

Clinical relevance of molecular genetics to paediatric sarcomas

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J Clin Pathol 2007;60:1187–1194. doi: 10.1136/jcp.2006.040113

The application of cytogenetic and molecular genetic analyses to paediatric sarcomas has identified a number of characteristic changes associated with types and subtypes of sarcomas. This has led to increased understanding of the underlying molecular biology of some sarcomas and provided an important adjunct to standard morphological and immunohistochemical diagnoses. Characteristic genetic abnormalities, particularly specific chromosome translocations and associated fusion genes, have diagnostic and in some cases prognostic value. There is also the potential to detect micrometastatic disease. Fusion genes are most readily detected by fluorescence in situ hybridisation and reverse transcription-PCR technologies. The expression profiles of tumours with specific fusion genes are characteristically similar and the molecular signatures of sarcomas are also proving to be of diagnostic and prognostic value. Furthermore, fusion genes and other emerging molecular events associated with sarcomas represent potential targets for novel therapeutic approaches which are desperately required to improve the outcome of children with certain categories of sarcoma, including rhabdomyosarcomas and the Ewing's family of tumours. Increased understanding of the molecular biology of sarcomas is leading towards more effective treatments which may complement or be less toxic than conventional radiotherapy and cytotoxic chemotherapy. Here we review paediatric sarcomas that have associated molecular genetic changes which can increase diagnostic and prognostic accuracy and impact on clinical management.

Sarcomas can pose particular challenges in terms of their differential diagnosis, and accurate diagnosis is important in optimising the clinical management of patients. A number of types and subtypes of sarcomas possess characteristic genetic abnormalities, including specific chromosome translocation and associated fusion genes, which have diagnostic or in some cases prognostic value. These genetic abnormalities and other emerging molecular events associated with sarcomas represent potential targets for novel therapeutic approaches which are desperately required to improve outcome in certain categories of sarcomas. Novel treatments that are less toxic than conventional radiotherapy and cytotoxic chemotherapy could reduce long-term damage and the risk of secondary malignancies as well as improve the rate of survival. Here we review paediatric sarcomas that have associated molecular genetic changes which can be used to aid diagnosis and the clinical management of patients (table 1). We also discuss the potential for future therapeutic options for children with specific sarcomas based on our increasing understanding of the aberrant signalling pathways driving sarcoma development and the identification of key molecular targets in tumour cells (fig 2).

PREDISPOSITION TO SARCOMAS

Germ-line genetic abnormalities are known to predispose to the development of sarcomas, in many cases through increasing susceptibility to DNA damage (table 2). Germ-line mutations of the *p53* tumour suppressor gene are associated with Li-Fraumeni syndrome and an increased risk of tumours including sarcomas. Ten per cent of children with rhabdomyosarcoma have been identified with *p53* mutations.^{18–19} Germ-line mutation and subsequent inactivation of a second copy of the *RBI* gene result in retinoblastoma through the classic two-hit mechanism. This genetic change is also associated with an increased frequency of osteosarcomas and rhabdomyosarcomas. Additionally, predisposition to osteosarcoma is also found in Rothmund-Thomson and Werner syndromes that are associated with mutations in the *RECQL4* and *RECQL2* genes, respectively, which are involved in genomic instability.²⁰ Costello syndrome is caused by mutation of the *HRAS* gene at 11p15.5, a locus of frequent allelic imbalances in sporadic embryonal rhabdomyosarcomas. Children with Costello syndrome have a high incidence of rhabdomyosarcoma,²¹ but significantly sporadic embryonal rhabdomyosarcomas show uniparental disomy at the same locus, which is not driven by

Sarcomas are a heterogeneous group of tumours that are generally classified according to the type of tissue that they resemble, such as rhabdomyosarcoma which resembles developing skeletal muscle. However, the cell type(s) that gives rise to particular sarcomas is not clear. Sarcomas represent a higher proportion of cancers in children compared to adults, with 11% of all childhood cancers being sarcomas compared with 1% in the adult population. Therefore, although relatively rare, they comprise a significant proportion of paediatric oncology practice, with an incidence of 11.0 per million in children under the age of 20 (fig 1).¹ In high-risk categories of sarcoma the overall outcome has not significantly improved in several decades, despite many clinical trials in different continents.²

