Musculoskeletal Oncology

Advances in Cytogenetics and Molecular Genetics and their Clinical Implications

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Although musculoskeletal malignancies comprise a small group of cancers, a vast number of histological subtypes have been identified attesting to the heterogeneity of this class of tumours and the growing interest in their development. The mode of management for both bone and soft tissue sarcomas has been examined extensively and treatment guidelines have been proposed. Despite the intensive study and multidisciplinary treatment, a substantial proportion of tumours remain recalcitrant to therapy and recur locally and systemically. Improved methods of characterising these tumours may help in understanding their biology. Cytogenetic and molecular genetic techniques allow a subcellular dissection of these malignancies which may aid the identification of mechanisms that are important in tumorigenesis. Already candidate genes have been isolated which may play an important role in the deregulation of proliferation and/or the adoption of a malignant phenotype, features which are fundamental in tumour development. By studying the molecular biology and cytogenetics of tumours it may be possible to improve diagnostic and prognostic accuracy thereby minimising over and under treatment.

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Malignancies of the musculoskeletal system comprise a small fraction (<1%) of all tumors. The identification of many histologic subtypes has been the basis for the extensive classifications now in use (1, 2). These classifications are based on the most differentiated form of the tumors, but give no indication of their origin and are less predictive of tumor behavior. Our current understanding of sarcoma biology permits cure in two thirds of patients by surgery and adjuvant radiotherapy (3). Difficulties specific to soft tissue sarcomas, however, include unclassifiable histotypes in 10% of cases (4), controversy over the validity of the most common diagnosis, malignant fibrous histiocytoma (MFH) (5), and a paucity of single or combination chemotherapy agents which effect overall survival (6–8). In bone tumors where the results of adjuvant chemotherapy are dramatic, especially for osteosarcoma and Ewing sarcoma, it is not possible to predict potential responders prior to commencing chemotherapy as histologic similarity does not necessarily equate with biological similarity. Thus, refinements in existing prognostic systems and the search for new, stronger prognostic factors appear fundamental.

Clonal non-random chromosomal aberrations which are tumor specific have potential diagnostic value, particularly in poorly differentiated malignancies which may also lack characteristic immunohistochemical or ultrastructural features. The persistence of these aberrations in metastatic and recurrent tumors highlights the diagnostic importance of these chromosomal markers at any stage during the natural history of the tumor (9). Secondary chromosomal changes which occur in addition to the primary abnormality are often related to tumor progression, and may also have prognostic significance (10).

The importance of the multistep nature of tumorigenesis was alluded to even as early as the turn of this century (11) and this was later elaborated by Nowell in the description of clonal evolution (10). Many cytogenetic abnormalities not only reflect the acquisition of the malignant phenotype, in some cases the rearrangements appear central to the process of malignant transformation. Molecular biological techniques have complemented cytogenetic analysis by identifying the genetic aberrations arising from such chromosomal rearrangements and thereby refining tumor diagnostics, by implicating specific genes and gene products in tumour formation, and eventually, elucidation of mechanisms for tumorigenesis can pave the way for novel treatments, which may even be directed at the gene level

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(12). This paper reviews recent advances in the cytogenetics and molecular genetics of musculoskeletal malignancies with an emphasis on clinical implications and applications.

GENETIC BASIS FOR CANCER

The genetic basis for sarcomas is similar to that found in other organ systems. Deregulated proliferation via abnormally induced stimulatory factors (oncogenes) or the loss of restraining factors (tumor suppressor genes) are thought to be central to tumorigenesis; a concept expounded over 80 years ago.

> The unlimited tendency to rapid proliferation in malignant tumour cells could result from a permanent predominance of the chromosomes that promote division... Another possibility to explain cancer is the presence of definite chromosomes which inhibit division... Cells of tumours with unlimited growth would arise if those "inhibiting chromosomes" were eliminated... Since each kind of chromosomes is represented twice in the normal cell, the depression of only one of these two might pass unnoticed...
> Boveri, 1914; translation published in 1929 (13)

Three major mechanisms have been implicated in tumorigenesis. In the first, a protooncogene is translocated into a position adjacent to an activating sequence, e.g., on an immunoglobulin or T-cell receptor gene in lymphocytes. An example of this is the juxtapositioning of MYC and immunoglobulin heavy or light chain genes in Burkitt lymphoma (14) through, as for example, the translocation t(8;14)(q24;q32). At present, this mechanism has not been identified in any musculoskeletal tumor and will not be discussed further.

