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Advances in Brief

Fusion of the *EWS*-related Gene *TAF2N* to *TEC* in Extraskeletal Myxoid Chondrosarcoma[1]

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Abstract

Extraskeletal myxoid chondrosarcomas (EMCs) are characterized by a recurrent t(9;22)(q22;q12) translocation, resulting in the fusion of the *EWS* gene in 22q12 and the *TEC* gene in 9q22. Here we report that a third member of the *EWS*, *TLS/FUS* gene family, *TAF2N*, can replace *EWS* as a fusion partner to *TEC* in EMC. Two tumors, one with a novel t(9;17)(q22;q11) variant translocation and one with an apparently normal karyotype, expressed *TAF2N-TEC* fusion transcripts. In both cases, the chimeric transcripts were shown to contain exon 6 of *TAF2N* fused to the entire coding region of *TEC*. This transcript is structurally and functionally very similar to the *EWS-TEC* fusions. The exchange of the EWS NH_2-terminal part with the TAF2N NH_2-terminal part in EMC further underscores the oncogenic potential of these protein domains as partners in fusion genes.

Introduction

EMC[3] is a distinctive soft tissue sarcoma closely resembling embryonic cartilage (1). Recent studies have shown that it has a unique but more aggressive clinical course than originally described, characterized by a high rate of local recurrences, metastases, and tumor-related deaths, despite prolonged survival (2). Cytogenetically, EMCs have been found to have a unique t(9;22)(q22;q12) translocation detectable in approximately 75% of studied cases (3, 4). The translocation results in a fusion of the *EWS* gene at 22q12 to the *TEC/CHN* gene at 9q22 (5, 6). The deduced chimeric protein consists of the NH_2-terminal transactivation domain of EWS linked to the entire TEC protein. *EWS*, which was originally identified as the gene rearranged in Ewing's sarcoma with t(11;22)(q24;q12), encodes a putative RNA-binding protein (7), and *TEC* encodes a novel orphan nuclear receptor belonging to the steroid/thyroid receptor gene superfamily (5, 6). Two major types of *EWS-TEC* fusion transcripts have been identified in EMC. In the type 1 fusion, *EWS* exon 12 is fused to position −2 of the *TEC* cDNA, and in the type 2 fusion, *EWS* exon 7 is linked to position −176 of the *TEC* cDNA (5).

The gene *TAF2N* (also named *TAF68/RBP56*), which shows extensive similarities to the *EWS* and *TLS/FUS* genes, was recently identified (8, 9). The two latter genes were originally cloned as the 5′-parts of translocation-generated fusion genes in Ewing's sarcomas and myxoid liposarcomas (7, 10, 11). *EWS* was subsequently found to be involved in different fusion genes in several tumor types, including clear cell sarcoma of soft parts, desmoplastic small round cell tumors, and EMC (12). *TAF2N* encodes a ubiquitously expressed protein that contains a putative RNA-binding RNP-1 motif and a possible DNA-binding zinc finger motif (8, 9, 10, 13). The gene has provisionally been localized to chromosome 17q11.2–q12 (9).

Here we report that *TAF2N* can replace *EWS* as a fusion partner to *TEC* in EMC. Two tumors, one with a novel t(9;17)(q22;q11) variant translocation and one with an apparently normal karyotype, expressed *TAF2N-TEC* fusion transcripts. Nucleotide sequence analysis revealed a fusion of *TAF2N* exon 6 to the entire coding region of *TEC* in both tumors. These findings demonstrate that *TAF2N-TEC* gene fusions are recurrent in EMC and that they occur not only in tumors with the t(9;17) variant translocation but also in tumors with an apparently normal karyotype.

