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Novel *FUS-KLF17* and *EWSR1-KLF17* Fusions in Myoepithelial Tumors

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Abstract

Myoepithelial (ME) tumors of soft tissue and bone display a heterogeneous histologic spectrum and in about half of the cases harbor *EWSR1* gene rearrangements. Despite rare case reports, the prevalence of *FUS* gene abnormalities and its related fusion partners remains undetermined among ME tumors. Therefore, we screened 66 *EWSR1*-negative ME tumors for *FUS* abnormalities by fluorescence in situ hybridization (FISH). In an index *FUS*-rearranged case, 3'-Rapid Amplification of cDNA Ends (RACE) was applied to identify the fusion partner. Results were further confirmed by RT-PCR, followed by FISH screening the entire cohort of *FUS*-rearranged and *EWSR1*-positive ME lesions lacking a known fusion partner. The correlation between genotype and clinicopathological features was also investigated. As a result, six (9%) *FUS*-rearranged cases were identified, spanning divergent age groups, tumor locations, and morphologic features. A novel *FUS-KLF17* fusion was identified by 3'RACE in an 11 year-old girl with a foot lesion associated with locoregional metastases. Three additional cases with *FUS-KLF17* fusions were identified and one *KLF17* rearrangement (6.3%) was found among the 16 *EWSR1*-positive cases tested. The *KLF17*-related ME tumors affected younger patients and often exhibited trabecular growth in a myxohyaline stroma, but this genotype did not correlate with a malignant phenotype. In conclusion, a small subset of ME tumors harbor *FUS* rearrangements, two thirds of them being associated with *KLF17* fusion. *FUS* FISH analysis is recommended in *EWSR1*-negative lesions in which a ME diagnosis is suspected. *KLF17* is also a rare gene fusion partner to *EWSR1*-rearranged ME tumors.

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INTRODUCTION

Myoepithelial (ME) tumors of soft tissue and bone are rare tumors resembling their counterparts in salivary gland (Kilpatrick et al., 1997; Hornick and Fletcher, 2003; Rekhi et al., 2012). They show a wide morphologic spectrum with occasional duct formation or chondroid differentiation, but consistently co-express epithelial markers and S100 protein. While pleomorphic adenomas of salivary gland frequently exhibit *PLAG1* and *HMGA2* abnormalities (Kas et al., 1997; Martins et al., 2005; Persson et al., 2009), *PLAG1* rearrangements were mainly identified in a subset of cutaneous and superficial soft tissue ME tumors, often displaying ductal structures, consistent with benign mixed tumors (Bahrami et al., 2012; Antonescu et al., 2013). Instead, most deep-seated soft tissue and bone ME tumors harbor different molecular alterations. A handful of early case reports described *EWSR1* gene rearrangements, including t(1;22)(q23;q12) resulting in an *EWSR1-PBX1* fusion and t(19;22)(q13;q12) involving an *EWSR1-ZNF444* fusion (Gleason and Fletcher, 2007; Brandal et al., 2008; Brandal et al., 2009). A subsequent detailed molecular study of 66 ME tumors from soft tissue, bone, and lung revealed *EWSR1* rearrangements occurred in 45% of cases, with different fusion partners including *POU5F1*, *PBX1*, and *ZNF444* (Antonescu et al., 2010). The *EWSR1*-positive ME tumors were often seen in children and young adults, involving soft tissue of the extremities. Five ME tumors with *EWSR1-POU5F1* fusion shared a predominantly nested growth pattern with distinctive clear cell morphology. *EWSR1-PBX1* translocations were found in 5 ME tumors associated with a bland sclerotic appearance and *EWSR1-ZNF444* was identified in a pulmonary ME tumor. More recently our group described novel *EWSR1-PBX3* gene fusions in 3 cases of ME tumors, occurring with predilection in skeletal locations (Agaram et al., 2014). Of the 30 *EWSR1*-negative ME tumors, only one pulmonary case showed *FUS* gene rearrangement (3%). Two more recent case reports described *EWSR1-ATF1* and *FUS-POU5F1* fusion transcripts in a pelvic ME tumor of 57 year-old woman and in a sacral lesion of a 54 year-old man, respectively (Flucke et al., 2012; Puls et al., 2014).

As a significant proportion of ME tumors lack *EWSR1* and *PLAG1* alterations, we sought to investigate the prevalence of *FUS* rearrangement by FISH and to analyze the clinicopathologic features of such lesions in a larger cohort of ME tumors. Additionally, we performed 3'-RACE in a *FUS*-rearranged ME tumor in order to identify possible novel gene partners and to determine their recurrent potential among *FUS* and *EWSR1*-positive ME tumors.

MATERIALS AND METHODS

Tumor Samples and Patients Cohort

We retrieved 66 cases of ME tumors lacking *EWSR1* and *PLAG1* gene rearrangements, 30 of which were included in our earlier study (Antonescu et al., 2010). The cases were

selected from the MSKCC Surgical Pathology and consultation files of the senior authors (CRA, CDF). The slides and corresponding immunohistochemical stains were re-reviewed to confirm the diagnosis according to the standard criteria: tumors were composed of epithelioid, plasmacytoid, or spindled cells forming solid, reticular, or trabecular architectures in a variably myxoid or hyalinized stroma. All tumors showed immunohistochemical evidence of cytokeratin or epithelial membrane antigen (EMA) and S100 protein reactivity, as previously described (Hornick and Fletcher, 2003). The study was approved by the Institutional Review Board 02-060.

Fluorescence in situ Hybridization (FISH)

FISH for *FUS* and *EWSR1* break-apart assay was applied on formalin-fixed and paraffin-embedded 4-micron sections in all cases. FISH was performed by applying custom probes using bacterial artificial chromosomes (BACs), covering and flanking the *FUS* and *EWSR1* gene. Additional BAC clones were designed flanking the novel gene identified in one case by 3'RACE assay. BAC clones were chosen according to UCSC genome browser (<http://genome.ucsc.edu>), see Supplementary Table 1. The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI) (Oakland, CA)(<http://bacpac.chori.org>). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution, as previously described (Antonescu et al., 2010). The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A positive score was interpreted when at least 20% of the nuclei showed a break-apart signal. Nuclei with incomplete set of signals were omitted from the score.

