Leader

Diagnostic and prognostic implications of the unfolding molecular biology of bone and soft tissue tumours

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Abstract
Sarcomas account for approximately 1–2% of human malignant disease and are relatively uncommon. Histopathological study of these mesenchymal tumours at light microscopic and ultrastructural level may not always provide an unambiguous diagnosis. It has become apparent with the identification of increasing numbers of tumour specific genetic alterations that (cyto)genetic evaluation could become a very helpful adjunct to histopathological assessment in reaching a correct diagnosis. Thus, once the different tumour types can be accurately identified and classified, more meaningful clinical trials can be initiated to evaluate and select optimal methods of management. In addition, an increasing awareness and understanding of the molecular changes associated with, and the genetic variability in, the various tumour groups is beginning to provide important new information about clinical progression and prognosis.
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Bone and soft tissue tumours are a heterogeneous and complex group of lesions arising as a result of neoplastic transformation within structures derived from the embryological mesoderm and neuroectoderm,[1] and currently classified on a histogenetic basis according to the mature tissues they might resemble.[2] They represent a major group of tumours distinct from those neoplasms having an epithelial, haematopoietic, or central neurogenic origin. Benign lesions seen in a hospital setting outnumber their malignant counterparts by a margin of approximately 100:1, with an annual incidence of about 300/100 000 of the population.[3] This is almost certainly a conservative estimate since many of the benign lesions in the general population are not biopsied. Malignant tumours (sarcomas) are rare and represent about 1–2% of all cancers and cancer deaths in adults; in the USA roughly 5000 new cases are diagnosed annually, as compared with 90 000 cases of lung cancer and 180 000 of breast cancer.[4] In children, however, sarcomas are relatively more common, accounting for approximately 10% of all malignant solid tumours.[4] Furthermore, there appears to be an increasing incidence of soft tissue sarcomas, although this may merely represent improved diagnostic capability and an increasing interest in this group of lesions.

Primary malignant bone tumours are considerably rarer than soft tissue sarcomas, accounting for less than 1% of all cancer deaths[4]; involvement of the skeletal tissues by metastatic disease, however, is seen far more frequently (own observations).

Bone and soft tissue tumours often present diagnostic difficulties for clinician and pathologist alike and, despite the many advances in diagnostic histological techniques, the histogenesis of many of these neoplasms remains difficult to establish. Published work has shown that disparity between the original histopathological diagnosis and subsequent expert review could be present in as many as 25% of malignant mesenchymal lesions.[5] Thus our ability to initiate meaningful clinical trials to evaluate optimal methods of treatment—as well as to identify possible prognostic variables—for the various tumour types may be limited.

It has become apparent over the last decade that many malignant bone and soft tissue tumours carry chromosomal aberrations detectable at a cytogenetic or molecular level, several of which appear to be non-random and associated with specific tumour types.[6–9] These alterations, especially when compared with the genetic aberrations described in the far more prevalent carcinoma group, appear to be less complicated and consequently more accessible to study, resulting in a rapid acquisition of new information about the molecular genetics of sarcomas.

Since many high grade sarcomas may present morphologically as poorly differentiated tumours lacking distinctive phenotypic features, the presence of chromosomal changes could serve as an important aid in alleviating some of the diagnostic problems encountered. The identification of consistent genetic alterations associated with certain lymphomas and

Table 1 Small round cell tumours of bone and soft tissue

Ewing sarcoma/primitive neuroectodermal tumour
Intra-abdominal desmoplastic small round cell tumour
Alveolar rhabdomyosarcoma
Neuroblastoma
Small cell variant osteosarcoma
Small round cell variant malignant peripheral nerve sheath
tumour
Metastatic melanoma without a known primary
Non-Hodgkin lymphoma/leukaemia
Metastatic anaplastic small cell carcinoma

Table 2 Spindle cell tumours of bone and soft tissues

Monophasic synovial sarcoma
Malignant peripheral nerve sheath tumour
Malignant fibrous histiocytoma
Fibrosarcoma
Leiomyosarcoma
Congenital fibrosarcoma

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leukaemias has facilitated the diagnosis of haematological malignancy and helped to direct treatment and determine prognosis[10]; it is envisaged that a similar route will emerge in the management of bone and soft tissue tumours.

Cytogenetic and molecular analysis appears to be of particular value in two groups of soft tissue neoplasms: the small round cell and spindle cell tumours (tables 1 and 2). These groups are composed of fairly primitive round or spindle shaped cells, respectively, often lacking distinctive phenotypic features; despite the sophisticated histopathological methods available, chromosomal analysis aimed at detecting specific alterations appears to be the most effective variable in resolving the frequent diagnostic dilemmas.