Materials and Methods

Tumor Material and Cytogenetic Analysis. Fresh tumor tissue was obtained from two patients with EMC (one locally recurrent tumor and two metastatic tumors). One of the patients (case CG801) was a 72-year-old man who had a 10-cm locally recurrent EMC of the right thigh 3 years after removal of the primary tumor. The other patient (case CG923I and II) was a 47-year-old man who had two retroperitoneal metastases, 4 and 10 cm in size, removed 3 months after excision of the primary tumor in the right thigh. All primary, locally recurrent, and metastatic tumors from these two patients had typical light microscopic, immunohistochemical, and ultrastructural features of EMC (Fig. 1). Primary cultures were established from fresh, unfixed tumor tissues as described previously (4). Chromosome preparations were made from exponentially growing primary cultures, and these were subsequently G-banded and analyzed according to the 1995 guidelines of the International System for Human Cytogenetic Nomenclature.

Isolation of PAC Clones and FISH Analysis. Three PAC clones specific for *TAF2N* (9B20, 183K14, and 187P15) were obtained by screening a human genomic PAC library (Genome Systems, St. Louis, MO) with a 535-bp cDNA probe generated by PCR using the primers TAF477U24-GAGCAGTCAAATTATGATCAGCAGC and TAF.1012L23-TGGAATTCTTTTCCATCAAACCA. PAC DNA was prepared using the QIAGEN Plasmid Kit (Qiagen) and subsequently labeled with biotin by nick translation and cohybridized with digoxigenin-labeled α-satellite probes specific for chromosomes 9 and 17 (D9Z1 and D17Z1; Oncor) to normal metaphase spreads and metaphases from EMC CG801. Whole chromosome painting probes for chromosomes 9 and 17 (WCP 9 and 17; Vysis) were also hybridized to metaphases from tumor CG801. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole. Fluorescence signals were digitalized, processed, and analyzed using the PowerGene FISH image analysis system (Perceptive Scientific International, Chester, Great Britain).

RNA Isolation, RT-PCR, and Nucleotide Sequence Analyses. Total RNA was extracted from frozen tumor tissue using the Trizol (Life Technologies, Inc.) method. For cDNA synthesis, 5 μg of total RNA were reverse-transcribed using the SuperScript Preamplification System according to the manufacturer's manual (Life Technologies, Inc.). An aliquot of 0.25 μg of the resulting first-strand cDNA was amplified using the appropriate primer sets. Thirty-five cycles of PCR (30 s at 96°C, 30 s at 55°C, and 30 s at 72°C) were performed with 0.5 μl of cDNA in 50-μl reaction volumes. The AmpliTaq Gold (Perkin Elmer Applied Biosystems) DNA-polymerase was used for the amplification reactions. The following *TAF2N*- and *TEC*-derived primers were used: (*a*) TAF.314U23-TCATATAGCCAGCAACCATATAA (exon 5); (*b*) TAF.444U24-CAGGCTATGATCAACAT-

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[3] The abbreviations used are: EMC, extraskeletal myxoid chondrosarcoma; RT-PCR, reverse transcription-PCR; FISH, fluorescence *in situ* hybridization.

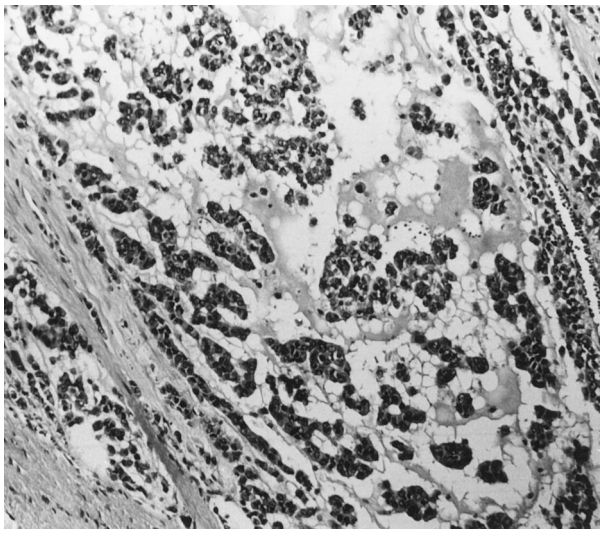

Fig. 1. A metastatic EMC (case CG923) with the characteristic growth pattern of tumor cells arranged in nests and cords in an abundant myxoid matrix.

