



Fusion of the *RBP56* and *CHN* genes in extraskelatal myxoid chondrosarcomas with translocation t(9;17)(q22;q11)

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Although most extraskelatal myxoid chondrosarcomas (EMC) are cytogenetically characterized by the translocation t(9;22)(q22;q12), another subset has recently been identified carrying a t(9;17)(q22;q11). Whereas the t(9;22) is known to result in fusion of the *CHN* (*TEC*) gene from 9q22 with the *EWS* gene from 22q12, creating a chimeric *EWS/CHN*, the genes involved in the t(9;17) of EMC are unknown. We examined two EMC with t(9;17)(q22;q11) and found that the *CHN* gene was recombined with the *RBP56* gene from 17q11 to generate a chimeric *RBP56/CHN*. *RBP56* has not previously been shown to be involved in tumorigenesis but it encodes a putative RNA-binding protein similar to the *EWS* and *FUS* (*TLS*) proteins known to play a pathogenetic role in several sarcomas. The presence of the *RBP56/CHN* chimeric gene in EMC with t(9;17)(q22;q11) shows that the N-terminal parts of *EWS* and *RBP56* have similar oncogenic potential making them pathogenetically equivalent in oncoproteins arising from fusions with certain transcription factors.

Keywords: chondrosarcoma; gene fusion; translocation genetics; karyotyping

Introduction

Extraskelatal myxoid chondrosarcoma (EMC) is a malignant soft tissue tumour found mostly in the extremities, but also occasionally in the tongue, chin, epiglottis, brachial plexus, chest wall, pleura, abdomen, buttock, inguinal region, testis and synovia (Liu-Shindo *et al.*, 1989; Steffen *et al.*, 1992; Elizalde *et al.*, 1993). The disease is more common in middle-aged and elderly men but also occurs in women and children (Klijanienko *et al.*, 1990). Long-term follow-up studies have shown that metastases can develop, especially in the lungs.

A translocation t(9;22)(q22;q12) has been found in several EMC but never in other tumours and, hence, seems to be pathognomonic for this disease (Mitelman, 1998). Recently, however, another translocation not previously described, t(9;17)(q22;q11) (Figure 1), was reported in two EMC, thus identifying a second cytogenetic subgroup of this tumour type (Bjerkehagen *et al.*, 1999). The 9;22-translocation fuses the *EWS*

gene from chromosome 22 with the *CHN* gene (also named *TEC*, *NOR-1* or *MINOR*), which encodes an orphan nuclear receptor, from 9q22 (Labelle *et al.*, 1995; Clark *et al.*, 1996). The genes involved in t(9;17)(q22;q11) are unknown, but obviously *CHN* would be expected to be one of them.

The three genes *EWS*, *FUS* (or *TLS*) and *RBP56* (also called *hTAFii68* or *TAF2N*) are related inasmuch as they all encode similar RNA-binding proteins containing an RNP box in the central part and a degenerated repeat with the consensus sequence Ser-Tyr-Gly-Gln-Gln-Ser in the N-terminal region (Delattre *et al.*, 1992; Crozat *et al.*, 1993; Rabbitts *et al.*, 1993; Bertolotti *et al.*, 1996; Morohoshi *et al.*, 1996). Whereas *FUS* (in 16p11) and *EWS* (in 22q12) are known to be arranged in several types of neoplasia, the tumorigenic involvement of *RBP56* (in 17q11-12) has hitherto not been demonstrated.

