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Original Article

Somatic Copy Number Alterations and Mutation Landscape in Before and Post-treatment Malignant Rhabdoid Tumor

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Abstract

Background and objectives: Malignant rhabdoid tumor (MRT) is an aggressive malignancy driven by pathogenic variants of SMARCB1/INI1 or, rarely, SMARCA4/BRG1. The heterogeneity of MRT suggests that other genomic alterations might contribute to tumor behavior. This study aimed to evaluate somatic copy number alterations (SCNAs) and mutation landscapes in MRT before and after treatment. ***Methods:*** With IRB approval, five patients underwent normal-tumor paired whole exome sequencing. Subsequently, the results were further analyzed using MuTect v1.1 for variant DNA and cn.mops for SCNA. ***Results:*** Our study revealed recurrent SCNAs harboring genes known to be involved in tumorigenesis. These include 2q37.3 gain (4/5, 80%, programmed death 1, TWIST2), 7q32.1 gain (3/5, 60%), 11q12.2 gain (3/5, 60%), 14q32.3 gain (4/5, 80%), 19p13.2 loss (SMARCA4, 4/5, 80%), 21q22.3 gain (3/5, 60%), and 22q11.1 loss (2/5, 40%, involving SMARCB1). Alterations more common in posttreatment MRTs included 11p15.4 gain (3/3, 100%) and 11q12.2 gain (2/3, 67%). No actionable pathogenic variants were observed. PD-1 immunohistochemistry correlated with 2q37.3 gain. ***Conclusion:*** Our study revealed recurrent SCNAs in MRT. Genes within these regions are known to be associated with the tumor immune response and metastasis. This preliminary study demonstrated the potential value of SCNAs in furthering the understanding of this highly malignant tumor.

Keywords: Malignant rhabdoid tumor; Somatic copy number alteration; Whole exome sequencing; PD-1.

Abbreviations: ASCL1, achaete-scute family bHLH transcription factor 1; AT, atypical teratoid; CRC, colorectal cancer; DAGLA, diacylglycerol lipase alpha; DDX11, DEAD/H-box helicase 11; FFPE, formalin-fixed paraffin-embedded; H&E, ematoxylin and eosin; IHC, immunohistochemical; LMO1, LIM domain only 1 (rhombotin 1); MMP, matrix metalloproteinase; MRT, malignant rhabdoid tumor; MTA1, metastasis-associated gene 1; MTA1, metastasis-associated gene 1; PCR, polymerase chain reaction; PD-1, programmed death 1; PD-L1, programmed cell death ligand 1; RCC, renal cell carcinoma; RT, rhabdoid tumor; SCNA, somatic copy number alteration; SLC9A3R1, solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1; SMARCA4, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator; SNV, single nucleotide variant; TRAF3, TNF receptor associated factor 3; TRIAP1, TP53 regulated inhibitor of apoptosis 1; TWIST2, twist family BHLH transcription factor 2; UPD, uniparental disomy; WES, whole exome sequencing; XIAP, X-linked inhibitor of apoptosis.

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Introduction

Malignant rhabdoid tumor (MRT) and related INI1-deficient neoplasms are present in the kidney and various extrarenal sites, including the central nervous system (atypical teratoid/rhabdoid tumor). These occurrences predominantly affect infants and young children and are genetically characterized by pathogenic variants of *SMARCB1* (>95%), or rarely, of *SMARCA4* (<5%).[1–7] These genes encode proteins that are components of the chromatin remodeling complex SWI/SNF, a highly conserved transcription regulator that recruits other transcription factors for target genes or alters nucleosome positions to modulate target gene expression.[8,9] The multimodal approach to management yielded disappointing outcomes.[10,11] The latter experience has motivated the pursuit of studies to better understand this tumor and its microenvironment. Technical advances have provided methods for dissecting the molecular and genomic landscape to gain insight into the events of tumorigenesis, tumor progression, and molecular alterations during treatment.

The molecular heterogeneity of MRT has been documented over the past several years.[12] Our hypothesis is that in addition to driver mutations, other genomic alterations in MRT might also contribute to tumorigenesis, tumor progression, and response or lack thereof to management and poor outcome. This study utilized whole exome sequencing to analyze somatic copy number alterations (SCNAs) and somatic variants in MRT patients. The results demonstrated genomic alterations, in addition to driver mutations, providing an additional layer of insight into one of the most aggressive neoplasms of childhood.

Material and methods

The sample selection and experimental studies were performed as previously described.[13] All case and case identification numbers were the same as previously described.[13]

Patient samples

This study was approved by the Washington University In-

stitutional Review Board (IRB # 201102311 "Nextgen Sequencing Approaches" and 201705056 "Expression of Tumor Immunotherapy Related Markers in Pediatric Malignancies"; informed consent was waived). The present study was retrospective and included chart review and residual tissue study following relevant guidelines and regulations. The terms "malignant rhabdoid tumor" and "atypical teratoid/rhabdoid tumor" were searched in the departmental archives of the Lauren V. Ackerman Laboratory of Surgical Pathology, Barnes-Jewish Hospital and St. Louis Children's Hospital from 1990 to 2017. Hematoxylin and eosin (H&E) and related immunohistochemical staining slides were reviewed, and patients with sufficient tissue (formalin-fixed paraffin-embedded (FFPE) tissue from biopsy/resection specimens) were included.

