Molecular and cytogenetics of soft tissue sarcomas

Mef Nilbert

Department of Oncology and Clinical Genetics, University Hospital, S-221 85 Lund, Sweden.
Tel +46-46-177569. Fax +46-46 147327. e-mail: mef.nilbert@onk.lu.se

Genetic studies of soft tissue sarcomas have revealed tumor-specific alterations with striking similarities between the diverse tumor types within this entity. Cytogenetics, i.e., the study of chromosomes, has identified about 1500 soft tissue tumors with chromosomal aberrations (Mitelman 1996), including tumor-specific aberrations and phenomena such as intratumor heterogeneity and clonal evolution. These aberrations may be numerical and/or structural and are classified as primary, secondary and cytogenetic noise. Primary chromosomal aberrations are usually tumor type specific, often occur as the sole anomaly and play an important role in tumorigenesis. Secondary aberrations are non-random but less specific, occur in addition to the primary abnormality and may be of importance for tumor progression. Cytogenetic noise can be seen as multiple numerical and structural changes and is the ultimate consequence of genetic instability. Recognition of cytogenetic abnormalities indicates mutated genes at the loci involved. Molecular genetics has in soft tissue sarcomas revealed gene amplification, isolated genetic segments frequently lost and identified mutated genes and novel gene products. This molecular and cytogenetic knowledge provides insight into soft tissue sarcoma histopathogenesis, refines tumor diagnostication, may be prognostic and perhaps paves the way for new treatment modalities.

Characteristic molecular and cytogenetic alterations

Malignant fibrous histiocytomas (MFH). Clonal chromosomal aberrations have been demonstrated in about 80 cases (Mitelman 1996). Most of these tumors have complex rearrangements with chromosome numbers in the triploid range with duplicate marker chromosomes. Several cases, however, also exhibit near-haploid or hypodiploid metaphases containing single copy markers. These findings suggest that structural rearrangements and chromosomal loss preceed polypolidization. No specific chromosomal abnormality has been identified, much because of the difficulties in distinguishing primary and secondary aberrations from cytogenetic noise and probably also because of the histologic heterogeneity within this tumor type. 15% of MFH show cytogenetic signs of gene amplification, either as homogenously staining regions (hsr) or as double minute chromosomes (dmin). The pattern of chromosomal aberrations is, however, non-random; of the karyotypically aberrant tumors 40% have rearrangements of 1q11-12, 25% carry aberrations of 19p13 and other bands frequently involved in rearrangements are 1p36, 1q21, 11p11, 17p11 and 19q13 (Mandahl et al. 1989, Örndal et al. 1994b). One third of MFH show ring chromosomes, which occur in two different contexts; as supernumerary rings with few numerical or no other changes in the 12% of the reported cases that represent MFH of low malignancy grade, and together with multiple other aberrations in MFH with complex karyotypes (Mandahl et al. 1989, Mandahl 1996). These rings contain genetic material from chromosome 12 and about 20% of MFHs show amplification of genes in the 12q13-15 region (Oliner et al. 1992a, Forus et al. 1993, Nilbert et al. 1994). Studies of recurrent MFH indicate that the acquisition of rings is an early event in tumorigenesis whereas 19p rearrangements occur later during tumor progression (Örndal et al. 1994b).

Dermatofibrosarcoma protuberans. Of the 15 cases reported, 13 had supernumerary ring chromosomes, in several tumors as the sole abnormality (Bridge et al. 1990). These rings have by Fluorescence *in situ* Hybridization (FISH) analysis been shown to contain material from chromosomes 17 and 22 (Pedeutour et al. 1993), but whether the rearrangements in the rings lead to gene amplification or represent a specific chromosomal recombination is unknown. Trisomy 8 has also been found repeatedly.

Infantile (congenital) fibrosarcomas have shown chromosomal aberrations in 10 cases. These tumors show hyperdiploid karyotypes with whole chromosome gains, in particular of chromosomes 8, 11, 17 and 20 (Sankary et al. 1993, Heim and Mitelman 1995, Mitelman 1996). The 4 cases of *adult fibrosarcomas* that have been characterized (Mitelman 1996) had hypodiploid karyotypes with multiple structural

chromosomal aberrations, which include addition of genetic material to 5p and loss of chromosome 21 (Örndal et al. 1993a, Heim and Mitelman 1995).

Liposarcomas have been chromosomally characterized in about 100 cases (Mitelman 1996).

Well-differentiated liposarcomas (also diagnosed as atypical lipoma) often show supernumerary markers, either as rings or as giant chromosomes (Mandahl et al. 1994). These markers contain chromosome 12 material, and in addition variable sequences from chromosomes 1, 4 and 16 (Pedeutour et al. 1994). Gene amplification studies and comparative genomic hybridization (CGH) have shown amplification of the segments 12q14-15 and 12q21-22 (Suijkerbuijk et al. 1994).

