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REVIEW ARTICLE

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Cytogenetic and molecular genetic techniques as adjunctive approaches in the diagnosis of bone and soft tissue tumors

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Abstract Important and meaningful advances have been made in mesenchymal tumor cytogenetics during the last two decades. A number of bone and soft tissue tumors have been shown to have recurrent, if not specific, chromosomal changes, particularly translocations. These changes not only serve as aids in the diagnosis and classification of bone and soft tissue tumors, especially in the differential diagnosis of those of confusing nature, but have also guided molecular studies in establishing the underlying genes involved. To date, a number of tumor-specific gene fusions have been identified and many have been shown to encode aberrant transcription factors. These key biological events in bone and soft tissue tumors are crucial not only to our understanding of the sarcomagenetic processes leading to the various tumors, but also ultimately in the design of specific therapies tailored to the genetic events in mesenchymal neoplasms.

Key words Cytogenetics · Chromosome · Karyotype · Ewing's sarcoma · Rhabdomyosarcoma · Sarcoma

Introduction

The dawn of cancer cytogenetics and subsequently of cancer molecular genetics started in 1956 when Tijo and Levan [1] published what ultimately proved to be the correct number of chromosomes in the human. The past developments which made this key advance possible included methodologies for cell and tissue culture to yield dividing cells, the use of colchicine for metaphase arrest and the application of hypotonic techniques for swelling the cells thus affording the examination of spreads in which the chromosomes could be visualized individually and their number reliably counted. Since the metaphase stage of the cells is the only one in which the chromosomes can be counted and examined, the importance of the developments just mentioned is readily appreciated.

Initial progress in cancer cytogenetics was achieved in hematological disorders, particularly the leukemias, since the affected cells can be easily obtained from the bone marrow and/or blood. An advantage in studying he-

matological disorders, in addition to the easy accessibility of the affected cells, is related to the nature and manifestations of these diseases. In contrast to many solid tumors whose development is often rather advanced when diagnosed, hematological disorders produce symptoms and manifestations which are recognized in the early stages. Thus, the cytogenetic findings frequently obtained in these conditions are confined to one event or a few at the most. This differs from the karyotypic findings in most cancers, which tend to be complex and/or numerous, probably due to the advanced stage at which these cancers are seen. An exception to this is bone and soft tissue tumors.

The chromosome changes in bone and soft tissue tumors were not realized until relatively late in the era of cancer cytogenetics. The description in the early 1980s of a specific change in Ewing's sarcoma spurred on studies on other tumors of mesenchymal origin [2]. The gist of these studies may be summarized as follows: a significant number of these tumors are associated with specific translocations resulting in the production of chimeric genes encoding for novel transcription factors involved in the causation of the tumors, while others are associated with multiple and complex changes (akin to those in epithelial tumors) pointing to a succession of changes required for tumor development. These aspects of the nature and significance of the chromosomal changes of bone and soft tissue tumors will be enlarged upon in this review.

Methodologies

Cytogenetic analysis

A mesenchymal tumor sample submitted for cytogenetic analysis should be representative of the neoplastic process and preferably be part of the specimen submitted for pathological examination. Necrotic and non-neoplastic tissues should be dissected from the sample. Larger samples are preferable (1–2 cm^3), but specimens obtained by needle biopsy can also be analyzed successfully. The tumor tissue should be transported to the laboratory in sterile culture medium or buffer solution as soon as possible following surgical removal. Specimens sent to cytogenetic laboratories over long distances (requiring 24–48 h for delivery) can be transported at room temperature or refrigerated (not frozen) in sterile isotonic saline or preferably culture medium containing serum.

Single cells or small cell clusters required for culture are obtained by mechanical and enzymatic disaggregation of the submitted specimen. For some high-grade tumors, metaphase cells can be extracted directly from disaggregated tissue if less than 1 h has passed from the time of biopsy, obviating the need for culture [3]. The duration of culture is individualized for each tumor, flask and dish, but typically ranges from 1 to 10 days. Harvest or culture arrest is carried out when "peak" mitotic activity is observed [4].

Fluorescence in situ hybridization (FISH)

Specific nucleic acid sequences can be visualized in individual metaphase and interphase cells by means of specially developed chromosome-specific probes and fluorescence in situ hybridization (FISH). This technique can be performed on fresh or aged samples (such as blood smears or cytological touch preparations), paraffin-embedded tissue sections, and disaggregated cells retrieved from fresh, frozen or paraffin-embedded material [5–8]. FISH is usually a same day or overnight procedure depending on the probes utilized. Paraffin-embedded material, however, may require more prolonged pretreatment, resulting in a slightly longer turn-around time.