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Accepted 29 March 2007
Published Online First
27 April 2007

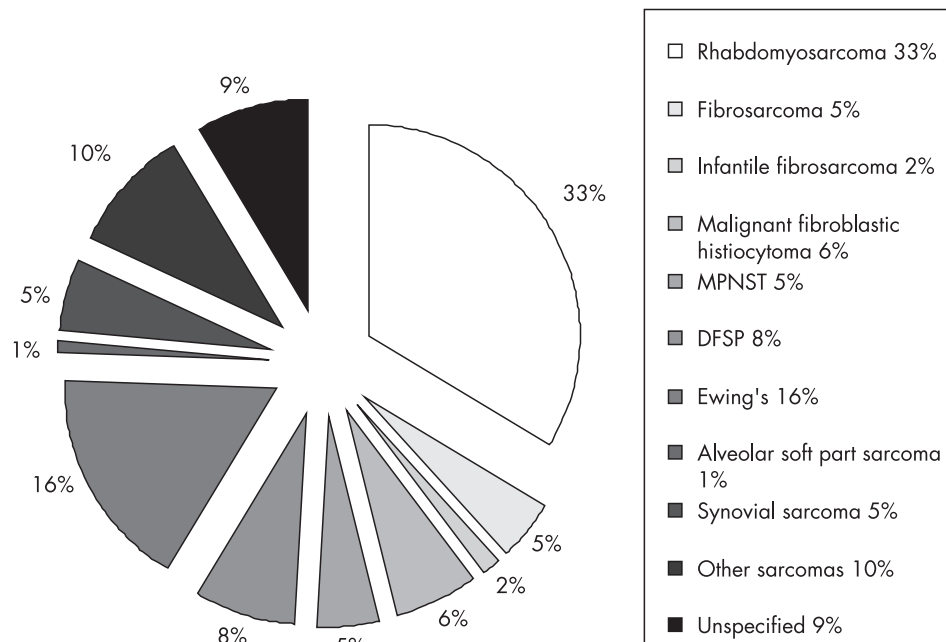


Figure 1 Distribution of childhood sarcomas.¹ MPNST, malignant peripheral nerve sheath tumour; DFSP, dermatofibrosarcoma protuberans.

HRAS mutation.²² Beckwith–Wiedemann syndrome also involves the 11p15.5 locus although the gene involved is not yet clear. This syndrome is associated with overgrowth, malformations and predisposition to embryonic tumours including rhabdomyosarcomas²³

APPROACHES TO THE DIAGNOSIS AND PROGNOSIS OF PAEDIATRIC SARCOMAS

Accurate diagnosis of paediatric sarcomas involves rational integration of clinical parameters, morphological features and investigation of tumour samples by appropriate immunohistochemistry and genetic analyses (table 1). Standard cytogenetic analysis can be used to identify chromosome translocations but it requires fresh material. Preparation and analysis of chromosomes can be both technically difficult and time consuming. The advantage of karyotype analysis is that it gives a global view of chromosome aberrations and can be combined with chromosome painting or fluorescence in situ hybridisation (FISH) approaches to aid definition of changes. The most convenient and widely used approaches for inferring the presence of specific fusion genes are reverse transcription PCR (RT-PCR) and interphase FISH. Using these methods fusion genes can be detected in fresh, snap frozen and formalin fixed paraffin embedded tumour material, including fine needle biopsy samples. RT-PCR and FISH detect specific fusion gene transcripts and disruption or juxtaposing of specific DNA segments associated with a translocation, respectively. The list of variant translocations has grown over the years and therefore a negative result for a particular gene fusion may not exclude a particular diagnosis. Also, although the specificity of particular gene fusions is high it is not exclusive. For example, the *TMP3-ALK* and *CTLC-ALK* gene fusions can be associated with both myofibroblastic tumours and anaplastic large cell lymphomas.^{12 13 33 34} Translocations involving the *EWS* gene are associated with several tumour types and therefore identification of disruption of the *EWS* gene using FISH analysis is not specific, although useful when variation in fusion partners occurs (table 1). The exons which fuse in, for example, the *EWS-FLII* fusion genes associated with Ewing's sarcomas vary and therefore RT-PCRs need to be designed in order to detect these. The sensitivity of RT-PCR allows the detection of

micrometastases; this has been demonstrated in a number of sarcomas although the clinical significance of this is not yet clear. Quantification of RT-PCR products through real time RT-PCR analyses may ultimately become useful in clinical management.³⁵