CAAGGCT (exon 6); (*c*) TAF.477U24-GAGCAGTCAAATTATGATCAG-CAGC (exon 6); (*d*) TAF.575U24-CGTCGTGATGTGAGTAGGTATGGA (exon 7); (*e*) TEC RevA-CCTGGAGGGGAAGGGCTAT; and (*f*) TEC RevC-GGTGGCTGTAGCCGTGATCT. As control for intact RNA and cDNA, an RT-PCR reaction for expression of the housekeeping gene *GAPDH* was performed on all cDNAs used. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and subsequently sequenced with an ABI PRISM 377 DNA Sequencer (Perkin Elmer Applied Biosystems) using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems). The resulting sequences were analyzed using GeneJockey (Biosoft) and basic local alignment search tool (BLAST) searches (National Center for Biotechnology Information).

Results and Discussion

Previous cytogenetic and molecular genetic studies have shown that approximately 75% of EMCs have a t(9;22) or a *EWS-TEC* fusion detectable by RT-PCR (5, 6, 14). To identify novel translocations and fusion genes characterizing the remaining 25% of the cases, we have cytogenetically analyzed six new cases of EMC, including a tumor with a novel t(9;17) variant translocation. The karyotype of this tumor was 47,XY, +8, t(9;17)(q22;q11) (Fig. 2*A*). FISH analysis using painting probes confirmed that this was a reciprocal rearrangement involving only chromosomes 9 and 17 (Fig. 2*B*). The previously reported similarity between the *TAF2N* and *EWS* genes and the fact that *TAF2N* has been provisionally localized to 17q11.2–q12 (8, 9) prompted us to search for possible involvement of *TAF2N* and *TEC* in the t(9;17) translocation.

To confirm the chromosomal localization of *TAF2N*, we isolated three genomic PAC clones and mapped these by FISH to normal metaphase chromosomes. All three clones were localized to 17q11 (Fig. 2*C*), *i.e.*, to the same band as the breakpoint on 17q in the t(9;17). We then hybridized PAC clone 9B20, which gave the most intense signal at the 17q11 locus, to metaphase chromosomes from the EMCs with the t(9;17). However, there was no visible signal on the der(9) chromosome in any of the analyzable metaphases, suggesting that the PAC clone might not span the 17q11 breakpoint or that the breakpoint maps close to one end of this PAC.

To search for a possible *TAF2N-TEC* fusion transcript in EMC CG801, we performed RT-PCR experiments with primers located in exons 5, 6, and 7 of *TAF2N* and in the 5′ region of *TEC*. PCR with the *TAF2N* primer 314U23 (located in exon 5) and the *TEC* primer RevA gave rise to an amplification product of 300 bp, indicating the presence of a chimeric *TAF2N*-TEC transcript (Fig. 3*A*). Similarly, amplifications with the *TAF2N* primers 444U24 and 477U24 (located in exon 6) and the *TEC* primer RevA resulted in products of 170 and 137 bp (Fig. 3*A*), respectively. In contrast, PCR analysis using a *TAF2N* primer specific for the first nucleotides of exon 7 (575U24) and the *TEC* primer RevA failed to produce an amplification product. Amplification using the three *TAF2N* exon 5 and 6 primers together with *TEC* RevC located 113 bp downstream of the *TEC* RevA primer produced products that were 113 bp larger than those obtained with *TEC* RevA (data not shown). These observations are consistent with the presence of a chimeric transcript in which exon 6 of *TAF2N* is fused to a position located 2 nucleotides upstream of the *TEC* ATG

