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BRIEF REPORT

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Transposable element insertion as a mechanism of *SMARCB1* inactivation in atypical teratoid/rhabdoid tumor

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Abstract

Atypical teratoid/rhabdoid tumor (AT/RT) is a malignant brain tumor predominantly occurring in infants. Biallelic *SMARCB1* mutations causing loss of nuclear SMARCB1/INI1 protein expression represent the characteristic genetic lesion. Pathogenic *SMARCB1* mutations comprise single nucleotide variants, small insertions/deletions, large deletions, which may be also present in the germline (rhabdoid tumor predisposition syndrome 1), as well as somatic copy-number neutral loss of heterozygosity (LOH). In some SMARCB1-deficient AT/RT underlying biallelic mutations cannot be identified. Here we report the case of a 24-months-old girl diagnosed with a large brain tumor. The malignant rhabdoid tumor showed loss of nuclear SMARCB1/INI1 protein expression and the diagnosis of AT/RT was confirmed by DNA methylation profiling. While FISH, MLPA, Sanger sequencing and DNA methylation data-based imbalance analysis did not disclose alterations affecting *SMARCB1*, OncoScan array analysis revealed a 28.29 Mb sized region of copy-number neutral LOH on chromosome 22q involving the *SMARCB1* locus. Targeted next-generation sequencing did also not detect a single nucleotide variant but instead revealed insertion of an AluY element into exon 2 of *SMARCB1*. Specific PCR-based Sanger sequencing verified the Alu insertion (SMARCB1 c.199_200 Alu ins) resulting in a frame-shift truncation not present in the patient's germline. In conclusion, transposable element insertion represents a hitherto not widely recognized mechanism of *SMARCB1* disruption in AT/RT, which might not be detected by several widely applied conventional diagnostics assays. This finding has particular clinical implications, if rhabdoid predisposition syndrome 1 is suspected, but germline *SMARCB1* alterations cannot be identified.

KEYWORDS
Alu element, atypical teratoid/rhabdoid tumor, SMARCB1, transposable element insertion

Martin Hasselblatt and Reiner Seibert contributed equally

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1 | INTRODUCTION

Atypical teratoid/rhabdoid tumor (AT/RT) is a highly malignant central nervous system tumor predominantly occurring in infants.[1] Biallelic *SMARCB1* mutations causing loss of nuclear SMARCB1/INI1 protein expression represent the characteristic genetic lesion. Pathogenic *SMARCB1* mutations comprise single nucleotide variants (SNVs), small insertions/deletions (indels) and large deletions, which may be also present in the germline (rhabdoid tumor predisposition syndrome 1; OMIM # 609322), as well as somatic copy-number neutral loss of heterozygosity (LOH).[2-5] Loss of nuclear SMARCB1/INI1 protein expression can be readily assessed by immunohistochemistry and has been established as a robust diagnostic marker in surgical neuropathology.[6,7] Using a combination of Fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing, we have been able to identify underlying biallelic *SMARCB1* mutations in the vast majority of AT/RT patients of the European Rhabdoid Tumor Registry EU-RHAB.[2] However, AT/RT with loss of SMARCB1/INI1 protein expression in which underlying biallelic *SMARCB1* mutations cannot be identified are occasionally encountered.[3,8] Here we report a transposable element insertion not detectable by conventional methods as a mechanism of *SMARCB1* disruption in AT/RT.

2 | MATERIALS AND METHODS

2.1 | Materials

Formalin-fixed paraffin-embedded tumor tissue was analyzed in the context of the European Rhabdoid Tumor Registry (EU-RHAB). EU-RHAB has received continuous approval by the ethics committee of the University of Münster (ID 2009-532-f-S, latest amendment 12/2016) and parents had given informed consent for scientific use of archival samples. Immunohistochemistry for SMARCB1/INI1 was performed using the streptavidin-biotin method on an automated staining system (Omnis, DAKO).

2.2 | DNA methylation profiling

After DNA isolation from formalin-fixed paraffin-embedded tumor samples, purification and bisulfite conversion using standard protocols provided by the manufacturer, the sample was analyzed using the MethylationEPIC BeadChip array (Illumina) as previously described.[9] DNA methylation-based classification was performed using the Heidelberg Brain Tumor Classifier (version v11b4).[10]

2.3 | Molecular genetic examinations

FISH of the *SMARCB1* locus, MLPA using the SALSA MLPA P258 (*SMARCB1*) kit (MRC-Holland) and *SMARCB1* sequencing were performed as described previously.[3,8] Tumor and peripheral blood mononuclear cell-derived DNA was analyzed using the OncoScan array (Affymetrix). Briefly, DNA was hybridized on an OncoScan array, normalization and analysis was done using the Chromosome Analysis Suite 4.0 (ChAS 4.0) from ThermoFisher Scientific.