Different fusion gene products are associated with the same sarcoma type, involving either different genes or different exons of the same genes (table 1). This may affect the molecular biology of the tumour cells and the clinical behaviour of the tumour. These different fusion proteins and other aberrantly expressed proteins associated with tumours may have profound effects on the overall RNA expression profile associated with a tumour. Both the fusion gene types and the expression profiles of tumours are emerging as having prognostic significance in sarcomas and are included in the discussion below on individual sarcoma types.

RHABDOMYOSARCOMA

Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas in children and are thought to be derived from a primitive mesenchymal cell committed to the skeletal lineage but arrested in the processes of differentiation.³⁶ The main histological subtypes are alveolar (ARMS) (20%) and embryonal (ERMS) (60%); survival rates vary from <25% to >95% respectively for these subtypes.³⁷ Molecular genetics has increased diagnostic accuracy of these tumours and is anticipated to increasingly impact on the management of patients.

Seventy per cent of ARMS harbour the translocation t(2;13)(q35;q14) which fuses the 5' end of *PAX3* with the 3' end of the *FOXO1a* gene.³ A further 10% of ARMS are associated with fusion of *PAX7* to the *FOXO1a* gene.³ The remaining 20% of ARMS do not have these fusion genes detectable by routine RT-PCR and comprise cases with a very low expression of a fusion gene, a rare variant fusion, or are true fusion negative cases.⁴ In addition to being of diagnostic relevance, the fusion status correlates with clinical outcome in RMS.

Evaluating clinical features in 34 patients with RMS, Kelly *et al* found significantly longer overall and event free survival in patients with tumours harbouring *PAX7-FOXO1a* fusion gene in comparison with the *PAX3-FOXO1a* group of patients.³⁸ In

Rare germline predisposing genetic change

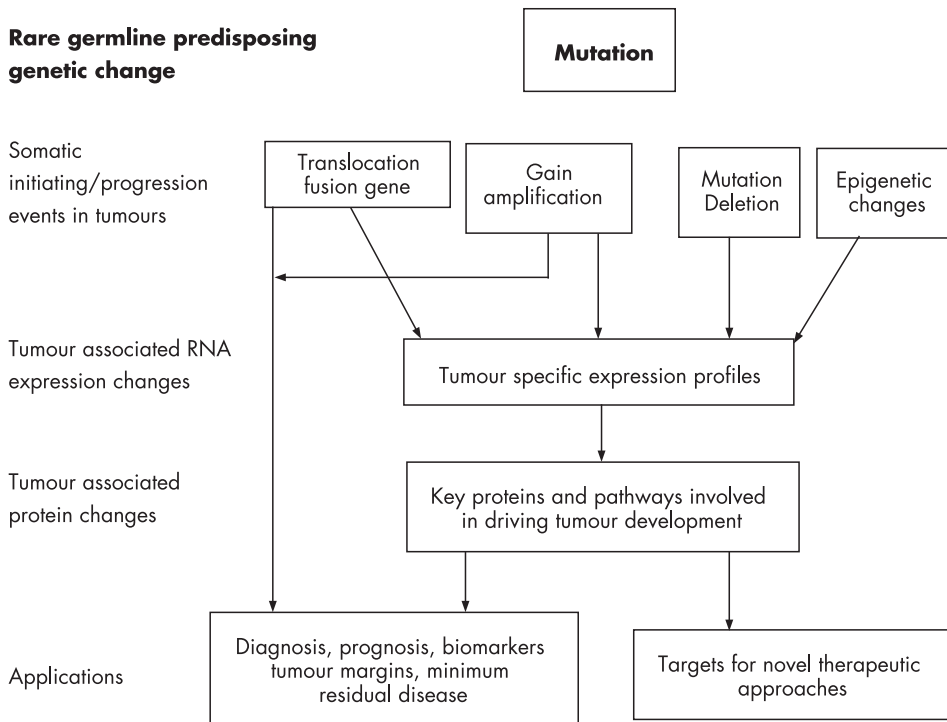


Figure 2 Application of molecular genetics to tumour development, diagnosis and treatment.