DNA extraction and whole-exome sequencing

Cases with sufficient amounts of both normal and tumor tissue underwent whole exome sequencing (WES). Genomic DNA was extracted from FFPE tissue from both the normal and neoplastic tissues using standard methods as detailed below.

DNA extraction

Genomic DNA of both normal and neoplastic tissue was extracted from the FFPE tissue at the Washington University Genome Technology Access Center (GTAC), St. Louis, MO, using the AllPrep DNA/RNA FFPE Kit (Qiagen, cat#80234). The extracted DNA was qualified using a Tapestation 4200 (Agilent).

Whole-exome sequencing

Whole-exome sequencing was performed at the Washington University GTAC facility as mentioned earlier. The genomic DNA was sonicated to an average size of 175 bp; the DNA fragments were ligated to the ends of Illumina sequencing adapters. The ligated DNA fragments were amplified for 7 cycles. DNA fragments were then hybridized to biotinylated RNA oligos specific to regions of interest (Clinical Research Exome [Agilent] and selected from the remaining fragments using streptavidin beads. The enriched library was amplified for 14 cycles with primers incorporating a unique indexing sequence tag. The resulting libraries were sequenced using the Illumina HiSeq-3000 platform to obtain 150 bp paired-end reads. Sequencing data revealed 25–30 M reads for normal tissue and 45–50 M reads for neoplastic tissue.

Somatic single nucleotide variant (SNV) determination

The raw sequencing data were processed, including variant score recalibration following the Genome Analysis Toolkit (GATK) v 3.3.0 best practices recommendations. Mutation (variation) analysis and somatic mutation (variation) discovery for SNVs were performed using MuTect v1.1.4. Indel calling and somatic indel identification were performed using the GATK IndelGenotyper tool v2. The SNVs and indels were subsequently annotated using ANNOVAR. The tumor mutational burden was calculated as the total number of missense somatic variants/54 mb, representing the size of the Agilent clinical exome. The detected variants were subsequently evaluated for clinical significance, including pathogenicity, following published guidelines.[14]

SCNAs

The presence of SCFAs (short-chain fatty acids) was determined by comparing the number of aligned reads per gene obtained by WES in tumors with that in normal tissues (controls) via the cn.mops tool. Gain or loss was defined by abs_CN, where 0 or 1 = deletion and 3 or more = amplification.

Immunohistochemistry (IHC)

Representative sections of tumor tissue from each case (one section per case) were selected. IHC staining with appropriate controls was performed on FFPE tissue samples for the following IHC markers such as PD-1 (2.97 µg/mL, mouse monoclonal, clone NAT105, Ventana, Tucson, AZ, USA), following standard protocols on a Ventana automated stainer (Ventana Medical Systems, Tucson, AZ, USA) in the AMP (Anatomic and Molecular Pathology) Core Lab, Department of Pathology & Immunology, Washington University School of Medicine. IHC staining was evaluated as follows: PD-1 expression was assessed semiquantitatively, and the number of stained cells per high-power field (400×) was calculated based on staining intensity (1+, 2++, and 3+++).

Results

Demographic data of the MRT patients

Five patients with adequate material were subjected to tumor-normal paired whole-exome sequencing (Table 1). All five patients had germline *SMARCB1/INI1* gene mutations to corroborate the pathological interpretation.[13] Among these patients, two had paired primary (pre-treatment, designated as C) and metastatic (post-treatment, designated as M) tissue samples, two had primary tissue samples (only pre-treatment tissue). Additionally, one had both pre-treatment and post-treatment relapsed tissue samples (designated as R). In total, there were three patients with post-treatment samples.

SCNAs

SCNAs were analyzed in both the pre-treatment and post-treatment groups. Twenty-one loci with SCNAs were identified at the whole-exome level in both groups, while the post-treatment group had seven unique foci with SCNAs (Fig. 1a and b, Table 2). Ten foci of SCNAs harbored malignancy-related genes.

Several recurrent SCNAs were identified that harbored gene alterations previously detected in malignancies. These alterations included 2q37.3 gain (4/5, 80%, PD-1, TWIST2), 7q32.1 gain (3/5, 60%), 11q12.2 gain (3/5, 60%), 14q32.3 gain (4/5, 80%), 19p13.2 loss harboring SMARCA4 (4/5, 80%), 21q22.3 gain (3/5, 60%), and 22q11.1 loss (2/5, 40%) involving SMARCB1. Alterations more frequently encountered in post-treatment MRTs were 11p15.4 gain (3/3, 100%) and 11q12.2 gain (2/3, 67%).

In the post-treatment group, seven unique foci of SCNAs were detected, including gain of copy number in four genes known to be involved in tumorigenesis: chromosome 11p15.4 harbors *LMO1* (LIM domain only 1) and *MMP26* (matrix metallopeptidase 26); chromosome 12q24.31 has *TRIAP1* (TP53 Regulated Inhibitor of Apoptosis 1); chromosome 14q32.32, *TRAF3* (TNF Receptor Associated Factor 3) with amplification; and chromosome 17q25.1 has *SLC9A3R1* (solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1).

