Pathology of soft tissue sarcomas with emphasis on molecular diagnostic techniques

Pancras C.W. Hogendoorn

Department of Pathology, Leiden University Medical Centre, 2300 RC Leiden, the Netherlands

Introduction

Soft tissue sarcomas (STS) are rare, accounting for less than 1% of all neoplasms. As a result, progress in recognising specific morphological and clinical subsets and application of modern techniques in meaningful numbers can only be achieved in a multicentred approach, combining (inter)national experience. In recognition of this prerequisite for progress, more and more patients are currently diagnosed and treated in specialised sarcoma centres.

As a result of increased interest in these tumours and the centralisation of diagnosis and treatment, the expertise and the ability to recognise subsets of tumours have increased. The diagnosis and classification of STS have been influenced greatly over the past decades by developments in cytogenetics and molecular genetics. Consequently, a substantial number of new clinicopathological entities are recognised, whereas those previously recognised as separate tumours are now considered to be part of a morphological spectrum based upon a shared molecular genetic make-up. This type of information is increasingly more important (along with the importance of molecular genetic information in sub-classifying tumours) in predicting the response to pre-operative treatment. This is a result not only of the fundamental genetic changes in the tumour, but also the identification of drug-able targets: molecules that are overexpressed as a result of down-stream activation by initial genetic events such as translocations.

Genetics and tumour classification

It has been recognised over the past decade that a substantial number of sarcomas harbour tumour-specific genetic abnormalities. These abnormalities are tumour-specific in that they occur only in specific tumour types and not in normal non-neoplastic cells, or unrelated tumours. The most important tumour-specific genetic abnormalities identified so far are given in Table 1. The specificity of these genetic events gives not only substantial insight into tumorigenesis, but offers possibilities for molecular diagnosis as well. This has resulted in a substantial impact of cytogenetic data on tumour classification, leading to the recent 2002 World Health Organization (WHO)

Table 1
Translocations of diagnostic significance in soft tissue tumours

Tumour	Translocation(s)	Genes involved
Ewing's sarcoma/atypical ES/	t(11; 22) (q24; q12)	*EWS/FLI1*
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/Round cell liposarcoma	t(12; 16) (q13; p11)	*CHOP/TLS (FUS)*
Congenital fibrosarcoma	t(12; 15) (p13; q25)	*ETV6/NTRK3*

PNET, primitive neuroectodermal tumour.

classification of tumours of soft tissue and bone. This classification combines (for the first time) clinical, epidemiological, morphological and genetic information. As a result, for instance, Ewing's sarcoma, primitive neuroectodermal tumour (PNET) and Askin tumour are now categorised within the morphological spectrum of one entity harbouring a unifying set of tumour-specific translocations (see Table 1). Also myxoid and round cell liposarcoma are now grouped together based upon their common translocation, t(12;16), as one tumour entity with a diverging morphological spectrum. Other tumours such as clear cell sarcoma — previously known as melanoma of the soft parts — are now defined by their specific translocation, t(12;22) (q13;q12), which is never found in cutaneous melanoma [1,2]. This knowledge has obvious consequences for differential diagnosis in daily practice. Analogous alveolar rhabdomyosarcoma, along with its rare solid variant, harbour the characteristic translocation, t(2;13). The rare solid variant of alveolar rhabdomyosarcoma, in particular, can be difficult to separate from embryonal rhabdomyosarcoma, with subsequent consequences for predicting prognosis and choice of treatment. Given the persistence of t(2;13), even in the solid variant of alveolar rhabdomyosarcoma, it readily sets it apart from embryonal rhabdomyosarcoma, which does not have this chromosomal rearrangement. A previously popular diagnosis like haemangiopericytoma has now virtually ended its contentious existence, based upon the recognition of the morphological overlap with synovial sarcoma, solitary fibrous tumour and mesenchymal chondrosarcoma, each of them either defined by specific cytogenetic aberrations or immunohistochemical expression pattern [3,4].

The aforementioned structural chromosomal aberrations are detectable at a cytogenetic and/or molecular level, and appear to be, especially when compared to the genetic aberrations described in the far more prevalent carcinoma group, less complicated and consequently more accessible for study. This has resulted in a rapid acquisition of new information concerning the molecular genetics of sarcomas. New entities are born; others are no longer in use. Since many high-grade sarcomas may present morphologically as poorly differentiated tumours lacking distinctive phenotypic features, the presence of chromosomal changes serves as an important aid in solving some of the encountered diagnostic problems and thus subclassifying the large group of high-grade tumours into more specific entities, with distinct prognosis. This development will certainly lead to the virtual abolishment of descriptive non-specific "entities" like pleomorphic malignant fibrous histocytoma [5]. In fact in the 2002 edition of the WHO's tumour classification [6], based upon the consensus view of a group of multinational experts, this once so common tumour description is now only justified in a very narrow restricted category of cases, accounting for less than 5% of adult sarcomas.