New studies are beginning to provide increasing, and convincing, evidence that genetic variability within specific tumour groups, such as Ewing sarcoma and synovial sarcoma, may also be of prognostic value.[11–14]

An increasing variety of techniques is now available to detect genetic alterations in bone and soft tissue tumours, including immunohistochemistry for the detection of products of altered gene expression, karyotype analysis (fig 1), fluorescence in situ hybridisation (FISH) of chromosome spreads (see cover illustration of the May issue of this journal) to assess ploidy status and identify certain genetic rearrangements, Southern blotting and the polymerase chain reaction (PCR) for genomic DNA analysis, reverse transcriptase polymerase chain reaction (RT-PCR) for detecting altered mRNA products (figs 2 and 3), and DNA sequencing.[15] Although a discussion of these techniques will not be attempted here, no single technique is capable of demonstrating all the genetic alterations present, and each technique serves to fulfil a specific requirement.

It is our aim in this short review to summarise our current knowledge of specific chromosomal aberrations, in particular those associated with the small round cell and spindle cell tumours, with regard to their possible diagnostic and prognostic value in clinical practice.

The small round cell tumours
The small round cell tumours of bone and soft tissues, a group of malignant tumours sharing many morphological features at a light microscopic level, comprise skeletal and extraskeletal Ewing sarcoma (including the Askin variant) and the related primitive neuroectodermal tumour (PNET), intra-abdominal desmoplastic small round cell tumour, embryonal rhabdomyosarcoma, neuroblastoma, the small round cell variant of skeletal and extraskeletal osteosarcoma, a small round cell variant of malignant peripheral nerve sheath tumour, and mesenchymal chondrosarcoma.

The differential diagnosis may be extended by the inclusion of metastatic melanoma without a known primary, non-Hodgkin lymphoma (NHL)/leukaemia, and metastatic anaplastic small cell carcinoma, all of which may have a
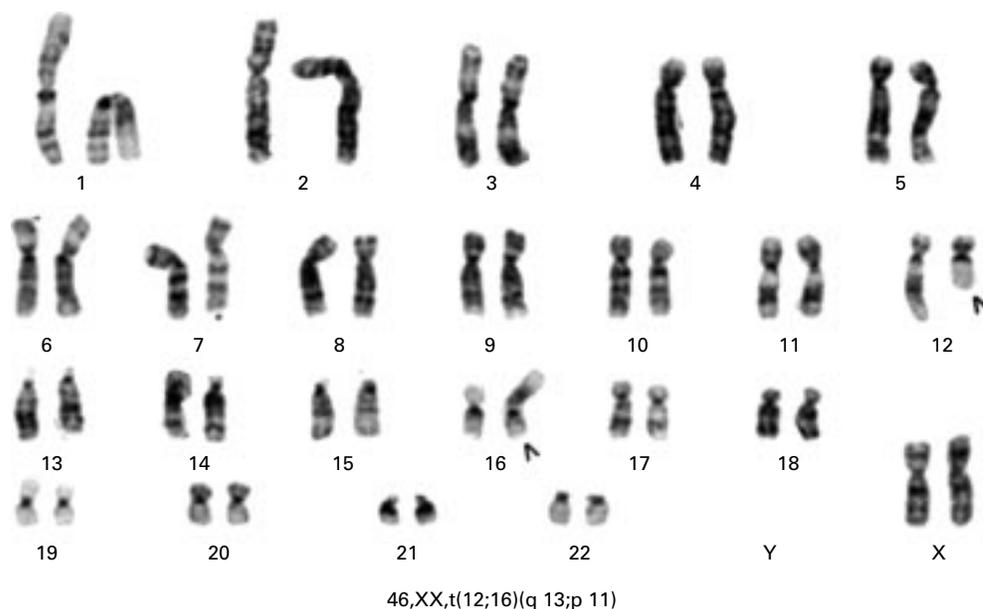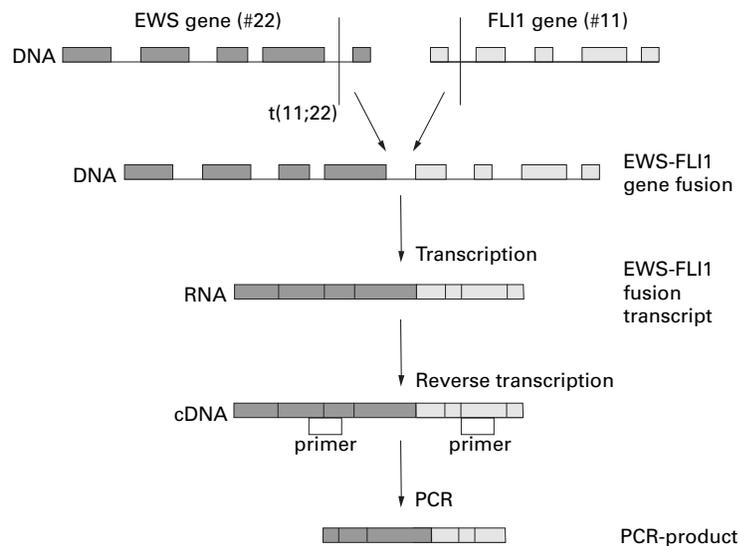

46,XX,t(12;16)(q 13;p 11)

Figure 1   Karyotype analysis of a myxoid liposarcoma, showing the classical t(12;16) reciprocal translocation (arrowheads). Courtesy of S L van Zelderen-Bhola, Department of Clinical Cytogenetics, Leiden University Medical Centre.

