



Adrienne Anderson. *The Birth of Passion*, 1995. Oil on canvas, 6' × 6' (feet).

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Genetic and Molecular Abnormalities in Tumors of the Bone and Soft Tissues

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Background: *Malignant transformation requires the accumulation of multiple genetic alterations such as chromosomal abnormalities, oncogene activation, loss of tumor suppressor genes, or abnormalities in genes that control DNA repair and genomic instability. Sarcomas are a heterogeneous group of malignant mesenchymal tumors of difficult histologic classification and strong genetic predisposition. This article provides a comprehensive review of the cytogenetic abnormalities observed in bone and soft-tissue tumors, emphasizing known downstream molecular changes that may play a role in oncogenesis.*

Methods: *The database of the National Library of Medicine was searched for literature relating to genetic and molecular mechanisms in sarcomas in general and in each of the main tumor entities.*

Results: *Recent techniques in chromosome analysis and molecular cytogenetics have improved our ability to characterize genetic changes in mesenchymal tumors. Some changes are so characteristic as to be virtually pathognomonic of particular histologic types, while others are complex, difficult to characterize, and of unknown relevance to pathogenesis. The implications to the cell of some of these abnormalities are now being recognized.*

Conclusions: *The study of sarcomas will benefit from the information derived from genetic studies and translational research. The human genome project and new methodologies, such as computer-based DNA microarray, may help in the histogenetic classification of sarcomas and in the identification of molecular targets for therapy.*

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Introduction

The field of cytogenetics has provided insight into the underlying genetic abnormalities of mesenchymal lesions. Techniques that have been successfully applied to the study of sarcomas include (1) polymerase chain reaction (PCR) to detect known gene abnormalities, (2) reverse transcriptase PCR (RT-PCR) to identify mRNA transcripts, (3) fluorescence in situ hybridization (FISH) to detect known genetic loci, (4) comparative genomic hybridization to detect chromosomal differences between neoplastic tissue and its normal counterpart, (5) chromosome painting to identify individual chromosomes, (6) spectral karyotyping (SKY) to identify the chromosomal location of DNA sequences in metaphase spreads, (7) representational differential analysis to compare expression libraries, and (8) loss of heterozygosity (LOH). Some of the genetic abnormalities observed in sarcomas are characteristic of certain tumor types (Table 1), while others (Table 2) are complex and less reproducible. Whether these changes are due to an inherent genomic instability or the result of a carefully orchestrated oncogenic machinery remains to be seen. The latter hypothesis seems to be supported by the fact that some of these alterations are found across histologic subtypes. New advances in molecular biology and signal transduction will help us not only to understand the biology of the tumors, but also to establish a molecular classification of sarcomas, identify prognostic indicators, and detect minimal residual disease.

This article reviews the molecular changes that occur downstream to DNA and that provide a link

between genetic abnormalities and the dynamic oncogenic machinery. The study of these molecular pathways will help us not only to appreciate commonalities and differences among tumors, but also to anticipate potential trends for future translational research that ultimately will lead to novel therapeutic approaches. The entities described have been grouped according to presumed histogenetic origin, and they have been selected because of their clinicopathologic importance or because they share abnormalities with other entities supporting a paradigm in the oncogenesis of sarcoma.

Adipose Tissue Tumors

Benign

Studies in lipomas have revealed that benign tumors, previously considered cytogenetically normal, are characterized not only by specific numerical and structural changes, but also by complex chromosome rearrangements that are generally considered a hallmark of malignancy. A subgroup of lipomas characterized by recurrent chromosome translocations involving the chromosome segment 12q13-q15 and either 3q27-q28 or other genes, such as lipoma-preferred partner gene (LPP) in chromosome 3, have been found as translocation partners.¹ Also, as in leiomyoma, the transcriptional regulator HMGI-C at the 12q14-q15 locus has been found to be abnormally regulated. Rearrangements of chromosome 13 with loss of the band 13q14, the site of retinoblastoma and osteosarcoma tumor suppressor genes, have also been observed.²

Table 1. — Chromosomal Translocations Frequently Observed in Sarcomas

Tumor	Translocation	Fusion Protein	References
Myxoid/round-cell liposarcoma	t(12;16)(q13;p11) t(2;22)(q13;q12) t(12;22;20)(q13;q12;q11)	TLS(FUS)-CHOP EWS-CHOP EWS-CHOP	7, 8, 9
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	COL1A1-PDGF-B	15
Fibrosarcoma	t(12;15)(p13;q25)	ETV6-NTRK3	17, 18
Desmoplastic small round-cell tumor	t(11;22)(p13;q12)	EWS-WT1	33
Ewing's sarcoma family	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12)	EWS-FLI1 EWS-ERG EWS-ETV1	34, 35, 49
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) t(1;13)(p36;q14)	PAX3-FKHR PAX7-FKHR	39, 40
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11.2)	EWS-CHN (TEC) RBP56 (HTAF1168) -CHN(TEC)	62
Clear cell sarcoma	t(12;22)(q13-q14;q12)	EWS-ATF1	68
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SYT-SSX1 or SSX2	69