univariate analysis of 80 patients with localised disease, comparing patients with *PAX3-FOXO1a* and *PAX7-FOXO1a*, the presence of the *PAX3-FOXO1a* fusion gene was an adverse prognostic factor, implying that these patients might benefit from treatment intensification.³⁹ Sorensen *et al* evaluated 78 ARMS tumours, reporting an overall survival rate of 8% for patients with metastatic disease and *PAX3-FOXO1a*, whereas survival was 75% for patients with *PAX7-FOXO1a* tumours.⁴⁰ Therefore the presence of a *PAX3-FOXO1a* fusion gene is an indicator of adverse outcome in RMS, but independence of alveolar histology has not been tested in any clinical trials.

The fusion genes encode chimeric transcription factors with the DNA binding domain from *PAX3/PAX7* fused to the potent transactivation domain of *FOXO1a*. Wild type *PAX3* and *PAX7* are transcription factors required for primary myoblast migration and specification of muscle satellite cells, respectively.⁴¹ The fusion proteins are 10 to 100-fold more potent as transcription factors than the wild type *PAX3* or *PAX7* gene products.⁴² Although the fusion protein can transform NIH3T3 cells *in vitro*,⁴³ this rarely results in tumours *in vivo*,⁴⁴ without additional changes such as disruption to the *INK4a/ARF* and *TP53* pathways.⁴⁵

Wild type *FOXO1a* regulates myoblast differentiation and cell fusion. Loss of a complete copy of the *FOXO1a* gene as a result of the translocation event in RMS results in reduced *FOXO1a* expression.⁴¹ Artificial restoration of *FOXO1a* expression has been shown to induce G₂/M cell cycle arrest, morphological changes resembling muscle cell differentiation and apoptosis through increased transcription of caspase 3.⁴¹ Restoration of *FOXO1a* function may represent a potential pathway for therapeutic intervention. *Trp53/Fos* double knockout mice develop highly proliferative and invasive ERMS of face and neck with 90% penetrance providing an excellent model for RMS development in humans.⁴⁶

A number of downstream targets of the fusion gene have been shown through expression analyses, such as *EN2*, *BVES*, *FLT1*, *Itam2A* and *MET*.⁴⁷⁻⁴⁸ *MET* encodes the HGF/SF receptor (hepatocyte growth factor/scatter factor); silencing *MET* expression in both ERMS and ARMS cell lines impaired cell replication, survival, invasiveness and anchorage independent

growth.⁴⁹ Furthermore, RMS were induced in mice at a high frequency and with short latency through simultaneous loss of *INK4a/ARF* function and disruption of *MET*.⁵⁰ *MET* represents a possible therapeutic target in RMS and in gastric tumours, which overexpress *MET* through amplification events, a *MET* inhibitor has recently been shown to be effective.⁵¹

Recurrent translocations have not been reported in ERMS. However, ERMS and ARMS in addition to the presence of translocations have characteristic chromosomal imbalances including amplification events. Furthermore, ARMS and the rare anaplastic variant of ERMS both exhibit a high frequency of amplification events involving similar genomic regions, which may contribute to their similar adverse clinical outcome.³⁶⁻⁵²⁻⁵³ Amplification of *MYCN* is used in the stratification of neuroblastoma and has been described in RMS.⁵⁴⁻⁵⁵ Significantly, amplification and overexpression of *MYCN* in ARMS has been associated with adverse clinical outcome in ARMS, but not ERMS, and *MYCN* also represents a possible molecular therapeutic target.⁵⁶ The hallmark of ERMS tumours is recurrent loss of heterozygosity, loss of imprinting or paternal disomy at the 11p15 locus which leads to overexpression of the *IGFII* gene.⁵⁷⁻⁵⁸ Coupled with a report of amplification of the 15q25–26 region encompassing the *IGFRI* locus in an ERMS case,⁵³ this suggest a role for the IGF pathway in ERMS development.

