Fusion of the FUS and BBF2H7 genes in low grade fibromyxoid sarcoma

Clelia Tiziana Storlazzi1,2, Fredrik Mertens1, Antonio Nascimento3, Margareth Isaksson1, Johan Wejde4, Otte Brosjö5, Nils Mandahl1 and Ioannis Panagopoulos1,*

1Department of Clinical Genetics, University Hospital, SE-221 85 Lund, Sweden, 2DAPEG, Section of Genetics, University of Bari, Bari, Italy, 3Division of Surgical Pathology, Mayo Clinic, Mayo Graduate School of Medicine, Rochester, New York, USA, 4Department of Pathology, Karolinska Hospital, Stockholm, Sweden and 5Department of Orthopedics, Karolinska Hospital, Stockholm, Sweden


The FUS gene at 16p11 fuses with DDIT3 and ATF1 as the result of translocations with chromosome band 12q13 in myxoid liposarcoma and angiomatoid fibrous histiocytoma, respectively, and with ERG as the result of a t(16;21)(p11;q22) in acute myeloid leukemia. We here show that a t(7;16)(q33;p11) in two cases of low grade fibromyxoid sarcoma fuses the FUS gene to BBF2H7, a previously uncharacterized gene that is homologous to the Drosophila Bbf-2 gene. BBF2H7 spans more than 120 kbp genomic DNA, is composed of 12 exons and contains a 1560 bp open reading frame. It codes for a 519 amino acid protein that contains a basic DNA binding and leucine zipper dimerization (B-ZIP) motif, highly similar to that in the OASIS, CREB-H, CREB4 and CREB3 transcription factors, followed by a hydrophobic region predicted to be an α-helical transmembrane domain. Reverse transcription–polymerase chain reaction (RT–PCR), using FUS forward and BBF2H7 reverse primers, amplified FUS/BBF2H7 chimeric transcripts composed of the first five exons and part of exon 6 of FUS and part of exon 5 and exons 6–12 of BBF2H7. The FUS/BBF2H7 chimera codes for a protein containing the N-terminus of FUS and the B-ZIP domain and the C-terminus of BBF2H7.

INTRODUCTION

Soft tissue sarcoma (STS) is the collective term used for malignancies arising in muscles, fat, vessels, the peripheral nervous system and fibrous tissue. Histopathologic examination of such tumors has revealed a large, and steadily increasing, number of distinct entities, each displaying its own morphologic and clinical characteristics (1). Cytogenetic and molecular genetic analyses have shown that many types of STS are characterized by specific translocations resulting in chimeric fusion genes. Not only are these translocations and fusion genes of clinical importance, as they may serve as differential diagnostic markers, but they have also provided important clues to the cellular mechanisms behind STS development. A recurrent theme among the STS-associated fusion genes is that they result in chimeric transcription factors. One of the partners often codes for a DNA-binding protein while the other one activates transcription through transactivating domains (2). It has also been noted that the same gene may be involved in different STS types, but then with different partner genes. One such example is the FUS (also known as TLS) gene, which consists of 15 exons covering 11 kb of genomic DNA in chromosome band 16p11, and encodes a glycine-rich, nuclear RNA-binding protein (3).

The FUS gene was originally shown to be rearranged in myxoid liposarcomas harboring a t(12;16)(q13;p11) translocation. Through this translocation, the part of FUS encoding the RNA-binding domain is replaced by the basic DNA binding and leucine zipper dimerization (B-ZIP) domain of DDIT3 (also known as CHOP) (4,5). Later, FUS has been shown to be involved as the 5′ partner in two other recombinations: with ERG in acute myeloid leukemia carrying a t(16;21)(p11;q22) (6,7) and with ATF1 in band 12q13 in angiomatoid fibrous histiocytoma (8,9). So far, exons 1–5 of FUS have invariably been present in reported tumor-associated fusion genes.