Table 2. — Chromosomal Abnormalities in Tumors of Bone and Soft Tissues

Tumor	Translocations	Chromosomal Gains	Chromosomal Losses	Loss of Heterozygosity	References
Lipoma	12q13-q15; 13q14				1
Spindle lipoma			16q13; 13q; 16		3
Lipoblastoma	8q11-q13				4
Atypical lipomatous tumor	12q13-15				5
Nodular fasciitis		3q21			10
Proliferative myositis		2			11
Fibroma of tendon sheath	t(2;11)(q31-32;q12)				12
Desmoplastic fibroblastoma		11q12in			13
Solitary fibrous tumor	t(9;22)(q31;p13)				14
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)				15
Sclerosing epithelioid fibrosarcoma		10, 18, 12q13, 12q15, 9, 20			18
Malignant fibrous histiocytoma		7q32; 1p31; 12q13-q14; 12q12-q15; 3; 4q31; 5p; 6; 7; 14q22; 1q21-q22; 17q28; 20q; 7p15r	9p21; 10q; 11q23; 13q10-q31; 13q21; 13q22		19-22
Leiomyoma			7q		24
Leiomyosarcoma			10		24
Neurofibroma			17q11.2		25
Schwannoma			22q12		26-28
Acoustic neuroma		22			26
Malignant peripheral nerve sheath tumors		17q24-17	7p22; 1p21; 7p11,14q11; 12p13; 1; 8; 16; 22; Xq26; 11q22; 13p; 9p22; 11p13; 17p; 17q11-21; 1p22-32; 1p34; 6q25		29, 30
Malignant triton tumors	t(1;13)(q10;q10)	8; 1			31
Embryonal rhabdomyosarcoma		2; 7; 8; 11; 12; 13q21; 20	6; 17; 14q21-32; 1p35-36.3; 9q22	16q23-16; 11p15.5	41
Rhabdoid tumors	t(11;22)(p15.5;q11.23)				43
Neuroblastoma		17q21; 1q21-q25	11; 1p36; 11q23; 14q23; 18q21.1, 18p-q12.3		44-49
Giant cell tumors		11; 16; 19; 20; 21			50
Osteosarcoma		1q21, (8q21.3-q22), 8-q1314, q24; Xp11.2-p21; 1p21-31; 3q25; 6p12-21; 8q12; 12p11-12; 12q12-15; 0q12; 20p; 8q24.1	6q16; 6q21-q22; 3p; 10q; 11p; 13	3q; 13q; 17p; 18q; Xq21; 6-q22; 18-q11.2	52-54
Chondroid lipoma	t(11;16)(q13;p12-p13)				56
Chondroid hamartoma	t(12;14)(q15;q24)				57
Chondromyxoid fibroma		Inv(6)(p25q13)			57
Chondrosarcoma		20q; 17p; 20p; 1-q24; 14q23; 6-q22; 7; 5q14-q32; 6p; 12q; 20q12; 20q; 8q24.1	X-q21; 6-q22; 18-q11.2	9p21; 10; 13q14; 17p13	59-61
Chordoma		Dic(1;9)(p36.1;p21)	1p	1p36	65
Malignant mesenchymoma		1q21-q25; 12q14-q15			66
Epithelioid sarcoma	t(6;8)(p25;q11.2); t(8;22)(q22;q11)	Add(7)(p15)			70
Alveolar soft-part sarcoma		1q; 8q; 12q; 16p; 12	1; 2; 3; 10; 11; 14; 15; 16; 17		67

In spindle/pleomorphic lipoma, loss of genetic material from the region 16q13 and 13q deletions have been reported. Similar abnormalities have been found in mammary myofibroblastomas, suggesting a link between these two tumors.³ Lipoblastoma shows a characteristic rearrangement in 8q11-q13.⁴ Similar changes in the 11q13 region have been reported in hibernoma.

Atypical Lipomatous Tumors

A molecular spectrum from lipomas to atypical lipomatous tumor (ALT) has been proposed. At one end are lipomas, characterized by 12q13-q15 rearrangements and HMGI-C (high mobility group) activation and, at the other end, ALT with ring chromosomes, 12q13-q15 amplification, and overexpression of HMGI-C, CDK4, or the murine double-minute type 2 gene (MDM2) genes.⁵

Liposarcoma

It has been suggested that there are three distinct pathogenic groups of liposarcoma: (1) the retroperitoneal group, where MDM2-mediated inactivation of p53 may play a role in pathogenesis, (2) the non-retroperitoneal group, where TP53 mutations (17p13.1) appear to correlate with dedifferentiation, and (3) the myxoid group, characterized by its own unique cytogenetic profile without apparent involvement of the TP53 or MDM2 genes. It has also been reported that the absence of MDM2 and p53 immunoreactivity in lipomas may be used in the differential diagnosis from well-differentiated liposarcoma lipoma-like (WDLPS).⁶ The WDLPS/atypical lipoma group is characterized by an extra ring and/or an extra giant chromosome marker. Myxoid liposarcoma has a t(12;16) translocation or, more rarely, a t(12;22) translocation, resulting in fusion of the transcription factor gene CHOP (GADD153) in chromosome 12q13 with TLS-FUS on chromosome 16p11 or with Ewing's sarcoma (EWS) on chromosome 22. The translocation t(12;16)(q13;p11) is shared with round-cell liposarcoma. The resulting two fusion protein variants, TLS-CHOP and FUS-CHOP, have different lengths and seem to activate a variety of genes with high similarity to glial-derived nexin, neuronatin, and the *RET* protooncogene.⁷ TLS (translocated in liposarcoma) is a 65 kDa protein (p65) recently identified as a member of a family of nuclear RNA-binding proteins involved in RNA processing. The N-terminus of TLS binds the DNA-binding domain in estrogen, thyroid, and glucocorticoid receptors and contains a potent transactivation domain that, when fused with the DNA-binding protein CHOP, converts it into a powerful transforming oncogene and transcription factor. In the case of FUS, the RNA-binding domain is replaced with the DNA-binding and leucine zipper dimerization domain of CHOP,⁸ while translin-

binding sequences and consensus topoisomerase II cleavage sites have been detected at both of TLS-FUS and CHOP breakpoints. Of interest is that if the FUS-CHOP transgene is introduced into the mouse genome, most of the histologic features of human liposarcoma, such as lipoblasts with round nuclei and accumulation of intracellular lipid, are reproduced.⁹ In some cases, however, only minor genetic alterations have been observed, suggesting that secondary cytogenetic aberrations are not a prerequisite for the development of round-cell morphology.

