarcoma, leiomyosarcoma and myxofibrosarcoma, were subsequently analyzed. The most common mutated genes in GISTs were *KIT*, *CDKN2A* and *CDKN2B*, and the most commonly mutated genes in osteosarcoma were *TP53*, *NCOR1*, *RB1*, *GID4*, *LRP1B*, *PTEN*, *ATRX*, *CCND3*, *MAP2K4* and *RICTOR*. In liposarcoma, *CDK4*, *MDM2*, *FRS2*, *LRP1* and *TP53* were the most frequently mutated, as were *TP53*, *MAP2K4*, *GID4*, *KDM6A* and *MCL1* in leiomyosarcoma. The most commonly mutated genes in myxofibrosarcoma were *TP53*, *CDKN2A*, *CDKN2B*, *FAM135B*, *AKT2* and *JUN* (Fig. 3).

Statistical analysis revealed that mutations in *TP53*, *AKT2*, *FAM135B*, *CDKN2A*, *JUN*, *CDKN2B*, *ROS1*, *AXL*, *SETD2* and *CCNE1* were significantly associated with myxofibrosarcoma (Table IV). The primary mutation type in GISTs was SNV, and mutations in *KIT* and *TP53* were significantly associated with GISTs. Gene amplifications were the most common mutations in osteosarcoma and liposarcoma (Fig. 2). Mutations in *NCOR1*, *GID4*, *LRP1B*, *RB1*, *AURKB*, *GLI2*, *RICTOR*, *MAP2K4*, *STK24*, *TNFSF13B*, *CCNE1*, *PRKDC*, *PTEN*, *CCND3*, *FGF10*, *BRD4*, *PRKACA*, *RET* and *IL7R* were significantly associated with osteosarcoma, while those in *CDK4*, *MDM2*, *FRS2*, *FUS*, *LRP1*, *MYB*, *PTPN11* and *TYK2* were significantly associated with liposarcoma (Table IV). Furthermore, mutations in *MAP2K4*, *TP53* and *KDM6A* were significantly associated with leiomyosarcoma (Table IV). Except for the negative association between *TP53* mutations and GISTs, the majority of these frequently-mutated genes significantly occurred in corresponding sarcomas. Although only 9 synovial sarcomas and 7 Ewing's sarcomas were identified in the present study, the mutations in *SS18* and *EWSR1* significantly occurred in synovial sarcoma and Ewing's sarcoma, respectively. Notably, the mutation of *TP53* was also significantly negatively associated with synovial sarcoma (Table IV).

Association between TMB value, sarcoma subtype and patient demographics. The associations between sarcoma subtype, TMB value and patient sex and age were further analyzed. Based on age distribution, the patients we categorized into 4 groups: i) 1-19 years; ii) 20-39 years; iii) 40-59 years; iv) and 60-86 years of age. Osteosarcoma and Ewing's sarcoma commonly occurred in younger patients (1-19 years old), accounting for 40.91% (9/22) and 57.14% (4/7), respectively; GISTs and liposarcomas were more common in elderly patients (60-86 years old), accounting for 39.13% (9/23) and 71.43% (10/14), respectively; and synovial sarcoma commonly occurred in young patients (20-39 years old), accounting for 66.67% (6/9). Statistical analysis showed that osteosarcomas, Ewing's sarcomas and synovial sarcomas significantly occurred in younger patients, while liposarcomas and GISTs significantly occurred in older patients (Fig. 4).

Most of the sarcoma subtypes had comparable frequencies in male and female patients, while 11 of the 13 leiomyosarcomas

