Genetic changes in bone and soft tissue tumors

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Background

In 1983, it was well known that acquired, clonal chromosome rearrangements were a characteristic feature of human malignancies. At that time almost 4 000 neoplasms with chromosome aberrations had been published and systematically surveyed in the first edition of the Catalogue of Chromosome Aberrations in Cancer (Mitelman 1983). It was obvious that not only were many of the chromosome changes nonrandom but some of them were also specifically associated with a particular tumor subtype. The first specific chromosome change in a neoplastic disorder, the Philadelphia (Ph) chromosome in chronic myeloid leukemia (CML), had been reported already in 1960 by Nowell and Hungerford. However, this long remained the only example of such a relationship, in fact until the chromosome banding techniques were developed, during 1968–1973 (Caspersson et al. 1970; reviewed in Hsu 1973). This was a major breakthrough allowing not only each chromosome pair to be identified, but also facilitating the analysis of structural changes involving parts of chromosomes. The Ph chromosome, initially thought to represent a deletion of a G–group chromosome, was by banding analysis shown by Rowley in 1973 to be the result of a reciprocal translocation, t(9;22)(q34;q11). During the following decade, an increasing number of specific and nonrandom, primary as well as secondary, chromosome aberrations were identified in hematological malignancies (Heim and Mitelman 1995).

Among the 3 844 cases listed in the data base in 1983 no less than 78% were hematological disorders, 10% were lymphomas and only 12% were solid tumors (Mitelman 1983). Of the 451 solid tumors only 10 were mesenchymal neoplasms: 1 case each of liposarcoma, leiomyosarcoma and rhabdomyosarcoma, 3 sarcomas NOS and 4 malignant mesotheliomas. The reasons for these disproportionate amounts of cytogenetic data on hematological malignancies versus solid tumors, which obviously not reflect their impact in terms of morbidity and mortality in the population, were purely technical. The single cells obtained from bone marrow or peripheral blood samples were well suited for existing cytogenetic techniques, whereas chromosome preparations made directly from fresh, minced solid tumor biopsies frequently showed a poor mitotic yield and/or poor chromosome morphology. Improved techniques, including mechanical and enzymatic disaggregation of tumor tissue prior to short–term culturing, were developed in the 1980s (Gibas et al. 1984; Limon et al. 1986a) and aroused a renewed interest in this area of research, as evidenced by the first International Conference on Chromosomes in Solid Tumors in Tucson in 1985.

In 1983, two reports on chromosome aberrations in Ewing sarcoma were published (Aurias et al. 1983; Turc–Carel et al. 1983), demonstrating for the first time in a sarcoma, the presence of a characteristic balanced translocation, t(11;22)(q24;q12). This finding in combination with the new technical advances in short–term culturing inspired several research groups to start investigating the cytogenetic changes present in orthopedic bone and soft tissue tumors (BSTT). In Scandinavia, researchers in Skövde and Gothenburg had, already in 1982, reported a case of rhabdomyosarcoma with chromosome aberrations (Seidal et al. 1982), and in 1984 the group in Lund initiated a broad, systematic study of cytogenetic changes in BSTT. The first two surgical specimens, both osteosarcomas, were obtained in September and during the remaining months of 1984 and in 1985, 46 samples were investigated. Of these, 18 cases showed clonal chromosome aberrations, 18 cases had a normal karyotype and in 10 cases no dividing cells were obtained. These and similar studies were soon integrated into the multidisciplinary work of SSG as part of the overall goal to improve classification and increase the knowledge on the behavior and biology of BSTT.

It was rapidly discovered that the association between Ewing sarcoma and the 11;22–translocation was not an exceptional finding among BSTT. In 1986, it was found that also myxoid liposarcomas were associated with a balanced translocation, t(12;16)(q13;p11), and that synovial sarcomas were characterized by yet another translocation, t(X;18)(p11;q11) (Limon et al. 1986b; Turc–Carel et al. 1986a; Smith et al. 1987). In the single case of alveolar rhabdomyosarcoma mentioned above (Seidal et al. 1982) recombination between chromosome bands 2q37 and 13q14 had been identified and a second case with a balanced t(2;13)(q37;q14) was reported four years later (Turc–Carel et al. 1986b). Additional cases confirming this particular cytogenetic–histopathologic correlation were reported shortly afterwards (references in Heim and Mitelman 1995).

