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Biology of Human Tumors

Clinical Cancer Research

Recurrent *PRDM10* Gene Fusions in Undifferentiated Pleomorphic Sarcoma

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Abstract

Purpose: Undifferentiated pleomorphic sarcoma (UPS) is defined as a sarcoma with cellular pleomorphism and no identifiable line of differentiation. It is typically a high-grade lesion with a metastatic rate of about one third. No tumor-specific rearrangement has been identified, and genetic markers that could be used for treatment stratification are lacking. We performed transcriptome sequencing (RNA-Seq) to search for novel gene fusions.

Experimental design: RNA-Seq, FISH, and/or various PCR methodologies were used to search for gene fusions and rearrangements of the *PRDM10* gene in 84 soft tissue sarcomas.

Results: Using RNA-Seq, two cases of UPS were found to display novel gene fusions, both involving the transcription factor *PRDM10* as the 3′ partner and either *MED12* or *CITED2* as the 5′ partner gene. Further screening of 82 soft tissue sarcomas for rearrangements of the PRDM10 locus revealed one more UPS with a MED12/PRDM10 fusion. None of these genes has been implicated in neoplasia-associated gene fusions before.

Conclusions: Our results suggest that *PRDM10* fusions are present in around 5% of UPS. Although the fusion-positive cases in our series showed the same nuclear pleomorphism and lack of differentiation as other UPS, it is noteworthy that all three were morphologically low grade and that none of the patients developed metastases. Thus, *PRDM10* fusion-positive sarcomas may constitute a clinically important subset of UPS. *Clin Cancer Res; 21(4); 864–9. ©2014 AACR.*

Introduction

Undifferentiated sarcoma is defined as a sarcoma with no identifiable line of differentiation, excluding dedifferentiated types of specific sarcomas (1). Undifferentiated sarcomas, accounting for approximately 20% of all soft tissue sarcomas, may be further subdivided according to cellular shape (round cell, spindle cell, epithelioid, or pleomorphic). The pleomorphic variant (undifferentiated pleomorphic sarcoma, UPS) is particularly common among adults, and most frequently arises in the lower extremities (2). It is typically a high-grade lesion with a local recurrence rate ranging between 19% and 31%, a metastatic rate of 31% to 35%, and a five-year survival of 65% to 70% (3). UPS have a highly variable morphology, all sharing a marked pleomorphism often admixed with spindle cells and bizarre multinucleated giant cells. Treatment is based on the same strategy as for most other soft tissue sarcomas, that is, surgery with wide margins. Depending on surgical margins, location, and tumor-associated risk factors, adjuvant treatment, including radiotherapy and chemotherapy, is considered.

The genetic aspects of UPS are still poorly defined, partly due to shifting diagnostic criteria; although many sarcomas now diagnosed as UPS were previously classified as malignant fibrous histiocytoma (MFH), a substantial subset of MFH tumors was shown to constitute poorly differentiated forms of other sarcomas, such as leiomyosarcoma or liposarcoma (1). The karyotypes and copy-number profiles for UPS tend to be highly complex, with extensive intercellular variation, and a complete description of all chromosomal aberrations is rare (4–7). However, the level of cytogenetic complexity varies considerably, with a subset showing only a few structural and/or numerical aberrations. Still, no specific recurrent aberration has so far been identified, and there are no good genetic markers that could be used for treatment stratification.

In an attempt to identify clinically and biologically relevant subgroups of UPS, we performed transcriptome sequencing (RNA-Seq), and we here report the finding of two novel gene fusions in UPS, both involving the transcription factor *PRDM10* as the 3′ partner and either *MED12* or *CITED2* as the 5′ partner gene.

