Fusion of the Genes *PHF1* and *TFE3* in Malignant Chondroid Syringoma

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Abstract. *Background/Aim:* Malignant chondroid syringoma is a rare tumor of unknown pathogenesis. *Materials and Methods:* Genetic analyses were performed on a malignant chondroid syringoma. *Results:* G-banding analysis of short-term cultured tumor cells yielded the karyotype 46,Y,t(X;6)(p11;p21)[15]/46,XY[2]. RNA sequencing detected an in-frame fusion of *PHF1* from 6p21 with *TFE3* from Xp11, verified by RT-PCR and Sanger sequencing. Genomic PCR showed that the *PHF1*-*TFE3* junction was identical to the fusion found by RNA sequencing and RT-PCR. *Conclusion:* Malignant chondroid syringoma is genetically related to tumors with *PHF1* rearrangements such as low-grade endometrial sarcoma and ossifying fibromyxoid tumor, but also with tumors having *TFE3* rearrangements such as renal cell carcinoma, alveolar soft part sarcoma, *PEComa*, and epithelioid hemangioendothelioma. Further investigations on malignant chondroid syringomas are needed in order to determine whether genetic heterogeneity exists among them and the clinical impact of the *PHF1*-*TFE3* fusion.

Malignant chondroid syringoma, also known as malignant mixed tumor of the skin, is a very rare tumor with less than 50 cases being described in the relevant literature. It is considered to be the malignant counterpart of benign chondroid syringoma (benign mixed tumor) (1, 2). Malignant chondroid syringomas are twice as common in women as in

men and show predilection for the trunk and extremities (1-3). The clinical course is unpredictable (4). Half of the reported cases had local recurrences whereas nodal and distant metastases were observed in 39% and 36% of the cases, respectively. The most common sites for distant metastases are the lungs, bone, and brain (4). Malignant chondroid syringoma is composed of both epithelial and mesenchymal tissue. The epithelial component consists of neoplastic cells with hyperchromatic nuclei and abundant mitotic figures, with occasional areas of necrosis. The mesenchymal component may show myxoid, chondroid, osteoid, adipose or fibrous features enclosing clusters of epithelial cells (1-3).

Malignant and benign chondroid syringomas are cutaneous myoepithelial neoplasms (5-9). Genetic studies of both cutaneous and soft tissue myoepithelial neoplasms have demonstrated considerable genetic heterogeneity (10-14). In some of these tumors, *EWSR1-ZNF444*, *EWSR1-PBX1*, *EWSR1-PBX3* or *EWSR1-POU5F1* fusion genes have been found (10, 12, 13, 15) whereas others had rearrangements of *PLAG1* (11, 14). The differential diagnosis for myoepithelial tumor is extremely broad and may include ossifying fibromyxoid tumors (7, 16). In the latter, the PHD finger protein 1 gene (*PHF1*) is recurrently rearranged (17) with an *EP400-PHF1* fusion being detected in some tumors (17, 18).

Herein, we present a malignant chondroid syringoma which had a t(X;6)(p11;p21) as the sole karyotypic aberration. Using RNA sequencing and molecular methodologies, we demonstrated that the molecular consequence of the translocation was the fusion of the *PHF1* gene from 6p21 with the transcription factor binding to IGHM enhancer 3 gene (*TFE3*) from Xp11.

Materials and Methods

Ethics statement. The study was approved by the regional Ethics Committee (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, <http://helseforskning.etikkom.no>). Written informed consent was obtained from the patient for publication of the case details.

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Key Words: Malignant chondroid syringoma, *PHF1*, *TFE3*, *PHF1*-*TFE3* fusion gene, RNA sequencing.

Table I. Primers used for PCR amplification and Sanger sequencing analyses.

Name	Sequence (5'→3')	Position	Reference sequence	Gene
PHF1-1295F1	CTGGGGGAGGGGTCTCACGTCC	1295-1316	NM_002636.4	<i>PHF1</i>
TFE3-1431R1	GGTGCCCTTGTTCCAGCGCATC	1452-1431	NM_006521.5	<i>TFE3</i>
PHF1-Intr11-F2	AGGTCTTGGGGGTGTCCGGGAGG	33414902-33414924	NC_000006.12	<i>PHF1</i>
TFE3-Intr7-R2	GTGCTGTGCAGGCCTGCCTCACC	49033696-49033674	NC_000023.11	<i>TFE3</i>
PHF1-Intr11-F3	GGCTGGGGGATAAGGAGGCCTCT	33414926-33414949	NC_000006.12	<i>PHF1</i>
TFE3-Intr7-R3	GGGCAATGCACACGCTCTCTGGC	49033699-49033721	NC_000023.11	<i>TFE3</i>