The first benign BSTT were investigated in 1985, and all 5 benign adipose tissue tumors showed clonal chromosome aberrations; 3 of them had a hyperdiploid karyotype with supernumerary ring chromosomes and 2 had different rearrangements involving chromosome segment 12q13–15. One of the latter cases had an aberration that was similar to the one found independently by another group in a lipoma, a t(3;12)(q27–28;q13–14) (Heim et al. 1986; Turc–Carel et al. 1986c). Studies of larger series of lipomas revealed that most cases had chromosome changes and that they were cytogenetically heterogeneous, but with nonrandom changes (Mandahl et al. 1987, 1994; Sreekantaiah et al. 1991). These findings, together with results previously obtained in meningiomas (Mark 1977; Zang 1982) and pleomorphic adenomas of the salivary glands (Mark et al. 1980), clearly demonstrated that not only malignant tumors have chromosome aberrations. It is now clear that the acquisition of chromosome changes is a characteristic feature of every benign tumor that has been studied in sufficient number to permit conclusions.

The general view that emerged from these early studies was that at least a subset of BSTT was cytogenetically similar to hematological malignancies, with relatively simple karyotypic changes including specific, sometimes even pathognomonic, rearrangements. The finding of recurrent structural chromosome rearrangements in hematological malignancies led to the idea that these recombinations might have consequences at the gene level, and that the genes of interest were located in the chromosomal breakpoints involved. Step by step, during the first half of the 1980s, data in favor of this idea were accumulated, and it could eventually be demonstrated that chimeric genes involving *ABL* in 9q34 and *BCR* in 22q11 were formed as a result of the t(9;22) in CML and that the *BCR/ABL* hybrid gene was transcribed as a novel mRNA (e.g., Stam et al. 1985). Additional examples of chimeric genes were identified, and it could also be shown that the same mechanism was active in BSTT. In 1992, it was reported that the t(11;22) in Ewing sarcoma resulted in chimeric genes involving *FLI1* in 11q24 and *EWS* in 22q12 (Delattre et al. 1992; Zucman et al. 1992). The second example of a chimeric gene in BSTT, the *FUS (TLS)/CHOP* fusion in myxoid liposarcomas with t(12;16), was reported shortly thereafter (Åman et al. 1992; Crozat et al. 1993; Rabbitts et al. 1993). The initial mapping of cytogenetic changes and identification of recurrent chromosomal breakpoints were a prerequisite for these and other successful molecular genetic studies.

Although the combination of cytogenetic and molecular genetic techniques has been highly rewarding, the gap in resolution level has been an obstacle. The rapid development of fluorescence in situ hybridization (FISH) techniques starting in the late 1980s represents a major advancement, bridging the gap between cytogenetics and molecular genetics (Gray and Pinkel 1992). There are multiple options for selection of probes that are suitable for different approaches, including for example chromosome specific centromere probes, whole chromosome or chromosome arm specific painting probes, and a variety of YAC and cosmid probes hybridizing to unique sequences. The latter have been extremely useful in mapping of breakpoints as a step towards the identification of tumor–associated gene rearrangements.

Through a particular variant of FISH, comparative genomic hybridization (CGH), net changes of DNA copy numbers in tumor cell populations can be studied (Kallioniemi et al. 1992). The major strength of this technique is that no tumor metaphase cells are needed and that the entire genome can be screened for DNA copy number changes. The latest contributions to FISH technology are various techniques for multicolor FISH producing all chromosome pairs in different pseudocolors or a specific, multi–colored fluorescence banding pattern (Schröck et al. 1996; Speicher et al. 1996; Ferguson–Smith 1997). These techniques offer a genome–wide screening of structural chromosome rearrangements, with, at least in some situations, a better precision than banding techniques, but a lower resolution.

Genetic changes in BSTT

A great body of knowledge on genetic changes in BSTT has been collected by SSG members at centra in Gothenburg, Helsinki, Lund, Oslo, and Stockholm and by many other research groups during the last 15

