Studies on the Molecular Pathogenesis of Extraskelletal Myxoid Chondrosarcoma—Cytogenetic, Molecular Genetic, and cDNA Microarray Analyses

Helene Sjögren,* Jeanne M. Meis-Kindblom,*
Charlotte Örndal,* Peter Bergh,[†]
Konrad Ptaszynski,* Pierre Åman,*
Lars-Gunnar Kindblom,* and Göran Stenman*

From the Department of Pathology,* Lundberg Laboratory for Cancer Research, and the Department of Orthopedic Surgery,[†] Sahlgrenska University Hospital, Göteborg University, Göteborg, Sweden

Extraskelletal myxoid chondrosarcomas (EMCs) are characterized by recurrent chromosome translocations resulting in fusions of the nuclear receptor TEC to various NH₂-terminal partners. Here we describe the phenotypic, cytogenetic, and molecular genetic characteristics of a series of 10 EMCs. Using spectral karyotyping and fluorescence *in situ* hybridization, clonal chromosome abnormalities were detected in all but one tumor. A t(9;22)(q22;q12) translocation was found in three cases; a del(22)(q12-13) in one case; and variant translocations, including t(9;17)(q22;q11-12), t(7;9;17)(q32;q22;q11), and t(9;15)(q22;q21), were detected in one case each. Recurrent, secondary abnormalities, including trisomy 1q, 7, 8, 12, and 19, were found in seven tumors. All tumors contained translocation-generated or cryptic gene fusions, including *EWS-TEC* (five cases, of which one was a novel fusion), *TAF2N-TEC* (four cases), and *TCF12-TEC* (one case). cDNA microarray analysis of the gene expression patterns of two EMCs and a myxoid liposarcoma reference tumor revealed a remarkably distinct and uniform expression profile in both EMCs despite the fact that they had different histologies and expressed different fusion transcripts. The most differentially expressed gene in both tumors was *CHI3L1*, which encodes a secreted glycoprotein (YKL-40) previously implicated in various pathological conditions of extracellular matrix degradation as well as in cancer. Our findings suggests that EMC exhibits a tumor-specific gene expression profile, including overexpression of several cancer-related genes as well as genes implicated in chondrogenesis and neural-neuroendocrine differentiation, thus distinguishing it from other soft tissue sarcomas. (*Am J Pathol* 2003, 162:781–792)

Extraskelletal myxoid chondrosarcoma (EMC) is a rare and unique soft tissue malignancy that is clinically characterized by involvement of the proximal extremities and limb girdles of adults and has a frequently prolonged course despite a high incidence of local recurrences and metastases.^{1,2} Light microscopically, ultrastructurally, and histochemically, EMC shows some resemblance to embryonal cartilage.^{1–3} The morphological spectrum of EMC is wide, thus the differential diagnosis based on histology alone is at times problematic.² The origin of EMC remains unclear and recent reports of neural-neuroendocrine differentiation in a subset of EMC has posed further questions regarding its histogenesis.^{4,5}

Recent cytogenetic and molecular genetic studies of EMC have revealed three pathogenetically relevant chromosome translocations. The t(9;22)(q22;q12) translocation, found in ~75% of the cases, results in a fusion of the 5' part of the *EWS* gene to the *TEC* gene.^{6–9} The deduced chimeric protein consists of the NH₂-terminal transactivation domain of EWS linked to the entire TEC protein. *EWS* encodes a putative RNA-binding protein and *TEC* (also called *NR4A3*, *NOR1*, *MINOR*, and *CHN*) encodes a novel orphan nuclear receptor belonging to the steroid/thyroid receptor gene super family.^{8–11} Similarly, the recurrent, less frequent (15% of cases), t(9;17)(q22;q11) results in a fusion of the NH₂-terminal part of the EWS-related gene *TAF2N* to *TEC*.^{12–14} We recently identified yet a third variant translocation t(9;15)(q22;q21) that generates a fusion of the NH₂-terminal part of the basic helix-loop-helix transcription factor TCF12 to *TEC*.¹⁵

The NH₂-terminal parts of EWS and the related protein, TLS (FUS), are also regular fusion partners of different transcription factors in several other sarcomas, including Ewing's sarcoma, clear cell sarcoma of tendons and aponeuroses, desmoplastic small round cell tumor, and myxoid liposarcoma.¹⁶ Presumably the TET family of fusion proteins (TLS, EWS, and TAF2N) exert their onco-

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Address reprint requests to Prof. Göran Stenman, Lundberg Laboratory for Cancer Research, Department of Pathology, Göteborg University, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden. E-mail: goran.stenman@lcr.med.gu.se.

Table 1. Clinical-Pathological Data on Nine EMC Cases

Case	Age/sex	Site	Size	Histology	EM	Chr/Syn/MIB1	Follow-up
1	42/M	IM, R lower leg	7 cm	Classical, hypocellular	MT	–/–/ <1%	LR 13 years*; AWD 15 years
2	57/M	IM, L gluteus maximus*	11 cm	Classical	MT; NSG	–/–/ <1%	Pulmonary mets at primary diagnosis; AWD 5 years
3	52/M	IM, L thigh	10 cm	Classical	MT; NSG	+ / + / 5%	LR 2 years*; A & W 6 years
4	71/M	Pelvic*	14 cm	Classical; cellular; pleomorphic [†]	MT; NSG	–/–/ 10%	NED 1 year
5	57/F	IM, R upper arm*	4 cm	Cellular-solid	MT	–/–/ <5%	NED 6 months
6	46/M	IM, R thigh	12 cm	Classical, rhabdoid	MT; NSG	+ / + / 25%	Pulmonary and 2 retroperitoneal mets* 1 year; AWD 4 years
7	55/F	IM, R thigh*	6 cm	Classical	MT	–/–/ <1%	LR & pulmonary mets 8 years; TRD 10 years
8	71/M	IM, R thigh*	22 cm	Cellular-solid	NSG	–/–/ 10%	LR & bone mets 1 year; TRD 2 years
9	69/M	IM, L thigh	20 cm	Classical	MT; NSG	–/–/ <1%	Pulmonary mets 6 months; LR 3 years*; AWD 6 years

Abbreviations: A & W, alive and well; AWD, alive with disease; Chr, chromogranin; Cl, classical; IM, intramuscular; L, left; LR, local recurrence; M, male; mets, metastases; MT, microtubular aggregates; NED, no evidence of disease; NSG, neurosecretory granules; R, right; Syn, synaptophysin; TRD, tumor-related death.

*Cytogenetically analyzed tumors.

[†]Three areas studied; area A, classical EMC morphology; areas B and C, cellular and pleomorphic areas with necrosis and increased proliferative activity.

genic effect by functioning as abnormal transcription factors.¹⁷ At least some fusion proteins, eg, EWS-FLI1 and EWS-TEC, may also function as dominant-negative inhibitors of splicing that affect splice site selection.^{18,19} Recently, it was shown that the AF2 (activation function-2) domain of TEC is essential for the transcriptional activity of the EWS-TEC fusion protein and that EWS-TEC, at least in part, interacts with the same transcriptional co-activators as the normal TEC protein.²⁰

To gain further insights into the molecular pathogenesis of EMC we have extended and supplemented our previous investigations^{7,8,12,15} with cytogenetic, fluorescence *in situ* hybridization (FISH), spectral karyotype (SKY), and molecular analyses of five new tumors as well as with FISH and SKY analyses of previously reported tumors. In addition, we have used cDNA microarray to study the gene expression pattern in EMCs with differing fusion genes and morphologies.