Somatic variation/mutation landscape

Somatic mutations (variations) are listed in Figure 2. These mutations included nonsense mutations, frame shift insertions, frame shift deletions, frame insertions, frame deletions, splice site deletions, missense mutations, and 5′ flank-

Table 1. Demographic data of the MRT patients

Case number	Sequencing number	Tumor status	Age at diagnosis	Gender	Location	Germline *SMARCB1* variations
Primary, pre-treatment						
3	40310_C	Primary, before treatment	9 weeks	F	Kidney	Intronic
6	40311_C	Primary, before treatment	9 months	M	Soft tissue	p.R332W
5	40312_C	Primary, before treatment	6 months	M	Liver	p.R40X, p.P221L
8	40307_C	Primary, before treatment	10 months	M	Kidney	Intronic
13	40309_C	Primary, before treatment	19 months	F	Kidney	pW197X
Post-treatment						
6	40311_R	Post-treatment residual tumor	11 months		Soft tissue	
5	40312_M	Post-treatment metastatic tumor	9 months		Lung	
8	40307_M	Post-treatment metastatic tumor	15 months		Lung	

MRT, malignant rhabdoid tumor.

ing regions. A search in ClinVar suggested that most of these variations are more likely benign. Notably, *DDX11 (DEAD/H-box helicase 11)* R186 W, was found in 2/5 cases (40%) and could possibly be actionable (Table 3).[14–17]

IHC analysis of PD-1

Gain of chromosome 2q37.3 in both the pre- and post-treatment groups suggested possible amplification of *PD-1* and *TWIST*2 (the twisted family BHLH transcription factor 2, Fig. 3a). To investigate whether *PD-1* locus gain correlated with protein expression, IHC was performed on *PD-1* (Fig. 3b). The *PD-1* immunostaining score was higher in tumors with 2q37.3 gain (Fig. 3c). With an immune score of 100 as the cutoff, a significant difference in the IHC score was observed between those cases with 2q37.3 gain versus no gain (3/3 vs 0/4, $P = 0.03$; Fisher's exact test).

Discussion

This study is the first effort to investigate SCNAs and somatic variations in primary, pre-treatment, and post-treatment metastatic or recurrent MRTs by normal-tumor whole-exome sequencing. Several recurrent SCNAs were identified and were shared by more than one patient, some of which harbored genes with alterations known tumorigenic alterations. These alterations included 2q37.3 gain (4/5, 80%; *PD-1, TWIST2*), 7q32.1 gain (3/5, 60%), 11q12.2 gain (3/5, 60%), 14q32.3 gain (4/5, 80%), 19p13.2 loss harboring *SMARCA4* (4/5, 80%), 21q22.3 gain (3/5, 60%), and 22q11.1 loss (2/5, 40%) involving *SMARCB1*. Alterations more common in post-treatment MRTs included 11p15.4 gain (3/3, 100%) and 11q12.2 gain (2/3, 67%). The recurrent 2q37.3 gain involving the *PD-1 g*ene correlated with *PD-1* IHC.

Previous genomic studies of MRT/AT/RT and comparisons with our findings

To date, genetic and genomic studies have investigated somatic variations, SCNAs, gene expression via pathway analysis, microRNAs, and methylation. These studies have revealed the presence of driver mutation(s), epigenetic dysregulations, and dysregulated pathways (Table 4).[3,12,18–21] In our study, loss of 22q11.1 was identified in two patients (40309_C and 40310_C), while loss of 22q11.23 was observed in two patients (40309_C and 40312_M) where *SMARCB1* was located. These findings are consistent with the findings of earlier studies.[3] *SMARCA4* mutations are present in MRTs. The heterozygous nonsense mutation c.3565C>T (p.Arg1189X) was found in *SMARCA4*, suggesting that either a severely truncated translation product or nonsense-mediated decay of mRNA were possible consequences.[4,18] SMARCA4 is located at 19p13.2 according to Ensembl. In our study, the 19p13.2 deletion was found in two patients (40309_C, 40312_C), and the 19p13.2 gain was observed in three patients (40307_M, 40311_R, 40312_M).

SCNAs in MRT

In addition to the findings of prior studies, the present study revealed several recurrent SCNAs that harbor genes with alterations involved in malignancy. These alterations included 2q37.3 gain (4/5, 80%; *PD-1, TWIST2*), 7q32.1 gain (3/5, 60%), 11q12.2 gain (3/5, 60%), 14q32.3 gain (4/5, 80%), 19p13.2 loss harboring *SMARCA4* (4/5, 80%), 21q22.3 gain (3/5, 60%), and 22q11.1 loss (2/5, 40%) involving *SMARCB1.*

