

# PROGNOSTIC RELEVANCE OF MOLECULAR GENETICS IN SOFT TISSUE TUMORS

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In recent years it has become apparent that, from a genetic perspective, sarcomas broadly segregate into two major types: (a) those sarcomas with specific genetic and typically simple karyotypes, including reciprocal translocations giving rise to gene fusions (e.g. *PAX3-FKHR* and *PAX7-FKHR* in alveolar rhabdomyosarcomas) and specific point mutations (e.g. *KIT* mutations in GIST), and (b) those sarcomas with no specific genetic alterations but demonstrating complex karyotypes, multiple unbalanced translocations, and numerous genomic losses and gains. Generally, the latter group is more often characterized by older age groups and alterations of the p53, p16<sup>CDKN2A</sup>, and p14<sup>ARF</sup> pathways (1). Sarcomas with complex unbalanced karyotypes lacking specific translocations include osteosarcoma, high grade undifferentiated pleomorphic sarcoma (formerly termed MFH), liposarcoma (other than myxoid type), angiosarcoma, leiomyosarcoma, adult fibrosarcoma, and skeletal chondrosarcoma. This category will not be further discussed during the seminar, and only diagnostic and prognostic data on gene fusion positive sarcomas with simple karyotypes will be summarized in the lecture.

## **Molecular Abnormalities with Prognostic Significance in Gene Fusion Positive Sarcomas**

Chromosomal translocations constitute the majority of specific genetic alterations associated with sarcomas harboring simple karyotypes. Most of the specific recurrent chromosomal translocations have been cloned and the resulting gene fusions identified (see Table 1). Gene fusion-related sarcomas appear to account for about one third of all sarcomas (2).

In addition to providing specific and powerful diagnostic markers, gene fusions resulting from these translocations encode chimeric oncoproteins that appear to drive the biology of the tumors. These include abnormal transcription factors or deregulated tyrosine kinases that affect multiple downstream genes and pathways (3). The key role of these chimeric proteins in the pathogenesis of different sarcomas is supported by their absolute requirement for *in vitro* growth of corresponding sarcoma cell lines, and the impact of variability in the structure of these chimeric proteins (due to variant cytogenetic or molecular breakpoints) on tumor phenotype and clinical behavior (4). Several of these gene fusion positive sarcomas will be discussed in the lecture.

### ***1. EWS-ETS Gene Fusions in Ewing Family Tumors (EFTs)***

A consistent feature of EFTs is the presence of a common set of genetic rearrangements, with approximately 85% of cases showing a t(11;22)(q24;q12) chromosomal translocation (5). Molecular cloning of the translocation breakpoint identified an in-frame fusion of the *EWS* gene from chromosome 22q12 with *FLI1* from chromosome 11q24, a member of the *ETS* family of transcription factors (6). This alteration can be detected by a simple EWS-FLI1 RT-PCR reaction, which reveals multiple fusion variants. An additional 10-15% of ESFTs show a variant t(21;22)(q22;q12) translocation in which *EWS* is fused to another *ETS* gene, *ERG* from chromosome 21q22 (7). More rarely ( $\leq 1\%$  of tumors), *EWS-FEV*, *EWS-EVII*, and *EWS-ETV1* gene fusions resulting from t(2;22), t(7;22) and t(17;22) translocations, respectively, as well as

several other rearrangements that been recently reported. It is now thought that virtually all EFTs carry some form of *EWS-ETS* gene fusion, and that these rearrangements are pathognomonic of the EFT family (8). For this reason, the preferred diagnostic PCR reaction utilizes 5' *EWS* primers coupled with a generic *ETS*-domain 3' primer. Such rearrangements can also be detected by *EWS* fluorescence in situ hybridization (FISH). The description of gene fusions between the *FUS (TLS)* gene on chromosome 16p11 with the *ERG* gene in EFTs further highlights the theme that the transactivation domains of *EWS* and related proteins are important in EFT oncogenesis (9). It will be important to assess the incidence of *FUS-ERG* gene fusions in *EWS-ETS* fusion negative EFTs.