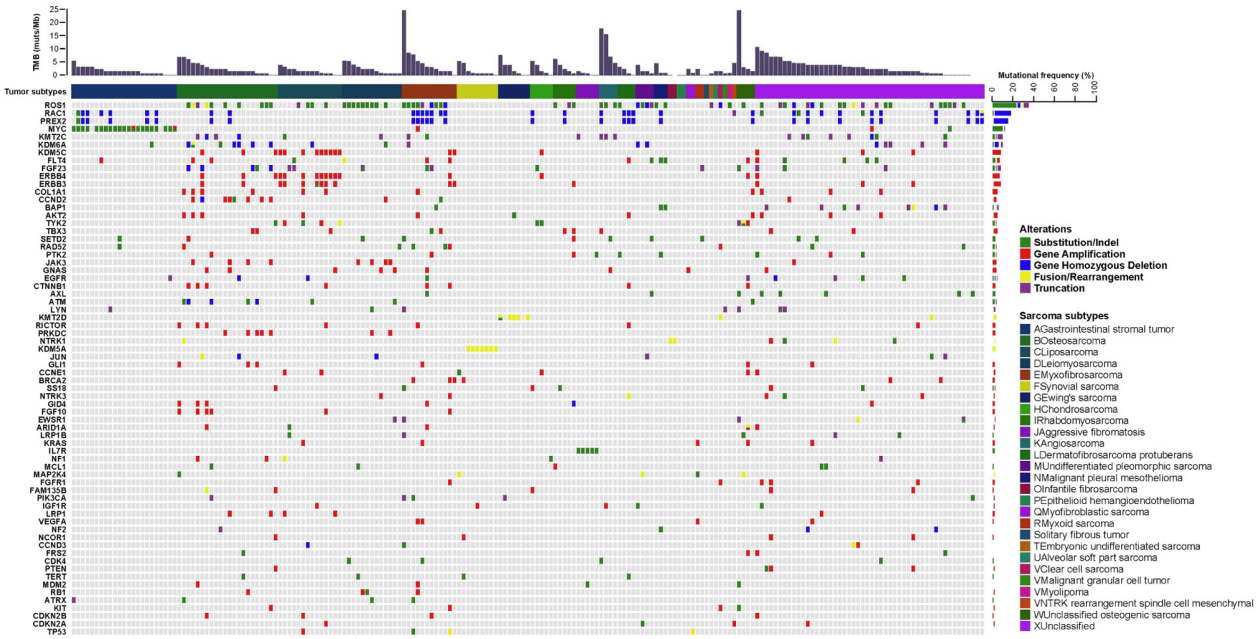

Figure 2. Mutational profiling of 199 patients with sarcoma. X-axis represents each case sample, and the Y-axis represents each mutated gene. Bar graphs to the right and above show the gene mutation frequency of each sample, and the TMB value of all samples, respectively. Green represents substitution/insertion-deletion polymorphism, red represents gene amplification, blue represents gene homozygous deletion, yellow represents fusion/rearrangement, and purple represents truncation. TMB, tumor mutation burden.