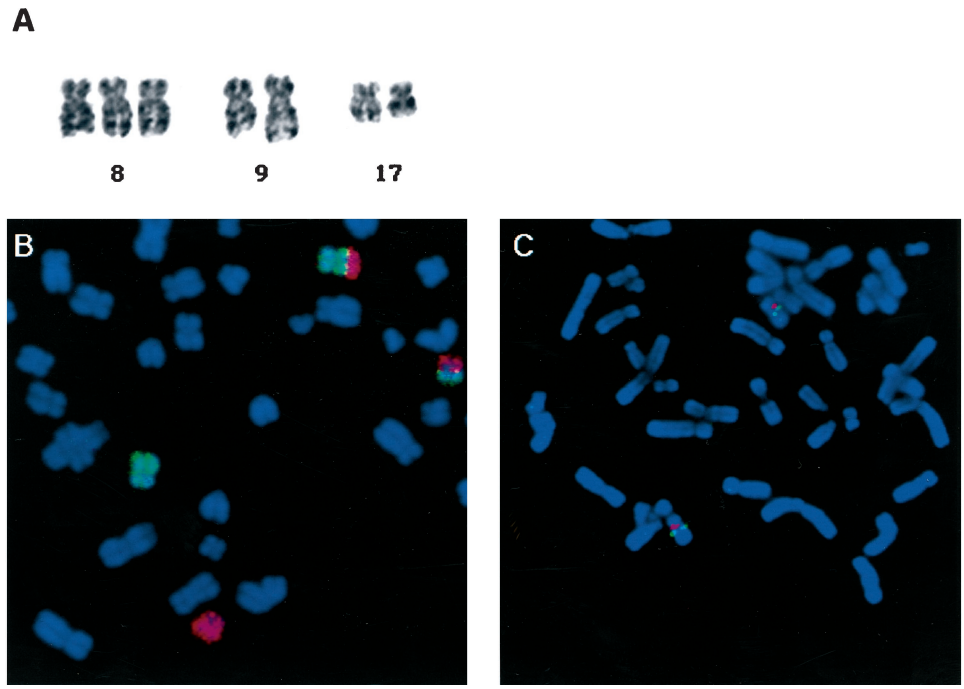

Fig. 2. *A*, partial G-banded karyotype of EMC CG801 showing trisomy 8 and the t(9;17)(q22;q11) variant translocation. *B*, FISH analysis demonstrating the t(9;17) translocation using painting probes for chromosomes 9 (*green signal*) and 17 (*red signal*). *C*, chromosomal localization of the *TAF2N* gene. PAC clone 9B20 containing the *TAF2N* gene (*green signals*) was cohybridized with α-satellite probes specific for chromosome 17 (*red signals*). *TAF2N*-specific hybridization signals were found on both chromosomes 17 at a location corresponding to band 17q11.

