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Molecular investigation of Ewing sarcoma: about detecting translocations

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Ewing sarcoma is a highly aggressive neuroectodermal tumour affecting young patients and characterized by adverse outcome. Recently our understanding of molecular pathogenesis of Ewing sarcoma has greatly progressed, and there have been some promising therapeutic advances. The cytogenetic hallmark of Ewing sarcoma is balanced translocation t(11;22)(q24;q12), first described by Aurias et al (1983) and Turc-Carel et al (1983). The t(11;22)(q24;q12) was the first sarcoma associated translocation to be characterized at the molecular level. The translocation fuses the Ewing sarcoma breakpoint region 1 (EWSR1) in 22q12 with the Friend leukemia virus integration 1 (FLI1) gene in 11q24 to generate a novel hybrid gene. The EWSR1 gene encodes a multifunctional protein, member of the ten-eleven translocation (TET) family of proteins, that is involved in various cellular processes, including gene expression, cell signalling and ribonucleic acid (RNA) processing and transport. The protein encoded by FLI1 is a member of the E-twenty six (ETS) family of transcription factors that target deoxyribonucleic acid (DNA) sequences through structural motif in their DNA binding region (Riggi et al, 2007). The t(11;22)(q24;q12) joins the 5′portion of the EWSR1 gene to the DNA binding region of FLI1, thus resulting in the replacement of its transcription activation domain by EWSR1 sequences. The breakpoints in the two genes vary, but the most common fusions are between EWSR1 exon 7 and FLI1 exon 5 or 6.

Several alternative gene fusions involving the EWSR1 gene and a different member of the ETS family of transcription factors than FLI1, have been described in Ewing sarcomas (Table 1). These alternate translocations result in fusions of the EWSR1 gene with one of four different ETS genes including ERG (ETS-related gene), ETV1 (ETS-variant gene 1), ETV4 (ETS variant gene 4) or FEV (fifth Ewing sarcoma variant). The most common of these is the EWSR1-ERG fusion, seen in 5–15% of the cases (Sankar & Lessnick, 2011). Despite their genetic diversities, the alternate fusions are structurally very similar to EWS/FLI. Moreover the retrospective study comparing EWS-ERG Ewing sarcoma cases with EWS-FLI cases revealed no significant differences in clinical presentation, overall survival and event-free survival. This study confirms that EWS-FLI and EWS-ERG fusion proteins function similarly to drive the process of oncogenesis in Ewing sarcoma (Ginsberg et al, 1999). Chromosomal translocations between the EWSR1 gene and various genes encoding transcription factors result in the production of chimeric proteins that are involved in tumorigenesis through theirs function as an aberrant transcription factor.

In addition, rare cases of Ewing sarcoma where EWSR1 becomes fused to another type of transcription factor have been reported: inv(22)(q12q12) resulted in an EWSR1-PATZ1 (POZ/BTB and A-T-hook containing zinc finger 1) fusion gene, t(6;22)(p21;q12) fused EWSR1-POU5F1 (POU class 5 homeobox 1) chimera, t(2;22)(q31;q12) resulted in an EWSR1-SP3 fusion, and t(20;22)(q13;q12) achieved an EWSR1-NFATc2 (nuclear factor of activated T-cells, cytoplasmic, calcineurin-

Table 1. Chromosomal translocations in Ewing sarcoma

Translocation	Fusion product	Frequency (%)	Protein family
t(11;22)(q24;q12)	FLI1-EWSR1	85	ETS
t(21;22)(q22;q12)	ERG-EWSR1	5–15	
t(7;22)(p22;q12)	ETV1-EWSR1	<1	
t(17;22)(q12;q12)	E1AF-EWSR1	<1	
t(2;22)(q33;q12)	FEV-EWSR1	<1	
t(20;22)(q13;q12)	NFATC2-EWSR1	<1	NFAT
t(6;22)(p21;q12)	POU5FI-EWSR1	<1	POU
t(4;22)(q31;q12)	SMARCA5-EWSR1	<1	SWI/SNF
inv(22)(q22q22)	ZSG-EWSR1	<1	Zinc finger protein
t(1;22)(p36.1;q12)	ZNF278-EWSR1	<1	
t(2;22)(q31;q12)	SP3-EWSR1	<1	
t(16;21)(p11;q22)	ERG-FUS	<1	
t(2;16)(q35;p11)	FEV-FUS	<1	