The FUS protein shows extensive sequence similarity with EWSR1 (previously called EWS), another protein appearing as the 5′ partner in a number of sarcomas (10). Further evidence

*To whom correspondence should be addressed. Tel: +46 46172889; Fax: +46 46131061; Email: ioannis.panagopoulos@klingen.lu.se

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for functional similarities between these two proteins may be found in the facts that the tumor breakpoints in the EWSR1 and FUS genes are all localized in the same regions of the genes, upstream of the RNP encoding exons, and that, in all described fusion genes, FUS and EWS have been linked to transcription factor encoding genes (2). Moreover, a subset of myxoid liposarcoma, histopathologically indistinguishable from the larger proportion of cases showing a chimeric FUS/DDIT3 protein, instead displays an EWSR1/DDIT3 fusion (11). The present paper describes the detection of a novel fusion gene involving FUS and BBF2H7, a homolog to Drosophila Bbf-2, in two cases of low grade fibromyxoid sarcoma (LGFMS), an STS that previously has not been characterized at the genetic level.

**RESULTS**

**G-banding and fluorescence in situ hybridization (FISH)**

G-banding analysis of metaphase cells from case 1 revealed the karyotype 46,XY,t(7;16)(q33;p11.2)/46,idem,-10,+r. COBRA-FISH verified the t(7;16) translocation and showed that the ring was entirely composed of chromosome 10 material (data not shown). Subsequent FISH experiments using contigs of BAC clones revealed that the breakpoints were located within BAC clones RP11-388M20 (AC009088) in band 16p11.2, and RP11-29B3 (AC022173) and RP11-377B19 (AC009263) in band 7q33; all three clones gave split signals on the derivative chromosomes 7 [der(7)] and 16 [der(16)] (Fig. 1). To delineate

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*Figure 1.* (A and B) Contig maps of BAC clones of the breakpoint regions in chromosome bands 16p11.2 and 7q33, respectively; (C) FISH cohybridizations using clones RP11-388M20 (red), RP11-29B3 (green) and RP11-377B19 (blue) on a metaphase from case 1 (a) and on chromosome 16 and der(16) in case 2 (b).
further the breakpoint region on chromosome 7, additional BAC clones were used: RP11-351B12 (AC009245) and CTD-2546I18 (Fig. 1B) gave signals on both the normal 7 and the der(16), while CTD-2375H21 gave rise to a split signal (Fig. 1). Regarding the breakpoint on chromosome 16, BAC clones were used: RP11-351B12 (AC009245) and CTD-2375H21 (Fig. 1). The clone RP11-261H5 was shown to be retained on the der(16) (Fig. 1C). Blast analysis showed that these 33 nt originated from intron 7 of FUS (accession number NM_004960) in exon 6 was fused with nt 1026 of FUS (accession number NM_004960) in exon 1 (Fig. 1C). Molecular genetic findings

RT–PCR with various combinations of FUS forward and BBF2H7 reverse primers (Table 1) successfully amplified cDNA fragments from both cases, strongly suggesting the presence of a FUS/BBF2H7 chimeric gene. The primer combinations TLS0F and BBF2-1967R, TLS391F and BBF2-1967R, TLS165F and BBF2-1435R, and TLS165F and BBF2-1435R yielded bands of size 1680, 1270, 750, 450 bp, respectively (Fig. 2). RT–PCR with the primers BBF2-1396R and BBF2-1435R, TLS165F and BBF2-1435R, TLS165F and BBF2-1435R, and TLS165F and BBF2-1435R each amplified two cDNA fragments of 970 and 870 bp, 580 and 480 bp, and 450 and 350 bp in case 1, whereas no BBF2H7/FUS cDNA fragments were amplified from case 2 (Fig. 2).

