Consistent Rearrangement of Chromosomal Band 6p21 with Generation of Fusion Genes JAZF1/PHF1 and EPC1/PHF1 in Endometrial Stromal Sarcoma

Francesca Micci, Ioannis Panagopoulos, Bodil Bjerkehagen, and Sverre Heim

Departments of Cancer Genetics and Pathology, The Norwegian Radium Hospital; Faculty of Medicine, University of Oslo, Oslo, Norway; and Department of Clinical Genetics, University Hospital, Lund, Sweden

Abstract

Endometrial stromal sarcomas (ESS) represent <10% of all uterine sarcomas. Cytogenetic data on this tumor type are limited to 32 cases, and the karyotypes are often complex, but the pattern of rearrangement is nevertheless clearly nonrandom with particularly frequent involvement of chromosome arms 6p and 7p. Recently, a specific translocation t(7;17)(p15;q21) leading to the fusion of two zinc finger genes, juxtaposed with another zinc finger (JAZF1) and joined to JAZF1/JJAZ1, was described in a subset of ESS. We present three ESS whose karyotypes were without the disease-specific t(7;17) but instead showed rearrangement of chromosomal band 6p21, twice as an unbalanced t(6p;7p) and once as a three-way 6;10;10 translocation. All three tumors showed specific rearrangement of the PHD finger protein 1 (PHF1) gene, located in chromosomal band 6p21. In the two tumors with t(6;7), PHF1 was recombined with the JAZF1 gene from 7p15, leading to the formation of a JAZF1/PHF1 fusion gene. The third tumor showed a t(6p;10q;10p) as the sole karyotypic abnormality, leading to the fusion of PHF1 with another partner, the enhancer of polycomb (EPC1) gene from 10p11; EPC1 has hitherto not been associated with neoplasia. The PHF1 gene encodes a protein with two zinc finger motifs whose involvement in tumorigenesis and/or tumor progression has not been reported before, but its rearrangement clearly defines a new pathogenetic subgroup of ESS. (Cancer Res 2006; 66(1): 107-12)

Introduction

Malignant tumors mimicking the differentiation of endometrial stromal cells, endometrial stromal sarcoma (ESS), are rare and account for <10% of uterine sarcomas. ESS were traditionally divided into low-grade and high-grade tumors according to the classification of Norris and Taylor (1), but because the so-called high-grade ESS usually lack specific differentiation and often present little or no histologic resemblance to endometrial stroma, they are now mostly referred to as undifferentiated endometrial sarcomas (2). ESS therefore consist of only low-grade ESS and variants thereof, regardless of the mitotic index, as long as the lesional cells resemble nonneoplastic proliferative endometrial stroma (3–5).

Little is known about the genetic background of ESS as only 32 such tumors have been karyotyped and reported scientifically (6–8). The pattern of rearrangements thus detected is nevertheless clearly nonrandom with particularly frequent involvement of chromosome arms 6p and 7p (7). Recently, a specific translocation t(7;17)(p15q21) leading to the fusion of two zinc finger genes, juxtaposed with another zinc finger (JAZF1) and joined to JAZF1/JJAZ1, was described in a subset of ESS (9). Both genes, the JAZF1 at 7p15 and JJAZ1 at 17q21, contain sequences encoding zinc finger motifs characteristic of DNA-binding proteins. The gene fusion results in expression of a tumor-specific mRNA transcript containing 5’-JAZF1 and 3’-JJAZ1 sequences but retaining the zinc finger motifs from both genes. Because wild-type JAZF1 is expressed in normal endometrium, it has been suggested that the JAZF1/JJAZ1 fusion gene creates a chimeric protein that disrupts transcription in a lineage-specific manner (9).