Materials and Methods

Patients and tumors

RNA-Seq of two UPS (cases 1 and 2), selected on the basis of their simple karyotypes, showed that they harbored gene fusions involving the *PRDM10* gene. To evaluate the frequency and distribution of *PRDM10* fusions in UPS and other soft tissue sarcomas, a cohort of 82 additional soft tissue sarcomas was analyzed (26 UPS, 22 myxofibrosarcomas, 10 leiomyosarcomas,

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Undifferentiated pleomorphic sarcoma (UPS) is one of the most common subtypes of soft tissue sarcomas. The clinical behavior is unpredictable, and metastases occur in about one third of the patients. Treatment is based on surgery with wide margins. Depending on surgical margins, location, and tumor-associated risk factors, adjuvant treatment, including radiotherapy and chemotherapy, is considered. Biomarkers that could distinguish UPS from other types of sarcoma as well as improve treatment stratification are needed. Previous genetic analyses have failed to reveal any consistent or tumor-specific aberrations. We here describe the finding of novel, and so far tumor-specific, gene fusions—*MED12*/*PRDM10* and *CITED2*/*PRDM10*—in a subset of UPS. None of the patients with these gene fusions has developed any metastases and all tumors were diagnosed as low-grade malignant at morphologic re-review, suggesting that fusion-positive tumors may represent a less aggressive subset of UPS.

5 low-grade fibromyxoid sarcomas, 5 myofibroblastic sarcomas, 3 myxoid liposarcomas, 2 malignant peripheral nerve sheath tumors, 1 solitary fibrous tumor, 4 spindle cell sarcomas, 1 fibroblastic sarcoma, and 3 unclassifiable sarcomas). The tumors in this extended cohort were partly selected on the basis of their karyotypes. Thus, tumors with structural rearrangements of chromosome arms Xq, 6q, and 11q, that is, the locations of the *MED12*, *CITED2*, and *PRDM10* genes, respectively, at G-banding analysis were retrieved from the archives of the Department of Clinical Genetics in Lund; Xq, 6q, and/or 11q rearrangements were present in 12, 15, and 29 cases, respectively. We also specifically retrieved 16 tumors that had been diagnosed as low-grade malignant UPS, myxofibrosarcoma, or leiomyosarcoma by querying the Scandinavian Sarcoma Group registry. All tumors were diagnosed according to established criteria (1, 8). Clinical, morphologic, and cytogenetic data are presented in Supplementary Table S1. All samples were obtained after written consent and all studies were approved by the institutional ethical committees.

Cytogenetic and FISH analyses

Cell culturing, harvesting, and G-banding were performed as described, and the karyotypes were written following the recommendations of the International System for Human Cytogenetic Nomenclature (9, 10).

FISH was performed on interphase nuclei from cases 2, 27, 29, 52, and 75 using bacterial artificial chromosome (BAC) clones flanking the *PRDM10* locus obtained from the BAC PAC resources. 5′ probes were RP11–664J16, RP11–237N19, and RP11–61J24 and 3′ probes were RP11–1104M18, RP11–121M22, and RP11–110K10. Clone preparation, hybridization, and analysis were performed as described previously (11). No material for FISH was available from case 1.

RNA-Seq

RNA-Seq and bioinformatic analysis to identify candidate fusion transcripts were performed on cases 1, 2, 35, 36, 44, and 49. mRNA libraries were prepared for sequencing using the Truseq RNA Sample Preparation Kit v 2 (Illumina) as previously described (12). Briefly, poly-A–tailed RNA was enriched from total RNA using magnetic oligo-dT beads. RNA was fragmented to a median size of 200 nucleotides and cDNA was synthesized from these fragments using Superscript II reverse transcriptase (Invitrogen). Double-stranded cDNA was produced using DNA polymerase I and RNase H. Oligonucleotide adaptors were ligated to the double-stranded cDNA, and the adaptor-bound fragments were enriched using a 15 cycle PCR. Paired-end 101-bp reads were generated from the mRNA libraries using the HiScanSQ System (Illumina).

To identify candidate fusion transcripts from the sequence data, analyses were performed on fastq files using Chimerascan (13) version 0.4.5, SOAPfuse (14) version 1.26, and TopHat (15) version 2.0.7. The GRCh37/hg19 build was used as the human reference genome.

Quantitative real-time PCR

To evaluate differences in the expression levels of the 5′ and 3′ parts of *PRDM10*, indicative of a chromosomal breakage within the gene, TaqMan gene-expression assays were performed with: Hs00360640 (*PRDM10* 5′) covering exons 5–6 and Hs000999748 (*PRDM10* 3′) covering exons 20–21. The *TBP* gene was used as endogenous control. Quantitative real-time PCR (qPCR) was performed according to the manufacturer's instructions, and all reactions were run in triplicate (Applied Biosystems). Calculations were done using the comparative C_t method (i.e., $\Delta\Delta C_t$ method; 16) using the SDS software 1.3.1 (Applied Biosystems).