For targeted next-generation sequencing of *SMARCB1* exons and flanking intronic sequences, the TruSight DNA target enrichment was used for library preparation and sequencing was performed on the MiSeq platform (Illumina). The generated fastq files were analyzed by SeqPilot software version 5.1.0 (Module SeqNext, JSI Medical Systems) for alignment and variant calling. The *SMARCB1* gene was moreover manually inspected using the integrative genomics viewer (IGV). In addition, raw reads were aligned to the hg38 human reference genome (GRCh38_full_analysis_set_plus_decoy_hla) with the Burrows-Wheeler Aligner algorithm (v0.7.17). Base quality score recalibration was performed using the GATK v4.1.4 suite. Duplicate reads were removed using sambamba v0.7.0. Samtools v1.1.0 was used for BAM file handling. Sequence similarity search was performed using NCBI nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/). For confirmatory automatic transposable element insertion detection, we used SCRAMble (Soft Clipped Read Alignment Mapper)[11] (https://github.com/GeneDx/scramble) with the following non-default parameter adjustment: "–pct-align 20". The candidate transposable element insertion in *SMARCB1* was verified by Sanger sequencing after specific nested PCR. All primers are listed in Table S1.

3 | RESULTS

3.1 | Case presentation

A 24-months-old girl presented with a large intracerebral mass involving the left frontal and parietal lobes. On histopathological examination, a malignant rhabdoid tumor with brisk mitotic activity and areas of necrosis was encountered (Figure 1(A)). The tumor cells showed loss of nuclear SMARCB1/INI1 protein expression (Figure 1(B)) and a diagnosis of AT/RT was established according to current WHO criteria. After subtotal resection, conventional chemotherapy according to the EU-RHAB protocol was started (nine courses). After the first course of chemotherapy, second-look surgery was performed and gross total resection could be achieved. The patient received adjuvant local proton beam radiotherapy. At the time of writing, the patient is in complete remission for 25 months.

3.2 | Molecular findings

DNA methylation-based classification classified the tumor as AT/RT of the molecular subgroup SHH (calibrated score: 0.99). The copy-number profile derived from the DNA methylation array data did not reveal any chromosomal gains or losses. In line with this finding, FISH and MLPA also did not disclose any imbalances of the *SMARCB1* locus. Sanger sequencing showed wild-type sequences of all nine