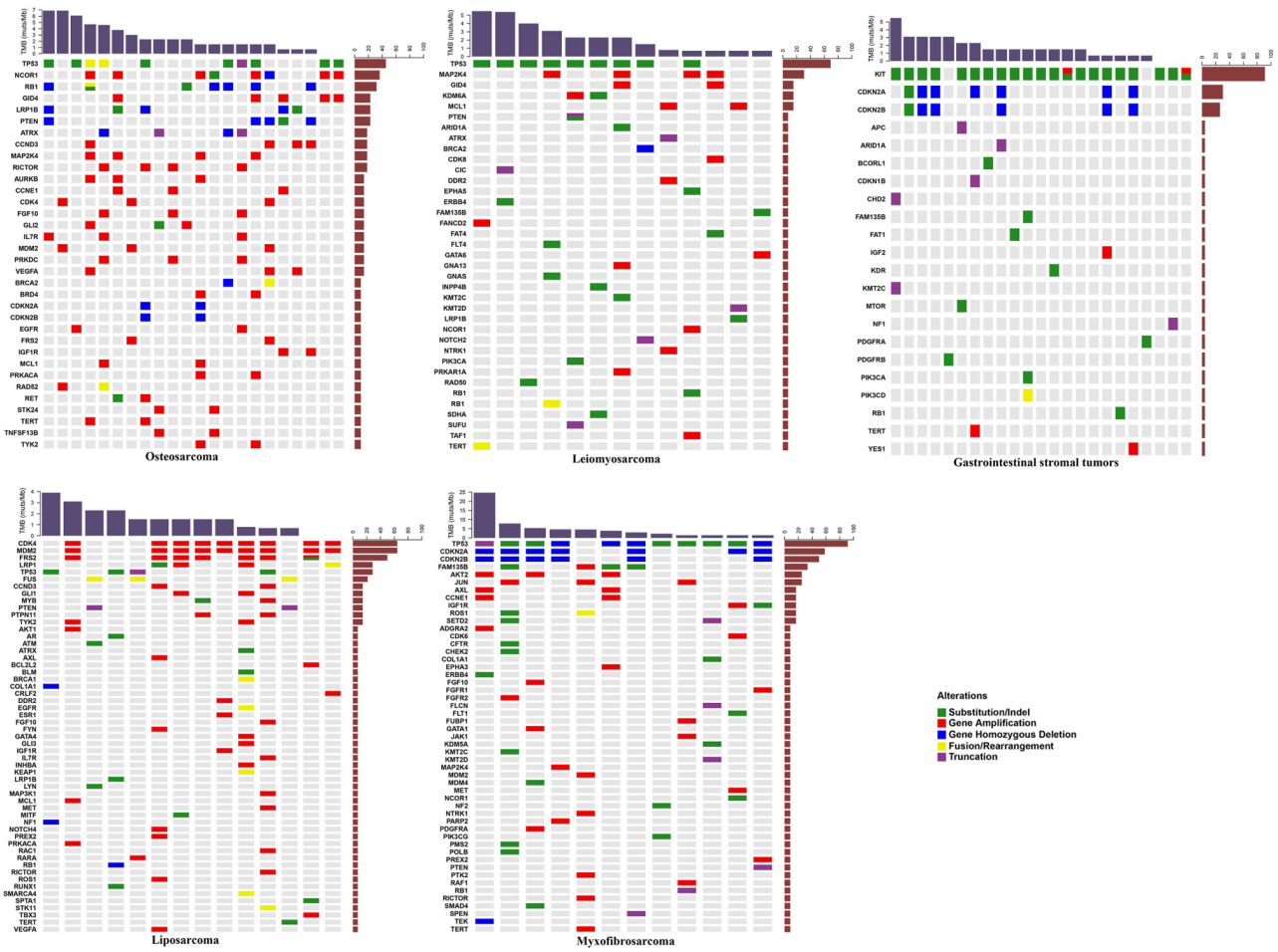

Figure 3. Mutational profiling of sarcoma subtypes. X-axes represent each case sample and the Y-axes represent the mutated genes. Bar graphs to the right and above show the gene mutation frequency of each sample, and the TMB value of each subtype, respectively. Green represents substitution/insertion-deletion polymorphism, red represents gene amplification, blue represents gene homozygous deletion, yellow represents fusion/rearrangement, and purple represents truncation. TMB, tumor mutation burden.

Table II. Comparison of sarcoma subtypes identified by histochemistry- and NGS-based methods.

Sarcoma subtype	Histochemistry-based, n	NGS-based, n
Gastrointestinal stromal tumor	23	23
Osteosarcoma	22	22
Liposarcoma	11	14
Leiomyosarcoma	11	13
Myxofibrosarcoma	12	12
Synovial sarcoma	9	9
Ewing's sarcoma	4	7
Chondrosarcoma	5	5
Rhabdomyosarcoma	5	5
Aggressive fibromatosis	5	5
Angiosarcoma	4	4
Dermatofibrosarcoma protuberans	1	4
Undifferentiated pleomorphic sarcoma	4	4
Malignant pleural mesothelioma	3	3
Infantile fibrosarcoma	0	2
Epithelioid hemangioendothelioma	2	2
Myofibroblastic sarcoma	2	2
Myxoid sarcoma	2	2
Solitary fibrous tumor	1	1
Embryonic undifferentiated sarcoma	1	1
Alveolar soft part sarcoma	1	1
Clear cell sarcoma	1	1
Malignant granular cell tumor	1	1
Myolipoma	1	1
NTRK rearrangement spindle cell mesenchymal tumors	0	1
Unclassified osteogenic sarcoma	4	4
Unclassified	64	50

NGS, next-generation sequencing; NTRK, tropomyosin-related kinase.

occurred in females. Statistical analysis showed that leiomyosarcomas occurred significantly more often in women than in men (Fig. 5A). TMB values were obtained from 194 of the 199 enrolled patients. High TMB (TMB-H), which was defined as a TMB value >10 muts/Mb, was observed in 5 patients, including 1 with fibrosarcoma, 2 with angiosarcoma and 2 patients with unclassified soft-tissue sarcoma. Notably, only 4 angiosarcomas were identified in the cohort, and statistical analysis showed that angiosarcoma was significantly associated with TMB-H (Fig. 5B).

