Cancer Letters 347 (2014) 75–78

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

MEAF6/PHF1 is a recurrent gene fusion in endometrial stromal sarcoma

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ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form 22 January 2014
Accepted 24 January 2014

Keywords:
MEAF6/PHF1
Fusion gene
Endometrial stromal sarcoma
RNA sequencing
Reverse transcriptase-PCR

ABSTRACT

The chimeric transcripts described in endometrial stromal sarcomas (ESS) are *JAZF1/SUZ12*, *YWHAE/FAM22*, *ZC3H7/BCOR*, *MBTD1/CXorf67*, and recombinations of *PHF1* with *JAZF1*, *EPC1*, and *MEAF6*. The *MEAF6/PHF1* fusion had hitherto been identified in only one tumor. We present two more ESS with *MEAF6/PHF1* detected by transcriptome sequencing (case 1) and RT-PCR (case 2), proving that this fusion is recurrent in ESS. The transcript of both cases was an in-frame fusion between exon 5 of *MEAF6* and exon 2 of *PHF1*. Both genes are involved in epigenetic modification, and this may well be their main pathogenetic theme also in ESS tumorigenesis.

1. Introduction

Endometrial stromal tumors (EST) are composed of cells resembling those of proliferative phase endometrial stroma. Endometrial stromal sarcomas (ESS) account for less than 10% of uterine sarcomas. Traditionally, ESS have been divided into low- and high-grade type based on mitotic count [1]. However, as the high-grade tumors often lack evidence of endometrial stromal cell differentiation and are clinically more aggressive, it has been generally accepted that they should rather be classified as undifferentiated endometrial or uterine sarcoma (UUS) [2,3].

Only relatively few ESS and/or UUS have been subjected to cytogenetic analysis and different chromosomal aberrations have been detected. The most frequent rearrangement, found in ESS of classical histology (i.e., low-grade tumors), is a t(7;17) translocation leading to the fusion of the *JAZF1* and *SUZ12* (previously known as *JJAZ1*) genes that map to chromosomes 7 and 17, respectively [4]. Following in frequency are rearrangements of chromosome arm 6p [5] targeting the *PHF1* gene in 6p21 and recombining it with *JAZF1* from 7p15 [6], *EPC1* from 10p11 [6] or *MEAF6* from 1p34 [7] (only one tumor with this change has been reported). Lately, a *ZC3H7/BCOR* fusion transcript was detected in ESS with X;22-rearrangements [8] and an *MBTD1/CXorf67* chimera was found as the product of a t(X;17) in another two ESS [9]. Furthermore, Lee et al. [10,11] described a *YWHAE/FAM22* chimeric fusion brought about by a t(10;17) translocation found in an ESS-subtype with more aggressive clinical behavior. None of these molecular changes is presently used on a regular basis for the diagnosis of ESS.

We present here two more cases of ESS with *MEAF6/PHF1* fusion, detected by transcriptome sequencing (case 1) and RT-PCR (case 2), proving that this, too, is a recurrent fusion in this tumor type.

2. Materials and methods

2.1. Case history

2.1.1. Case 1

A 76-year-old woman presented in 2008 with a large tumor, 15 cm in diameter, of the pelvis. The patient had been diagnosed with ESS in 1983 and had then undergone a supravaginal hysterectomy. The case was later reviewed and the diagnosis confirmed by morphology and immunohistochemistry in a large study of uterine sarcomas in Norway during the years 1970–2000 [12,13]. A new operation in

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http://dx.doi.org/10.1016/j.canlet.2014.01.030
0304-3835/

2008 removed a tumor that was macroscopically enveloped by a pseudo-capsule. The cut surface was heterogeneous with necrotic areas. Microscopic examination showed a cellular tumor with monomorphic spindle cells typical of ESS, and with areas of fibrosis and necrosis. The mitotic count was 0–2/10 high-power fields (HPF). Vascular invasion was seen (Fig. 1). The patient has not had additional recurrences since and was alive at last follow-up in 2012.