FISH is a valuable technique for defining chromosomal rearrangements in bone and soft tissue tumors [9–25]. Sarcoma translocations (defined as an exchange of chromosomal material between two or more chromosomes) can be visualized in interphase cells by the use of site-specific probes labeled with fluorescent dyes. Bicolor FISH with translocation breakpoint "flanking" or "spanning" cosmid or yeast artificial chromosome (YAC) probes (labeled unique sequences using large-insert probes) or whole chromosome paint probes can be used diagnostically. A significant advantage of FISH is that it is not dependent on the procurement of metaphase cells and can be performed on tissues of limited quantity such as cytological touch preparations. With this approach, cryptic or masked translocations may be seen, a phenomenon similar to that described in chronic myelogenous leukemia [26]. This highly sensitive and reliable technology has been adopted into the routine service of many clinical laboratories and is an effective alternative to reverse transcriptase polymerase chain reaction (RT-PCR) analysis [27]. Turn-around times usually range from 1 to 3 days.

Reverse transcriptase polymerase chain reaction (RT-PCR)

The polymerase chain reaction (PCR) technique uses specific synthetic oligonucleotides to amplify a section of a given gene in vitro. With the additional step of reverse transcription (messenger RNA→complementary DNA), PCR can be carried out on DNA. This technique can be used to detect chimeric or fusion genes created by translocation events such as the 11;22 translocation [t(11;22)(q24;q12)] in Ewing's sarcoma. Ideally, snap-frozen tissue is preferred for RNA extraction and RT-PCR analysis, but this procedure can also be performed

on archival (paraffin-embedded) material if the RNA is of sufficient quality.

RT-PCR analysis is remarkably sensitive. It may allow for the detection of abnormalities present in cells too few to be identified by traditional cytogenetic or FISH methods. This method may be suitable for detection or monitoring of minimal (microscopic) residual disease. Also, RT-PCR analysis is not dependent on successful cell culture, and similar to FISH, RT-PCR analysis is rapid with a short turn-around time (2–4 days). The greatest disadvantage of RT-PCR analysis, in comparison with cytogenetic analysis, is its inability to detect chromosomal anomalies other than those for which the test was designed. With conventional cytogenetic analysis, all major chromosomal abnormalities, including those that were not initially anticipated by the clinician or laboratorian, may be uncovered.

Discussion

Cytogenetic analysis plays a direct, potentially decisive role in the examination of benign and malignant bone and soft tissue tumors. The histopathological classification of mesenchymal neoplasms, particularly "high-grade" lesions, often presents a challenge to the surgical pathologist. Electron microscopy may be helpful, but sampling errors can occur with the technique. Many lesions defy histological classification, even with the aid of immunohistochemistry. A distinct advantage of cytogenetic and molecular genetic analyses as diagnostic tools is that most primary chromosomal aberrations are not lost as a neoplasm becomes less differentiated. Also, primary or characteristic chromosomal abnormalities persist in metastatic and previously treated lesions. This is in contrast to some immunohistochemical and ultrastructural features.

Small round cell neoplasms

It is often difficult, with traditional histological approaches, to classify a group of tumors that have been morphologically categorized as "small round cell tumors", such as Ewing's sarcoma and peripheral primitive neuroectodermal tumors, rhabdomyosarcoma, lymphoma, desmoplastic small round cell tumor and neuroblastoma, because of their homogeneous appearance by light microscopy and their frequent lack of organ specificity. An accurate diagnosis is essential for purposes of treatment and prognosis as each neoplasm is biologically different. Fortunately, separate or distinct chromosomal anomalies have been recognized in most of these neoplasms (Table 1) [4, 5, 27, 28]. Identification of these anomalies is an important aid in establishing an exact diagnosis. These anomalies can be detected by conventional karyotypic analysis, FISH with probes from loci spanning or flanking the translocation breakpoints and RT-PCR analysis (Figs. 1–3).