3'- RACE and Reverse Transcription-PCR (RT-PCR)

Total RNA was extracted from the frozen tissue available in the index case (case #1), using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA quality was determined by Eukaryote Total RNA Nano Assay and cDNA quality was tested for *PGK* housekeeping gene (247 bp amplified product). One microgram of total RNA was prepared for 3'-RACE cDNA synthesis by using the SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA). Reverse transcription was initiated at the poly(A) tail of mRNA with 3'-RACE CDS Primer A for making 3'-RACE-Ready cDNA according to the manufacturer's manual. The first round PCR was done by Clontech Advantage 2 PCR Enzyme System kit with the Universal Primer A and *FUS_Ex1/2* Fwd primer (5'-CCTCAAACGATTATACCCAACAAGC-3'), followed by nested PCR using the Nested Universal Primer A and *FUS_Ex4* Fwd primer (5'-CCAATCGTCTTACGGGCAG CAG-3'). The PCR products were analyzed by electrophoresis, TA cloning, and direct sequencing. Subsequent RT-PCR was performed to validate the RACE results using Clontech Advantage 2 PCR Enzyme System kit for 33 cycles at a 64.5°C annealing temperature, using the above *FUS_Ex1/2* Fwd primer and *KLF17_Ex2* Rev (5'-GGCTGCTCTGGTAGAAATG GG-3') primer. The amplicon was confirmed by Sanger

sequencing. Frozen tissue was available from an additional tumor (case #2) and the *FUS-KLF17* fusion transcript was confirmed by RT-PCR using similar primers as above. The cDNA synthesis was performed with SuperScript® III First-Strand Synthesis Kit (Invitrogen), followed by a PCR step. The PCR amplicons were confirmed by direct sequencing.

RESULTS

Clinical and Pathologic Characteristics of *FUS*-Positive ME Tumors

A total of 6 *FUS*-rearranged cases (9%) were identified from the 66 ME tumors lacking abnormalities in *EWSR1* or *PLAG1* genes. There were 4 males and 2 females with a mean age at diagnosis of 28 years (range 8–50; median 32). Most tumors occurred in soft tissue, including extremities, trunk and neck. One lesion involved the tibial periosteum and one was cutaneous. Only one case arose in a visceral location, involving the lung. The tumors demonstrated a broad histologic spectrum, as summarized in Table 1. The tumors were composed of a mixture of spindled, ovoid, or epithelioid cells, with amphophilic to eosinophilic cytoplasm. Their growth patterns varied from trabecular or reticular within a myxohyaline or hyaline background in 3 cases (Cases #1–3, Figs. 1A–C), to a solid sheet-like arrangement with scant stromal component in 2 cases (Cases #4 and 5, Figs. 1D, 1E). The remaining case displayed a nested architecture separated by sclerotic septa (Case #6, Fig. 1F). Focal microcystic change was noted in myxoid areas of case #1. Case #4 showed prominent cytoplasmic vacuolation, imparting a chordoma-like appearance, while case #3 had focal clear cell changes. No ductal differentiation or osteochondroid matrix formation was present in any of the cases.

Two of the cases were designated as malignant (cases #1 and 5). Case #1 had brisk mitotic activity (5/10 HPFs) without significant nuclear pleomorphism, and designated as malignant due to presentation with loco-regional metastatic disease. Case #5 showed significant nuclear atypia, increased mitotic activity (4/10 HPFs), and obvious necrosis (Fig. 1H). This lesion occurred in chest wall soft tissue and was associated with rib invasion (Fig. 1E). The remaining 4 cases with no nuclear atypia or increased mitotic activity were diagnosed as benign in spite of two cases showing focal necrosis.

The fusion negative ME group encompassed 38 benign and 22 malignant ME tumors with a male to female ratio of 1.6 and an average age of 43.2 years (median 45, range 2 months to 85 years) after excluding 3 cases without available data. The most common location was lower extremity (20), followed by trunk (13), viscera (10), upper extremity (9), head & neck (4), and body cavity (4).

Novel *FUS-KLF17* fusions identified in more than half of *FUS*-rearranged ME tumors

From the index case, a 11 year-old girl (case #1) who presented with a 6 cm foot mass and multiple small nodules in her lower leg and thigh, frozen tissue was available to perform 3'-RACE PCR. The amplified sequence revealed a chimeric transcript composed of *FUS* exon 4, followed by a small fragment of *FUS* intron 4, fused with the 5'-untranslated region (5'-UTR) of *KLF17* (Fig. 2A). Subsequent RT-PCR (Fig. 2B) using primers flanking this

breakpoint confirmed the fusion transcript by Sanger sequencing. This result was further validated by FISH analysis, using *KLF17* specific break-apart probes (Figs. 2C, 2D). FISH screening for *KLF17* abnormalities was performed in the remaining 5 *FUS*-rearranged ME tumors. Three additional cases (cases # 2–4) were found to have *FUS-KLF17* fusions. In case # 2 frozen tissue was also available to perform RT-PCR analysis, which showed *FUS* exon 6 fused to the 5'-UTR of *KLF17* gene (Figs. 2E).

