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Expanding the Molecular Signature of Ossifying Fibromyxoid Tumors with 2 Novel Gene Fusions: *CREBBP-BCORL1* and *KDM2A-WWTR1*

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Abstract

Ossifying fibromyxoid tumor (OFMT) is an uncommon mesenchymal neoplasm of uncertain differentiation and intermediate malignant potential. Recurrent gene fusions involving either *PHF1* or *BCOR* have been found in 85% of OFMT, including typical and malignant examples. As a subset of OFMT still lack known genetic abnormalities, we identified two OFMTs negative for *PHF1* and *BCOR* rearrangements, which were subjected to transcriptome analysis for fusion discovery. The RNA sequencing found a novel *CREBBP-BCORL1* fusion candidate in an axillary mass of a 51 year-old male and a *KDM2A-WWTR1* in a thigh mass of a 36 year-old male. The gene fusions were validated by RT-PCR and FISH in the index cases and then screened by FISH on 4 additional OFMTs lacking known fusions. An identical *CREBBP-BCORL1* fusion was found in an elbow tumor from a 30 year-old male. Both OFMTs with *CREBBP-BCORL1* fusions had areas of typical OFMT morphology, exhibiting uniform round to epithelioid cells arranged in cords or nesting pattern in a fibromyxoid stroma. The OFMT with *KDM2A-WWTR1* fusion involved dermis and superficial subcutis, being composed of ovoid cells in a fibromyxoid background with hyalinized giant rosettes. The S100 immunoreactivity ranged from very focal to absent. Similar to other known fusion genes in OFMT, *BCORL1*, *CREBBP* and *KDM2A* are also involved in histone modification. In summary, we expand the spectrum of molecular abnormalities in OFMT with 2 novel fusions, *CREBBP-BCORL1* and *KDM2A-WWTR1*, further implicating the epigenetic deregulation as the leading pathogenetic mechanism in OFMT.

Keywords

ossifying fibromyxoid tumor; CREBBP; BCORL1; KDM2A; WWTR1; endometrial stromal sarcoma

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INTRODUCTION

Ossifying fibromyxoid tumor (OFMT) is a rare soft tissue neoplasm of uncertain histogenesis and intermediate risk of malignancy (rarely metastasizing). (Fletcher et al., 2013) It often affects middle-aged adults, with a median age of 49 years and a male-to-female ratio of 1.5:1. (Schneider et al., 2016) The most common presentation of OFMT is in the superficial or deep soft tissues of the extremities, and less commonly in the trunk or head and neck regions. First described in 1989 by Enzinger et al., OFMT is a well-circumscribed neoplasm often associated with an incomplete peripheral shell of bone, multilobular growth pattern, and uniform round to oval tumor cells arranged in cords or nests in a fibromyxoid stroma. (Enzinger et al., 1989) Following the recognition of this entity, tumors with atypical histology or more aggressive clinical behavior, including higher recurrent and metastatic rates, have been reported. (Kilpatrick et al., 1995; Zamecnik et al., 1997; Folpe and Weiss, 2003; Miettinen et al., 2008) However, the diagnostic criteria of atypical or malignant OFMT have been controversial, since they often lack the characteristic immunophenotype and/or peripheral ossification. Recent advances in our understanding of genetic abnormalities of OFMTs have shown that at least 85% of cases, including typical/benign, atypical, and malignant, harbor recurrent genetic translocations. (Gebre-Medhin et al., 2012; Graham et al., 2013; Antonescu et al., 2014) The most common molecular alterations are the *PHF1*-related fusions, involving various gene partners, such as *EP400*, *MEAF6*, and *EPC1*. (Gebre-Medhin et al., 2012; Graham et al., 2013; Antonescu et al., 2014) Rare cases harboring *ZC3H7B-BCOR* fusion have also been described. (Antonescu et al., 2014) A *JAZF1-PHF1* fusion was also reported in a likely case of OFMT, described as a peculiar cardiac sarcoma showing ossification and monotonous oval to short spindle cell proliferation. (Schoolmeester et al., 2013) *PHF1* rearrangements were found in both benign and malignant OFMTs without a conventional typical component (so-called de novo malignant OFMT). (Graham et al., 2013) The identification of recurrent gene rearrangements in OFMT has not only provided a useful diagnostic tool in challenging cases, such as atypical morphology or non-specific immunoprofile, but also helped clarify the dispute over the existence of malignant OFMTs. However, there still remains a small subset of OFMTs lacking known gene rearrangement. (Antonescu et al., 2014) In this study, we take advantage of transcriptome sequencing in two index OFMT cases with frozen material for further fusion gene characterization.

