Short communication

Fusion of the *FUS* and *CREB3L2* genes in a supernumerary ring chromosome in low-grade fibromyxoid sarcoma

Hammurabi Bartuma[a*], Emely Möller[a], Anna Collin[a], Henryk A. Domanski[b], Fredrik Vult Von Steyern[c], Nils Mandahl[a], Fredrik Mertens[a]

[a]Department of Clinical Genetics, University and Regional Laboratories, Lund University Hospital, Lund University, SE-221 85 Lund, Sweden
[b]Department of Cytology and Pathology, University and Regional Laboratories, Lund University Hospital, Lund University, SE-221 85 Lund, Sweden
[c]Department of Orthopedics, Lund University Hospital, SE-221 85 Lund, Sweden

[*]Corresponding author: Tel: +46-46-173398; fax: +46-46-131061: e-mail address: hammurabi.bartuma@med.lu.se (H. Bartuma)

Key words: Low-grade fibromyxoid sarcoma, supernumerary ring chromosome, *FUS/CREB3L2* fusion.

Abstract

Low-grade fibromyxoid sarcoma (LGFMS) is a rare, low-grade malignant soft tissue tumor that is often mistaken for either benign or more malignant tumor types. Commonly, this tumor affects young adults and typically arises in the deep proximal extremities or trunk with frequent recurrences and can metastasize to the lungs many years later. Most cases have a recurrent balanced translocation involving chromosomes 7 and 16, t(7;16)(q32-34;p11), which leads to the fusion of the *FUS* and *CREB3L2* genes. However, supernumerary ring chromosomes have been identified in a subset of *FUS/CREB3L2*-positive LGFMS, but it has not yet been formally demonstrated that such ring chromosomes harbor the *FUS/CREB3L2* fusion gene. Here, we report the genetic findings of a supernumerary ring chromosome from an LGFMS from a 77-year-old man. Chromosome banding analysis revealed a supernumerary ring chromosome, and further studies with fluorescence in situ hybridization and RT-PCR showed that the ring contained material from chromosomes 7 and 16, that the *FUS* gene was present in two normal copies and one rearranged copy, and that it expressed the *FUS/CREB3L2* fusion gene. Moreover, a comparison of previously reported cases suggests that tumors with ring chromosomes have a higher risk of relapse compared to tumors with a balanced t(7;16).

1. Introduction

Low-grade fibromyxoid sarcoma (LGFMS) is a rare, low-grade malignant tumor that typically occurs in the proximal extremities and the trunk of children and young adults. The tumor was first described by Evans, who emphasized its deceptively benign appearance [1]. Metastasis is unusual but may occur many years after the diagnosis of the primary tumor. A recurrent balanced translocation between chromosomes 7 and 16 has been identified and is present in most LGFMS cases, indicating that this translocation is important for the development of LGFMS [2]. The translocation t(7;16)(q32-34;p11) has been shown to lead to the expression of the fusion gene *FUS/CREB3L2* [2-5]. However, in five of 18 reported cases including LGFMS that were *FUS/CREB3L2*-positive, a supernumerary ring chromosome was found, without any sign of a t(7;16). Here, we describe the molecular cytogenetic and molecular genetic findings in an LGFMS with a supernumerary ring chromosome and compare previously published tumors with translocation with tumors with ring chromosomes.

2. Materials and methods

2.1. Case report

The patient, a 77-year-old male, presented with a 5 cm tumor in the upper right lung lobe. No other tumor sites were found. The tumor was surgically removed with radical margins. The tumor morphology was consistent with LGFMS, specifically the subtype known as hyalinizing spindle cell tumor with giant rosettes. The tumor was strikingly hyper cellular with a mitotic count of 3 per 10 high power fields. No atypical mitoses were seen, and there was no necrosis. Immunohistochemistry showed positive staining for bcl2 and patchy positivity for the epithelial membrane-associated antigen (EMA). The tumor cells were negative for CD31, CD34, S-100, cytokeratin CKAE1/3, desmin and smooth muscle actin.