Several sarcoma-specific translocations exist, but some of them display considerable molecular diversity both in terms of intragenic variant fusions and the involvement of alternative translocation partners in pathogenetically equivalent variant translocations (10, 11). Accordingly, the failure to detect the JAZF1/JJAZ1 fusion transcript in ESS not showing the 7;17 translocation (7, 12) suggests the existence of pathogenetic, cytogenetic, and molecular variants of the ESS-specific t(7;17) and JAZF1/JJAZ1. Indicative of the same possibility is also the fact that chromosomal band 7p15 was found rearranged in 5 of the 32 ESS with karyotypic changes but without the aforementioned 7;17 translocation (8). The second most common cytogenetic abnormality registered in ESS involves chromosomal band 6p21, rearranged in eight tumors; this too hints at pathogenetic, cytogenetic, and molecular genetic ESS-specific features that have not yet been sufficiently examined and understood.

We therefore subjected to detailed analysis three ESS showing rearrangement of chromosomal band 6p21. In all tumors, specific involvement of the PHD finger protein 1 (PHF1) gene in 6p21 could be shown. PHF1 was found recombined with two different partners, with the JAZF1 gene in the two tumors showing a 6p;7p rearrangement and with the enhancer of polycomb (EPC1) gene from 10p11 in the third tumor, which had a 6;10;10 translocation as the sole karyotypic abnormality.

Materials and Methods

Tumors. Samples from three surgically removed ESS were subjected to histologic and genetic analyses. The patient of case 1 was a 33-year-old woman who underwent hysterectomy after a biopsy of a uterine tumor had shown an ESS. The tumor cells were small and spindle shaped with a mitotic count of 0.1 per high-power field. Only small areas with fibrous or myxoid tissue were seen. The tumor infiltrated extensively through the uterine wall and into the parametrium and vessels (Fig. 1). The patient of
Fluorescence in situ hybridization analyses. Different fluorescence in situ hybridization (FISH) analyses were done depending on which types of chromosome aberrations were discovered by G-banding. Multiplex FISH (15) was done on cases 1 and 2; in case 1 on a previously G-banded slide according to the protocol described by Teixeira et al. (16), whereas in case 2, fresh chromosome preparations were available for multiplex FISH.

In case 3, locus-specific probes derived from bacterial artificial chromosomes (BAC) and P1 artificial chromosome clones were prepared and hybridized to detect the exact breakpoint of the 10;10 translocation originally thought to be the only chromosomal abnormality present in this tumor (see below). The BAC clones were retrieved from the RPCI-4 Human BAC library and CalTech human BAC library; the P1 artificial chromosome clones were from the RPCI-4 Human P1 artificial chromosome library (P. de Jong libraries, http://bacpac.chori.org/home.htm). They were selected according to physical and genetic mapping data on chromosomes 6, 7, and 10 (see below) reported in the Human Genome Browser at the University of California, Santa Cruz website (May 2005, http://genome.ucsc.edu/).

The clones initially used were RP11-414H17 and RP11-74N14 mapping to 10p15 and RP11-3E5 and RP11-7D5 mapping to 10q24. The identification of a three-way translocation involving also chromosome 6 and the redefinition of the breakpoints on 10p and 10q (see below) led us to perform additional analyses using BAC clones RP11-61SA19, RP11-51SH15, and RP11-75H10, mapping to 6p21 (from centromere to telomere); RP11-32F2 and RP11-2H102 mapping to 10p11; and 433J16 mapping to 10q21 (resources for Molecular Cytogenetics, Bari, Italy; http://www.biologia.uniba.it/rmc/). In case 2, clones RP11-81H15 and RP1-78A18, mapping to 7p15 and covering the JAZF1 locus, and CTD-2307H19, mapping to 6p21 and covering the PHF1 locus, were used to detect JAZF1/PHF1 fusion at the molecular cytogenetic level. The clones were chosen in selective media, and DNA was extracted according to standard methods (17). DNA probes were directly labeled with a combination of FITC-12-dCTP and FITC-12-dUTP, Texas Red-6-dCTP and Texas Red-6-dUTP (New England Nuclear, Boston, MA), or indirectly with biotin-dUTP (Molecular Probes, Invitrogen, Carlsbad CA) by nick translation and detected with streptavidin-diethylaminocoumarin (Invitrogen). The subsequent hybridization conditions as well as the detection procedure were according to standard protocols (18).