Figure 2   Schematic representation of the reverse transcriptase polymerase chain reaction (RT-PCR) reaction used for the t(11;22) chromosomal translocation detection in a case of the Ewing sarcoma. The chimaeric gene fusion product contains a portion of the EWS gene on chromosome 22 and the Fli1 gene on chromosome 11. Following RNA extraction from the tumour tissue, a single strand of DNA (copy-DNA or cDNA) is reverse transcribed from the mRNA template using either a site specific primer or a non-specific oligo-dT primer. The cDNA strand is subsequently amplified using the PCR technique. The enzyme DNA polymerase amplifies a selected segment of DNA situated between two site specific primers, one on either side of the chosen translocation to be detected. The PCR reaction will consequently only be positive if the chimaeric gene product is present.
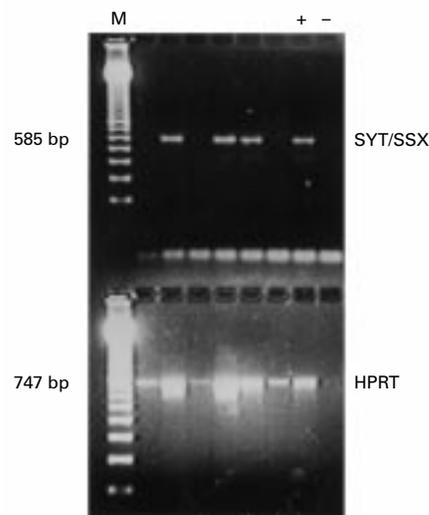
Figure 3   Reverse transcriptase polymerase chain reaction (RT-PCR) assay used to screen for the t(X;18) translocation present in synovial sarcoma. Agarose gel electrophoresis of amplified DNA following RT-PCR to screen histologically suspected cases of synovial sarcoma for the presence of the t(X;18) translocation, and visualised using ethidium bromide. Lane 1 of both the top and bottom gel contains a standard marker to control for PCR fragment size. While all the lanes in the bottom gel show the presence of a 747 base pair fragment derived from a ubiquitous gene used as a positive control for each RT-PCR reaction, lanes 3, 5, 6, and 8 in the top gel show a 585 base pair fragment supporting the presence of the described translocation and subsequently confirmed by in situ oligonucleotide mediated hybridisation (not illustrated). The lane marked + represents a positive control, known to carry a cytogenetically proven t(X;18) translocation; the lane marked—represents a negative control without the required template.

often unremarkable, nuclei, usually lacking distinguishing microscopic features which might aid in facilitating histological classification. Although special stains and immunohistochemistry may be of use in determining tumour type, especially in identifying NHL/leukaemia, and metastases from a malignant melanoma or small cell anaplastic carcinoma, it has become apparent that tumour specific genetic alterations may be very helpful in aiding the classification of those tumours that remain diagnostic dilemmas.

EWING SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOUR

Ewing sarcoma of bone predominantly afflicts adolescents and young adults, showing a predilection for the pelvis and long tubular bones of the extremities.[16] Primary extraskeletal Ewing sarcoma affects a similar age group, occurring predominantly in the paravertebral region, chest wall, and lower extremities.[17] A specific clinical entity in the Ewing sarcoma group, the Askin tumour, is a malignant small round cell tumour of the thoracopulmonary region occurring predominantly in children, having a locally aggressive course and poor prognosis.[18] The skeletal/extraskeletal Ewing sarcoma/primitive neuroectodermal tumour (PNET) family of tumours is generally believed to represent a single spectrum of neoplasms, differing only in their degree of neural differentiation. The Ewing sarcoma group lacks any degree of differentiation at a light microscopic level, while at the atypical Ewing sarcoma/PNET end, increasing neural differentiation is present, as assessed at a microscopic, immunohistochemical, and ultrastructural level.[19]

Immunohistochemically this family of tumours expresses p30/p32 encoded by the MIC2 gene and detectable using the monoclonal antibody O13.[20] Tumours within the sarcoma group, as well as unrelated tumours, have increasingly been found to show immunoreactivity for this epitope, limiting its diagnostic use.[21] Three cytogenetic translocations specific for this group have been identified to date, all involving the **Ew**ing **s**arcoma gene (EWS) on the long arm of chromosome 22 (table 3).[22] In approximately 90% of cases material is exchanged with the long arm of chromosome 11 resulting in a fusion of EWS with the **f**riend **l**eukaemia virus **i**ntegration site **1** gene (FLI1) present at this site on chromosome 11. In the remaining 10% of cases the translocation results in fusion of the EWS gene with the **E**TS **r**elated **g**ene (ERG) on chromosome 21 or the **E**TS **t**ranslocation **v**ariant **1** gene (ETV1) gene on chromosome 7.[22–25]