RT-PCR

Total RNA was extracted from frozen tumor samples using the RNeasy Lipid Tissue Kit (Qiagen). Reverse transcription and PCR amplifications were performed as described previously (11, 17). Primers specific for *MED12*, *CITED2*, and *PRDM10* were designed to detect possible fusion transcripts (Supplementary Table S2). Transcripts were amplified using an initial denaturation for 2 minutes at 94°C, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 58°C, and 3 minutes at 72°C, and a final extension for 3 minutes at 72°C. Amplified fragments were purified from agarose gels and directly sequenced using the Big Dye v1.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI-3130 genetic analyzer (Applied Biosystems). The BLASTN software (http://www.ncbi.nlm.nih.gov/blast) was used for the analysis of *MED12*, *CITED2*, and *PRDM10* sequence data.

Results

Genetic findings in the two index cases

RNA-Seq resulted in 13,955,975 reads in case 1 and 12,758,033 reads in case 2. In case 1, Chimerascan identified a *MED12*/*PRDM10* fusion, supported by three unique flanking reads, and in case 2 SOAPfuse identified a *CITED2*/*PRDM10* fusion supported by two spanning reads and six junction reads. In both cases, the genes implicated in the fusions map to breakpoints identified at G-banding analysis: *MED12* maps to Xq13, *PRDM10* to 11q24, and *CITED2* to 6q24. Thus, both fusions were in agreement with the karyotypes, that is, a t(X;1;11)(q13;p36;q23) in case 1 and a t (6;11)(q24;q24) in case 2 (Fig. 1; Supplementary Table S1). Additional detected potential fusion transcripts were considered read-through transcripts or other artefacts.

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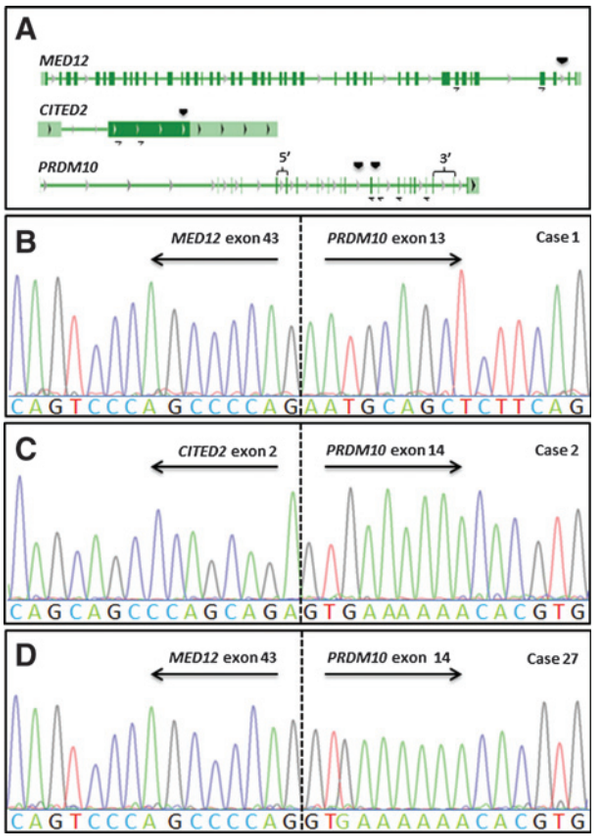

Figure 1.
A, illustration of the *MED12*, *CITED2*, and *PRDM10* genes with vertical arrow heads indicating the breakpoint locations, horizontal arrows indicating the locations of PCR primers, and braces indicating the locations of probes for quantitative real-time PCR. The coding parts of the genes are indicated in dark green color. B to D, partial chromatograms of amplified fragments corresponding to in-frame *MED12/PRDM10* and *CITED2/PRDM10* fusion transcripts.

RT-PCR and subsequent sequencing of amplified products from cases 1 and 2 identified in-frame *MED12/PRDM10* and *CITED2/PRDM10* fusions, respectively (Fig. 1). No reciprocal transcript, that is, *PRDM10/MED12* or *PRDM10/CITED2*, could be detected (data not shown). FISH with *PRDM10*-specific probes in case 2 verified the break in *PRDM10* also at the genomic level (Fig. 2).

