Received: 27 June 2017 | Revised: 19 July 2017 | Accepted: 21 July 2017

DOI: 10.1002/gcc.22485

RESEARCH ARTICLE

WILEY

Fusion of the genes *BRD8* and *PHF1* in endometrial stromal sarcoma

Francesca Micci[1] | Marta Brunetti[1] | Paola Dal Cin[2] | Marisa R. Nucci[2] | Ludmila Gorunova[1] | Sverre Heim[1,3] | Ioannis Panagopoulos[1]

[1]Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Norway

[2]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts

[3]Faculty of Medicine, University of Oslo, Norway

Correspondence
Dr. Francesca Micci, Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, 0310 Oslo, Norway.
Email: francesca.micci@labmed.uio.no

Funding information
Radium Hospital Foundation

Abstract

We present a new endometrial stromal sarcoma (ESS)-associated genomic rearrangement involving chromosome arms 5p and 6p and leading to the formation of a *BRD8-PHF1* fusion gene. The *PHF1* (PHD finger protein 1) gene, from 6p21, is known to be rearranged in ESS in a promiscuous way inasmuch as it has been shown to recombine with *JAZF1*, *EPC1*, *MEAF6*, and now also with *BRD8*, in tumors of this type. In all rearrangements of *PHF1*, including the present one, a recurrent theme is that the entire coding part of *PHF1* constitutes the 3′ end of the fusion. *BRD8* (bromodomain containing 8) encodes a protein which is involved in regulation of protein acetylation and/or histone acetyl transferase activity. All the genetic fusions identified so far in ESS appear to recombine genes involved in transcriptional regulation, that is, polycomb group complex-mediated and aberrant methylation/acetylation genes. This adds to the likelihood that the new *BRD8-PHF1* shares the same pathogenetic mechanism as the other ESS-specific rearrangements.

1 | INTRODUCTION

Endometrial stromal sarcoma (ESS) is a mesenchymal malignancy composed of cells that resemble those of proliferative-phase endometrial stroma. According to the World Health Organization tumor classification system,[1] ESS are divided into low-grade (LG-ESS) and high-grade (HG-ESS) tumors, which differ histologically, genetically, and clinically. Several specific chromosomal rearrangements and the genes behind them have been identified for both entities.[2] In the LG-ESS subgroup, the translocation t(7;17)(p15;q21) is the most common, followed by rearrangements of chromosomal band 6p21, the balanced t(X;17)(p11;q23), and X;22-rearrangements.[2] The *JAZF1-SUZ12* transcript (previously known as *JAZF1-JJAZ1*) was the first ESS-specific fusion to be identified, as a result of the 7;17-translocation,[3] but later *JAZF1* was shown to rearrange also with the PHD finger protein 1 gene (*PHF1*) from 6p21.[4] *PHF1* is promiscuous in the sense that it has been found rearranged with several different partners in ESS, namely *EPC1* (10p11), *MEAF6* (1p34), and the already mentioned *JAZF1* (7p15).[4–6] The *MBTD1-CXorf67* fusion is brought about by the t(X;17)[7] whereas *ZC3H7B-BCOR* was identified in cells carrying a X;22-rearrangement.[8] Recently, Allen and coworkers identified a new fusion variant in LG-ESS, *JAZF1-BCORL1*.[9]

HG-ESS are characterized by a balanced 10;17-translocation leading to the formation of a *YWHAE-NUTM2* fusion transcript (formerly known as *YWHAE-FAM22*).[10]

A review of the literature shows 18 reported ESS with other cytogenetic rearrangements, strongly indicating that several tumor-specific gene hybrids still remain to be detected.[11] Here, we describe a novel partner of the *PHF1* gene detected by G-banding analysis followed by transcriptome sequencing of an LG-ESS with rearrangement of chromosome band 6p21.

2 | MATERIALS AND METHODS

2.1 | Patient history

A 50-year-old woman underwent hysterectomy and bilateral salpingo-oophorectomy and was diagnosed with FIGO stage I ESS for which she subsequently received radiation therapy. A CT scan of the chest at the time revealed a solitary small left lung nodule. A follow up CT scan 3 years later showed that the nodule had remained stable in size.