MATERIALS AND METHODS

Index Cases and Patient Selection

The index case 1 was a malignant OFMT arising in a 51 year-old man, as an 11 cm left axillary mass. The case was included in our previous study (Case# 38), lacking gene rearrangements in *PHF1*, *EP400*, and *BCOR* genes by FISH. (Antonescu et al., 2014) The patient subsequently developed a local recurrence 2 years after the initial diagnosis and received radiotherapy followed by resection. One year later he developed bilateral pulmonary metastases, for which he received multiple wedge resections and chemotherapy. Fresh tissue was available from a pulmonary metastasis and submitted for RNA sequencing.

Due to subsequent disease progression, the patient succumbed of disease 4 years after diagnosis.

The second index case was a benign OFMT arising in a 36 year-old man presenting with a superficial thigh mass. The tumor was negative for *PHF1* and *BCOR* rearrangements by FISH. Upon re-excision of the primary lesion, fresh frozen tissue was collected for RNA sequencing.

In order to establish the prevalence of the novel gene fusions identified by transcriptome sequencing in the index cases, we searched our files for OFMT lacking known genetic alterations. We selected 4 additional OFMTs which fit the above criteria and had available materials for further molecular testing, 3 of them being included in our previous study (Case No. 34, 35, 37).(Antonescu et al., 2014) The remaining case was a recent case arising in the elbow of a 30 year-old man (Table 1). Similar diagnostic criteria for malignancy were applied as previously described.(Antonescu et al., 2014) Tumors with increased cellularity, mitotic activity more than 2 per 50 high power fields, and/or nuclear pleomorphism or necrosis were classified as malignant OFMT; while cases showing increased cellularity but lacking increased mitotic activity, necrosis or nuclear pleomorphism were defined as atypical OFMTs. Clinical and pathologic information was collected from the electronic medical records. The study was approved by the Institutional Review Board.

RNA sequencing and analysis by FusionSeq

Total RNA was extracted using RNeasy Plus Mini (Qiagen), followed by mRNA isolation with oligo(dT) magnetic beads and fragmentation by incubation at 94°C in fragmentation buffer (Illumina) for 2.5 minutes. To reduce the inclusion of artifact chimeric transcripts into the sequencing library due to random priming, an additional gel size-selection (350–400bp) was introduced before the adapter ligation step.(Quail et al., 2008) The adaptor ligated library was then enriched by PCR for 15 cycles and purified. The library was sized and quantified using DNA1000 kit (Agilent) on an Agilent 2100 Bioanalyzer according to the manufacturer's instructions. Paired-end RNA sequencing at read lengths of 50 or 51 bp was performed with the HiSeq 2000 (Illumina). All reads were independently aligned with STAR (ver 2.3)(Dobin and Gingeras, 2015) against the human reference genome (hg19). The reads were then analyzed by FusionSeq for potential gene fusions.(Sboner et al., 2010) Paired-end reads mapped to different genes were first used to identify potential chimeric candidates. A cascade of filters, each taking into account different sources of noise in RNA-sequencing experiments, was then applied to remove spurious fusion transcript candidates. The mRNA expression levels of the partner genes involved in the translocations were evaluated.

In addition, we also analyzed the gene expression profiles of 5 OFMT cases, each harboring *ZC3H7B-BCOR*, *MEAF6-PHF1*, *EP400-PHF1*, *CREBBP-BCORL1*, and *KDM2A-WWTR1*. The first 4 cases were previously published (Case No. 1, 3, 39, 38 in prior report), (Antonescu et al., 2014) while the last one represented the index case 2 from this study. The quantile normalized and log₂ transformed RPKM (reads per kilo base per million mapped reads) values were compared to those of a wide array of other sarcomas in our RNA sequencing data to get a differentially expressed gene list of OFMT by a log₂ fold change threshold of positive 1 or negative 5 and p-value less than 0.05.

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed on the index cases to confirm the fusion transcripts identified by RNA sequencing. An aliquot of the RNA material extracted above for RNA sequencing was reverse transcribed by SuperScript III First-Strand Synthesis System (Invitrogen) into complementary DNA. PCR was performed by Advantage 2 PCR kit (Clontech, Mountain View, CA). PCR primers were designed according to the reads from RNA sequencing (Supplementary table 1), using annealing temperatures at 64.5°C for *CREBBP-BCORL1* and 66°C for *KDM2A-WWTR1*. Since *WWTR1* was described as a 5' fusion partner gene in epithelioid hemangioendothelioma, (Errani et al., 2011) we have additionally designed primers to exclude the possibility of *WWTR1-KDM2A* fusion (Supplementary table 1). The PCR products were then analyzed by agarose gel electrophoresis and purified by QIAquick PCR Purification Kit (Qiagen) for Sanger sequencing.

