Soft tissue sarcoma at the turn of the millennium
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Chapter 6

Prognostic relevance of cytogenetic changes in soft tissue sarcomas

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submitted
Introduction

The prognosis of soft tissue sarcomas (STS) largely depends on tumor-specific characteristics, as histological (sub) type, tumor grade, size and site [1-3]. In recent years, significant progress has been made in identifying chromosomal abnormalities in solid tumors. Characteristic cytogenetic alterations have been demonstrated in several STS, and often have diagnostic relevance [4-7]. Well-known rearrangements include the translocation t(11;22)(q24;ql1.2-12) in Ewing’s sarcoma and primitive neuroectodermal tumors, t(12;16)(q13;p11) in myxoid liposarcomas (MXLPS), t(X;18)(p11.2; q11.2) in synovial sarcomas, and t(2;13)(q35-37;ql4) in alveolar rhabdomyosarcomas [7-9]. Most studies deal with the specific chromosomal rearrangements within histological tumor types and refer to their diagnostic relevance, whereas only a few report on prognosis of such aberrations [10-14].

The present study analyzes the prognostic significance of cytogenetic changes observed in soft-tissue sarcomas, using a computer-assisted cytogenetic analysis [13]. A database was constructed, which permits the detection of statistically significant, non-random chromosomal aberrations and allows direct comparison of different karyotypes. Special attention was paid to cytogenetic differences between metastatic and non-metastatic STS and between patients who died of the disease and who did not.

Materials and methods

For this study, material for cytogenetic analysis was obtained from consecutive STS specimens submitted for pathologic examination at the Department of Pathology of the Groningen University Hospital from 1984-1993. All STS were reviewed by a pathologist with special interest and experience in STS (WMM), and histopathologically classified according to the criteria described by Enzinger and Weiss [6]. Cytogenetic analysis was performed at the department of Medical Genetics at the University of Groningen. The criteria for inclusion in the current study were 1) a (reviewed) histological diagnosis of a primary or locally recurrent malignant mesenchymal tumor, located in the soft tissues, which had not been previously treated with radiotherapy and/or chemotherapy, 2) a successful karyotype, and 3) the availability of complete clinical follow-up. Mesenchymal proliferations of parenchymal organs were excluded.

For genetic analysis, part of the tissue specimen was minced with scalpels, incubated in a collagenase-DNAse solution and cultured in RPMI 1640 supplemented with 16% FCS, glutamine and antibiotics. After short-term culture, cells were harvested, chromosomes were G-banded using trypsin/pancreatin, and karyotypes were described according to the ISCN 1995 Guidelines for Cancer [15]. If more than one tumor per patient was described, it was decided to use the karyotype of one tumor, preferably the primary tumor, to avoid overrepresentation.

The database consisted of four main parts related to the described karyotype: 1) patient’s characteristics, 2) histopathological data, 3) gain and loss of chromosomal material, and 4) structural rearrangements. After interpretation of the karyotype, the gains and losses of chromosomal material were entered, as described by Plaat et al [13]. Each chromosome was divided according to the ideogram at 400 bands level as described by the ISCN, in such a...
way that the net gains and losses in 1p11-13 were summarized in 1p1, changes in 1p21-22 were summarized in 1p2, etc. In case of loss in a particular region a \(-1\) was entered. If the same region was lost in both chromosomes, a \(-2\) was entered. Similarly, if gain occurred in one of the chromosomal regions a \(+1\) was entered, etc. Only changes as compared to the constitutional karyotype were evaluated.

Data were analyzed for the number of tumors with gains or losses in a particular chromosomal region, and the mean change in chromosomal material per chromosomal region. Mean change in chromosomal material in each of the 86 chromosomal regions was expressed as a chromosomal change ratio (CCR), which was defined as the change in a specific chromosomal region as compared to a normal diploid karyotype. Only full abnormal karyotypes were used for the analysis of the over- or under representation of chromosomes or chromosomal regions. In the analysis of over- or under representation, marker-chromosomes were not included, because of the fact that these are structurally rearranged chromosomes, in which no part can be identified. Chromosomal changes were compared between patients with metastasizing STS and those with STS that had not metastasized, and between patients with no evidence of disease and patients who had died from their disease. Graphs were constructed to visualize the change in chromosomal material, and the differences between selected groups.