2.2. Cytogenetic and FISH analyses

Culturing and harvesting of the tumor cells and chromosome banding of metaphase spreads was performed as described [6]. Karyotypes were described according to ISCN (2009). Metaphase FISH to determine the chromosomal origin of the ring chromosomes was done using whole chromosome painting probes (wcp 7 and wcp 16; Applied Spectral Imaging, Migdal Haemek, Israel) and a dual-color break-apart probe for *FUS* (Abbott Molecular, Des Plaines, USA). Slides were prepared and analyzed as previously described [7].

2.3. Molecular analyses

Total RNA from fresh frozen tumor biopsy kept at -80 ºC was extracted using the RNeasy Lipid Tissue Mini kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). Two and a half microgram of total RNA were reverse transcribed in a volume of 50 µl for cDNA synthesis, as described previously [8]. RT-PCR and sequencing were performed to detect and study the *FUS*/*CREB3L2* transcript. The PCR reaction was performed as described [8]. The PCR products were analyzed on a 2% agarose gel. Forward primer TLS-165F and reverse primer BBF2H7-1435R [4] were used as well as forward primer FUS-797F (5'CTA TGA ACC CAG AGG TCG TG) and reverse primer CREB3L2-1201R (5'GAT CAG GGT CCT CTT CTC CT). All transcripts identified were verified by sequencing, and the corresponding nucleotide sequences were analyzed using the Chromas software (http://www.technelysium.com.au/chromas.html). The origin of the sequences was verified by BLAST search (http://www.ncbi.nlm.nih.gov/blast). As reference, the sequences with accession numbers NM_004960 (*FUS*) and NM_194071 (*CREB3L2*) were used.

3. Results

3.1. Chromosome banding and FISH

G-banding revealed the karyotype 48,XY,+r,+mar[22]/48,XY,+2r[2] (Fig. 1A). FISH showed that the supernumerary ring chromosomes mainly consisted of material from chromosome 7, and a smaller piece of chromosome 16 (Fig. 1B). Metaphase FISH with the *FUS*-specific break-apart probe showed normal signals on the two chromosomes 16 and two normal signals and one rearranged signal on the supernumerary ring chromosome (Fig. 1C).

3.2. RT-PCR and sequencing

A 1076 bp fragment and a 215 bp fragment were found with RT-PCR using primer combinations TLS-165F with BBF2H7-1435R and FUS-797F with CREB3L2-1201R, respectively. Sequencing showed that the 1076 bp fragment was an out-of-frame fusion transcript while the 215 bp was an in frame fusion transcript. The breakpoint occurred in *FUS* exon 7, position 852 (NM_004960), in both transcripts. The breakpoint in *CREB3L2* occurred in exon 5, position 1047, in the out-of-frame transcript (data not shown) and in exon 5, position 1042 (NM_194071), in the in frame transcript (Fig. 1 D). A point mutation, exchanging a cytosine for an adenine was found at position 848 in *FUS* exon 7 in both transcripts, giving rise to a silent mutation in both transcripts.

4. Discussion

Excluding the present case, only 18 cases of LGFMS with abnormal karyotypes have been reported previously, 12 of which had the t(7;16), or variants thereof [2, 4, 5, 9-12]. In the six other tumors, no rearrangement of either chromosome 7 or chromosome 16 was found. Five of these, however, had a supernumerary ring chromosome [4, 10]. Mezzelani [10] showed by metaphase CGH that one case with such a ring chromosome displayed gain of material from 7p14-pter, 7q31-33 and 16p, and concluded that the gained segments most likely corresponded to the supernumerary ring chromosome. Further indirect support for the hypothesis that ring chromosomes in LGFMS are composed of material from chromosomes 7 and 16, and that they contain the *FUS/CREB3L2* fusion, came from the finding of fusion gene transcripts in cases with ring chromosomes as the sole aberration [4]. Here, by combining chromosome banding analysis, FISH and RT-PCR, we could conclusively show that the *FUS/CREB3L2* fusion gene can be present in ring chromosomes in LGFMS.