Materials and Methods

Tumor Material

Ten EMCs from nine patients were analyzed (Table 1). The tumors from five of the patients (cases 1 to 5) have not been previously reported. The remaining five tumors from four patients (cases 6-I and 6-II as well as cases 7 to 9) have been previously reported regarding the expression of EMC-specific fusion transcripts, and all but two of these (cases 5 and 6-I) have also been cytogenetically analyzed.^{7,12,15} For SKY analysis, frozen, cultured tumor cells from three of the previously reported tumors were thawed, explanted, and harvested after 10 to 14 days *in vitro*.^{7,12} In addition, total RNA and/or cDNA were available for analyses in six EMCs. As a reference tumor for the cDNA microarray analysis, fresh frozen tumor tissue from a myxoid liposarcoma was used. Archival material from four additional myxoid liposarcomas was used for

validation of the microarray results. All five myxoid liposarcomas expressed *TLS-CHOP* fusion transcripts.²¹

Light and Electron Microscopy and Immunohistochemistry

The 10 tumors were histologically reviewed and classified as either classical or cellular-solid variants of EMC as previously described.^{2,15} Tumors from all nine patients were obtained fresh, fixed in glutaraldehyde, and routinely processed for electron microscopy. Using standardized antigen retrieval techniques, all cases were analyzed immunohistochemically with regard to cytokeratins (AE1/AE3, KL1, and CAM 5.2), S100 protein, neuron-specific enolase, chromogranin A, synaptophysin, glial fibrillary acidic protein (GFAP), and Ki-67 (MIB1), as previously described.² For validation of the cDNA microarray results the following antibodies and dilutions were used: RELB (1:25), COL3A1 (1:10), XRCC3 (1:50) (all from Santa Cruz Biotechnology, Santa Cruz, CA), and GSTM-4 (1:500; Novocastra, Newcastle, UK). Antigen retrieval techniques, using citrate buffer (pH 6.0) or Tris-ethylenediaminetetraacetic acid (pH 9.0) and microwave heating (17 minutes) were applied. For detection, the Techmate (ChemMate; DAKO, Glostrup, Denmark) detection kit systems were used.

Cytogenetic and FISH Analyses

Primary cultures were established from fresh, unfixed specimens of four of the new EMC cases as previously described.¹⁵ Chromosome preparations were made from exponentially growing primary cultures or early passage cells (tumor cells from the three previously cultured EMCs), and these were subsequently G-banded and analyzed using standard procedures. Cytogenetic anal-

ysis of a second metastasis from a previously harvested EMC was also completed (case 6-I).¹²

FISH analysis was performed on metaphase chromosomes using wcp probes specific for the Y chromosome and chromosomes 9 and 17 (Vysis, Inc., Downers Grove, IL), and α -satellite probes specific for chromosomes 1, 2 (Vysis), and 14/22 (Appligene Oncor; Qbiogene, Carlsbad, CA). Chromosomes were counterstained with 4',6'-diamidino-2'-phenylindole dihydrochloride (DAPI). Interphase FISH analysis of formalin-fixed, paraffin-embedded tissue sections was performed using a paraffin pretreatment kit (Vysis) as recommended by the manufacturer. Five- μ m sections were independently hybridized with α -satellite probes for chromosomes 7, 8, and 12, and telomeric probes for 1q and 19q (Vysis). The sections were counterstained with DAPI, and evaluated using a Zeiss Axioplan 2 Imaging fluorescence microscope (Carl Zeiss GmbH, Göttingen, Germany). At least 500 nuclei were scored with the exception of four sections hybridized with the 1q probe in which only 200 to 250 nuclei per section could be scored. All analyses were performed without knowing the case identity or cytogenetic findings. Ten sections were randomly selected, analyzed, and re-analyzed by two independent reviewers; the results obtained fell within a 5% range for each section studied. Five- μ m sections of normal tonsil used as controls indicated that the basal background level of three signals per nuclei was in the range of 1 to 5% for all five probes tested. A specimen was therefore considered to be trisomic for a given chromosome if three separate signals for each respective probe could be detected in more than 10% of the nuclei analyzed.

SKY Analysis

SKY analysis was performed in 7 of 10 EMCs (there was no material available for SKY analysis in cases 5 and 9, case 8 had been previously analyzed).¹⁵ The conditions for pretreatment, hybridization, posthybridization washes, and detection were essentially as recommended by the manufacturer (ASI-Applied Spectral Imaging Ltd., Migdal Ha'Emek, Israel). Image acquisition was achieved with the SpectraCube system (ASI) mounted on a Zeiss Axioplan 2 Imaging microscope equipped with a custom-designed optical filter cube (SKY-1; Chroma Technology, Brattleboro, VT) and a DAPI filter.²² Analysis of spectral images was performed using the SkyView software (ASI).

Isolation of RNA, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), and Nucleotide Sequence Analyses

Total RNA was extracted from frozen tumor tissue using the Trizol (Invitrogen Life Technologies, Carlsbad, CA) method. For cDNA synthesis, 5 μ g of total RNA were reverse-transcribed using the SuperScript Preamplification System according to the manufacturer's manual (Invitrogen). An aliquot of 0.25 μ g of the resulting first-strand cDNA was amplified using the appropriate primer sets. Thirty-six cycles of PCR (30 seconds at 95°C, 30 seconds

Table 2. Primers Used for RT-PCR and Nucleotide Sequence Analyses

Primer designation	Sequence 5' $\rightarrow$ 3'
EWS exon 7 fwd	CCCCTAGTTACCCACCCCA
EWS exon 12 fwd	AAGGCGATGCCACAGTGTC
TAF477U24 fwd	GAGCAGTCAAATATGATCAGCAGC
TAF314U23 fwd	TCATATAGCCAGCAACCATATAA
TAF575U24 fwd	CGTCGTGATTGA GTAGGTATGGA
TCF12.1 fwd	GCAACAACGCATGGCCGCTAT
TCF12.2 fwd	GGACTTCAGTGCATGATTTT
TEC fwd α	GGCTCACTTTGCAACGCTGA
TEC fwdF	TCTCGGCTGTGCTCTCCCA
TEC fwd β	GTGCAGATTTCCGGACAGCT
TEC RevA	CCTGGAGGGAAGGGCTAT
TEC RevC	GGTGGCTGTAGCCGTGATCT
TEC RevD	ACACGCAGGAAGGCTTGAGTT
CHI3L1 fwd	TGCCCTTGACCGCTTCTCTGTACC
CHI3L1 rev	CATCCTCTGACCTCGGAACAGG
CHI3L1 fwd 1	TGGCTCTACCCTGGACGGA
CHI3L1 rev 1	ATCCTCTGACCTCGGAAC
GAPDH fwd	GTGAAGGTCGGAGTCAACG
GAPDH rev	GGTGAAGCGCCAGTGGACTC
GAPDH fwd 1	GGTGAAGGTCGGAGTCAACGG
GAPDH rev 1	GGTCATGAGTCCTTCCACGAT

at 55°C, and 30 seconds at 72°C) were performed with 1 μ l of cDNA in 50- μ l reaction volumes. The AmpliTaq Gold (Applied Biosystems, Foster City, CA) DNA polymerase was used for the amplification reactions. PCR primers used for amplification of normal *TEC* and *CHI3L1* transcripts, *TEC*-containing fusion transcripts, and nucleotide sequence analysis are shown in Table 2.^{8,12,15,23,24} As a control for intact RNA and cDNA, a RT-PCR reaction for expression of the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was performed on all cDNAs used (Table 2). PCR products were purified and sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) and the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The resulting sequences were analyzed using basic local alignment search tool (BLAST) searches (National Center for Biotechnology Information).