The 1270, 750 and 450 bp fragments detected using TLS391F and BBF2-1967R, TLS165F and BBF2-1435R, TLS165F and BBF2-1396R, TLS427F and BBF2-1435R, and TLS427F and BBF2-1396R yielded bands of size 1680, 1270, 750, 450 bp, respectively (Fig. 2). RT–PCR with the primers BBF2-1396R and BBF2-1435R, TLS165F and BBF2-1435R, TLS165F and BBF2-1435R, and TLS165F and BBF2-1435R each amplified two cDNA fragments of 970 and 870 bp, 580 and 480 bp, and 450 and 350 bp in case 1, whereas no BBF2H7/FUS cDNA fragments were amplified from case 2 (Fig. 2).

The 1270, 750 and 450 bp fragments detected using TLS391F and BBF2-1967R, TLS165F and BBF2-1435R, and TLS427F and BBF2-1396R were analyzed by direct sequencing. In case 1, nt 700 of FUS (accession number NM_004960) in exon 6 was fused with nt 1026 of BBF2 in exon 5 (Fig. 3). At the junction, there was an insertion of 33 nt which maintained an open reading frame of the chimeric transcript (Fig. 3). Blast analysis showed that these 33 nt originated from intron 7 of FUS, but in reverse orientation (complementary to

**Table 1. Primers for PCR and sequencing**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence (5'—3')</th>
<th>Position</th>
<th>Gene</th>
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<td>TLS0F</td>
<td>CCGCGGGTGTCTACGAGTGT</td>
<td>18–38</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS35F</td>
<td>GCCGCCACATGCGCTCAAAAG</td>
<td>71–91</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS165F</td>
<td>AGCCAGCTACGCCACATCTACAGCC</td>
<td>202–225</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS391F</td>
<td>CTCAGAGACGAGCTATGCGGAG</td>
<td>428–450</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS427F</td>
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<td>464–487</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS905R</td>
<td>CCAATCTCTGTTGAATCAAGCCG</td>
<td>981–1004</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS1025R</td>
<td>CTTCTCCTCTCTACGAGTCCTTC</td>
<td>1063–1087</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS1677R</td>
<td>GGAATTTAGAGCTACAAATAACAGG</td>
<td>1714–1741</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS1314R</td>
<td>CATACAGCTCTGCTCTCC</td>
<td>1330–1351</td>
<td>FUS</td>
</tr>
<tr>
<td>TLS1163R</td>
<td>CATGTCACACACCCAGAATTAG</td>
<td>1200–1223</td>
<td>FUS</td>
</tr>
<tr>
<td>BBF2-1396R</td>
<td>GGCGGACAGTGGGTCTGGGAC</td>
<td>202–225</td>
<td>FUS</td>
</tr>
<tr>
<td>BBF2-1218R</td>
<td>GTGAGTGGAACTTCTGGTTCC</td>
<td>545–567</td>
<td>BBF2H7</td>
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<td>BBF2-1625F</td>
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<td>976–996</td>
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<tr>
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<td>938–957</td>
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<tr>
<td>BBF2-1151R</td>
<td>GTGGACCACCTGCATTTGCC</td>
<td>1337–1360</td>
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<tr>
<td>BBF2-1151F</td>
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<tr>
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<td>1005–1025</td>
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<tr>
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<td>BBF2H7</td>
</tr>
<tr>
<td>BBF2-1550F</td>
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<td>1270–1290</td>
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<tr>
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<td>976–996</td>
<td>BBF2H7</td>
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<tr>
<td>BBF2IN5-70R</td>
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<tr>
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<td>GATTTTCTCGGAGGATACTTG</td>
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<td>BBF2H7</td>
</tr>
<tr>
<td>BBF2IN5-70R</td>
<td>GATTTTCTCGGAGGATACTTG</td>
<td>1625–1648</td>
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<tr>
<td>BBF2IN5-70R</td>
<td>GATTTTCTCGGAGGATACTTG</td>
<td>1065–1065</td>
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<tr>
<td>BBF2IN5-70R</td>
<td>GATTTTCTCGGAGGATACTTG</td>
<td>1184–1204</td>
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</tr>
<tr>
<td>BBF2IN5-70R</td>
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<td>BBF2H7</td>
</tr>
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<td>BBF2IN5-70R</td>
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<td>1967–1989</td>
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<td>BBF2IN5-70R</td>
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<tr>
<td>BBF2IN5-70R</td>
<td>GATTTTCTCGGAGGATACTTG</td>
<td>1783–1804</td>
<td>BBF2H7</td>
</tr>
</tbody>
</table>

aF denotes forward; R denotes reverse.