We found two different fusion transcripts in the present LGFMS; one in-frame fusion transcript joining parts of the *FUS* exon 7 and the *CREB3L2* exon 5, and one out-of-frame transcript that is predicted to result in the expression of a truncated *FUS* protein. Different fusion transcripts have been reported before in LGFMS, and it was suggested that this might be due to clonal evolution [2, 13]. The FISH findings in our case, i.e. that several copies of *FUS* were present in the ring chromosome, suggest that the origin of different transcripts might also be due to two or more different fusion events, or, reorganization of the ring through break-fusion-bridge.

Gene fusions, in leukemias and lymphomas as well as in solid tumors, typically arise through balanced translocations. In some bone and soft tissue tumors, however, the fusion is associated with concomitant imbalances, especially amplifications. For example, dermatofibrosarcoma protuberans is characterized by a *COL1A1/PDGFB* fusion gene. Occassionally, especially in children and young adults, the fusion is due to a reciprocal t(17;22), but more often, the fusion gene is found in supernumerary ring chromosomes containing material from chromosomes 17 and 22 [14]. Another example is alveolar rhabdomyosarcoma (ARMS) with the *PAX7/FOXO1* fusion. This fusion is sometimes caused by a reciprocal t(1;13), but it is also found to be amplified in the form of double minutes. This

is in contrast to the other characteristic gene fusion in ARMS, *PAX3/FOXO1*, which almost exclusively is the result of a t(2;13). This suggests that the transforming capacity of *PAX7/FOXO1* is copy number-dependent and thus weaker, than that of *PAX3/FOXO1* [14-17].

Whether the *FUS/CREB3L2* fusion gene, resulting from the t(7;16), is sufficient for the development of LGFMS is not known, but the fact that there are more cases with a balanced t(7;16) than with supernumerary ring chromosomes strongly indicates that amplification of the fusion gene and/or other parts of chromosomes 7 and 16 is not necessary for tumor formation. This does not exclude the possibility that ring formation might have an additional effect on the tumor phenotype. When comparing all reported LGFMS with t(7;16) with those with ring chromosomes, we could not find any significant differences with regards to gender, age, location, size or depth. However, tumor relapse was more common among cases with ring chromosomes than among cases with t(7;16): out of five cases with ring chromosomes, two metastasized and one recurred locally, whereas none of the 12 tumors with t(7;16) relapsed ($p<0.05$; Fisher's exact test). For two reasons, we did not include the present case in this comparison. First, the follow-up time was very short and, second, the location of the tumor, i.e., in the lungs, raises the question of whether the tumor was a metastasis and not a primary tumor. Careful clinical examination has, however, not revealed any additional tumor site, indicating that it may be a rare case of primary pulmonary LGFMS. Nevertheless, the preliminary data reported here, based on small patient numbers with short follow-up periods, indicate that it might be of clinical interest to characterize LGFMS not only with regard to type of fusion gene, but also with regard to the absence/presence of ring chromosomes or other accompanying genomic imbalances.

Acknowledgments

The authors wish to thank Prof Christopher DM Fletcher for reviewing the pathological slides and Margareth Isaksson for technical assistance. This study was supported by grants from the Swedish Cancer Society and the Swedish Research Council.