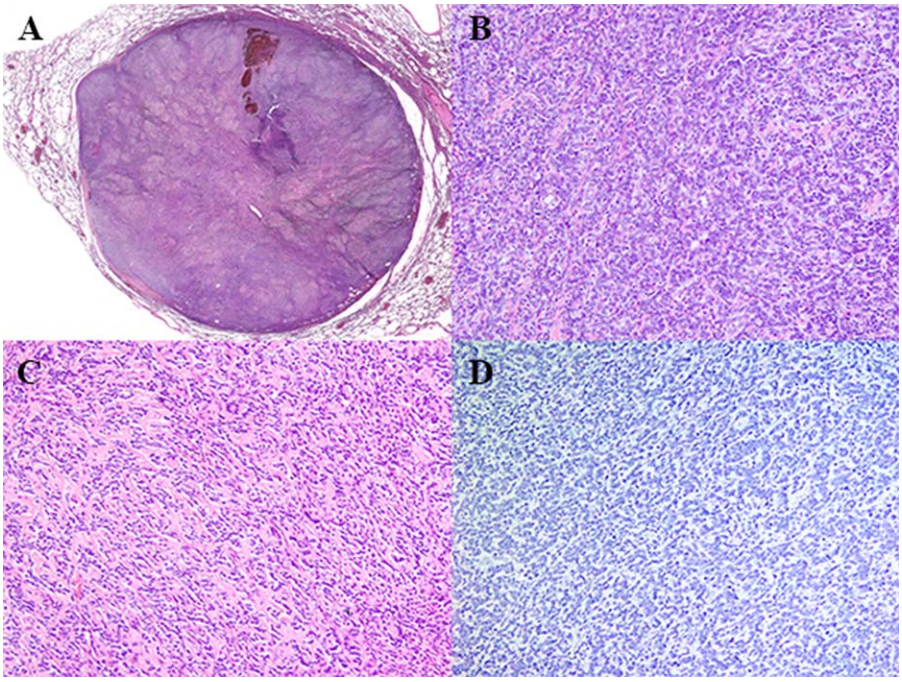

FIGURE 1 Pathologic examination of the metastatic LG-ESS. The tumor forms a well circumscribed mass in lung parenchyma and is composed of cells which morphologically resemble proliferative phase endometrial stroma (A). In areas, the tumor showed a corded growth indicative of sex-cord like differentiation (B) while other foci had a dense collagenous matrix (C). The tumor cells were negative for markers of sex-cord like differentiation, including SF-1 (D) [Color figure can be viewed at wileyonlinelibrary.com]

However, a new 0.8 cm nodule in the right lower lobe was now seen; this was resected and diagnosed as metastatic ESS based on morphology in combination with strong expression for CD 10 (Figure 1); of note, the tumor was remarkable for sex cord-like differentiation (Figure 1B) as well as dense collagenous matrix deposition (Figure 1C). The sex-cord like areas were negative for markers of sex cord differentiation, including calretinin, inhibin, and SF-1 (Figure 1D). One year later, another suspicious 5 mm nodule in the right lower lobe was noted, which remained stable for another 2 years but then slowly increased in size leading to a pulmonary wedge resection 5 years after it was initially identified. It, too, was diagnosed as metastatic ESS. Six months later, she underwent three additional pulmonary wedge resections of remaining tumor nodules involving the left upper and lower lobe, all confirmed as metastatic ESS and one of which was sampled for cytogenetic analysis. She is at the time of writing without evidence of disease for 1 year.

2.2 | Cell culturing and karyotyping

The sample was sent to the cytogenetic laboratory at Brigham and Women's Hospital for diagnostic purposes. Cell culturing, harvesting, and G-banding analysis were performed according to standard methods.[12] The karyotype was written following the recommendation of the International System for Human Cytogenomic Nomenclature.[13]

2.3 | RNA extraction and transcriptome sequencing

Total RNA was extracted from cells using miRNeasy (Qiagen, Hilden, Germany) and QIAcube (Qiagen). The RNA quality was evaluated using the Experion Automated Electrophoresis System (Bio-Rad Laboratories, Oslo, Norway). One microgram of total RNA was sent for high-throughput paired-end RNA-sequencing at the Norwegian Sequencing Centre, Ullevål Hospital (http://www.sequencing.uio.no/). Detailed information about the RNA sequencing was given elsewhere.[11] The software FusionCatcher (version 0.99.3a beta-April 15, 2014) was used for the discovery of fusion transcripts (https://code.google.com/p/fusioncatcher/).