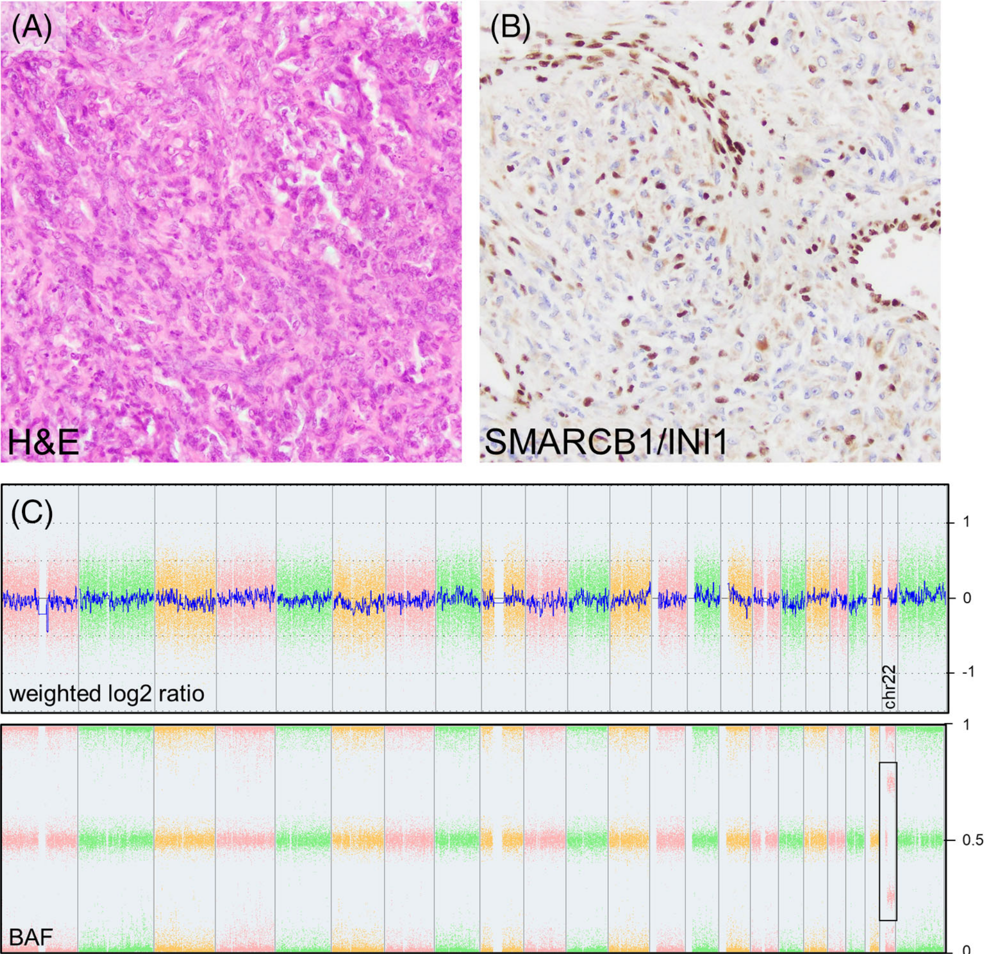

FIGURE 1 Histopathology and OncoScan array analysis. On histopathological examination, the malignant tumor is composed of rhabdoid tumor cells (A). Tumor cells show loss of nuclear SMARCB1/INI1 protein expression, while SMARCB1/INI1 is retained in the nuclei of non-neoplastic cells (internal positive control, (B). OncoScan array analysis reveals a 28.29 Mb copy-number neutral LOH on chromosome 22q involving the *SMARCB1* locus (C)

exons of the *SMARCB1* gene. OncoScan array analysis, however, revealed a 28.29 Mb (chr22:22923807-51213826, hg19) copy-number neutral LOH on chromosome 22q involving the *SMARCB1* locus (Figure 1(C)). As the copy-number neutral LOH encompassing the *SMARCB1* locus along with the lack of SMARCB1/INI1 protein expression in the tumor cells strongly suggested a *SMARCB1* mutation on the retained allele, we performed targeted enrichment and next-generation sequencing of all exons and neighboring intronic sequences of *SMARCB1*. In line with Sanger sequencing results, the bioinformatic pipelines did not identify any pathogenic SNVs or indels. Nevertheless, further inspection of aligned reads revealed a total of 108 soft-clipped reads at position c.199 (transcript NM_003073) in exon 2 of *SMARCB1* (Figure 2(A)). These soft-clipped bases at this position were subsequently shown to represent a non-reference Alu element. The consensus sequence (80 nt) of the clipped-reads demonstrated 99% similarity with the reference AluY element sequence (Dfam accession number # DF0000002) and 100% similarity with AluYm1 (Dfam accession number #DF0001154) (Figure 2(B)). The Alu insertion at this position (c.199_200 Alu ins) was further verified by Sanger sequencing after specific PCR (Figure 2(C)). According to the Human Genome Variation Society (HGVS) nomenclature, the insertion is predicted to result in a frame-shift truncating mutation NP_003064.2:p.Ser67Trpfs*32 (Figure 2(D)). The Alu insertion was not identified in the patient´s germline (data not shown), and thus most likely represents the primary somatic event. Which was followed by copy-number neutral LOH as secondary event.

4 | DISCUSSION

Our findings suggest that transposable element insertion represents a mechanism of *SMARCB1* disruption in AT/RT. In SMARCB1-deficient AT/RT, the majority of underlying *SMARCB1* alterations can be resolved using FISH, MLPA, array-based imbalance profiling and/or Sanger sequencing.[2,8,12] The present case showed a large copy-number neutral LOH on chromosome 22q, but a second hit was not apparent. We therefore set out to perform targeted next-generation sequencing, which plays an increasing role in the diagnosis of central nervous system tumors.[13] Bioinformatic pipelines that are not taking into account SVs but focusing on SNVs and indels in aligned reads might miss detection of transposable element insertions, as well as some pipelines in which repetitive elements are filtered out. However, further inspection revealed an abrupt drop of sequencing coverage within exon 2 due to a large number of soft-clipped reads that were confirmed as transposable element insertion using SCRAMble, a bioinformatic tool developed for identification of transposable element insertions in clinical exome sequencing data.[11] PCR-based Sanger sequencing using exon flanking primers failed to detect the mutated