Table 1. The data base on chromosome aberrations in bone and soft tissues

Benign tumors	No. of cases	Malignant tumors	No. of cases
Benign mesenchymal tumor, NOS	1	Sarcoma, NOS	14
Fibroma	3	Fibrosarcoma	22
Fibromatosis/Desmoid tumor	68	Malignant fibrous histiocytoma	55
Lipoma	251	Dermatofibrosarcoma protuberans	27
Lipoblastoma	7	Clear cell sarcoma	19
Hibernoma	7	Liposarcoma	140
Leiomyoma	8	Leiomyosarcoma	30
Rhabdomyoma	1	Rhabdomyosarcoma	94
Hemangioma	1	Angiosarcoma	6
Chondroma/Chondroblastoma/ Chondromyxoid fibroma	16	Hemangiopericytoma	11
Osteocartilaginous exostosis	15	Kaposi sarcoma	4
Osteoid osteoma/Osteoblastoma	2	Chondrosarcoma	81
Tenosynovial giant cell tumor	14	Osteosarcoma	100
Neurofibroma	2	Giant cell tumor of bone	34
Neurilemoma	30	Synovial sarcoma	98
Benign mesenchymal tumor, special type [a]	62	Neurofibrosarcoma	42
		Epithelioid cell sarcoma	5
		Desmoplastic small round cell tumor	8
		Malignant mesenchymal tumor, special type [a]	60
		Primitive neuroectodermal tumors	181
Total	488	Total	1031

[a] Includes a variety of rare histopathologic entities.

Table 2. Soft tissue tumors with characteristic genetic changes

Tumor type	Cytogenetic aberration	Molecular genetic aberration
Lipoma	der(12)(q13–15)	*HMGIC* rearrangements
	t(3;12)(q27–28;q14–15)	*HMGIC / LPP* fusion
Lipoblastoma	der(8)(q11–13)	
Hibernoma	der(11)(q13)	
Spindle cell lipoma / pleomorphic lipoma	del(16q), del(13q)	
Atypical lipomatous tumor	supernumerary ring chromosome or giant marker	Amplification of 12q sequences, often *MDM2*
Myxoid liposarcoma	t(12;16)(q13;p11)	*FUS / CHOP* fusion
	t(12;22)(q13;q12)	*EWS / CHOP* fusion
Synovial sarcoma	t(X;18)(p11;q11)	*SYT / SSX1* fusion
		SYT / SSX2 fusion
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	*FKHR / PAX3* fusion
	t(1;13)(p36;q14)	*FKHR / PAX7* fusion
Infantile fibrosarcoma	+8, +11, +17, +20,	
	t(12;15)(p13;q25–26)	*ETV6 / NTRK3* fusion
Dermatofibrosarcoma protuberans	r(17;22)	*COL1A1 / PDGFB* fusion
	t(17;22)(q22;q13)	
Schwannoma	–22, del(22q)	Loss of *SCH*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWS / ATF1* fusion
Desmoplastic small–cell round–cell tumor	t(11;22)(p13;q12)	*EWS / WT1* fusion

years. At present the data base on cytogenetic aberrations in BSTT comprise 1 519 cases: 488 benign tumors and 1 031 malignant tumors (Table 1; Mitelman 1998). Below follows a brief summary of the major genetic data, including some recent information on CGH findings. Detailed references may be found in several reviews covering the cytogenetic and molecular genetic findings (e.g., Sandberg and Bridge 1994; Ladanyi 1995; Mandahl 1996; Mitelman et al. 1997b; Sreekantaiah 1998); some very recent references are provided in the text. Characteristic chromosome and gene rearrangements in soft tissue and bone tumors are listed in Tables 2 and 3, respectively.

Table 3. Bone tumors with characteristic genetic changes

Tumor type	Cytogenetic aberration	Molecular genetic aberration
Ewing tumors	t(11;22)(q24;q12)	*EWS /FLI1* fusion
	t(21;22)(q22;q12)	*EWS /ERG* fusion
	t(7;22)(p22;q12)	*EWS / ETV1* fusion
	t(2;22)(q33;q12)	*EWS / FEV* fusion
	t(17;22)(q12;q12)	*EWS /EIAF* fusion
Parosteal osteosarcoma	Supernumerary ring chromosomes	Amplification of 12q sequences
Osteocartilaginous exostosis	del(8)(q24)	Loss of *EXT1*
Myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWS / CHN(TEC)* fusion

Adipose tumors

Adipose tissue tumors are exceptionally well characterized genetically, and several specific associations between genetic changes and histopathologic subtypes have been identified. The characteristic cytogenetic feature of the childhood lipoblastomas is rearrangements of chromosome segment 8q11–13, whereas hibernomas are characterized by aberrations involving 11q13. In both tumor types, these chromosome regions may recombine with a variety of other chromosomes. Both spindle cell lipomas and pleomorphic lipomas show unbalanced chromosome aberrations with loss of material from the long arms of chromosomes 16 and 13. Ordinary lipomas are cytogenetically fairly heterogeneous, but 2/3 of the cases show aberrations involving 12q13–15. This segment may recombine with numerous other chromosome bands, but several recurrent translocations have been observed, the most common one being t(3;12)(q27–28;q13–15). The high mobility group protein gene, *HMGIC*, in 12q15 has been shown to be affected frequently and in tumors with t(3;12) a fusion gene, *HMGIC/LPP*, has been identified. Also aberrations involving 6p21–22 and 13q have been seen recurrently. A candidate gene in 6p is *HMGIY*, which has been found to be rearranged in uterine leiomyomas with 6p changes.