Ewing's sarcoma (a tumor of unknown histopathogenesis) has been shown to share a functionally identical chromosomal rearrangement, t(11;22)(q24;q12), with peripheral primitive neuroectodermal tumors (pPNET, tumors of neural origin), indicating a common mechanism of oncogenesis and a similar tissue of origin for these distinct clinicopathological entities [29–31]. The 11;22

Table 1 Characteristic and variant chromosomal translocations and associated fusion genes in bone and soft tissue sarcomas

Neoplasm	Translocation	Fusion gene(s)
Alveolar rhabdomyosarcoma	t(2; 13)(q35; q14) t(1; 13)(p36; q14)	*PAX3/FKHR* *PAX7/FKHR*
Clear cell sarcoma	t(12; 22)(q13; q12)	*EWS/ATF1*
Congenital fibrosarcoma[a]	t(12; 15)(p13; q25)	*ETV6/NTRK3*
Desmoplastic small round cell tumor	t(11; 22)(p13; q12)	*EWS/WT1*
Ewing's sarcoma/pPNET	t(11; 22)(q24; q12) t(21; 22)(q22; q12) t(7; 22)(q22; q12) t(17; 22)(q21; q12) t(2; 22)(q33; q12)	*EWS/FLI1* *EWS/ERG* *EWS/ETV1* *EWS/EIAF* *EWS/FEV*
Synovial sarcoma	t(X; 18)(p11.2; q11.2)	*SYT/SSX1* *SYT/SSX2*
Myxoid/round cell liposarcoma	t(12; 16)(q13; p11) t(12; 22)(q13; q12)	*TLS*[b] */CHOP* *EWS/CHOP*
Extraskeletal myxoid chondrosarcoma	t(9; 22)(q22–31; q12) t(9; 17)(q22; q11)	*EWS/CHN*[c] *TAF2N/CHN*[c]
Alveolar soft part sarcoma	t(X; 17)(p11.2; q25.3)	?
Malignant hemangiopericytoma	t(12; 19)(q13; q13)	?

[a] This translocation is also seen in congenital mesoblastic nephromas confirming a relationship with congenital fibrosarcoma (Cancer Genet Cytogenet, in press [81], Cancer Res 1998; 58:5046–5048 [82] and Am J Pathol 1998; 153:1451–1458 [83])
[b] Also referred to as *FUS*
[c] Also referred to as *TEC*