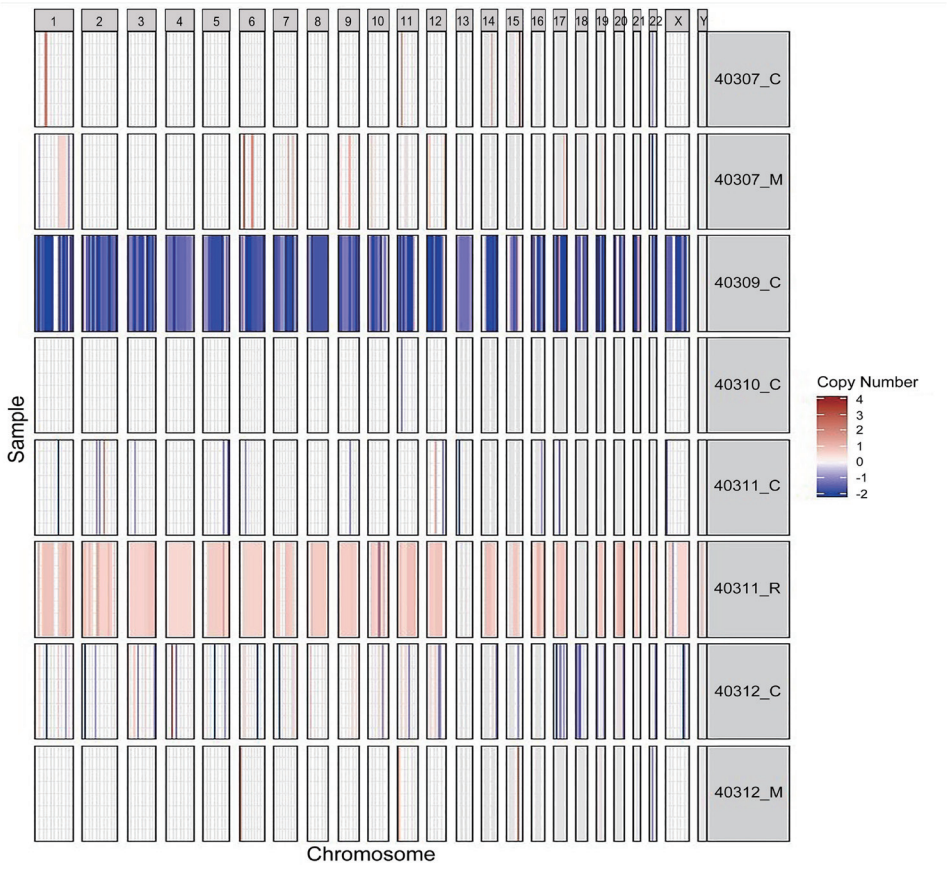
Alterations more frequently observed in posttreatment MRTs included 11p15.4 gain (3/3, 100%) and 11q12.2 gain (2/3, 67%). 11p15.4 was the only SCNA in two metastatic patients (40307_M, 40312_M), suggesting that a gain of 11p15.4 might indicate the possibility of metastasis.

Potential pathogenic roles of gene amplification in MRT

In our study, SCNAs were also found in regions where several other important tumorigenesis-related genes are located (Table 2). A gain in chromosome 2q37.3 was found in four patients (40307_M, 40309_C, 40311_R, and 40312_C), in which the *PD-1* gene and *TWIST2* (twist family BHLH transcription factor 2) gene were located. In the CD8+ cytotoxic antitumor response, receptor-ligand interactions between molecules such as programmed cell death 1/programmed cell death ligand 1 (*PD-1*/*PD-L1*) suppress the CD8+ cytotoxic response. The *PD-1* gene encodes *PD-1* (also known as CD279), which is a negative stimulator of the immune system with potent inhibitory effects on T and B lymphocytes as well as the monocyte response.[22–25] The expression levels of these genes during persistent antigen exposure are observed in chronic infections and cancer. IHC staining for *PD-1* was performed, revealing a significantly higher *PD-1* immunostaining score in tumors from patients with 2q37.3 gain.

Overexpression of the *TWIST1* and *TWIST2* proteins has

a



b

Chromosomal location	40307_C	40307_M	40309_C	40310_C	40311_C	40311_R	40312_C	40312_M	Associate genes	
2q37.3		3,4,7	5			3	3		PD-1	TWIST2
7q32.1			5			3	3			
11q12.2		3				3	3			
12q24.31		3				3	1		TRIAP1	
13q12.11			1		1					
14q23.1	3					3,7				
14q32.32		4				3				
14q32.33			4			3	3		MTA1	
15q15.1			1			1				
15q23			1				1			
16q22.1			1		1					
17p13.1			0,1				1			
19p13.2			1,4				1		SMARCA4	
19q13.33			1				0			
21q22.3		3	8			4				
22q11.1			1	0						
22q11.23			1					1	SMARCB1	
22q13.2			0,1				1			
22q13.31			1				1			
Xq23			1				1			
Xq25			1				1		XIAP	
Post-treatment specific										
11p15.4		4				3,4,7		3	LMO1	MMP26
11q12.2		3				3				
12q24.31		3				3			TRIAP1	
14q32.32		4				3			TRAF3	
17q25.1		3				3,4			SLC9A3R1	
19p13.3		3				3				
19q13.12-19q13.13		3				4				

Copy number alterations	
	Loss
	Gain

Fig. 1. Somatic copy number alterations (SCNA) in malignant Rhabdoid tumor. (a) SCNA plot. (b) SCNAs seen in more than one sample, with corresponding genes. The numbers in this figure represent various copy numbers. 2 is the normal copy number for diploid samples. 1 is a heterozygous deletion 0 is a homozygous deletion. 3 through 8 are amplifications. LMO1, LIM domain only 1 (rhombotin 1); MMP, matrix metalloproteinase; MTA1, metastasis-associated gene 1; PD-1, programmed death 1; SLC9A3R1, solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1; SMARCA4, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator; TRAF3, TNF receptor associated factor 3; TRIAP1, TP53 regulated inhibitor of apoptosis 1; TWIST2, twist family BHLH transcription factor 2; XIAP, X-linked inhibitor of apoptosis.