cDNA Microarray Analysis

Total RNAs from case 7 (classical EMC) and case 8 (cellular-solid variant of EMC) were selected for gene expression profiling using the Human Named Genes GeneFilter (GF211) Microarray Release 1 (Research Genetics, Huntsville, AL) containing ~4000 known genes and expressed sequence tags. As a reference tumor, total RNA from a myxoid liposarcoma with a *TLS-CHOP* fusion was selected. Three μ g of total RNA was used as template in a cDNA synthesis reaction using the superscript first-strand synthesis system (Invitrogen) according to the manufacturer's recommendations but with the following modifications: the dCTP was replaced with 10 μ l of ³²P-dCTP (NEN, Applied Biosystems), the mixture was subsequently incubated for 1 hour at 37°C and for 1 hour at 42°C. The cDNA probes were separated from free nucleotides and small molecules using a SpinX 200 spin column (Amersham Pharmacia Biotech, Uppsala, Swe-

den) according to the manufacturer's recommendations. The cDNA probe mixture was subsequently denatured for 3 minutes at 96°C and added to the GF211 filters in 5 ml of hybridization solution (Research Genetics). Prehybridization and hybridization conditions were according to the manufacturer's recommendations. After overnight incubation at 42°C, the filters were washed in 20 ml of washing solution (2× standard saline citrate, 1% sodium dodecyl sulfate) at 50°C for 2 × 20 minutes, followed by a high stringency wash (0.5× standard saline citrate, 1% sodium dodecyl sulfate) at 55°C for 2 × 15 minutes. The filters were wrapped in plastic film and the pattern of bound reactivity was scanned using a Molecular Imager FX Pro instrument (BioRad, Stockholm, Sweden).

Pairwise comparative analyses of the resulting images were performed using the software Pathways 4.0 (Research Genetics). Normalization was performed using the default data point protocol (Pathways 4.0). All sample data point intensities were divided by the mean sample intensity of all clones. All selected hybridization spots were inspected visually and data points that were difficult to interpret were omitted from further analysis. The control data points containing total genomic DNA showed up to four times difference between the samples. We therefore considered genes with at least eight times differential signal strength and at least two times above background to be differentially expressed. Expression ratios were calculated by dividing the normalized values of the selected genes from the two EMC tumors by the normalized value of the same genes from the reference tumor.

To verify the cDNA microarray results we studied the expression of four differentially overexpressed genes in EMC (*CHI3L1*, *RELB*, *GSTM4*, and *XRCC3*) and one gene that was differentially overexpressed in the myxoid liposarcoma reference tumor (*COL3A1*). *CHI3L1* was assessed by RT-PCR, and *RELB*, *GSTM4*, *XRCC3*, and *COL3A1* by immunohistochemistry. The expression of the five genes was studied in all 10 EMCs as well as in the five myxoid liposarcomas (including the reference tumor).

Results

Clinicopathological Characteristics

Seven men and two women between 42 and 71 years of age were included in this series. Six primary tumors were intramuscular and located in the lower extremity (five, thigh; one, lower leg), one was intramuscular in the upper arm, one was intragluteal, and the remaining tumor involved the soft tissues around the symphysis pubis and inguinal regions. Tumor sizes ranged from 4 to 22 cm in greatest dimension.

Seven of nine primary tumors were classified as classical EMC (Figure 1A and Table 1). One of these cases had hypocellular foci, one had cells with rhabdoid features, and another had in addition to classical areas (case 4, Figure 1B) cellular areas with rare pleomorphic foci (Figure 1C) associated with increased mitoses and focal necrosis. The remaining two cases, cases 5 and 8 (Figure 1D), were classified as cellular-solid variants of

EMC. Local recurrences and metastases were morphologically similar to the primary tumors.

Tumors from all nine patients were analyzed ultrastructurally. In addition to demonstrating the characteristic features of EMC,² eight tumors revealed abundant microtubules; these were packed in intracisternal aggregates in five cases and freely distributed in the cytoplasm in three cases. Six of nine tumors had dense core neurosecretory granules (Figure 1E and Table 1).

Chromogranin A and synaptophysin were detected immunohistochemically in two tumors (Figure 1, F and G), S100 protein was found in three tumors, and all but one tumor were positive for neuron-specific enolase. Cytokeratins and GFAP were not detected in any case. Ki67 immunolabeling with MIB1 ranged from <1 to 25%. Clinical follow-up data for all patients is tabulated in Table 1.

Cytogenetic, SKY, and FISH Analyses

Cytogenetic analysis was performed in five previously not analyzed EMCs (cases 1 to 4 and 6-I). Diploid or near-diploid karyotypes predominated in all tumors. The karyotypic findings based on G-banding in combination with FISH and/or SKY are summarized in Table 3. Clonal structural aberrations were detected in four tumors; a fifth tumor (case 6-I) showed trisomy 7 as the sole clonal change. Two tumors had a t(9;22)(q22;q12) and a third tumor had a del(22)(q12-13) (Figure 2; A to C). SKY analysis did not reveal any hidden rearrangements of chromosomes 9 or 22 in the latter case. In addition, both tumors with t(9;22) had several other structural and numerical abnormalities. Notably, one case had a jumping translocation in which the segment 1q12-qter was translocated onto dissimilar chromosome segments, ie, Yq, 3p, 15p, and 22q (Figure 2A). Because all four clones also contained two intact chromosomes 1, the net result of these rearrangements is partial trisomy for 1q. This case had also a single cell with a t(1;17)(p36;q12) as the sole anomaly. All of the remaining cells analyzed had the t(9;22). Three different areas sampled from case 4 showed a complex t(7;9;17)(q23;q22;q11) and a del(10)(q21) in all areas (Figure 2, D and E); areas A and B also contained several other more or less complex rearrangements (Figure 2E). SKY analysis of case 7 confirmed the dup(1) and t(9;22) originally identified on the basis of G-banding (Figure 2F).⁷

FISH analysis showing the *in vivo* frequencies of trisomy 1q, 7, 8, 12, and 19 in tissue sections revealed the presence of one or more trisomies in seven of nine EMCs (Table 3). Using conventional cytogenetics, trisomies were detected in five of nine tumors. Thus, the combination of these two methods detected trisomies in all but one tumor. The most common gains were +7 (five tumors), +1q (four tumors), and +12 (four tumors). Five tumors had multiple trisomies and four cases had single trisomies.