bPosition in FUS is based on the sequence with accession no. NM_004960. Position in BBF2H7 is based on the sequence in Figure 4. Position for BBF2IN5-70R and BBF2IN5-22R is based on the sequence with accession no. AC022173 (clone RP11-29B3).
position 8676–8709 in the sequence with accession number AF071213). In case 2, part of exon 6 of \textit{FUS} was also fused in frame with a sequence from exon 5 of \textit{BBF2H7} (Fig. 3). However, a precise determination of the fusion point was not possible due to the existence of an identical CGC trinucleotide at the junction (position 724–726 in \textit{FUS} and 1016–1018 in \textit{BBF2H7} cDNA).

Sequence analysis of the two fragments amplified with \textit{BBF2H7} forward and \textit{FUS} reverse primers in case 1 showed that both were \textit{BBF2H7/FUS} cDNA chimeras in which exon 1 of \textit{BBF2H7} was fused in frame to exon 8 of \textit{FUS}. In the 100 bp shorter fragment, exon 9 of \textit{FUS} was alternatively spliced out (Fig. 3).

PCR with TLS427F forward and BBF2IN5-70R reverse primers on genomic DNA, extracted from the tumor of case 1, amplified a 1000 bp fragment. Direct sequencing showed that it was a hybrid \textit{FUS/BBF2H7} genomic DNA fragment containing part of exon 5 (from the TLS427F primer position), the entire intron 5, the same part of exon 6 as found in the cDNA \textit{FUS/BBF2H7} chimera, and the inverse oriented 33 bp insertion of intron 7 of \textit{FUS}, as well as the part of exon 5 of \textit{BBF2H7} found in the cDNA \textit{FUS/BBF2H7} chimera and part of intron 5 to the position of the BBF2IN5-70R reverse primer (accession number AJ549097).

\textbf{BBF2H7 characterization}

Searching the UCSC Human Genome Browser (24 June 2002, www.genome.ucsc.edu/) and the Ensembl Database (17 September 2002, www.ensembl.org), we found that the only gene mapped to the BACs RP11-29B3 (AC022173) and RP11-377B19 (AC009263) was a \textit{Bbf-2} (\textit{Drosophila}) homolog (LOC155008). Through an EST homology search using the ensemblgene ID ENSG00000146854, the human EST clones BU55391, BG390143, BU189408, BG008814, and XM_088116 containing various parts of the \textit{BBF2H7} gene were retrieved. Similarly, searches in the DNA databases (GenBank + EMBL + DDBJ + PDB sequences) retrieved the human sequences with accession number XM_088116, XM_114557 and BC037414, and six sequences from \textit{Mus musculus} that seem to be the mouse homolog of \textit{BBF2H7}: BC043466 (clone MGC:49250 IMAGE:5052843, mRNA, complete), AK041669 and AK042418 (3 days neonate thymus cDNA), AK041050 (adult male aorta and vein cDNA), AK036712 (adult male bone cDNA) and AK035695 (adult male urinary bladder cDNA).

Using the above-mentioned sequences, and by performing PCR with the \textit{BBF2-347F} and \textit{BBF2-1967R} primer combination on cDNA from lung and placenta and direct sequence analysis, a 2400 bp cDNA was eventually compiled containing a 1560 bp open reading frame coding for a 519 amino acid protein with an estimated molecular weight of 57 kDa (Fig. 4). A termination codon is located 33 bp upstream of and in-frame with the first methionine codon of the cDNA, suggesting that this cDNA includes the complete protein sequence. The putative initiating methionine codon is located in a region with similarity to a Kozak consensus sequence (CCGCCATGG) (14).