The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Case description. A 61 year old male had noticed a tender, growing lump in the left deltopectoral fold, during the last 6 months. MRI revealed a subcutaneous heterogeneous tumor whose appearance evoked suspicion of a soft tissue sarcoma. On macroscopic examination following surgery, the tumor was partly chondroid with myxoid and necrotic areas. Viable tumor tissue was removed for culturing prior to formalin fixation. Microscopically, the tumor was composed of chondroid areas, spindle cells, epithelioid cells, and necrotic areas (Figure 1A). The tumor cells were positive for cytokeratins and also weakly for S-100. The tumor was classified as a malignant chondroid syringoma. Because of the molecular findings (see below), immunohistochemistry was performed using TFE3 (MRQ-37) rabbit monoclonal antibody (Cell Marque, CA, USA) according to the company's recommendations. The TFE3 antibody showed strong nuclear positivity (Figure 1B).

G-banding and karyotyping. Fresh tissue from a representative area of the tumor was short-term cultured and analyzed cytogenetically as previously described (19).

RNA sequencing. Total RNA was extracted from frozen (−80°C) tumor tissue adjacent to that used for cytogenetic analysis and histologic examination using miRNeasy Mini Kit (Qiagen Nordic, Oslo, Norway). One µg of total RNA was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital (<http://genomics.no/oslo/>) for high-throughput paired-end RNA-sequencing using the Illumina TruSeq Stranded mRNA protocol. The software FusionCatcher (20, 21) was used to find fusion transcripts.

Reverse transcription (RT) and genomic PCR analyses. The primers used for PCR amplifications and Sanger sequencing analyses are shown in Table I. One µg of total RNA was reverse-transcribed in a 20 µl reaction volume using iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad, Oslo, Norway). Genomic DNA was extracted using the Maxwell RSC Instrument and the Maxwell RSC Tissue DNA Kit (Promega, Madison, WI, USA) and the concentration was measured using the Quantus Fluorometer and the QuantiFluor ONE dsDNA System (Promega).

For amplification of the *PHF1-TFE3* fusion transcript, the primers used were the forward PHF1-1295F1 and the reverse TFE3-1431R1. For amplification of genomic *PHF1-TFE3* fragments, the primer combinations were PHF1-Intr11-F2/TFE3-Intr7-R2 and

PHF1-Intr11-F3/TFE3-Intr7-R3. All PCR amplifications were performed in 25 µl reaction volume which contained 12.5 µl Premix Ex Taq™ DNA Polymerase Hot Start Version (Takara Bio Europe/SAS, Saint-Germain-en-Laye, France), template (1 µl cDNA or 100 ng of genomic DNA), and 0.4 µM of each of the forward and reverse primers. PCR amplifications were run on a C-1000 Thermal cycler (Bio-Rad) and the cycling was performed at 94°C for 30 sec followed by 35 cycles of 7 sec at 98°C, 30 sec at 68°C, 30 sec at 72°C, and a final extension for 5 min at 72°C. Three µl of the PCR products were stained with GelRed (Biotium, VWR International, Oslo, Norway), analyzed by electrophoresis through 1.0 % agarose gel, and photographed. The remaining PCR products were purified using the MinElute PCR Purification Kit (Qiagen) and direct sequenced using the dideoxy procedure with the BigDye terminator v1.1 cycle sequencing kit (ThermoFisher Scientific, Waltman, MA, USA) on the Applied Biosystems Model 3500 Genetic Analyzer sequencing system. The BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for computer analysis of sequence data.

Results

G-banding. The G-banding analysis yielded a karyotype with a single chromosome abnormality: 46,Y,t(X;6)(p11;p21) [15]/46,XY[2] (Figure 2).

RNA sequencing and molecular genetic confirmation of the *PHF1-TFE3* fusion. Using the FusionCatcher software with the fastq files from the RNA sequencing, a fusion of *PHF1* from 6p21 with *TFE3* from Xp11 was found: GGAAAGTGGAGGAGCTGGGGCCACCCTCAGCAGTGC GCAATCAGCCCCGAG*CAGAGACCGAGGCAAAGGCCCTTTTGAAGGAACGGCAGAAGAAAGACAAT.

RT-PCR with the primer combination PHF1-1295F1/TFE3-1431R1 amplified strongly a 286 bp cDNA fragment and weakly two other fragments of larger sizes, which were not further studied (Figure 3A). Direct sequencing of the 286 bp PCR fragment showed that it was a *PHF1-TFE3* chimeric cDNA fragment. The fusion point was identical to that found by analysis of the RNA sequencing data using the FusionCatcher software. Thus, in the *PHF1-TFE3* chimeric transcript, exon 12 of *PHF1* (nt 1420 in sequence with accession number NM_002636.4) was fused in frame to exon 7 of *TFE3* (nt 1135 in NM_006521.5) (Figure 3B). Two extra

Figure 1. Microscopic examination of the malignant chondroid syringoma. A) H&E-stained section showing tumor cells with epithelioid/myoepithelioid features embedded in hyaline stroma, 100 $\times$. B) Immunoexpression of TFE3 showing strong nuclear positivity, 200 $\times$.

nt “AG” were found at the junction maintaining the open reading frame. They most probably came from the acceptor/donor splicing site of intron 6/exon 7 of *TFE3* (Ensemble; ENSG00000068323; ENST00000315869.7).