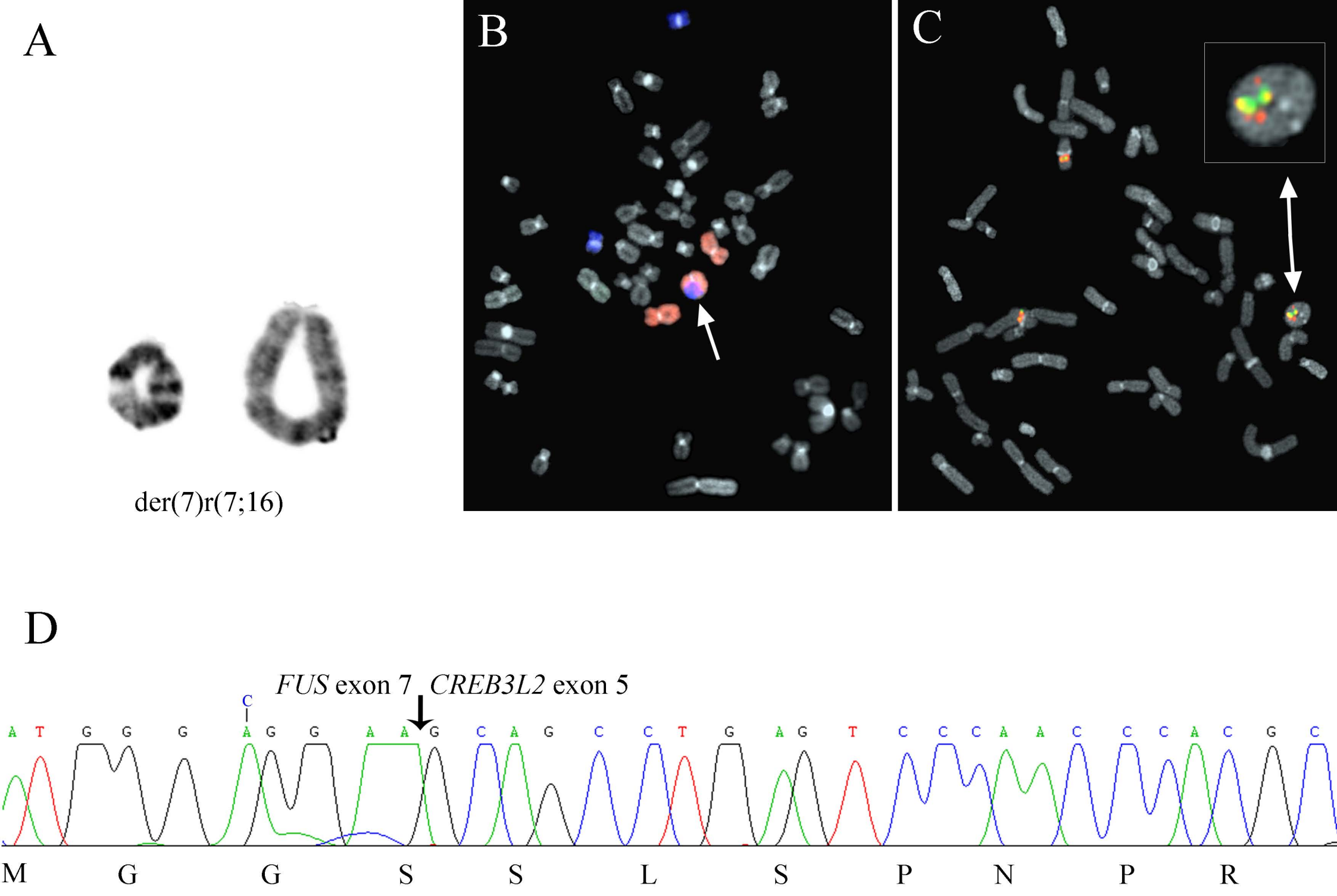
References

1. Evans HL. Low-grade fibromyxoid sarcoma. A report of two metastasizing neoplasms having a deceptively benign appearance. Am J Clin Pathol 1987;88:615-9.
2. Mertens F, Fletcher CDM, 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;85:408-15.
3. Panagopoulos I, Möller E, Dahlén A, Isaksson M, Mandahl N, Vlamis-Gardikas A, Mertens F. Characterization of the native CREB3L2 transcription factor and the FUS/CREB3L2 chimera. Genes Chromosomes Cancer 2007;46:181-91.
4. Panagopoulos I, Storlazzi CT, Fletcher CDM, Fletcher JA, Nascimento A, Domanski HA, Wejde J, Brosjö 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;40:218-28.
5. Storlazzi CT, Mertens F, Nascimento A, Isaksson M, Wejde J, Brosjö O, Mandahl N, Panagopoulos I. Fusion of the FUS and BBF2H7 genes in low grade fibromyxoid sarcoma. Hum Mol Genet 2003;12:2349-58.
6. Mandahl N, Heim S, Arheden K, Rydholm A, Willén H, Mitelman F. Three major cytogenetic subgroups can be identified among chromosomally abnormal solitary lipomas. Hum Genet 1988;79:203-8.
7. Dahlén A, Mertens F, Rydholm A, Brosjö O, Wejde J, Mandahl N, Panagopoulos I. Fusion, disruption, and expression of HMGA2 in bone and soft tissue chondromas. Mod Pathol 2003;16:1132-40.
8. Bartuma H, Hallor KH, Panagopoulos I, Collin A, Rydholm A, Gustafson P, Bauer HC, Brosjö O, Domanski HA, Mandahl N, Mertens F. Assessment of the clinical and molecular impact of different cytogenetic subgroups in a series of 272 lipomas with abnormal karyotype. Genes Chromosomes Cancer 2007;46:594-606.
9. Bejarano PA, Padhya TA, Smith R, Blough R, Devitt JJ, Gluckman JL. Hyalinizing spindle cell tumor with giant rosettes-a soft tissue tumor with