The amino acid sequence spanning residues 291–356 of the predicted human \textit{BBF2H7} protein contains a consensus B-ZIP domain highly similar to that in the OASIS, CREB-H, CREB4, CREB3 and \textit{Drosophila} Bbf-2 transcription factors with 80, 60, 59, 56 and 71% identity, respectively (Fig. 5). It also contains the amino acid sequence RRKKKEY which is exactly...
conserved among CREB, CREM, ATF1, ATF6 and CREBL1 (15). The leucine zipper motif of BBF2H7 is similar to that in CREB-H and CREB4 (pattern L-X₀-C-X₀-L-X₀-L-X₀-L-X₀-L; Fig. 5). It contains six repeats and consists of five leucines and one cysteine at the second heptad position (amino acid 328) of the leucine zipper. Downstream of the B-ZIP domain, BBF2H7 also contains a hydrophobic region, which was predicted to be an α-helical transmembrane domain (position 376–397; GTCLMVVVLCFAVAGSFFQGY) by the prediction program PSORT II (http://psort.ims.u-tokyo.ac.jp/form2.html). This structural feature is also seen in the other members of the family, i.e. OASIS, CREB-H, CREB3 and CREB4 (16–19).

To investigate the expression pattern of BBF2H7 in human tissues, we performed RT–PCR analysis using cDNAs from 24 tissues, including eight fetal ones, and two primer combinations (BBF2-347F and BBF2-1218R, and BBF2-628F and BBF2-1151R). The results were similar for the two amplifications and showed that BBF2H7 is expressed in most of the examined tissues (Fig. 6). The strongest expression was seen in placenta, lung, spleen and intestine, and the weakest in heart, brain, skeletal muscle, thymus, colon and leukocytes. In fetal tissues, the weakest expression was detected in brain and heart. A splice variant, lacking exon 2, was found in placenta, spleen and fetal liver (Fig. 6). Since northern blot analysis was not performed, the possibility of additional splice variants and the actual size of the normal BBF2H7 transcript could not be determined.

An exon–intron map of the BBF2H7 gene was constructed by alignment of the BBF2H7 cDNA with the genomic sequences of the RP11-29B3 (AC022173) and RP11-377B19 (AC009263) clones. The entire BBF2H7 gene was found to span more than 120 kbp genomic DNA and to be composed of 12 exons (Fig. 7). Exon 1, containing the initiation ATG, is the largest (454 bp) and exon 7 the smallest (59 bp). Exon 12 includes the termination TAA codon. Introns 1 and 9 are the largest (73132 bp) and smallest (281 bp), respectively.
DISCUSSION

The FUS gene has previously been reported as the 5′-partner in three neoplasia-associated fusion genes. In myxoid liposarcomas, where FUS forms a chimeric protein with the full-length B-ZIP transcription factor DDIT3, exon 5 (type 2 FUS/DDIT3 chimera) of FUS is the most frequent site of fusion, followed in frequency by exon 7 (type 1) and exon 8 (type 3) (4,5,20–22). Exon 5 of FUS is also the fusion point in the formation of chimeras in angiomatoid fibrous histiocytoma (8,9), where it is fused to the B-ZIP domain of the ATF1 gene. However, in the subset of acute myeloid leukemia with t(16;21)(p11;q22), it is most commonly exon 7 of FUS that is fused to the DNA binding and C-terminal encoding parts of ERG, a member of the ETS gene family (6,7). Nevertheless, the theme is the same in the three above-mentioned hybrid genes; the RNA binding domain and C-terminal part of FUS are replaced by the DNA binding and C-terminal parts of a transcription factor.