Molecular genetic investigations. Tumor tissue adjacent to that used for cytogenetic analysis and histologic examination had been frozen and stored at −80°C. Total RNA was extracted from the three tumors using Trizol reagent according to the manufacturer’s instructions (Invitrogen). The primers used for PCR amplification and sequencing are listed in Table 2. cDNA quality was checked in each case using ABL-specific primers (19).

5′-Rapid amplification of cDNA ends (RACE)-PCR was done in case 2 with primers JAZF1-182F and JAZF1-357F. 5′-RACE-PCR was done in case 3 with primers PHF1-327R and PHF1-250R (Table 2). In both cases, the cDNA synthesis, the RACE-PCR, the subsequent NESTED-PCR were done using the BD SMART RACE kit following the manufacturer’s recommendations (BD Biosciences, San Jose CA). The PCR cycles were as follows: 1 minute at 94°C and 25 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 3 minutes at 72°C.

cDNA was synthesized using 5 μg of total RNA in 20 μl reaction mixture [50 mmol/L Tris-HCl (pH 8.3) at 25°C, 75 mmol/L KCl, 3 mmol/L MgCl₂, 108

---

Table 1. Karyotypic data on the three ESS based on G-banding and FISH analysis

<table>
<thead>
<tr>
<th>Case no.</th>
<th>G-banding karyotype</th>
<th>FISH karyotype</th>
</tr>
</thead>
</table>
10 mmol/L DTT, 1 mmol/L each deoxynucleotide triphosphate, 20 units RNase inhibitor (RNA guard, Amersham Biosciences, Piscataway, NJ), 0.5 pmol/L random hexamers, and 400 units Moloney murine leukemia virus reverse transcriptase (Invitrogen). The reaction was carried out at 37°C for 60 minutes, heated for 5 minutes at 65°C, then kept at 4°C until analysis.

The PCR amplification was done using 1 μL of the cDNA as template in 50 μL reaction volume containing 20 mmol/L Tris-HCl (pH 8.4) at 25°C, 50 KCl, 1.25 mmol/L MgCl₂, 0.2 mmol/L of each deoxynucleotide triphosphate, 1 unit platinum Taq polymerase (Invitrogen), and 0.5 μmol/L of each of the forward and reverse primers used. The JAZF1/PHF1 fusion transcript was detected using JAZF1-182F and PHF1-250R and JAZF1-182F and PHF1-327R primer combinations, whereas the EPC1/PHF1 transcript was detected using EPC1-1651F and PHF1-327R and EPC1-1860F and PHF1-250R primer combinations. The PCR was done with the following cycles: 5 minutes at 94°C and 30 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute at 72°C, and a final extension for 10 minutes at 72°C. The PCR products were analyzed by electrophoresis through 1.2% agarose gel, stained with ethidium bromide, and purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany). Both strands were subjected to sequence analysis using the Applied Biosystems, Foster City, CA). For cases 1 and 2, the JAZF1-182F and PHF1-250R were used. Both strands were subjected to sequence analysis using the Applied Biosystems, Foster City, CA). For cases 1 and 2, the JAZF1-182F and PHF1-250R were used. The analysis was done on the Applied Biosystems Model 3100-Avant DNA sequencing system. The BLAST software (http://www.ncbi.nlm.gov/BLAST) was used for computer analysis of sequenced data.

### Results

Cases 1 and 2 showed complex karyotypes, which could not be described completely after G-banding analysis only; therefore, multiplex FISH was used to identify the chromosomes involved in different rearrangements (Table 1; Fig. 2). In case 3, FISH with locus-specific probes was done to identify the breakpoint positions on the short and long arms of chromosome 10, as a t(10p11q12) was thought to be the only rearrangement based on the G-banded karyotype. The FISH analysis, however, showed that the probe from 10p11 mapped to a cytogenetically seemingly normal 6p; thus, eventually, a three-way translocation t(6p11q10q12p) was identified (Table 1; Fig. 2). None of the three tumors showed the disease-specific 7;17 translocation. However, as all three ESS presented rearrangements involving chromosome arm 6p and in cases 1 and 2 in recombination with 7p15, we decided to study these tumors at the molecular level to identify possible variant partners of the JAZF1 gene located in 7p15 and already known to be rearranged in the subgroup of ESS with t(7;17) (9, 12).