A variety of techniques to detect specific genetic abnormalities are available. These include immunohistochemistry for the detection of products of altered gene expression, karyotype analysis of chromosome spreads, fluorescence *in situ* hybridisation (FISH) of interphase nuclei to identify certain genetic rearrangements, polymerase chain reaction (PCR) for genomic DNA analysis, and reverse transcriptase and/or real-time polymerase chain reaction (RT–PCR) for detecting altered mRNA products and DNA sequencing [7,8]. While a number of techniques are laborious and time-consuming, restricting them to research settings, others, like immunohistochemistry, RT–PCR (see Fig. 1), and interphase FISH are rapid, reliable and relatively cheap, making them ideal for clinicopathological use. Using these techniques, a specific diagnosis is possible using a minimal amount of material [7,9]. As the core needle biopsy technique provides only limited material, these techniques support its use. Furthermore, core needle biopsy techniques are being used more frequently and are more economical and patient-friendly when compared with open biopsies.

Genetics and biological behaviour

Genes like *p53*, *Rb*, *ras*, *myc*, *MDM2*, *CDK4*, *GLI*, etc. have been implicated in STS development. None of these however has proven to be tumour-specific; they more or less reflect general ongogenic pathways operative in the development of STS. A number of tumour types have been reported to occur on a familial or inherited basis [10]. In these syndrome-related tumours, *TP53* (Li–Fraumeni), *NF* (neurofibromatosis), *RB1* (retinoblastoma), or *APC* (Gardner's syndrome) play a crucial role. In gastrointestinal stromal tumours (GIST), constitutive activation of *KIT* has been demonstrated — the hallmark of this tumour often results from oncogenic point mutations involving the *KIT* cytoplasmic juxtamembrane region (for a review, see Ref. [11]). Only a few GIST lack *KIT* mutations. Interestingly, recent data indicate that the site and nature of the *KIT* mutations appear to be related to the clinical behaviour and response to treatment. Karyotypic analyses have so far not found tumour-specific abnormalities for GIST, although deletions of chromosome 14 and 22 are quite commonly found.