The second mechanism is the loss of tumor suppressor gene function. The two most important examples are the retinoblastoma and the *TP53* genes.

Retinoblastoma gene

The retinoblastoma susceptibility gene, *RB1*, was the prototype tumor suppressor gene for Knudson's two-hit hypothesis in the development of retinoblastoma (15). Normal individuals have two wild-type *RB1* genes located in each of chromosome bands 13q14 (16). A somatic or germ-line mutation causes the first hit which induces a cancer prone state, and the second hit, by inactivating the remaining allele, results in loss of function and retinoblastoma development. The association between *RB1* loss and osteosarcoma was recognised when patients with germ-line mutations of *RB1* were noted to have a higher incidence of second malignancies, of which osteosarcoma was the commonest (17, 18). An earlier study had alluded to this relationship by showing a higher incidence of osteosarcoma in families with retinoblastoma (19). Expression of *RB1* results in a 110kD nuclear phosphoprotein (20) that in part, functions as a cell cycle regulator by inhibiting transcription of *FOS*, *IL6*, and *MYC* (21–23); three genes that have been implicated in bone formation and tumorigenesis. Up to 80% of osteosarcomas in some series show loss of expression of RB1 (24). *RB1* deletions have been reported in other tumors including MFH of bone, chondrosarcoma, Ewing sarcoma, malignant giant cell tumor and chordoma and a variety of soft tissue sarcomas including MFH, liposarcoma, synovial sarcoma, peripheral neuroepithelioma, leiomyosarcoma, hemangiopericytoma and the extraskeletal forms of chondrosarcoma and osteosarcoma (25).

TP53

The *TP53* gene is located in chromosome sub-band 17p13.1 and was first identified as a cellular protein that bound to SV40 T antigen (26). Initially mistaken to be an oncogene, its role as a tumor suppressor gene is now clearly recognised. This gene has been extensively studied and is regarded as one of the most important tumor related genes known (27). Examples of mutations involving this gene are available in many tumor types (28, 29). *TP53* encodes a factor which positively or negatively regulates the expression of genes governing the progression of cells through the late G_1 phase of the cell cycle, as well as having an integral role in the repair of DNA damage induced by ionizing radiation (30). Mutated TP53 may act in a dominant negative way which means inactivation may follow deletion of one or both alleles (31). Amplification of the *MDM2* gene, which is located at 12q13, results in overexpression of another factor which forms complexes with TP53 and may thereby inactivate the latter (27, 32). Whether these complexes inhibit binding of TP53 to specific binding sites or whether they allow binding to these sites but inhibit transcriptional activation is unknown (27).

TP53 was the second gene to be linked with the development of osteosarcoma in which the mutation frequency is high (33). This association came from studies of cancer families with the Li-Fraumeni syndrome where patients with a germ-line mutation of *TP53* exhibit a high risk for malignancies of the breast, brain, adrenal cortex, bone, soft tissue and blood (34, 35). Germ-line mutations in sarcoma patients, however, appear to be rare ($< 2\%$) (36). The role of *TP53* mutation in the development of bone and soft tissue sarcomas has been strengthened by the observation of point mutation and gross defects in a wide array of these tumors (32, 37–39).

The third mechanism of tumorigenesis follows chromosomal translocation and the consequent formation of a fusion gene that encodes a chimeric protein. This chimeric protein participates in tumorigenesis as an aberrant transcriptional activator. The first reported example of this was the fusion of the *BCR* and *ABL* genes on the Philadelphia chromsome resulting from the t(9;22)(q34;q11) found in chronic myeloid leukemia (40). All the known tumor-specific translocations in soft tissue sarcomas form chimeric proteins (Table).