Myxoid and round cell liposarcomas show the specific translocation t(12;16)(q13;p11) in 90% of the tumors and less than 10 % carry alternative translocations involving 12q13, e.g., as t(12;22)(q13;q12) (Sreekantaiah et al. 1992, Heim and Mitelman 1995). Trisomy 8 and isochromosome 7q occur as nonrandom secondary changes in about 10 % of the tumors (Heim and Mitelman 1995). The molecular consequence of the t(12;16) is a fusion between the C/EBP homologous protein (*CHOP*) gene on chromosome 12 and the fusion (*FUS*) gene on chromosome 16 (also referred to as *TLS* for translocated in liposarcoma) (Åman et al. 1992, Rabbits et al. 1993). The *CHOP* gene possesses a leucine zipper dimerization domain, whereby the protein can bind to C/EBP-like proteins and block their interaction with DNA. CHOP thus acts as a dominant inhibitor of transcription. The *FUS* gene carries an RNA binding domain, which probably provides transcriptional activation. In the new hybrid gene, *FUS/CHOP*, the RNA-binding domain from *FUS* is fused to the entire *CHOP* coding region (Crozat et al. 1993). In variant translocations involving 12q13 and 22q12 the *CHOP* gene has been found to be fused with the Ewing sarcoma (*EWS*) gene on chromosome 22 (Panagopoulos et al. 1996). How these novel proteins contribute to tumorigenicity is not known.

Pleomorphic liposarcomas show complex aberrations. Rearrangements of 19q13 have been found repeatedly and no t(12;16) has been reported (Mitelman 1996).

Synovial sarcomas are cytogenetically well characterized; more than 70 aberrant tumors have been reported (Mitelman 1996). 90% of these carry the translocation t(X;18)(p11;q11), in one third of the cases as the sole anomaly (Limon et al. 1991, Heim and Mitelman 1995). Variant translocations, involving X or 18 with another partner, have been observed. The X;18-translocation involves the synovial sarcoma translocation (*SYT*) gene in 18q11, which is fused to either of the two related synovial sarcoma X chromosome breakpoint genes (*SSX1* and *SSX2*) located in Xp11 (Clark et al. 1994, de Leeuw et al. 1994a). The SSX proteins show homology to the Krüppel-associated box, but seem to lack DNA-binding regions. The *SYT* gene on chromosome 18 contains several putative SH2 and SH3 binding domains, which may be of importance for intracellular signalling (Clark et al. 1994). The derivative X-chromosome is believed to be required for tumor formation, whereas the derivative 18 is sometimes lost and has as yet failed to show any fusion gene transcript (Limon et al. 1991). Secondary numerical aberrations include +7, +8, +12 and -3.

Leiomyosarcomas have been reported to carry chromosomal aberrations in about 50 cases, one third of which were of soft tissue origin (Mitelman 1996). The pattern of aberrations is complex with considerable intertumor variability. Cytogenetic signs of gene amplification—dmin and/or hsr—are present in 20% and structural changes of 1q32, 14p11-13 and 19q13 in 15–20 % of leiomyosarcomas (Heim and Mitelman 1995).

Rhabdomyosarcomas have shown cytogenetic abnormalities in about 80 tumors; 60% of the alveolar, 20% of the embryonal and 20% of unspecified histopathologic subtype (Mitelman 1996).

Alveolar rhabdomyosarcomas carry the specific translocation t(2;13)(q35;q14) in 75 % of the cases, usually together with other aberrations (Whang-Peng et al. 1992). The variant translocation t(1;13) (p36; q14) occurs in 5% of the tumors (Biegel et al. 1991). The forkhead-domain (*FKHR*) gene on chromosome 13q14 is fused to either of the paired box genes *PAX3* in 2q35 or *PAX7* in 1p36 (Barr et al. 1993, Davis et al. 1994). The *PAX* genes encode developmentally regulated trancription factors with DNA-binding domains (paired box and homeodomain) located at the 5'end of the genes. The *FKHR* gene encodes a transcription factor of the fork head domain family, containing DNA-binding motifs related to those of the Drosophila homeotic fork head gene. The chimeric protein encoded by the der (13) consists of the *PAX3* or *PAX7* DNA-binding domains and a truncated *FKHR* fork head and C-terminal.

Embryonal rhabdomyosarcomas often contain multiple numerical chromosomal aberrations; most commonly +2, + 8, +12 and +20 (Whang-Peng et al. 1992, Mitelman 1996). Loss of heterozygosity from 11p15 and structural rearrangements of 8q11-13 and 12q13 also occur repeatedly (Heim and Mitelman 1995).