2.1.2. Case 2

A 53-year-old woman was subjected to hysterectomy and bilateral salpingo-oophorectomy for a low-grade endometrial stromal sarcoma in 1988. The tumor protruded into the endometrial cavity as a mass of 4 cm in largest diameter and infiltrated the entire myometrial wall. Upon microscopy, a monotonous proliferation of ovoid to spindle cells with minimal cytoplasm was seen with tongue-like infiltration of the myometrium. Areas of edema and hyalinization were present, and there were prominent arterioles. Mitotic index was 3/10 HPF. There was focal presence of sex cord like differentiation. Tumor cells were positive for CD-10 and steroid hormone receptors. There was prominent lympho-vascular space invasion. No evidence of recurrence was seen during the first ten years after surgery after which follow-up was discontinued. Twenty-five years after initial surgery, in 2013, the patient experienced an increased abdominal perimeter and pain. CT scan showed a large abdominal mass, 11 cm in largest diameter, and multiple peritoneal nodules. After a diagnostic fine-needle aspiration biopsy, the abdominal masses were removed (Fig. 1). Microscopic examination demonstrated multiple foci of metastatic low-grade endometrial stromal sarcoma with smooth muscle differentiation (Fig. 1). Mitotic index was 1/10 HPF.

2.2. G-banding and karyotyping

Fresh tissue from a representative area of the tumor (case 1) was received and analyzed cytogenetically as part of our diagnostic routine. The sample was disaggregated mechanically and enzymatically with collagenase II (Worthington, Freehold, NJ, USA). The resulting cells were cultured and harvested using standard techniques. Chromosome preparations were G-banded with Wright stain and examined. The karyotype was written according to The International System for Human Cytogenetic Nomenclature (ISCN) 2009 guidelines [14].

2.3. Fluorescence in situ hybridization (FISH) analyses

Hybridization with bacterial artificial chromosomes (BAC) probes RP11-81H15 and RP4-781A18, mapping on 7p21, and CTD-3145B2, mapping on 17q21, was performed in search of cryptic rearrangement of *JAZF1* and *SUZ12*, known to be present in the great majority of ESS. No such fusion was detected. BAC clone CTD-2307H19, mapping on 6p21, was used to test for involvement of *PHF1*, but no split signal of this probe was noted. A total of 19 clones spanning from 5q22.3 to 5q31.1 were tested without finding the precise breakpoint of the sole chromosomal rearrangement detected (see below; a pericentric inversion of one of the two copies of chromosome 5 was seen in all 13 metaphases examined). The BAC clones were retrieved from the RPCI-11 Human BAC library and the CalTech human BAC library D (P. de Jong libraries, http://bacpac.chori.org/home.htm). They were selected according to the physical and genetic mapping data on chromosome 5 reported in the Human Genome Browser at the University of California, Santa Cruz website (March 2006, http://genome.ucsc.edu/). All clones were grown in selective media and DNA was extracted according to standard methods [15]. DNA probes were directly labeled with a combination of fluorescein isothiocyanate (FITC)-12-deoxycytidine triphosphate (dCTP) and FITC-12-2-deoxyuridine triphosphate (dUTP), Texas Red-6-dCTP and Texas Red-dUTP (New England Nuclear, Boston, MA, USA), and Cy3-dCTP (GE-healthcare, UK), or indirectly with Biotin-dUTP (Molecular Probes, Invitrogen, Carlsbad, CA, USA) by nick translation, and detected with streptavidin-diethylaminocoumarin (DEAC; Invitrogen). The hybridization was performed on destained slide after karyotypic analysis. The hybridization conditions as well as the detection procedure were according to a published protocol [16]. The hybridizations were analyzed using a CytoVision system (Applied Imaging, Newcastle, UK). We were forced to discontinue this approach due to the fact that it was not possible to hybridize more on the same slide that had been stripped several times. We then chose an alternative approach to investigate the possible fusion behind the inv(5) by using the newest transcriptome sequencing methodology.

2.4. Molecular investigations

RNA was extracted from representative areas of both tumors using the Trizol reagent (Life Technologies) with a homogenizer (Omni THQ Digital Tissue Homogenizer, Kennesaw, GA, USA). The RNA quality was evaluated using the Experion Automated Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA). cDNA was synthesized using the iScript kit and random primers (Bio-Rad Laboratories). All procedures were done according to the manufacturers' recommendations.