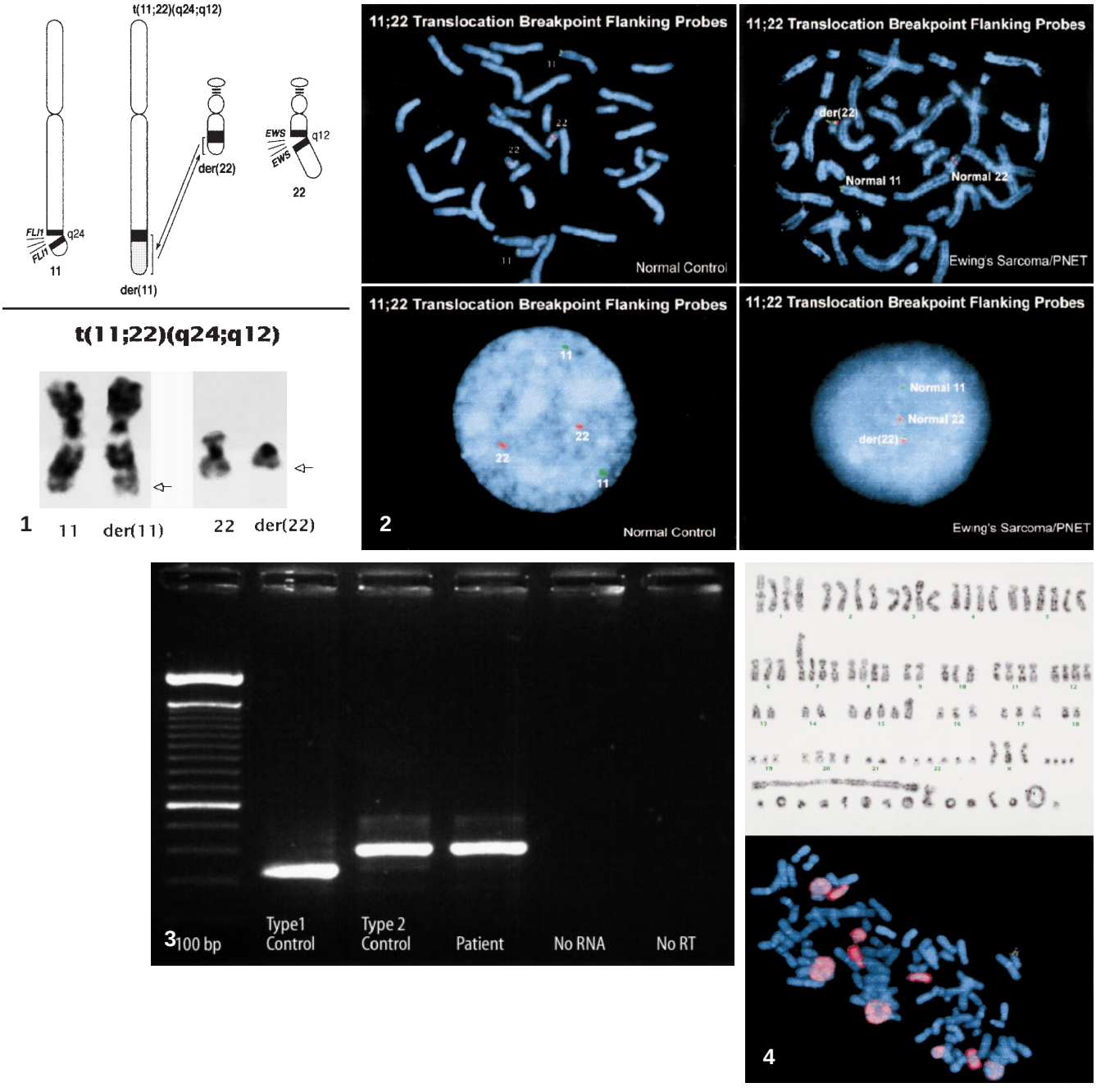

Fig. 1 *Top*: Schematic illustration of the 11; 22 translocation [t(11; 22)(q24; q12)] characteristic of Ewing's sarcoma and peripheral primitive neuroectodermal tumors. *Bottom*: G-banded (Giemsa stain) partial karyotype illustrating the 11; 22 translocation in a Ewing's sarcoma. (Reproduced from [81] with the permission of *Cancer Genetics and Cytogenetics*)

Fig. 2 *Top left*: Bicolor fluorescence in situ hybridization (FISH) studies performed on normal control metaphase cells with a chromosomal probe distal to the 11q24 translocation breakpoint (green) and a chromosomal probe proximal to the 22q12 translocation breakpoint (red) showing localization of the two probes to the normal chromosome 11 and 22 homologues. *Top right*: FISH performed on Ewing's sarcoma metaphase cells with the 11; 22 translocation breakpoint flanking probes, described in the top left, confirming the presence of this translocation. *Bottom left*: FISH performed on normal control interphase cells with the 11; 22 translocation breakpoint flanking probes. Two distinct green and two distinct red signals are seen as expected in a normal cell. *Bottom right*: FISH performed on a Ewing's sarcoma cytological touch preparation with 11; 22 translocation breakpoint flanking probes. The derivative chromosome 22 of the 11; 22 translocation is labeled. (Reproduced from [81] with the permission of *Cancer Genetics and Cytogenetics*)

Fig. 3 Reverse transcriptase polymerase chain reaction (RT-PCR) studies performed on a patient with Ewing's sarcoma. *Lane 1* 100 bp ladder, *lane 2* positive type 1 *EWS/FLI1* cell line control, *lane 3* positive type 2 *EWS/FLI1* cell line control, *lane 4* patient sample showing a type 2 *EWS/FLI1* fusion product, *lanes 5 and 6* negative controls, no RNA and no reverse transcriptase (RT), respectively. (Reproduced from [81] with the permission of *Cancer Genetics and Cytogenetics*)

Fig. 4 *Top*: G-banded metaphase cell of a well-differentiated liposarcoma demonstrating multiple ring chromosomes and a giant marker chromosome. *Bottom*: FISH analysis performed on a metaphase cell of the case illustrated above with a chromosome 12 paint probe (red signals). The ring chromosomes are composed of chromosome 12 material as well as some of the marker chromosomes and normal chromosome 12 homologues. These findings are typical of well-differentiated liposarcoma

translocation or variants thereof are detectable cytogenetically or by molecular approaches in more than 95% of Ewing's sarcomas and pPNETs [32–38]. As a consequence of the 11;22 translocation, the 5' portion of the *EWS* gene from 22q12 is fused to the 3' portion of the *FLI1* gene (a member of the ETS family of transcription factors) from 11q24. The fusion results in the formation of a tumor-specific chimeric RNA which encodes a novel transcription factor that retains ETS domain-specific DNA binding capability in combination with EWS transactivational properties [39, 40].