Prognostically, it has been reported that the type of *EWS-FLI1* fusion transcript in EFTs can affect patient outcome; i.e. the type I transcript of the *EWS-FLI1* translocation is associated with a better outcome (10). However, there is some controversy on this point. There is no obvious difference in outcome when the different *EST* translocation partners of *EWS* are compared in EFTs (11). In contrast, it appears clear that *p53* overexpression or *INK4A* deletion portends a poorer prognosis in EFTs (12).

## **2. *PAX-FKHR* Gene Fusions in Alveolar Rhabdomyosarcoma (ARMS)**

Childhood RMS is generally subdivided into embryonal RMS (ERMS, ~65% of cases), and more primitive forms including alveolar RMS (ARMS, ~20% of cases), and so-called undifferentiated sarcoma (~15% of cases) (13). The diagnosis of RMS is based on tumor architecture, cellular morphology and myogenic differentiation in tumor cells by desmin and muscle-specific actin immunostaining, and expression of myogenin and MyoD family of myogenic transcription factors. However, these markers can be expressed in many tumor types. Of RMS subtypes, only ARMS is characterized by tumor-defining diagnostic rearrangements. Approximately 60% of ARMS cases demonstrate the t(2;13)(q35;q14) translocation which fuses the *PAX-3* gene from 2q35 with the *FKHR* gene from 13q14. A smaller proportion of ARMS (15-20%) have t(1;13)(p36;q14) translocations that fuse *PAX7* from 1p36 with *FKHR* (14, 15). *PAX3* and *PAX7* are members of the *PAX* family of transcription factors, while *FKHR* is a member of the forkhead family of developmentally regulated transcription factors. Resulting *PAX3/FKHR* and *PAX7/FKHR* fusion transcripts can be detected in tumor tissue by RT-PCR or FISH. Although several early molecular studies indicate that *PAX3-FKHR* and *PAX7-FKHR* gene fusions are present in a large majority of ARMS cases, others have reported that significantly higher percentages of ARMS cases lack these fusions, indicating that a proportion of ARMS cases have molecular alterations that are yet to be defined. Recently, 171 Children's Oncology Group (COG) rhabdomyosarcoma cases were analyzed for *PAX-FKHR* gene fusions. *PAX3-FKHR* or *PAX7-FKHR* gene fusions were only expressed in ARMS and not in ERMS cases. While *PAX3-FKHR* and *PAX7-FKHR* fusion transcripts were detected in 55% and 22% of ARMS patients, respectively, of interest ~23% were fusion-negative (16). *PAX3-FKHR* and *PAX7-FKHR* chimeric oncoproteins, like *EWS-ETS* chimera, function as aberrant transcription factors (17). These fusion proteins activate transcription of genes containing *PAX*-binding sites, but with higher potency than the corresponding wild-type *PAX* proteins. Expression of *PAX3-FKHR* in NIH3T3 cells activates myogenic transcription, including the myogenic transcription factors MyoD and myogenin, demonstrating that this chimeric oncoprotein induces myogenesis (18). Similar to *EWS-FLI1* in human fibroblasts, *PAX3-FKHR* transformation requires the presence of an intact IGF1 receptor axis (19).

Prognostically, we previously reported that in a study of COG RMS patients, those with *PAX7-FKHR* positive tumors had significantly improved outcomes in comparison to patients with *PAX3-FKHR* positive tumors (16). *PAX7-FKHR* expression was shown to correlate with