The breakpoints in the two 5′ genes (*MED12* and *CITED2*) were located toward the ends of their coding parts. In *MED12*, the breakpoint was located in the intron between exons 43 and 44. *MED12* thus only loses two of its 45 exons in the fusion event. *CITED2* has two exons, and the breakpoint was located within exon 2, at nucleotide position 1047 (NM_006079.4), which is only 9 nucleotide from the stop codon. The shared 3′ partner, *PRDM10*, has 22 exons. In case 1, the fusion breakpoint was located between exons 12 and 13 and in case 2 between exons 13 and 14.

qPCR showed higher expression of the 3′ part of *PRDM10* in both cases. The ratios between the expression levels of the 3′ and 5′ probes were 1.82 and 4.15 in cases 1 and 2, respectively.

Genetic findings in an extended cohort of soft tissue sarcomas

Because of the possibility of multiple 5′ partners to *PRDM10* and the finding of differential expression of the 5′- and 3′-parts of *PRDM10* in the two fusion-positive index tumors (cases 1 and 2), 78 additional soft tissue sarcomas were analyzed by qPCR. Neither the 3′ nor the 5′ expression levels were consistently higher among fusion-positive tumors than among fusion-negative tumors (Supplementary Fig. S1). None of the tumors showed a 3′:5′ ratio above 1.2, whereas six had ratios below 0.7. All these six cases were analyzed by RT-PCR for *MED12/PRDM10* and *CITED2/PRDM10* fusion transcripts, using multiple primer pairs (Supplementary Table S2), revealing a *MED12/PRDM10* fusion in one (case 27, an UPS). Sequencing confirmed a fusion between the last nucleotide of *MED12* exon 43 and the first nucleotide of *PRDM10* exon 14 (Fig. 1). Three of the five RT-PCR negative cases could be analyzed also by interphase FISH using a break-apart probe for *PRDM10*; all were negative. Finally, four myxofibrosarcomas were subjected to RNA-Seq, but did not display any fusion transcript involving *PRDM10*. Thus, only one additional *PRDM10* gene fusion was detected among the 82 soft tissue sarcomas, including 26 UPS, in the extended cohort (Supplementary Table S1).

Morphology of *PRDM10*-positive tumors

All three cases showed features of an UPS with neither morphologic nor immunophenotypic evidence of any specific line of differentiation (Fig. 3). Each consisted of eosinophilic spindled, ovoid, or multinucleate cells with bizarre, irregular, vesicular nuclei. Each had a variably prominent collagenous stroma containing multifocally scattered lymphocytes. In contrast with most pleomorphic sarcomas, in each case, mitoses numbered less than 1 per 10 high power fields and there was no necrosis. These unusual tumors were graded subjectively as low grade based on the experience of one of the authors (C.D.M. Fletcher). Aside from this finding, there were no features that distinguished these tumors from other UPS in general. One case each had focally

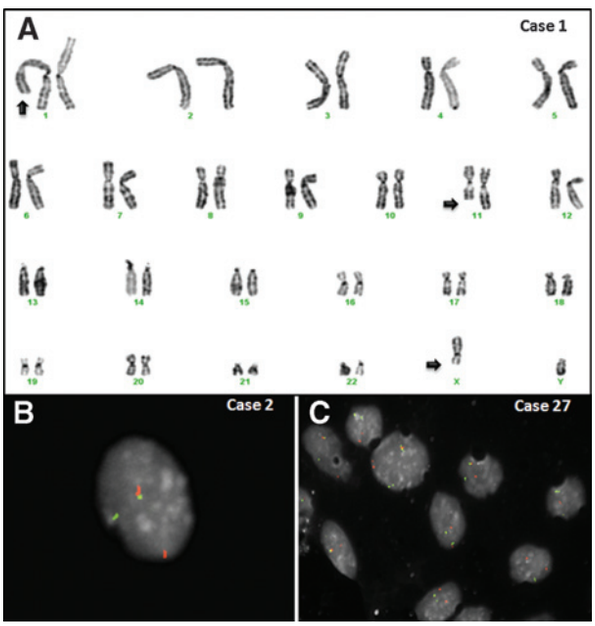

Figure 2.
A, representative karyotype of case 1 showing a translocation t(X;1;11)(q13;p36;q23) correlating with the genomic location of the *MED12* and *PRDM10* genes. B and C, interphase FISH analysis revealing split signals with BAC probes covering 5′and 3′ regions neighboring the *PRDM10* gene.