Fibrous Lesions

Benign

Abnormalities in 3q21 have been reported in nodular fasciitis¹⁰ and trisomy 2 and the haplotype 46,XX,t(6;14)(q23;q32) in proliferative myositis.¹¹ Superficial fibromatosis exhibits trisomy 8, which is also observed in deep fibromatosis in association with trisomy 20. In fibroma of tendon sheath, a t(2;11)(q31-32;q12) has been reported, suggesting that this entity is a neoplasm instead of a reactive process.¹² Clonal chromosomal abnormalities involving the long arm of chromosome 11, 11q12in, have been described as collagenous fibroma (desmoplastic fibroblastoma). Since 11q12 is also rearranged in fibromas of tendon sheath, this region might be a common genetic denominator of benign fibroblastic lesions.¹³

Intermediate Malignancy and Malignant

In solitary fibrous tumor, the translocation t(9;22)(q31;p13) and a rearrangement of 12q13-15 have been reported. This latter abnormality is shared with a subset of hemangiopericytomas, suggesting a histogenetic relationship between the two tumors.¹⁴ Dermatofibrosarcoma protuberans and its juvenile form, giant cell fibroblastoma, are related tumors with unique cytogenetic abnormalities, such as supernumerary ring chromosomes containing sequences from chromosomes 17 and 22 and the translocation t(17;22)(q22;q13), a new tumor-associated chromosome rearrangement.¹⁵ These aberrations lead to gene fusions between the platelet-derived growth factor B-chain (PDGF-B) gene, the cellular equivalent of the *v-sis* oncogene, and the collagen type 1 alpha 1 gene (COL1A1) that codes for the major protein constituent of the extracellular matrix in skin connective tissue. The location of breakpoints within COL1A1 is limited to the region encoding the alpha-helical domain. The PDGF-B segment of the chimeric transcript always starts with exon 2, placing PDGF-B under the control of the COL1A1 promoter and removing all known elements negatively controlling PDGF-B

transcription and translation. The aberrant transcripts may function in an autocrine loop in fibroblasts.¹⁶ This fusion gene COL1A1-PDGF-B has not been detected in dermatofibroma, malignant fibrous histiocytoma, or fibrosarcomatous areas of dermatofibrosarcoma protuberans. The congenital (or infantile) form of fibrosarcoma (CFS) is histologically identical to adult-type fibrosarcoma (ATFS), but it has an excellent prognosis and a low metastatic rate.

A recently described t(12;15)(p13;q25) rearrangement in CFS fuses the helix-loop-helix protein dimerization domain of the ETV6 gene (also known as TEL) in 12p13 with the protein tyrosine kinase domain of NTRK3, neurotrophin-3 receptor gene (also known as TRKC) in 15q25. ETV6-NTRK3 chimeric transcripts are not detected in ATFS or infantile fibromatosis.¹⁷ The TEL-TRKC fusion variants are also associated with acute myelogenous leukemia (AML) and are potent activators of the MAP kinase pathway. A gene located in the long arm of chromosome 1 and involved in cellular senescence has also been implicated in the pathogenesis of fibrosarcoma. Sclerosing epithelioid fibrosarcoma is a recently described low-grade fibrosarcoma. In one case,¹⁸ 40-45,XY,add(9)(p13),add(10)(p11),-12,-13,-18,add(18)(q11),add(20)(q11) was the observed karyotype. Both the add(10) and the add(18) contained amplified sequences from 12q13 and 12q15, including the HMGI-C gene. This karyotype is unrelated to that of other tumors such as synovial sarcoma or chondrosarcoma, suggesting that sclerosing epithelioid fibrosarcoma is a low-grade variant of fibrosarcoma.

Fibrohistiocytic Tumors

Malignant Fibrous Histiocytoma

Multiple chromosomal structural and numerical aberrations (eg, marker chromosomes, translocations, telomeric associations, double minutes, and ring chromosomes) have been reported in malignant fibrous histiocytoma (MFH), predominantly in the storiform/pleomorphic variant.¹⁹ Certain chromosomal gains such as 7q32 and 1p31 have been associated with decreased metastasis-free survival and overall survival.²⁰ Amplification of the MDM2 gene has been reported in one third of cases but, in contrast to TP53, it does not have a significant effect on either disease-free or overall survival.²¹ In the myxoid variant, ring chromosomes and trisomy of chromosome 2 have been reported in up to 50% of cases. The amplification unit in 12q13-q14 containing the sarcoma-amplified sequence (SAS) observed in many soft-tissue tumors has also been reported.²² Some childhood MFH share abnormalities with adult MFH such as co-amplification of MDM2, CDK4, SAS, and

CHOP. A genetic linkage of the rare autosomal-dominant bone dysplasia/cancer syndrome, diaphyseal medullary stenosis with malignant fibrous histiocytoma (DMS-MFH), has been mapped to a region on chromosome bands 9p21-q22, suggesting the presence of a tumor suppressor gene in the DMS-MFH critical region and a common genetic etiology for autosomal dominant and sporadic forms of MFH.²³

Smooth Muscle Tumors

Transformation of leiomyoma into leiomyosarcoma is yet to be conclusively confirmed, but the absence of specific anomalies common to benign and malignant tumor argues against their being benign-malignant counterparts. In leiomyoma, deletion of 7q, a potential site for a tumor suppressor gene, is commonly found. Almost 60% of leiomyosarcomas show LOH for at least one marker on chromosome 10. It has recently been suggested that in leiomyosarcoma, restoration of wild-type p53 not only enhances cell cycle control in vitro and inhibits tumor growth, but also inhibits angiogenesis via transcriptional repression of vascular endothelial growth factor (VEGF), a key mediator of tumor angiogenesis.²⁴