Detection of EMC-Specific Fusion Transcripts

To search for possible *EWS-TEC*, *TAF2N-TEC*, and *TCF12-TEC* fusion transcripts in cases 1 to 5, we per-

Figure 1. **A:** Classical EMC, case 2. **B:** Classical areas with uniform, bland spindle cells in a myxoid matrix, corresponding to area A in case 4. **C:** Same case, area C showing prominent pleomorphism and associated with an increased proliferative index. **D:** Unusual, entirely cellular and solid variant of EMC, case 8. **E:** Ultrastructural detection of scattered tumor cells with dense core granules of neurosecretory type, case 3. **F** and **G:** Distinct immunoreactivity for chromogranin (**F**) and synaptophysin (**G**), case 6. **H** and **J:** Strong immunoreactivity for RELB (predominantly cytoplasmic) (**H**) and GSTM4 (cytoplasmic and nuclear) (**J**) in case 7. **I** and **K:** The myxoid liposarcoma reference tumor is predominantly negative for RELB (**I**) and focally, weakly positive for GSTM4 (**K**).

formed RT-PCR experiments using the appropriate primer combinations. Amplification with the primer set EWS ex. 7 fwd and TEC RevC resulted in a product of 745 bp in cases 1 and 2, corresponding to a type 1 fusion in which EWS exon 12 is fused in frame to TEC exon 3 (Figure 3A). In cases 3 and 5 amplification with the same primer set resulted in shorter fragments of 415 bp and 567 bp, respectively (Figure 3A). The 415-bp fragment in case 3 corresponds to a type 2 fusion in which EWS exon 7 is fused to TEC exon 2. The 567-bp fragment in case 5 represents a novel fusion in which EWS exon 10 is fused to a 72-bp sequence derived from intron 2 of the TEC gene (EMC type 5 fusion, GenBank accession no. AF524261) (Figure 3B). This sequence corresponds to a

previously unrecognized TEC exon, which we have designated exon 2b. Analysis of the genomic sequence surrounding this exon revealed the presence of the highly conserved GT/AG sequences at the donor and acceptor splice sites, respectively. The fact that a sequence identical to exon 2b previously has been recognized in mitogen-induced TEC transcripts in various cell types,²⁵ indicates that it represents an alternative TEC exon. In case 4 amplification with the primers TAF2N 314U23 and TEC RevA generated a 300-bp fragment (Figure 3A) consistent with a fusion of TAF2N exon 6 to TEC exon 3. The identities of the putative fusion transcripts were confirmed by nucleotide sequence analysis (data not shown). RT-PCR analysis revealed that the normal TEC

Table 3. Summary of Cytogenetics, SKY, FISH, and RT-PCR Analyses in 10 EMCs from Nine Patients

Case	Karyotype based on G-banding and SKY	Relative gains of # 1q, 7, 8, 12, and 19 (based on FISH) [‡]	Fusion transcript	Expression of the unrearranged TEC allele
1	46,XY,t(9;22)(q22;q12)[8]/46,X,der(Y)t(Y;1)(q11.2;q12),t(9;22)(q22;q12)[17]/46,XY,t(9;22)(q22;q12),der(15)t(1;15)(q12;p13)[17]/46,XY,der(3)t(1;3)(q12;p26),t(9;22)(q22;q12)[5],46,XY,t(9;22)(q22;q12),der(22)t(1;22)(q12;q11.2)[2]	+1q (24), +7 (12)	<i>EWS-TEC</i> type 1	+
2	46,XY,t(6;13)(q23-24;q14),t(9;22)(q22;q12)[15]/48,XY,der(5)t(5;17)(q31-32;q22),t(6;13)(q23-24;q14),+7,der(9)t(9;22)(q22;q12),del(17)(q22),+19,der(22)(22pter→22q12::9q22→9q34::5q31-32→5qter)[10]	+12 (18)	<i>EWS-TEC</i> type 1	+
3	46,XY,del(22)(q12-13)[12]/46,XY,t(2;14)(q33-34;q32)[7]/46,XY[19]	+12 (14)	<i>EWS-TEC</i> type 2	+
4A*	88-91,XXYY,der(2)t(2;19)(p13-15;q13.1-3)del(2)(q33),del(6)(q16),t(7;9;17)(q32;q22;q11),del(10)(q21),-11,der(11)t(2;11)(?;p13),der(19)t(11;19)(p13;p13.3)t(2;19)(p13-15;q13.1-3)	—	<i>TAF2N-TEC</i>	+
4B*	47,XY,der(2)t(2;19)(p13-15;q13.1-3)del(2)(q33),t(7;9;17)(q32;q22;q11),del(10)(q21),der(11)t(2;11)(?;p13),+12,der(19)t(11;19)(p13;p13.3)t(2;19)(p13-15;q13.1-3)	+1q(13), +7 (24), +12 (39)	<i>TAF2N-TEC</i>	+
4C*	47,XY,t(7;9;17)(q32;q22;q11),del(10)(q21),+12	+7 (13), +8 (20), +12 (54)	<i>TAF2N-TEC</i>	+
5	N.A.	+1q (17), +7 (10)	<i>EWS-TEC</i> type 5	+
6-I [†]	46,XY[25]/44-47,XY,+7	—	<i>TAF2N-TEC</i>	+
6-II [†]	46,XY[17]	—	<i>TAF2N-TEC</i>	+
7	46,XX,dup(1)(q12q44),t(9;22)(q22;q12)[7]/46,XY,dup(1)(q12q44),t(9;22)(q22;q12),del(6)(q22q24)[5]	+1q (12)	<i>EWS-TEC</i> type 1	+
8	48,X,-Y,t(9;15)(q22;q21),+12,der(15)t(9;15)(q22;q21),+19[18]	N.D.	<i>TCF12-TEC</i>	+
9	47,XY,+8,t(9;17)(q22;q11-12)[9]	+8 (42)	<i>TAF2N-TEC</i>	+

*Case 4 A, B, and C represent different parts of the same tumor.

[†]Case 6 I and II represent two separate metastases from one patient.

[‡]The percentage of nuclei with three signals is shown in parentheses.

N.A., Fresh tumor material was not available for cytogenetic analysis; N.D., not determined.

transcript (amplified as a 517-bp fragment using the primer set *TEC fwdF* and *TEC RevC*) encoded by the unrearranged *TEC* allele was expressed in all 10 cases of EMC (data not shown).

Gene Expression Profiles of EMCs with Different Morphologies and Fusion Transcripts

The gene expression profiles of two EMCs with differing histologies (classical myxoid *versus* cellular-solid variant) and fusion transcripts (*EWS-TEC versus TCF12-TEC*) were compared with the profile of the reference tumor, a myxoid liposarcoma with a known *TLS-CHOP* fusion transcript. We chose myxoid liposarcoma as a reference tumor because this tumor may simulate EMC and expresses related fusion transcripts (*TLS-CHOP* or *EWS-CHOP*). Analysis of scatter plots revealed that the expression profiles of the two EMCs were remarkably similar despite differing histologies and expression of different fusion transcripts (Figure 4A). None of the analyzed genes in the two tumors showed a difference in expression ratio that was >3. In contrast, the expression profiles of the two EMCs were very different from that of the myxoid liposarcoma (Figure 4B). Among the genes with an expression ratio >8, there were 66 genes common to both EMCs, two genes overexpressed in case 7, and 22 genes overexpressed in case 8. A list of the 35 genes

with the highest expression ratios in both EMCs is shown in Table 4 (a complete list of the expression data are available on request). The two genes with the highest expression ratios in both tumors were *CHI3L1* and *METTL1*. As previously mentioned, EMC may show ultrastructural and immunohistochemical evidence of neural-neuroendocrine differentiation (Table 1). Among the genes with an expression ratio ≥8, there were at least four genes encoding neural-neuroendocrine markers, including *SCG2* (chromogranin C), *NEF3* (neurofilament 3), *GFAP*, and *GAD2* (glutamate decarboxylase 2). The latter gene was only overexpressed in case 8.