FUS is rearranged in the t(12;16)(q13;p11) that characterizes myxoid liposarcoma (MLS) and in a subset of acute myeloid leukaemia (AML) with t(16;21)(p11;q22) (Crozat *et al.*, 1993; Rabbitts *et al.*, 1993; Ichikawa *et al.*, 1994; Panagopoulos *et al.*, 1994). In MLS, where the translocation results in the hybrid gene *FUS/CHOP*, the central and C-terminal parts of *FUS* are replaced by the full-length *CHOP* protein, a member of the bZIP family containing a leucine zipper structural motif (Crozat *et al.*, 1993; Rabbitts *et al.*, 1993). In AML with t(16;21), the N-terminal part of *FUS* is fused to the *ETS* DNA-binding domain and C-terminal part of *ERG*, a member of the *ETS* gene family, resulting in the formation of two hybrid genes, *FUS/ERG* and *ERG/FUS* (Ichikawa *et al.*, 1994; Panagopoulos *et al.*, 1994).

EWS is rearranged in Ewing sarcoma (ES) and related primitive neuroectodermal tumours (PNET) by means of a t(11;22)(q24;q12) or one of four variant translocations, t(21;22)(q22;q12), t(7;22)(p22;q12), t(17;22)(q12;q12) and t(2;22)(q33;q12) (Delattre *et al.*, 1992; Zucman *et al.*, 1993b; Sorensen *et al.*, 1994; Jeon *et al.*, 1995; Kaneko *et al.*, 1996; Urano *et al.*, 1996; Peter *et al.*, 1997). In all five translocations, the same N-terminal part of *EWS* is fused to the *ETS* DNA-binding domains of either *FLI1* from 11q24, *ERG* from 21q22, *ETV1* from 7p22, *ETV4/E1AF* from 17q12 or *FEV* from 2q33, all of which are members of the *ETS* gene family (Delattre *et al.*, 1992; Zucman *et al.*, 1993b; Giovannini *et al.*, 1994; Sorensen *et al.*, 1994; Jeon *et al.*, 1995; Kaneko *et al.*, 1996; Urano *et al.*, 1996; Peter *et al.*, 1997). In addition, the N-terminal part

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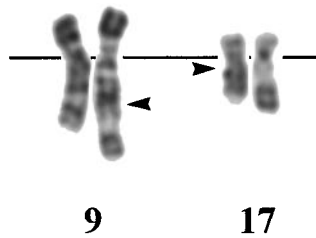


Figure 1 Partial karyotype of homologue pairs 9 and 17 from the EMC of case 2 showing the t(9;17)(q22;q11). The rearranged chromosome 9 is to the right and the rearranged chromosome 17 to the left. Arrowheads indicate breakpoints

of *EWS* is fused with the DNA-binding domain of *ATF1*, a member of the bZIP family, in clear cell sarcomas of tendons and aponeuroses with t(12;22)(q13;q12), with the DNA-binding domain of *WT1* in the intra-abdominal desmoplastic small round cell tumour with t(11;22)(p13;q12), and with the *CHOP* gene in myxoid liposarcomas with translocations between 12q13 and 22q12 (Zucman *et al.*, 1993a; Ladanyi and Gerald, 1994; Panagopoulos *et al.*, 1996).

In the examples given above, the N-terminal part of *FUS* or *EWS* was fused to the DNA-binding domain of the genes *CHOP*, *ATF1*, *FLI1*, *ERG*, *ETV1*, *ETV4*, *E1AF*, *FEV* or *WT1* in tumorigenic, specific translocations affecting the respective gene loci. Since *RBP56* maps to the proximal region of 17q which harbours one of the breakpoints of the t(9;17), we decided to find out if this gene was fused with *CHN* in EMC carrying a t(9;17)(q22;q11).

Results

PCR with a TAF308F and CHN823R primer combination amplified a 422 bp fragment from the cDNA from both tumours. Nested PCR with a TAF348F and CHN789R primer combination amplified a 382 bp fragment and PCR with a TAF444F and CHN789R primer combination amplified a 253 bp cDNA fragment (Figure 2). To verify the presence of an *RBP56/CHN* chimeric transcript, the 382 bp and 253 bp PCR amplified fragments were analysed by direct sequencing. This showed that, in both tumours, exon 6 of the *RBP56* gene was fused in frame to position -2 of the *CHN* cDNA immediately before the initiating methionine (Figure 3).