Here, we show that FUS is involved in yet another type of neoplasia, LGFMS and again fused to a transcription factor, BBF2H7. The detected FUS/BBF2H7 chimeric transcripts were composed of the first five exons and part of exon 6 from FUS and part of exon 5 and exons 6–12 from BBF2H7 (Figs 2 and 3). Thus, as in the three previously identified fusion genes involving FUS, the FUS/BBF2H7 chimera is coding for a protein containing the N-terminus of FUS, and the B-ZIP domain and the C-terminus of a transcription factor, but in contrast to the previously described chimeras, the two cases presented here had intra-exonic fusions. Whether this is coincidental or necessary for generating a functional fusion gene is presently unknown.

The fusion points in the present two cases were different, but were in both cases located within exon 6 of FUS (Fig. 3). In case 1, the sequence of exon 6 was followed by a small inverted sequence from intron 7 of FUS, and, in the reciprocal BBF2H7/FUS chimera, exon 1 of BBF2H7 was found to be fused to exon 8 of FUS. Attempts to amplify other chimeric BBF2H7/FUS transcripts using forward primers in exon 2 or 3 of BBF2H7 failed. Thus, the translocation t(7;16) was in case 1 accompanied by an inversion in FUS and a deletion in BBF2H7. The fact that the reciprocal BBF2H7/FUS transcript was found only in case 1 strongly implies that it is not of pathogenetic importance. The FUS gene is transcribed from telomere to centromere and, according to the Ensembl Database, the transcription of BBF2H7 proceeds in the same direction. Hence, the formation of the FUS/BBF2H7 chimera is possible on the derivative chromosome 7 by a simple balanced chromosomal translocation.

BBF2H7 is a member of the OASIS B-ZIP family of transcription factors together with OASIS, CREB-H, CREB3, and CREB4 (16–19,23,24). These proteins can dimerize with each other, but not with transcription factors belonging to other B-ZIP families (15), can bind CRE DNA sequences, can activate transcription through box-B and ATF6 elements, and contain a transcriptional activation domain at the N-terminal part (16–19,23,24). In the FUS/BBF2H7 chimera, all the above-mentioned functions may be influenced. The B-ZIP encoding domain of BBF2H7 comes under the control of the FUS promoter, which in turn may cause deregulation of genes normally controlled by BBF2H7. In addition, the B-ZIP domain of BBF2H7 becomes fused to the N-terminal part of FUS, which displays transactivating and oncogenic properties (25–27), and one might speculate that the ability to dimerize with other members of the OASIS family could be affected by replacing the normal N-terminal part with that from FUS.
LGFMS is probably a rare type of STS, but the exact incidence is unknown (28). LGFMS typically occurs in young adults, but children account for up to one-fifth of the cases. Immunohistochemistry and ultrastructural studies have indicated that the tumor cells show a strictly fibroblastic differentiation. Lack of positivity for other immunohistochemical markers makes LGFMS difficult to diagnose, and it is likely that some cases have been misdiagnosed as other forms of low-grade malignant STS, e.g. myxofibrosarcoma. In this context it could be noted that previous cytogenetic analyses of LGFMS are restricted to three cases, but that a supernumerary ring chromosome was identified as the sole anomaly in two of them. Interestingly, one of those cases was further investigated by comparative genomic hybridization, revealing gain of material from chromosomes 7 and 16 (29), and it may be speculated that also this case harbored a FUS/BBF2H7 fusion gene. If so, the cytogenetic data would imply that additional cases with FUS/BBF2H7 fusion genes may be found in the relatively large subgroup of low grade malignant STS showing supernumerary ring chromosomes at chromosome banding analysis.