Genomic PCR with PHF1-Intr11-F2/TFE3-Intr7-R2 and PHF1-Intr11-F3/TFE3-Intr7-R3 amplified a 308 bp fragment and a 259 bp fragment, respectively (Figure 3C). Direct sequencing of the PCR products showed that they were genomic *PHF1-TFE3* chimeric fragments and that the genomic *PHF1-TFE3* junction was identical to the fusion point found by RNA sequencing and RT-PCR (Figure 3A-C).

Discussion

We identified a *PHF1-TFE3* fusion gene in the cells of a malignant chondroid syringoma. To the best of our knowledge, this is the first time an acquired genetic change, let alone a fusion gene, is described in this type of tumor.

PHF1 encodes a polycomb group (PcG) protein that contains a tudor domain, PHD zinc finger domains, and a polycomb-like MTF2 factor 2 domain (22). PcG proteins are thought to form a multimeric complex that modifies local chromatin structure and establishes a heritable repression state at the particular loci. The PHF1 protein is a component of the histone H3 lysine-27 (H3K27) specific methyltransferase complex and is important for Hox gene expression *in vivo* (23, 24). PHF1 is also recruited to DNA double strand breaks and interacts physically with many proteins which are involved in DNA damage response (25). Recently, PHF1 was found to be a reader for histone H4R3 symmetric demethylation and to interact with the PRMT5-WDR77/CRL4B complex (26).

Figure 2. Cytogenetic analysis of the malignant chondroid syringoma. Partial karyotype showing the *der(X)t(X;6)(p11;p21)*, *der(6)t(X;6)(p11;p21)*, and the normal chromosome 6. Breakpoint positions are indicated by arrows.

PHF1 was first reported as a neoplasia-associated fusion partner in low-grade endometrial stromal sarcomas in which rearrangements of the chromosomal band 6p21 generated the *JAZF1-PHF1* and *EPC1-PHF1* fusion genes (27). Furthermore, *MEAF6-PHF1*, *BRD8-PHF1*, and *EPC2-PHF1* fusions have been reported in low-grade endometrial stromal sarcomas (28-31). Recurrent rearrangement of the *PHF1* gene and the fusion genes *EP400-PHF1*, *EPC1-PHF1*, and *MEAF6-PHF1* have also been reported in ossifying fibromyxoid tumors (17, 18, 32) and a *JAZF1-PHF1* and an *AFF3-PHF1* fusion gene have been reported in an unusual ossifying sarcoma of the heart and a myxofibrosarcoma,

Figure 3. Molecular genetic analysis of the malignant chondroid syringoma. A) Gel electrophoresis showing the amplified cDNA fragment using the PHF1-1295F1/TFE3-1431R1 primer combination (lane 1). B) Partial sequence chromatogram of the cDNA amplified fragment showing the junction position of the PHF1 and TFE3 genes (arrow). C) Amplification of genomic PHF1-TFE3 fragments using the primer combinations PHF1-Intr11-F2/TFE3-Intr7-R2 (lane 1) and PHF1-Intr11-F3/TFE3-Intr7-R3 (lane 2). M, 1 Kb DNA ladder (GeneRuler, ThermoFisher Scientific). D) The predicted PHF1-TFE3 fusion protein. The tudor domain is in lilac, the two PHD finger domains are in green, the helix-loop-helix domain is in yellow, the domain of unknown function is in brown, and the PHF1-TFE junction is in red.

respectively (33, 34). In all the above-mentioned fusions, the entire *PHF1* coding region became the 3' terminal part of the fusion gene.

The *TFE3* gene encodes a basic helix-loop-helix domain-containing transcription factor that binds MUE3-type E-box sequences in the promoter of genes and activates transcription (35, 36). The *TFE3* gene was first reported as a neoplasia-associated fusion partner in papillary renal cell

carcinomas carrying a t(X;1)(p11.2;q21.2) chromosome translocation which fused *PRCC* to *TFE3* (37, 38). Since then, *TFE3* was found to be a partner in more than 10 different fusion genes in various neoplasms amongst which are *NONO*, *SFPQ*, and *DVL2* in papillary renal cell carcinomas and PEComas (39-41), *ASPC1* in alveolar soft part sarcoma and papillary renal cell carcinomas (42, 43), and *YAP1* in epithelioid hemangioendothelioma (44).