The t(11;22) translocation was the first sarcoma translocation to be cloned and characterised at the molecular level and remains the paradigm for oncogenesis in sarcomas.[23] The EWS gene encodes a ubiquitous protein of unknown function containing RNA binding domains, while the FLI1, ERG, and ETV1 genes all belong to the **e**rythroblastosis virus **t**ransforming **s**equence (ETS) family of transcription factor encoding genes.[9] The resulting hybrid (chimaeric) protein product contains

comparable histological phenotype (table 1). These tumours are generally composed of small primitive round to oval cells with round,

Table 3 Translocations of diagnostic significance in the sarcoma group

Tumour	Translocation	Genes involved
Ewing sarcoma/atypical Ewing	t(11;22)(q24;q12)	EWS/FLI1
sarcoma/Askin tumour/PNET	t(21;22)(q22;q12)	EWS/ERG
	t(7;22)(p22;q12)	EWS/ETV1
Intra-abdominal desmoplastic small		
round cell tumour	t(11;22)(p13;q12)	EWS/WT1
Clear cell sarcoma	t(12;22)(q13;q12)	EWS/ATF-1
Myxoid chondrosarcoma	t(9;22)(q22;q11–12)	EWS/CHN
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	PAX3/FKHR
	t(1;13)(p36;q14)	PAX7/FKHR
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SYT/SSX1;SSX2
Myxoid liposarcoma	t(12;16)(q13;p11)	CHOP/TLS (FUS)
Congenital fibrosarcoma	t(12;15)(p13;q25) ?	ETV6/NTRK3

PNET, primitive neuroectodermal tumour.

the N-terminal domain derived from EWS and a C-terminal domain of a transcription factor derived from the ETS family.[26] A general hypothesis is that the chimaeric proteins represent deregulated activated transcription factors with sequence specific DNA binding domains directed towards tumour specific binding sites in the genome, and directly or indirectly influence genes responsible for the neoplastic phenotype through the EWS portion of the chimaera.[9] Analysis of the predominant EWS/ FLI1 translocation has identified various breakpoints in EWS clustered between exons 7 and 10—with FLI1 showing a similar variability in breakpoints clustered between exons 5 and 8—resulting in a diversity of fusion products, of which two (types I and II) account for roughly 80% of the EWS/FLI1 hybrids.[9 26] Similarly, several EWS/ERG variants have been identified.[26 27]

Recent work is beginning to suggest that the variability in chimaeric products may be of essential use in identifying distinct risk groups within the general group of Ewing sarcoma patients and would thus impart important independent prognostic information.[13 14 28 29]

The translocations described in the Ewing sarcoma/PNET family are readily demonstrable using karyotype analysis of chromosome spreads, FISH, or RT-PCR and are important for aiding or confirming accurate tumour diagnosis.[22 30]

Recently, the t(11;22) translocation has been identified in a group of biphenotypic sarcomas showing both neural and myogenic features.[31] Although this group of malignant ectomesenchymomas may thus appear to be related to the Ewing sarcoma/PNET group, the precise nature of the relation with respect to histogenesis and to biological and clinical behaviour is still unclear.[9] Interestingly, both a putative small cell variant of osteosarcoma and a mesenchymal chondrosarcoma have been reported containing a t(11;22)(q24;q12) translocation at a cytogenetic level.[32 33] The breakpoint has not as yet been determined and the pathological implications and significance with respect to tumour histogenesis remain to be determined.

Numerous other non-random chromosome alterations have been identified in the Ewing sarcoma/PNET group, including trisomy 8 and trisomy 12—although these do not appear to be tumour specific and thus are not of known diagnostic or prognostic significance as yet.[7]

INTRA-ABDOMINAL DESMOPLASTIC SMALL ROUND CELL TUMOUR

The intra-abdominal desmoplastic small round cell tumour is a primitive sarcoma occurring predominantly in adolescent males, particularly on the serosal abdominal surfaces. It shows extreme desmoplasia and immunohistochemical positivity for epithelial, neural, and myogenic markers.[34] Cytogenetic analysis has identified an invariable, non-random t(11;22)(p13;q12) translocation which appears to be restricted to this tumour, generating a fusion between EWS on chromosome 22 and the **W**ilms **t**umour gene (WT1) on chromosome 11,[35] detectable by RT-PCR.[36]