Neural Tumors

Neurofibroma

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder affecting 1 in 3,000 people and characterized by multiple peripheral nerve tumors containing mainly Schwann cells and fibroblasts. The NF1 is a tumor suppressor gene located on chromosome 17q11.2 that encodes for "neurofibromin," a Ras GTPase-activating protein (RasGAP) that functions as a negative regulator of Ras-controlling cell growth and differentiation. However, since Ras activity is detected in only a fraction of Schwann cells, neurofibromin may not be an essential regulator of ras activity. LOH of NF1 has been reported in 25% of neurofibromas. The S-100 positive cells in neurofibromas typically lack the NF1 transcript, while the fibroblasts carry at least one normal NF1 allele and express both NF1 mRNA and protein. This suggests that additional molecular events besides NF1 inactivation in Schwann cells and/or other neural crest derivatives are necessary for the development of neurofibromas.²⁵

Neurilemoma (Schwannoma)

Neurofibromatosis type 2 (NF2) was separated from NF1 when the NF1 gene was located to chromo-

some 17 and the NF2 gene to chromosome 22.²⁶ NF2 is an uncommon autosomal dominant disorder in which patients are predisposed to neoplastic lesions of Schwann cells (schwannomas and schwannosis), meningeal cells (meningiomas and meningioangiomatosis), and glial cells (gliomas and glial hamartomas). Vestibular schwannomas, the hallmark of NF2, do not occur with increased frequency in NF1 patients. Schwannomas carry inactivating mutations of the NF2 tumor suppressor gene on chromosome 22q12. The NF2 gene encodes a widely expressed protein, "merlin," that links the cytoskeleton to the cell membrane. Chromosome 22 deletions have been detected in over 80% of the cases, suggesting that genes other than the NF2 locus are involved in the development of schwannomas. In some cases, a putative locus on 22q has been suspected.²⁷ Alterations in 1p similar to those observed in meningiomas, another NF2-associated neoplasm, have been reported. Furthermore, the nerve growth factor (NGF), which binds to a specific cell surface receptor (NGFR), exists in high affinity (trk) and low affinity (p75NGFR) forms. During early development and after nerve injury, Schwann cells express only p75NGFR, suggesting that transcriptional repression plays a major role in the regulation of these genes and that the markedly different regulation of NGFI-A, NGFI-B, and *c-fos* may guide Schwann cell differentiation.²⁸

Malignant Peripheral Nerve Sheath Tumors

Malignant peripheral nerve sheath tumors (MPNSTs) exist in sporadic forms and in association with neurofibromatosis. Since the genes involved in peripheral and central neurofibromatosis are located in chromosomes 17q and 22, respectively, these two regions are of particular interest to MPNST pathogenesis. In NF1, MPNSTs may arise in a preexisting neurofibroma or may be the initial manifestation of the disease. MPNSTs are often triploid, and recent studies have shown inactivation of both NF1 gene alleles during the development of MPNSTs in patients with NF1. All chromosome arms, except 22p and the Y chromosome, have been reported in recombinations. Inactivation of the TP53 gene has been reported in a few tumors. It has been suggested that the NF1 gene may function as a tumor suppressor gene and that both the NF1 and the TP53 genes may be critical for the progression to malignancy. Overexpression of p53 is found in 60% of MPNSTs, while neurofibromas are typically p53 immunonegative. Half of the NF1 patients with p53-positive MPNSTs develop recurrence, metastases, or a second malignancy within 2 years of diagnosis, whereas patients with p53-positive sporadic MPNSTs are free of disease for up to 7 years.²⁹ The NF1 gene may also contain a mutation that results in either greatly reduced transcription or message insta-

bility.³⁰ In malignant triton tumors, which are rare variants of MPNSTs showing muscle differentiation and often seen in patients with NF1, an isochromosome for the long arm of chromosome 8 and an unbalanced translocation (1;13) (q10;q10) leading to a gain of the long arm of chromosome 1 have been observed.³¹

Small Round-Cell Tumors

Desmoplastic Small Round-Cell Tumor

Desmoplastic small round-cell tumor (DSRT) is a multiphenotypic, primitive tumor characterized by the t(11;22)(p13;q12) chromosomal translocation. This translocation produces a chimeric protein in which the transactivation domain of the EWS protein is fused to zinc-fingers 2-4 of WT1, the Wilms' tumor suppressor and transcriptional repressor. One of the consequences is the induction of platelet-derived growth factor A chain (PDGF-A), a potent fibroblast growth factor that may contribute to the characteristic fibrosis observed in DSRT. Novel molecular variants of the specific EWS-WT1 gene fusion involving an in-frame junction of the exon 9 or exon 10 of EWS to exon 8 of WT1 have been reported.³²

Ewing's Sarcoma Family

The recently defined Ewing's sarcoma family of tumors (EWSFT) includes EWS, extraosseous EWS, primitive neuroectodermal tumor (PNET), neuroepithelioma, and Askin's tumor. These entities share balanced chromosomal translocations involving the EWS gene. A hallmark of EWS and PNET is the immunohistochemical expression of CD99/MIC2, a cell surface glycoprotein encoded by a pseudoautosomal gene on chromosomes X and Y. Two translocations — t(11;22)(q24;q12) and t(21;22)(q22;q12) — that are observed in more than 85% of the tumors are considered characteristic of EWSFT. They result in the fusion of the 5' portion of the EWS gene (22q12) to either the FLI1 gene (11q24) or the ERG gene (21q22). The EWS gene encodes an RNA-binding protein of unknown function, and the FLI1 gene encodes an Ets-1-related transcriptional activator. The translocation t(11;22) consists of a series of breakpoints in central introns of EWS and FLI1 that result in various fusion proteins differing in the sequence surrounding the breakpoint where the transcriptional regulation domain is located. EWS-FLI1 upregulates the expression of manic fringe (MFNG) reported to be involved in neuroectodermal differentiation and in leukemogenesis via the Notch signaling pathway.³³ EWS-FLI1 is also a transactivator of the *c-myc* promoter and activates genes such as type I