RNA of good quality was extracted from fresh frozen samples of all three ESS. To investigate for a cryptic rearrangement of chromosomes 7 and 17, reverse transcription-PCR was done using specific primer combinations for JAZF1-182F and JAJZ1-885R (Table 2; ref. 7), but no specific transcript was found.

To test if tumors 1 and 2 (i.e., those with cytogenetic aberrations of 7p15) presented some other fusion transcript than the JAZF1/JAZ1, we did 3′-RACE-PCR analysis for the JAZF1 gene. This investigation detected a specific transcript in which the JAZF1 gene was fused with the PHF1 gene, and the fusion was then confirmed using combinations of specific primers for the aforementioned genes. In case 1, two PCR fragments were present, of 170 and 250 bp, respectively, whereas in case 2, a single 45-bp PCR fragment was detected (Fig. 3) using a specific JAZF1-182F/PHF1-250R primer combination. Direct sequencing of the transcripts revealed that the fragments from case 1 were JAZF1/PHF1 chimeric fragments, both containing a sequence of intron 2 of JAZF1 (Fig. 3). Fragment 1 contained an open reading frame for the JAZF1/PHF1 fusion, whereas fragment 2 was an out-of-frame fusion of the JAZF1/PHF1 fusion. In case 2, direct sequencing revealed that the fragment contained sequences from intron 3 of JAZF1 fused with sequences of a noncoding region from PHF1 intron 1 (Fig. 3). The fusion transcript had an open reading frame. FISH analysis of this case with two JAZF1-specific and one PHF1-specific probe unequivocally showed that the PHF1 gene was fused with the JAZF1 gene (Fig. 2).

In the third ESS, FISH with locus-specific probes, RP11-414H17 and RP11-74N14 mapping to 10p15 and RP11-34E5 and RP11-7D5 mapping to 10q22, identified a t(6;10;10)(p21;q22;p11) as the sole karyotypic abnormality (Fig. 2). We again used locus-specific probes to better characterize the breakpoints of the translocation, but due to lack of material, we could not find out which BAC clones spanned the breakpoints. In addition, we tested for possible cryptic rearrangements between chromosomes 6 and 7 by reverse transcription-PCR using a JAZF1/PHF1-specific primer combination, but no specific transcript was identified. The PHF1 gene, located in chromosomal band 6p21, was then tested for involvement in this tumor using 5′-RACE-PCR with primers for the PHF1 gene. A specific transcript was detected identifying a fusion between the EPC1 and PHF1 genes. Direct sequencing of this transcript revealed in-frame fusion of exon 10, codon 581, of the EPC1 mRNA to exon 2, 17 bp upstream the ATG, of the PHF1 mRNA (Fig. 4).

### Discussion

All three ESS examined in this report showed rearrangement of chromosomal band 6p21 but no disease-specific t(7;17); that was the reason why these tumors were selected for study. In cases 1 and 2, 6p21 was recombined with 7p15, whereas in case 3, the recombination was with chromosomal band 10p11. Molecular