Discussion

The present study demonstrates the formation of an *RBP56/CHN* fusion gene in two EMC carrying the translocation t(9;17)(q22;q11) (Figure 1). It appears, therefore, that the translocations t(9;22)(q22;q12), giving rise to an *EWS/CHN* fusion gene, and t(9;17)(q22;q11), giving rise to an *RBP56/CHN* fusion gene, are pathogenetically equivalent in EMC and that both are pathognomonic for this tumour type. Cytogenetically (Mitelman, 1998; Bjerkehagen *et al.*, 1999) as well as at the molecular level, the former rearrangement seems to be the more

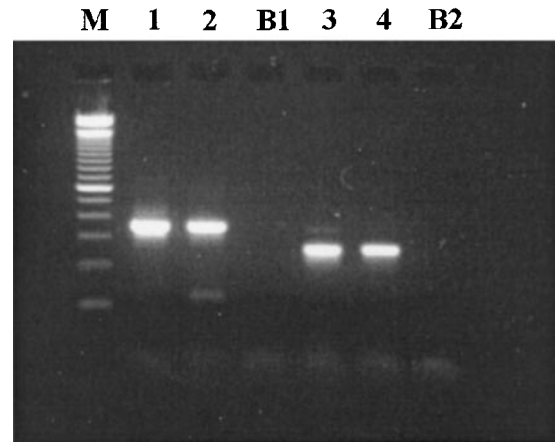


Figure 2 RT-PCR analysis of the two EMC with t(9;17)(q22;q11). Total RNA was reversely transcribed and cDNA was used as template in a first PCR amplification using TAF308F and TEC823R primer combination. The first PCR products were diluted 1:100 and 2 μ l of this dilution were subsequently nested PCR amplified. In both samples, PCR with TAF348F and CHN789R primer combination amplified a 382 bp fragment (lanes 1 and 2) and PCR with TAF444F and CHN789R primer combination amplified a 253 bp cDNA fragment (lanes 3 and 4). B1, blank for the TAF348F and TEC789R primer combination. B2, blank for the TAF444F and TEC789R primer combination. M, 100 bp DNA ladder

common: Labelle *et al.* (1995) detected the *EWS/CHN* gene in five out of seven EMC, Brody *et al.* (1997) found it in six of eight EMC, and Antonescu *et al.* (1998) described it in seven of the nine EMC cases examined by them. It is tempting to speculate that some, if not all, of the EMC cases that were negative for the *EWS/CHN* fusion gene in these series had the alternative *RBP56/CHN* chimeric gene.

Two main *EWS/CHN* transcripts have been reported for the standard t(9;22)(q22;q12) in EMC. In type 1, *EWS* exon 12 is fused to position -2 of the *CHN* cDNA. In type 2, exon 7 of the *EWS* gene is fused to position -176 of the *CHN* cDNA resulting in a novel open reading frame with the addition of 59 amino acids between the fusion point and the initiating methionine of *CHN* (Labelle *et al.*, 1995; Clark *et al.*, 1996). In the *RBP56/CHN* transcripts detected in the two cases of EMC with t(9;17), exon 6 of *RBP56*, which corresponds to exon 7 of *EWS*, is fused to position -2 of the *CHN* cDNA. Thus, the putative *RBP56/CHN* chimeric protein contains the N-terminal part of *RBP56*, a region rich in Ser, Gly, Gln and Tyr, and the full-length normal *CHN* protein. In analogy with what is presumed to be the case for the *EWS/CHN* fusion protein, *RBP56/CHN* is likely to be a potent transcriptional activator wielding its tumorigenic influence by action on as yet unknown target genes involved in cell proliferation. It seems, therefore, that the N-terminal parts of the *RBP56* and *EWS* proteins are functionally equivalent when fused with the transcription factor *CHN*, leading in both instances to the same malignant phenotype (EMC). This implies a situation parallel to that in myxoid liposarcomas, in which the N-terminal parts of the *FUS* and *EWS* proteins are functionally equivalent

when fused to the transcription factor CHOP (Zinszner *et al.*, 1994; Panagopoulos *et al.*, 1996). The observed role of RBP56, EWS and FUS in the oncogenic activation of transcription factors CHOP

(FUS or EWS in MLS) and CHN (RBP56 or EWS in EMC) (Figure 4) raises the question of whether they may also be interchangeable in other tumour types.