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dependent 2) (Mastrangelo et al, 2000; Szuhai et al, 2009). Recently described, t(4;22)(q31;q12) encodes for a somewhat different type of fusion. In this case, the EWSR1 gene is fused to the chromatin-remodelling gene SMARCA5. Members of this family have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes (Sumegi et al, 2010).

To further add to the complexity of Ewing sarcoma, EWSR1 may occasionally be exchanged for another member of the TET family of proteins: the t(16;21)(p11;q22) fused the FUS (fusion involved in t(12;16) in malignant liposarcoma) gene with ERG (v-ets erythroblastosis virus E26 oncogene like) and t(2;16)(q35;p11) results in FUS-FEV (ETS oncogene family) chimeric transcript (Table 1). Both fusion proteins are found in <1% of Ewing sarcoma cases (Romeo et al, 2010).

In addition to many reported TET-ETS translocation, a number of other fusion event have been described: t(4;19)(q35;13), t(15;19)(q13;q13) and ins(4;X)(q31-32;p11p22) (Kawamura-Saito et al, 2006; Mertens et al, 2007; Surace et al, 2005).

In spite of the molecular heterogeneity of Ewing sarcoma, genetic analysis may provide important differential diagnostic information. Although Ewing sarcomas are characterized by typical morphological figures and immunohistochemical markers, such as CD99, differential diagnosis may be extremely difficult to achieve. The detection of fusion genes usually includes chromosome-banding analysis followed by fluorescence *in situ* hybridization (FISH) studies and molecular analyses based on the reverse transcriptase polymerase chain reaction (RT-PCR). Karyotyping requires the availability of fresh, vital cells for short-term culturing, and can be used to detect large chromosomal abnormalities. But some of translocation, in particular t(21;22) are difficult to identify by chromosome-banding analysis. Moreover the material available for genetic diagnosis is often limited to cells from fine needle or core needle biopsies. Directed FISH analyses of the EWSR1 locus or RT-PCR for the most common gene fusions come across as the fastest and reliable methods to verify Ewing sarcoma. These methods are precise and highly specific for the analysis of one or a few candidate fusion genes at predefined breakpoints. However, the approach is highly dependent on prior information. Recent developments of sequencing technologies enable genome-wide identification of fusion transcripts at an unprecedented level of resolution, but these technologies are yet limited by the number of samples that can be analyzed within a reasonable timeframe and at an acceptable cost. A few studies have utilized oligo-microarrays targeting junction sequences to detect fusion transcripts (Skotheim et al, 2009).

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Luo et al (2012) in their paper 'Antibody detection of translocations in Ewing sarcoma' developed a new sensitive approach called antibody detection of translocations (ADOT) to detect chromosomal translocations and to avoid the shortcomings of current techniques. ADOT combines custom oligonucleotide microarrays with S9.6 antibody to identify chromosomal translocations. Compared to traditional microarray techniques, this new method utilizes total RNA. Using the S9.6 antibody it is possible to recognize small RNA–DNA hybrid. This method is useful to detect translocations from patients' fresh tumour specimens, cell lines, frozen tumours and formalin fixed tumours. The main advantage of this method is that ADOT is capable of detecting known or unknown translocations in biological samples, including those most commonly encountered during the diagnostic work-up of a patient. After additional labour the ADOT could be moved to the diagnostic work-up. Furthermore with additional design, ADOT could also be used in different types of cancer to detect chromosomal translocations.

The author declares that she has no conflict of interest.

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