Table 2. Genes and functions associated with somatic copy number alterations (SCNAs)

Cytogenetic band	Samples and changes (_C: primary; _M, _R: post-treatment)	Gene and functions	Roles in tumorigenesis
Overall in all samples			
2q37.3	40309_C (gain), 40312_C (gain),40307_M (gain), 40311_R (gain)	PD-1(programmed death 1)	The programmed death 1 (PD-1) gene encodes for PD-1 (also known as CD279), a negative costimulator in the immune system. PD-1 renders potent inhibitory effects on T and B lymphocytes as well as monocyte responses. Expression of PD-1 ligands by tumor cells has been reported to contribute in immune system evasion.
		TWIST2 (twist family BHLH transcription factor 2). This gene is hypermethylated and overexpressed in multiple human cancers, and the encoded protein promotes tumor cell invasion and metastasis, as well as metastatic recurrence.	Overexpression of TWIST1 and TWIST2 proteins are seen in soft tissue sarcomas.
14q32.33	40309_C (gain), 40311_R (gain), 40312_C (gain)	MTA1 (metastasis-associated gene 1). Expression of this gene has been correlated with the metastatic potential.	Cancer invasion and metastasis
19p13.2	40307_M (gain), 40309_C (loss), 40311_R(gain), 40312_C (loss), 40312_M (gain)	SMARCA4 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4)	Bona fide tumor suppressor gene and clearly implicated in cancer development
22q11.23	40309_C (loss), 40312_M (loss)	SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1). The protein encoded by this gene is a member of the SWI/SNF family of proteins. Members of this family have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes.	Encoding subunit of the SWI/SNF complex and subject to biallelic mutations (germinal and somatic) in rhabdoid tumors
Xq25	40309_C (loss), 40312_C (loss)	XIAP (X-linked inhibitor of apoptosis). This gene encodes a protein that belongs to a family of apoptotic suppressor proteins.	Seen in variable cancers
SCNA in post-treatment samples			
11p15.4	40307_M (gain), 40311_R (gain), 40312_M (gain)	LMO1 (LIM domain only 1 (rhombotin 1)). LMO1 encodes a protein containing a cysteine-rich LIM domain involved in protein–protein interactions. Recent studies have shown that LMO1 functions as an oncogene in several cancer types.	Neuroblastoma, colorectal cancer, lung cancer, T-ALL
		MMP26 (matrix metallopeptidase 26). Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis.	Seen in variable cancers
12q24.31	40307_M (gain), 40311_R (gain)	TRIAP1 (TP53 regulated inhibitor of apoptosis 1). Its related pathways are Gene Expression and TP53 Regulates Transcription of Cell Death Genes.	Solid tumors
14q32.32	40307_M (gain), 40311_R (gain)	TRAF3 (TNF Receptor Associated Factor 3). This protein is found to be a critical component of the lymphotoxin-beta receptor (LTbetaR) signaling complex, which induces NF-kappaB activation and cell death initiated by LTbeta ligation.	Hodgkin disease, Lymphomas
17q25.1	40307_M (gain), 40311_R (gain)	SLC9A3R1 (solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1). The protein also interacts with proteins that function as linkers between integral membrane and cytoskeletal proteins.	There is growing evidence SLC9A3R1 plays an important role in cancer progression.
11q12.2	40307_M (gain), 40311_R (gain)	DAGLA (diacylglycerol lipase alpha). Its product is a hydrolytic enzyme that yields 2-AG and free fatty acids.	DAGLA is found in aggrevating malignant phenotypes and cancer progression in hepatocellular carcinoma.