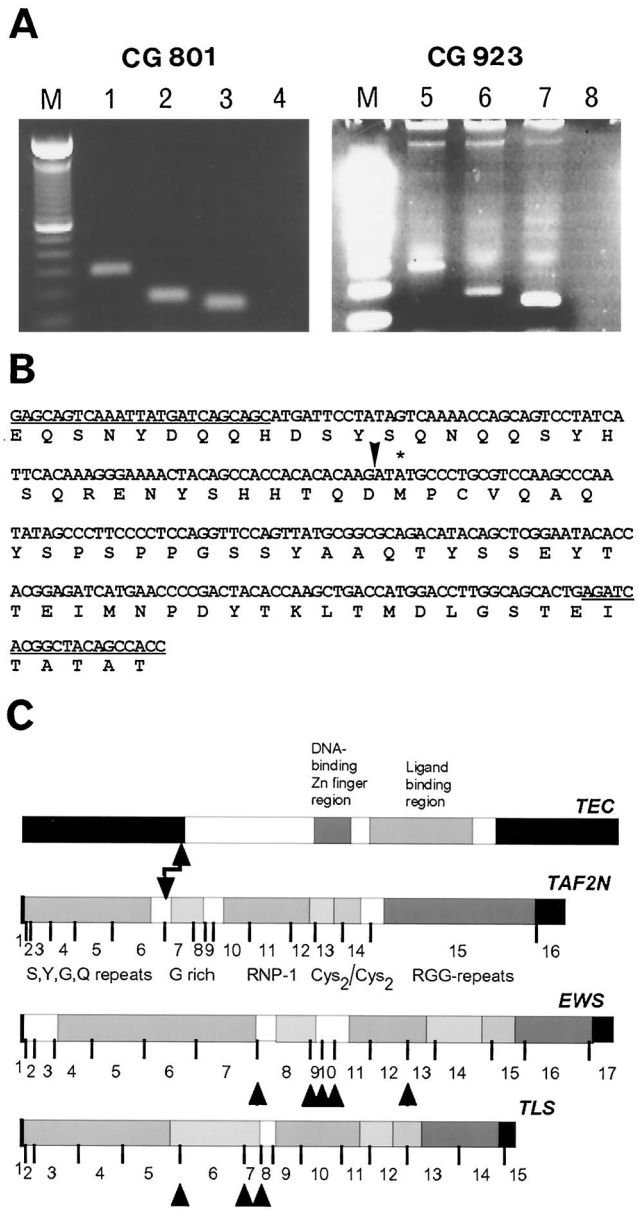

Fig. 3. *A*, detection of chimeric *TAF2N-TEC* transcripts by RT-PCR in EMC CG801 and CG923I. Primer sets specific for exon 5 (*Lanes 1* and *5*) and exon 6 (*Lanes 2*, *3*, *6*, and *7*) of *TAF2N* and the 5′ noncoding region of *TEC* give rise to products of 300, 170, and 137 bp, respectively, consistent with a fusion of exon 6 of *TAF2N* to *TEC* sequences 2 nucleotides upstream of the ATG initiation codon. *Lanes 4* and *8* are blanks (PCRs with primers TAF477U24 and TEC Rev A but without cDNA). A 100-bp ladder was used as a DNA molecular weight marker (*M*). (Amersham Pharmacia Biotech). *B*, nucleotide and deduced amino acid sequences of parts of the *TAF2N-TEC* cDNA fragment amplified using the TAF.477U24 and TEC RevC primer set. The ATG initiation codon in *TEC* is indicated by an *asterisk*, and the fusion point is indicated by a *vertical arrow*. Primer sequences are *underlined*. *C*, schematic representation of the TEC, TAF2N, EWS, and TLS/FUS transcripts. The location of the exons in the three latter are shown. Breakpoints are indicated by *arrows* and *arrowheads* (modified from Ref. 13); untranslated regions are shown in *black*.

start codon. The latter breakpoint corresponds to one of the two known fusion points in *TEC* (5). Analysis of control RNA from an EMC (CG431) with a t(9;22)(q22;q12) and a known fusion of *EWS* exon 12 to the 5′ noncoding region of TEC (5) failed to show a *TAF2N-TEC* fusion transcript. The identity of the putative hybrid transcripts was also confirmed by nucleotide sequence analysis. A 252-bp fragment was sequenced using the TAF.477U24 and TEC RevC primers and was shown to consist of *TAF2N* exon 6 fused to TEC sequences 2 nucleotides upstream of the ATG initiation codon (Fig. 3, *B* and *C*), GenBank accession number AJ245932. The sequence data reveal that the *TAF2N* and *TEC* sequences are linked so that their normal reading frames are maintained. In analogy with the EWS-TEC fusion protein, the putative TAF2N-TEC protein is expected to consist of the first NH_2-terminal 159 amino acids of TAF2N, followed by the entire TEC protein.