Malignant peripheral nerve sheath tumors (MPNS)

have in the 40 chromosomally aberrant cases reported shown complex abnormalities, often with near-triploid chromosome numbers. Loss of the X chromosome and rearrangements of 7p22, 17p11, 17q11 and 22q11 have been seen repeatedly, but no specific aberration has been identified (Glover et al. 1991, Mertens et al. 1995). Loss of genetic material from 17p, which is the locus for the *TP53* tumor suppressor gene and loss of material from 17q, locus of the neurofibromatosis type 1 *(NF1)* gene responsible for von Recklinghausen's disease, both occur in one third to half of MPNS (Glover et al. 1991, Mertens et al. 1995). No systematic differences have been detected between the aberrations in MPNS from patients with and without von Recklinghausen's disease.

Hemangiopericytomas have been characterized cytogenetically in 15 cases (Mitelman 1996). These tumors show near-diploid karyotypes, half of which contain aberrations of 12q13-15 (Mandahl et al. 1993). Also 19q13 and 7p15 have recurrently been involved in the alterations, which in the majority of hemangiopericytomas have led to unbalanced karyotypes.

Extraskeletal myxoid chondrosarcomas with chromosomal aberrations have been reported in only 4 cases, 2 of which had identical t(9;22)(q22;q12) and the third tumor had a variant translocation involving the same regions (Heim and Mitelman 1995, Mitelman 1996). The genes involved in this translocation are, again, the *EWS* gene on chromosome 22 and a recently identified gene on chromsome 9 termed translocated in extraskeletal chodrosarcoma *(TEC)* (Labelle et al. 1995). *TEC* is a member of the subfamily of orphan nuclear receptors and may play a role in cell proliferation and differentiation by modulating growth factor response or by interfering in the retinoic acid signalling pathway. The fusion gene created by the 9;22-translocation is composed of part of *EWS* and the whole *TEC* coding region, and thus resemble the *FUS/CHOP* fusion in myxoid liposarcomas.

Parosteal osteosarcomas appear to have a characteristic cytogenetic profile. The 4 aberrant cases reported have all shown a supernumerary ring chromosome consisting of chromosome 12 material (Mertens et al. 1993).

Clear cell sarcomas of tendons and aponeuroses (also referred to as malignant melanoma of soft parts) have displayed aberrations in 15 cases; an extra chromosome 8 was found in 11 of these tumors and a translocation t(12;22)(q13;q12) in 9 tumors (Bridge et al. 1995). This translocation results in the fusion of the *EWS* gene on chromosome 22 and the cAMP regulated transcription factor gene *(ATF1)* on chromosome 12 (Zucman et al. 1993). The fusion gene creates a hybrid protein consisting of the N-terminal of *EWS*, which carries transcriptional activation ability, linked to the functionally important DNA-binding bZIP domain of *ATF1*. The consequence of the 12;22-translocation thus resembles those of the *EWS/FLI1* and *EWS/ERG* fusions in Ewing sarcoma.

Desmoplastic small round cell tumors have been characterized cytogenetically in only 5 cases (Mitelman 1996). Interestingly, 3 of these carry an identical t(11;22)(p13;q12) translocation and the remaining two show variants of this anomaly (Heim and Mitelman 1995). The 11;22-translocation fuses the *EWS* gene in 22q12 with the *WT1* gene in 11p13, which is involved in Wilm's tumor development (Ladanyi et al. 1994). In the *EWS-WT1* fusion gene the RNA-binding domain of *EWS* is replaced by the C-terminal DNA-binding domain of *WT1*. Thus, the breaks within the *EWS* gene occur in the same region as in Ewing sarcoma.

Only few cases of epithelioid cell sarcomas, hemangiosarcomas, lymphangiosarcomas and Kaposi's sarcomas have been cytogenetically characterized and no recurrent changes have yet been recognized (Mitelman 1996).

Hereditary sarcomas

Li-Fraumeni syndrome patients carry germ-line mutations in the *TP53* gene and have an increased risk of developing malignancies of the breast, brain, adrenal cortex, blood, bone and soft tissue (Malkin et al. 1990). Constitutional *TP53* mutations have been found in 1–2% of "sporadic" sarcoma patients (Toguchida et al. 1992). Molecular genetic diagnostication of Li-Fraumeni families raises difficult ethical questions considering the wide array of tumors associated with the disease and the limited possibilities to screen for these tumor types.