ALVEOLAR RHABDOMYOSARCOMA

Rhabdomyosarcoma is the most common malignant mesenchymal tumour occurring under the age of 25 years. Rhabdomyosarcoma has a highly variable morphology and is subdivided into embryonal, alveolar, and pleomorphic types. Immunohistochemically these tumours may be distinguishable from other small round cell tumours by positivity for the myogenic markers and expression of the MyoD1 gene.[37] Of the three subtypes generally recognised, a specific non-random genetic translocation—t(2;13)(q35;q14)—has been identified in approximately 80–90% of the alveolar group, including the solid variant,[38] while cytogenetic analysis of the other subtypes has not as yet identified non-random alterations. The translocation results in a fusion between the **pa**ired bo**x 3** gene (PAX3) on chromosome 2 and the transcription factor **fo**r**kh**ead **r**elated gene (FKHR), also referred to as the **alv**eolar rhabdomyosarcoma gene (ALV), on chromosome 13.[39] The translocation results in an activated transcription factor containing exons from the 5' end of PAX3, encoding a DNA binding domain, joined to a portion of the forkhead DNA binding domain and additional C-terminal regions. A variant translocation—t(1;13)(p36;q14)—has been recently described in alveolar rhabdomyosarcoma involving the same FKHR gene but another gene in the PAX family, PAX7.[40] Additionally, amplification of N-myc has been detected in a large proportion of the alveolar rhabdomyosarcoma group, although the diagnostic or prognostic significance of this finding remains uncertain.[41] Recent work by Kelly *et al* suggests that the type of PAX/FKHR transcription factor hybrid influences the clinical presentation and clinical course, that is the prognosis, within the alveolar rhabdomyosarcoma group.[42]

The clinical management of alveolar and embryonal rhabdomyosarcoma is somewhat different, particularly with respect to the chemotherapy regimen used, and embryonal rhabdomyosarcoma appears to have a generally better prognosis than its alveolar counterpart.[43] Consequently, it is tempting to speculate about the possible prognostic significance of the lack of the t(2;13) translocation and lack of N-myc amplification in this group. While N-myc amplification has also been detected in neuroblastoma and appears to correlate with an

advanced disease state, the absence of the specific t(2;13) translocation may facilitate in the distinction between these lesions at a molecular level in difficult cases, such as between the solid variant of alveolar rhabdomyosarcoma and embryonal rhabdomyosarcoma.[44 45] Additionally, embryonal rhabdomyosarcoma often has a deletion of the short arm of chromosome 11, a second feature aiding the clinically important distinction between the alveolar and embryonal types of rhabdomyosarcoma.[6 44]

NEUROBLASTOMA

Neuroblastoma is one of the most common malignant tumours of childhood, exceeded only by leukaemia and brain tumours.[46] The peak age of presentation is around 18 months and roughly a quarter of the cases are congenital. The majority are sporadic and only a small percentage occurs in a familial setting.[46] Sites of predilection follow the distribution of the sympathetic nervous system, occurring predominantly in the paramidline between the skull and pelvis. Neuroblastoma can be clinically divided into two groups based on disease progression, and this shows a good correlation with certain genetic alterations.[45 47] The first group invariably demonstrates deletion of the short arm of chromosome 1 between 1p32 and 1pter, while roughly half of the tumours are associated with N-myc amplification.[45 47] These patients are seldom cured, even with intensive treatment. The second group has neither a deletion of the short arm of chromosome 1 nor N-myc amplification and generally has a far better prognosis, even occasionally undergoing spontaneous regression.[47] The absence of the t(2;13) translocation present in alveolar rhabdomyosarcoma, coupled with the presence of N-myc amplification, should facilitate distinction between these two round cell tumours. Although the described genetic alterations in neuroblastoma are not diagnostically useful owing to a lack of tumour specificity, they are of proven prognostic value and a multiplex PCR is available to assess loss of heterozygosity on chromosome 1.[48]

OTHER SMALL ROUND CELL TUMOURS

The additional tumours listed in table 1 may represent diagnostic dilemmas when encountered in unusual clinical settings. Using the current histopathological methods available, including immunohistochemistry and electron microscopy, the correct diagnosis is generally apparent. Recently a novel report has appeared documenting five cases of primitive, small round cell tumours with histochemical and ultrastructural features supporting a neural line of differentiation, and without evidence of EWS chimaeric transcripts as analysed using RT-PCR; cogent arguments were put forward proposing these poorly differentiated lesions as possible variants of malignant peripheral nerve sheath tumours which may further complicate the histological assessment of the small round cell tumour group.[49]

The spindle cell tumours

This represents a group of histologically poorly or undifferentiated tumours including monophasic synovial sarcoma, malignant peripheral nerve sheath tumour (MPNST), so called "malignant fibrous histiocytoma (MFH)," fibrosarcoma, and leiomyosarcoma (table 2). The spindle cell tumours are composed of generally spindled cells with elongated, cigar shaped, or pointed nuclei, often making distinction on histological grounds difficult. Immunohistochemistry and electron microscopy can aid in reaching the correct diagnosis in a proportion of cases, but tumour specific genetic alterations may be particularly useful in those cases hardly classifiable on a histopathological basis alone.[6–9]