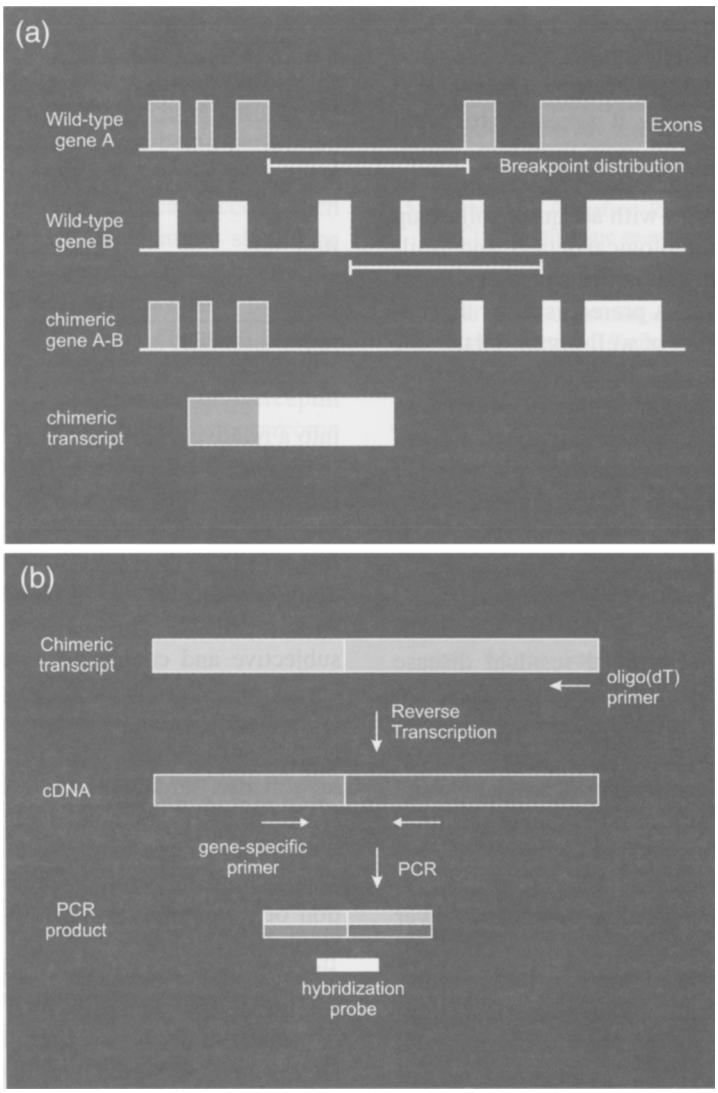

Fig. 1. Principle of RT–PCR. (a) The wild-type genes *A* and *B* constituting the chimeric gene of the translocation *A–B*. Coding sequences (exons) are indicated by solid boxes. (b) The principle of RT–PCR *in vitro*: cDNA is constructed following the RT reaction with oligo (dT) primer. This is followed by PCR with gene-specific primers flanking the breakpoint. The PCR product formed is hybridised with a specific probe to ensure specificity. A PCR product is formed only when the specific breakpoint is present.

Recent studies are beginning to provide increasing, and convincing, evidence that genetic variability within specific tumour groups, such as Ewing's sarcoma and synovial sarcoma, may also be of prognostic value, implying that transcript type determines clinical behaviour. Kawai et al. have recently shown a significant correlation between the genotype (*SYT/SSX1* or *SYT/SSX2*) and histological morphology [12]. Biphasic tumours tend to carry the *SYT/SSX1* hybrid, while monophasic tumours tend to carry the *SYT/SSX2* variant. Additionally, these authors have shown that the translocation type in translocation-positive tumours has important prognostic value: the *SYT/SSX2* group appears to show significantly improved metastasis-free survival time. Although confirmed in a subsequent paper of this group of distinguished authors [13], and by others [14], these findings were later challenged by a large multi-institute study from France [15]. Originally, research focused on the influence of the primary genetic event i.e. the tumour-specific translocation; it has now been recognised that these translocations activate the transcription of specific sets of genes and/or molecular pathways. It has, for instance, been shown that *EWS–FLI1* fusion in the Ewing family of tumours activates the transcription of target genes such as *PDGF*, *C-myc* and *MFNG* [16]. In synovial sarcoma, *SYT–SSX1* and/or *SYT–SSX2* rearrangements activate distinct target genes such as *HER*1 and other HER-family of tyrosine kinase growth factor receptors [17]. Molecular fingerprints

of STS (based upon the rapid evolvement of cDNA microarray technology [18]) should be available soon, which will help unravel these pathways. With the rapid maturation of RNA technology, it is possible to obtain RNA for microarray analysis, even from very paucicellular and matrix-rich sarcomas [19]. The need for meaningful patient numbers with adequate follow-up and comparable clinicopathological details, and available access to upfront technology, clearly calls for international collaboration. A prerequisite for this development is the availability of well-organised tumour banks, either "virtual" or real [20]. It will, however, require effort to overcome national ethics rules and laws to have these "well-guarded treasures in the interest of patients and research" multinationally available.

Minimal residual disease/bone marrow staging

The tumour specificity of certain translocations opens the possibility for minimal residual disease detection in specific sarcomas which are prone to metastasise to bone. The most challenging example in this regard at present is Ewing's sarcoma. RT–PCR for EWS–ETS fusion products is a sensitive and specific method for the indention of small numbers of circulating tumour cells not yet visible to the eye by microscopy. The current Euro–Ewing trial has a molecular side-study looking at the diagnostic power and prognostic impact of these as yet morphological unrecognised distant bone marrow localisations. Given, however, the large number of clinical prognostical parameters, a large number of patients will be needed before a prognostic implication can be stated for the individual patient.

Grading and revised categorisation of biological behaviour

As stated above, the recent developments in tumour genetics and recognition of tumour-specific expression profiles by immunohistochemistry have led to the 2002 WHO classification. This new classification also includes a revised categorisation of clinico-biological behaviour. The tumours were divided into four distinct categories reflecting their clinico-biological potential:

(1) benign
(2) intermediate (locally aggressive)
(3) intermediate (rarely metastasising)
(4) malignant.