Expression profiling at the chromosomal level has shown discriminating patterns of expression in ARMS and ERMS.⁵⁹ Higher resolution expression analysis has also demonstrated distinctive expression profiles in fusion gene positive and negative tumours. A subset of genes were able to identify three ARMS risk groups with very different overall survival rates (7%, 48% and 95% overall survival).⁶⁰ Further discriminatory patterns and key genes are likely to emerge from this and similar data.

EWING'S SARCOMA FAMILY OF TUMOURS

The Ewing's family of tumours (ESFTs) encompasses Ewing's sarcoma, peripheral neuroectodermal tumour, Askin tumour and neuroepithelioma. For Ewing's sarcoma, 5-year survival with combination treatment of surgery, chemotherapy and

Table 1 Chromosomal rearrangements in childhood sarcoma

Tumour type	Chromosomal rearrangement	Genes involved	Prevalence	Reference
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	PAX3-FOXO1a	~70%	3
	t(1;13)(p36;q14)	PAX7-FOXO1a	~10%	4
Alveolar soft part sarcoma	t(X;17)(p11;q25)	ASPL-TFE3	~100%	5
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	FUS-ATF1	NA	6
Clear cell sarcoma/malignant melanoma of the soft parts	t(12;22)(q13;q12)	EWS-AFT1	NA	7
Congenital fibrosarcoma/mesoblastic nephroma	t(12;15)(p13;q25)	ETV-NTRK3	~100%	8
Dermatofibrosarcoma protuberans	Ring chromosome with sequences from chromosome 17 and 22 t(17;22)(q22;q13)	COL1A1-PDGFB	92%	9
Desmoplastic small round cell tumour	t(11;22)(p13;q12)	EWS-WT1	93%	10
Ewing's sarcoma family of tumours	t(11;22)(q24;q12)	EWS-FLI1	85%	11
	t(21;22)(q22;q12)	EWS-ERG	10%	11
	t(7;22)(p22;q12)	EWS-ETV1	~1%	
	t(7;22)(q21;q12)	EWS-E1AF	~1%	
	t(2;22)(q33;q12)	FUS-ERG EWS-FEV	~1% ~1%	
Giant cell fibroblastoma (juvenile form of DFSP)	t(17;22)(q22;q13)	COL1A1-PDGFB	100%	9
Inflammatory myofibroblastic tumour	t(2;19)(p23;q13)	TPM4-ALK	NA	12
	t(1;2)(q22;p23)	TPM3-ALK		12
	t(2;17)(p23;q23)	CLTC-ALK		13
	t(2;2)(p23;q13)	RANBP2-ALK		14
Rhabdoid tumour	t(1;22)(p36;q11.2)	SNFS/INI1	NA	15
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SSX1/SYT	63%	16
		SSX2/SYT	37%	16
		SSX4/SYT	rare	17

NA, not available.

radiotherapy is 55–60% in localised disease, while patients with metastatic disease have a 5-year survival of only 30%. The ESFTs share genetic alterations consisting of a number of translocations, most frequently (80–85%) the translocation t(11;22)(q24;q12) which results in the fusion of the 5' end of the *EWS* gene to the 3' end of *FLI1*.^{61, 62} A further 5–10% of these tumours are associated with the t(21;22)(q22;q21) and *EWS-ERG* fusion gene.^{7, 63} In addition there are rare variant translocations in which *EWS* is fused to other members of the *ETS* family genes (table 1). Depending on genomic breakpoints and the exons fused, there are two types of *EWS-FLI1* transcripts: *EWS* exon 7 is most frequently fused to either *FLI1* exon 6 (type-1 transcript (60%)) or *FLI1* exon 5 (type-2 transcript (25%)).^{64, 65} Patients with a type-1 *EWS-FLI1* fusion transcript have been reported to have a better disease-free survival compared with those with other fusion transcripts types.⁶⁶ The type-1 fusion encodes a less active chimeric transcription factor⁶⁷ and is associated with a lower proliferative index.⁶⁴ However, other authors report a lack of evidence for the *EWS-FLI1* type 1 fusion impacting on disease-free or overall survival, but in comparison with *EWS-ERG* tumours.⁶⁸