Fluorescence in situ hybridization (FISH)

FISH was performed on 4 µm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections to confirm the RNA sequencing fusion candidates and subsequently screen the additional cases. Custom probes using bacterial artificial chromosomes (BAC) clones (Supplementary Table 2) were designed flanking the *CREBBP*, *BCORL1*, *KDM2A* and *WWTR1* genes, according to UCSC genome browser (<http://genome.ucsc.edu>) and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA; <http://bacpac.chori.org>). DNA from each BACs was isolated according to the manufacturer's instructions. The BAC clones were labeled with fluorochromes by nick translation and validated on normal metaphase chromosomes. The slides were deparaffinized, pretreated, and hybridized with denatured probes. After overnight incubation, the slides were washed, stained with DAPI, mounted with an antifade solution, and then examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) controlled by Isis 5 software (Metasystems). Fusion FISH assay was also performed on index case 2, combining the BAC clones centromeric to *KDM2A* and flanking both sides of *WWTR1* (Supplementary Table 2).

RESULTS

OFMT with *CREBBP-BCORL1* fusion show upregulation of *BCORL1*

RNA sequencing in case 1 identified a *CREBBP-BCORL1* fusion candidate composed of *CREBBP* (16p13.3) exon 30 fused to *BCORL1* (Xq26.1) exon 4 (Fig. 1). The fusion transcript was further confirmed by RT-PCR and break-apart FISH assay of both genes (Fig. 1B,C). The predicted amino acid sequence was in-frame and the protein domains of the chimeric protein are illustrated in Figure 1. The *BCORL1* mRNA expression showed up-regulation of the downstream exons distal to the breakpoint (exon 4) (Fig. 1E). In contrast, *CREBBP* showed no increased expression, either at the whole transcript or individual exon levels (data not shown). Interestingly, investigating a wide array of sarcoma types from our RNA sequencing database, *BCORL1* up-regulation was also identified in undifferentiated round cell sarcomas with *BCOR* genetic alterations, i.e. *BCOR* internal tandem duplication (ITD) or *BCOR-MAML3* gene fusion, which also demonstrated *BCOR* overexpression. (Kao et al., 2016) In contrast to the OFMT with *CREBBP-BCORL1* fusion, tumors with *BCOR*

genetic abnormalities showed overexpression of the entire *BCORL1* gene (Fig. 1D,E), suggesting a different underlying molecular mechanism of *BCORL1* up-regulation.

***CREBBP-BCORL1* occurs in both benign and malignant OFMT**

Histologically, the index case 1 showed typical OFMT features, with uniform round cells, arranged in cord-like pattern in a fibromyxoid background (Fig. 3A). Foci of ossification, both in the center and at the periphery of the tumor, were also seen and were accompanied by chondroid metaplasia in areas. The tumor showed variegated cellularity, ranging from hypocellular areas with hyalinized stroma (Fig. 3B) to hypercellular solid areas. The latter showed increased mitotic activity (7/10 high power fields) (Fig. 3C) and focal necrosis, findings in keeping with a malignant OFMT. The tumor wrapped around axillary artery and vein, and lymphovascular invasion was present. Immunohistochemically, the tumor showed very focal S100 positivity, while desmin was negative. The local recurrence showed extensive hyalinization (> 90%) secondary to radiotherapy and the subsequent lung metastases resembled the histology of the primary lesion.

FISH screening of the additional 4 cases identified a second case with similar *CREBBP-BCORL1* gene fusion, which occurred in the elbow of a 30 year-old man. Grossly the lesion was well defined and surrounded by a thin fibrous pseudocapsule. Microscopically, the tumor was composed of alternating collagenous and myxoid areas (Fig. 3D). The tumor cells were arranged in cords, reticular pattern, or embedded within the fibrous stroma (Fig. 3E,F). The lesional cells had a round to oval morphology with bland nuclear features and low mitotic activity (<2/50HPFs), in keeping with a benign OFMT. No ossification was present. Immunohistochemically, only rare cells were S100 protein positive and desmin was negative.

The remaining 3 fusion-negative OFMTs included in our previous study were negative for *CREBBP*, *BCORL1*, *KDM2A*, and *WWTR1* break-apart by FISH.