Next to this categorising of tumour entities, STS are graded in order to separate malignant tumours into a relatively favourable and poor prognosis group [21]. The so-called French grading system (FNLCC) has proven to be superior to other grading systems such as Broder's system and the National Cancer Institute (NCI) system. As a result, the WHO recommends the use of the FNCLCC grading system, while acknowledging the fact that grading remains subjective and cannot be a substitute for appropriate tumour classification. Some tumours cannot be graded reliably and grading of these tumour types is thus not recommended [6]. These include alveolar soft part sarcoma, clear cell sarcoma, epithelioid sarcoma, angiosarcoma, malignant peripheral nerve sheath tumour and extra-skeletal myxoid chondrosarcoma. Uniformisation of grading systems and definition of exceptions to the rules makes it possible to compare trial results, as in the past groups of clearly different grades were discussed when comparing trial results. A separate issue which needs to be addressed in the near future is the lack of reliable morphological factors predicting prognosis in GIST. In an attempt to design a tumour-specific risk assessment scheme for these tumours, a proposal was formulated including tumour size and mitotic activity per 50 high-power fields (HPFs) as seen in Table 2 [22]. Whether this scheme proves useful for trial and individual patient management, independent of *KIT* mutation status and origin/site of the tumour, remains to be proven.

Table 2
Risk assessment in GIST taken from Ref. [22]

Risk	Size	Mitotic count
Very low	<2 cm	<5/50 HPF
Low	2–5 cm	<5/50 HPF
Intermediate	<5 cm	6–10/50 HPF
	5–10 cm	<5/50 HPF
High	>5 cm	>5/50 HPF
	>10 cm	Any mitotic rate
	Any size	>10/50 HPF

HPF = high-power field; no surface area defined yet.

Identification of protein expression and drug targeting

As mentioned above, several primary genetic events in tumours, such as translocations or mutations subsequently lead to expression of downstream drug-able targets such as C-kit, EGFR-1, Her-2 Neu. The expression of these molecules can be readily monitored either at the RNA level, or (even simpler and maybe more effective) at the protein expression level by immunohistochemistry. Immunohistochem-

istry, however, has its limits with regard to specificity, both as a result of primary antibody cross-reactivity as well as detection techniques such as antigen retrieval. Currently, clinical trials are being performed based on selective interactions with these molecules. Aside from trials with obvious clinical success, such as those targeting C-kit in GIST, caution should be one's guide. In osteosarcoma, for instance, despite original reports on HER2 overexpression [23–26], more and more critical papers have appeared [27,28] challenging the original observation. Phase II trials, however, have tested the efficacy of Herceptin in patients with relapsed or refractory osteosarcoma [24,29]. Obviously, standardisation in technology is necessary to avoid these caveats presented by overoptimistic interpretation of preliminary results. It opens a future use for pathology, not only in the careful diagnosis of tumours, but also in identifying potentially useful treatment options.

Conclusions

The rapid evolvement in our knowledge on the molecular biology of sarcomas has had an enormous impact on the understanding, classification and treatment of these rare tumours over the past ten years. In this respect, these rare tumours have acted as an example for other much more common tumours, both by presenting their pathogenetic mechanisms, as well as by unravelling potential targets for treatment. The role of pathology in future diagnostic and therapeutic strategies will become important; molecular biological techniques will be standard in routine work-ups in the near feature. As these tumours are rare, a worldwide network of both fundamental researchers as well as treating physicians has been formed, which has certainly contributed to increasing our knowledge. As such, these rare experiments of nature have taught the medical community much more than could have been foreseen by their relatively infrequent occurrence.

References

1 Langezaal SM, Graadt van Roggen JF, Cleton-Jansen AM, Baelde JJ, Hogendoorn PCW. Malignant melanoma is genetically distinct from clear cell sarcoma of tendons and aponeurosis (malignant melanoma of soft parts). Br J Cancer 2001, 84(4): 535–538.

2 Graadt van Roggen JF, Mooi WJ, Hogendoorn PCW. Clear cell sarcoma of tendons and aponeuroses (malignant melanoma of soft parts) and cutaneous melanoma: exploring the histogenetic relationship between these two clinicopathological entities. J Pathol 1998, 186: 3–7.

3 Fletcher CDM. Haemangiopericytoma — A dying breed? Reappraisal of an 'entity' and its variants: a hypothesis. Curr Diagn Pathol 1994, 1: 19–23.

4 Nappi O, Ritter JH, Pettinato G, Wick MR. Hemangiopericytoma: histopathological pattern or clinicopathologic entity? Semin Diagn Pathol 1995, 12: 221–232.