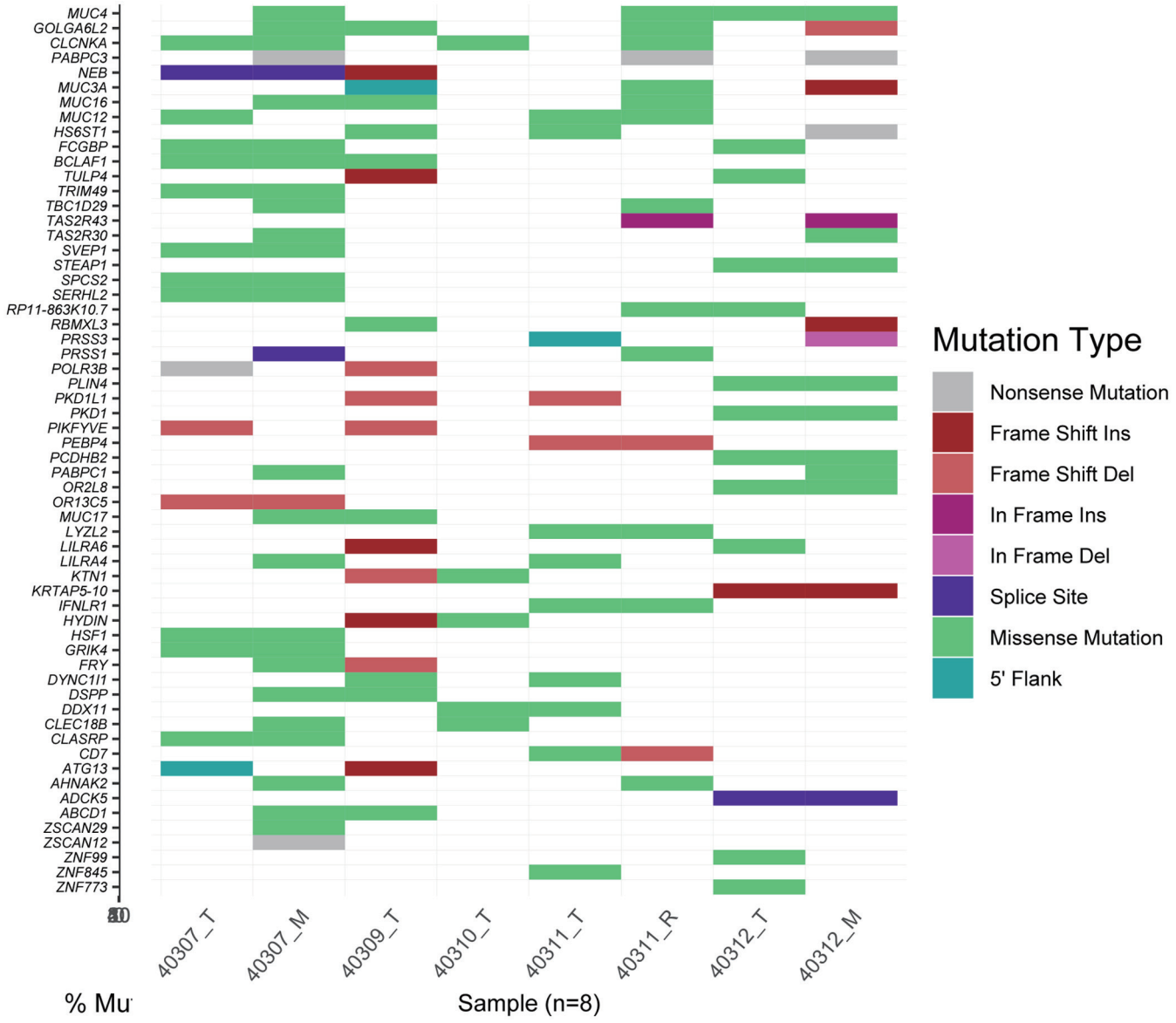


Fig. 2. Somatic mutation landscape in malignant rhabdoid tumors.

been reported in other soft tissue sarcomas, with *TWIST2* amplification observed in rhabdomyosarcoma, contributing to the repression of myogenesis and promotion of oncogenesis.[26] Chromosome rearrangement at 14q32.33 is noted in multiple myeloma with variable partner sites, including 11q13.3, 8q24.1, 18q21.3, and 6p21.1.[27] In our study, chromosome 14q32.33 was found in three patients (40309_C, 40311_R, 40312_C), where *MTA1* (metastasis-associated gene 1) is located. MTA1 is related to invasion and metastasis.[28–32] Chromosome Xq25 is lost in two patients (40309_C and 40312_C), in which the *XIAP* gene (X-linked inhibitor of apoptosis) resides. The XIAPs regulate cell death signaling pathways through binding and inhibiting caspases and also participate in cancer initiation, promotion, and progression.[33–36]

SCNA in post-treatment MRT

In our study, several SCNAs were identified in posttreatment tumors with chromosomal gains. Chromosome 11p15.4 was found in three patients (40307_M, 40311_R, 40312_M), in which *LMO1* (LIM domain only 1 or rhombotin 1) and *MMP26* (matrix metallopeptidase 26) were located. *LMO1* functions as a neuroblastoma oncogene and is implicated as an oncogene in colorectal and lung cancer.[37,38] Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling. Additionally, they are involved in disease processes such as arthritis and metastasis and have been reported as biomarkers of various cancers.[39–41] Chromosome 12q24.31 is gained in two patients (40307_M and 40311_R), hosting the TP53-regulated inhibitor of apoptosis 1 (TRIAP1) gene. TRIAP1 is a novel apoptosis inhibitor that binds HSP70 in the cytoplasm and inhibits apoptosome and caspase-9 activation. *TRIAP1* has been shown to be upregulated in various cancer types.[42,43] Chromosome

Table 3. Somatic variants found in more than one malignant rhabdoid tumor sample

Gene name	Samples	Mutations (AA change)	Actionable	GnomAD allele frequency	Functions	Roles in neoplasms
CLCNKA	40307, 40310 (also found in 40311_R 40307_M?)	A287V	Possibly Benign (1 Submission in ClinVar though) https://www.ncbi.nlm.nih.gov/clinvar/variation/773389/?oq=CLCNKA[gene]+AND+A287V[varname]+&m=NM_004070.4(CLCNKA):c.860C%3ET%20(p.Ala287Val)	Allele Frequency 0.005537 (exomes) https://gnomad.broadinstitute.org/variant/1-16354394-C-T?dataset=gnomad_r2_1	Chloride voltage-gated channel Ka	RNA expression ratios based on the four-gene panel can accurately classify subtypes of RCC as well as help distinguish some oncocytomas from chromophobe RCC.[15]
DDX11	40310, 40311	R186 W	Possibly actionable as the mutation is located on a functional Helicase ATP-binding domain. No variant evidence in ClinVar though.	Allele Frequency 0.1208 (Exomes)	DEAD/H-box helicase 11	DDX11 was significantly upregulated and predicted poor prognosis in lung adenocarcinoma.[16]
FCGBP	40307, 40312	M1617V (40307_T) K3848E (40307_M D3847E (40307_M) R300W (40312_T)	M1617V – Possibly benign as GnomAD exomes allele frequency is high(0.092) and position is not conserved; K3848E – Possibly Benign as GnomAD exomes allele frequency = 0.396 and position is not conserved; D3847E- Possibly benign as not inconserved region. Variant not found in gnomAD exomes; R300W – Uncertain Significance – not conserved but is absent in controls in GnomAD with good coverage in region. Extremely rare?	M1617V - 0.092 (Exomes); K3848E – 0.396 (Exomes); D3847E – Absent in exomes; R300W - Absent in exomes	Fc fragment of IgG binding protein	Differentially expressed in paired tumor-benign tissue samples from patients with stage II CRC.[17]

CRC, colorectal cancer; RCC, renal cell carcinoma.