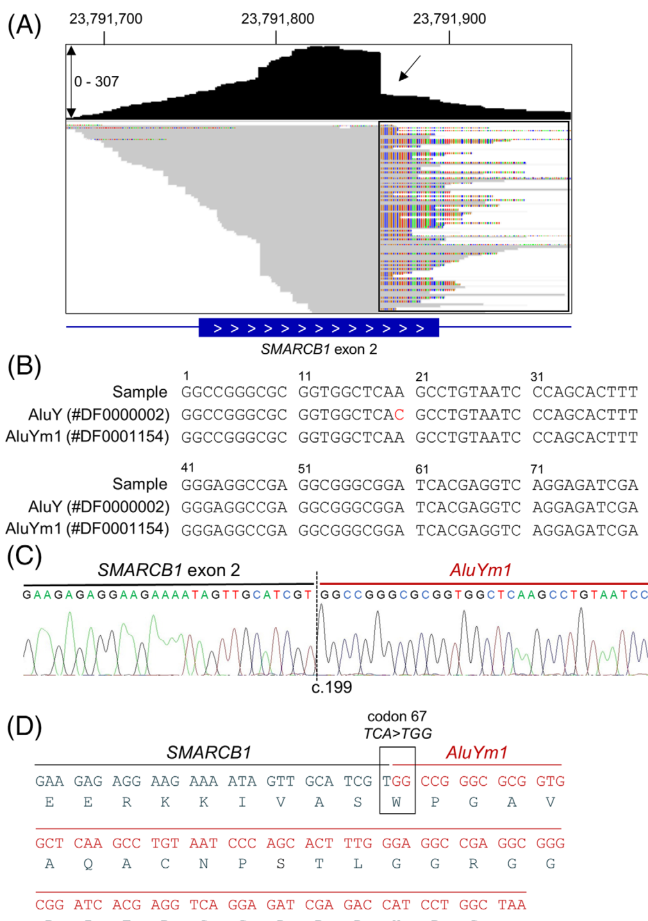

FIGURE 2 Next Generation Sequencing. Inspection of aligned reads reveals a drop of coverage due to a high frequency of soft-clipped reads at position c.199 in exon 2 of the *SMARCB1* gene (NM_003073, A). Sequence of the consensus sequence of soft-clipped reads (80 nt) shows 99% similarity of the AluY reference sequence (Dfam accession number # DF0000002) and 100% similarity with the AluYm1 subtype (Dfam accession number #DF0001154) (B). Confirmation of the AluY element insertion by Sanger sequencing specific primers (C). Predicted protein sequence (D)

allele because the insertion either led to deletion of one primer binding site or separated it that far away from the other primer binding site that PCR did not amplify over the insertion.

Retrotransposons are genomic elements that mobilize via an RNA intermediate in a copy-and-paste mechanism and comprise nearly half of the human genome. Although the majority of these elements are inactive ancient insertions, a small proportion retains its retrotransposition capacity,[14,15] serving as a powerful source of genetic variation,[16] but also causing disease.[17] Somatic retrotransposon insertions have been identified in cancer, including Long INterspersed Element (LINE-1) insertions within the coding sequence of the *APC* tumor suppressor gene in colorectal cancer.[18,19] Taking advantage of newly developed algorithms to detect retrotransposon insertions in whole-genome sequencing data of 43 tumors from The Cancer Genome Atlas (TCGA), variable insertion frequencies were observed with highest prevalence in colorectal, prostate and ovarian cancers.[20] In that study malignant gliomas showed little evidence of somatic retrotransposition,[20] but the frequency of transposable element insertions in other central nervous system tumors was not examined. Furthermore, transposable element insertions are not routinely detected in clinical diagnostic testing and currently available tools show low performance in terms of sensitivity and precision.[21] It is therefore reasonable to assume that a number of pathogenic retrotransposition events remain undetected.

In the present case, the Alu element insertion caused a frame-shift truncation mutation with interruption of the protein-coding sequence of *SMARCB1*. Other biological consequences of retrotransposition include alterations of splicing causing loss-of-function alleles, disruption of non-coding DNA regulatory elements and antisense Alu insertion within introns leading to a new exon sequence in a process known as exonization.[22] Of note, the latter two mechanisms can only be detected when non-coding DNA is sequenced and should be considered when no alterations within the coding sequence can be detected.

The majority of germline *SMARCB1* alterations occurs de novo, but *SMARCB1* mutations may be also inherited from apparently healthy parents.[5,23,24] Even though in our patient the Alu insertion was not identified in the germline, the possibility of germline transposable element insertions affecting the *SMARCB1* locus needs to be taken into account. This holds especially true for patients in whom rhabdoid predisposition syndrome 1 is clinically suspected, but germline *SMARCB1* alterations cannot be identified using conventional methods.

Taken together, transposable element insertion represents a hitherto probably under recognized mechanism of *SMARCB1* inactivation in AT/RT. Our findings may have clinical implications, especially if rhabdoid tumor predisposition syndrome 1 is suspected, but germline *SMARCB1* alterations cannot be identified.

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CONFLICTS OF INTERESTS

The authors declare there is no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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