insulin-like growth factor receptor (IGF-IR) and EAT-2, which contain a functionally active Src-homology domain. An autocrine pathway involving IGF-I and IGF-IR may also be responsible for blocking apoptosis in tumor cells. Thus, antisense oligonucleotides against EWS-FLI1 prevent the growth of EWS cells in vitro and in vivo. Although no differences in survival have been shown for EWS-FLI1 and EWS-ERG, type 1 EWS-FLI1 seems to be a weaker transcriptional activator than type 2 EWS-FLI2.³⁴ Furthermore, type 1 EWS-FLI1 fusions, with a breakpoint between exon 7 of EWS and exon 6 of FLI1, seem to be associated with better prognosis, possibly due to a differential regulation of the IGF-IR pathway.³⁵ While EWS-ETS fusion proteins may function as dominant negative forms of ETS transcription factors, the EWS-FLI1 protein seems to target the transforming growth factor beta (TGF- β) type II receptor (T β R-II), a putative tumor suppressor gene. In fact, transcriptional repression of TGF- β appears to be an important target of all three oncogenes. Thus, fibroblasts transfected with EWS-FLI1, EWS-ERG, or EWS-ETV1 gene fusions express reduced levels of T β R-II mRNA and protein and are less sensitive to TGF- β -mediated effects. Furthermore, co-transfection of these fusion genes and the T β R-II promoter suppresses the activity of T β R-II, FLI1, ERG, and ETV.

The molecular mechanism responsible for these translocations is not completely understood. However, the observation that different translocation partners of the EWS gene are specifically associated with several distinct types of sarcomas suggests a model in which the translocation partner supplying the DNA-binding domain confers the target specificity of the transcriptional activation mediated by these chimeric proteins, whereas the partner supplying the N-terminal domain and promoter region determines their transactivation potential and expression level. Furthermore, the adenovirus early region 1A (E1A) gene has been shown to induce the fusion transcript EWS-FLI1, suggesting a direct link between viral genome and chromosome translocations.³⁶

Chromosomal amplification has been detected in EWSFT with various frequencies. Thus, 1q21-q22 amplification is detected in 18% of the tumors and amplification of chromosome 8 in 38%. Amplification of chromosome 12 is observed in 12% of cases and, if 6p is coamplified, distant disease-free and overall survival is decreased. Secondary genetic alterations (eg, overexpression of the *c-myc* gene and amplification of N-*myc* and *c-erb B-1*) are rare in EWS. Aberrant p53 expression, present in 11% of patients, appears to define a small clinical subset of patients with markedly poor outcome.³⁷ Interestingly, hybrid cells of a PNET cell line and normal human fibroblasts, or HeLa cells, are

nontumorigenic, while hybrid cells of the PNET cell line and other soft-tissue sarcoma cell lines are highly tumorigenic. The genetic abnormality responsible for this conversion has been mapped to chromosome 17, suggesting the presence of a tumor suppressor gene on this chromosome.³⁸

Alveolar Rhabdomyosarcoma

Alveolar rhabdomyosarcoma is characterized by two variant translocations, t(2;13)(q35;q14) and t(1;13)(p36;q14). In t(2;13)(q35;q14), the NH2-terminal paired box and homeodomain DNA-binding domains of PAX3 on chromosome 2 are fused in frame to COOH-terminal regions of the FKHR gene, a novel member of the fork-head DNA-binding domain family located on chromosome 13. The sequences that flank the t(2;13) breakpoint are similar to the DNA-binding site for a protein termed "translin," also known to flank other chromosomal translocations. The PAX3 gene seems to function upstream of genes that control skeletal muscle differentiation. This effect on differentiation appears to be supported by the fact that MDM2 has also been found as amplified in association with this translocation. MDM2 seems to block MyoD, which drives terminal differentiation of skeletal muscle cells. Furthermore, amplification of the ATR gene has been also observed. ATR, a protein kinase that regulates DNA repair, has been shown to block skeletal muscle differentiation. The PAX3-FKHR fusion protein may function as an oncogenic transcription factor by enhancing activation of normal PAX3 target genes and by an antiapoptotic mechanism due, at least in part, to direct transcriptional modulation of Bcl_{XL}.³⁹ Furthermore, the twist gene, which is overexpressed in rhabdomyosarcoma and known to block skeletal muscle differentiation, may also contribute to oncogenesis by blocking *myc*-induced apoptosis. Cells with the translocation t(2;13) often overexpress the *MET* protooncogene, which encodes for the hepatocyte growth factor (HGF) receptor. The combination of MET/HGF is believed to increase cell motility and metastatic potential. In the variant translocation t(1;13), a novel fusion gene, PAX7-FKHR, is formed. While PAX7 is virtually inactive, PAX7-FKHR is comparable to PAX3-FKHR.⁴⁰ Microarray studies have revealed 37 genes consistently expressed and related to both primary (PAX3-FKHR) and secondary (CDK4) genetic alterations.⁴⁰

Embryonal Rhabdomyosarcoma

Loss of 1p36 affects the locus for PAX7, the paired box characteristically altered in embryonal rhabdomyosarcoma, and the region 9q22 corresponds to the locus of a putative tumor suppressor gene. LOH has been reported at 16q23-16qter and 11p15.5, a region containing several genes — RRM1, GOK, Nup98,