mesenchymal and neuroendocrine features. An immunohistochemical, ultrastructural, and cytogenetic analysis. Arch Pathol Lab Med 2000;124:1179-84.

10. Mezzelani A, Sozzi G, Nessling M, Riva C, Della Torre G, Testi MA, Azzarelli A, Pierotti MA, Lichter P, Pilotti S. Low grade fibromyxoid sarcoma: a further low-grade soft tissue malignancy characterized by a ring chromosome. Cancer Genet Cytogenet 2000;122:144-8.
11. Reid R, de Silva MV, Paterson L, Ryan E, Fisher C. Low-grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes share a common t(7;16)(q34;p11) translocation. Am J Surg Pathol 2003;27:1229-36.
12. Sawyer JR, Binz RL, Gilliland JC, Nicholas RW, Thomas JR. A novel reciprocal (10;17)(p11.2;q23) in myxoid fibrosarcoma. Cancer Genet Cytogenet 2001;124:144-6.
13. Guillou L, Benhattar J, Gengler C, Gallagher G, Ranchere-Vince D, Collin F, Terrier P, Terrier-Lacombe MJ, Leroux A, Marques B, de Saint Aubain Somerhausen N, Keslair F, Pedeutour F, Coindre JM. Translocation-positive low-grade fibromyxoid sarcoma: clinicopathologic and molecular analysis of a series expanding the morphologic spectrum and suggesting potential relationship to sclerosing epithelioid fibrosarcoma: a study from the French Sarcoma Group. Am J Surg Pathol 2007;31:1387-402.
14. Sirvent N, Maire G, Pedeutour F. Genetics of dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment. Genes Chromosomes Cancer 2003;37:1-19.
15. Barr FG, Nauta LE, Davis RJ, Schafer BW, Nycum LM, Biegel JA. In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. Hum Mol Genet 1996;5:15-21.
16. Möller E, Isaksson M, Mandahl N, Mertens F, Panagopoulos I. Comparison of the proximal promoter regions of the PAX3 and PAX7 genes. Cancer Genet Cytogenet 2007;178:114-9.
17. Xia SJ, Pressey JG, Barr FG. Molecular pathogenesis of rhabdomyosarcoma. Cancer Biol Ther 2002;1:97-104.

Fig 1 A) Partial karyotype visualizing the supernumerary ring chromosomes. **B)** Metaphase FISH, with wcp 7 (red) and wcp 16 (blue) showing that the supernumerary ring chromosomes consisted of material from chromosomes 7 and 16 (arrow). **C)** Metaphase FISH with dual-color break-apart probe for *FUS*. Normal signals are seen on the two chromosomes 16. On the supernumerary ring chromosome two normal signals and one rearranged signal for *FUS* were seen. Note the single red signal indicating a rearrangement of *FUS*. In the upper right corner an enlargement of the ring chromosome is made to better visualize the rearrangement (arrow). **D)** Partial chromatogram showing the junction (arrow) of the *FUS/CREB3L2* in frame fusion transcript. C indicates the substitution mutation, replacing a cytosine (C) for an adenine (A) in position 848 in *FUS* exon 7. The predicted amino acid sequence is indicated below.


A
B
C
der(7)r(7;16)
D
FUS exon 7
CREB3L2 exon 5
C
A T G G G A G G A A G C A G C C T G A G T C C C A A C C C A C G C
M G G S S L S P N P R