MATERIALS AND METHODS

Patients

Case 1 was a 38-year-old man who for 20 years had noted a slowly growing soft tissue mass at his right shoulder. An MRI showed a 9 cm tumor located deep of the trapezius muscle. A preoperative fine needle aspiration biopsy was suggestive of a benign fibroblastic tumor, and the tumor was removed by marginal excision. Histopathologic analysis revealed a well circumscribed lesion that on cut sections exhibited a solid, grayish-white, firm, homogeneous cut surface, without evidence of necrosis. The tumor was composed of bland spindle-shaped cells displaying small hyperchromatic nuclei, finely clumped chromatin, inconspicuous nucleoli and indistinct, pale cytoplasm. Some mild nuclear atypia and scant mitotic activity could be observed. The cells were arranged in a vague whorling or fascicular pattern, and there was an alternation of more dense, fibrous areas with loose, myxoid stroma (Fig. 8A). Furthermore, the tumor showed the characteristic 'hyalinizing spindle cell pattern with giant rosettes' phenotype (Fig. 8B). Thus, the diagnosis was LGFMS with giant collagen rosettes. Nine months after surgery, the patient remains disease-free.

Case 2 was a 17-year-old girl with a 1-year history of a growing tumor in the right axilla. An MRI showed an 8 cm, extracompartmental and well demarcated soft tissue tumor with central necrosis. After a fine needle aspiration biopsy and a complementary open biopsy, a preoperative diagnosis of hemangiopericytoma was established. The tumor was removed by local excision with a wide surgical margin. Histopathologic examination of the excised tumor showed features that were very similar to those in case 1, but without giant collagen rosettes. The diagnosis was classical LGFMS. There is no evidence of disease 2 years after surgery.

Cytogenetic analysis and FISH

Culturing and harvesting of the tumor cells and chromosome banding were performed as described (30). Karyotypes were described according to the recommendations of ISCN (31). Bacterial artificial chromosome (BAC) probes for FISH were obtained from the RPCI-11 P. de Jong library (www.chori.org/bacpac/) and the CITB Human BAC D library (CTD; Research Genetics; www.resgen.com). The location of the clones on chromosomes 7 and 16 was based on the latest update of the UCSC Human Genome Browser (November 2002; www.genome.ucsc.edu/) and the Ensembl Database (2 December 2002 www.ensembl.org). Probes were directly

Figure 5. (A) Alignment of human BBF2H7 with OASIS proteins. (B) Alignment of the B-ZIP domain of BBF2H7 with the other members of the OASIS family of transcription factors, OASIS, CREB-H, CREB3 and CREB4.
labeled with Cy3-dUTP (Amersham, Amersham Place, UK), FluorX-dCTP (Amersham) or indirectly labeled with Biotin-dUTP using Amersham’s mega Prime kit and detected with streptavidin-diethylaminocoumarin (DEAC). FISH was performed as described previously (32). The hybridizations were analyzed with the Chromofluor System (Applied Imaging, Newcastle, UK). All WCPs used for combined binary ratio labeling (COBRA) FISH were supplied by Professor H. Tanke and Dr J. Wiegant, Leiden University, The Netherlands. COBRA-FISH analysis was performed as described (33).

**PCR and sequence analysis**

Tumor tissue pieces adjacent to those used for cytogenetic analysis and histological examination had been frozen and stored at −80°C. Total RNA was extracted from the frozen specimens using the Trizol reagent according to the manufacturer’s instructions (Gibco BRL, Stockholm, Sweden). DNA was extracted using standard methods (34). Five micrograms of total RNA were reverse-transcribed in a 20 μl reaction volume containing 50 mM Tris–HCl pH 8.3 (at 25°C), 75 mM

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**Figure 6.** Expression pattern of BBF2H7. RT–PCR analysis was performed using cDNAs from 16 adult and eight fetal human tissues. The highest expression of BBF2H7 is seen in placenta, and adult lung, spleen and small intestine, and the lowest in heart, brain, skeletal muscle, thymus, colon and leucocytes. A splice variant, lacking exon 2, was found in placenta, spleen and fetal liver.