To identify chromosomal regions with significant gains or losses, 95% confidence intervals (CI) were determined. Survival curves were calculated by the method of Kaplan and Meier. Cox’s proportional hazards regression model was used to assess the importance of specific chromosomal alterations in overall and metastasis-free survival. A P-value <0.05 was considered statistically significant.

Results

Patients

Forty-three patients met the inclusion criteria, 23 males and 20 females (53% and 47%, respectively), with a mean age of 45 (range 2-81) years. Most STS (n=38) were primary tumors (88%), the others (n=5) were local recurrences (12%). Liposarcoma (LPS) was the most common histological type (n=20, 47%), followed by synovial sarcoma (n=5, 12%), malignant fibrous histiocytoma (MFH) (n=4, 9%), and rhabdomyosarcoma (n=2, 5%) [Table 1].

Table 2 presents the patients’ status of disease after a median and mean follow-up of 55 and

<table>
<thead>
<tr>
<th>Table 1. Distribution according to histopathology (n=43).</th>
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<tbody>
<tr>
<td><strong>Histopathology</strong></td>
</tr>
<tr>
<td>Liposarcoma</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
</tr>
<tr>
<td>Sarcoma nos</td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Sarcoma nos: sarcoma with no other specification</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>5</td>
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<tr>
<td>4</td>
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<tr>
<td>2</td>
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<tr>
<td>7</td>
</tr>
</tbody>
</table>
70 months, respectively (range 2-238 months). Twenty patients (47%) showed no evidence of disease, after a median and mean follow-up of 71 and 79 months, respectively (range 22-145 months). Sixteen patients had died from their disease (37%), after a median and mean follow-up of 34 and 50 months, respectively (range 2-238 months). Fourteen of these sixteen patients died from distant metastatic disease; the other two from irresectable local recurrences. One patient had died from a cerebral hemorrhage. Six patients (14%) were alive with recurrent disease, two of which had pulmonary metastases, whereas the other four (all retroperitoneal liposarcomas) had local recurrences. The mean and median metastasis free period in the metastasis group was 15 and 28 months, respectively, with a range of 0-128 months. The lung was the most common site of distant failure (n=13, 81%), followed by bone (n=3, 19%), liver (n=2, 13%), and soft tissues (n=1, 6%).

Chromosomal loss and gain

Construction of 95% confidence intervals (CI) revealed no statistically significant loss or gain of chromosomal material, expressed as chromosomal change ratio (CCR). Although not statistically significant, notable loss was seen in the chromosomal regions 15p, 21p, and 22q. Notable gain in chromosomal material was observed in region 7p1, 7p2, and 7q1 [Fig. 1].

The difference in CCR between patients still alive without evidence of disease, and patients who died from (recurrent) disease, is presented in Fig. 2. At univariate analysis, overall survival was influenced negatively by gain of chromosomal material in region 1q1-4, and by loss of chromosomal material in region 18q1-2, 14p, 18p, 10q1, Yp, Yq1, 2q2-3, 12p, 9p2, 17p, 17q1-2, and 4p (Table 3). At stepwise backward multivariate analysis, chromosomal gain in 1q (RR 38.7, P<0.001), and loss in 4p (RR 6.3, P=0.002) were the only negative prognostic factors regarding overall survival. Survival curves according to chromosomal change in the significant regions are presented in Fig. 3 a- c.

The difference in CCR between patients who developed metastases during follow-up (n=16) and patients without distant metastases (n=27) is presented in Fig. 4. At univariate analysis, significant poor prognostic factors regarding distant relapse were gain of chromosomal material in region 1q1-2, and loss in regions 18p, 18q1-2, 10q1, 2q2-3, Yp, Yq, 10q2, 14p, and 22p (Table 4). At stepwise backward multivariate analysis, chromosomal loss in 18p (RR 8.3, P<0.001) remained the only significant negative prognostic factor regarding metastasis-free survival (Fig. 5). Furthermore, there was a strong association between loss in 18p and gain in 1q in patients with the shortest survival.