2.4 | Reverse transcriptase-PCR (RT-PCR) and Sanger sequencing

The primers used for validation of the *BRD8-PHF1* fusion and subsequent Sanger sequencing are listed in Table 1. For RT-PCR, 200 ng of total RNA was reverse-transcribed in a 20 μL reaction volume using iScript Advanced cDNA synthesis Kit for RT-PCR according to the manufacturer's instructions (Bio-Rad Laboratories, Oslo, Norway). The 25 μL PCR volume contained 12.5 μL Premix Ex Taq DNA Polymerase Hot Start Version (Takara Bio Europe/SAS, Saint-Germain-en-Laye, France), 1 μL of cDNA, and 1 μL of each of the forward and reverse primers. The primer combinations BRD8F1-PHF1Ex2R1 and BRD8F2-PHF1Ex2R2 were used to validate presence of *BRD8-PHF1* fusion transcript by PCR and NESTED-PCR, respectively. The PCR amplifications were run on a C-1000 Thermal cycler (Bio-Rad Laboratories) with an initial denaturation at 94°C for 30 seconds, followed by 35 cycles at 98°C for 7 seconds, 55°C for 30 seconds, 1 minute at 72°C, and a final extension at 72°C for 5 minutes. Three microliter of the PCR product were stained with GelRed (Biotium, Hayward, CA), analyzed by electrophoresis through 1.0% agarose gel, and photographed. The remaining

TABLE 1 Primers used for PCR and Sanger sequencing analyses

Name	Sequence	Position	Gene	Accession number
BRD8 F1	5′-GCAGAGACTCAGCACAAGTT-3′	2069–2088	*BRD8*	NM_006696.3
PHF1-Ex2 R1	5′-GCAGGGCTAATGTCTTTCCA-3′	459–478	*PHF1*	NM_002636.4
BRD8 F2	5′-GGAAGATGGTGTCAGTGAAGC-3′	2170–2190	*BRD8*	NM_006696.3
PHF1-Ex2 R2	5′-ATCTTGACCCTCCCAAAGCC-3′	325–344	*PHF1*	NM_002636.4

22 μL PCR product were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced using 3500 Genetic Analyzer (Applied Biosystems). The BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and BLAT (http://genome.ucsc.edu/cgi-bin/hgBlat) softwares were used for computer analysis of sequence data.

3 | RESULTS

The G-banding analysis of the tumor cells showed an abnormal karyotype with material of unknown origin on the short arm of chromosome 6 as the sole aberration, that is, 46,XX,add(6)(p21) (Figure 2A). Since