Gene amplification and tumor suppressor gene mutations

Gene amplification increases the copy number of the amplified segment. These segments contain several genes, one or more of which are believed to be the target of the amplification. Surrounding genes may be co-amplified, i.e. constitute part of an amplicon. A selective advantage as a result of an increased expression of these genes is believed to be the mechanism behind amplification. The oncogene *MYCN* on chromosome 2p23-24 is a member of the *MYC* gene family, which encodes proteins that play a role in the control of cell proliferation and differentiation. Aberrant expression of *MYCN* has been detected in several childhood tumors, including neuroblastoma where it is associated with a poor outcome. *MYCN* amplifica-

tion occurs in almost half of the alveolar rhabdomyosarcomas investigated, but has only occasionally been detected in embryonal rhabdomyosarcomas (Driman et al. 1994). The biological significance of *MYCN* amplification is unknown. Amplification of genes on chromosome 12q in soft tissue sarcomas have been associated with the presence of marker ring chromosomes and the amplification is seen in two different contexts; in tumors with simple aberrations and supernumerary ring chromosomes such as atypical lipomas and well-differentiated liposarcomas, and in tumors with complex aberrations without specific changes, but sometimes displaying ring chromosomes, such as pleomorphic liposarcomas and MFHs (Örndal et al. 1992, Mandahl et al. 1994, Nilbert et al. 1994). In mixed series of soft tissue sarcomas 15-25% of the tumors have shown amplification of the human homolog of the murine double minute type two *(MDM2)* gene (Oliner et al. 1992a, Nilbert et al. 1994, Örndal et al. 1994). The amplicon usually includes the segment 12q13-15, but in well-differentiated liposarcomas a second amplicon located in 12q21-22 has also been found (Pedeutour et al. 1994, Suijkerbuijk et al. 1994). The amplified segments contain several different genes that may be important in tumor development, but among the candidate target genes are the sarcoma amplified sequence *(SAS)*, the *MDM2* gene and the cyclin-dependent kinase gene *(CDK4)* (Forus et al. 1993, Nilbert et al. 1995). The *MDM2* gene product functionally interacts with TP53; when overexpressed the MDM2 protein complex binds TP53 and inhibits the function of the latter (Oliner et al. 1992b). Whether this occurs by inhibiting TP53 binding to specific binding sites or by inhibiting its transcriptional activation is unknown, but importantly, *MDM2* amplification and *TP53* deletion may in the cell be alternative pathways. In a series of 24 soft tissue sarcomas one third of the tumors carried *MDM2* amplification and one third had *TP53* mutations, but no tumor contained alteration of both genes (Leach et al. 1993). In addition to the 12q amplicon, CGH studies of soft tissue sarcomas, including leiomyosarcoma, liposarcoma, MFH and neurofibrosarcoma, have revealed amplification of sequences from 1q21-22 in one fourth of the tumors, but the target gene in this novel amplicon is unknown (Forus et al. 1995).

Two important tumor suppressor genes in sarcoma tumorigenesis are the *TP53* gene (see pages 68–73) and the retinoblastoma gene (*RB1*). *TP53* abnormalities, including point mutations, deletions and overexpression have been detected in one third of soft tissue sarcomas, most commonly in MFH, leiomyosarcomas and liposarcomas (Andreassen et al. 1993, Leach et al. 1993, Cordon-Cardo et al. 1994). The spectrum of *TP53* mutations in soft tissue sarcomas is similar to that observed in other tumor types. Patients with retinoblastoma have an increased risk of osteosarcoma and second malignancies and loss of heterozygozity corresponding to the retinoblastoma gene locus in 13q has been detected in half of osteosarcomas and in one third of soft tissue sarcomas, including as MFHs, synovial sarcomas and leiomyosarcomas (Stratton et al. 1989). Altered expression of the RB1 protein have been reported in more than half of soft tissue sarcomas (Cance et al. 1990). Childhood sarcomas, other than osteosarcoma, rarely display *RB1* alterations.

Genetic heterogeneity

Tumor progression is a multistep process during which the proliferating cells gradually acquire new changes. A tumor thus contains subclones that are heterogenous with respect to a number of properties. Mutations and most likely also epigenetic phenomena such as methylation and imprinting drives the clonal evolution. Intratumor heterogeneity was found in 23/29 tumors in a series of soft tissue sarcomas, most of which were MFH (Örndal et al. 1994a). This implies that the molecular and cytogenetic picture derived from a single tumor sample analysis may be incomplete. However, in all cases where a primary aberration could be identified this was present in all the abnormal clones. Clonal evolution over time (multiple local recurrences and/or metastases from the same patient) was in the same series detected in 10/12 cases. In some of these tumors the cytogenetic complexity also paralleled clinical and histopathological tumor progression. In the initiation of metastatic growth the new lesion will be dominated by a subclone from the primary tumor, but gradually new subclones appear in the metastasis. Cytogenetic analysis is one way of determining the origin of metastasis. In a case of MFH, cytogenetic data showed two different lineages in the primary tumor, one of which gave rise to a local recurrence and the other to a pulmonary metastasis, whereas in a synovial sarcoma the cytogenetic findings suggested that a pulmonary metastasis, rather than the local recurrence, gave rise to a second pulmonary metastasis (Örndal et al. 1993b). The notion that metastases may metastasize needs further investigation, but may influence the timing and the extent of surgery for metastatic disease.