***KDM2A-WWTR1* was identified in an OFMT with collagenous giant rosettes**

In the index case 2, RNA sequencing revealed chimeric reads between *KDM2A* (11q13.2) exon 14 and *WWTR1* (3q23–q24) exon 3. Break-apart FISH showed a typical *KDM2A* rearrangement, while FISH for *WWTR1* showed a constant split, in keeping with an inversion pattern. Fusion FISH assay confirmed the signal association of the 5'-centromeric probe of *KDM2A* to the 3'-centromeric probe of *WWTR1* (Fig. 2A). RT-PCR further demonstrated the in-frame fusion between *KDM2A* exon 14 to *WWTR1* exon 3 (Fig. 2B). No reciprocal *WWTR1-KDM2A* transcript was amplified by the RT-PCR. As exons 1 and 2 of *WWTR1* remain untranslated, the predicted fusion protein would include the entire coding sequence of *WWTR1* (Fig. 2B). No up-regulation of *KDM2A* and *WWTR1* mRNA expression levels were noted, compared to other OFMTs or a variety of other sarcomas.

The index case 2 occurred in the dermis and superficial subcutis of the thigh in a 36 year-old male (Fig. 3G). The tumor appeared relatively circumscribed and showed a multilobulated growth pattern. The tumor cells had a monomorphic ovoid phenotype, lacking atypia or mitotic activity (Fig. 3H). The lesion showed a distinctive stromal component, with collections of collagenous giant rosettes set in a loose fibromyxoid background (Fig. 3I).

There was no ossification noted. Immunohistochemically, tumor was negative for S100, CD34, and only focally positive for desmin. Due to the presence of giant rosettes a diagnosis of low grade fibromyxoid sarcoma was considered in the differential, but MUC4 immunostain was negative and FISH showed no *FUS* and *EWSR1* gene rearrangements.

Most OFMTs form a tight genomic cluster regardless of the fusion type

Using RNAseq data, unsupervised clustering showed a distinct genomic group of the 4 OFMTs harboring *EP400-PHF1*, *MEAF6-PHF1*, *CREBBP-BCORL1*, and *ZC3H7B-BCOR*, among other sarcomas (Supplem Fig. 1). OFMT with *KDM2A-WWTR1* clustered nonspecifically with other tumors types. A list of 257 differentially expressed genes was identified in the 4 OFMTs compared to a large spectrum of sarcomas. The gene list included various genes normally expressed in the central nervous system (i.e. *ECEL1*, *C1QL4*, *C1QL1*, *DNER*, *BAII*, *KCNH3*, *HCN2*, *NPAS1*, *KIF1A*, *GLII*, etc.) and some genes related to bone or cartilage formation (i.e. *CRTAC1*, *PTN*, *BMP7*, *PTH1R*, *TMEM119*, etc).

DISCUSSION

In this study we report two novel gene fusions, *CREBBP-BCORL1* and *KDM2A-WWTR1*, expanding the genetic signature of OFMT. Recurrent *PHF1* related gene fusions have been identified in the majority of OFMTs, with *EP400-PHF1* being the most common variant, but several fusion partners have been reported.(Gebre-Medhin et al., 2012; Graham et al., 2013; Antonescu et al., 2014) A common theme among these fusion partners, including *PHF1*, *EP400*, *MEAF6*, *EPC1*, and *BCOR*, is their function implicated in histone modification and epigenetic regulation. Based on the common core function of these genes it appears that epigenetic dysregulation might be a critical event in the pathogenesis of OFMTs. In concordance with this hypothesis, the novel genes identified in this study, including *CREBBP*, *BCORL1* and *KDM2A*, are also associated with histone modification. *CREBBP* (CREB binding protein, also known as CBP) encodes an ubiquitously expressed histone acetyltransferase (HAT) and functions as a transcriptional co-activator. *CREBBP* and its paralog, *EP300*, are involved in diverse physiological processes, including cell cycle progression, differentiation, apoptosis and DNA repair.(Iyer et al., 2004; Wang et al., 2013) With its HAT activity, CREBBP can acetylate both histones and non-histone proteins, including some transcription factors (e.g. p53, CREB). Germline mutations of *CREBBP* are associated with Rubinstein-Taybi syndrome, a congenital autosomal dominant disease with a heterogeneous clinical presentation including mental retardation, growth impairment, craniofacial dysmorphism and skeletal abnormalities, as well as an increased predisposition to neoplasms.(Iyer et al., 2004; Roelfsema et al., 2005) Somatic inactivating mutations of *CREBBP* have been reported in certain types of lymphoma,(Lunning and Green, 2015) while a *MYST3-CREBBP* fusion was described in acute myeloid leukemia with t(8;16). (Iyer et al., 2004; Diaz-Beya et al., 2013) In our case #1 harboring a *CREBBP-BCORL1* fusion, the *CREBBP* transcript retained most of its coding sequence, except for the last exon. The breakpoint presumably disrupts the zinc finger structure (ZZ domain, Fig. 1) and truncates the C-terminal TAZ2, NCBD and LXXL domains, which are responsible for interaction with many transcription factors (e.g. TFIIB, p53, FOXO3a, STAT1, MEF2, IRF-3), co-activators (e.g. PCAF, GCN5, ACTR), and nuclear receptors (e.g. retinoid X