### Table 2. Primers used for PCR and sequencing

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence</th>
<th>Position</th>
<th>Gene</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC1-1651F</td>
<td>5'-CGCTGGAAGGCTTACTGGA-3'</td>
<td>1651-1673</td>
<td>EPC1</td>
<td>NM_025209</td>
</tr>
<tr>
<td>EPC1-1860F</td>
<td>5'-GCCGGAGACATCGCTTCTGGA-3'</td>
<td>1860-1883</td>
<td>EPC1</td>
<td>NM_025209</td>
</tr>
<tr>
<td>JAZF1-182F</td>
<td>5'-CCGACACCTATGTCGCCCTGAG-3'</td>
<td>182-205</td>
<td>JAZF1</td>
<td>NM_175061</td>
</tr>
<tr>
<td>JAZF1-357F</td>
<td>5'-CAGCAGCTGGAAAGCCTT-3'</td>
<td>335-354</td>
<td>JAZF1</td>
<td>NM_175061</td>
</tr>
<tr>
<td>JAZF1-357F</td>
<td>5'-CGCGGTTTTGTTGATGAG-3'</td>
<td>848-868</td>
<td>JAZF1</td>
<td>NM_013535</td>
</tr>
<tr>
<td>PHF1-250R</td>
<td>5'-AAGCTGCTCCAAAAGTGAAGG-3'</td>
<td>250-273</td>
<td>PHF1</td>
<td>NM_024165</td>
</tr>
<tr>
<td>PHF1-327R</td>
<td>5'-AGCCCATCAGTCCTACCGAC-3'</td>
<td>327-349</td>
<td>PHF1</td>
<td>NM_024165</td>
</tr>
</tbody>
</table>
investigation revealed that the PHF1 gene in 6p21 was split in all three tumors. PHF1 encodes a protein with significant sequence similarity to the protein coded for by the Drosophila polycomblike (PCL) gene. The region of similarity between PHF1 and PCL includes the sequences coding for the two PHD fingers, the region between them, and sequences 3’-terminal to the PHD fingers (20). The Drosophila polycomb group of genes (PcG) has been shown to be required for the maintenance of repression of a number of key developmental regulatory genes, including the homeotic genes (20–22). PcG proteins are thought to form a multimeric complex that modifies local chromatin structure and establishes a heritable repression state at particular loci. The JAZF1/PHF1 chimeric fusion in case 1 led to an open reading frame containing 63 amino acid residues from JAZF1, 27 additional amino acid residues from intronic sequences in frame with the initial methionine in PHF1, and the entire PHF1 protein consisting of 567 amino acids. This gives a predicted fusion protein of 657 amino acids. In case 2, the fusion led to an open reading frame containing the first 126 amino acid residues from JAZF1 and 54 additional residues from intronic sequences from both genes, in frame with the initial methionine in PHF1, and the entire PHF1 protein sequences. This gives a predicted chimeric protein of 727 amino acids. The putative proteins in both cases retain one zinc finger domain from the JAZF1 gene and the two zinc finger domains from PHF1. In addition, as a result of the t(6;7) the entire coding region of PHF1 is brought under the control of the JAZF1 promoter. The specific functions of the JAZF1 and PHF1 and why they are involved in a neoplasia-specific gene fusion are not directly apparent, but the fact that regions in their sequences encode zinc finger motifs suggests that deregulation of normal transcription processes in the tumor cells may be the crucial result.

In two tumors, cases 1 and 2, the PHF1 gene was fused with the JAZF1 gene from 7p15. The involvement of JAZF1 in ESS has previously been reported by Koorntz et al. (9) but then with another translocation partner, the JAZF1 gene from 17q21. Both JAZF1 and JJAZ1 encode zinc finger proteins. In this fusion transcript, too, the chimeric mRNA retains zinc finger motifs from both genes. Because wild-type JAZF1 is expressed in normal endometrium, it has been suggested that the JAZF1/JJAZ1 fusion creates a chimeric protein that disrupts transcription in a lineage-specific manner (9); however, the role of the JAZF1 gene in the regulation of its downstream products and its involvement in tumorigenesis have not been clarified. JAZF1 has been shown to interact physically and specifically with TAK1, which is a positive as well as a negative regulator of transcription. JAZF1 acts as a strong repressor of DRI-dependent transcriptional activation by TAK1 (23).