New neurotrophic tyrosine kinase (NTRK)1/3 fusions in the current cohort. *NTRK1/3* mutations were detected in 11 of the 199 patients with sarcoma. Among these patients, 4 *NTRK1* and 1 *NTRK3* mutations were gene amplifications, and 2 *NTRK1* and 4 *NTRK3* mutations were gene fusions, including 1 *LMNA-NTRK1*, 1 *IRF2BP2-NTRK1*, 1 *MEF2A-NTRK3*, 1 *ITFG1-NTRK3* and 2 *ETV6-NTRK3* fusions (Table V). Similar to previous reports (33,34), gene fusions of *ETV6-NTRK3* and *LMNA-NTRK1* were detected in 2 infantile fibrosarcoma cases and 1 unclassified sarcoma, respectively. To the best of our knowledge, the present study is the first to describe the fusion of *IRF2BP2-NTRK1*, *MEF2A-NTRK3* and *ITFG1-NTRK3* in sarcoma. Therefore, sarcoma patients with *NTRK* fusions or amplifications may potentially benefit from NTRK inhibitor therapy.

Discussion

The tumorigenesis of sarcoma is characterized by genomic abnormalities, manifested as multiple phenotypic changes and divided into various subtypes (35). To date, histological examination remains the primary method of sarcoma diagnosis (36). The histological and molecular heterogeneity of sarcoma make it particularly difficult to diagnose, though with the rapid development of NGS technology, increasing numbers of sarcoma genome sequencing studies have emerged (37-40). In the present study, the most commonly mutated genes were identified in 199 patients with sarcoma, and included *TP53*, *CDKN2A*, *CDKN2B*, *KIT*, *ATRX* and *RB1*. *TP53* encodes the p53 protein and functions in the p53 pathway, while the *CDKN2B* and *CDKN2A* genes are associated with the regulation of p53 pathways (41). These findings suggest that p53 pathway mutations frequently occurred in the present cohort,

Table III. List of cases diagnosed by next-generation sequencing.

Case	Sarcoma subtype	Mutated genes	Mutation type
1	DFSP	*COL1A1-PDGFB*	FUS
2	DFSP	*COL1A1-PDGFB*	FUS
3	DFSP	*COL1A1-PDGFB*	FUS
4	Ewing's sarcoma	*EWSR1* (*EWSR1-FLI1*)	FUS
5	Ewing's sarcoma	*EWSR1* (*EWSR1-ERG*)	FUS
6	Ewing's sarcoma	*EWSR1* (*EWSR1-Intergenic*)	FUS
7	Liposarcoma	*CDK4*	CNV
		MDM2	CNV
8	Liposarcoma	*CDK4*	CNV
		MDM2	CNV
9	Liposarcoma	*CDK4*	CNV
		MDM2	CNV
10	Leiomyosarcomas	*RB1*	SNV
		TP53	SNV
11	Leiomyosarcomas	*TP53*	SNV
12	Infantile fibrosarcoma	*ETV6-NTRK3*	FUS
13	Infantile fibrosarcoma	*ETV6-NTRK3*	FUS
14	GIST	*PDGFRA*	SNV
15	Spindle cell mesenchymal tumor	*NTRK1* (*LMNA-NTRK1*)	FUS

DFSP, dermatofibrosarcoma protuberans; GIST, Gastrointestinal stromal tumor FUS, fusion; CNV, copy number variant; SNV, single nucleotide variant.

which is consistent with a previous report (42). Somatic mutations of *TP53* are associated with poor prognosis and low chemotherapy response rates in various tumor types (43,44). Poor patient prognosis is associated with *TP53* mutations in various sarcoma subtypes, such as gliosarcoma (45), osteosarcoma (46), Ewing's sarcoma (47), chondrosarcoma (48) and liposarcoma (49). In the present study, the highly frequent *TP53* mutations were identified in osteosarcoma, fibrosarcoma, liposarcoma and leiomyosarcoma, suggesting an association with poor prognosis in these subtypes.