SYNOVIAL SARCOMA

Synovial sarcoma, a highly malignant mesenchymal tumour predominantly of adolescents and young adults, accounts for approximately 5–10% of soft tissue sarcomas, and occurs preferentially in the extremities in the vicinity of joints, usually around the knee and in the thigh region.[50] The classical biphasic variant contains both epithelioid and spindle cell components, while the more unusual monophasic variant may be composed of either epithelial or spindle cells. Additionally, roughly 20% of the tumours may be very poorly differentiated, complicating the diagnosis using routine histopathology, especially in biopsy specimens. Cytogenetic analysis has shown a non-random and highly specific t(X;18)(p11;q11) translocation in 90–95% of all cases, irrespective of subtype.[51 52] The translocation generates a fusion between the **sy**novial sarcoma **t**ranslocation gene (SYT) on chromosome 18 and either of the **s**ynovial **s**arcoma **X** breakpoint **1** or **2** genes (SSX1 or SSX2), two highly homologous genes on band Xp11 (53) (table 3). Several other changes appear to accompany the described translocation,[51] although these are variable and not of apparent diagnostic use. Two cases of undifferentiated mesenchymal tumours, not diagnosed as synovial sarcoma, have been reported containing the t(X;18) translocation, a fibrosarcoma, and a malignant fibrous histiocytoma.[53] Since the histological distinction between various poorly differentiated sarcomas may be difficult, these reports probably represent variants of synovial sarcoma.[8] Kawai *et al* have recently shown a significant correlation between the genotype (SYT/SSX1 or SYT/SSX2) and histological morphology,[12] supporting similar earlier observations[54 55]: translocation positive biphasic tumours tend to carry the SYT/SSX1 hybrid, while translocation positive monophasic tumours tend to carry the SYT/SSX2 variant. Additionally, Kawai *et al* have shown for the first time that the translocation type in translocation positive tumours has important prognostic value: the SYT/SSX2 group appear to show significantly improved metastasis-free survival time.[12] The described genetic alterations are detectable using karyotype analysis and FISH, but the development of a RT-PCR

assay is now the method of choice for establishing the correct diagnosis.[56]

MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR

Both benign and malignant neural tumours occur sporadically or in association with the hereditary neurofibromatosis syndromes. MPNST is an aggressive tumour occurring sporadically as well as in about 1–2% of patients with neurofibromatosis type I (von Recklinghausen disease, NF1).[57] Although no tumour specific genetic alterations have been detected in this group, aberrations of the NF1 locus at 17q11 occur relatively often in this group,[58 59] while loss of heterozygosity on chromosome 17 in malignant tumours in NF1 patients occurs in more than 50% of cases.[60] The p53 tumour suppressor gene, although a non-specific variable, appears to be mutated in a large number of cases, the direct significance of which is unknown.[61] Consequently, no diagnostic genetic methods are as yet available to identify these lesions unambiguously, and histology remains the bastion of excellence in this group.

MALIGNANT FIBROUS HISTIOCYTOMA

This remains a controversial histological soft tissue entity. A thorough and in depth review of a large group of such tumours indicated that, with extensive investigation, many of them showed some degree of differentiation, allowing classification into one of the other sarcoma groups.[62] The prevailing impression is that the remaining unclassifiable lesions represent undifferentiated tumours in which possible differentiation is not identifiable using current techniques.[62] p53 Mutation analysis in several of these tumours suggests that malignant fibrous histiocytoma (MFH) of bone carries a different subset of mutations from “MFH” of the soft tissue, lending support to the concept that MFH of bone is a separate clinical entity.[63] Attempts at identifying prognostic variables in “MFH” tumours have indicated that lesions in this heterogeneous group bearing additional material on chromosome 19p appear to have a worse prognosis with a higher relapse rate.[64]

FIBROSARCOMA, LEIOMYOSARCOMA

(Non-congenital) fibrosarcomas occur anywhere in the body where fibrous tissue is found and are clustered in patients between the ages of 30 and 55 years, although any age group may be affected. Leiomyosarcomas are tumours of adults, more commonly affecting women and with a preference for retroperitoneal and abdominal sites. These malignant tumours contain a large number of random alterations which cannot be used in the clinical setting at present.[6] Interestingly, a t(12;14) translocation has been identified in approximately 20% of uterine leiomyomas but not in its malignant counterpart.[65] It remains to be ascertained whether the loci involved on chromosome 12 or 14 in these leiomyomas are altered by alternative mechanisms in leiomyosarcomas.

CONGENITAL FIBROSARCOMA

Congenital (infantile) fibrosarcoma is a rapidly growing but low grade malignant tumour occurring in neonates, infants, and small children under the age of two years and found predominantly in the extremities. The lesion has a similar morphology to its adult counterpart but has a significantly less aggressive course and a very low metastatic potential. Distinction from fibromatosis, a benign fibrous tumour, may be difficult.