Cytogenetic studies of Ewing's sarcoma and pPNETs have revealed karyotypic rearrangements that differ slightly from the characteristic 11;22 translocation [t(11;22)(q24;q12)] in a subset of these tumors. These are referred to as "cytogenetic variant translocations." These variants exhibit rearrangements of 22q12 with a chromosomal partner other than 11q24. An example is the 21;22 translocation [t(21;22) (q22;q12)] that fuses *EWS* with another ETS family member, *ERG* (localized to 22q22), seen in approximately 5% of Ewing's sarcomas and pPNETs [35, 41]. At least four variant translocations (including *EWS/ERG*) have been defined cytogenetically and molecularly (Table 1) [27, 41].

In addition to the variably involved chromosomes in Ewing's sarcoma/pPNET rearrangements, considerable heterogeneity also exists with respect to specific *EWS/FLI1* molecular breakpoints. "Molecular variants" in this text refer to alternative forms of a given fusion product reflecting differences in exon composition. To date, all *EWS/FLI1* chimeric transcripts have consistently included exons 1–7 of *EWS* and exons 8 and 9 of *FLI1*. All have shown an intact *FLI1* DNA-binding domain, suggesting that this function is necessary for biologic activity [35, 42]. Approximately 85% of *EWS/FLI1* fusion transcripts are characterized by fusion of exon 7 of *EWS* to exon 6 of *FLI1* (referred to as a type 1 *EWS/FLI1* chimeric transcript) or fusion of exon 7 of *EWS* to exon 5 of *FLI1* (referred to as a type 2 *EWS/FLI1* chimeric transcript) [35].

Recent studies suggest not only that identification of the 11;22 translocation or *EWS/FLI1* fusion transcript is useful diagnostically in Ewing's sarcoma and pPNETs, but also that distinguishing type 1 and type 2 *EWS/FLI1* transcripts may be important prognostically [43, 44]. Specifically, two large clinical studies have both shown that the most common type of *EWS/FLI1* fusion (type 1), seen in approximately two-thirds of cases, is associated with a significantly better survival [43, 44]. Interestingly, the better clinical behavior of Ewing's sarcomas and pPNETs with the *EWS/FLI1* type 1 fusion is correlated with functional differences between the type 1 fusion protein and other alternative forms of *EWS/FLI1* [45].

RT-PCR, a sensitive method for detecting the presence of sarcoma-specific fusion transcripts, is capable of consistently detecting one t(11;22)-bearing cell among 10^5 non-t(11;22)-bearing cells [44]. For this reason, the RT-PCR technique has been considered a potential strategy for monitoring minimal residual disease in patients undergoing sarcoma therapy or in identifying micrometastatic disease [46–52]. This assay could potentially permit early identification of patients who may benefit from alternative therapy or who may be spared possible overtreatment [47]. For example, several studies have been conducted at examining peripheral blood (including peripheral blood progenitor cell collections) and/or bone marrow specimens of patients with Ewing's sarcoma/pPNET, rhabdomyosarcoma and myxoid liposarcoma for molecular evidence of circulating tumor cells at the time of diagnosis [46–54]. RT-PCR detection of circulating Ewing's sarcoma or pPNET cells in 23 patients (all with clinically localized disease) in a study performed by de Alava et al. [50] is in accordance with the markedly poor outcome of surgery alone for Ewing's sarcoma or pPNET. Moreover, preliminary studies suggest that minimal marrow involvement by Ewing's sarcoma or pPNET cells as determined by RT-PCR is associated with a poor clinical outcome [51, 52]. At present, it is not known whether reinfusion of translocation-positive peripheral blood progenitor cell collections contributes to eventual relapse [46].