Table

Characteristic cytogenetic and molecular genetic findings in malignant musculoskeletal tumours

Type	Cytogenetics	Molecular genetics
Osteosarcoma		
Classic	Complex	Loss of *RB1* (40%) and *TP53* (30%), amplification of *MDM2* (30%)
Parosteal	Ring chromosomes	
Ewing sarcoma PNET	t(11;22)(q24;q12)	*EWS/FLI1* (85%)
	t(21;22)(q22;q12)	*EWS/ERG* (5%)
	t(7;22)(p22;q12)	*EWS/ETV1* (< 5%)
Chondrosarcoma	Complex	
Extraskeletal myxoid	t(9;22)(q22;q12)	*EWS/TEC*
Chordoma	−3, −4	
Clear cell sarcoma	+8, t(12;22)(q13;q12)	*EWS/ATF1*
Dermatofibrosarcoma protuberans	r(17;22), t(17;22)(q22;q13)	*COL1A1/PDGFB*
Fibrosarcoma		
Infants	+11, +20, +17, +8	
Adults	Complex	
Giant cell tumour	Telomeric associations	
Hemangiopericytoma	12q13–15 aberrations	
Leiomyosarcoma	Complex	
Liposarcoma		
Well-differentiated	Ring chromosomes	Amplification of 12q14–15 and 12q21–22
Myxoid	t(12;16)(q13;p11)	*FUS/CHOP*
Pleomorphic	t(12;22)(q13;q12)	*EWS/CHOP*
	Complex	
MFH*		
Myxoid	Ring chromosomes	
Storiform-pleomorphic	Complex	
Neurofibrosarcoma	Complex	
Rhabdomyosarcoma		
Alveolar	t(2;13)(q35;q14)	*PAX3/FKHR* (70%)
	t(1;13)(p36;q14)	*PAX7/FKHR* (5%)
Embryonal	+2, +8, +13, +20	
Synovial sarcoma	t(X;18)(p11;q11)	*SSX/SYT*
Desmoplastic small round cell tumour	t(11;22)(p13;q12)	*EWS/WT1*

* malignant fibrous histiocytoma

GENETIC ABERRATIONS IN MUSCULOSKELETAL TUMORS

Characteristic chromosomal aberrations

Ewing sarcoma. Ewing sarcoma occurs in both skeletal and extraskeletal forms and its karyotypic abnormalities have been extensively studied. In the majority of cases (85%), a characteristic translocation of the long arms of chromosome 11 and 22, t(11;22)(q24;q12), has been observed (41). In 5–10% of tumors, variant translocations have also been observed by molecular genetic and cytogenetic analyses, most commonly as t(21;22)(q22;q12) (42–44). Non-random aberrations such as trisomy 8 and translocations involving chromsomes 1 and 16 have also been reported (45). The fact that an identical t(11;22)(q24;q12) has been observed in neuroepithelioma, Askin tumors and esthesioneuroblastomas (46–49) provides a strong argument for a neural crest origin of Ewing sarcoma.

The t(11;22)(q24;q12) aberration in Ewing sarcoma and other neuroectodermal tumors results in the fusion of the Ewing sarcoma (*EWS*) gene on chromosome 22 with the ETS-like DNA binding domain of the Friend Leukemia Virus (*FLI1*) gene on chromosome 11 (48, 50). The EWS/FLI1 chimeric protein has been shown to function as a transcriptional activator (51), and the molecular mechanism for this appears to be the replacement of the N-terminal transcriptional activation domain of the FLI1 protein with the regulatory domain of EWS which results in the activation of the C-terminal transcriptional activation domain of FLI1. Additional work has also identified other Ewing sarcoma translocations (43, 44), t(21;22)(q22;q12) and t(7;22)(p22;q12) that fuses *EWS* to other members of the *ETS* gene family, the *ERG* and *ETV1* genes, respectively, both encoding an ETS-like DNA binding domain

related to FLI1. All three fusion transcripts are believed to act via a similar mechanism on a similar repetoire of target genes.

Liposarcoma

Myxoid liposarcoma is a soft tissue sarcoma which is characterised by a reciprocal translocation of chromosomes 12 and 16, t(12;16)(q13;p11), in 90% of cases (52–54). The remarkable histotype specificity of this aberration as depicted by its incidence and the lack of such a translocation in any other subtype of liposarcoma or myxoid sarcoma places it amongst the more important diagnostic markers in soft tissue sarcomas. Pleomorphic liposarcomas are usually typified by high chromosome counts and complex karyotypes with multiple rearrangements and translocations (54). There is no abnormality that is distinctive for this variant and it shares a similar level of karyotypic complexity as seen in other high grade tumors. Supernumerary giant marker chromosomes or ring chromosomes seem to be characteristic of well-differentiated liposarcomas, which are usually near-diploid (54, 55). The giant marker and ring chromosomes represent DNA amplification and include material from chromosome 12 (55, 56).