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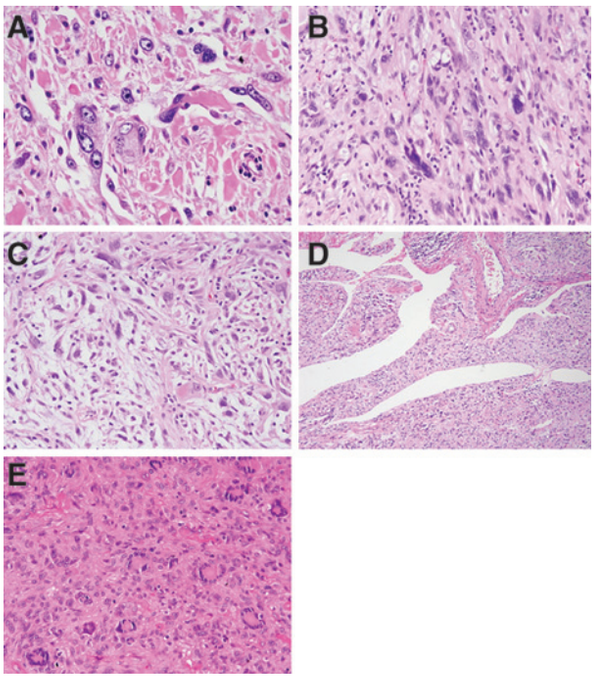

Figure 3.
Morphology of *PRDM10* fusion-positive UPS. A, case 2 showing nondistinctive pleomorphic spindle and ovoid cells. B, high power of case 1 highlights bizarre nuclear morphology. C to E, individual cases showing focally myxoid stroma (case 2), pseudovascular clefts (case 1), and prominent tumor giant cells (case 27).

myxoid matrix (case 2), prominent pseudovascular clefts (case 1) and numerous multinucleate giant cells (case 27), respectively. Tumor cells expressed only CD34, which is not lineage specific.

Discussion

Although UPS is one of the most common sarcoma subtypes, its genetic features remain poorly explored. Marked differences in clinical outcome that cannot be explained merely by differences in tumor size or location, combined with a lack of targeted treatments, provide compelling arguments for more comprehensive attempts to delineate genetic subgroups of UPS. We have in this study been able to identify a small but significant subset of UPS showing gene fusions in which either *MED12* or *CITED2* is fused with *PRDM10*. None of these gene fusions has been described in any other neoplasm, suggesting that they are specific for UPS.

The cohort studied here included a total of 84 soft tissue sarcomas, 28 of which were diagnosed as UPS. The three sarcomas that were positive for *PRDM10* fusions were all diagnosed as UPS, but we find it unlikely that *PRDM10* fusions are present in as much as 10% (3/28) of UPS. Only one of the 26 UPS cases in the extended cohort was positive, and that case had been selected because it had been classified as a low-grade malignant tumor; low-grade malignant lesions constitute a minority of all UPS (1). Also, a comparison between the features of the present cases and previous cytogenetic data on UPS indicate that *PRDM10* fusions are rare events. In the present study, two of three fusion-positive cases had simple karyotypes with a balanced translocation, either as the sole change or together with a few numerical aberrations; the cytogenetic analysis failed in the third case. Abnormal karyotypes have been described in 85 cases of UPS, the majority (57/85) showing highly complex karyotypes with 50 to 100 chromosomes and multiple structural and numerical changes (4); only 13 of the cases had a near-diploid karyotype with less than five structural rearrangements and without any sign of gene amplification (ring chromosomes or double minutes). Thus, we estimate the frequency of *PRDM10* fusion-positive tumors to be around 5% of all UPS.