**Figure 7.** Exon–intron structure of the BBF2H7 gene. The borders and the size of the exons and introns as well as the positions of exons in the two clones RP11-29B3 (AC022173) and RP11-377B19 (AC009263) are shown. The beginnings and ends of the exons correspond to the nucleotide positions in the cDNA of BBF2H7 shown in Figure 4.
phenotype (case 1, HE 250×), and 2 of each dNTP, 1 unit Platinum Taq DNA polymerase (Gibco BRL), 0.5 μl RNA guard (Pharmacia, Uppsala, Sweden), 10 pmol random hexamers, 1 μg Oligo (dT)10, and 400 units M-MLV Reverse Transcriptase (Gibco BRL). The reaction was carried out at 37°C for 60 min, heated for 10 min at 65°C, and then kept at 4°C.

The primers used for PCR amplification and sequence analyses are listed in Table 1. A one-step PCR was performed for amplification of both the FUS/BBF2H7 and the BBF2H7/FUS chimeric transcripts. The 50 μl reaction volume contained 20 mM Tris–HCl pH 8.4 (at 25°C), 50 mM KCl, 1.25 mM MgCl2, 0.2 mM of each dNTP, 1 unit PlatinumTag DNA polymerase (Gibco BRL), 0.5 μM of each of the forward and reverse primers (Table 1), and 2 μl of the cDNA. The FUS/BBF2H7 fusion transcript was detected using the TLS165F and BBF2-1435R, TLS165F and BBF2-1396R, TLS427F and BBF2-1435R, TLS427F and BBF2-1396R, TLS05F and BBF2-1967R, and TLS391F and BBF2-1967R primer combinations. The reciprocal BBF2H7/FUS transcript was detected using the BBF2-347F and TLS1677R, BBF2-347F and TLS1314R, and BBF2-347F and TLS1163R primer combinations.

A one-step PCR was also performed for the detection of the FUS/BBF2H7 genomic hybrid. The 50 μl PCR reaction volume had the same composition, 0.5 μM of each of the TLS427F forward and BBF2IN5-70R reverse primers and 0.5 μg DNA extracted from tumor biopsy and peripheral blood lymphocytes from a healthy individual (negative control).

BBF2H7 expression analyses were performed using cDNA multiple tissue (BD Biosciences, Stockholm, Sweden) panels I (K1420-1; brain, heart, kidney, liver, lung, pancreas, placenta and skeletal muscle), and II (K-1421-1; colon, ovary, peripheral blood leucocyte, prostate, small intestine, spleen, testis and thymus), and a fetal panel (K1425-1; brain, heart, kidney, liver, lung, skeletal muscle, spleen and thymus), according to the manufacturer’s instructions. The primer combinations were BBF2-347F and BBF2-1218R, and BBF2-628F and BBF2-1151R. Amplification of the entire open reading frame was performed using cDNA from lung and placenta (from panel I) and the BBF2-347F and BBF2-1967R primer combination.

For all PCR, after an initial denaturation at 94°C for 5 min, 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C were run using a PCT-200 DNA Engine (MJ Research, Waltham, MA, USA), followed by a final extension for 10 min at 72°C. Fifteen microliters of the PCR products were analyzed by electrophoresis through 1.5% agarose gels, stained with ethidium bromide and photographed.

For sequence analysis, the amplified fragments were run on 1.0% agarose gels, purified using Qiagen gel extraction kit (Qiagen, Hilden, Germany), and directly sequenced using the dideoxy procedure with an ABI Prism BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, CA, USA) with various primers (Table 1) on the Applied Biosystems Model 310 DNA sequencing system. The BLAST software (www.ncbi.nlm.nih.gov/ BLAST/) tools and software packages from ExPASy molecular biology server (www.expasy.org/) and the PSORT II (http://psort.im.u-tokyo.ac.jp/form2.html) were used for computer analysis of sequence data.

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REFERENCES