ME tumors harboring *FUS-KLF17* occurred in 3 males and one female, with a mean age of 21.5 years (range 8–37; median 20.5). The 2 pediatric tumors arose in the lower extremity, while the lesions occurring in adult patients were located in the lung and the skin of the back. Both pediatric *FUS-KLF17* ME tumors were composed of cords or nests of epithelioid to spindled tumor cells embedded in a myxoid or hyaline stroma (Fig. 1A, 1B). One pediatric tumor (case #1) demonstrated brisk mitotic activity (Fig. 1A) and developed multiple soft tissue metastases in the ipsilateral thigh. The cutaneous lesion (case #3) showed epithelioid cells with prominent cytoplasmic vacuoles, reminiscent of "physaliferous cells", arranged in trabeculae or clusters in an alternating myxoid and sclerotic stroma (Fig. 1C). The pulmonary ME tumor (case #4) showed increased cellularity (Fig. 1D), focal nuclear atypia and necrosis (Fig. 1G); however, no increased mitotic activity was noted.

Rare *EWSR1-KLF17* Gene Fusions Identified in ME Tumors

We additionally tested 19 *EWSR1*-rearranged ME cases lacking a known gene partner, including 9 soft tissue ME cases from our previous study (Antonescu et al., 2010) and 4 cases of cutaneous syncytial myoepithelioma described in another study (Jo et al., 2013). Excluding three failed cases, only one tumor (case #7) showed an *EWSR1-KLF17* fusion among the 16 cases tested (Fig. 3B, 3C). This case occurred in the left foot soft tissue of a 20 year-old male. Microscopically, the tumor had a lobular architecture and consisted of interconnecting cords or clusters of epithelioid to spindled cells in a myxohyaline stroma (Fig. 3A). No mitotic activity, nuclear atypia, or necrosis was observed in this case.

Discussion

Since first being described by Dr. Stout in 1959 as cutaneous mixed tumors of the salivary gland type (Stout and Gorman, 1959), the subsequent studies of ME tumors of soft tissue and bone suggested a heterogeneous histologic spectrum (Kilpatrick et al., 1997; Hornick and Fletcher, 2003). The critical denominator among these different lesions is the consistent immunoexpression of cytokeratin/EMA and S100 protein, in conjunction with variable degrees of calponin, GFAP, smooth muscle actin, and p63 immunoreactivity, which indicates myoepithelial differentiation (Kilpatrick et al., 1997; Hornick and Fletcher, 2003; Rekhi et al., 2012). Genetically, the most common abnormality is *EWSR1* gene rearrangement, present in approximately half of ME tumors (Antonescu et al., 2010; Flucke et al., 2011), which is seen most often among deep soft tissue, bone or lung lesions. The most common gene fusions are *EWSR1-POU5F1* and *EWSR1-PBX1*, each accounting for 17% of *EWSR1*-rearranged tumors (Antonescu et al., 2010). Alternative fusions have been documented mainly as case reports or small case series, including *EWSR1-ZNF444*, *EWSR1-ATF1*, and *EWSR1-PBX3* (Brandal et al., 2009; Antonescu et al., 2010; Flucke et al., 2012;

Agaram et al., 2014). Recently, a distinctive variant of cutaneous ME tumor, designated as syncytial myoepithelioma, was found to have a higher percentage (82%) of *EWSR1* gene rearrangement (Jo et al., 2013). However, despite an extensive FISH work-up for known gene candidates, no fusion partner was detected.

The second common molecular finding in ME lesions is the presence of *PLAG1* gene rearrangements, detected in 37% of cases lacking *EWSR1* and *FUS* abnormalities, with a rare case demonstrating *LIFR-PLAG1* fusion (Antonescu et al., 2013). All 13 *PLAG1*-rearranged ME cases were benign and occur in dermis or subcutis and 12 of them showed tubulo-ductal differentiation. Among 11 ME tumors tested, Bahrami et al. found 5 cutaneous and 3 soft tissue cases exhibiting *PLAG1* rearrangement, which were associated with prominent ductal structures and *PLAG1* immunoreactivity (Bahrami et al., 2012). Conversely, a different study showed that cutaneous mixed tumors could also harbor *EWSR1* abnormalities (Flucke et al., 2011). No *HMG2* gene abnormalities were discovered so far in soft tissue or bone ME tumors (Hallor et al., 2008; Antonescu et al., 2013).

FUS rearrangement in ME tumors has only rarely been documented in literature. One such example was described in a pulmonary lesion of a 30 year-old man (Antonescu et al., 2010), also included in our current study (case #4). The second case is that of an intraosseous sacral ME tumor from a 54 year-old man (Puls et al., 2014). Morphologically, this latter case was composed of epithelioid cells arranged in a reticular and nested pattern in a densely hyalinized matrix. The tumor showed mild nuclear pleomorphism and a mitotic count of 3/10 HPFs. Cytogenetic analysis revealed a 46,XY,del(6)(p21),der(16)t(6;16)(p21;p11) karyotype. The SNP array confirmed a 6p22.3-6p21.2 deletion, affecting the *POU5F1* gene, and a 16p deletion, with one of the breakpoints in the *FUS* gene. Subsequent FISH and RT-PCR confirmed a *FUS-POU5F1* transcript involving *FUS* exon 5 and *POU5F1* intron 1 and exon 2.

The current work investigates the prevalence of *FUS* gene abnormalities and its potential fusion partners in a large cohort of 66 *EWSR1* and/or *PLAG1*-negative ME tumors. Our results from combined FISH and 3'RACE/RT-PCR assays found 6 *FUS*-rearranged ME tumors (9%). As seen with most *EWSR1*-positive lesions, the *FUS*-rearranged ME tumors had wide clinical presentations, morphologic patterns, and consistently lacked ductal differentiation or osteochondroid matrix formation. Remarkably, 4 of the 6 *FUS*-rearranged ME tumors showed novel *FUS-KLF17* fusion. *KLF17* (Krüppel-like factor 17), a.k.a. *ZNF393* (Zinc Finger Protein 393), located at chromosome 1p34.1 region, is the human homologue to murine *Zfp393* and encodes a protein containing 389 amino acids (van Vliet et al., 2006). The Sp/KLF family consists of 25 known members and is characterized by a highly conserved C-terminal DNA-binding domains having three C₂H₂ Krüppel-like zinc motifs, which bind G/C-rich sites in DNA such as GC-box and CACCC-box sequences (Swamynathan, 2010). Thereby, *KLF17* protein acts as a transcription factor involved in regulation of different genes. In breast cancer, *KLF17* protein acts as a negative regulator of epithelial-mesenchymal transition and metastasis (Gumireddy et al., 2009), by binding its 5'-CACCC-3' sequence to the *ID1* promoter, a key metastasis regulator in breast cancer, and repressing its expression. Similarly reduced *KLF17* levels correlates with tumor growth, distant metastasis and poor prognosis in lung carcinoma, hepatocellular carcinoma, gastric