Like Ewing's sarcoma and pPNETs, alveolar rhabdomyosarcomas are also characterized by specific chromosomal translocations that appear to have a relationship with clinical behavior [55]. The vast majority of alveolar rhabdomyosarcomas exhibit one of two chromosomal translocations: t(2;13)(q35;q14) associated with a *PAX3/FKHR* fusion transcript or t(1;13)(p36;q14) associated with a *PAX7/FKHR* fusion transcript [56]. The 2;13 translocation has been observed in approximately 75% of alveolar rhabdomyosarcomas examined and the 1;13 translocation in 10%. A comparison of the clinical features of 18 patients with *PAX3/FKHR* alveolar rhabdomyosarcomas and 16 patients with *PAX7/FKHR* alveolar rhabdomyosarcomas revealed a trend toward improved overall survival and a significantly longer event-free survival in the *PAX7/FKHR* group. These findings suggest that, similar to Ewing's sarcoma and pPNET, an association with fusion transcript type and distinct clinical phenotypes may exist in alveolar rhabdomyosarcoma [55].

Ring chromosomes in sarcomas of borderline or low-grade malignancy

The cytogenetic findings in well-differentiated liposarcoma, myxoid malignant fibrous histiocytoma (MFH) and parosteal osteosarcoma differ from those in other types of liposarcoma, MFH and osteosarcoma and provide a unique marker for distinguishing these histological subtypes [4, 5, 25, 57, 58]. Each of these neoplasms is characterized by the presence of supernumerary ring chromo-

Table 2 Ring chromosomes in sarcomas of borderline or low-grade malignancy

Neoplasm	Ring chromosome composition
Dermatofibrosarcoma protuberans	17q and 22q or t(17; 22)(q22; q13)[a]
Myxoid malignant fibrous histiocytoma	?
Well-differentiated liposarcoma	12q13–15 12q21.3–22
Parosteal osteosarcoma	12q13–15

[a] The 17; 22 translocation results in the fusion of *COL1A1* and *PDGFB* (Nat Genet 1997; 15:95–98 [84])

somes, frequently as the sole karyotypic anomaly (Table 2). The ring chromosomes in well-differentiated liposarcoma and parosteal osteosarcoma have been shown to be composed of chromosome 12 sequences, particularly 12q13–15 (Figure 4) [59, 60]. This region contains several oncogenes known to be amplified in sarcomas, including *MDM 2* and *CDK4* [61–63]. Similarly, comparative genomic hybridization analysis (a molecular cytogenetic approach to screen gains and losses of DNA sequences in a tumor specimen) has been performed on a series of low-grade central osteosarcomas revealing recurrent gain of 12q13–14 as the sole imbalance in several cases [64]. Prognostically, patients with one of these histological subtypes fare better than patients with other forms of liposarcoma, MFH and osteosarcoma. Hence, the observation of a ring chromosome may not only be of diagnostic utility but also may serve as an important prognostic indicator of a neoplasm of low-grade malignant potential.

Mesenchymal tumors with overlapping clinicohistopathological features or unusual clinical presentations

Mesenchymal neoplasms have traditionally been classified into categories based on patterns of differentiation and biological potential. Establishing the correct diagnosis of a mesenchymal neoplasm is dependent on the assimilation of clinical, radiographic and pathological findings [65]. Difficulties may arise in discriminating certain benign tumors or conditions from their malignant counterparts. Benign mesenchymal tumors are also characterized by distinct chromosomal abnormalities which may assist in establishing a correct diagnosis. For example, chondromyxoid fibroma may occasionally be difficult to distinguish from chondroblastoma or chondrosarcoma, particularly when the tissue quantity is limited as applies to some biopsy specimens (Fig. 5). Inversions or other rearrangements involving chromosome 6 have been repeatedly observed in chondromyxoid fibroma (Fig. 6) [66, 67]. Thus, this possible adjunctive marker as well as others reportedly characteristic of separate benign mesenchymal tumors listed in Table 3 could be of considerable diagnostic value [4, 68, 69].

Not infrequently, an atypical clinical presentation is encountered in a bone or soft tissue tumor. The anatomi-

Fig. 5 Anteroposterior (**A**) and lateral (**B**) radiographs of a 55-year-old white woman with an 18 month history of recurring tenderness over the left proximal tibia. There was no history of previous trauma, low-grade fever or night sweats, weight loss or systemic symptoms. Histopathologically and genetically, the lesion was consistent with a chondromyxoid fibroma

Fig. 6 Partial karyotype of a chondromyxoid fibroma illustrating the following translocation: t(6; 9)(q25; q22). Abnormalities of chromosome 6 appear recurrent in chondromyxoid fibroma