CARS, hNAP2 (NAP1L4), H19, IGF-II, WT2, p57KIP2 (CDKN1C), KVLQT1, TAPA-1, TH, ASCL2, TSSC1, TSSC2, and TSSC3.⁴¹ TSSC1 shows homology to the Rb-associated protein p48 and the chromatin assembly factor CAF1. TSSC2 is homologous to the *Caenorhabditis elegans* beta-mannosyl transferase, and TSSC3 shows homology to the mouse TDAG51, implicated in FasL-mediated apoptosis. The 5' region of the human tyrosine hydroxylase gene (TH) on chromosome band 11p15.5 contains several important loci for disease phenotypes such as the congenital overgrowth disorder, Beckwith-Wiedemann syndrome, and Wilms' tumor, and it appears to be involved in rhabdomyosarcoma. In rhabdomyosarcoma, p53 has been shown to inhibit the activity of the P3 and P4 promoters of IGF-II, thus decreasing endogenous IGF-II gene expression. Cells with a high expression of IGF-II have shortened cycling time and diminished G₁ checkpoint associated with increases in cyclin D1, p21, and p53 protein levels, as well as mitogen-activated protein kinase activity.⁴²

Rhabdoid Tumors

Malignant rhabdoid tumors are extremely aggressive soft-tissue sarcomas that tend to be widely metastatic at diagnosis. These tumors, first described as variants of Wilms' tumor, have been found in a variety of extrarenal sites. A chromosomal translocation t(11;22) (p15.5;q11.23) encoding a tumor suppressor gene, INI1, has been reported. The chromosome 11 breakpoint has been mapped to a region in the vicinity of genes involved in rhabdomyosarcoma, Wilms' tumor, and Beckwith-Wiedemann syndrome. Two other tumor-associated loci, EWS1 and NF2, mapped to 22q11.2 do not appear to be involved.⁴³ In a rhabdoid tumor cell line, the chromosome 11 breakpoint of the translocation has been mapped to the vicinity of IGF-II.

Neuroblastoma

Gain of genetic material from chromosome arm 17q (17q21) is the most frequent cytogenetic abnormality and is associated with advanced disease. A novel chromosomal gain at 1q21-q25 has been found in 50% of advanced cases.⁴⁴ Amplification of *N-myc* is also a prominent genetic abnormality and suggests the involvement of IGF-IR in neuroblastoma pathogenesis. In some cases, *N-myc* amplification may be due to the formation of double minutes and loss of chromosome 11. Amplification of *N-myc* and telomerase activity are predictors of poor prognosis. A complex translocation of chromosome 17 has also been found to be associated with poor prognosis.⁴⁵ A putative novel zinc-finger gene (zf5-3) has been mapped to chromosome 19. During development of the fetal human brain, high levels of zf5-3 mRNA are restricted to the mitotically active, undif-

ferentiated neuroblasts, and morphologic evidence of differentiation is accompanied by a marked loss in zf5-3 expression.⁴⁶ Consistent areas of chromosomal loss, including 1p36, 11q23, 14q23, 18q21.1 and possibly at 18-q12.3, may identify the location of neuroblastoma suppressor genes. A suppressor gene associated with *N-myc*-amplified tumors probably maps to a region that harbors genes such as FGR, SLC9A1, HMG17, EXTL1, AML2, RH, and OP18 with roles in cell growth, differentiation, and morphogenesis.⁴⁷ The chromosome 18 is frequently deleted in neuroblastoma, and a distinct common region of allelic imbalance at 18q21.1 has been shown to include the DCC (deleted in colon carcinoma) gene but not the Smad2 and Smad4 genes. The DFFB gene, encoding the active subunit of the apoptotic nuclease DNA fragmentation factor (DFF40), maps to 1p36, near the imprinted putative tumor suppressor gene TP73. The DFFA gene, encoding the inhibitory DFF45 subunit, also maps to 1p36.2-36.3, and a processed DFFB pseudogene maps to chromosome 9. However, DFFB does not seem to be the neuroblastoma tumor suppressor gene TP73.⁴⁸ In olfactory neuroblastoma, a t(11;22) translocation identical to that of EWS has been observed, and some tumors have been shown to produce the EWS-FLI1 fusion protein. Recent studies,⁴⁹ however, have failed to confirm a relationship of olfactory neuroblastoma to EWSFT.

Bone-Forming Tumors

Giant Cell Tumor of the Bone

Giant cell tumor of the bone is a benign, primary skeletal neoplasm with variable biologic aggressiveness that demonstrates telomeric associations of chromosomes 11, 16, 19, 20, and 21, reduction of telomere length, marker chromosomes, double minutes, chromosome fragments, ring chromosomes, and polyploidy. Overexpression of TGF- β , but not TGF- β , has been reported.⁵⁰

Adamantinoma

Adamantinoma of long bones is a biphasic malignant tumor composed of epithelial and fibroblastic cells. Results of one study, based on flow cytometric and genetic analysis, suggest that in adamantinoma the epithelial component is malignant, while the fibroblastic component is part of an osseofibrous reaction.⁵¹

Osteogenic Sarcoma

Karyotypes in osteosarcoma (osteogenic sarcoma, OS) are usually complex with extensive numerical and structural changes, particularly in high-grade tumors. The highest frequencies of LOH have been reported on

chromosomes 3q, 13q, 17p, and 18q. The high incidence of LOH on chromosome 3q suggests the presence of a novel tumor suppressor gene.⁵² The most frequent aberrations found by comparative genomic hybridization are copy number increases at 1q21 and 8q (8q21.3-q22) and 8cen-q13, followed by copy number increases at 14q24-qter and Xp11.2-p21. The most common losses are detected at 6q16 and 6q21-q22. Patients with copy number increases at 8q21.3-q22, 1q21, and 8cen-q13 have diminished distant disease-free survival and show a trend toward short overall survival.