Oncogenic activity of some *TFE3* gene fusions has been shown in cancer models. The *PRCC-TFE3* and *SFPQ-TFE3* fusions transformed NIH3T3 mouse fibroblast cells (45, 46) and the alveolar soft part sarcoma-associated fusion *ASPCSCR1-TFE3* was shown to drive tumorigenesis in mice (47, 48). The widely accepted model for oncogenic activity of *TFE3* gene fusions is that the 5'-partner gene has a stronger or less tightly controlled promoter than *TFE3*, one that is not regulated in the same manner as the wild-type *TFE3* promoter (49). Thus, in the fusion proteins, the basic helix-loop-helix domain and the carboxyl terminal part of *TFE3* are upregulated (49). Consistent with this model, all *TFE3* fusion partners have constitutively active gene promoters leading *TFE3* fusion proteins to be expressed at dramatically higher levels than wild-type *TFE3* (49).

Based on the reference sequences NM_002636.4/NP_002627.1 for *PHF1* and NM_006521.5/NP_006512.2 for *TFE3*, the fusion *PHF1-TFE3* we describe is predicted to code for a 636 amino acid residues chimeric transcription factor consisting of the first 395 and the last 241 (position 335-575) amino acid residues of the *PHF1* and *TFE3* proteins, respectively (Figure 3D). The *PHF1-TFE3* protein contains the tudor domain and the two PHD zinc finger domains of *PHF1*, and the helix-loop-helix domain and a carboxyl domain of unknown function of *TFE3* (22, 36). The expression of the helix-loop-helix domain and the carboxyl domain of *TFE3* is therefore regulated by the *PHF1* promoter.

Although the tumor cells of the malignant chondroid syringoma carried a simple t(X;6)(p11;p21) translocation, we believe that additional submicroscopic rearrangements took place that facilitated formation of the *PHF1-TFE3* fusion gene. At the genomic level, *PHF1* (on chromosome band 6p21) is transcribed from telomere to centromere whereas transcription of *TFE3* (on chromosome band Xp11) proceeds in the opposite direction, from centromere to telomere. Hence, formation of a *PHF1-TFE3* fusion is not possible through a simple t(X;6)(p11;p21) alone but would require an additional genomic aberration, such as inversion, on one of the derivative chromosomes, der(X) or der(6).

Hallor *et al.* (14) reported a case of myoepithelioma/mixed tumor with der(6)ins(6;X)(p21;p11p22) among several other aberrations and another case in which del(X)(p11) was part of a complex karyotype. In another study reporting recurrent *PHF1* rearrangements in ossifying fibromyxoid tumors, two of the cytogenetically analyzed cases had karyotypes in which chromosome bands Xp11 and 6p21 had also been targeted (17). The first tumor had the karyotype 46,Y,ins(X;6)(p11;p21p25),t(2;12)(q31;q22),+12 whereas the second tumor had the more complex karyotype 45,Y,add(X)(p11),del(1)(p35),der(3)t(3;16)(p12;p11), der(6) t(6;7)(p21;p15),der(7)t(X;7)(p2?p15)t(X;6)(p11;p21), del(13)(q?21), der(17)t(?13;17)(q?2?2;q?25),-20. Both tumors had *PHF1* rearrangements but did not have the *EP400-PHF1* fusion.

Based on the transcription orientation of the *PHF1* and *TFE3* genes, the involvement of chromosome bands Xp11 and 6p21 as visualized by karyotyping, the aberration of *PHF1* in ossifying fibromyxoid tumors (17) and the fact that the cases reported by Hallor *et al.* (14) were myoepithelioma/mixed tumors (14), it is likely that the above-mentioned tumors carried a *PHF1-TFE3* fusion gene.

The present study shows that malignant chondroid syringoma on the one hand is genetically related to tumors with *PHF1* rearrangements such as low-grade endometrial sarcoma and ossifying fibromyxoid tumor, and on the other hand to tumors carrying *TFE3* rearrangements such as renal cell carcinoma, alveolar soft part sarcoma, PEComa, and epithelioid hemangioendothelioma. Further investigations of malignant chondroid syringomas and myoepithelial tumors are needed in order to determine the frequency of *PHF1-TFE3* and, in particular, to find out what kind of genetic heterogeneity exists.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest exist.

Authors' Contributions

IP performed experiments, was the primary Author of the manuscript, produced experimental design, performed the bioinformatics analysis, conceived the study, and drafted the manuscript. LG performed cytogenetic analysis, ML-I performed the pathological examination, AB performed the pathological examination, SH assisted with experimental design and writing of the manuscript. All Authors read and approved the final manuscript.

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