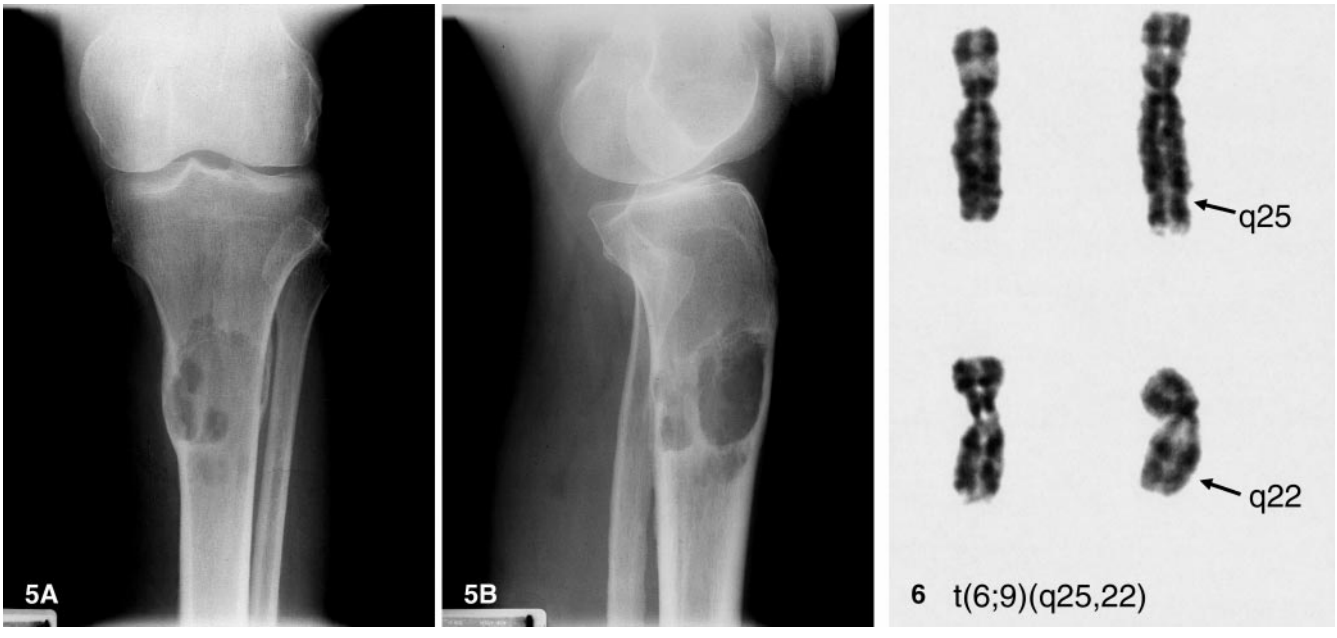

Table 3 Chromosomal abnormalities characteristic of benign mesenchymal tumors

Neoplasm	Chromosomal abnormality (recurrent breakpoints)
Benign schwannoma	–22
Chondromyxoid fibroma	inv(6)(p25q13) 6q25
Desmoid tumor (aggressive fibromatosis)	loss of 5q21–22 +8 and/or +20
Giant cell tumor of tendon sheath	1p11, 16q24
Hibernoma	11q13
Leiomyoma (uterus)	t(12; 14) (q14–15; q23–24) loss of 7q or 13q
Lipoma (typical, subcutaneous)	12q14–15, 6p21 loss of 13q
Lipoma (spindle cell or pleomorphic)	loss of 16q13-qter
Osteochondroma	loss of 8q24 or 11p11–12

cal location may seem to be at odds with the histopathological appearance, or the immunohistochemical findings do not support the light microscopic portrait. The spectrum of presentations associated with certain diagnoses has expanded with the help of confirmatory cytogenetic and molecular genetic data [27]. The following is one example: adamantinoma of extragnathic bones, a low-grade malignant neoplasm with epithelial features, is not typically considered in the differential diagnosis of Ewing's sarcoma. Recently, one of the authors studied three Ewing's sarcomas with histological, immunohistochemical and/or ultrastructural epithelial features by RT-PCR and sequencing studies for the Ewing's sarcoma molecular rearrangement (Fig. 7) [70]. (Two of the three cases were originally described as adamantinomas prior to the availability of genetic characterization.) In addition, conventional cytogenetic analysis and a unique combined interphase molecular cytogenetic/immunohistochemical approach with bicolor 11;22 translocation breakpoint flanking probes and pancytokeratin antibodies were performed on one neoplasm. A type II *EWS/FLI1* fusion transcript was detected in all three cases and the combined genetic/immunocytochemical approach revealed the presence of the 11;22 translocation in the nuclei of cytokeratin-immunoreactive cells. These genotypic and phenotypic findings delineated a novel Ewing's sarcoma histological variant, "adamantinoma-like Ewing's sarcoma" [70].