Atypical lipomatous tumors (ALT), borderline malignant or low malignant tumors including primarily atypical lipoma and well differentiated liposarcoma,

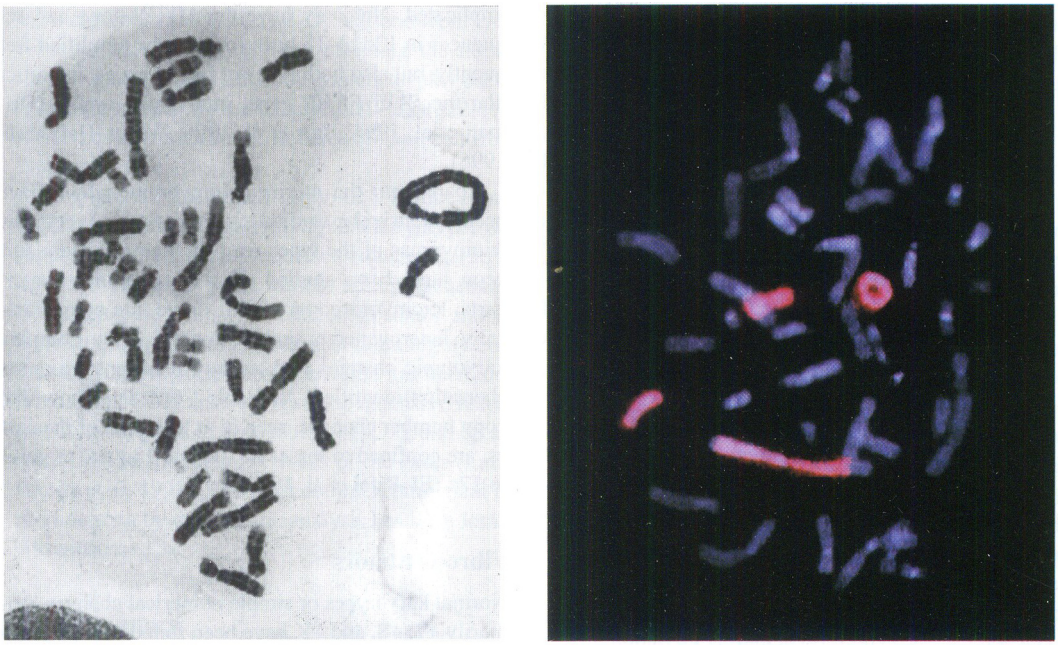


Figure 1. Metaphase cells from atypical lipomatous tumors. Left: G–banding showing a large supernumerary ring chromosome. Right: FISH using a whole chromosome painting probe for chromosome 12. Signals are seen all over the normal chromosomes 12 and the ring, whereas the long marker chromosome is only partly positive.

are characterized by one or more supernumerary ring and/or giant marker chromosomes, the origin of which was not possible to identify by banding techniques. FISH analysis was used to solve this problem and the rings and markers were shown to contain sequences from chromosome segment 12q13–15 (Figure 1). Several studies using FISH and molecular techniques have shown that these particular aberrations lead to amplification of a variable number of genes with oncogenetic potential localized to 12q, most often including *MDM2*, a known inhibitor of the tumor suppressor TP53 activity. The genomic amplification of *MDM2* is associated with over-expression of the gene (Flrenes et al. 1994).

Close to 90% of the myxoid liposarcomas, including mixed myxoid and round cell tumors, display the specific aberration t(12;16)(q13;p11), often as the sole cytogenetic anomaly. In a few cases a t(12;22)(q13;q12), or complex variants thereof, have been found. Both of these translocations lead to the formation of fusion genes, *FUS(TLS)/CHOP* in t(12;16) and *EWS/CHOP* in t(12;22). *FUS* and *EWS* belong to the same gene family and show extensive sequence homologies. In pleomorphic liposarcomas, showing complex chromosomal changes, no characteristic aberration has been identified.