better patient outcome than PAX3-FKHR expression in ARMS, but this only reached statistical significance for patients with metastatic disease (16). However, this notion is not universally accepted. In recent studies, gene expression microarray analysis has been used to determine not only diagnostic subgroups in RMS, but also to define a novel prognostic classification scheme. Using small metagene subsets for analysis, investigators have been able to derive highly reproducible molecular classes of RMS based solely on genomic analysis at diagnosis. Adoption of these molecular criteria may offer a more clinically relevant diagnostic scheme, thus potentially improving patient management and therapeutic outcomes in RMS. Furthermore, these studies were extended to produce a metagene cohort whereby the expression pattern of a small number of genes was highly predictive of outcome. This was independent of individual clinical risk factors such as patient age, stage, tumor size and histology, but was correlated with risk classification used by COG as well as biologic subsets of ARMS tumors. This metagene analysis scheme is highly predictive of outcome and appears to provide additional information about outcome for intermediate risk patients. This scheme will be discussed in the lecture.

### ***3. SYT-SSX Gene Fusions in Synovial Sarcoma***

Tumor-specific translocations and resulting gene fusions have also been reported in several spindle cell tumors, namely synovial sarcoma, congenital infantile fibrosarcoma, and congenital mesoblastic nephroma. Synovial sarcoma (SS) is a malignant neoplasm of children and young adults showing variable combinations of spindle cell and epithelial-glandular components. The epithelial component stains positively for cytokeratin and EMA, causing diagnostic confusion with epithelial tumors if SS is not included in the differential diagnosis. A tumor-defining t(X;18)(p11.2;q11.2) translocation is present in greater than 90% of either biphasic or monophasic forms of SS (20). Molecular cloning of the translocation breakpoints identified two different rearrangements, namely the fusion of the *SYT* gene from 18q11.2 with either of two closely mapped Xp11.2 genes, *SSX1* or *SSX2* (21, 22). The *SYT-SSX2* gene fusion is associated with a monophasic phenotype and the *SYT-SSX1* gene fusion is more commonly seen with a biphasic phenotype (23), but this has not been validated in other studies. Several other variants have since been reported.

Prognostically, analysis of metastasis-free survival showed that there are differences in clinical outcome depending on the *SYT-SSX* fusion type, in that cases with *SYT-SSX2* fusion had superior outcome (24, 25). However, after the first two years, the survival curve of patients with tumors containing *SYT-SSX2* began to drop and became almost parallel to that of patients with tumors containing *SYT-SSX1*. These findings indicate that patients with tumors positive for *SYT-SSX2* had a lower risk of early relapse than those with an *SSX1*-specific translocation, but that the cumulative risk of distant metastasis may be similar in both groups. While it is therefore unclear if translocation subtype portends better or worse diagnosis, large tumors, neurovascular invasion, p53 overexpression, high Ki67 expression, and poorly differentiated subtype all portend a poorer outcome.

### ***4. ETV6-NTRK3 Gene Fusions in Diagnosis of Spindle Cell Tumors of Infancy***

Congenital infantile fibrosarcoma (CFS) is a cellular spindle cell tumor of the soft tissues that generally presents before the age of 2 years (26). As the name implies, many cases are congenital. While CFS shows frankly malignant cytology and a high recurrence rate, it has a very good prognosis with an 80-90% overall survival and only a 10% metastatic rate. Knezevich et al identified a t(12;15)(p13;q25) translocation in CFS and showed that the rearrangement fuses 5' exons from the chromosome 12p13 *ETV6* gene of with 3' exons of the 15q25 *NTRK3* neurotrophin-

3 receptor gene (27). Morphologic, cytogenetic and biologic evidence supports a relationship between congenital fibrosarcoma and cellular congenital mesoblastic nephroma (CMN) of the kidney. Both tumors are characterized by the t(12;15) translocation and associated *ETV6-NTRK3* gene fusion, and both are associated with polysomies of chromosomes 8, 11, 17 and 20 (28, 29). Greater than 90% of CFS and cellular CMN cases express the *ETV6-NTRK3* gene fusion, which has led to the development of diagnostic molecular assays for these tumors based on fusion transcript detection. *ETV6-NTRK3* chimeric transcripts encode the helix-loop-helix protein tyrosine kinase domain of ETV6 fused to the protein tyrosine kinase domain of NTRK3. Studies indicate that the chimeric protein tyrosine kinase expressed is a transforming protein and likely contributes to oncogenesis by dysregulation of NTRK3 signal transduction pathways. Expression of this oncoprotein may also somehow underlie the sensitivity of ETV6-NTRK3 to pro-apoptotic pathways as induced by chemotherapeutic agents, since CFS or CMN cases expressing *ETV6-NTRK3* gene fusions tend to be highly chemosensitive.

