Cancer Genetics 205 (2012) 55–60

Cancer Genetics

BRIEF COMMUNICATION

A t(17;22)(q21;q12) with partial *ETV4* deletion in a soft tissue Ewing sarcoma

Anne-Laure Rougemont [a,b,*], Dorothée Bouron-Dal Soglio [a,c], Natacha Patey-Mariaud de Serre [a,c], Raouf Fetni [a,c], Li Fan [d], Stéphane Barrette [d], Jean-Christophe Fournet [a,c]

[a] *Department of Pathology, Centre Hospitalier Universitaire Sainte-Justine, Montréal, Québec, Canada;* [b] *Division of Clinical Pathology, Geneva University Hospitals, Geneva, Switzerland;* [c] *Department of Pathology and Cellular Biology, Université de Montréal, Québec, Canada;* [d] *Department of Pediatrics, Hematology-Oncology Service, Centre Hospitalier Universitaire Sainte-Justine, Montréal, Québec, Canada*

Cytogenetic analysis of a lumbar soft tissue Ewing sarcoma (ES) in a 7-month-old female child showed a t(17;22)(q21;q12), a rare translocation leading to an *EWSR1-ETV4* chimeric transcript. These findings were confirmed by reverse transcription–polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) techniques. The breakpoints were characterized by direct sequencing of the chimeric fusion gene. Tumor genotyping using the Affymetrix Genome-Wide Human single nucleotide polymorphism (SNP) array 6.0 Genechip identified deletions of both chromosomal regions involved in the translocation, resulting in partial deletion of *ETV4*, but an uninvolved *EWSR1* gene. The creation of a fusion between *EWSR1* and an *ETS* family gene consecutive to a chromosomal translocation is characteristic of the Ewing family of tumors (EFT). This is the first report of a deletion involving the two breakpoints in an *EWS-ETS* translocation. To date, only two cases of t(17;22)(q21;q12) in Ewing sarcoma have been reported, with no associated deletion. Interestingly, both cases had also occurred in soft tissue tumors, which are less common than their bone-involving counterparts.

Keywords Pediatric small-round-cell tumor, single nucleotide polymorphism–array genotyping, *ETS* transcription factor family, *EWSR1*, *ETV4*

Ewing sarcoma (ES) and primitive neuroectodermal tumor (PNET) are round-cell sarcomas with varying degrees of neuroectodermal differentiation and are together considered a single neoplastic entity known as the Ewing family of tumors (EFT), or as ES/PNET (1). They represent the second most common malignant bone tumor in children and young adults, after osteosarcoma (1,2), with nearly 80% of cases occurring in the first two decades of life (3).

Most members of the EFT express a translocation-induced fusion between *EWSR1*, located on 22q12, and a member of the *ETS* family of transcription factors (1,3,4). In more than 85% of cases, a recurrent t(11;22)(q24;q12) fuses the *EWSR1* and *FLI1* genes (1–4). A t(21;22)(q22;q12), which creates a chimeric *EWSR1*/*ERG* gene, is observed in another 10–15% of cases. In the remaining cases, representing less than 5% of EFT cases, a t(2;22)(q36;q12), with an *EWSR1*/*FEV* fusion gene, or a t(7;22)(p22;q12) that fuses *EWSR1* and ETV1 (1–4) are observed. Recently, a novel t(20;22)(q13;q12) resulting in a *EWSR1/NFATC2* gene fusion was described in four patients (5). In rare instances, *FUS* can substitute for *EWSR1*, resulting in either a t(16;21)(p11;q24) or a t(2;16)(q35;p11), with subsequent *FUS*/*ERG* or *FUS*/*FEV* gene fusions, in the absence of any *EWS* rearrangement (6,7).

A rare t(17;22) variant creating a chimeric *EWSR1*/*ETV4* transcript has been reported so far in only two cases of EFT (8,9). We report the cytogenetic and molecular characterization of a soft tissue ES/PNET with a t(17;22)(q21;q12) and deletions adjacent to both chromosomal breakpoints at 17q21 and 22q12, with partial deletion of *ETV4* on 17q21.

Received June 12, 2010; received in revised form January 13, 2012; accepted January 16, 2012.

* Corresponding author.
E-mail address: anne-laure.rougemont@hcuge.ch

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doi:10.1016/j.cancergen.2012.01.007

Materials and methods

Morphological and immunohistochemical studies

Representative sections of the mass were routinely fixed in 10% buffered formalin and embedded in paraffin. For routine histological examination, 3-μm-thick sections were stained with hematoxylin phloxine saffron (HPS).