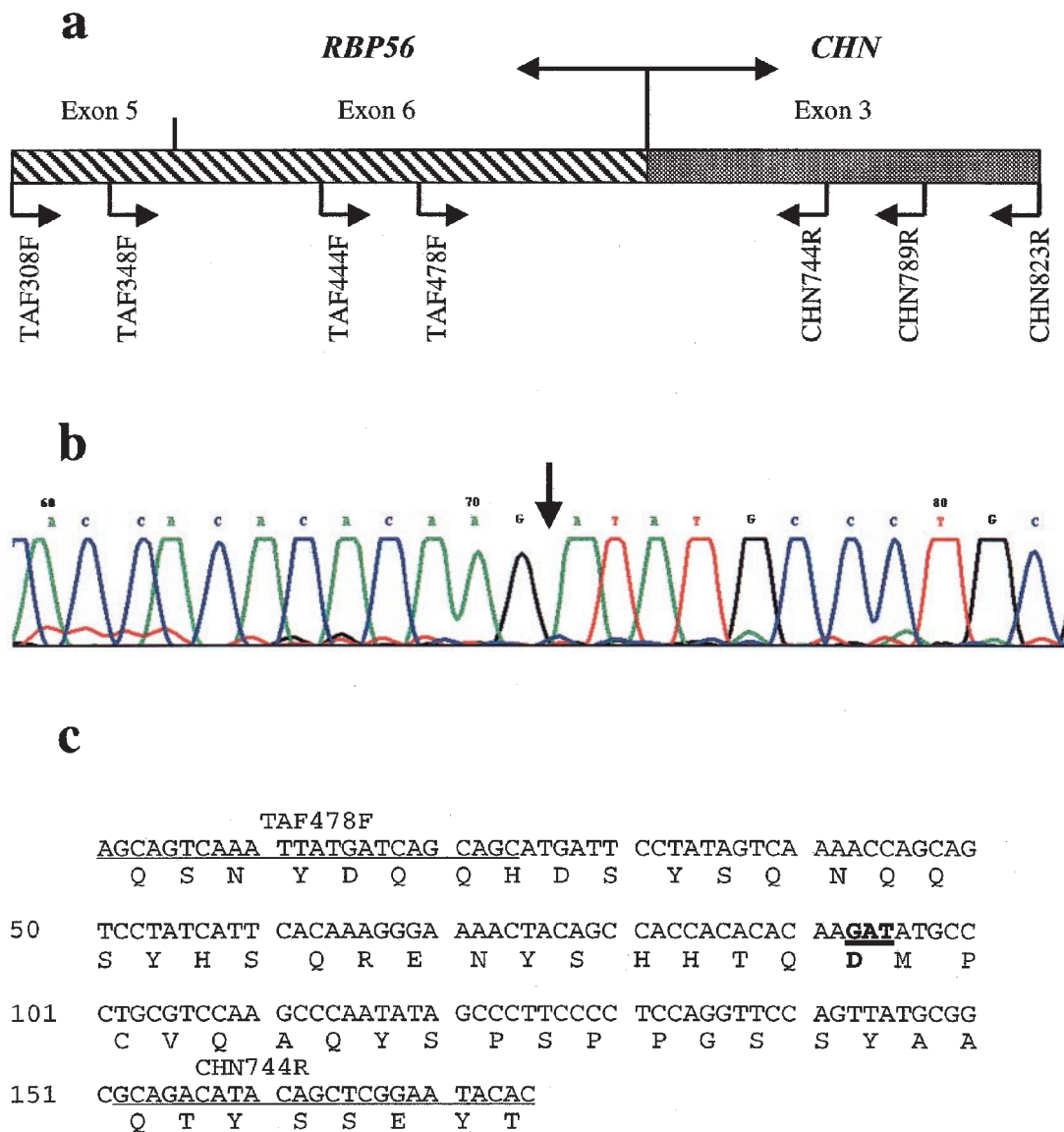


Figure 3 (a) Schematic representation of the chimeric cDNA fragment of *RBP56/CHN* detected using RT-PCR in both EMC with t(9;17)(q22;q11). Numbers refer to exons of the *RBP56* and *CHN* genes. The position and direction of the primers are indicated. (b) Partial sequence chromatogram showing the junction (arrow) of the *RBP56* and *CHN* genes. (c) Nucleotide sequence of the amplified *RBP56/CHN* chimeric cDNA fragment determined after direct sequencing of the fragment using the TAF478F and CHN444R primers. Double underlining shows the junction of the *RBP56* and *CHN* genes. In both cases, the exon 6 of the *RBP56/hTAFi68* gene is fused, in frame, to position -2 of the *CHN* cDNA immediately before the initiating methionine. The primers TAF478F and CHN744R used for sequence are underlined

Table 1 Primers used for PCR and sequencing

Designation	Sequence	Direction	Position*	Gene
TAF308F	5'-GCAGAGCTCATATAGCCAGCAACC-3'	Forward	308-331	<i>RBP56</i>
TAF328F	5'-CAGCAGCAAACATGGAATCATC-3'	Forward	348-370	<i>RBP56</i>
TAF444F	5'-CAGGCTATGATCAACATCAAGGC-3'	Forward	445-467	<i>RBP56</i>
TAF478F	5'-AGCAGTCAAATTATGATCAGCAGC-3'	Forward	478-501	<i>RBP56</i>
CHN823R	5'-TGGTGGCTGTAGCCGTGATCTC-3'	Reverse	823-844	<i>CHN</i>
CHN789R	5'-AGGTCCATGGTCAGCTTGGGTAG-3'	Reverse	789-812	<i>CHN</i>
CHN744R	5'-GTGTATTCCGAGCTGTATGTCTGC-3'	Reverse	744-767	<i>CHN</i>

*Position of *RBP56* (*hTAFi68*) primers is based on *RBP56* (*hTAFi68*) sequence with accession number U51334. Position of *CHN* (*TEC*) primers is based on *CHN* (*TEC*) sequence with accession number X89894