cancer, and papillary thyroid carcinoma (Cai et al., 2012; Liu et al., 2013; Peng et al., 2014; Ye et al., 2014). In endometrioid-type uterine cancer, *KLF17* was implicated in epithelial to mesenchymal transition via direct activation of *TWIST1* and its overexpression was associated with high tumor grade and loss of hormone receptors (Dong et al., 2014). *KLF17* may also function as a germ cell-specific transcription factor involving in spermatid differentiation and oocyte development (Yan et al., 2002). As the *KLF17* breakpoint in ME tumor was located in the 5'-UTR, which is a critical region controlling mRNA translation, degradation, and localization (Mignone et al., 2002), the subsequent *FUS-KLF17* or *EWSR1-KLF17* chimeric transcript may dysregulate the translation or function of the *KLF17* protein.

Half of the *FUS-KLF17*-positive ME tumors occurred in children, involving the soft tissue of lower extremities and exhibited uniform epithelioid to spindled cell morphology, arranged in trabecular or reticular patterns in a myxohyaline stroma. In contrast, *EWSR1-KLF17* gene fusion was found only in one of the 16 *EWSR1*-rearranged cases tested. This case occurred in the lower extremity soft tissue of a young patient and demonstrated classic morphologic features similar to those of *FUS-KLF17* fusion positive ME tumors.

Based on their similar function, it is not surprising that *EWSR1* and *FUS* can be involved interchangeably as oncogenic fusion partners in ME neoplasms. The *FUS* (fused in sarcoma) gene, also known as *TLS* (translocated in liposarcoma), is a member of the TET family together with *EWSR1* (EWS RNA-binding protein 1 or Ewing sarcoma breakpoint region 1) and TATA-binding protein-associated factor 15 (*TAF15*) (Aman et al., 1996; Morohoshi et al., 1998; Paronetto, 2013). Similar to *EWSR1* gene, the *FUS* gene participates in multiple chimeric transcripts not only in mesenchymal tumors but also in hematologic malignancies (Ichikawa et al., 1994). There are many notable examples where *EWSR1* and *FUS* can substitute for one another as fusion partners in sarcomas, such as myxoid liposarcoma (*FUS/EWSR1-DDIT3*), low-grade fibromyxoid sarcoma (*EWSR1/FUS* related fusions either with *CREB3L2* or *CREB3L1*), and angiomatoid fibrous histiocytoma (*EWSR1/FUS-ATF1*) (Rabbitts et al., 1993; Waters et al., 2000; Panagopoulos et al., 2004; Mertens et al., 2005). However, there is rarely equal involvement, with either *EWSR1* or *FUS* prevalence predominating in a particular type of sarcoma, while, in some sarcoma types only one of the genes is implicated in their pathogenesis (i.e. clear cell sarcoma, desmoplastic small round cell tumor) (Fisher, 2014). In keeping with these results, the *EWSR1*-related fusions are more frequent events compared to *FUS* alterations in ME neoplasms. One possible explanation why *FUS-KLF17* fusions outnumber *EWSR1-KLF17* fusions in ME tumors is that both *FUS* and *KLF17* have the same orientation (centromeric) and their fusion can be achieved by a simple balanced translocation.

In summary, we identified a small subset (9%) of *FUS*-rearranged cases from studying a large cohort of *EWSR1*-negative ME tumors. The *FUS*-rearranged ME tumors spanned a heterogeneous clinical and pathologic spectrum, including both benign and malignant/metastatic examples. The most common fusion partner was *KLF17* seen in 4 cases (67%), resulting in novel *FUS-KLF17* fusions, which occurred mainly in the deep soft tissue of extremities in children. Their morphology showed epithelioid to ovoid cells arranged in a trabecular growth in a myxohyaline stroma. Thus, *FUS* FISH analysis is recommended in *EWSR1*-negative lesions in which a ME diagnosis is suspected. Additionally, *KLF17* was