We validated the microarray data by studying the expression of four highly overexpressed genes in EMC and one gene that was overexpressed in the myxoid liposarcoma reference tumor. RT-PCR analysis of the expression of *CHI3L1* revealed that it was expressed in cases 7 and 8 as well as in the other eight EMCs but not in the five myxoid liposarcomas analyzed (Figure 5). The authenticity of the amplified product was confirmed by nucleotide sequence analysis. Similarly, high expression of *RELB*, *GSTM4*, and *XRCC3* was confirmed by strong immunoreactivity in cases 7 and 8 as well as in the eight other EMCs. The *RELB* immunostaining was predominantly cytoplasmic (Figure 1H) (*REL* proteins may be found in either the cytoplasm or nucleus depending on the cell type and state of the cell),²⁶ the *GSTM4* reaction was

Figure 2. Partial G-banded and partial and complete SKY karyotypes of case 1 (A), case 2 (B), case 3 (C), case 4C (D), case 4B (E), and case 7 (F). Translocated chromosome segments are indicated. Breakpoints are denoted by **arrowheads**.

cytoplasmic and nuclear (Figure 1J), and the XRCC3 staining nuclear in six EMCs and predominantly cytoplasmic in three cases (data not shown). The reference tumor was only focally positive for RELB and GSTM4 (Figure 1, I and K) and negative for XRCC3. Of the other four myxoid liposarcomas two were focally positive and two negative for RELB; three were focally positive and one

negative for GSTM4, and one was positive and three negative for XRCC3. COL3A1 was strongly expressed (cytoplasmic staining) in the reference tumor as well as in the other four myxoid liposarcomas. In contrast, the EMC cases 7 and 8 were only focally positive for COL3A1. Of the other eight EMCs, six were entirely negative and two focally positive for COL3A1 (data not shown).

Figure 3. A: Detection of *TEC*-containing chimeric transcripts by RT-PCR using *EWS-TEC*-specific primers in case 1 (lane 1), case 3 (lane 2), case 2 (lane 3), case 5 (lane 6), and *TAF2N-TEC*-specific primers in case 4, areas A, B, and C (lanes 7, 8, and 9). Lanes 4, 5, and 10 are controls (PCR reactions with *EWS-TEC*- and *TAF2N-TEC*-specific primers but without cDNA template). The DNA Molecular Weight Marker VIII (Boehringer Mannheim) was used as a molecular weight marker (lane M). **B:** Nucleotide and deduced amino acid sequences of the *EWS-TEC* breakpoint junction in case 5. The fusion point is indicated by a vertical line and the ATG initiation codon in *TEC* is indicated by an asterisk. The previously unrecognized *TEC* exon 2b is underlined.

Discussion

Previous cytogenetic studies of EMC have been primarily confined to conventional chromosome analysis.^{6,7,27–33} Here we have used a combination of chromosome banding, FISH, and SKY to obtain detailed information about the karyotypic profiles of EMC. Detailed comparison of the G-banded karyotypes with the SKY and DAPI-band images allowed us to map the breakpoints in all but one of the marker chromosomes recorded. Including the 10 EMCs presented here, cytogenetic information regarding 26 tumors (25 patients) is available in the literature.^{6,27–33} Collectively, the most striking observation is the finding of recurrent rearrangements of chromosome 9 with consistent breakpoints at 9q22 in 22 tumors. Fifteen of the 26 tumors had a t(9;22)(q22;q12) or variants thereof, six a t(9;17)(q22;q11-12) or variants thereof, and one case a t(9;15)(q22;q21). Of the four remaining tumors, three had apparently cryptic rearrangements resulting in *EWS-TEC* or *TAF2N-TEC* fusions. Thus, all but 1 of 26 cases of EMC with known karyotypes have shown translocations with recurrent breakpoints at 9q22 or cryptic rearrangements resulting in *TEC*-containing fusion transcripts. The only case deviating from this pattern is a tumor described by Bridge and colleagues³⁴ that had a t(2;13)(q32;p12) as the sole anomaly. Whether this case expressed any of the EMC-

specific fusion transcripts is not known. Because the translocations t(9;22)(q22;q12), t(9;17)(q22;q11-12), t(9;15)(q22;q21), and variants of these have not been found in any other tumors (Mitelman Database of Chromosome Aberrations in Cancer. Edited by F Mitelman, B Johansson, F Mertens. <http://cgap.nci.nih.gov/chromosomes/mitelman>, 2001) they can be regarded as pathognomonic for EMC.

We detected a fusion transcript in all 10 EMCs; *EWS-TEC*, *TAF2N-TEC*, and *TCF12-TEC* fusions were found in tumors from five, three, and one patient, respectively.^{12,15} Tumors lacking typical EMC-specific translocations may also express fusion transcripts, as illustrated in cases 3 and 6. The former had a del(22)(q12) and two apparently normal chromosome 9 homologues and expressed an *EWS-TEC* transcript, whereas both tumors in the latter case had apparently normal chromosomes 9, 17, and 22 and expressed *TAF2N-TEC* transcripts. Cryptic fusions have recently been detected also in Ewing's sarcoma.³⁵ From a practical, diagnostic standpoint, these results further emphasize the specificity, sensitivity, and utility of demonstrating *TEC*-containing fusion transcripts in the diagnosis of EMC. This is especially apparent with cases lacking typical EMC-specific translocations and with cellular-solid variants of EMC, which are very difficult to recognize on a purely histological basis.

We also found expression of the unrearranged, normal *TEC* allele in all 10 EMCs with fusion transcripts. This is in contrast to Brody and colleagues²⁴ who found that the normal *TEC* allele was not expressed in two EMCs with *EWS-TEC* fusions. Experiments using a semiquantitative RT-PCR approach have, however, indicated that the expression level of the normal *TEC* transcript is very low compared to that of the *TEC* fusion transcripts (unpublished observations), suggesting that the strong expression of the *TEC* fusion genes is likely to be driven by the promoter regions of the 5'-partner genes.

Secondary chromosome abnormalities seem to be relatively common in EMC. In the total material of 26 cytogenetically analyzed cases, 50% had recurrent, secondary abnormalities, notably partial trisomy for 1q and/or single or multiple trisomies. In this study, FISH analysis of tissue sections of nine EMCs revealed that the *in vivo* frequency of trisomies was more common than suggested by cytogenetics (Table 3). In the 26 collective cases of EMC, the most frequent trisomies (based on cytogenetic and FISH analyses) are +1q (seven cases), +7 (seven cases), +12 (six cases), +19 (four cases), and +8 (three cases). Because in-

Figure 4. Comparison of gene expression profiles by means of scatter plots of log intensities. Comparisons of the expression profiles of the two EMC cases 7 and 8 (A), case 7 (B) versus the myxoid liposarcoma reference tumor. Each point in a scatter plot represents 1 of the 4000 genes and expressed sequence tags analyzed.