14q32.32 was found in two patients (40307_M, 40311_R), in which TNF receptor-associated factor 3 (TRAF3) was located. TRAF3 is expressed in Hodgkin disease and lymphomas.[44,45] Chromosome 17q25.1 was found in two patients (40307_M, 40311_R), in which solute carrier family 9 (sodium/hydrogen exchanger) was located. There is growing evidence that *SLC9A3R1* plays an important role in cancer progression.[46,47]

Overall, our study revealed that in addition to driver mutations, there are recurrent SCNAs in different cases of MRT. These alterations are associated with malignancy-related genes, including *SMARCB1, SMARCA4, PD-1, TWIST2, TRIAP1, MTA1,* and *XIAP*. Notably, specific alterations, including those in *LMO1, MMP26, TRIAP1, TRAF3,* and *SLC9A3R1,* are specific for post-treatment MRTs. The identification of 11p15.4 as the only recurrent SCNA in two metastatic patients (40307_M, 40312_M) suggests its potential as an indicator of metastasis. There are various treatments available for patients with MRT, and our study may contribute to the development of treatments based on SCNAs.[48] Importantly, our study is the first to report specific SCNAs in post-treatment MRTs.

Somatic mutation landscape

No obvious pathogenic variants were found in the current study, with the exception of *DDX11* (DEAD/H-box helicase 11) R186 W, which was found in two of five patients. DDX11 was found to be substantially upregulated in lung adenocarcinoma and to predict poor prognosis.[49] This mutation may be actionable because it is located within a functional helicase ATP-binding domain, while there is no variant evidence in ClinVar.

Limitations

Due to the limited tissue available for clinical trials, a major limitation is the small sample size. Two of the three post-treatment samples were derived from metastatic sites; there is a possibility that these differences were due to changes in tumor metastasis rather than to clonal evolution specifically from external therapeutic pressures. Moreover, the possibility that posttreatment alterations may be artifactual due to poor sample quality or necrosis cannot be ruled out.

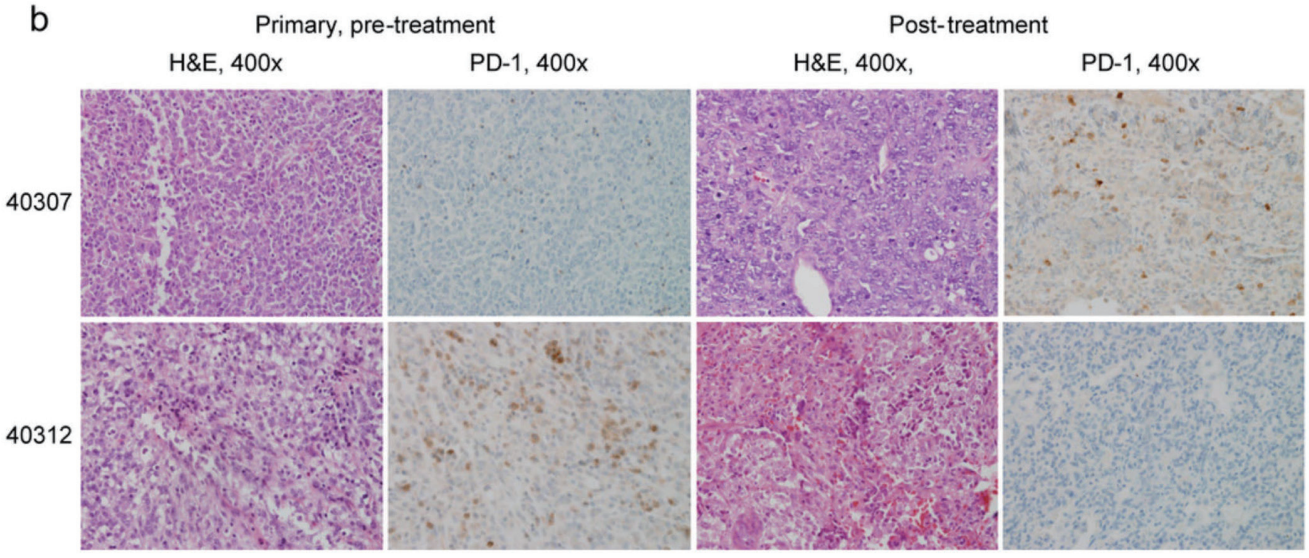
Future directions

We previously reported the expression of PD-L1, PD-1 and CD8 as well as the tumor mutational burden in patients with malignant rhabdoid tumors.[13] Recently, Forrest's study demonstrated similar results for a significant proportion of INI1-negative tumors expressing PD-L1.[50] These prior studies suggested future directions for further genomic and immunologic characterization of malignant rhabdoid tumors, such as those harboring mutation-specific neoantigens and in the tumor microenvironment. With more cases of MRT studied, we hope to gain more understanding of the clinical significance of these genomic alterations, especially those with SCNAs.