EWS belongs to a family of genes that encode proteins involved in RNA processing, while *FLI1* is part of the *ETS* family of DNA-binding transcription factors. *EWS-FLI1* is a more potent transcriptional activator than *FLI1*.⁶² Furthermore, *EWS-FLI1* promotes transforming and tumorigenic activities,^{69, 70} which are abrogated when either *EWS* or *FLI1* are mutated. The fusion protein affects the cell cycle, disrupts signal transduction pathways, affects cell differentiation and changes the status of p53 tumour suppressor.³ Mutation of p53 and homozygous deletion of p16/p14ARF have been found in 25% of 60 patients

with Ewing's sarcoma. This subgroup is defined by highly aggressive tumours which have poor response to chemotherapy.⁷¹

Gene expression profiling reveals an association of the *EWS-FLI1* fusion gene with overexpressed genes encoding cell cycle regulators, genes associated with invasion and metastasis and down-regulated genes including tumour suppressor genes and inducers of apoptosis.⁷²

Modulation of the tumorigenic properties of *EWS-FLI1* may also take place through the basic fibroblast growth factor pathway.⁷³ *EWS-FLI1* suppresses TGF-type II receptor transcription and histone deacetylase inhibitors can reverse this effect. Restoring TGF signalling in this way has been shown to suppress the growth of Ewing's cells.⁷⁴ Another promising agent that down-regulates *EWS-FLI1* protein and restores TGF-receptor II expression is rapamycin which acts by inhibiting the intracellular protein kinase mTOR.⁷⁵ IGF1 is also a downstream target of the *EWS-FLI1* protein.⁷⁶ IGF1 and IGF1R trigger growth, proliferation and antiapoptotic signals in tumour cells. The insulin-like growth factor binding protein (IGFBP3) is expressed at low levels in ESFTs and influences regulation of IGF1. Silencing *EWS-FLI1* with siRNA is associated with increased levels of IGFBP3 and apoptosis, and exogenous IGFBP3 significantly inhibits the growth of Ewing's cell lines in culture. Recombinant IGFBP3 could therefore have therapeutic potential in ESFTs.^{77, 78}

DESMOPLASTIC SMALL ROUND CELL TUMOUR

The desmoplastic small round cell tumour (DSRCT) is a rare, poorly understood neoplasm primarily affecting adolescents

Table 2 Syndromes which predispose to paediatric sarcoma

Cancer syndrome	Locus	Gene	Characteristic malignancy	Sarcoma type	Reference
Beckwith–Wiedemann syndrome	11p15.5	Unknown	Wilms' tumour, hepatoblastoma, adrenocortical carcinoma	Rhabdomyosarcoma	23
Bloom syndrome	15q26.1	RECQL3/BLM	All common malignancies with increased frequency		24
Costello syndrome	11p15.5	HRAS	Rhabdomyosarcoma, neuroblastoma, transitional cell carcinoma		21
Li–Fraumeni syndrome	17p13.1	TP53	Breast carcinoma, CNS tumours	Rhabdomyosarcoma Osteosarcoma	25, 26
Hereditary retinoblastoma	13q14	RB1	Retinoblastoma	Osteosarcoma Rhabdomyosarcoma	27
Neurofibromatosis type 1	17q11.1	NF1	Glioma, neurofibroma	Rhabdomyosarcoma Malignant peripheral nerve sheath tumour	28
Noonan syndrome	12q14.13	PTPN11	Juvenile myelomonocytic leukaemia, neuroblastoma	Rhabdomyosarcoma	29
Gorlin syndrome	9q22	PTCH	Basal cell carcinoma, medulloblastoma	Rhabdomyosarcoma	30
Rapadilino and Rothmund–Thomson syndrome	8q24.3	RECQL4	Osteosarcoma		20
Werner syndrome	8p11.2	RECQL2/WERN	Osteosarcoma		20
Mosaic variegated aneuploidy	15q15	BUB1B	Wilms' tumour, leukaemia	Rhabdomyosarcoma	31, 32

and young adult males. It presents with widespread intra-abdominal serosal involvement not related to a particular organ system.⁷⁹ DSRCT is usually a disseminated tumour at diagnosis and most patients die within 2 years despite aggressive treatment. In children the 3 and 5 year survival is 44% and 15%, respectively.⁸⁰