receptor, estrogen receptor, androgen receptor).(Goodman and Smolik, 2000; Vo and Goodman, 2001)

BCORL1 (BCL6 corepressor-like 1), in contrast to *CREBBP*, encodes a transcriptional corepressor homolog to *BCOR*, with which shares some degree of amino acid sequences homology, including the ankyrin repeats.(Pagan et al., 2007) However, *BCORL1* does not interact with BCL-6, as *BCOR* does, and has a different mechanism of gene repression by interacting with class II histone deacetylase (HDAC) and C-terminal-binding protein (CtBP). (Pagan et al., 2007) *BCORL1* gene rearrangement was previously reported in hepatocellular carcinoma, where *BCORL1* exon 11 was fused to *ELF4* (E74-like factor 4) exon 8, resulting from an intra-chromosomal inversion of the long arm of chromosome X.(Totoki et al., 2011) Similar to *BCOR*, inactivating mutations of *BCORL1* have been reported in small subsets of acute myeloid leukemia, myelodysplastic syndrome, and chronic myelomonocytic leukemia. (Li et al., 2011; Damm et al., 2013; Yamamoto et al., 2014) The presence of *CREBBP-BCORL1* fusion in our OFMT case#1 was associated with mRNA overexpression of the 3' end of *BCORL1*, which was represented in the fusion transcript. Interestingly, whole transcript *BCORL1* up-regulation was also identified in a group of undifferentiated round cell sarcomas harboring *BCOR* genetic abnormalities, including *BCOR* ITD and *BCOR-MAML3* fusion.(Kao et al., 2016) Additional functional studies are needed to elucidate the mechanism of concurrent *BCORL1* upregulation in tumors overexpressing the *BCOR* homolog, as well as to investigate the biologic consequences of the *BCORL1* truncated versus whole transcript upregulation.

KDM2A (lysine demethylase 2, previously known as *JHDM1A* or *FBXL11*) is a histone demethylase, which specifically demethylates H3K36me1/me2, affecting chromatin structure and gene transcription.(Cheng et al., 2014) *KDM2A* plays an important role in cell proliferation of mesenchymal stem cells and can negatively regulate the differentiation toward osteogenic/dentinogenic, adipocytic, and chondrogenic lineages in mesenchymal stem cells. (Dong et al., 2013; Du et al., 2013; Gao et al., 2013) *WWTR1* (*TAZ*) is a transcriptional coactivator of the Hippo pathway and can activate / de-repress the TEAD transcription factor and result in growth limitation.(Mohamed et al., 2015) In epithelioid hemangioendothelioma, *WWTR1* is the 5' gene of the *WWTR1-CAMTA1* fusion transcript,(Errani et al., 2011) while in OFMT case 2, *WWTR1* was the 3' partner. In contrast to *CREBBP-BCORL1*, the *KDM2A-WWTR1* fusion did not result in mRNA up-regulation of either constituent genes.

At transcriptional level, OFMTs with various fusion variants, including *ZC3H7B-BCOR*, *MEAF6-PHF1*, *CREBBP-BCORL1*, and *EP400-PHF1*, clustered tightly together, suggesting that despite different genetic events, they share a common core of downstream targets and gene expression involved in the pathogenesis of this distinct tumor entity. A prior study using gene microarrays showed upregulation of *DLK1*, *EAAT4*, *HHLA2* and *MUC4* genes and downregulation of *PMP22* and *MYEF2* in OFMT compared to other S100-positive neoplasms, such as schwannoma and nerve sheath myxoma.(Graham et al., 2011) Our RNAseq data could not reproduce these results.