Table 4. List of the 35 Genes with the Highest Expression Ratios in EMC Cases 7 and 8, Relative to the Myxoid Liposarcoma Reference Tumor

Gene symbol	Description	Expression ratio	
		Case 7	Case 8
<i>CHI3L1</i>	Chitinase 3-like 1 (YKL-40, cartilage glycoprotein-39)	51	46
<i>METTL1</i>	Methyltransferase-like 1	49	44
<i>RNF15</i>	Ring finger protein 15	27	23
<i>HSF2</i>	Heat shock transcription factor 2	22	25
<i>SPINK2</i>	Serine protease inhibitor, Kazal type, 2	18	22
<i>DGKD</i>	Diacylglycerol kinase delta (130 kD)	19	21
<i>ABR</i>	Active BCR-related gene	18	21
<i>ELA3A</i>	Elastase 3A, pancreatic (protease E)	16	22
<i>RELB</i>	V-rel avian reticuloendotheliosis viral oncogene homolog B	14	24
<i>GARS</i>	Glycyl-tRNA synthetase	17	21
<i>GSTM4</i>	Glutathione S-transferase M4	16	21
<i>DBP</i>	D site of albumin promoter (albumin D-box) binding protein	17	20
<i>AANAT</i>	Arylalkylamine N-acetyltransferase	18	18
<i>AOC2</i>	Amine oxidase, copper containing 2	16	19
<i>METAP2*</i>	Methionyl aminopeptidase 2	17 (15)	18 (15)
<i>CRSP9</i>	Co-factor required for Sp1 transcriptional activation, subunit 9	14	19
<i>PPM1G</i>	Protein phosphatase 1G, magnesium-dependent, gamma isoform	14	19
<i>HLALS</i>	Major histocompatibility complex, class I-like sequence	17	15
<i>PEX13</i>	Peroxisome biogenesis factor 13	16	16
<i>KCNN4</i>	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	13	18
<i>CYBB</i>	Cytochrome b-245, beta polypeptide	14	16
<i>PPIB</i>	Peptidyl-prolyl isomerase B	13	17
<i>ADRB2</i>	Adrenergic, beta-2-, receptor, surface	14	16
<i>DCI</i>	Dodecenoyl-Coenzyme A delta isomerase	11	19
<i>EIF5</i>	Eukaryotic translation initiation factor 5	14	15
<i>ITGB3</i>	Integrin beta 3	13	16
<i>XRCC3</i>	X-ray repair complementing defective repair in Chinese hamster cells 3	11	17
<i>MIPEP</i>	Mitochondrial intermediate peptidase	11	16
<i>SFRS1</i>	Splicing factor, arginine/serine-rich 1	14	13
<i>CHST1</i>	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	13	14
<i>SCG2</i>	Secretogranin II (chromogranin C)	13	14
<i>POU6F1</i>	POU domain, class 6, transcription factor 1	11	15
<i>ATP5J</i>	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit F6	12	14
<i>POLR2A</i>	Polymerase II, RNA, subunit A	10	14
<i>HLA-DMB</i>	Major histocompatibility complex, class II, DM beta	9	14

*cDNA present in duplicate on the microarray filter.

formation about clinical data, follow-up, and morphology are unfortunately lacking in most of the previously published EMCs, there is little if anything known about possible correlations between clinicopathological pa-

Figure 5. RT-PCR analysis of the expression of *CHI3L1* in EMC cases 7 (lane 1), 8 (lane 2), 4B (lane 3), and 5 (lane 4) as well as in four myxoid liposarcomas (lanes 5 to 8). Lane 9 is a control (PCR reactions with *CHI3L1*- and *GAPDH*-specific primers but without cDNA template) and lane M is a molecular weight marker (DNA Molecular Weight Marker VIII, Boehringer Mannheim). A 550-bp *GAPDH* fragment was co-amplified with the 270-bp *CHI3L1* fragment as an internal standard. Expression of *CHI3L1* was only detected in the four EMC samples (lanes 1–4).

rameters and the genetics of these tumors. In our series, we found no apparent correlation between any of the trisomies, histological features, type of translocation/fusion gene, or prognosis. We also found that classical *versus* cellular-solid subtype of EMC and evidence of neural-neuroendocrine differentiation did not correlate with cytogenetic and molecular characteristics. Further studies in larger series with long-term follow-up are, however, necessary to evaluate the presence or absence of correlations among the above-mentioned factors.

In EMCs with partial trisomy for 1q, the breakpoints were at 1q12-21 (four cases) and 1q25 (one case). Notably, all EMCs with an extra copy of 1q also had a t(9;22). In one of the current cases an extra copy of 1q was involved in a jumping translocation. To the best of our knowledge this is the first example of a jumping translocation in EMC and the second example of this aberration in a solid tumor.³⁶ Previous molecular studies of jumping translocation breakpoints in neoplasia have shown that they might involve oncogenes, resulting in amplification of the affected gene, eg, *ABL* and/or *CD3-MLL*.³⁷ Whether the 1q21 breakpoint in the jumping trans-

locations in our case affects an oncogene is not known. There are several candidate genes in this region of 1q, including *PRUNE*, *JTB*, and *EAT/mcl-1*.^{38–40} One of these, *PRUNE*, is of particular interest since it was recently shown to be amplified and overexpressed in sarcomas.³⁹ *PRUNE* encodes a protein that negatively regulates nm23-H1, a known metastasis suppressor in, for example, colon and breast cancers.

Microarray analyses of the gene expression profiles of two EMCs revealed a remarkably distinct and uniform expression profile in both tumors despite the fact that they had different histological appearances (one classical and one cellular-solid type) and expressed different fusion transcripts (one *EWS-TEC* and one *TCF12-TEC*). In contrast, the expression profile of the reference tumor, a myxoid liposarcoma, was quite different, suggesting that EMC exhibits a tumor-specific gene expression profile that distinguishes it from myxoid liposarcoma and probably other soft tissue sarcomas. This conclusion is supported by recent studies of the pattern of global gene expression in several other types of soft tissue sarcomas, including synovial sarcoma, gastrointestinal stromal tumors, and subgroups of leiomyosarcoma, all of which have rather consistent and homogeneous expression profiles.^{41,42} Our findings suggest that differences in morphology and/or expression of fusion transcripts are not associated with any major differences in gene expression profiles in EMC.

Among the genes with the highest expression ratios in EMCs were several potentially significant genes such as *CHI3L1*, *METTL1*, and *RELB*. *CHI3L1* encodes a chitinase-like protein (YKL-40, cartilage glycoprotein-39), which is a major secreted protein of human articular chondrocytes and synovial fibroblasts and a marker of late macrophage differentiation.^{23,43} Elevated serum levels of CHI3L1 have been observed in patients with rheumatoid arthritis and hepatic fibrosis and cirrhosis.^{44,45} It has been suggested that the protein may function in remodeling or degradation of extracellular matrix, and could therefore play a role also in tumor invasion.^{23,46} High serum levels of this protein have been associated with short survival in patients with colorectal and recurrent breast cancers.^{46,47} Recently, two microarray analyses also identified *CHI3L1* as highly overexpressed in high-grade malignant gliomas⁴⁸ and papillary thyroid carcinomas.⁴⁹ Using RT-PCR, we confirmed that *CHI3L1* was expressed in all 10 EMCs of our study but not in any of the five myxoid liposarcomas, indicating that overexpression of *CHI3L1* is a consistent finding in EMC. Because CHI3L1 is a secreted protein, it could conceivably be useful as a serum marker monitoring disease progression in EMC patients.

METTL1 encodes a methyltransferase-like protein, which is also of potential interest in relation to cancer because abnormalities of, for example, DNA methylation are consistently found in human neoplasia.^{50,51} *RELB*, a member of the nuclear factor- κ B family of transcription factors, has been implicated in the control of cell proliferation, differentiation, and apoptosis.^{52,53} Overexpression of *RELB* has previously been observed in chondrosarcoma cells stimulated with interleukin-1 β .⁵⁴ Immunohistochemical analysis

of RELB expression confirmed our microarray data by demonstrating intense staining of all 10 EMCs. Interestingly, the microarray also identified overexpression of *MYB*, a target gene of *RELB*,⁵⁵ in both EMCs.