Prognostically, detection of *ETV6-NTRK3* fusion transcripts allow CFS and cellular CMN to be differentiated from other childhood spindle cell tumors. These include malignant tumors such as SS, spindle cell RMS, and so-called adult-type fibrosarcoma (ATFS) of older children. ATFS has a poor prognosis similar to that for fibrosarcomas occurring in adults (30). Gene fusion detection also distinguish CFS and CMN from benign spindle cell lesions such as infantile fibromatosis and myofibromatosis, which lack similar rearrangements (30). The prognostic significance of the t(12;15) associated *ETV6-NTRK3* gene fusion therefore lies in its utility for ensuring a correct diagnosis of CFS/cellular CMN and therefore appropriate management for patients with these diseases.

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**Table 1: Partial list of fusion genes in human sarcomas**

TUMOUR	CYTOGENETIC EVENT	MOLECULAR EVENT	FREQUENCY	DIAGNOSTIC UTILITY?
Alveolar soft part sarcoma	t(X;17)(p11;q21)	<i>ASPL-TFE3</i> fusion	>90%	Yes
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	<i>FUS-ATF1</i> fusion	?	Yes
Clear cell sarcoma	t(12;22)(q13;q12)	<i>EWS-ATF1</i> fusion	>75%	Yes
Congenital fibrosarcoma	t(12;15)(p13;q25)	<i>ETV6-NTRK3</i> fusion	>90%	Yes
Congenital mesoblastic nephroma	t(12;15)(p13;q25)	<i>ETV6-NTRK3</i> fusion	>90%	Yes
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	<i>COL1A1-PDGFB</i> fusion	?	Yes
Desmoplastic small round cell tumour	t(11;22)(p13;q12)	<i>EWS-WT1</i> fusion	95%	Yes
Endometrial stromal tumour	t(7;17)(p15;q21)	<i>JAZF1-JJAZ1</i> fusion	30%	Yes
Ewing tumour	t(11;22)(q24;q12) t(21;22)(q12;q12) t(2;22)(q33;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) t(16;21)(p11;q22)	<i>EWS-FLI1</i> fusion <i>EWS-ERG</i> fusion <i>EWS-FEV</i> fusion <i>EWS-ETV1</i> fusion <i>EWS-EIAF</i> fusion <i>FUS-ERG</i> fusion	>85% 10-15% <1% <1% <1% ?	Yes Yes Yes Yes Yes ?
Gastrointestinal stromal tumour		<i>KIT</i> or <i>PDGFRA</i> mutations	>85%	Yes
Inflammatory myofibroblastic tumour	t(2;var)(p23;var)	<i>ALK</i> fusion genes	>50%	Yes
Myxoid liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	<i>TLS-CHOP</i> fusion <i>EWS-CHOP</i> fusion	95% 5%	Yes Yes
Rhabdoid tumour	Deletion of 22q	<i>INI1</i> inactivation	>90%	Yes
Rhabdomyosarcoma Alveolar	t(2;13)(q35;q14) t(1;13)(q36;q14) t(X;3)(q13.1;q35) t(2;2)(q35;p23)	<i>PAX3-FKHR</i> <i>PAX7-FKHR</i> <i>PAX3-AFX</i> <i>PAX3-NCOA1</i>	~55% ~20% ? ?	Yes Yes ? ?
Synovial sarcoma	t(X;18)(p11;q11)	<i>SYT-SSX1</i> or <i>SYT-SSX2</i> fusion	>90%	Yes