In contrast to most other translocation-associated sarcomas, the fusion transcripts in OFMT do not occur through a simple balanced rearrangement due to the opposite directions of transcription of the gene partners, including *EP400-PHF1*, *EPC1-PHF1*, *MEAF6-PHF1* and even *JAZF1-PHF1*.(Antonescu et al., 2014) Thus most OFMTs, including typical and malignant examples, harbor complex structural rearrangements and numerical chromosomal changes by conventional karyotyping.(Sovani et al., 2001; Nishio et al., 2002; Folpe and Weiss, 2003; Kawashima et al., 2007; Gebre-Medhin et al., 2012) Opposite orientation of transcription is also predicted for the *KDM2A-WWTR1*, but not for *CREBBP-BCORL1* fusion.

Quite intriguingly, OFMTs show a significant genetic overlap with low grade endometrial stromal sarcoma (ESS), including identical *EPC1-PHF1*, *MEAF6-PHF1* and *ZC3H7-BCOR* gene fusions.(Micci et al., 2006; Panagopoulos et al., 2012; Panagopoulos et al., 2013; Micci et al., 2014). More recently, a *KDM2B-CREBBP* fusion was reported in a low grade ESS. (Micci et al., 2016) However, the *CREBBP* breakpoint in ESS is located at the beginning of exon 2, while in OFMT at the end of exon 30, although most of the cDNA is retained in both variants. Similar to *KDM2A*, *KDM2B* belongs to the same family of F-box proteins, both being involved in differentiation of mesenchymal stem cells via histone modification.(Deng et al., 2015)

In summary, we expand the genetic spectrum of OFMTs with 2 novel gene fusions, *CREBBP-BCORL1* and *KDM2A-WWTR1*. In keeping with other translocations reported in OFMT, these partner genes play a role in histone modification, reinforcing the emerging role of epigenetic deregulation in the oncogenesis of OFMT. This new report further reinforces prior studies suggesting that the presence of gene fusions in OFMTs are not associated with the risk of malignancy, being present in both benign and malignant examples. However, these rare variant fusions appear more prone to occur in lesions with unusual morphology, lack of ossification or non-specific immunoprofile.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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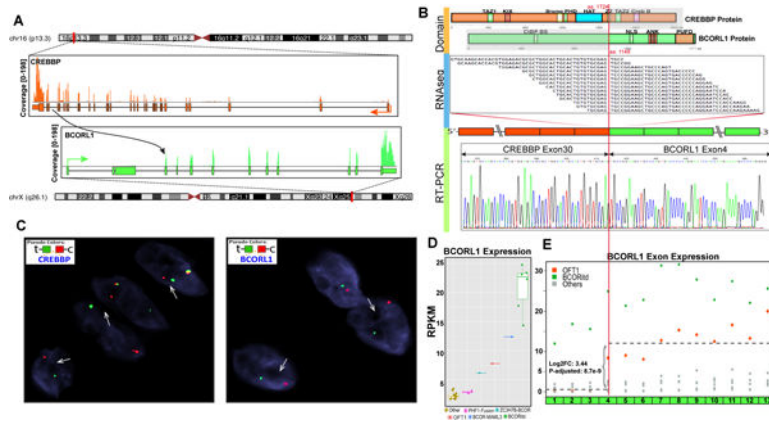


Figure 1. CREBBP-BCORL1 fusion in OFMT

RNA sequencing fusion discovery showed fusion junction reads mapped to the exon 30 of *CREBBP* (16p13.3) and exon 4 of *BCORL1* (Xq26.1); results further confirmed by RT-PCR and Sanger sequencing (A,B). The predicted CREBBP chimeric protein reveals the histone acetyltransferase (HAT) domain being retained in the fusion and joined to the C-terminal part of BCORL1 (B). FISH analyses showed *CREBBP* and *BCORL1* break-apart signals (green, telomeric and red, centromeric, arrows, C). *BCORL1* on Xq26 shows split-apart of the only allele in this male patient. High *BCORL1* mRNA expression (distal to the exon 4 breakpoint) was noted in the *CREBBP-BCORL1* positive OFMT, compared to OFMTs with other gene fusions, including *MEAF6-PHF1*, *EP400-PHF1*, and *ZC3H7B-BCOR* (D,E). Additionally full cDNA *BCORL1* overexpression was also identified in round cell sarcomas with *BCOR*-gene abnormalities (*BCOR-MAML3* or *BCOR ITD*) (D).