a

Chromosomal Location	40307_C	40307_M	40309_C	40310_C	40311_C	40311_R	40321_C	40312_M	Associated Genes	
2q37.3									PD-1	TWIST2

b



c

Case #	PD1 IHC	PD1/CD8 %	PD1#/HPF	PD1 intensity	Score
8	40307_C	<1	40	1	40
8	40307_M	50	60	3	180
13	40309	30	120	3	360
3	40310	<1	0	0	0
6	40311_C	20	0	0	0
6	40311_R	30	No more tumor tissue		
5	40312_C	30	140	3	420
5	40312_M	20	0	0	0

Fig. 3. ***PD-1* gain and PD-1 immunohistochemistry (IHC) in malignant rhabdoid tumors.** (a) Somatic copy number alterations (SCNAs) at chromosomal region 2q37.3 where the *PD-1* gene is located. (b) Representative images of H&E and PD-1 immunohistochemistry (all taken as 400×). (c) PD-1 IHC score summary. H&E, ematoxylin and eosin; PD-1, programmed death 1; TWIST2, twist family BHLH transcription factor 2.

Conclusions

Our study demonstrated that in addition to the driver mutations *SMARCB1* and *SMARCA4*, MRT patients exhibit recurrent SCNAs. As demonstrated by PD-1 immunohistochemistry, the expression of genes within these chromosomal loci correlates with tumor progression. Nevertheless, genes within these regions may be worthy of further studies for their role in tumorigenesis and tumor progression including metastasis, their potential as treatment targets, and their response to treatment.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (MH, JP), acquisition of data (MH), analysis and interpretation of data (MH, MK), drafting of the manuscript (MH, YM, LPD, JP), administrative, technical, or material support (MH), and study supervision (MH, JP). All authors have made significant contributions to this study and have approved the final manuscript.

Ethics statement

This study was carried out in accordance with the ethical guidelines of the Helsinki Declaration (as revised in 2013).

Table 4. Literature review related to genomic alterations in malignant rhabdoid tumors

Chromosome locus	Alterations	Genes	Functions and oncogenesis	Methods	References
22q11.2 (Cytogenetic band: 22q11.23 by HGNC 22q11.23|22q11 by Entrez Gene 22q11.23 by Ensembl)	Deletion	SMARCB1	hSNF5/INI1 encodes a member of the SWI/SNF complexes, which are thought to facilitate the transcriptional activation of inducible genes through the remodeling of chromatin	PCR, Southern blot	3
	MRT cell of origin is related to neural crest cells, and that the consequences of SMARCB1 loss in MRT are heterogeneous at the epigenetic and gene expression levels.	SMARCB1	Same as above. The study revealed gene expression and methylation subgroups and focused on dysregulated pathways, including those involved in neural crest development.	Whole-genome sequencing, whole-genome bisulfite sequencing, whole transcriptome (RNA-seq) and microRNA sequencing (miRNA-seq), and histone modification profiling to characterize extracranial MRTs.	12
	Regulator of NOTCH signaling	ASCL1	Transcriptional analyses identified two atypical teratoid rhabdoid tumor subgroups with differential enrichment of genetic pathways, and distinct clinicopathological and survival features. Expression of ASCL1, a regulator of NOTCH signaling, correlated with supratentorial location ($p = 0.004$) and superior 5-year overall survival.	259 rhabdoid tumors from 37 international institutions and assessed transcriptional profiles in 43 primary tumors and copy number profiles in 38 primary tumors to discover molecular subgroups of atypical teratoid rhabdoid tumors. Gene and pathway enrichment analyses were used to discover group-specific molecular markers and did immunohistochemical analyses on 125 primary tumors to evaluate clinicopathological significance of molecular subgroup and ASCL1-NOTCH signaling.	20
	21 MRT specimens. In eight samples, uniparental disomy (UPD) of 22q segments caused homozygous mutations/deletions of SMARCB1. Ten samples had homozygous focal deletions commonly involving a 175-kb region (ch22:22,353,181-22,528,353), which exclusively included SMARCB1.	SMARCB1 CCNL1 (3q25.31), POT1 (7q31.33), CNTNAP2, and PRPTD	Same as above	SNP-chip analysis	19
	mutations	SMARCB1	Alterations affecting the SMARCB1 locus could be demonstrated by MIP SNP in 15 out of 16 evaluable cases (94%). Remarkably, MIB SNP analysis did not yield any further recurrent chromosomal gains, losses, or copy neutral LOH. On MIP SNP screening for somatic mutations, the presence of a SMARCB1 mutation (c.472C>T p.R158X) was confirmed, but no recurrent mutations of other cancer relevant genes could be identified.	molecular inversion probe single-nucleotide polymorphism (MIP SNP) assay (Affymetrix OncoScan formalin-fixed paraffin-embedded express)	18
N/A	N/A	N/A	N/A	Cytogenetic and molecular studies of an AT/RT on a 15-month-old boy. The tumor showed a complex karyotype with one cell line showing monosomy 22 and another near-tetraploid one with additional chromosomal abnormalities, involving chromosomes 2, 3, 5, 6, and Y.	21

ASCL1, achaete-scute family bHLH transcription factor 1; AT, atypical teratoid; MRT, malignant rhabdoid tumor; PCR, polymerase chain reaction; RT, rhabdoid tumor; SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator.

The protocol was approved by the Washington University Institutional Review Board (IRB # 201102311 "Nextgen Sequencing Approaches" and 201705056 "Expression of Tumor Immunotherapy Related Markers in Pediatric Malignancies"). The individual consent for this retrospective analysis was waived.

Data sharing statement

All the data are included within the article.

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