Molecular diagnosis based on NGS detection can accurately characterize sarcomas according to molecular characteristics, which is a powerful complement to histological identification (50) since pathologists often provide descriptions such as 'probable' or 'possible' during sarcoma diagnosis. The molecular features of different sarcoma subtypes have been extensively studied. For example, *PDGFB* rearrangement in DFSP, *MDM2* and *CDK4* amplification in liposarcoma, *EWSR1* translocation in Ewing's sarcoma, and *SS18* translocation in synovial sarcoma (51-54). With the additional assistance of NGS detection, 15 sarcomas that were difficult diagnose by histological examination were further classified. Notably, one misclassified sarcoma subtype was also successfully corrected. These results suggest that NGS technology can effectively assist in the diagnosis and classification of sarcoma subtypes. However, 54 cases were still not well classified, which may be due to the fact that the corresponding molecular characteristics or biomarkers of sarcoma are still not clearly understood. Therefore, the identification of sarcoma biomarkers is important for further diagnostic advancements.

A number of specific mutations have been used for the classification of sarcoma. For example, Pierron *et al* (55) defined a novel type of bone sarcoma by identifying the *BCOR-CCNB3* gene fusion. Yoshida *et al* (56) identified that CIC-rearranged sarcomas were distinctly different from Ewing's sarcomas, clinically, morphologically and immunohistochemically. Furthermore, Michal *et al* (57) reported a *EWSR1-SMAD3*-rearranged fibroblastic tumor that represented a novel subtype, and Chiang *et al* (58) identified a novel tumor type with the features of fibrosarcoma by *NTRK* fusion. However, few reports have focused on the association between gene mutations and different sarcoma subtypes. In the present study, the associations between GAs and tumor subtypes, patient demographics and TMB values were analyzed, which may provide potential biomarkers for the future diagnosis of sarcoma.

KIT mutations are a significant phenotypic feature of GISTs (59). As predicted, the association between *KIT* mutations and GISTs was also identified in the present study. In addition, a significant negative association was observed between *TP53* mutations and GISTs. These results suggest that mutations in both *KIT* and *TP53* may be used as biomarkers for GIST diagnosis.

In osteosarcoma, the frequent mutation of *RB1* was highly prevalent, and was thus proposed as a potential prognostic biomarker (60). With the exception *RB1*, the association between *NCOR1* mutation and osteosarcoma was also identified in the present study. *NCOR1* is a transcription factor that regulates various biological functions (61). As a tumor suppressor gene, mutation in *NCOR1* was confirmed to be associated with the prognostic prediction of numerous cancers, such as breast cancer,

Table IV. Association between mutated genes and sarcoma subtypes.

Sarcoma subtype	Mutated gene	Mutation frequency within subtype, %	Mutation frequency outside of subtype, %	P-value
Osteosarcoma	*NCOR1*	36.36	1.69	8.14×10^{-7}
	GID4	22.73	1.13	1.92×10^{-4}
	LRP1B	22.73	1.69	4.75×10^{-4}
	RB1	31.82	6.21	1.12×10^{-3}
	AURKB	13.64	0.00	1.19×10^{-3}
	GLI2	13.64	0.00	1.19×10^{-3}
	RICTOR	18.18	1.13	1.15×10^{-3}
	MAP2K4	18.18	2.82	9.90×10^{-3}
	STK24	9.09	0.00	0.012
	TNFSF13B	9.09	0.00	0.012
	CCNE1	13.64	1.69	0.019
	PRKDC	13.64	1.69	0.019
	PTEN	22.73	6.21	0.020
	CCND3	18.18	3.95	0.022
	FGF10	13.64	2.26	0.031
	BRD4	9.09	0.56	0.033
	PRKACA	9.09	0.56	0.033
	RET	9.09	0.56	0.033
	IL7R	13.64	2.82	0.046
Myxofibrosarcoma	*TP53*	91.67	31.02	4.28×10^{-5}
	AKT2	25.00	0.53	6.57×10^{-4}
	FAM135B	33.33	2.67	8.32×10^{-4}
	CDKN2A	58.33	16.04	1.77×10^{-3}
	JUN	25.00	1.60	3.06×10^{-3}
	CDKN2B	50.00	12.83	3.48×10^{-3}
	ROS1	16.67	1.07	0.019
	AXL	16.67	1.60	0.030
	SETD2	16.67	1.60	0.030
	CCNE1	16.67	2.14	0.044
Leiomyosarcoma	*MAP2K4*	30.77	2.69	1.17×10^{-3}
	TP53	69.23	32.26	0.013
	KDM6A	15.38	1.08	0.022
GIST	*KIT*	91.30	1.70	4.00×10^{-23}
	TP53	0.00	39.20	3.36×10^{-5}
Liposarcoma	*CDK4*	64.29	3.78	1.72×10^{-8}
	MDM2	64.29	4.86	6.96×10^{-8}
	FRS2	50.00	4.32	7.70×10^{-6}
	FUS	21.43	0.00	2.81×10^{-4}
	LRP1	28.57	3.24	2.58×10^{-3}
	MYB	14.29	0.00	4.62×10^{-3}
	PTPN11	14.29	0.54	0.013
	TYK2	14.29	1.62	0.041
Synovial sarcoma	*SS18*	77.78	0.00	1.63×10^{-11}
	TP53	0.00	36.32	0.029
	CREB3L1	11.11	0.00	0.045
	PDK1	11.11	0.00	0.045
	TET1	11.11	0.00	0.045
Ewing's sarcoma	*EWSR1*	71.43	1.04	1.75×10^{-7}
	EPHB1	14.29	0.00	0.035
	FEV	14.29	0.00	0.035
	VGLL3	14.29	0.00	0.035