The NH_2-terminal regions of EWS and TLS were previously found to be parts of several fusion proteins in a variety of sarcomas (Figs. 3C and 4) (12), and the extensive similarity between their protein sequences and the TAF2N NH_2-terminal suggests an analogous role for this protein. EWS and TLS fusion partners are invariably DNA-binding proteins of the transcription factor type. TEC belongs to the family of steroid receptors and contains a DNA-binding region but differs from the other fusion partners by its putative ligand binding domain. The NH_2 terminal part of TLS was shown to bind a conserved region found in many steroid receptors (15). Binding of the highly homologous NH_2-terminals of EWS and TAF2N to the TEC protein or to other steroid receptors is therefore plausible but remains to be experimentally verified. The NH_2-terminal parts of EWS and TLS and the homologous *Drosophila* Cabeza protein are functionally exchangeable in myxoid liposarcomas and in certain experimental transformation assays (16–18). Recently, it was also shown that the EWS-TEC fusion protein acts as a strong transcriptional activator (19). The exchange of the EWS NH_2-terminal part with the TAF2N NH_2-terminal part in EMC further underscores the oncogenic potential of these proteins domains as partners in fusion.

To determine whether the *TAF2N-TEC* gene fusion is recurrent in EMCs, RT-PCR experiments were performed using RNA from five new EMCs (the cytogenetic findings of these experiments will be published elsewhere). Amplifications with primers specific for *TAF2N* exon 6 and the 5′ noncoding part of *TEC* revealed fusion transcripts in two separate metastases of EMC from one patient (case CG923I and II). In both tumors, products of the same size as those found in EMC CG801 were detected (Fig. 3*A*), consistent with a fusion of exon 6 of *TAF2N* to 5′ noncoding sequences of *TEC* in these tumors also. Preliminary cytogenetic analysis of one of these tumors has revealed an apparently normal karyotype, suggesting that there may also be cryptic *TAF2N-TEC* fusions in addition to those caused by the t(9;17)(q22;q11) variant translocations. Cryptic rearrangements resulting in gene fusions have also been recently found in several other types of neoplasms, including those with apparently normal karyotypes (20).

The TAF2N-TEC fusion protein is most likely encoded by the der(17) chromosome. This presumption is supported by the fact that

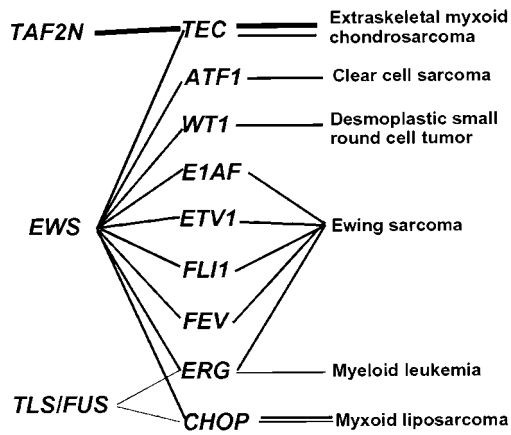

Fig. 4. Involvement of *TAF2N*, *EWS*, and *TLS/FUS* and different transcription factor genes in neoplasia.

the transcriptional orientation of the closely related *EWS* gene is directed toward the telomere (7) and that the entire coding region of *TEC* is fused to exons 7 or 12 of *EWS* in the t(9;22) (5, 6). It is therefore very likely that *TAF2N* is also transcribed from centromere to telomere.

Our finding of a novel *TAF2N-TEC* gene fusion in EMC could account for those 25% of cases that lack t(9;22) and *EWS-TEC* fusions (5, 6, 14). However, whether all of these cases have *TAF2N-TEC* fusions or whether additional variant fusions exist remains to be determined. The identification of a second characteristic gene fusion in EMC increases the possibility of establishing this diagnosis using molecular techniques. The correct diagnosis is particularly important in view of the distinctive biological behavior of EMC. Its wide morphological spectrum, coupled with relatively nondiscriminatory immunohistochemical and ultrastructural features, makes the diagnosis extremely difficult at times. The differential diagnosis of EMC may include benign myxoid lesions as well as high-grade sarcomas, depending on a specific lesion's cellularity and other morphological features. Therefore, an objective technique that confirms or establishes the diagnosis of EMC is extremely important.

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