We also observed expression of at least two genes implicated in chondrogenesis (*CHI3L1* and *CHST1*) and several genes encoding neural-neuroendocrine markers (*SCG2*, *NEF3*, *GFAP*, and *GAD2*). In addition, prominent immunoreactivity for chromogranin A and synaptophysin were found in 3 of 10 tumors and neurosecretory granules in six of nine tumors. These observations are in line with the recent detection of partial neural-neuroendocrine differentiation in a subset of EMC.^{4,5} The presence of abundant microtubules in eight of our nine cases further suggests neural-neuroendocrine differentiation, since microtubule-associated protein-2 (MAP-2) and class III β -tubulin, major components of microtubules found in neurons and their derivatives, have been recently detected in EMC.⁵⁶ Whether or not the *TEC*-containing fusion genes are responsible for the induction of a neural-neuroendocrine differentiation program or whether this is an inherent feature of the progenitor cell of EMC is at present unclear. Notably, *EWS-ETS* fusion genes are able to induce epithelial and neuroectodermal differentiation in NIH 3T3 cells.⁵⁷

References

1. Enzinger FM, Shiraki M: Extraskelletal myxoid chondrosarcoma: an analysis of 34 cases. *Hum Pathol* 1972, 3:421–435
2. Meis-Kindblom JM, Bergh P, Gunterberg B, Kindblom L-G: Extraskelletal myxoid chondrosarcoma: a reappraisal of its morphologic spectrum and prognostic factors based on 117 cases. *Am J Surg Pathol* 1999, 23:636–650
3. Kindblom LG, Angervall L: Histochemical characterization of mucosubstances in bone and soft tissue-tumors. *Cancer* 1975, 36:985–994
4. Harris M, Coyne J, Tariq M, Eyden BP, Atkinson M, Freemont AJ, Varley J, Attwooll C, Telford N: Extraskelletal myxoid chondrosarcoma with neuroendocrine differentiation: a pathologic, cytogenetic, and molecular study of a case with a novel translocation t(9;17)(q22;q11.2). *Am J Surg Pathol* 2000, 24:1020–1026
5. Okamoto S, Hisaoka M, Ishida T, Imamura T, Kanda H, Shimajiri S, Hashimoto H: Extraskelletal myxoid chondrosarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 18 cases. *Hum Pathol* 2001, 32:1116–1124
6. Sciort R, Dal Cin P, Fletcher C, Samson I, Smith M, De Vos R, Van Damme B, Van den Berghe H: t(9;22)(q22-31;q11-12) is a consistent marker of extraskelletal myxoid chondrosarcoma: evaluation of three cases. *Mod Pathol* 1995, 8:765–768
7. Stenman G, Andersson H, Mandahl N, Meis-Kindblom JM, Kindblom L-G: Translocation t(9;22)(q22;q12) is a primary cytogenetic abnormality in extraskelletal myxoid chondrosarcoma. *Int J Cancer* 1995, 62:398–402
8. Labelle Y, Zucman J, Stenman G, Kindblom L-G, Knight J, Turc-Carel C, Dockhorn-Dworniczak B, Mandahl N, Desmaze C, Aurias A, Delattre O, Thomas G: Oncogenic conversion of a novel orphan nuclear receptor by chromosome translocation. *Hum Mol Genet* 1995, 4:2219–2226
9. Clark J, Benjamin H, Gill S, Sidhar S, Goodwin G, Crew J, Gusterson BA, Shipley J, Cooper CS: Fusion of the EWS gene to CHN, a member of the steroid/thyroid receptor gene superfamily, in a human myxoid chondrosarcoma. *Oncogene* 1996, 12:229–235
10. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Kovar H, Joubert I, de Jong P, Rouleau G, Aurias A, Thomas G: Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumors. *Nature* 1992, 359:162–165
11. Ohkura N, Hijikuro M, Yamamoto A, Miki K: Molecular cloning of a