Synovial sarcomas

Another tumor type showing a highly specific cytogenetic profile is synovial sarcoma, with the t(X;18)(p11;q11), including simple and complex variant translocations, found in 90% of the cases. Additional chromosome aberrations are found in 2/3 of the cases, but rarely complex changes. Cytogenetic and CGH analyses have shown partly similar and partly different results (Szymanska et al. 1998). Both methods show gain of material from chromosomes 2, 8, and 12 and loss of sequences from chromosome 3 to be frequent secondary changes. The CGH studies revealed that the average copy number changes differed by a factor of two between mono- and biphasic tumors, being more frequent among the former. Molecular genetic studies have shown that two different chimeric genes may be formed as a consequence of cytogenetically indistinguishable X;18-translocations. Common to both is the involvement of *SYT* in 18q11. This gene is fused with either of two related genes, *SSX1* and *SSX2*, in Xp11. In a study of 45 synovial sarcomas it was found that *SYT/SSX1* fusion was present in all 12 biphasic tumors, whereas *SSX1* and *SSX11* were involved in equal proportions of the monophasic tumors (Kawai et al 1998).

Tumors of synovia

For tenosynovial giant cell tumors less data are available. The localized forms have frequently shown rearrangements involving 1p11–13, in a few cases as a seemingly balanced t(1;2)(p11;q35–36). The diffuse forms seem to be characterized primarily by gain of chromosomes 5 and 7.

Muscle tumors

Among the skeletal muscle tumors, alveolar and embryonal rhabdomyosarcomas show distinctly different karyotypic profiles. Numerical aberrations dominate in the embryonal tumors and no recurrent structural aberration has been identified. Cytogenetic and CGH data are partly at variance, but both techniques show gain of chromosomes 2 and 12 in half of the cases (Weber-Hall et al. 1996). In the alveolar subtype two alternative, specific translocations, often present together with several other chromosome aberrations, have been identified, t(2;13)(q35;q14) in 70% of the cases and t(1;13)(p36;q14) in 5%. As in many other BSTT, the molecular genetic consequences are the formation of chimeric genes, involving two members of the *PAX* gene family, *PAX7* in 1p36 and *PAX3* in 2q35, and the *FKHR* gene in 13q14. Cytogenetic signs of gene amplification are seen in about 1/5 of rhabdomyosarcomas, and several genes have been implicated. Not only have the *MYCN* gene and sequences in 12q13–15 been found to be amplified frequently, but also both types of fusion genes, in particular the *PAX7/FKHR* gene, in alveolar tumors (Driman et al. 1994; Barr et al. 1996; Weber-Hall et al. 1996).

Apart from the uterine leiomyomas, which have been extensively studied cytogenetically, very few leiomyomas of the types seen by the orthopedic surgeon have been studied. Their malignant counterparts, leiomyosarcoma of soft tissue, are cytogenetically heterogeneous and frequently show complex karyotypic changes without any recurrent structural aberration identified. Available CGH data show that copy number changes, seen in at least half of the cases, are confined to losses in 10q and 13q and to gains in 17p (El-Rifai et al. 1998).

Fibrous tumors

Normal karyotypes or simple numerical changes, primarily +7, +8, and –Y, have been found in superficial fibromatoses, including Dupuytren contracture. Deep fibromatoses, desmoid tumors, show a more complex picture with gain of chromosomes 8 and 20, and

structural changes of 5q, often leading to loss of material. Trisomy 8 has been found more frequently by interphase FISH analysis, using chromosome 8 specific centromere probes, than by banding analysis. The essential outcome of the 5q aberrations may be inactivation of the *APC* gene that is associated with familial adenomatous polyposis. Infantile or congenital fibrosarcomas are characterized by simple numerical aberration, most often involving chromosomes 8, 11, 17, and/or 20. Recently, the finding in a few cases of structurally rearranged chromosomes 12 and 15 led to the identification of a chimeric gene, involving *ETV6* in 12p13 and *NTRK3* in 15q25–26 (Knezevich et al. 1998). It is possible that the numerical changes represent common secondary aberrations and that the gene fusion frequently is cytogenetically cryptic. The few adult fibrosarcomas investigated have had more complex changes and no similarities with the infantile tumors.

Fibrohistiocytic tumors

Most cases of dermatofibrosarcoma protuberans show supernumerary ring chromosomes unidentifiable by chromosome banding techniques. FISH analyses revealed that they were composed of material from chromosomes 17 and 22. Combined FISH and molecular studies showed that the essential outcome of the ring formation is the fusion of two genes, one from chromosome 17, *COL1A1*, and one from chromosome 22, *PDGFB*, without affecting the normal chromosomes 17 and 22. Some tumors display pseudiploid karyotypes with a balanced t(17;22)(q22;q13), which has also been found in cases of giant cell fibroblastoma.