found as a rare fusion partner with *EWSR1*, with only one of the 16 (6%) *EWSR1*-positive ME cases lacking a known gene partner being positive for this gene fusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Agaram NP, Chen HW, Zhang L, Sung YS, Panicek D, Healey JH, Nielsen GP, Fletcher CD, Antonescu CR. *EWSR1*-*PBX3*: A novel gene fusion in myoepithelial tumors. *Genes Chromosomes Cancer*. 2014 [Epub ahead of print].
- Aman P, Panagopoulos I, Lassen C, Fioretos T, Mencinger M, Toresson H, Hoglund M, Forster A, Rabbitts TH, Ron D, Mandahl N, Mitelman F. Expression patterns of the human sarcoma-associated genes *FUS* and *EWS* and the genomic structure of *FUS*. *Genomics*. 1996; 37:1–8. [PubMed: 8921363]
- Antonescu CR, Zhang L, Chang NE, Pawel BR, Travis W, Katabi N, Edelman M, Rosenberg AE, Nielsen GP, Dal Cin P, Fletcher CD. *EWSR1*-*POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone, and visceral lesions, showing common involvement of the *EWSR1* gene. *Genes Chromosomes Cancer*. 2010; 49:1114–1124. [PubMed: 20815032]
- Antonescu CR, Zhang L, Shao SY, Mosquera JM, Weinreb I, Katabi N, Fletcher CD. Frequent *PLAG1* gene rearrangements in skin and soft tissue myoepithelioma with ductal differentiation. *Genes Chromosomes Cancer*. 2013; 52:675–682. [PubMed: 23630011]
- Bahrami A, Dalton JD, Krane JF, Fletcher CD. A subset of cutaneous and soft tissue mixed tumors are genetically linked to their salivary gland counterpart. *Genes Chromosomes Cancer*. 2012; 51:140–148. [PubMed: 22038920]
- Brandal P, Panagopoulos I, Bjerkehagen B, Gorunova L, Skjeldal S, Micci F, Heim S. Detection of a t(1;22)(q23;q12) translocation leading to an *EWSR1*-*PBX1* fusion gene in a myoepithelioma. *Genes Chromosomes Cancer*. 2008; 47:558–564. [PubMed: 18383210]
- Brandal P, Panagopoulos I, Bjerkehagen B, Heim S. t(19;22)(q13;q12) Translocation leading to the novel fusion gene *EWSR1*-*ZNF444* in soft tissue myoepithelial carcinoma. *Genes Chromosomes Cancer*. 2009; 48:1051–1056. [PubMed: 19760602]
- Cai XD, Zhou YB, Huang LX, Zeng QL, Zhang LJ, Wang QQ, Li SL, Feng JQ, Han AJ. Reduced expression of Kruppel-like factor 17 is related to tumor growth and poor prognosis in lung adenocarcinoma. *Biochem Biophys Res Commun*. 2012; 418:67–73. [PubMed: 22240024]
- Dong P, Kaneuchi M, Xiong Y, Cao L, Cai M, Liu X, Guo SW, Ju J, Jia N, Konno Y, Watari H, Hosaka M, Sudo S, Sakuragi N. Identification of *KLF17* as a novel epithelial to mesenchymal transition inducer via direct activation of *TWIST1* in endometrioid endometrial cancer. *Carcinogenesis*. 2014; 35:760–768. [PubMed: 24220291]
- Fisher C. The diversity of soft tissue tumours with *EWSR1* gene rearrangements: a review. *Histopathology*. 2014; 64:134–150. [PubMed: 24320889]
- Flucke U, Mentzel T, Verdijk MA, Slootweg PJ, Creyten DH, Suurmeijer AJ, Tops BB. *EWSR1*-*ATF1* chimeric transcript in a myoepithelial tumor of soft tissue: a case report. *Hum Pathol*. 2012; 43:764–768. [PubMed: 22154050]
- Flucke U, Palmedo G, Blankenhorn N, Slootweg PJ, Kutzner H, Mentzel T. *EWSR1* gene rearrangement occurs in a subset of cutaneous myoepithelial tumors: a study of 18 cases. *Mod Pathol*. 2011; 24:1444–1450. [PubMed: 21725291]

- Gleason BC, Fletcher CD. Myoepithelial carcinoma of soft tissue in children: an aggressive neoplasm analyzed in a series of 29 cases. *Am J Surg Pathol.* 2007; 31:1813–1824. [PubMed: 18043035]
- Gumireddy K, Li A, Gimotty PA, Klein-Szanto AJ, Showe LC, Katsaros D, Coukos G, Zhang L, Huang Q. KLF17 is a negative regulator of epithelial-mesenchymal transition and metastasis in breast cancer. *Nat Cell Biol.* 2009; 11:1297–1304. [PubMed: 19801974]
- Hallor KH, Teixeira MR, Fletcher CD, Bizarro S, Staaf J, Domanski HA, von Steyern FV, Panagopoulos I, Mandahl N, Mertens F. Heterogeneous genetic profiles in soft tissue myoepitheliomas. *Mod Pathol.* 2008; 21:1311–1319. [PubMed: 18604193]
- Hornick JL, Fletcher CD. Myoepithelial tumors of soft tissue: a clinicopathologic and immunohistochemical study of 101 cases with evaluation of prognostic parameters. *Am J Surg Pathol.* 2003; 27:1183–1196. [PubMed: 12960802]
- Ichikawa H, Shimizu K, Hayashi Y, Ohki M. An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. *Cancer Res.* 1994; 54:2865–2868. [PubMed: 8187069]
- Jo VY, Antonescu CR, Zhang L, Dal Cin P, Hornick JL, Fletcher CD. Cutaneous syncytial myoepithelioma: clinicopathologic characterization in a series of 38 cases. *Am J Surg Pathol.* 2013; 37:710–718. [PubMed: 23588365]
- Kas K, Voz ML, Roijer E, Astrom AK, Meyen E, Stenman G, Van de Ven WJ. Promoter swapping between the genes for a novel zinc finger protein and beta-catenin in pleomorphic adenomas with t(3;8)(p21;q12) translocations. *Nat Genet.* 1997; 15:170–174. [PubMed: 9020842]
- Kilpatrick SE, Hitchcock MG, Kraus MD, Calonje E, Fletcher CD. Mixed tumors and myoepitheliomas of soft tissue: a clinicopathologic study of 19 cases with a unifying concept. *Am J Surg Pathol.* 1997; 21:13–22. [PubMed: 8990137]
- Liu FY, Deng YL, Li Y, Zeng D, Zhou ZZ, Tian DA, Liu M. Down-regulated KLF17 expression is associated with tumor invasion and poor prognosis in hepatocellular carcinoma. *Med Oncol.* 2013; 30:425. [PubMed: 23325444]
- Martins C, Fonseca I, Roque L, Pereira T, Ribeiro C, Bullerdiek J, Soares J. PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry. *Mod Pathol.* 2005; 18:1048–1055. [PubMed: 15920557]
- Mertens F, Fletcher CD, Antonescu CR, Coindre JM, Colecchia M, Domanski HA, Downs-Kelly E, Fisher C, Goldblum JR, Guillou L, Reid R, Rosai J, Sciot R, Mandahl N, Panagopoulos I. Clinicopathologic and molecular genetic characterization of low-grade fibromyxoid sarcoma, and cloning of a novel FUS/CREB3L1 fusion gene. *Lab Invest.* 2005; 85:408–415. [PubMed: 15640831]
- Mignone F, Gissi C, Liuni S, Pesole G. Untranslated regions of mRNAs. *Genome Biol.* 2002; 3 REVIEWS0004.
- Morohoshi F, Ootsuka Y, Arai K, Ichikawa H, Mitani S, Munakata N, Ohki M. Genomic structure of the human RBP56/hTAFII68 and FUS/TLS genes. *Gene.* 1998; 221:191–198. [PubMed: 9795213]
- Panagopoulos I, Storlazzi CT, Fletcher CD, Fletcher JA, Nascimento A, Domanski HA, Wejde J, Brosjo O, Rydholm A, Isaksson M, Mandahl N, Mertens F. The chimeric FUS/CREB3L2 gene is specific for low-grade fibromyxoid sarcoma. *Genes Chromosomes Cancer.* 2004; 40:218–228. [PubMed: 15139001]
- Paronetto MP. Ewing sarcoma protein: a key player in human cancer. *Int J Cell Biol.* 2013; 2013:642853. [PubMed: 24082883]
- Peng JJ, Wu B, Xiao XB, Shao YS, Feng Y, Yin MX. Reduced Kruppel-like factor 17 (KLF17) expression correlates with poor survival in patients with gastric cancer. *Arch Med Res.* 2014; 45:394–399. [PubMed: 24947617]
- Persson F, Andren Y, Winnes M, Wedell B, Nordkvist A, Gudnadottir G, Dahlenfors R, Sjogren H, Mark J, Stenman G. High-resolution genomic profiling of adenomas and carcinomas of the salivary glands reveals amplification, rearrangement, and fusion of HMGA2. *Genes Chromosomes Cancer.* 2009; 48:69–82. [PubMed: 18828159]
- Puls F, Arbajian E, Magnusson L, Douis H, Kindblom LG, Mertens F. Myoepithelioma of Bone with a Novel FUS-POU5F1 Fusion Gene. *Histopathology.* 2014