DSRCTs harbour a specific translocation t(11;22)(p13;q12) which juxtaposes the 5' end of the *EWS* gene to the 3' *WT1* (Wilms' tumour) tumour suppressor gene, resulting in formation of EWS-WT1 fusion protein.⁸¹ As in the ESFT, the *EWS-WT1* fusion gene includes up to exon 7 or more rarely exons 8–10 of the *EWS* gene.^{82–83} The fusion includes exons 8–10 of the *WT1* gene. *EWS* fusion products have been shown to be potent transcriptional activators and can transform NIH3T3 cells. The amino-terminal domain of EWS is required for both of these activities. The EWS-WT1 chimeric protein probably functions as an inappropriately expressed transactivator, whereas native WT1 is primarily a repressor.

The downstream targets for EWS-WT1 include exocytosis regulator BAIAP3, TALLA 1 (T-cell acute lymphoblastic leukaemia associated antigen), IL-2R, MLF1 and LRRC15. LRRC15 probably contributes to the invasive phenotype of DSRCT.⁸⁴ EWS-WT1 induces expression of platelet derived growth factor (PDGF) growth factor, which has weak transforming capacity but is a potent mitogen and chemo-attractant for fibroblasts and endothelial cells. PDGF contributes to the characteristic reactive fibrosis associated with DSRCT.⁸⁵ A phase I clinical trial using the PDGF inhibitor SU 101 (leflunomide) has been conducted with encouraging results.⁸⁶

GIANT CELL FIBROBLASTOMA

Giant cell fibroblastoma (GCF) represents the juvenile form of dermatofibrosarcoma protuberans (DFSP),^{87–88} occurring exclusively in the first two decades.⁹ Although histologically different these two diseases share a number of similarities: clinical localisation and course, CD34 positivity, and most importantly,

genetic background. Genetic changes in both diseases result in the COL1A1-PDGFB fusion protein. *COL1A1* is located at 17q22 encoding $\alpha 1$ chain of type 1 collagen, while the *PDGFB* gene located at 22q13 encodes the β chain of the PDGF ligand. The mechanism of genetic alteration is different and appears to be age related: in GCF this is mostly an unbalanced translocation t(17;22), while in DFSP it appears as supernumerary ring chromosome. In both disorders the fusion transcript is under the control of the regulatory sequences of the *COL1A1* gene, which leads to continuous activation of PDGFR receptor tyrosine kinase that promotes tumour growth. Imatinib mesylate is a small molecule inhibitor of tyrosine kinases including PDGFR, and the clinical response in adults has been dramatic.⁸⁹ However, there has not been a specific clinical trial for imatinib in children with GCF.⁹⁰ The use of other PDGFR inhibitors, such as sunitinib and sorafenib, has recently commenced in patients with metastatic DFSP.⁹¹

SYNOVIAL SARCOMA

Synovial sarcoma is the second most common soft tissue sarcoma in children and adolescents. It arises in the para-articular structures of the limbs, though it might occur in other locations. This is a spindle cell tumour which presents as two major histological subtypes, biphasic or monophasic, defined by the presence or absence of areas of glandular epithelial differentiation, respectively. Treatment is multimodal and 5 and 10 year survival rates are 60% and 50% respectively.⁹²

The main molecular event is a reciprocal translocation t(X:18)(p11;q11) present in more than 90% of synovial sarcomas.⁹³ The translocation results in fusion of the *SYT* gene at 18q11 to either the *SSX1* gene (Xp11.23) or the *SSX2* gene (Xp11.21),⁹⁴ or in very rare case to the *SSX4* gene.⁹⁵ Seventy-five per cent of synovial sarcomas involve the *SSX1* fusion type which is associated with biphasic histology, while 12% of tumours involve *SSX2* fusions in association with monophasic histology. Several studies have shown that the type of fusion