The t(12;16)(q13;p11) that characterises myxoid liposarcoma brings together the fusion (*FUS*) gene (also known as translocated in liposarcoma, *TLS*) on chromosome 16 and the CEBP homologous protein (*CHOP*) gene on chromosome 12 (57, 58). The latter gene transcribes a nuclear protein that is a member of the CCAAT/enhancer-binding protein (C/EBP) family, and which functions as a dominant negative inhibitor of gene transcription (59). The function of the *FUS* gene is unknown. Like the EWS protein, the FUS protein contains an RNA-binding domain at its C-terminal portion and fusion of the *FUS* and *CHOP* genes results in the replacement of the RNA-binding domain of FUS by the whole CHOP coding region. The FUS domain in the chimeric protein provides a transcriptional activation domain to a presumptive DNA binding activity of CHOP. The FUS/CHOP fusion product is thought to behave in a similar manner to that found in Ewing sarcoma.

Synovial sarcoma

The identification of a reciprocal translocation involving chromosomes X and 18, t(X;18)(p11;q11), in more than 90% of monophasic and biphasic synovial sarcomas is diagnostically important (60). The histotypes from which the monophasic variant of synovial sarcoma needs to be differentiated include fibrosarcoma, malignant fibrous histiocytoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, rhabdomyosarcoma and hemangiopericytoma (61). The specificity of t(X;18) for synovial sarcoma also makes it a reliable diagnostic tool when dealing with recurrent, metastatic or undifferentiated spindle cell tumors. Variant translocations have also been found but all involve the X or chromosome 18 with another partner (60). An interesting observation is that even in histologically more aggressive lesions, a near-diploid chromosome number is often encountered with few additional aberrations other than t(X;18) (60, 62).

The presence of t(X;18)(p11.2;q11.2) as the sole anomoly in several synovial sarcomas suggests a central role of this aberration in tumor development. The synovial sarcoma translocation (*SYT*) gene on chromosome 18 and the two closely linked and related genes, *SSX1* and *SSX2* on the X chromosome have been shown to form fusion genes, SYT/SSX, as a result of the t(X;18) (63). The function of the chimeric gene is still unknown (63, 64). However, several putative SH3 and SH2 domains have been identified in the SYT protein which may be important because of their role as intracellular signalling proteins (63). There is speculation that it is the formation of the der(X) rather than the der(18) chromosome that is required for malignant transformation (63). Examples supporting this hypothesis include the formation of the der(X) but not the der(18) chromosome in complex translocations involving three chromosomes (60), reports of tumors which have lost the der(18) (65), and the fact that some synovial sarcoma cell lines have lost the der(18) but never the der (X) chromosome (63). Furthermore, a SYT/SSX transcript encoded by the der(X) chromosome has been detected but not one encoded by der(18) (63).

Rhabdomyosarcoma

Rhabdomyosarcoma is the commonest soft tissue sarcoma in children and several different forms have been recognised (66). The two most common variants, alveolar and embryonal rhabdomyosarcoma, are associated with different chromosomal abnormalities. The most frequent abnormality in alveolar rhabdomyosarcoma is a reciprocal translocation, t(2;13)(q35;q14), that has not been identified in any other small round cell tumor (67, 68). Recently, a variant translocation, t(1;13)(p36;q14), has also been identified (69, 70) which may represent a subgroup that has a preference for younger patients than those associated with the t(2;13), although the numbers are too few to be conclusive. Embryonal rhabdomyosarcoma, by contrast, lacks the t(2;13), and exhibits other non-random abnormalities including trisomies of chromosomes 2, 8, 13 and 20 (68). These trisomies are frequently associated with deletions involving the short arm of chromosome 11. None are specific for embryonal rhabdomyosarcoma; similar changes having been noted in other embryonal-like tumors such as hepatoblastoma (71) and Wilms' tumor (72).