- Rabbitts TH, Forster A, Larson R, Nathan P. Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nat Genet.* 1993; 4:175–180. [PubMed: 7503811]
- Rekhi B, Sable M, Jambekar NA. Histopathological, immunohistochemical and molecular spectrum of myoepithelial tumours of soft tissues. *Virchows Arch.* 2012; 461:687–697. [PubMed: 23097005]
- Stout AP, Gorman JG. Mixed tumors of the skin of the salivary gland type. *Cancer.* 1959; 12:537–543. [PubMed: 13652101]
- Swamynathan SK. Kruppel-like factors: three fingers in control. *Hum Genomics.* 2010; 4:263–270. [PubMed: 20511139]
- van Vliet J, Crofts LA, Quinlan KG, Czolij R, Perkins AC, Crossley M. Human KLF17 is a new member of the Sp/KLF family of transcription factors. *Genomics.* 2006; 87:474–482. [PubMed: 16460907]
- Waters BL, Panagopoulos I, Allen EF. Genetic characterization of angiomatoid fibrous histiocytoma identifies fusion of the FUS and ATF-1 genes induced by a chromosomal translocation involving bands 12q13 and 16p11. *Cancer Genet Cytogenet.* 2000; 121:109–116. [PubMed: 11063792]
- Yan W, Burns KH, Ma L, Matzuk MM. Identification of Zfp393, a germ cell-specific gene encoding a novel zinc finger protein. *Mech Dev.* 2002; 118:233–239. [PubMed: 12351194]
- Ye WC, Gao L, Huang J, Fang XM, Xie G. Suppressed Kruppel-like factor 17 expression induces tumor proliferation, metastasis and a poor prognosis in papillary thyroid carcinoma. *Mol Med Rep.* 2014; 10:2087–2092. [PubMed: 25109837]