Diagnostic, prognostic and therapeutic possibilities

Primary chromosomal aberrations and their underlying molecular genetic alterations have potential diagnostic value. Several soft tissue sarcoma types are

Characteristic molecular and cytogenetic features of soft tissue sarcomas

Tumor type	Cytogenetics	Molecular genetics
Clear cell sarcoma	+8, t(12;22)(q13;q12)	*EWS/ATF1*
Dermatofibrosarcoma protuberans	+r(17;22)	17 and 22 amplification
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWS/WT1*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWS/TEC*
Fibrosarcoma		
infantile	+8, +11, +17, +20	
adult	complex	
Hemangiopericytoma	12q13-15 aberrations	
Leiomyosarcoma	complex	
Liposarcoma		
well-differentiated	+r(12)	12q14-15, 12q21-22 amplification
myxoid and round cell	t(12;16)(q13;p11)	*FUS/CHOP*
	t(12;22)(q13;q12)	*EWS/CHOP*
pleomorphic	complex	
Malignant fibrous histiocytoma		
myxoid	ring chromosome	12q14-15 amplification
storiform-pleomorphic	complex	1q21-22 amplification
Malignant peripheral nerve sheath tumor	complex	17p, 17q loss
Parosteal osteosarcoma	+r(12)	
Rhabdomyosarcoma		
alveolar	t(2;13)(q35;q14)	*PAX3/FKHR*
	t(1;13)(p36;q14)	*PAX7/FKHR*
		MYCN amplification
embryonal	+2, +8, +12, +20	
Synovial sarcoma	t(X;18)(p11;q11)	*SYT/SSX1 or SSX2*

characterized by highly specific chromosomal translocations. Molecular and cytogenetic studies can thus provide valuable information that may distinguish neoplastic from nonneoplastic conditions, differentiate between benign and malignant lesions, and suggest a specific diagnosis. Perhaps, genetically based classifications will in the future redefine the current histopathologic entities. Molecular and cytogenetic techniques can be applied on material derived from fine needle aspirates, tumor biopsies and fresh or frozen tumor tissue. DNA studies and CGH can be performed also on archival, paraffin embedded tissue, which opens new possibilities for retrospective studies.

Rhabdomyosarcoma accounts for half of the soft tissue sarcomas in children and adolescents. Reliable distinction between the embryonal and alveolar subtypes is necessary as each carries a different prognosis. Genetic analysis demonstrating numerical chromosome aberration and loss of 11p15 material (and in the future direct mutation testing of the underlying genetic defect) in embryonal rhabdomyosarcoma, and 2;13 or 1;13-translocations and/or their corresponding fusion proteins *PAX3* or *PAX7/FKHR* and *MYCN* amplification in alveolar rhabdomyosarcoma are valuable tools to distinguish between these two major subtypes. Synovial sarcomas, especially of the monophasic variant, may be difficult to distinguish from poorly differentiated spindle cell tumors. The identification of a t(X;18) or a *SYT/SSX* fusion can be helpful for diagnosis. Cytogenetically, the translocations in the monophasic and the biphasic histopathologic subtypes are indistinguishable. It is possible, however, that the exact Xp breakpoint at the molecular level influences the phenotype and determines whether the growth pattern be mono or biphasic; *SSX2* rearrangements seem to be commoner in monophasic synovial sarcomas (de Leeuw et al. 1994b). Cytogenetic studies of lipomatous tumors have revealed distinct patterns of aberrations. E.g., myxoid liposarcomas may be morphologically hard to indistinguish from lipoblastomas. Genetic analysis can give valuable diganostic information; the t(12;16) or a *FUS/CHOP* fusion is pathognomomic for myxoid and round cell liposarcomas, whereas a derivative chromosome 8q11-13 would argue for a lipoblastoma. Furthermore, atypical lipoma and well-differentiated liposarcoma both show karyotypes containing a supernumerary ring chromosome and giant markers involving chromosome 12 (Mandahl et al. 1994). The

cytogenetic data thus suggest that these two histopathologic entities are identical. The cytogenetic findings in typical lipomas are, however, completely different with recurrent aberrations of 12q13-15, 6p and 13q (Mitelman 1996). The tumor type specific translocations characteristic of several soft tissue sarcomas can also be used to detect occult tumor cells and may thus influence tumor staging (Peter et al. 1995).