Genomic amplification, especially of both the p53-binding MDM2 gene and the flanking SAS gene, plays an important role in the biology of these tumors. SAS is the human homolog of the MDM2. SAS amplification, however, is more common in surface OS than in conventional intramedullary OS. Overexpression of *c-erb* B-2, a cellular growth factor, is observed in approximately 40% of OS and correlates with decreased 4-year survival, early pulmonary metastases, and poor survival.⁵³ Alterations in *c-fos* occur more frequently in patients with recurrent or metastatic disease, and LOH of Rb has been identified as a negative prognostic marker. Alterations of *c-myc* and *N-myc* are also common, and mutations have been reported in p53, MDM2, DCC, p16^{INK4A}, and p19^{INK4D}. The cdk inhibitors p15, p16, and p18 are seldom mutated in OS, although p15 and p16 have been found co-deleted in some tumors. The INK4A gene, localized to human chromosome 9p21, encodes p16^{INK4A}, a tumor suppressor protein that functions at least in part through the inhibition of CDK4, a cyclin-dependent kinase encoded by a gene at 12q13.

The prevalence of these alterations, in conjunction with the reported inactivation of Rb in up to 80% of cases, suggests that genetic lesions deregulating the G₁ to S cell cycle checkpoint are a constant feature in OS pathogenesis. Of interest for the study of OS arising in Paget's disease is that in sporadic OS, LOH has identified a putative tumor-suppressor locus that maps to chromosome 18q in a region tightly linked to familial Paget's disease.⁵⁴ Parosteal OS, a less aggressive form of OS, seems to have consistent gains of genetic material on chromosome 12q13-q15. Double minutes and monosomy of 17p and 6p have also been reported. In low-grade central OS, translocation between chromosomes 14 and 15, balanced translocations involving chromosomes 5, 21, 12, and 20, and a supernumerary ring chromosome derived from chromosome 1 have been detected despite a near diploid karyotype. Other tumors display a range from diploid to peritetraploid DNA content. The low number of genetic alterations found in this type of tumor may explain its relatively low malignancy.

Cartilage-Forming Lesions

Hereditary multiple exostosis (EXT) is an autosomal dominant disorder characterized by the formation of osteochondromas in the growth centers of the long bones. EXT is genetically heterogeneous with three loci on chromosomes 8q24.1, 11p13, and 19q. Mutations of the EXT1 gene on chromosome 8q24.1 have been identified. Some mutations result in frameshifts and premature termination codons, while others result in amino acid substitutions. The EXT2 gene maps to the region containing the marker D11S903, suggesting that EXT genes may be tumor-suppressor genes and that the initiation of tumor development may follow a multistep model. In fact, mutations at EXT1 and other genetic events including LOH at other loci may be required for the development of the malignant phenotype.⁵⁵ The patterns of EXT gene mutation observed in EXT, in solitary and multiple osteochondromas, and in chondrosarcoma are analogous to those observed in tumor suppressor genes responsible for other cancers. In chondroid lipoma, a three-way rearrangement among chromosomes 1, 2, and 5, and an 11;16 translocation with a breakpoint in 11q13, proximal to the MEN1 region frequently rearranged in hibernoma, have been reported. The t(11,16)(q13;p12-p13), however, is different from that reported in myxoid/round-cell liposarcoma.⁵⁶ In chondroid hamartoma, rearrangement of 12q15 is common. A t(12;14)(q15;q24), similar to that observed in leiomyoma, has also been detected. Rearrangements of chromosome 6 involving the band 6q13 with a pericentromeric inversion 6 [inv(6)(p25q13)] seem to be relatively specific for chondromyxoid fibroma. Cytogenetic studies have also identified a complex karyotype and an unbalanced reciprocal translocation between the short arm of chromosome 3 and the long arm of chromosome 6. The rearrangement affects two genes that control growth and maturation of endochondral bone — the type X collagen gene (COL10A1), located at 6q21-q22, and the parathyroid hormone/parathyroid hormone-related peptide receptor gene (PTH/PTHrP), located at 3p21.1-p22.⁵⁷ In enchondroma, supernumerary ring chromosomes in chromosome 12 and chromosomal abnormalities involving the 12q13-q15 region, associated with a wide range of benign soft-tissue tumors and sarcomas, have been reported. Genes implicated in the control of the cell cycle such as SAS, the protooncogenes CHOP/GADD153, GLI, A2MR, and CDK4, and the high mobility group (HMGI-C) gene implicated in mesenchymal tumorigenesis are all located on the long arm of chromosome 12.⁵⁸

Chondrosarcoma

Chondrosarcomas may arise centrally in bone (central chondrosarcoma) or secondarily within the carti-

liginous cap of a hereditary or sporadic exostosis (peripheral chondrosarcoma). In one study,⁵⁹ the vast majority of peripheral chondrosarcomas were highly proliferative with LOH at many loci, while a small subgroup of central chondrosarcomas showed anomalies that were restricted to 9p21, 10, 13q14, and 17p13 and were peridiploid or near haploid. The cytogenetic analysis of a well-differentiated (grade I) chondrosarcoma shows numerous structural chromosome aberrations and a striking degree of genetic instability. In some studies,⁶⁰ genetic aberrations were found in 72% of chondrosarcomas and amplifications of small chromosome regions in only 17% of cases. The RET oncogene locus, implicated in the pathogenesis of multiple endocrine neoplasia types 2A and 2B, Hirschsprung disease, and medullary and papillary thyroid carcinomas, has been reported to be involved in chondrosarcoma. Amplification of the *c-myc* protooncogene has rarely been observed,⁶¹ and *fos/jun* has recently been implicated in chondrosarcoma pathogenesis.⁶²

Extraskelatal Myxoid Chondrosarcoma

The t(9;22)(q22;q12) translocation is common in extraskelatal myxoid chondrosarcoma. A newly identified chromosomal translocation, t(9;17)(q22;q11.2), seems to generate a fusion transcript consisting of an EWS-like gene (HTAF1168 or RBP56) and the CHN nuclear receptor gene (TEC).⁶³ As a result of the translocation, a protein that is analogous to the EWS-CHN is produced. CHN encodes a novel orphan nuclear receptor with a zinc-finger DNA-binding domain. Other studies have revealed t(9;22;15)(q31;q12.2;q25) and 10q21.1 rearrangements. Although some cases show neither an EWS-CHN fusion transcript nor EWS or CHN genomic rearrangement, extraskelatal myxoid chondrosarcoma is another tumor containing a gene fusion involving EWS.