Even if the *PRDM10* fusion-positive cases constitute a minority of all UPS, it may be clinically important to identify them. The high metastasis rate of UPS, approximately one third, calls for aggressive treatment. Possibly, *PRDM10* fusion-positive tumors have a lower propensity for metastasizing; none of our 3 patients has developed metastases and they were all in complete remission after 41 months to 21 years of follow-up. Furthermore, and in agreement with the favorable outcome, all three were classified as low-grade malignant tumors when re-reviewed; it should be emphasized, though, that two had initially been diagnosed as high-grade lesions. However, there were no distinct morphologic features among the *PRDM10* fusion-positive cases setting them apart from other UPS. Thus, fusions involving *PRDM10* could possibly function as a marker to identify a patient subset with favorable clinical outcome. Needless to say, however, the behavior of *PRDM10* fusion-positive tumors needs to be evaluated in a much larger series of cases, before it can be decided whether they should be treated in other ways than other UPS.

PRDM10 is a poorly studied member of the PRDM (PRDI-BF1 and RIZ homology domain containing) family of proteins. It lacks enzymatic activity and is believed to function as a transcriptional cofactor by recruiting histone-modifying enzymes to target promoters, and is suggested to have an important role during development of the central nervous system (18). The protein is characterized by multiple zinc-finger domains and an N-terminal PR domain (19) . Several other members of the PRDM family are associated with cancer and gene fusions involving *PRDM16* have been reported in cases of acute myelogenous leukemia and myelodysplastic syndrome. *PRDM16* can have several partner genes and all reported fusions lead to overexpression of parts of the gene, usually not containing the PR domain, or the complete gene by promoter swapping (20).

MED12 is part of a large multiprotein complex known as the mediator complex, which functions as a protein bridge between transcription factors and RNA polymerase II to initiate transcription (21). This complex also affects later stages of the transcription process, including elongation and termination. MED12, MED13, Cyclin C, and cyclin-dependent kinase 8 together form a dissociable part of the mediator complex known as the CDK8 module (22). The CDK8 module functions as a negative regulator of transcription by competing for the same binding site as RNA polymerase II on the core mediator complex. However, there are also reports implicating CDK8 as a transcriptional activator (23). This multifunctional module plays major roles in proliferation and differentiation and participates in various molecular pathways, including the p53 and Wnt/β pathways (24). MED12 regulates the kinase activity of the Cdk8 module and mutations in *MED12* are associated with several diseases, including neoplasia. Mutations, especially in exon 2, are found at high frequencies in uterine leiomyoma and fibroadenoma of the breast (25, 26), as well as in malignancies, such as colorectal cancer, leiomyosarcoma, and prostate cancer (21, 27).

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CITED2 is a non–DNA-binding transcriptional coactivator that affects the activity of multiple genes by recruiting CBP/p300 to chromatin via the DNA-binding transcription factor AP2. CITED2 also competitively inhibits the transcription of hypoxia-activated genes by blocking the interactions between HIF-A1 and CBP/p300 (28). It is a multifunctional protein best known for its importance during development but also in cancer. It has been reported to be overexpressed in breast cancer in which it modulates the transcriptional activity of the estrogen receptor (29).

It is difficult to make predictions on the functional outcome of fusion genes without further analysis at the protein level. However, it is reasonable to assume that both *MED12/PRDM10* and *CITED2/PRDM10* act as driver mutations; all previously identified recurrent gene fusions occurring in sarcomas with simple karyotypes, that is, with few or no additional aberrations other than the translocations underlying the fusions, have been shown to be strong driver mutations (30). It is also worth noting that all genes involved in the *PRDM10* fusions play important roles in gene regulation. The breakpoint in *PRDM10* reveals that the PR domain is lost, but nine of the 10 zinc-finger domains are included in the fusion. The breakpoints in *MED12* and *CITED2* are located close to the 3′end of the genes, which might indicate that the functions of these proteins are still intact despite the fusion events. Recruiting functional transcription regulators to a new set of target genes by fusing them to the zinc-finger domains of *PRDM10* could potentially be a mode of action to promote tumor development in these cases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J. Hofvander, F. Mertens
Development of methodology: J. Hofvander, J. Tayebwa
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): O. Brosjö, O. Larsson, F.V. von Steyern, N. Mandahl, C.D.M. Fletcher, F. Mertens
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Hofvander, J. Tayebwa, N. Mandahl, C.D.M. Fletcher, F. Mertens
Writing, review, and/or revision of the manuscript: J. Hofvander, J. Tayebwa, F.V. von Steyern, N. Mandahl, C.D.M. Fletcher, F. Mertens
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Hofvander, J. Nilsson, L. Magnusson, F.V. von Steyern, F. Mertens
Study supervision: F. Mertens

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