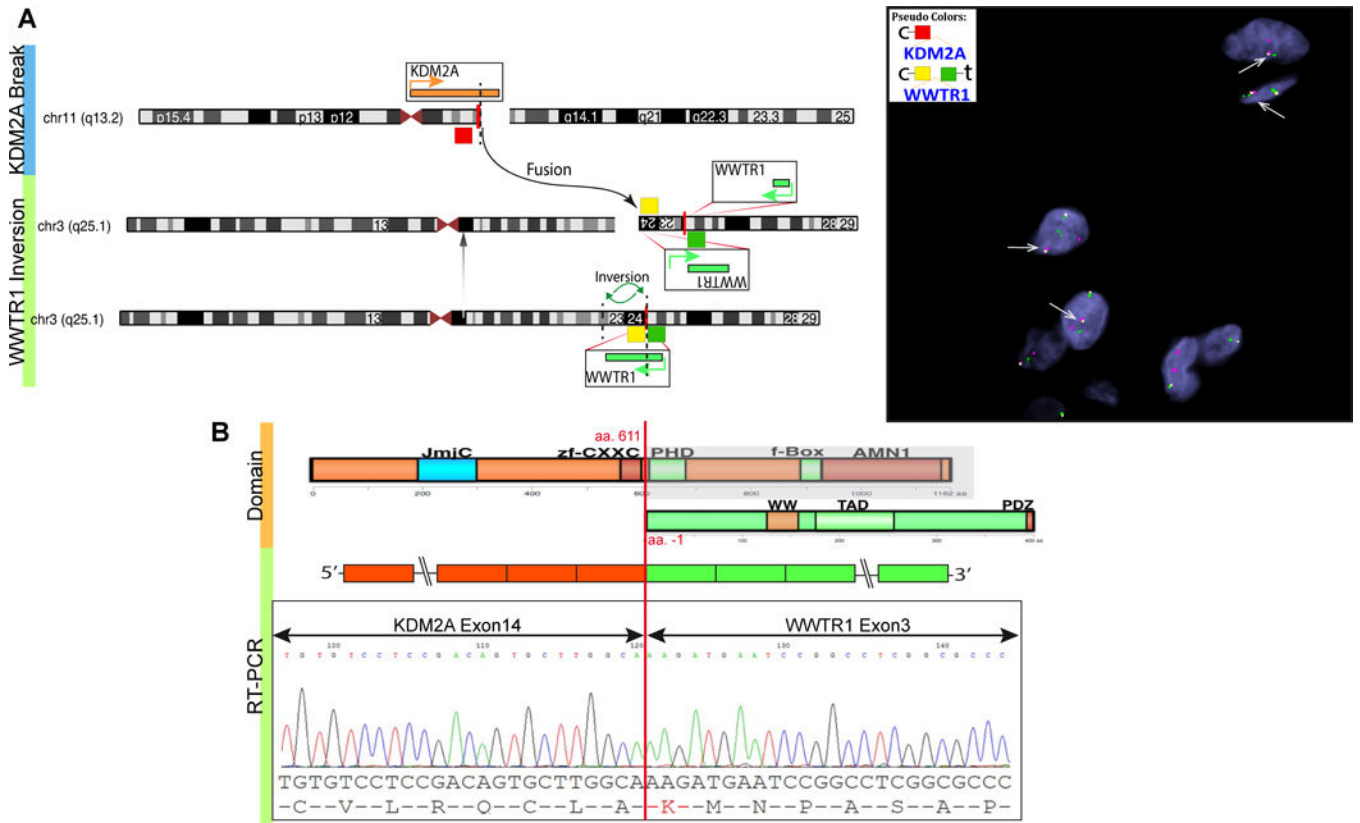


Figure 2. *KDM2A-WWTR1* fusion in OFMT

Schematic diagram and fusion FISH assay demonstrate the 5' *KDM2A* (centromeric, red) being fused to the inverted 3' *WWTR1* (centromeric, yellow) (A). RT-PCR confirms the *KDM2A-WWTR1* in-frame transcript (B). The chimeric protein retains the entire *WWTR1* coding region.