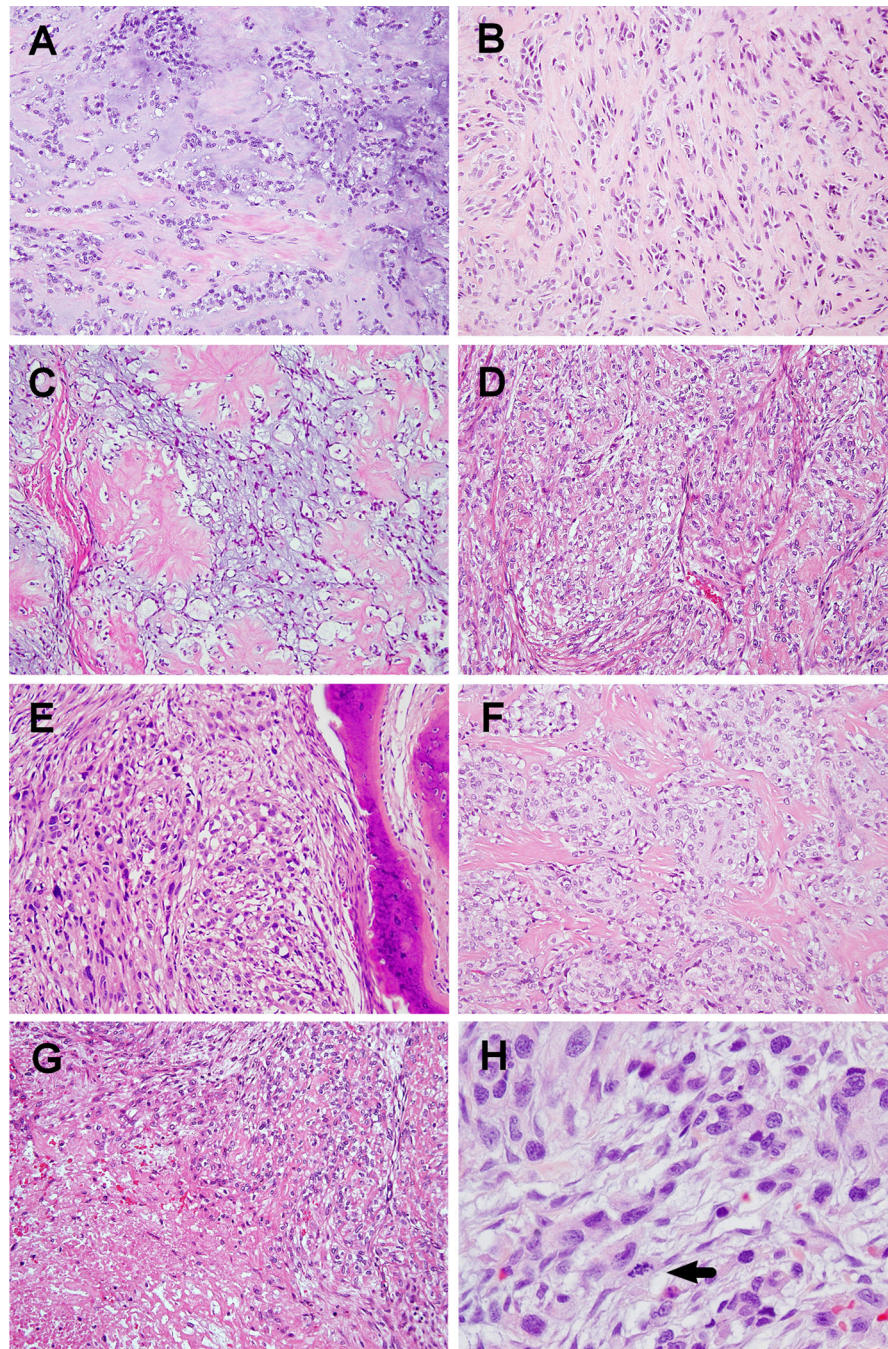


Figure 1. Pathologic findings of *FUS*-rearranged ME tumors

Case #1 (A, 200 \times), #2 (B, 200 \times), and #3 (C, 200 \times) showing cords or clusters of tumor cells set in variably myxohyaline background. Prominent cytoplasmic vacuolation was noted in case #3. Both case #4 (D, 200 \times) and case #5 (E, 200 \times) showed higher cellularity with less stromal component; nuclear atypia was evident in both cases. Nests of tumor cells separated by sclerotic bands in case #6 (F, 200 \times). Tumors exhibiting focal necrosis (G, case #4, 200 \times). Malignant ME tumor with significant nuclear pleomorphism and increased mitotic activity (H, case #5, 400 \times).