- novel thyroid/steroid receptor superfamily gene from cultured rat neuronal cells. *Biochem Biophys Res Commun* 1994, 205:1959–1965
12. Sjögren H, Meis-Kindblom J, Kindblom L-G, Åman P, Stenman G: Fusion of the EWS-related gene TAF2N to TEC in extraskeletal myxoid chondrosarcoma. *Cancer Res* 1999, 59:5064–5067
13. Attwooll C, Tariq M, Harris M, Coyne JD, Telford N, Varley JM: Identification of a novel fusion gene involving hTAFI168 and CHN from a t(9;17)(q22;q11.2) translocation in an extraskeletal myxoid chondrosarcoma. *Oncogene* 1999, 18:7599–7601
14. Panagopoulos I, Mencinger M, Dietrich CU, Bjerkehagen B, Saeter G, Mertens F, Mandahl N, Heim S: Fusion of the RBP56 and CHN genes in extraskeletal myxoid chondrosarcomas with translocation t(9;17)(q22;q11). *Oncogene* 1999, 18:7594–7598
15. Sjögren H, Wedell B, Meis-Kindblom JM, Kindblom L-G, Stenman G: Fusion of the NH₂-terminal domain of the basic helix-loop-helix protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9;15)(q22;q21). *Cancer Res* 2000, 6:6832–6835
16. Åman P: Fusion genes in solid tumors. *Semin Cancer Biol* 1999, 9:303–318
17. Bertolotti A, Bell B, Tora L: The N-terminal domain of human TAFI168 displays transactivation and oncogenic properties. *Oncogene* 1999, 18:8000–8010
18. Knoop LL, Baker SJ: EWS/FLI alters 5'-splice site selection. *J Biol Chem* 2001, 276:22317–22322
19. Ohkura N, Yaguchi H, Tsukada T, Yamaguchi K: The EWS/NOR1 fusion gene product gains a novel activity affecting pre-mRNA splicing. *J Biol Chem* 2002, 277:535–543
20. Maltais A, Filion C, Labelle Y: The AF2 domain of the orphan nuclear receptor TEC is essential for the transcriptional activity of the oncogenic fusion protein EWS/TEC. *Cancer Lett* 2002, 183:87–94
21. Meis-Kindblom JM, Sjögren H, Kindblom LG, Peydro-Mellquist A, Røijer E, Åman P, Stenman G: Cytogenetic and molecular genetic analyses of liposarcoma and its soft tissue simulators: recognition of new variants and differential diagnosis. *Virchows Arch* 2001, 439:141–151
22. Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T: Multicolor spectral karyotyping of human chromosomes. *Science* 1996, 273:494–497
23. Hakala BE, White C, Recklies AD: Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993, 268:25803–25810
24. Brody R, Ueda T, Hamelin A, Jhanwar S, Bridge J, Healey J, Huvos A, Gerald W, Ladanyi M: Molecular analysis of the fusion of EWS to an orphan nuclear receptor gene in extraskeletal myxoid chondrosarcoma. *Am J Pathol* 1997, 150:1049–1058
25. Hedvat CV, Irving SG: The isolation and characterization of MINOR, a novel mitogen-inducible nuclear orphan receptor. *Mol Endocrinol* 1995, 9:1692–1700
26. Nakayama K, Shimizu H, Mitomo K, Watanabe T, Okamoto S, Yamamoto K: A lymphoid cell-specific nuclear factor containing c-Rel-like proteins preferentially interacts with interleukin-6 kappa B-related motifs whose activities are repressed in lymphoid cells. *Mol Cell Biol* 1992, 12:1736–1746
27. Hinrichs SH, Jaramillo MA, Gumerlock PH, Gardner MB, Lewis JP, Freeman AE: Myxoid chondrosarcoma with a translocation involving chromosomes 9 and 22. *Cancer Genet Cytogenet* 1985, 14:219–226
28. Turc-Carel C, Dal Cin P, Rao U, Karakousis C, Sandberg AA: Recurrent breakpoints at 9q31 and 22q12.2 in extraskeletal myxoid chondrosarcoma. *Cancer Genet Cytogenet* 1988, 30:145–150
29. Örndal C, Carlén B, Åkerman M, Willén H, Mandahl N, Heim S, Rydholm A, Mitelman F: Chromosomal abnormality t(9;22)(q22;q12) in an extraskeletal myxoid chondrosarcoma characterized by fine needle aspiration cytology, electron microscopy, immunohistochemistry and DNA flow cytometry. *Cytopathology* 1991, 2:261–270
30. Hirabayashi Y, Ishida T, Yoshida MA, Kojima T, Ebihara Y, Machinami R, Ikeuchi T: Translocation (9;22)(q22;q12). A recurrent chromosome abnormality in extraskeletal myxoid chondrosarcoma. *Cancer Genet Cytogenet* 1995, 81:33–37
31. Rao UN, Surti U, Hoffner L, Howard T, Leger W, Contis L, Yaw K: Extraskeletal and skeletal myxoid chondrosarcoma: a multiparameter analysis of three cases including cytogenetic analysis and fluorescence in situ hybridization. *Mol Diagn* 1996, 1:99–107
32. Day S, Nelson M, Rosenthal H, Vergara G, Bridge J: Der(16)t(1;16)(q21;q13) as a secondary structural aberration in yet a third sarcoma, extraskeletal myxoid chondrosarcoma. *Genes Chromosom Cancer* 1997, 20:425–427
33. Bjerkehagen B, Dietrich C, Reed W, Micci F, Saeter G, Berner A, Nesland J, Heim S: Extraskeletal myxoid chondrosarcoma: multimodal diagnosis and identification of a new cytogenetic subgroup characterized by t(9;17)(q22;q11). *Virchows Arch* 1999, 435:524–530
34. Bridge JA, Sanger WG, Neff JR: Translocations involving chromosomes 2 and 13 in benign and malignant cartilaginous neoplasms. *Cancer Genet Cytogenet* 1989, 38:83–88
35. Batanian JR, Bridge JA, Wickert R, Vogler C, Gadre B, Huang Y: EWS/FLI-1 fusion signal inserted into chromosome 11 in one patient with morphologic features of Ewing sarcoma, but lacking t(11;22). *Cancer Genet Cytogenet* 2002, 133:72–75
36. Aledo R, Aurias A, Chretien B, Dutrillaux B: Jumping translocation of chromosome 14 in a skin squamous cell carcinoma from a xeroderma pigmentosum patient. *Cancer Genet Cytogenet* 1988, 33:29–33
37. Tanaka K, Arif M, Eguchi M, Kyo T, Dohy H, Kamada N: Frequent jumping translocations of chromosomal segments involving the ABL oncogene alone or in combination with CD3-MLL genes in secondary leukemias. *Blood* 1997, 89:596–600
38. Okita H, Umezawa A, Fukuma M, Ando T, Urano F, Sano M, Nakata Y, Mori T, Hata J: Acute myeloid leukemia possessing jumping translocation is related to highly elevated levels of EAT/mcl-1, a Bcl-2 related gene with anti-apoptotic functions. *Leuk Res* 2000, 24:73–77
39. Forus A, D'Angelo A, Henriksen J, Merla G, Maelandsmo GM, Florenes VA, Olivieri S, Bjerkehagen B, Meza-Zepeda LA, del Vecchio Blanco F, Muller C, Sanvito F, Kononen J, Nesland JM, Fodstad O, Raymond A, Kallioniemi OP, Arrighi G, Ballabio A, Myklebost O, Zollo M: Amplification and overexpression of PRUNE in human sarcomas and breast carcinomas—a possible mechanism for altering the nm23-H1 activity. *Oncogene* 2001, 20:6881–6890
40. Hatakeyama S, Osawa M, Omine M, Ishikawa F: JTB: a novel membrane protein gene at 1q21 rearranged in a jumping translocation. *Oncogene* 1999, 18:2085–2090
41. Allander SV, Nupponen NN, Ringner M, Hostetter G, Maher GW, Goldberger N, Chen Y, Carpten J, Elkahoul AG, Meltzer PS: Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. *Cancer Res* 2001, 61:8624–8628
42. Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX, Zhu S, Fero M, Sherlock G, Pollack JR, Brown PO, Botstein D, van de Rijn M: Molecular characterisation of soft tissue tumours: a gene expression study. *Lancet* 2002, 359:1301–1307
43. Krause SW, Rehli M, Kreutz M, Schwarzfischer L, Paulauskis JD, Andreesen R: Differential screening identifies genetic markers of monocyte to macrophage maturation. *J Leukoc Biol* 1996, 60:540–545
44. Johansen JS, Stoltzenberg M, Hansen M, Florescu A, Horslev-Petersen K, Lorenzen I, Price PA: Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. *Rheumatology* 1999, 38:618–626
45. Johansen JS, Moller S, Price PA, Bendtsen F, Junge J, Garbarsch C, Henriksen JH: Plasma YKL-40: a new potential marker of fibrosis in patients with alcoholic cirrhosis? *Scand J Gastroenterol* 1997, 32:582–590
46. Cinton C, Johansen JS, Christensen IJ, Price PA, Sorensen S, Nielsen HJ: Serum YKL-40 and colorectal cancer. *Br J Cancer* 1999, 79:1494–1499
47. Johansen JS, Cinton C, Jorgensen M, Kamby C, Price PA: Serum YKL-40: a new potential marker of prognosis and location of metastases of patients with recurrent breast cancer. *Eur J Cancer* 1995, 31A:1437–1442
48. Tanwar MK, Gilbert MR, Holland EC: Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma. *Cancer Res* 2002, 62:4364–4368
49. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A: Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. *Proc Natl Acad Sci USA* 2001, 98:15044–15049

50. Bahr A, Hankeln T, Fiedler T, Hegemann J, Schmidt ER: Molecular analysis of METTL1, a novel human methyltransferase-like gene with a high degree of phylogenetic conservation. *Genomics* 1999, 57: 424–428
51. Jones PA, Baylin SB: The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002, 3:415–428
52. Rayet B, Gélinas C: Aberrant *rel/nfkb* genes and activity in human cancer. *Oncogene* 1999, 18:6938–6947
53. Bren GD, Solan NJ, Miyoshi H, Pennington KN, Pobst LJ, Paya CV: Transcription of the *RelB* gene is regulated by NF-kappaB. *Oncogene* 2001, 20:7722–7733
54. Vincenti MP, Brinckerhoff CE: Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1beta. *Arthritis Res* 2001, 3:381–388
55. Suhasini M, Pilz RB: Transcriptional elongation of c-myc is regulated by NF-kappaB (p50/RelB). *Oncogene* 1999, 18:7360–7369
56. Hisaoka M, Okamoto S, Ishida T, Imamura T, Kanda H, Kameya T, Meis-Kindblom JM, Kindblom L-G, Hashimoto H: Microtubule-associated protein-2 and class III β -tubulin are expressed in extraskeletal myxoid chondrosarcoma. *Mod Pathol* (in press)
57. Teitell MA, Thompson AD, Sorensen PH, Shimada H, Triche TJ, Denny CT: EWS/ETS fusion genes induce epithelial and neuroectodermal differentiation in NIH 3T3 fibroblasts. *Lab Invest* 1999, 79: 1535–1543