Cytogenetic analysis of three cases has recently identified a novel t(12;15)(p13;q25) translocation involving the **E**TS **t**ranslocation **v**ariant **6** (ETV6) gene (also known as TEL) at 12p13 and the **n**eurotropic **t**yrosine **k**inase **r**eceptor type **3** (NTKR3) gene (formerly known as TRKC) at 15q25; the subsequent expression of a chimaeric protein tyrosine kinase is postulated to have an oncogenic potential through disruption of the signal transduction pathways.[66] The tumour specificity of this translocation remains uncertain and analysis of additional cases and other tumour groups needs to be done to confirm an aetiological role in congenital fibrosarcoma. Additionally, earlier work has shown the presence of non-random genetic changes, including trisomy of chromosomes 11, 17, and 20 in cases analysed, although these changes are not specific for congenital fibrosarcoma.[67]

DERMATOFIBROSARCOMA PROTUBERANS

This represents a cutaneous/subcutaneous fibrohistiocytic tumour, occurring predominantly in early and mid-adult life and with a locally aggressive mode of behaviour. It has a high local recurrence rate, probably because of inadequate local excision of the extensive infiltrative margins. Very few cases have been studied cytogenetically, but a recurring t(17;22)(q22;q13) translocation has been documented the significance of which is as yet uncertain.[68]

Genetic alterations in other sarcomas

CLEAR CELL SARCOMA OF TENDONS AND APONEUROSES

Described in 1965 by Enzinger,[69] clear cell sarcoma is now a well recognised clinicopathological entity, usually targeting young adults and occurring predominantly in the extremities, often in intimate relation to the tendons or aponeuroses. The tumour has a neuroectodermal origin and may create enormous diagnostic difficulties with other round cell tumours, especially metastatic malignant melanoma to the soft tissues or bone in the absence of a known primary cutaneous lesion.[70] Cytogenetic findings confirm the clinical impression that this tumour is a distinct entity; using karyotype analysis approximately 65–70% of cases contain a tumour specific translocation— t(12;22)(q13;q12)—which results in the fusion between the EWS gene on chromosome 22 and the **a**ctivating **t**ranscription **f**actor **1** gene (ATF-1, a transcription factor) on chromosome 12.[70 71] Additionally, it is likely that alterations at this locus may be found in a higher proportion of tumours if a more
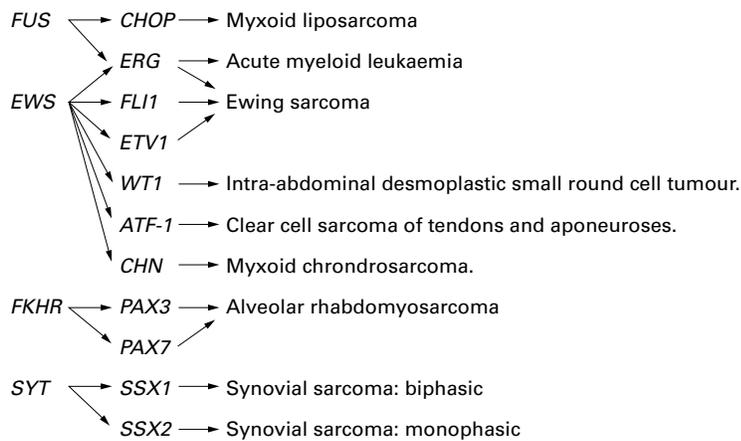
Figure 4 *Gene fusion partners and tumour specificity.*

sensitive assay such as RT-PCR is used. As yet, this translocation has not been detected in any other neoplasm (table 3) and is readily demonstrable using karyotype analysis, FISH, or RT-PCR.[70 71] As in the EWS family, there is variability in the exact site of the breakpoint and to date two variants have been identified, although any possible significance this may have clinically has not yet become apparent.[70]

LIPOSARCOMA

Liposarcomas generally arise in the retroperitoneum and thigh of adults and are divided into four groups: well differentiated, myxoid, round cell, and pleomorphic. It has been established that more than 75% of myxoid liposarcomas contain a t(12;16)(q13;p11) translocation, resulting in the fusion of the **C**CAAT/enhancer binding protein **ho**mologous **p**rotein gene (CHOP) on chromosome 12 and the **t**ranslocated in **l**ipo**s**arcoma gene (TLS, otherwise known as FU**S**: **fus**ion) on chromosome 16,[72 73] and not identified in any other group of myxoid sarcomas. The CHOP/TLS fusion gene in myxoid liposarcoma appears to encode an activated transcription factor, and the mechanism of action is postulated to be similar to the EWS/ETS fusion products.[9] Demonstration of the translocation is possible cytogenetically or by RT-PCR.[74] The other types of liposarcoma show a variety of random genetic alterations, the significance of which is as yet unknown.[7–9]