Low–grade malignant fibrous histiocytomas (MFH) frequently show hyperdiploid karyotypes with supernumerary ring chromosomes containing 12q sequences. The high–grade tumors are cytogenetically heterogeneous, both within the same tumor and between tumors, and among the most often highly complex karyotypes no specific aberration has been identified. Also CGH analysis revealed extensive heterogeneity with gains of sequences in 1p, 9q, and 5p and losses in 13q found in 1/3 to 1/5 of the cases (Larramendy et al. 1997). The most common numerical aberration seen by cytogenetic analysis has been loss of chromosome 13.

Neural tumors

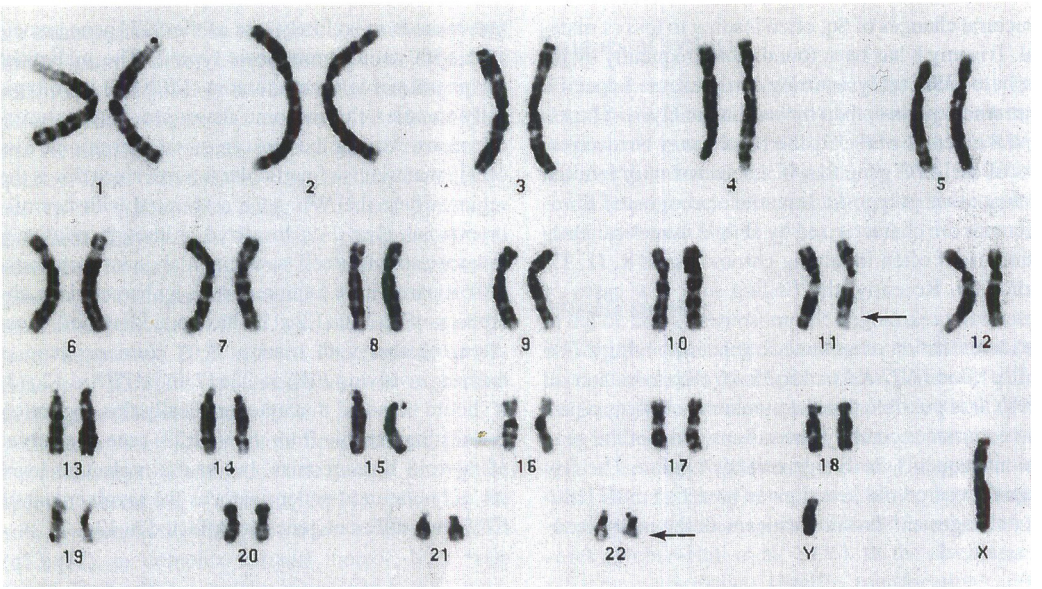
The benign schwannomas (neurilemomas) are characterized by monosomy 22 or deletions in the long arm of chromosome 22. The pathogenetically important event is most likely loss of the *SCH* gene associated with neurofibromatosis type 2. The malignant peripheral nerve sheath tumors (MPNST) have typically complex chromosome aberrations and are heterogeneous. Among the most frequent aberrations, loss of 17p material is of particular interest since this is the region where the *NF1* gene associated with neurofibromatosis type 1 is localized. Although relatively few cases of clear cell sarcoma (malignant melanoma of soft parts) have been reported, a characteristic aberration, t(12;22)(q13;q12), has been identified, most often together with trisomy 8. A chimeric gene is formed, involving *EWS* in 22q12 and *ATF1* in 12q13. It should be noted that cytogenetically this aberration is indistinguishable from the t(12;22) seen in a subset of myxoid liposarcomas, but at the molecular level the only common denominator is the involvement of *EWS*; two different genes are affected in 12q.

Miscellaneous soft tissue tumors

Despite the fact that few cases of intra–abdominal desmoplastic small–cell round–cell tumors have been investigated both a specific translocation and its molecular consequences have been identified. The t(11;22)(p13;q12) leads to the formation of a chimeric gene involving *EWS* and one of the Wilms tumor associated genes, *WT1*, in 11p13. In alveolar soft part sarcomas rearrangements of 17q25 are common. The aberrations have frequently been described as add(17)(q25) and the combined cytogenetic and FISH data indicate that chromosome 17 often recombines with the X chromosome.