In general, there seems to be a relationship between increasingly complex karyotypes and poor prognosis. Likewise are simple changes more commonly a feature of tumors of lower malignancy. Supernumerary ring chromosomes have been detected in e.g., atypical lipoma, parosteal osteosarcoma, dermatofibrosarcoma protuberans and low malignancy grade MFH (Örndal et al. 1992, Heim and Mitelman 1995). Some structural aberrations do, however, seem to be of importance for clinical tumor behaviour. In MFH, 19p13 abnormalities have been associated with a higher rate of local recurrence and with metastases, trisomy 8 more often occurs in relapsing MFH, whereas presence of ring chromosomes has been associated with a lower tendency for tumor relapse (Choong et al. 1996, Mandahl 1996). In alveolar rhabdomyosarcomas, an association was found between t(2;13) and older patient age and truncal location, but the genetic change did not seem to influence the tumor response rate after treatment (Douglass et al. 1993). Reduced expression of the RB1 protein have been detected more often in metastatic sarcomas (Cance et al. 1990) and an increased expression of the TP53 and MDM2 proteins have been shown to correlate with poor survival (Cordon-Cardo et al. 1994). With an increased understanding of the relation between the genetic status of the tumor cells and other tumor characteristics the genetic analysis add valuable information about the clinical outcome.

Some of the genes affected by translocations, such as *CHOP* and *ATF1* seem highly tumor type specific. *EWS*, on the other hand is involved in several different recombinations and tumor forms, e.g., in the t(9;22) of extraskeletal myxoid chondrosarcoma, the t(11;22) of desmoplastic small round cell tumors, the t(12;22) of clear cell sarcoma and in the t(12;22) variant of myxoid liposarcoma, but the exact fusions in which it participates are tumor type specific. For instance, *EWS* on chromosome 22 and *FUS* on 16 show strong homology and both genes, when recombined with *CHOP* on chromosome 12, result in a myxoid liposarcoma. The promiscuous pairing of the N-terminal parts of *EWS* and *FUS* with the DNA-binding domains of other genes, including *CHOP*, *ATF1*, *FLI1* and *WT1* seem to be necessary for *EWS* or *FUS*-associated oncogenesis and these fusions may perhaps determine the exact phenotype in some undifferentiated sarcomas.

Several of the type-specific translocations and subsequent gene fusions such as e.g., the t(12;16) - *FUS/CHOP* in myxoid and round cell liposarcomas, the t(12;22) - *EWS/ATF1* in clear cell sarcoma and the t(2;13) - *PAX3/FKHR* in alveolar rhabdomyosarcoma involve transcription factor genes. These novel transcription factor containing fusion genes presumably result in the activation of downstream target genes, which are still largely unknown. The fusion proteins generated are tumor specific antigens at which new therapeutic strategies could be directed. Unfortunately, these chimeric proteins are located inside the cell, and often even in the nucleus. Gene therapy directed at these would therefore have to interfere somewhere between transcription and protein function. Such an approach would, however, have the advantage that since presence of the fusion protein is necessary for tumor cell persistance, development of resistant cells seems less likely.

Summary

Molecular and cytogenetically soft tissue sarcomas are a well-characterized tumor entity with several tumor type specific changes understood also at the molecular level. Most likely, additional characteristic alterations will be detected, recurrent aberrations characterized at the molecular level, new clinical and histopathological correlations will be found, and little is known about how the genetic changes lead to tumor formation, promote progression and sometimes give tumor cells the ability to reccur locally or distantly. The possibility to detect cells with these acquired genetic alterations may increase the reproducibility of tumor classification, yields promising possibilities for early diagnosis of tumor recurrences and/or metastasis, and may allow development of therapeutic regimens targeted at the genetically alterated cell.

References

Åman P, Ron D, Mandahl N, et al. (1992). Rearrangement of the transcription factor gene CHOP in myxoid liposarcomas with t(12;16)(q13;p11). Genes Chrom Cancer 5: 278-85.

Andreassen Å, Oyjord T, Hovig E, et al. (1993). *p53* abnormalities in different subtypes of human sarcomas. Cancer Res 53: 468-71.

Barr FG, Galili N, Holick J, et al. (1993). Rearrangement of the PAX3 paired box gene in the paediatric solid tumor alveolar rhabdomyosarcoma. Nature Genet 3: 113-7.