Most solitary lipomas (60%), excluding angiolipomas, have been shown to harbour cytogenetic alterations often involving a breakpoint on chromosome 12(q13-15). Interestingly, this breakpoint lies distal to the region involved in myxoid liposarcoma, but targets the same gene that is implicated in many uterine leiomyomas.[6 65 75]

Angiolipomas do not, however, appear to show cytogenetic alterations, in contrast to other lipomas, supporting the proposal that this represents a distinct group of tumours.[76]

CHONDROSARCOMA

Cartilaginous skeletal and extraskeletal neoplasms are a diverse group of lesions with respect to their clinical and pathological presentation.[77] Of the various histological types

of chondrosarcoma, only the extraskeletal myxoid group has shown a non-random t(9;22)(q22;q11-22) translocation; this occurs in approximately 25% of cases in which the EWS gene is fused with the **t**ranslocated in **e**xtraskeletal **c**hondrosarcoma gene (TEC, also known as **ch**o**n**drosarcoma, CHN) present at 9q22 and believed to encode an orphan nuclear receptor protein containing a zinc finger DNA binding domain,[78] and detectable by RT-PCR analysis.[79]

Chondrosarcomas arising from pre-existing sporadic or hereditary exostoses appear to involve the **ex**os**t**oses (EXT) genes located at 8q24, 11p1̄2, and 19p which have also been implicated in the hereditary multiple exostosis syndrome, a heterogeneous autosomal dominant disease.[80 81] Recent work is beginning to indicate that p53 mutations may be correlated with increasing histological grade.[82 83]

GASTROINTESTINAL STROMAL TUMOUR

The gastrointestinal stromal tumour (GIST) group represents a common, usually solitary, mesenchymal tumour of the gastrointestinal tract, with variable and unpredictable biological and clinical behaviour. Recently, gain of function mutations has been identified within the c-kit proto-oncogene, which codes for a type III tyrosine kinase receptor protein (CD117), although tumour specificity remains to be determined.[84]

General considerations and discussion

The detection and description of apparently tumour specific genetic alterations within the sarcoma group is beginning to play an increasingly important role in unravelling and beginning to understand the molecular biology of tumorigenesis.

Interestingly, there are many structural similarities among the majority of tumour specific reciprocal translocations identified to date within the sarcomas. Gene fusion products are generated combining a gene with a putative RNA binding domain (EWS, TLS) or DNA binding domain (PAX3, PAX7) with a transcription factor gene (FLI1, ERG, ETV1, ATF-1, WT1, CHN, or FKHR). The promiscuous EWS gene on chromosome 22 is frequently involved, generating chimaeric gene fusion products with FLI1, ERG, ETV1 (Ewing sarcoma), WT1 (intra-abdominal desmoplastic small round cell tumour), ATF-1 (clear cell sarcoma), and CHN (myxoid chondrosarcoma) (fig 4). The CHOP/TLS chimaera (myxoid liposarcoma) has a similar construction. A generally held hypothesis is that while the EWS fusion partners specify DNA binding and hence tumour type, the EWS portion would regulate or deregulate RNA processing and synthesis, somehow contributing to tumorigenesis.

In addition to the genetic alterations described in this monograph, the role of tumour suppressor genes in the sarcomas is becoming clearer. p53 Gene mutations, although not tumour specific, have been detected in 30–70% of cases of so called "malignant fibrous histiocytoma," leiomyosarcomas, rhabdomyosarco-

mas, and liposarcomas, while these do not appear to play a role in neuroblastomas.[8 85] Mutations in the retinoblastoma gene (Rb) have been detected in a large proportion of high grade sarcomas unrelated to hereditary retinoblastoma, including leiomyosarcomas, "malignant fibrous histiocytomas," liposarcomas, and malignant peripheral nerve sheath tumours.[8] The coinactivation of p53 and Rb indicates that both genes may be involved in tumorigenesis of certain sarcomas.[8] Additionally the occurrence of soft tissue sarcomas as part of certain clinical syndromes such as in the Li-Fraumeni and Gardner syndromes, as yet poorly understood, is intellectually challenging and will probably generate much new information about tumour origin.

In the last decade the rapid developments in the field of molecular genetics and the increasing sophistication of laboratory techniques available to the clinician have underscored the value of the cytogenetic evaluation of bone and soft tissue tumours as an essential adjunct for accurate histopathological diagnosis. An accurate histological classification of tumour types is necessary to establish meaningful clinical trials of optimal management strategies. In addition, cytogenetic evaluation is an important tool in attempting to identify variables that may influence the clinical progression of disease and prognosis.

With the increasing identification of genes involved in translocation breakpoints, PCR and RT-PCR are increasingly becoming the methods of choice for rapid, sensitive, and specific detection of the cytogenetic and molecular alterations present in sarcomas.

For those bone and soft tissue tumours in which the non-random, specific molecular events are as yet uncrystallised, the search is continuing in awareness of the important contribution such new information will make to our ultimate understanding of tumour biology.

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