5 Fletcher CD, Gustafson P, Rydholm A, Willen H, Akerman M. Clinicopathologic re-evaluation of 100 malignant fibrous histiocy-tomas: prognostic relevance of subclassification. J Clin Oncol 2001, 19(12): 3045–3050.

6 World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon: IARC Press, 2002.

7 Graadt van Roggen JF, Bovee JVMG, Morreau J, Hogendoorn PCW. Diagnostic and prognostic implications of the unfolding molecular biology of bone and soft tissue tumours. J Clin Pathol 1999, 52: 481–489.

8 Graadt van Roggen JF, Bovee JVMG, Van der Woude HJ, Hogendoorn PCW. An update of diagnostic strategies using molecular genetic and magnetic resonance imaging techniques for musculoskeletal tumors. Curr Opin Rheumatol 2000, 12(1): 77–83.

9 Peter M, Gilbert E, Delattre O. A multiplex real-time pcr assay for the detection of gene fusions observed in solid tumors. Lab Invest 2001, 81(6): 905–912.

10 Congenital and inherited syndromes associated with bone and soft tissue tumours. In: Fletcher CDM, Unni KK, Mertens F (eds). World Health Organisation classification of tumours. Pathology and genetics of tumours of soft tissue and bone. Lyon: IARC Press, 2002: 348–367.

11 Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alternations. Hum Pathol 2002, 33: 484–495.

12 Kawai A, Woodruff J, Healy JH, Brennan MF, Antonescu CR, Ladanyi M. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. N Engl J Med 1998, 338: 153–160.

13 Antonescu CR, Kawai A, Leung DH, Lonardo F, Woodruff JM, Healey JH et al. Strong association of SYT-SSX fusion type and morphologic epithelial differentiation in synovial sarcoma. Diagn Mol Pathol 2000, 9(1): 1–8.

14 Panagopoulos I, Mertens F, Isaksson M, Limon J, Gustafson P, Skytting B et al. Clinical impact of molecular and cytogenetic findings in synovial sarcoma. Genes Chromosomes Cancer 2001, 31(4): 362–372.

15 Pelmus M, Guillou L, Hostein I, Sierankowski G, Lussan C, Coindre JM. Monophasic fibrous and poorly differentiated synovial sarcoma: immunohistochemical reassessment of 60 t(X;18) (SYT-SSX)-positive cases. Am J Surg Pathol 2002, 26(11): 1434–1440.

16 May WA, Arvand A, Thompson AD, Braun BS, Wright M, Denny CT. EWS/FLI1-induced manic fringe renders NIH 3T3 cells tumorigenic. Nat Genet 1997, 17(4): 495–497.

17 Helman L, Fletcher J, Baker L. The best targets in sarcomas. Proc Am Assoc Cancer Res, 2002 Program, 194.

18 Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX et al. Molecular characterisation of soft tissue tumours: a gene expression study. Lancet 2002, 359(9314): 1301–1307.

19 Baelde HJ, Cleton-Jansen A-M, Van Beerendonk H, Namba M, Bovee JVMG, Hogendoorn PCW. High quality RNA isolation from tumours with low cellularity and high extracellular matrix component for cDNA microarrays: application to chondrosarcoma. J Clin Pathol 2001, 54(10): 778–782.

20 Oosterhuis JW, Coebergh JW, van Veen EB. Tumour banks: well-guarded treasures in the interest of patients. Nat Rev Cancer 2003, 3(1): 73–77.
21 Graadt van Roggen JF. The histopathological grading of soft tissue tumours: current concepts. Curr Diagn Pathol 2001, 7(1): 1–7.
22 Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, et al. Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol 2002, 33(5): 459–465.
23 Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A et al. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 1996, 77: 71–78.
24 Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH et al. Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 1999, 17(9): 2781–2788.
25 Akatsuka T, Wada T, Kokai Y, Sawada N, Yamawaki S, Ishii S. Loss of ErbB2 expression in pulmonary metastatic lesions in osteosarcoma. Oncology 2001, 60(4): 361–366.
26 Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K et al. ErbB2 expression is correlated with increased survival of patients with osteosarcoma. Cancer 2002, 94(5): 1397–1404.
27 Maitra A, Wanzer D, Weinberg AG, Ashfaq R. Amplification of the HER-2/neu oncogene is uncommon in pediatric osteosarcomas. Cancer 2001, 92(3): 677–683.
28 Thomas DG, Giordano TJ, Sanders D, Biermann JS, Baker L. Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. Clin Cancer Res 2002, 8(3): 788–793.
29 Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH et al. Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod Pathol 2001, 14(12): 1277–1283.