Biegel JA, Meek RS, Parmiter AH, et al. (1991). Chromosomal translocation t(1;13)(p36;q14) in a case of rhabdomyosarcoma. Genes Chrom Cancer 3: 483-4.

Bridge JA, Neff JR, Sandberg AA (1990). Cytogenetic analysis of dermatofibrosarcoma protuberans. Cancer Genet Cytogenet 49: 199-202.

Bridge JA, Sreekantaiah C, Neff JR, Sandberg AA (1995). Cytogenetic findings in clear cell sarcoma of tendons and aponeuroses. Malignant melanoma of soft parts. Cancer Genet Cytogenet 52: 101-6.

Cance WG, Brennan MF, Dudas ME, et al. (1990). Altered expression of the retinoblastoma gene product in human sarcomas. New Engl J Med 323: 1457-62.

Choong PFM, Mandahl N, Mertens F, et al. (1996). 19p+ marker chromosome correlates with relapse in 69 malignant fibrous histiocytomas. Genes Chrom Cancer 16: 88-93.

Clark J, Rocques PJ, Crew AJ, et al. (1994). Identification of novel genes, *SYT* and *SSX*, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. Nature Genet 7: 502-8.

Cordon-Cardo C, Latres E, Drobnjak M, et al. (1994). Molecular abnormalities of *mdm2* and *p53* genes in adult soft tissue sarcomas. Cancer Res 54:794-9.

Crozat A, Åman P, Mandahl N, Ron D (1993). Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363: 640-4.

Davis RJ, D'Cruz CM, Lovell MA, et al. (1994). Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. Cancer Res 54: 2869-72.

Douglass EC, Shapiro DN, Valentine M, et al. (1993). Alveolar rhabdomyosarcoma with the t(2;13): Cytogenetic findings and clinicopathologic correlations. Med Pediatr Oncol 21: 83-7.

Driman D, Thorner PS, Greenberg ML, et al. (1994). MYCN gene amplification in rhabdomyosarcoma. Cancer 73: 2231-7.

Forus A, Florenes VA, Maelandsmoe GM, et al. (1993). Mapping of amplification units in the 12q13-14 region of chromosome 12 in human sarcomas: some amplica do not include MDM2. Cell Growth Different 4: 1065-70.

Forus A, Weghuis DO, Smeets D, et al. (1995). Comparative genomic hybridization analysis of human sarcomas: I. Occurrence of genomic imbalances and identification of a novel major amplicon at 1q21-q22 in soft tissue sarcomas. Genes Chrom Cancer 14: 8-14.

Glover TW, Stein CK, Legius E, et al. (1991). Molecular and cytogenetic analysis of tumors in von Recklinghausen neurofibromatosis. Genes Chrom Cancer 3: 62-70.

Heim S, Mitelman F (1995). Cancer Cytogenetics. 2nd Ed. Wiley-Liss, New York.

Labelle Y, Zucman J, Stenman G, et al. (1995). Oncogenic conversion of a novel orphan nuclear receptor by chromosome translocation. Hum Mol Genet 4: 2219-26.

Ladanyi M, Gerald W (1994). Fusion of the *EWS* and *WT1* genes in the desmoplastic small round cell tumors. Cancer Res 54: 2837-40.

Leach FS, Tokino T, Meltzer P, et al. (1993). *p53* mutation and *MDM2* amplification in human soft tissue sarcomas. Cancer Res 53: 2331-4.

de Leeuw B, Balemans M, Weghuis DI, et al. (1994a). Molecular cloning of the synovial sarcoma-specific translocation (X;18)(p11.2;q11.2) breakpoint. Hum Mol Gen 3: 745-9.

de Leeuw B, Suijkerbuijk RF, Olde Weghuis D, et al. (1994b). Distinct Xp11.2 breakpoint regions in synovial sarcoma revealed by metaphase and interphase FISH: Relationship to histologic subtypes. Cancer Genet Cytogenet 73: 89-94.

Limon J, Mrozek K, Mandahl N, et al. (1991). Cytogenetics of synovial sarcoma: Presentation of ten new cases and review of the literature. Genes Chrom Cancer 3: 338-45.

Malkin D, Li FP, Strong LC, et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233-8.

Mandahl N, Heim S, Willén H, et al. (1989). Characteristic karyotypic anomalies identify subtypes of malignant fibrous histiocytoma. Genes Chrom Cancer 1: 9-14.

Mandahl N, Örndal C, Heim S, et al. (1993). Aberrations of chromosome segment 12q13-15 characterize a subgroup of hemangiopericytomas. Cancer 71: 3009-13.

Mandahl N, Höglund M, Mertens F, et al. (1994). Cytogenetic aberrations in 188 benign and borderline malignant adipose tissue tumors. Genes Chrom Cancer 9: 207-15.