The Ewing family of tumors

The t(11;22)(q24;q12), originally found in Ewing sarcomas (Figure 2), has later been shown not to be restricted to this tumor subtype, but has also been identified in tumors classified as Askin tumor, primitive peripheral neuroectodermal tumor, and peripheral neuroepithelioma. In all of these, the t(11;22) leads to *EWS/FLI1* fusion. Although this aberration is the clearly dominating one, several alternative translocations and gene fusions have been found in frequencies from about 5% down to a few single cases (Table 2). A possible factor determining the frequency by which different translocations occur may be that for some of them complex chromosome rearrangements are required in order to arrange the fusion genes in frame. Common to all variant aberrations is the involvement of the *EWS* gene, whereas the fusion partners are members of the *ETS* family of transcription factor genes. At the molecular level there is some variation



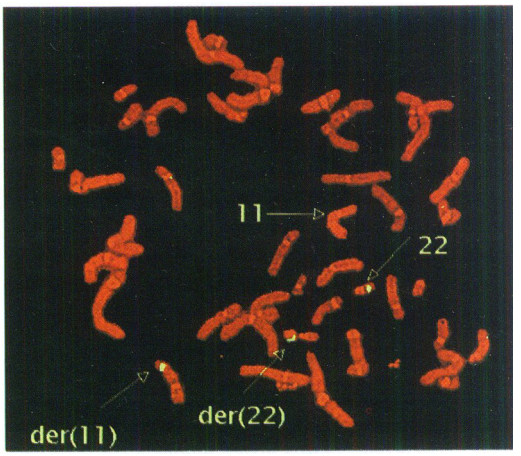


Figure 2. Metaphase cells from a case of Ewing sarcoma showing the characteristic translocation, t(11;22)(q24;q12). Top: G–band karyotype obtained from a fine needle aspiration sample; the derivative chromosomes 11 and 22 are indicated by arrows. Bottom: FISH with an EWS *probe localized to the breakpoint in chromosome 22. The translocation results in a split signal seen in the der(11) and der(22).*

in the structure of the chimeric gene; in the most common fusion mRNA variant, *EWS* exon 7 is linked in frame with exon 6 of *FLI1*.

Tumors of bone and cartilage

The low–grade parosteal osteosarcomas are, in similarity with ALT and low–grade MFH, characterized by supernumerary ring chromosomes containing sequences from 12q. The high–grade osteosarcomas typically show extremely complex karyotypic changes. Comparison of similarities between cytogenetic and CGH data indicate that loss of sequences from chromosome arms 3p, 6q, 10p, 13q, and 15q are among the most common imbalances in osteosarcomas (Tarkkanen et al. 1995).

The chromosome aberrations found in osteocartilaginous exostoses indicate that loss of segments from distal 8q is a recurrent change. One of three genes associated with multiple exostoses, *EXT1*, has been localized to 8q24 and it is possible that loss of one copy of this gene is important for the development of both hereditary and sporadic osteocartilaginous exostoses. Chondromas usually show fairly simple chromosome aberrations, in several cases with various rearrangements involving 12q13. Among the chondrosarcomas, the only specific aberration identified so far is the t(9;22)(q22;q12) in extraskeletal myxoid tumors. A chimeric gene involving *EWS* gene and *CHN* (*TEC*) is formed. Other chondrosarcomas are heterogeneous, but cytogenetically much less complex than osteosarcomas.

Clinical impact

As indicated above, many characteristic and specific, even some pathognomonic, chromosome aberrations have been identified in BSTT. This level of knowledge has been reached despite the fact that only 1 519 cases with chromosome aberrations have been reported. There is a lesson to be learned from the hematological malignancies where still new characteristic aberrations are reported despite the fact that the data

base is much larger. No doubt, many more rearrangements associated with particular tumor morphologies are to be found in BSTT.

Diagnosis

Already at this stage of knowledge, cytogenetic analysis may be an important adjunct to clinical data and histopathological investigation in order to establish a diagnosis (Mitelman et al. 1997a). At present, the prime example is the childhood small–cell round–cell tumors that offer great differential diagnostic dilemmas. The major alternatives, i.e., Ewing sarcoma, rhabdomyosarcoma, desmoplastic small round cell tumor, extraskeletal chondrosarcoma, neuroblastoma, and non–Hodgkin lymphoma, all show different cytogenetic profiles. Also adipose tissue tumors may pose diagnostic problems (Fletcher et al. 1996). The reliable associations between cytogenetic aberrations and morphological subtype may help differentiate between for example hibernoma vs. ALT, spindle cell and pleomorphic lipoma vs. ALT, ALT vs. myxoid liposarcoma, and lipoblastoma vs. myxoid liposarcoma. There are more examples, and it is expected that the usefulness of cytogenetics will increase the more the data base is expanded.