Dedifferentiated Chondrosarcoma

Dedifferentiated chondrosarcoma is a high-grade sarcoma adjacent to a low-grade malignant cartilaginous tumor. In one study,⁶⁴ numerous genetic alterations were found in both the high- and low-grade components. However, a shared loss of chromosome 13 suggests that both components originate from a common precursor.

Miscellaneous Tumors

Tenosynovial Tumors

Localized giant cell tumor of tendon sheath exhibits two different karyotypic changes. One involves 1p11 in

a translocation with chromosome 2 or with another chromosome. A second type involves 16q24.

Myxoma

In cardiac myxomas, karyotype abnormalities that perhaps are responsible for defective DNA repair have been reported. In patients with the autosomal dominant Carney complex, a major 17 cM locus on chromosome 17q2 containing the Carney complex disease gene has been identified.⁶⁵

Chordoma

Chordomas are rare skeletal sarcomas of the spine that originate from remnants of the notochord. The rearrangement dic(1;9)(p36.1;p21) has been reported.⁶⁶ Chromosome 1p appears to be involved in unbalanced translocations with different chromosomes, leading to variable losses of 1p. Microsatellite instability, an indirect marker of globally defective DNA mismatch repair, has also been demonstrated.

Malignant Mesenchymoma

In one study,⁶⁷ supernumerary ring- and rod-shaped marker chromosomes were observed next to an otherwise normal diploid karyotype. The chromosome 1q21-q25 and 12q14-q15 sequences were amplified, suggesting an association with the well-differentiated liposarcomatous component of the tumor.

Alveolar Soft-Part Sarcoma

Alveolar soft-part sarcoma is a rare, histologically distinctive soft-tissue sarcoma typically occurring in children and young adults. Comparative genomic hybridization studies have shown that gains are more common than losses.⁶⁸

Clear Cell Sarcoma (Melanoma of Soft Parts)

Clear cell sarcoma is characterized by the translocation t(12;22)(q13-q14;q12). Thus, the oncoprotein EWS-ATF1 is a highly specific marker for clear cell sarcoma and is a potent activator of several cAMP-inducible promoters, including the somatostatin promoter. As in EWS and multiple myxoid liposarcoma, trisomy/tetrasomy 8 represents a nonrandom secondary aberration.⁶⁹

Synovial Sarcoma

Synovial sarcoma (SS) is characterized by the translocation t(X;18)(p11.2;q11.2), which fuses the SYT

gene (SSXT) on chromosome 18 to the SSX gene on the X chromosome. Of the five variants of the SSX gene identified, SSX1, SSX2, and SSX4 have been shown to fuse with SYT. The SYT-SSX2 seems to be largely restricted to monophasic synovial sarcomas, while the SYT-SSX1 occurs in 80% of the tumors, either monophasic or biphasic, but not in other sarcomas.⁷⁰ This translocation seems to correlate with a higher proliferation rate (Ki-67 index) and a shorter metastasis-free survival. Two alternative breakpoint regions involving Xp11.2 — one in the ornithine aminotransferase-like 1 (OATL1) region and the other in the OATL2 region — have also been described. The IGF-IR plays a crucial role in proliferation and survival of transformed cells, and when over-expressed (in approximately 50% of cases), it is associated with a high incidence of lung metastases. No association has been found between IGF-IR and the type of fusion gene transcript (SYT-SSX1 or SYT-SSX2).

Epithelioid Sarcoma

Cytogenetic studies have revealed translocations t(6;8)(p25;q11.2), t(8;22)(q22;q11), and add(7)(p15). In one case,⁷¹ the band of chromosome 22 involved in the translocation is the same as is involved in the majority of EWS-PNET tumors.

Conclusions

Potentially diagnostic chromosomal translocations and associated fusion genes are (1) Ewing's sarcoma/primitive neuroectodermal tumor: t(11;22)(q24;q12), EWS-FLI1; t(21;22)(q22;q12), EWS-ERG; t(7;22)(p22;q12), EWS-ETV1, (2) desmoplastic small round-cell tumor: t(11;22)(p13;q12), EWS-WT1, (3) extraskelatal myxoid chondrosarcoma: t(9;22)(q22;q12), EWS-TEC(CHN), (4) malignant mesenchymoma: t(11;22)(q24;q12), EWS-FLI1, (5) alveolar rhabdomyosarcoma: t(2;13)(q35;q14), PAX-3-FKHR; t(1;13)(p36;q14), PAX-7-FKHR, (6) myxoid/round-cell liposarcoma: t(12;16)(q13;p11), CHOP-TLS(FUS), (7) synovial sarcoma: t(X;18)(p11.2;q11.2), SSX1-SYT, and (8) clear cell sarcoma: t(12;22)(q13-14;q12). Many other genetic abnormalities are currently being studied to ascertain their relationship to histogenesis and oncogenesis. The molecular pathways linking genetic aberrations to known and unknown signal transduction mechanisms are being completed at a steady pace. Transfection methodologies in combination with microarray technologies will soon reveal genes that may increase our knowledge in sarcoma oncogenesis and help us to establish new therapeutic modalities.

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