Mandahl N (1996). Cytogenetics and molecular genetics of bone and soft tissue tumors. Adv Cancer Res 69: 63-99.

Mertens F, Mandahl N, Örndal C, et al. (1993). Cytogenetic findings in 33 osteosarcomas. Int J Cancer 55: 44-50.

Mertens F, Rydholm A, Bauer HFC, et al. (1995). Cytogenetic findings in malignant peripheral nerve sheath tumors. Int J Cancer 61: 793-8.

Mitelman F (1996). Catalog of Chromosome Aberrations in Cancer. 6th Ed. Wiley-Liss, New York.

Nilbert M, Rydholm A, Willén H, et al. (1994). *MDM2* gene amplification correlates with ring chromosomes in soft tissue tumors. Genes Chrom Cancer 9: 261-5.

Nilbert M, Rydholm A, Mitelman F, et al. (1995). Characterization of the 12q13-15 amplicon in soft tissue tumors. Cancer Genet Cytogenet 83: 32-6.

Oliner JD, Kinzler KW, Meltzer PS, et al. (1992a). Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358: 80-3.

Oliner JD, Pietenpol JA, Thiagalingam S, et al. (1992b). Oncoprotein MDM2 conceals the activation domain of tumor suppressor p53. Nature 362: 857-60.

Örndal C, Mandahl N, Rydholm A, et al. (1992). Supernumerary ring chromosomes in five bone and soft tissue tumors of low or borderline malignancy. Cancer Genet Cytogenet 60: 170-5.

Örndal C, Mandahl N, Rydholm A, et al. (1993a). Cytogenetic heterogeneity and clonal evolution in a recurrent fibrosarcoma. J Exp Clin Cancer Res 12: 23-31.

Örndal C, Mandahl N, Willén H, et al. (1993b). Cytogenetic clonal evoultion in primary tumors, local recurrences, and pulmonary metastases of two soft tissue sarcomas. Clin Exp Metastasis 11: 401-8.

Örndal C (1994a). Cytogenetic heterogeneity and clonal evolution in bone and soft tissue tumors. Thesis. Lund University, Sweden .

Örndal C, Rydholm A, Willén H, et al. (1994b). Cytogenetic intratumor heterogeneity in soft tissue tumors. Cancer Genet Cytogenet 78: 127-37.

Panagopoulos I, Höglund M, Mertens F, et al. (1996). Fusion of the EWS and CHOP genes in myxoid liposarcoma. Oncogene 12: 489-94.

Peudeutour F, Coindre J-M, Nicolo G, et al. (1993). Ring chromosomes in dermatofibrosarcoma protuberans contain chromosome 17 sequences: Fluorescence in situ hybridization. Cancer Genet Cytogenet 67: 149.

Pedeutour F, Suijkerbuijk RF, Forus A, et al. (1994). Complex composition and co-amplification of *SAS* and *MDM2* in ring and giant rod marker chromosomes in well-differentiated liposarcoma. Genes Chrom Cancer 10: 85-94.

Peter M, Magdelenat H, Michon J, et al. (1995). Sensitive detection of occult Ewing's cells by the reverse transcriptase-polymerase chain reaction. Brit J Cancer 72: 96-100.

Rabbits TH, Forster A, Larson R, Nathan P (1993). Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcoma. Nature Genet 4: 175-180.

Sankary S, Dickman PS, Wiener E, et al. (1993). Consistent numerical chromosome aberrations in congenital fibrosarcoma. Cancer Genet Cytogenet 65: 152-6.

Sreekantaiah C, Karakousis CP, Leong SPL, Sandberg AA (1992). Cytogenetic findings in liposarcoma correlate with histopathologic subtypes. Cancer 69: 2484-95.

Stratton MR, Williams C, Fisher A, et al. (1989). Structural alterations of the RB1 gene in human soft tissue sarcomas. Br J Cancer 60: 202-5.

Suijkerbuijk RF, Olde Weghuis DEM, Van Den Berg M, et al. (1994). Comparative genomic hybridization as a tool to define two distinct chromosome 12-derived amplification units in well-differentiated liposarcomas. Genes Chrom Cancer 9: 292-5.

Toguchida J, Yamaguchi T, Dayton SH, et al. (1992). Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. New Engl J Med 326: 1301-8

Whang-Peng J, Knutsen T, Theil K, et al. (1992). Cytogenetic studies in subgroups of rhabdomyosarcoma. Genes Chrom Cancer 5: 299-310.

Zucman J, Delattre O, Desmaze C, et al. (1993). *EWS* and *ATF-1* gene fusion indiced by t(12;22) translocation in malignant melanoma of soft parts. Nature Genet 4: 341-5.