The consequence of the t(2;13) in alveolar rhabdomyosarcoma is the fusion of the paired-box homeo-domain region of the *PAX3* gene on chromosome 2 with a

forkhead-domain gene, *FKHR*, on chromosome 13 (73, 74). *PAX3* is of particular interest because it encodes a transcription factor with DNA-binding domains that activates specific target genes important for development (75). In situ hybridisation studies in mice have localised PAX3 expression to the developing nervous system, the developing limb buds and dermomyotomes (76). Similarly, *FKHR* belongs to a family of genes which have also been implicated in the control of embryonic development and adult tissue specific gene expression (77, 78). A growth regulatory role has been suggested by the identification of the avian retroviral oncogene qin as a forkhead-domain gene (79). The developmental and growth regulatory roles of these two genes may be relevant to the development and frequency of this tumor in the pediatric age group. A chimeric transcript encoded by the der(13) has been identified which consists of the DNA-binding domain of PAX3, a truncated DNA-binding domain of the C-terminal part of FKHR (73, 74, 79). This may bind to the normal PAX3 targets but aberrantly regulate transcription because of the modulatory effects of a truncated FKHR. In this regard, mutations of other forkhead-domain genes have been shown to inactivate or modulate DNA binding function (80). A further and simpler explanation for an increase in tumorigenicity by t(2;13) may be that the translocation increases the expression of PAX3 or FKHR (73), both of which have been shown to be tumorigenic when aberrantly expressed. The t(1;13) is associated with a fusion between the related *PAX7* gene and the *FKHR* gene (81, 82).

Clear cell sarcoma

Clear cell sarcoma of tendons and aponeuroses, is also known by the name malignant melanoma of soft parts, because of its histologic similarities with cutaneous malignant melanoma. Although rare, clear cell sarcoma can now be recognised by a specific reciprocal translocation between chromosomes 12 and 22, t(12;22)(q13;q12) (83, 84). The breakpoint on chromsome 22 in clear cell sarcoma involves the same band affected in the t(11;22) of Ewing sarcoma (48) which in part highlights earlier speculation of a neural crest origin of clear cell sarcoma based upon ultrastructural and immunohistochemical findings (85). The replacement of the RNA-binding domain on EWS with sequences from a DNA-binding protein also occurs in clear cell sarcoma where the t(12;22) results in a fusion between *EWS* and a gene encoding a cAMP-dependent transcription factor, *ATF1*, on chromosome 12 (48).

Extraskeletal myxoid chondrosarcoma

Extraskeletal myxoid chondrosarcoma is a low-grade malignancy with a prevalence for late relapse. A recurrent t(9;22)(q22;q12) has been reported (86, 87), and the *EWS* gene has been shown to be involved in the rearrangement (88, 89). A fusion gene has been demonstrated between *EWS* and a novel gene termed *TEC* (translocated in extraskeletal myxoid chondrosarcoma) (88). Unlike the other EWS fusion proteins, the putative EWS/TEC protein contains the whole coding sequence of TEC (88), which is reminiscent of the FUS/CHOP fusion protein. TEC appears to be a member of the subfamily of orphan nuclear receptors and shares a 98% homology with another orphan nuclear receptor called NURR1 (88). This latter protein is thought to control cell proliferation and differentiation by modulating the response to specific growth factors or by interfering with the signalling pathway of retinoic acid, a potent morphogen. The *EWS/TEC* gene fusion is only the second example of the oncogenic conversion of a nuclear receptor in human tumorigenesis.

Malignant fibrous histiocytoma

MFH is the commonest soft tissue sarcoma diagnosis. As yet, no primary chromosomal abnormalities have been identified that are specific for this tumor or its subtypes (90, 91). The karyotype is generally complex with numerous numerical and structural changes. Chromosomal aberrations include ring chromosomes, telomeric associations, dicentric chromosomes and evidence of gene amplification (90, 92). Aberrations involving bands 1q11–12 and 19p13 appear to be the commonest (93, 94). A less chaotic karyotype is usually encountered with the myxoid (lower grade) variant in comparison to the storiform-pleomorphic subtype, and ring chromosomes have been reported in up to 50% of myxoid tumors (91). The rings seem to contain chromosome 12 sequences (92).