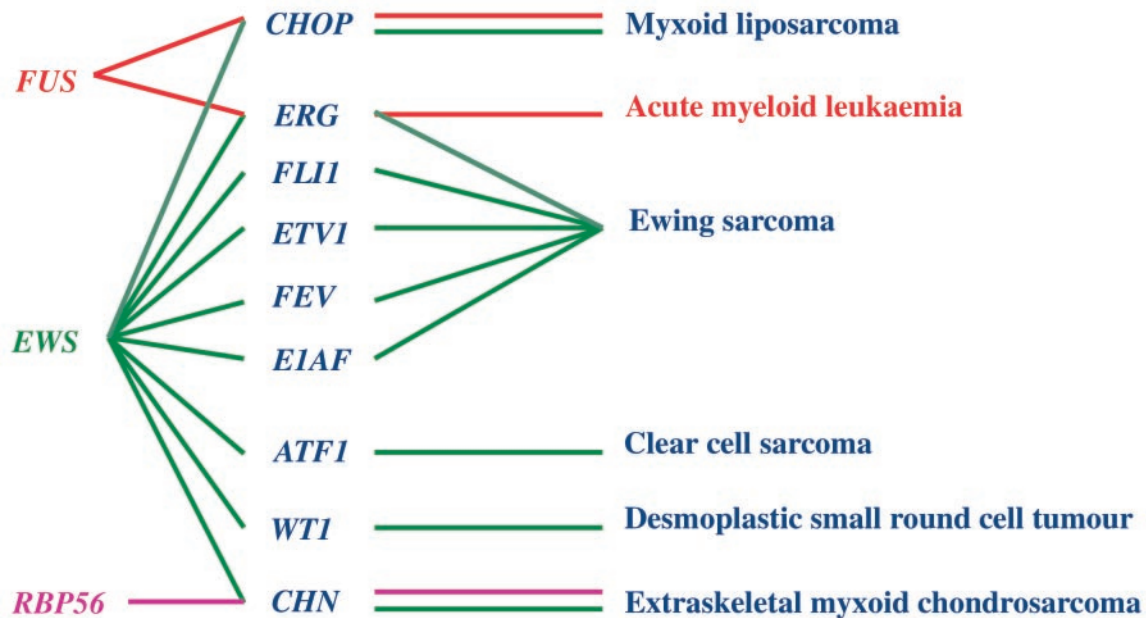


Figure 4 Diagram showing the currently known involvement of *EWS*, *FUS* and *RBP56* with different transcription factor genes in various types of neoplasia

Materials and methods

Cases

The clinicopathological description and cytogenetic findings of the two cases have previously been described in detail (Bjerkehagen *et al.*, 1999). Cases 1 and 2 in our report correspond to cases 3 and 4 in the series examined by Bjerkehagen *et al.* (1999). Both tumours were phenotypically EMC and both had a t(9;17)(q22;q11) (Figure 1) as their only chromosomal abnormality.

RT-PCR and sequencing analysis

The primers used for PCR amplification and sequence analysis are presented in Table 1. *RBP56* primers were based on the full length *RBP56* cDNA sequence (Bertolotti *et al.*, 1996; Morohoshi *et al.*, 1996) and *CHN* primers on the reported *CHN* cDNA sequence (Hedvat and Irving, 1995; Labelle *et al.*, 1995; Clark *et al.*, 1996; Ohkura *et al.*, 1996).

RT-PCR was carried out for the detection of *RBP56/CHN* chimeric transcripts using total RNA as the starting material. Total RNA was extracted using the Trizol reagent according to the manufacturer's instructions (Gibco-BRL). Four and 2 µg from cases 1 and 2, respectively, were reversely transcribed in 20 µl reaction volume containing 50 mM Tris-HCl pH 8.3 (at 25°C), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 1 mM of each dNTP, 37 units RNA guard (Pharmacia AB), 10 pmol random hexamers, 1 µg Oligo (dT)₁₇ and 200 units M-MLV Reverse Transcriptase (Gibco-BRL). The reaction was carried out at 37°C for 60 min, then heated for 5 min at 99°C and kept at 5°C.

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In the first PCR amplification, the 50 µl reaction volume contained 20 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 1 unit PlatinumTaq polymerase (Gibco-BRL), 0.5 µM of each of the outer primers (Table 2), and 2 µl of the cDNA. The first PCR products were diluted 1:100 and 2 µl of this dilution were amplified in a second 50 µl PCR with the same composition as in the first PCR, except that 1 mM MgCl₂ and the inner primers were used (Table 2). 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 MJ Research PCT-200 DNA Engine, followed by a final extension for 10 min at 74°C.

Ten µl of the PCR products were analysed by electrophoresis through 1.5% agarose gels, stained with ethidium bromide, and photographed.

For sequence analysis, the RT-PCR amplified *RBP56/CHN* cDNA fragments were run on 1.5% agarose gels, purified using Qiagen gel extraction kit and directly sequenced using the dideoxy procedure with Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) on the Applied Biosystems Model 373A DNA sequencing system.

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