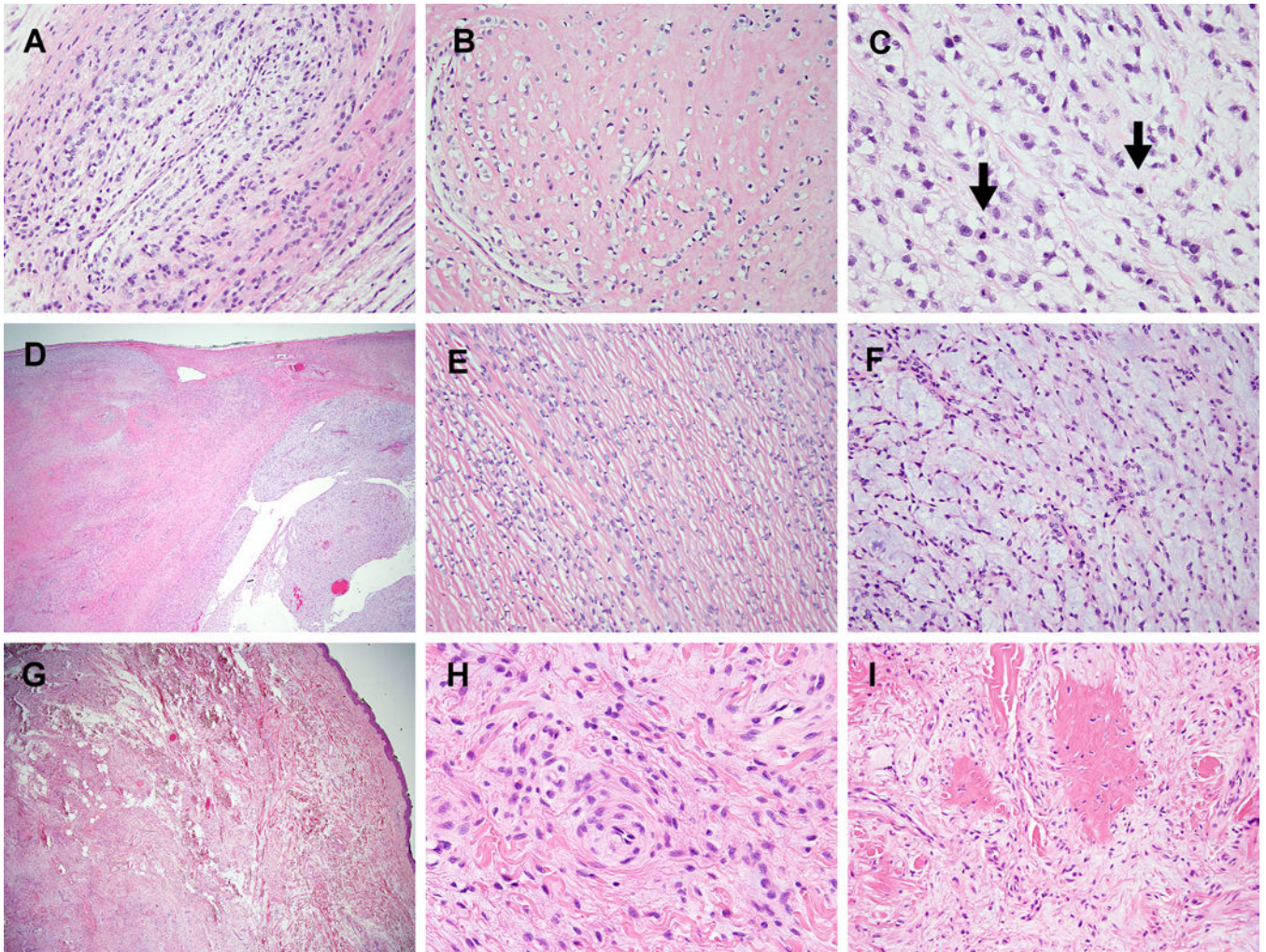


Figure 3. Pathologic findings of OFMTs with *CREBBP-BCORL1* (A–F) and *KDM2A-WWTR1* fusions (G–I)

Case#1 was composed of uniform evenly-spaced small round cells arranged in cord-like structures in a fibromyxoid to hyalinized stroma (A,B), which based on the increased cellularity and mitotic activity was classified as malignant (arrows, C). Case#3 showed alternating collagenous and myxoid areas with focal microcystic change (D), with bland round cells with uniform nuclei and scant cytoplasm (E) arranged in cord-like to reticular patterns (F). The *KDM2A-WWTR1* positive OFMT#2 was located within dermis (G) and superficial subcutis. It was composed of uniform ovoid tumor cells set in a loose fibromyxoid background (H). The tumor showed distinctive giant collagen rosettes throughout (I).

Clinicopathologic findings of OFMTs with *CREBBP-BCORL1* and *KDM2A-WWTR1* fusions

Table 1

Case	Age/Sex	Site	Tumor depth	Histology	S100	Desmin	No. in previous study ¹⁰
<i>CREBBP-BCORL1</i>							
Index case 1	51/M	Axilla	Subcutaneous	Malignant, ossifying	Fpos	Neg	38
Case 3	30/M	Elbow	Subcutaneous	Benign, non-ossifying	Rare	Neg	Not included
<i>KDM2A-WWTR1</i>							
Index case 2	36/M	Thigh	Dermal-Subcutaneous	Benign, non-ossifying	Neg	Fpos	Not included

M, male; Fpos, focally positive; Neg, negative.