Osteosarcoma

Despite its frequency, cytogenetic studies of osteosarcoma have been limited by the poor adaptability of osteosarcoma cells to in vitro culture and the frequent overgrowth of normal fibroblast. No distinctive aberration has been identified. Multiple complex numerical and structural abnormalities have been observed (95–97). While the karyotypic complexity may hamper the determination of primary cytogenetic abnormalities or even the frequency of various changes, there may be preferential involvement of chromosome 13. Of note, the locus of tumor suppressor gene, *RB1* maps to 13q14 and *RB1* deletions are common in osteosarcoma. Similarly, deletions of chromosome 17, which contains the tumor suppressor gene *TP53*, have also been observed in osteosarcoma.

Chondrosarcoma

Cytogenetic heterogenity is a feature of most chondrosarcomas (98). Although no distinctive abnormality has been

identified, recurrent changes are common and appear to involve numerical rather than structural aberrations (98, 99). Hyperhaploid or hypodiploid tumors are common and the losses frequently include chromosomes 10, 6 and 13, while gains are more commonly seen with chromosomes 7 and 20.

GENE AMPLIFICATION

Gene amplification is the increase in copy numbers of one or more genes, which can be localised extrachromosomally as paired chromatin bodies called double minutes (dmin) or intrachromosomally as homogeneously staining regions (hsr). Gene amplification is thought to be a mechanism to increase gene expression whereby cells acquire a selective advantage, particularly if the amplified genes would confer a proliferative advantage or resistance against chemotherapy drugs. Amplified DNA sequences often contain several genes, one or more of which represent the essential target gene(s), whereas other flanking genes are only co-amplified. Examples of oncogene amplification are recognised in several musculoskeletal sarcomas.

Amplification of *MYCN* has also been reported in alveolar and embryonal rhabdomyosarcoma. More complex examples involve the amplification of regions on the long arm of chromosome 12 which may include one or more genes such as the *CHOP*, *SAS*, *MDM2*, *CDK4* and *GLI* genes. Although these genes have been linked with the signal transduction in mitogenesis, none are consistently amplified in all tumors carrying 12q amplification. Sarcomas which are known to display gene amplification involving 12q include MFH (100), liposarcoma (56), chondrosarcoma and osteosarcoma (101, 102). MDM2 may be of specific interest because it appears to have a direct role in negatively regulating the transcriptional activity of TP53 (102). Furthermore, TP53 may also be part of a feedback loop where under normal conditions, it induces the transcription of MDM2 (103). Chromosome 17 and 22 sequences are regularly amplified at a low level in dermatofibrosarcoma protuberans and located within supernumerary ring chromosomes (104).

CLINICAL APPLICATIONS

Diagnosis

The reliability of cytogenetic and molecular genetic techniques for identifying tumor-specific chromosomal and molecular markers is an important factor in diagnosis. Both techniques are now used routinely at our institution on fine needle aspirates, open biopsies and surgical specimens.

Ewing's sarcoma, non-Hodgkin lymphoma, neuroblastoma, embryonal rhabdomyosarcoma, and hemangiopericytoma may be so similar that they have been referred to collectively as small, round, blue cell tumours. This description, however, belies their distinctive biological behaviour and the specificity of treatment required for each. Although most of these tumours can be identified by the combination of light microscopy, electron microscopy and immunohistochemistry, in some cases an accurate diagnosis may be difficult or even impossible. Specific chromosomal abnormalities for each of these tumours and the cloning of fusion genes from several translocations has improved the diagnostic accuracy.

The monophasic variant of synovial sarcoma can be difficult to differentiate from other poorly differentiated spindle cell tumors such as MFH, fibrosarcoma and leiomyosarcoma. Some have suggested that synovial sarcoma may be responsive to chemotherapy (105). Therefore, identifying the t(X;18) aberration by cytogenetic analysis or the SYT/SSX fusion RNA, by reversed transcriptase polymerase chain reaction, which are specific for synovial sarcoma may be particularly relevant. The epithelioid subtype of synovial sarcoma may also be histologically similar to clear cell sarcoma, malignant schwannoma and spindle cell melanoma. Apart from the t(X;18) translocation, the t(12;22) in clear cell sarcoma is a differentiating feature.