A potential problem with cytogenetics is that normal karyotypes, which are obtained in about 30% of BSTT samples, are inconclusive in terms of diagnosis. Some of the cases may represent overgrowth in culture of stromal cells, but cryptic gene rearrangements not detectable at the cytogenetic level may also be present. For quite a number of BSTT the knowledge of the genes involved in the formation of chimeric genes may be utilized. PCR analysis or RT–PCR analysis, which is used more often because of the problems associated with gene size and the variable breakpoint distribution in most tumor types, are highly sensitive techniques that may help provide a diagnosis. In series of Ewing tumors, RT–PCR, which can detect also cryptic gene changes, has proved to be more sensitive than cytogenetics. Although the majority of aberrations will be detected by a single RT–PCR, the rare variant chimeric genes may pass undetected. Search for a split *EWS* gene using suitable FISH probes may come in handy, since all variant fusion genes share the involvement of *EWS* (Figure 2). Although cytogenetics is less sensitive than RT–PCR and FISH it may have certain advantages. Reliable information is obtained only when RT–PCR or FISH show positive results with the probes used, whereas cytogenetics, if successful, may help provide an alternative diagnosis. Hence, it is not sufficient to include only one of these techniques in the diagnostic arsenal.

Prognostication

So far, the genetic changes identified in BSTT have not been proven to be useful in prognostication. Although some aberrations have been reported to correlate with survival or risk of recurrence, their strength as prognostic factors and their independence of other available prognostic factors have to be studied in more detail and thoroughly evaluated. The presence of a derivative 19p+ chromosome in MFH has been found to be associated with increased risk of both recurrence and distant metastases, in particular in high–risk patients with high–grade tumors above 5 cm in size (Choong et al. 1996). In desmoid tumors, extra copies of chromosome 8 have been reported to correlate with increased risk of recurrence (Fletcher et al. 1995). In a study of synovial sarcomas using RT–PCR, the data indicated that patients with the *SYT/SSX2* fusion gene had longer metastasis–free survival than those with *SYT/SSX1* (Kawai et al. 1998). Preliminary data from Ewing tumor patients indicate that the type of *EWS/FLI1* transcript may be a prognostic factor (Zoubek et al. 1996; de Alava et al. 1998). Patients with the most common chimeric transcript, fusing *EWS* exon 7 to *FLI1* exon 6, seem to fare better than those with other transcript variants. The significance, if any, of the identification of circulating tumor cells by RT–PCR techniques in patients with myxoid liposarcoma and Ewing tumor is unknown and has to be evaluated in prospective studies (Panagopoulos et al. 1996; West et al. 1997; Fagnou et al. 1998). In patients with Ewing tumors, the predictive value of identification of the fusion transcript in bone marrow samples is uncertain, but might be associated with an adverse prognosis (Fagnou et al. 1998; Zoubek et al. 1998).

CGH analysis has the potential to provide information of use in prognostication rather than for diagnosis, especially in tumor types characterized by genetic heterogeneity and complexity, where cytogenetics frequently results in incompletely described karyotypes and therefore, most likely, is a blunt instrument. These tumors include primarily high–grade liposarcoma, MFH, osteosarcoma, MPNST, and leiomyosarcoma. Preliminary data from MFH have for example indicated that there may be a correlation between gain of 7q32 sequences and shorter metastasis–free and overall survival, and possibly also that gain of 1p31 sequences is associated with decreased overall survival (Larramendy et al. 1997). In chondrosarcoma, gain of sequences from 8q24 may be associated with shorter overall survival (Larramendy, personal communication).

Future prospects

Future directions must be to continue the mapping of genetic changes in BSTT using all techniques available. Identification of specific translocations, fusion genes, numerical chromosome changes, DNA sequence copy number changes, and amplifications all have the potential of being useful for diagnosis and/or prognostication. However, the identification of such genetic changes will not necessarily give answers to basic tumor biological questions. Still, we know practically nothing about how and why the rearrangements that we can identify are formed. More important, we know very little about the biological consequences of even a simple balanced translocation giving rise to a chimeric transcript and a novel protein and how this relates to tumorigenesis and tumor evolution. In parallel with the necessary, and clinically useful, mapping work, these important questions must be addressed since they might provide us with knowledge that may lead to new treatment modalities.

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