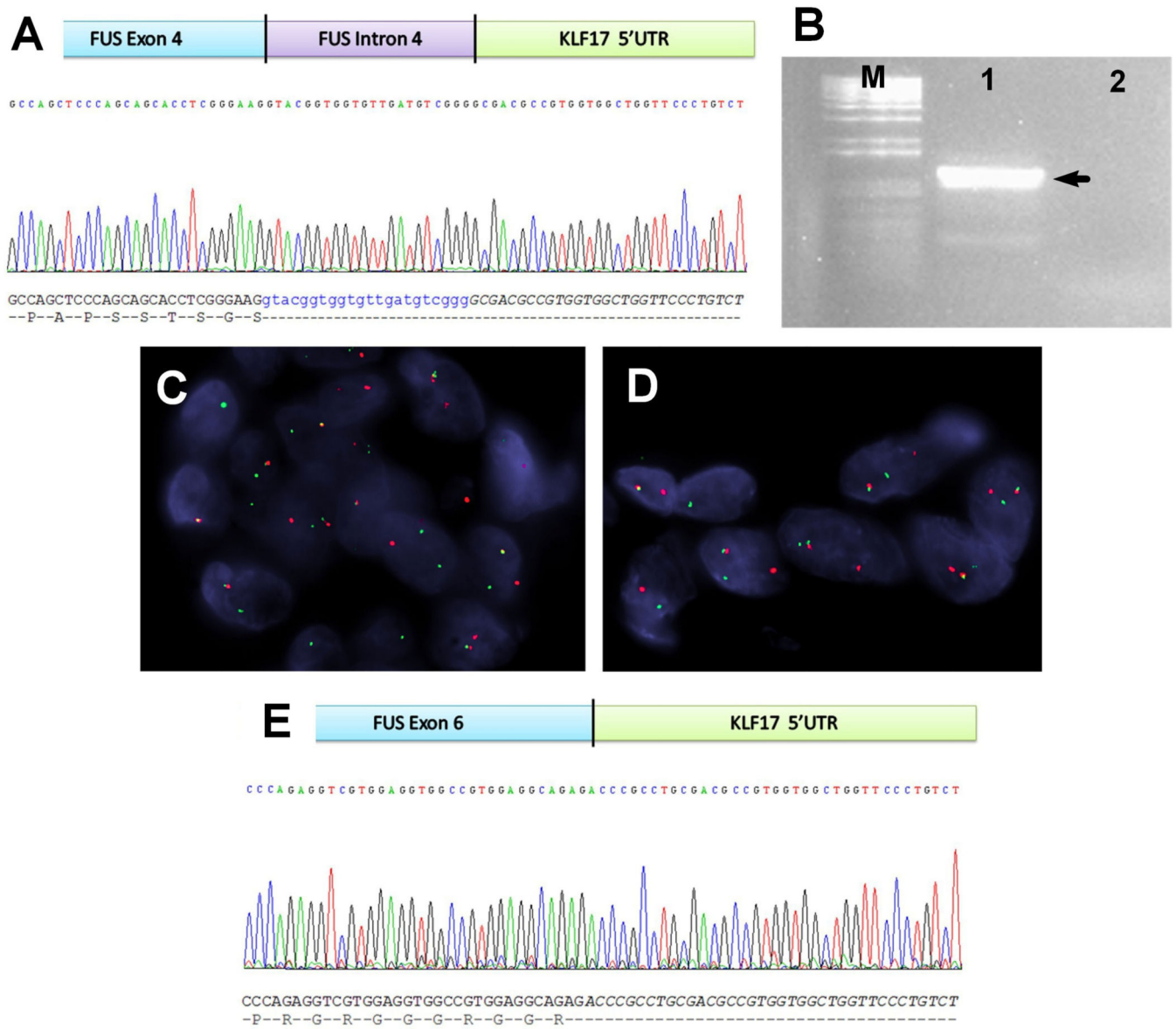


Figure 2. Novel *FUS-KLF17* and *EWSR1-KLF17* gene fusions in ME soft tissue tumors
 The 3'-RACE disclosed a transcript of exon 4 and partial intron 4 of *FUS* fused with 5'-UTR of *KLF17* (A), which was further confirmed by RT-PCR (M: size maker, lane 1: product, lane 2: negative control; the approximate size, 1100 bp) (B). FISH assays revealed split signals of *FUS* (C) and *KLF17* (D) probes in the index case (case #1). The RT-PCR and Sanger sequencing (E) of case #2 identified a variant of fusion pattern involving exon 6 of *FUS* and 5'-UTR of *KLF17*.

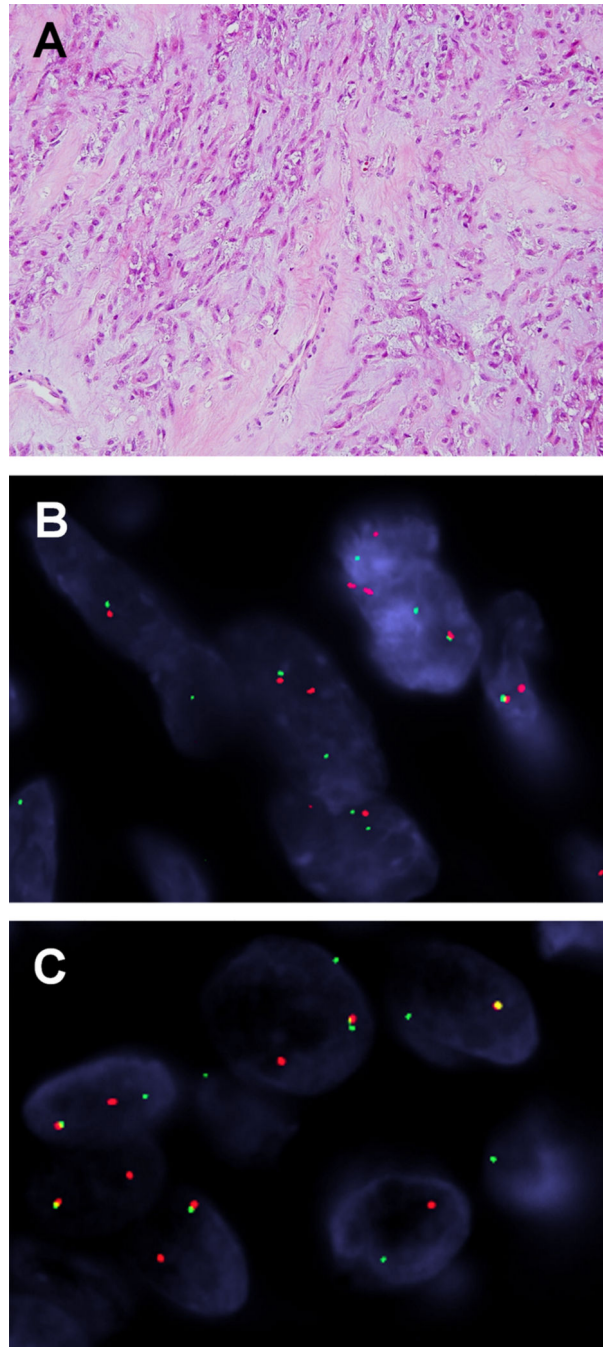


Figure 3. Morphologic appearance and FISH findings of *EWSR1-KLF17* positive ME tumor
Histologically, the spindled or ovoid tumor cells formed cords or clusters in myxohyaline stroma (A). FISH demonstrated break-apart signals involving *EWSR1* (B) and *KLF17* genes (C).

Table 1
Clinical and pathological features of myoepithelial tumors with *FUS* and/or *KLF17* translocations.

| Case | Age (years)/ Sex | Site | Malignant Potential | Morphological Features | Nuclear pleomorphism | Necrosis (%) | Mitosis (/10 HPF) | Genetic alteration | Metastasis |
|------|---------------------|-------------------|------------------------|---------------------------------|-------------------------|-----------------|----------------------|------------------------|------------|
| 1 | 11/F | Foot/soft tissue | Malignant | Trabecular, myxohyaline stroma | none | 0 | 5 | <i>FUS-KLF17</i> | Thigh |
| 2 | 8/M | Tibia/perosteum | Benign | Trabecular, myxohyaline stroma | none | 0 | 0 | <i>FUS-KLF17</i> | None |
| 3 | 37/M | Back/skin | Benign | Trabecular, myxohyaline stroma | none | 0 | 0 | <i>FUS-KLF17</i> | None |
| 4 | 30/M | Lung | Benign | Cellular nesting and trabecular | Mild | 10 | 0 | <i>FUS-KLF17</i> | None |
| 5 | 34/M | Chest/soft tissue | Malignant | Cellular nesting and trabecular | Severe | 40 | 4 | <i>FUS</i> -rearranged | None |
| 6 | 50/F | Neck/soft tissue | Benign | Nesting, sclerosing stroma | none | 10 | 0 | <i>FUS</i> -rearranged | None |
| 7 | 20/M | Foot/soft tissue | Benign | Trabecular, myxohyaline stroma | none | 0 | 0 | <i>EWSR1-KLF17</i> | None |