Other examples of bone or soft tissue tumors arising in unusual anatomical locations or presenting an atypical clinical picture, but demonstrating a characteristic cytogenetic or molecular genetic finding, include parosteal lipoma and primary lipoma of the lung [71–73]. Ewing's sarcomas or pPNETs arising in older adult patients [74] or unusual locations such as the ovary and pancreas [75, 76], primary synovial sarcomas of the lung or tongue [77, 78] and desmoplastic small round cell tumors (DSCRT) arising in the parotid gland and hand [79, 80]. Additional examples have been summarized in a recent review by Ladanyi and Bridge [27].

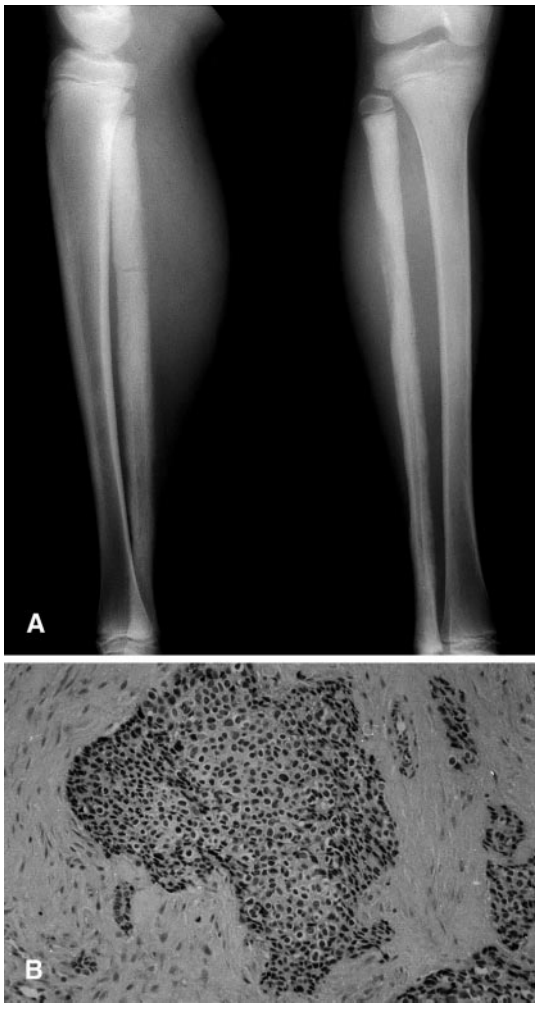

Fig. 7 **A** This anteroposterior and lateral radiograph of the right leg of a 13-year-old previously well and asymptomatic white male demonstrates a diffuse process encompassing the entire diaphysis of the fibula. A fat-suppressed T1-weighted MR image with gadolinium demonstrated an enhancing circumferential soft tissue mass. An open biopsy suggested Ewing's sarcoma with epithelial features. **B** The light microscopic appearance of this lesion revealed nests of epithelioid-like cells surrounded by a desmoplastic stroma

Conclusions

In the early phases of cancer cytogenetics it became apparent that chromosomal alterations were diagnostic of a number of malignancies, particularly leukemias. Over the years more and more entities became associated with specific cytogenetic anomalies and the usefulness of cytogenetic techniques has become particularly appreciated in tumors where the pathology is uncertain or confusing. This has been especially true of some bone and soft tissue sarcomas.

The identification of recurrently involved chromosomal breakpoints in bone and soft tissue tumors has enabled molecular biologists to determine the underlying genes involved in many of these rearrangements. In turn, this has facilitated the development of rapid and sensitive molecular assays (particularly for specific sarcoma gene fusions) which are now routinely utilized in many laboratories in the diagnostic investigation of a bone or soft tissue tumor. Future advancements should include further determination of the prognostic significance of many of these anomalies and the development of a new class of anti-neoplastic agents founded on underlying biological events in bone and soft tissue sarcomas for the treatment of patients with these malignancies.

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