In case 3, the PHF1 gene was fused with the EPC1 gene from 10p11. EPC1 is the parologue of the E(Pc) enhancer of polycorn gene of Drosophila, which is a member of the polycomb group of genes. E(Pc) is a chromatin protein of limited distribution which presumably participates in the regulation of specific gene loci and thus may have an indirect effect on nuclear architecture (24). Using homology cloning and FISH techniques, the human paralogue of JAZF1 has been shown to interact physically and specifically with TAK1. E(Pc) promoter; therefore, altered PHF1 regulation may possibly contribute to the pathogenetic effect of the fusion gene. The specific functions of the EPC1 and PHF1 and the reason for their involvement in a gene fusion associated with neoplasia are not directly apparent. Whether EPC1 is involved in other neoplastic contexts has to be further studied in a larger series of cases, possibly of different neoplasias but having cytogenetic rearrangement of 10p11 in common.

Figure 2. G-banding and FISH analyses of the three ESS. For detailed karyotypic description, see Table 1. Arrows, breakpoints of rearranged chromosomes. Normal chromosomes are included to facilitate comparison. A, partial G-banding (left) and multiplex FISH (right) karyogram from case 1. B, partial karyogram from case 2: G-banding (left), multiplex FISH (middle), and FISH cohybridization on the derivative chromosome 7 of clones RP11-81H15 (red signal) and RP4-781A18 (green signal) for the JAZF1 gene and clone CTD2507H19 (blue signal) for the PHF1 gene (right). C, partial G-banding karyogram from case 3. D, G-banding (left) and FISH image (right) of the same metaphase spread from case 3. Hybridization with locus-specific probes RP11-74N14 (green signal) mapping to 10p15 and RP11-54E3 (red signal) mapping to 10q24. Clone RP11-74N14 produces a specific hybridization signal to 6p.
The orientation of the genes, PHF1, JAZF1, and EPC1, found rearranged in these three tumors, was unexpected and worthy of further comment: JAZF1 was oriented from the centromere (5') to the telomere (3'); i.e., opposite of the PHF1 gene. These opposite orientations preclude the generation of a fusion gene by a simple balanced translocation, and yet a fusion product between the two genes was shown; similarly, opposite orientations of the involved genes has also been described in a subset of Ewing tumors by Desmaze et al. (26). A similar situation occurred for the EPC1 and PHF1 genes: the orientation of EPC1 was from the centromere (5') to the telomere (3'), whereas that of the PHF1 was from the telomere (5') to the centromere (3'). This indicates the presence of additional and cryptical genomic rearrangements, possibly an inversion, in the chromosomal region containing the EPC1 gene, whose detection was beyond the cytogenetic level. Unfortunately, we did not have spare material to perform FISH analysis with locus-specific probes to test this hypothesis.

A recurrent pathogenetic theme among mesenchymal tumors is that a fusion gene is generated by disease-specific translocations found in the cells of the tumor parenchyma resulting in the generation of chimeric transcription factors. This is clearly the case also for ESS, where three different and seemingly disease-specific fusion transcripts have now been identified (ref. 9; present study). The fusion transcripts hitherto shown in ESS all encode zinc finger domains and therefore have the possibility to deregulate the transcription of a number of genes. Further studies of JAZF1/JJAZ1, JAZF1/PHF1, and EPC1/PHF1 and their downstream effects are necessary to provide us with information that may help not only to design better and more specific diagnostic tools, but eventually, also to generate medicines that counteract the protein-level effect(s) of these cancer-specific fusion genes.

Acknowledgments

Received 7/15/2005, revised 9/14/2005; accepted 9/21/2005.

Grant support: Norwegian Cancer Society, COST Action B-19 (Molecular cytogenetics of solid tumors: Short-term scientific mission programme), and Gunnar Nilsson’s Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Prof. Mariano Rocchi (Department of Genetics, University of Bari, Bari, Italy) for kindly providing most of the BAC clones used in this study (resources for Molecular Cytogenetics, http://www.biologia.uniba.it/rmc/).
References


Consistent Rearrangement of Chromosomal Band 6p21 with Generation of Fusion Genes JAZF1/PHF1 and EPC1/PHF1 in Endometrial Stromal Sarcoma

Francesca Micci, Ioannis Panagopoulos, Bodil Bjerkehagen, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/1/107

Cited articles
This article cites 19 articles, 4 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/1/107.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/66/1/107.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.