GIST, gastrointestinal stromal tumor.

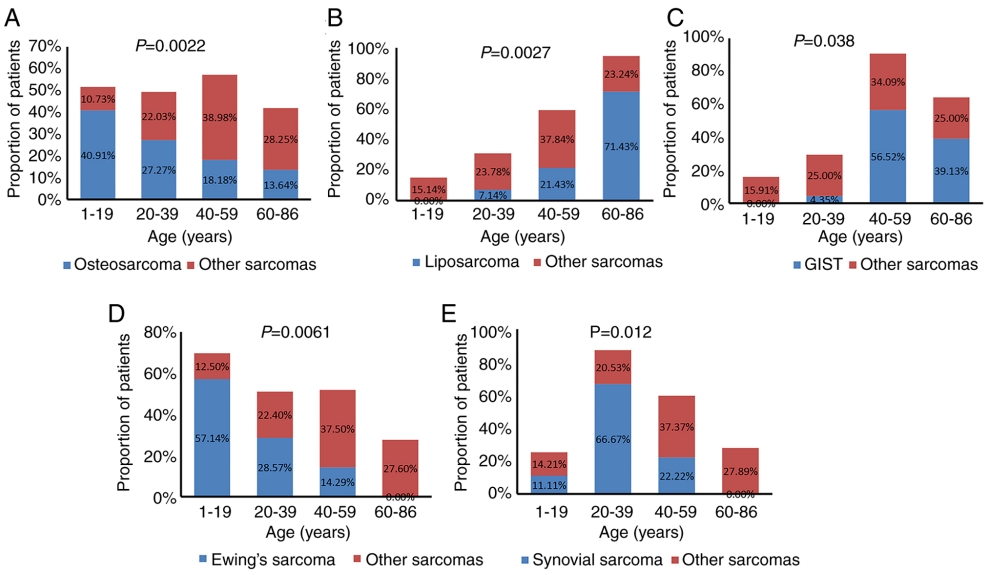

Figure 4. Association between patient age and sarcoma subtype for (A) osteosarcomas, (B) liposarcomas, (C) GISTs, (D) Ewing's sarcomas and (E) and synovial sarcomas. GIST, gastrointestinal stromal tumor.