Immunohistochemical evaluation included the following antigens: MIC2 mouse monoclonal antibody (Dako, Carpinteria, CA), Desmin mouse monoclonal antibody (Dako), and synaptophysin mouse monoclonal antibody (Dako). Immunohistochemistry was performed on paraffin-embedded sections using a labeled streptavidin-biotin (LSAB) kit with an automated Dako immunostainer (DakoCytomation, CA). First, for antigen retrieval, deparaffinized and rehydrated sections were treated using a pressure cooker (citrate buffer, pH 6, 1:10). Then the sections were mounted in the Dako autostainer and covered with hydrogen peroxide for 5 minutes. The slides were incubated for 60 minutes with the diluted antibody after a 5-minute application of Ultra V block (LabVision, Fremont, CA). We followed this step by applying the labeled streptavidin-biotin method according to the manufacturer's instructions (LSAB System HRP Kit, Dako). Diaminobenzodine (Dako) was used as a chromogen.

Cytogenetic and fluorescence in situ hybridization analyses

For karyotype analysis, fresh tissue fragments from the tumor were minced in culture medium and enzymatically digested overnight in 0.8% collagenase III (Biochrom, Berlin, Germany). Cells were washed prior to seeding in 25-cm^2 culture flasks with Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal calf serum. Cells were harvested according to standard procedure after 16 days in culture. Chromosome banding was performed using the trypsin-Giemsa technique of Seabright (10).

Metaphase spreads obtained from fresh tumor samples were hybridized overnight at 37°C with labeled Vysis probes (Abbott Molecular, Abbott Park, IL). The dual-color break-apart DNA probe flanking the *EWSR1* breakpoint region on chromosome 22 and the chromosome 17 centromere probe were applied. Following hybridization with these probes, the chromosomes were washed and counterstained.

Amplification of the fusion transcript and characterization and sequencing of the translocation breakpoints

Frozen tumor fragments were submitted for DNA analysis by reverse transcription–polymerase chain reaction (RT-PCR), using *EWSR1*, *FLI1,* and *ETV4* primers (11,12).

Total RNA from frozen tissue was extracted with a GenElute mammalian total RNA kit (Sigma, St. Louis, MO). This step was followed by a reverse transcription to complementary DNA (cDNA) using random primers. The fusion gene cDNA was then amplified by polymerase chain reaction (PCR) techniques using the following oligonucleotide primers, as previously described (11,12):

Primer EWSR1-F: 5′-TCCTACAGCCAAGCTCCAAGT C-3′
Primer ETV4-R: 5′-GCTGGCCGGTTCTTCTGGATG C-3′

PCR conditions consisted of 7 minutes of denaturation, followed by 35 PCR cycles at 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute, and completed by processing at 72°C for 7 minutes. A PCR amplicon of approximately 250 base pairs (bp) was produced and was further purified for DNA sequencing. The Ensembl database was used for computer analysis of the genomic data (http://www.ensembl.org).

High resolution single nucleotide polymorphism genomic profiling

After approval of the project by the Ethics Committee Board at our institution, the parents of the patient gave informed written consent for genetic analysis of the tumor. Prior to DNA extraction, the frozen tumor fragment was validated using a hematoxylin and eosin–stained slide. DNA extraction was performed using a Puregene Genomic DNA Purification Kit (Gentra Systems, Minneapolis). A high resolution genomic profiling of chromosomal aberrations was performed on DNA extracted from the frozen tumor specimen with the Genome-Wide Human SNP Array 6.0 Genechip (Affymetrix, Santa Clara, CA), which assesses 1.8 million genetic markers, including more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation (CNV). CNV and loss of heterozygosity analyses and visualization were performed using the Biodiscovery Nexus Copy Number software.

Results

Clinical case report

A previously healthy 7-month-old girl underwent surgery for a lumbar mass that had appeared 4 months earlier and was increasing in size. Magnetic resonance imaging showed a soft tissue lumbar mass, not involving the spine. The metastasis workup was negative.

On gross examination, the mass weighed 74.5 g, measured $6.5 \times 6.0 \times 3.5$ cm and was covered by a thin capsule except in its posterior aspect where the mass was in contact with the lumbar muscles. The cut surface showed a fleshy tan-grey mass, with areas of hemorrhage. Histology consisted of a lobular infiltration by sheets of poorly differentiated small- to medium-sized round blue cells set in a fibrovascular background (Figure 1A). Perivascular pseudorosettes were rare. Mitoses and karyorrhexis were abundant. Tumor necroses and dystrophic calcifications were observed. Tumor cells were reactive for the MIC2 surface antigen CD99 and had a strong and characteristic membranous and reticular pattern (Figure 1B). The tumors tested negative for desmin and synaptophysin. Tumor resection was complete, with histologically confirmed negative margins.

Chemotherapy with vincristine, doxorubicine and cyclophosphamide alternating with ifosfamide and etoposide was started. The patient is free of disease 4 years post-treatment.