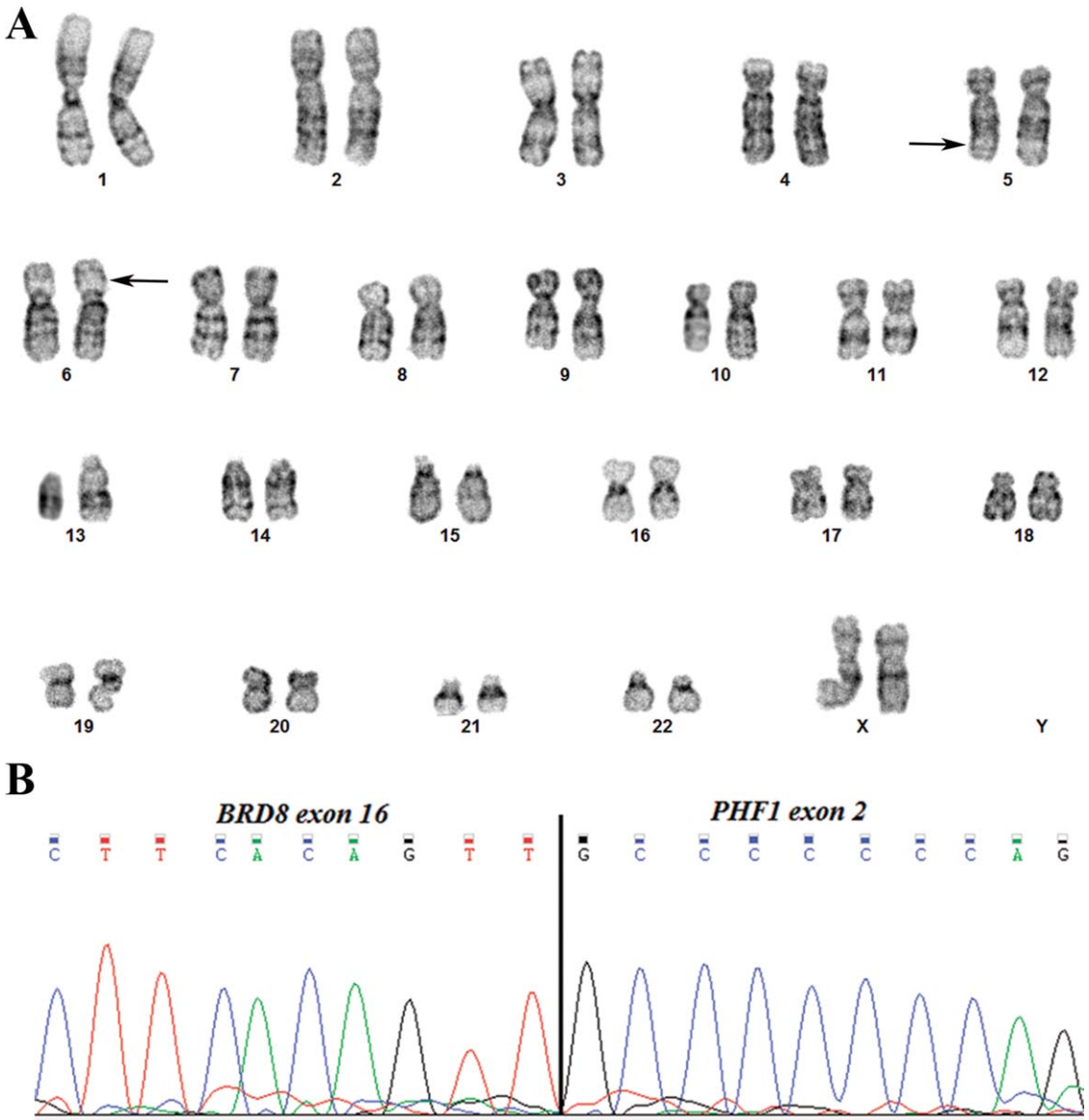

FIGURE 2 Karyogram of the ESS showing the 5;6-rearrangement. Arrows point at breakpoints (A). Partial sequence chromatogram of the *BRD8-PHF1* fusion (B) [Color figure can be viewed at wileyonlinelibrary.com]

the rearranged chromosomal band was 6p21, involvement of the *PHF1* gene was suspected. However, the origin of the additional material could not be identified by G-banding and material for FISH experiments was not available. To see if an already known fusion transcript was behind the 6p-aberration, or perhaps corresponding to a cryptic rearrangement on some other chromosome, a series of PCRs using specific primers for ESS-related fusions was performed.[11] No such fusion was identified (data not shown).

The data from transcriptome sequencing were analyzed with the FusionCatcher algorithm which found 997 potential fusion transcripts, among them a fusion between the bromodomain containing 8 gene (*BRD8*; from 5q31.2) and *PHF1*. RT-PCR with specific primers was performed and Sanger sequencing confirmed the presence of an in-frame fusion between exon 16 of *BRD8* (nucleotide 2361; accession number NM_006696.3) and exon 2 of *PHF1* (nucleotide 221; accession number NM_002636.4; Figure 2B).

A list of all putative fusion transcripts is provided in Supporting Information, Table S1. Except for *BRD8-PHF1*, all transcripts with more than two unique reads involved genes that were close to one another. These suggested transcripts were assumed to be the result of a read through and, hence, false positives. Besides, and in contrast to *BRD8-PHF1*, the karyotyping data did not give any indication that they might be for real.

The karyotype was consequently revised to 46,XX,t(5;6)(q31;p21) (Figure 2A).