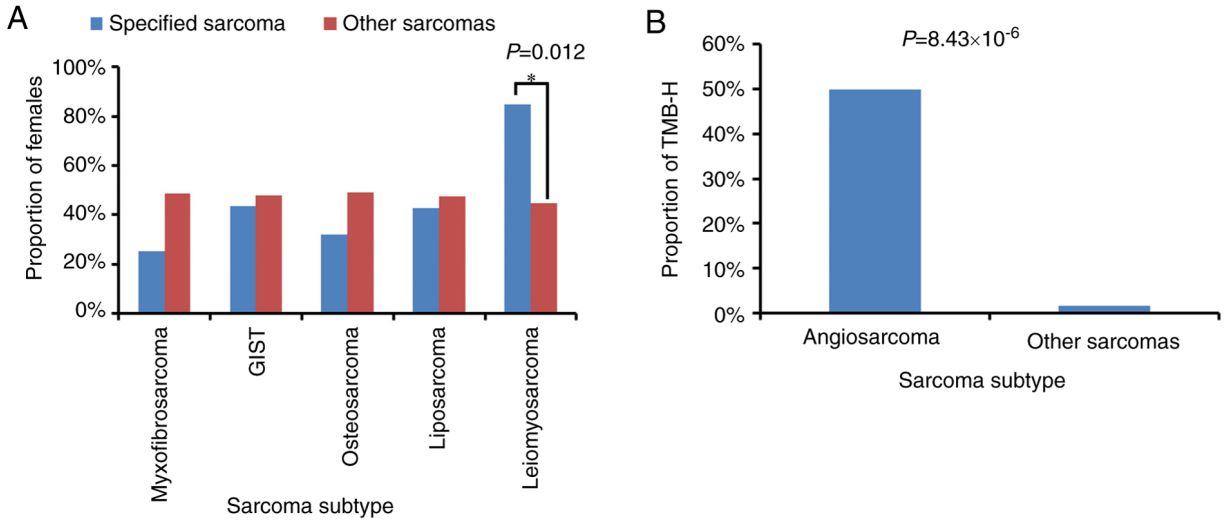

Figure 5. Association of sarcoma subtype with patient sex and TMB-H. (A) Association between specific sarcoma subtypes and the proportion of female patients. Each subtype (blue) was compared with the rest of the sarcoma subtypes (red). Leiomyosarcoma was the only subtype to be significantly associated with female patients; P=0.012. (B) Association between TMB-H and angiosarcoma compared with the association between TMB-H and other sarcoma subtypes. TMB-H, tumor mutational burden >10 muts/Mb; GIST, gastrointestinal stromal tumor.

lung adenocarcinoma and GISTs (62,63). These findings suggest that *NCOR1* mutations are a potential biomarker for the molecular diagnosis and prognosis of osteosarcoma. In the present study, genes such as *GID4*, *LRP1B* and *PTEN* were found to be significantly associated with osteosarcoma. These results have important relevance for guiding the diagnosis of osteosarcoma.

The amplification of *MDM2* and *CDK4* has been reported to occur in liposarcoma, and may therefore be considered as therapeutic targets (64), as well as used to assist the diagnosis of well-differentiated and dedifferentiated liposarcomas (65). The significant association between *CDK4* and *MDM2* amplification and liposarcoma was detected in the present study, and was able to successfully classify 2 cases of liposarcoma from soft-tissue sarcomas. These results support the significance of NGS detection in the diagnosis of liposarcoma. In addition to *CDK4* and *MDM2*, 6 additional mutated genes (including *FRS2*, *FUS*, *LRP1*, *MYB*, *PTPN11* and *TYK2)* were also associated with the liposarcoma subtype, indicating the potential diagnostic value of these genes in liposarcoma.

Fibrosarcoma can also be divided into multiple subtypes, such as myxofibrosarcoma, DFSP, solitary fibrous tumor and infantile fibrosarcoma. *COL1A1-PDGFB* fusion is a prominent molecular feature of DFSP (66). Mutations within the telomerase reverse transcriptase promoter were reported to be associated with the histologically malignant features of solitary fibrous tumors, and to some extent, to play an auxiliary role in their diagnosis and treatment (67). Although there are high mutational frequencies of *TP53*, *RB1*, *CDKN2A*, *CDKN2B*, *NF1* and *NTRK1*, few molecular predictors of myxofibrosarcoma have been identified (68). Due to the limited number of subtypes

Table V. List of NTRK fusions in the present cohort.

Case	Gene	Mutation type	DNA change
1	*NTRK1*	CNV	Gene amplification
2	*NTRK1*	CNV	Gene amplification
3	*NTRK1*	CNV	Gene amplification
4	*NTRK1*	CNV	Gene amplification
5	*NTRK3*	CNV	Gene amplification
6	*NTRK1*	FUSION	LMNA-NTRK1
7	*NTRK1*	FUSION	IRF2BP2-NTRK1
8	*NTRK3*	FUSION	MEF2A-NTRK3
9	*NTRK3*	FUSION	ITFG1-NTRK3
10	*NTRK3*	FUSION	ETV6-NTRK3
11	*NTRK3*	FUSION	ETV6-NTRK3

NTRK, tropomyosin-related kinase; CNV, copy number variation.