RT-PCR was used to investigate the presence of known fusion transcripts. The primers used are described in Table 1; all of them were used in previous publications. The primer combinations were JAZF1-357F and JJAZ1-843R, EPC1-1651F and PHF1-327R, MEAF6-322F and PHF1-380R, ZC3H7B-1190 and BCOR-3954R, and JAZF1-182F and PHF1-327R. The PCR cycles were as follows: initial denaturation at 94 °C for 30 s followed by 35 cycles of 7 s at 98 °C and 2 min at 68 °C, and a final extension was performed for 5 min at 68 °C. The PCR mix contained 2 μl of cDNA, 0.4 μM forward primer, 0.4 μM reverse primer, 12.5 μl Takara Premix, and a 25 μl volume was reached by adding water.

A total of 3 μg of RNA from case1 was sent for high-throughput paired-end RNA-sequencing to the Norwegian Sequencing Centre at Ullevål Hospital (http://www.sequencing.uio.no/). The Illumina software pipeline was used to process image data into raw sequencing data and only sequence reads marked as "passed filtering" were used in the downstream data analysis. A total of 105 million reads were obtained. The FASTQC software was used for quality control of the raw sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We used the fusion discovery software FusionMap (release date 2013-02-01) [17] and the pre-built Human B37 and RefGene from the FusionMap website (http://www.omicsoft.com/fusionmap/).

3. Results

The karyotype of the only tumor that was sent for cytogenetic analysis (case 1) showed an inv(5)(p13~p14q23~q31) as the sole chromosomal aberration in all 13 metaphases analyzed. To rule out that this could be a constitutional variant we analyzed also

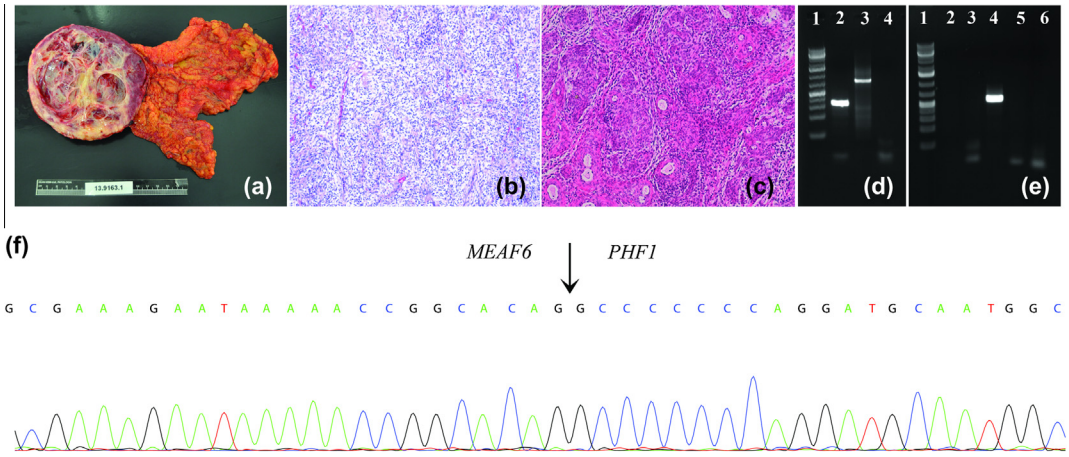

Fig. 1. (a) Macroscopic view of the mass removed from case 2; (b) microscopic examination of case 1 showed a cellular tumor with monomorphic spindle cells typical of low-grade ESS; (c) microscopic examination of case 2 showed a low-grade ESS with smooth muscle differentiation; (d) gel image of the PCR analysis performed with primers specific for the *MEAF6/PHF1* fusion in case 1 (lane 1: 1 Kb DNA ladder, lane 2: sample from case 1, lane 3: internal control for PCR reaction using primers specific for *ACTB*, lane 4: negative control); (e) gel image of the PCR analysis performed on case 2 (lane 1: 1 kb DNA ladder, lane 2–5: primers specific for *JAZF1/SUZ12*, *EPC1/PHF1*, *MEAF6/PHF1*, *ZC3H7B/BCOR*, and *JAZF1/PHF1*, respectively) and (f) partial sequence chromatogram showing the fusion between exon 5 of *MEAF6* and exon 2 of the *PHF1* gene.