Take-home messages

- Molecular genetic analyses of childhood sarcomas have identified characteristic genetic aberrations associated with tumour types.
- Chromosome translocations and their resultant gene fusion products are of particular diagnostic and prognostic value.
- These can be identified by cytogenetic analysis, interphase fluorescence in situ hybridisation and reverse transcription PCR.
- Genetic changes can also predispose to sarcoma development, frequently through increasing the probability of changes occurring to DNA.
- Increasing understanding of the molecular consequences of the genetic changes that are involved in the development of paediatric sarcomas is leading to identifying further clinically useful markers and targets for novel therapeutic approaches.

has prognostic implications.^{16 96 97} *SYT-SSX1* is associated with higher proliferation rate and has shorter progression-free survival.⁹⁶ *SYT-SSX* status has been demonstrated as the single most significant prognostic factor for overall survival in patients with localised disease at diagnosis in a multi-institutional study of 243 patients.¹⁶ In patients with metastatic disease, the tumour spread seems to outweigh any influence of the fusion protein.⁹⁸ However, a European study of 141 patients challenged these findings.⁹⁹

Identification of the fusion gene is a useful tool in difficult diagnostic cases, and may be valid for stratification. Blocking the fusion gene with antisense oligonucleotides results in decreased expression of the DNA repair gene *XRCC4* and *cyclin D1*.¹⁰⁰ The fusion protein may serve as target for tumour specific cytotoxic lymphocytes T,¹⁰¹ which a phase I pilot trial has confirmed.¹⁰² Alterations affecting cell cycle regulators involved in the G₁ checkpoint are also frequent events in synovial sarcomas and could be associated with poor outcome.¹⁰³ Bcl-2 is overexpressed in 79–94% of biphasic SS and likely to be related to protecting cells from apoptosis, which could contribute to their resistance to conventional chemotherapy.¹⁰⁴ Overexpression of epidermal growth factor receptor (EGFR) is found in 55.3% of tumours by immunohistochemistry¹⁰⁵; a phase II trial of the EGFR inhibitors in patients with both localised and metastatic synovial sarcomas that overexpress EGFR has commenced in Europe.¹⁰⁶ Quantitative RT-PCR analysis of *ErbB2* has shown that it is expressed in 73.3% of patients with synovial sarcoma.¹⁰⁵ cDNA microarray analysis indicates that, in contrast to other soft tissue sarcomas, *ErbB2* expression is found in synovial sarcoma.¹⁰⁷ A phase II clinical trial of trastuzumab, which targets ErbB2, in recurrent or metastatic synovial sarcoma is underway in adult patients.¹⁰⁸

CONCLUSIONS

In childhood sarcomas specific fusion genes have provided a sensitive and accurate approach to assist with diagnosis, treatment stratification and in some cases prognostication. However, inconsistencies have emerged from different studies supporting prognostic factors.^{16 38–40 64 65 68 99} Possible reasons for these discrepancies include different study designs and treatment protocols, confounding variables associated with retrospective analyses and use of diverse molecular methods. In order to resolve these issues, prognostic factors should be validated using uniform and multiple methods in both retrospective investigations and

prospective multinational multicentre studies.¹⁰⁹ The Euro Ewing's 99 Clinical Trial (in accrual at the moment) plans to prospectively study the prognostic significance of *EWS-FLII* transcripts as well as the value of detecting minimal residual disease.¹¹⁰

The advent of the human genome map and techniques to interrogate abnormalities in multiple genes has seen the identification of genes and pathways associated with the development sarcomas, both with and without fusion genes. Patterns of gene expression provide a new approach to classifying tumours and predicting clinical behaviour. In addition, understanding of the underlying molecular biology in paediatric sarcomas is leading to the identification of targets for novel therapeutic approaches. Targeted agents have already been used in some sarcoma patients, enabling treatment with improved efficacy and reduced toxicity and long-term side effects, which is of utmost importance in this young group of cancer patients.

ACKNOWLEDGEMENTS

The authors are very grateful to Kathy Pritchard-Jones for her critical comments in preparing this manuscript.

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Competing interests: None declared.

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