across the samples, only the association between the mutated genes and myxofibrosarcoma was analyzed in the present study, and the results showed that mutations in *TP53*, *AKT2*, *FAM135B*, *CDKN2A*, *JUN*, *CDKN2B*, *ROS1*, *AXL*, *SETD2* and *CCNE1* were significantly associated with myxofibrosarcoma. These results may be helpful for the diagnosis of myxofibrosarcoma. Although the number of cases was not large, the association between mutated genes and sarcoma subtype may still be used to guide molecular diagnoses. For example, based on 9 cases, a positive association was detected between the *SS18* mutation and synovial sarcoma, and based on 7 cases, a significant association was also detected between the *EWSR1* mutation and Ewing's sarcoma. However, studies with larger cohorts are required to identify potential biomarkers for the auxiliary diagnosis of sarcomas.

The incidence rate of different sarcoma subtypes varies with sex and age. Classical osteosarcoma and rhabdomyosarcoma frequently occur in children and adolescents, while myxofibrosarcoma, synovial sarcoma, angiosarcoma, DFSP and clear cell sarcoma are more common in patients >20 years of age (69,70). Also, myxofibrosarcoma, rhabdomyosarcoma and synovial sarcoma may be more likely to occur in men, while the occurrence of leiomyosarcoma was notably more common in female participants (70). The results of the present study support previous studies suggesting that osteosarcomas, Ewing's sarcomas, GISTs and liposarcomas are associated with patient age, and that leiomyosarcoma is associated with patient sex.

TMB is a novel biomarker for the prognosis of cancer patients treated with immune checkpoint inhibitors (ICPIs) (71,72). The majority of sarcomas (such as osteosarcomas, GISTs and Ewing's sarcomas) are reported to have a low TMB (73,74), while Trabucco (75) reported TMB-H in skin atypical fibroxanthoma and skin sarcoma. However, the results of the present study support that with the exception of 2 angiosarcoma cases, the TMB value of most sarcomas is low. Though only 4 cases were included in the current cohort, a significant association was detected between angiosarcomas and TMB-H, indicating that patients with angiosarcomas may benefit from ICPI therapy.

NTRK functions in the development, differentiation and metabolism of nerves and other tissues. NTRK inhibitors can be used as targeted agents for tumor therapy, thus the detection of *NTRK* fusions has important clinical significance (76-78). *ETV6-NTRK3* fusion is common in infantile fibrosarcoma, and the tropomyosin-related kinase inhibitor LOXO-101 was reported to benefit infantile fibrosarcoma patients harboring *ETV6-NTRK3* fusions (78). A metastatic infantile fibrosarcoma patient harboring *LMNA-NTRK1* showed a complete and durable response to crizotinib (79). Furthermore, Wong *et al* (77) presented a case of a reverse transcription PCR *ETV6-NTRK3*-negative congenital infantile fibrosarcoma harboring a *LMNA-NTRK1* gene fusion with a near-complete response to crizotinib. The data support the assumption that *NTRK* fusions are the drug target of LOXO-101 or crizotinib in sarcomas. In the present study, *ETV6-NTRK3* and *LMNA-NTRK1* fusions were successfully detected, indicating a potential treatment target for these patients. Follow-up information on the targeted treatment of patients with new *NTRK* fusions of *IRF2BP2-NTRK1*, *MEF2A-NTRK3* and *ITFG1-NTRK3* may also guide and expand the use of NTRK fusion therapy in patients with sarcoma.

In conclusion, the present study investigated the genomic mutation profiles of pan-sarcomas, identified potential biomarkers, and accurately classified sarcoma subtypes with the assistance of NGS. The identification of *NTRK* fusions in sarcoma provides important value for NTRK inhibitor therapy. The absence of FISH confirmation is a limitation of the present study. However, the results support that NGS targeting may effectively promote the accurate classification and diagnosis of sarcomas, and provide guidance for precise therapeutic strategies for bone and soft-tissue sarcomas.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

LX, XX and JW recruited the patients, collected the samples, analyzed the data and wrote the manuscript; XS, PZ and AL processed samples, conducted experiments, analyzed data and reviewed the manuscript; XS and PZ are responsible for the authenticity of the raw data. BZ designed and supervised the study. All authors read and approved the final manuscript.

Ethical approval and consent to participate

The present study was approved by the Ethics Committees of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical

College and the First Affiliated Hospital of Sun Yat Sen University. Written informed consent for participation was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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