4 | DISCUSSION

The promiscuity of *PHF1* is well known in ESS as the gene has been shown to recombine with *JAZF1* from 7p15, *EPC1* from 10p11, and *MEAF6* from 1p34; however, also additional genes must be involved since FISH analysis of a cohort of ESS with *PHF1* rearrangement demonstrated that neither *JAZF1* nor *EPC1* was the partner gene in a significant subset of cases.[14–16] We here present *BRD8* as a new partner for *PHF1* fusion in such tumors. *BRD8* maps to chromosome band 5q31 and encodes a protein which interacts with thyroid hormone receptor in a ligand-dependent manner to enhance thyroid hormone-dependent activation from thyroid response elements.[17] BRD8 contains a bromodomain which is an acetylated lysine binding domain thought to be involved in regulation of protein acetylation and/or histone acetyl transferase activity.[18,19] BRD8 is part of the signal pathway that begins with thyroid hormone or retinoid X; through interaction with the hormone, BRD8 is recruited to activate the NuA4HAT complex that regulates chromatin remodeling and transcription.[19] It has been suggested that drugs targeting BRD8 would improve therapy against aggressive/metastatic colorectal cancers.[19]

The chimeric transcript retains the entire coding region of *PHF1* but loses the conserved bromodomain sequence from *BRD8*. The predicted protein therefore consists of only the conserved PHF1 domains. Loss of bromodomain from BRD8 may result in alteration of protein acetylation and/or histone acetyl transferase activity. Additional studies of this chimeric protein should shed more light on its role in ESS tumorigenesis.

So far, all the *PHF1* fusion partners, *JAZF1*, *EPC1*, *MEAF6*, and now also *BRD8*, function as transcription regulators, either through formation of zinc finger motifs or in altering acetylation of histone proteins.[6,20]

PHF1, as well as SUZ12 and MBTD1, are members of the polycomb repressive complex family.[21,22] BCOR was found to be a key transcriptional regulator,[23] so it appears that all genes rearranged in ESS have a unifying role in epigenetic regulation, either through polycomb mediated gene silencing or post-transcriptional covalent modification of histone proteins.

In this case, transcriptome sequencing was used to identify the partner of *PHF1*. A renewed scrutiny of the tumor karyotype after the NGS analysis showed an aberrant 5q but with a size of the chromosome arm and a banding pattern similar to that of the normal one (Figure 2A), which is why it had been overlooked/misread in the initial analysis. Rearrangements of chromosomal band 6p21 characterize one-fifth to one-fourth (23.5%) of hitherto reported LG-ESS with karyotypic information.[11] The most frequent partner for *PHF1* is *JAZF1* through an unbalanced 6;7-translocation, followed by *EPC1* and *MEAF6* from rearrangements of chromosomes 10 and 1, respectively. We retrieved literature data[11] searching for ESS whose karyotypes showed the presence of a 5;6-rearrangement. No recombinations between these two chromosomes were identified; however, three cases showed a 6p21-rearrangement that did not target any known ESS-related partner chromosome.[10,24,25] These cases may hide the involvement of an additional new partner for *PHF1*. Furthermore, we have tested the *BRD8-PHF1* fusion in a cohort of ESS collected in our laboratories that were negative for the known ESS-related fusion finding no recurrence (data not shown). Speculative though this may seem, we nevertheless trust that additional studies on ESS will establish the recurrence of the newly reported fusion.

In all *PHF1*-targeting rearrangements, including the present one, a recurrent theme has been that the entire coding part of *PHF*1 constitutes the 3′-end of the fusion.[4,6] This adds to the likelihood that the pathogenetic mechanism behind the rearrangements is similar despite the different partners.

PHF1 is promiscuously involved in ESS, but the same gene is also found rearranged in non-ESS and non-endometrial stromal tumors such as cardiac ossifying sarcoma[26] as well as benign, atypical, and malignant ossifying fibromyxoid tumors,[27–29] leading to fusion genes *EPC1-PHF1*, *MEAF6-PHF1*, and *EP400-PHF1*. The finding of similar fusions in different tumor types is a well known phenomenon in cancer, and evidently ESS-related fusions are no exception. Of note, a recent study has suggested that *ZCH7B-BCOR* gene fusion may represent a novel type of high grade ESS; however, the examined tumors were histologically similar to myxoid leiomyosarcoma and the possibility that this, too, represents a rearrangement that is not unique to stromal tumors, comes across as a distinct possibility.[30]

ACKNOWLEDGMENTS

This work was supported by grants from the Radium Hospital Foundation. It was approved by the institutional research protocol # 201P002544.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Francesca Micci http://orcid.org/0000-0002-2194-556X

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Micci F, Brunetti M, Dal Cin P, et al. Fusion of the genes *BRD8* and *PHF1* in endometrial stromal sarcoma. *Genes Chromosomes Cancer*. 2017;56:841–845. https://doi.org/10.1002/gcc.22485