Aneurysmal bone cyst, giant cell tumour and telangiectatic osteosarcoma can present radiologic dilemmas. Occasionally, even histologic examination may not be able to discern between aggressive but benign lesions and malignant tumors. Cytogenetic analyses have identified multiple complex chromosomal abnormalities in osteosarcoma (95, 97), telomeric associations in giant cell tumours (106) and a normal karyotype in aneurysmal bone cysts, and these may be helpful for arriving at a diagnosis. Parosteal osteosarcoma can sometimes mimic another benign bone lesion, the osteochondroma. While histologic evaluation can occasionally be difficult, the finding of a ring chromosome as the sole anomaly or as one of a few aberrations would make the diagnosis of parosteal osteosarcoma more probable (107).

The clinical significance of chromosomal abnormalities in benign tumours remains unclear. Specific aberrations have been found in several benign lesions which may help to distinguish them from their malignant counterparts. For example, the distinction between lipoma and atypical lipoma or well-differentiated liposarcoma should be possible because of the presence of ring chromosomes in the latter two tumors and its absence in the former (108). Some classify atypical lipoma as a locally aggressive but benign lesion, while others prefer a diagnosis of well-differentiated liposarcoma with all the attendant risks of local recurrence and dedifferentiation. In this regard, ring chromosomes which are characteristic of well-differentiated liposarcoma are also found in atypical lipoma, thus, suggesting a much closer biological relation between the two entities than previously thought. Myxoid lipoma and myx-

oid liposarcoma should also be distinguishable from each other because of the t(12;16) translocation among the latter.

Prognosis

The major prognostic or staging systems rely to a large part on the histological grading of tumors, and the criteria employed are a combination of cellularity, differentiation, pleomorphism, mitotic rate and necrosis. There are, however, clear difficulties in achieving consensus between pathologists because of the subjective nature of grading. To address this problem, a wide variety of prognostic factors have been elucidated for primary lesions and after local recurrence, including size and compartmental location of tumors (109), specific histological features such as microscopic vascular invasion and spontaneous tumor necrosis (3), DNA content of tumors (110), the expression of cell-cycle related nuclear antigens (111, 112), and the growth rate of local recurrences (113). To these, can be added potential cytogenetic and molecular genetic markers.

In general, there seems to be a relationship between increasingly complex karyotypes and poor prognosis. Simple karyotypic changes including supernumerary ring chromosomes are more commonly a feature of low grade tumours or those of borderline malignancy, e.g. parosteal osteosarcoma, dermatofibrosarcoma protuberans, myxoid MFH, and well-differentiated liposarcoma/atypical lipoma (107, 114) In chondromatous tumours, increasingly complex aberrations have been found with an increasing tumour grade (115). While there are no cytogenetic abnormalitites that are characteristic for MFH, we have observed that an abnormality of chromosome 19 at band p13 is associated with a higher rate of metastasis, while patients with supernumerary ring chromosomes appear to have a lower incidence of relapse (91, 94). Increasing cytogenetic complexity have also been shown to accompany the clinical progression of a tumor.

Synovial sarcoma may express some sensitivity to chemotherapy (105). Accurate diagnosis with cytogenetic and/or molecular genetic techniques can be important for verifying this assumption. The association between survival and the subtypes of synovial sarcoma is controversial (61). Recent studies have correlated specific breakpoints to the monophasic and biphasic subtypes and if the delineation between these two varieties can be improved by identifying which breakpoint is involved within band Xp11.2, a more accurate analysis of outcome may be possible (64). Myxoid liposarcoma, like synovial sarcoma may show preferential response to chemotherapy (116). Identification of t(12;16) tumours may help to select those that have a greater potential to respond to current therapy. *RB1* loss or damage can be used to detect predisposition to tumor formation in the kindred of those afflicted with retinoblastoma (117). Altered expression of RB1 has also been correlated with tumor grade (118) and metastasis (119) leading to the suggestion that RB1 status should be assessed as an independent prognostic indicator. Accumulation of mutant TP53 has been linked with tumor aggression in a variety of soft tissue sarcomas (32), osteosarcoma and chondrosarcoma (37).

Although it has not been attempted, putative genetic mechanisms for the aetiology of musculoskeletal sarcomas may provide a future means for therapy at the gene level. Tumor suppressor genes may be candidates for genetic manipulation (120), but the multilevel nature of carcinogenic mutations appears too complex to rely on the correction of a single gene for cure. Unfortunately, the results of gene therapy has been disappointing in other malignancies.

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