### Table 2. Clinical status at follow-up.

|                | AWD  
|----------------|---------------------------------------------|
|                | (34-195; 95; 116)′ | DOC  
|                | (85)′ | DOD  
|                | (2-238; 34; 50)′ | NED  
|                | (22-145; 71; 79)′ | Total  
|                | (2-238; 55; 73)′ |
| Primary STS    | 3     | 1     | 15   | 19   | 38  |
| Local recurrence | 3   | -     | 1    | 1    | 5   |
| Total          | 6     | 1     | 16   | 20   | 43  |

AWD: Alive with disease; DOC: Dead of other cause; DOD: Dead of disease; NED: No evidence of disease.

′ Follow-up in months (range; median; mean).
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Figure 1. Mean CCR with 95% Confidence Interval of all cases

Figure 2. Mean CCR in patients with no evidence of disease (NED) versus patients who died of the disease (DOD)
Table 3. Overall Survival: statistically significant chromosomal changes at univariate analysis.

<table>
<thead>
<tr>
<th>Chromosomal Change</th>
<th>Relative Risk (RR)</th>
<th>P-value</th>
<th>Chromosomal Change</th>
<th>Relative Risk (RR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain of 1q1</td>
<td>7.7</td>
<td>&lt;0.001</td>
<td>Loss of 2q3</td>
<td>5</td>
<td>0.013</td>
</tr>
<tr>
<td>Loss of 14p</td>
<td>6.3</td>
<td>&lt;0.001</td>
<td>Loss of 12p</td>
<td>3</td>
<td>0.016</td>
</tr>
<tr>
<td>Loss of 18p</td>
<td>6.3</td>
<td>&lt;0.001</td>
<td>Loss of 18q2</td>
<td>4.8</td>
<td>0.029</td>
</tr>
<tr>
<td>Gain of 1q3</td>
<td>6.3</td>
<td>0.001</td>
<td>Loss of 9p2</td>
<td>2.2</td>
<td>0.037</td>
</tr>
<tr>
<td>Gain of 1q2</td>
<td>5</td>
<td>0.006</td>
<td>Loss of 17p</td>
<td>2.2</td>
<td>0.037</td>
</tr>
<tr>
<td>Loss of 10q1</td>
<td>5.6</td>
<td>0.009</td>
<td>Loss of 17q1</td>
<td>2.2</td>
<td>0.037</td>
</tr>
<tr>
<td>Gain of 1q4</td>
<td>4.4</td>
<td>0.01</td>
<td>Loss of 18q1</td>
<td>7.7</td>
<td>0.044</td>
</tr>
<tr>
<td>Loss of Yp</td>
<td>5.6</td>
<td>0.01</td>
<td>Loss of 4p</td>
<td>3</td>
<td>0.045</td>
</tr>
<tr>
<td>Loss of Yq</td>
<td>5.6</td>
<td>0.012</td>
<td>Loss of 17q2</td>
<td>2.1</td>
<td>0.045</td>
</tr>
<tr>
<td>Loss of 2q2</td>
<td>5</td>
<td>0.013</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 3a. Overall survival and gain in chromosome 1q

Figure 3b. Overall survival and loss in chromosome 4p
Figure 3c. Overall survival and loss in chromosome 18p

Figure 4. Mean CCR in patients with metastasizing STS versus STS that did not metastasize

<table>
<thead>
<tr>
<th>Chromosomal Change</th>
<th>Relative Risk (RR)</th>
<th>P-value</th>
<th>Chromosomal Change</th>
<th>Relative Risk (RR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain of 1q1</td>
<td>9.7</td>
<td>&lt;0.001</td>
<td>Loss of Yq</td>
<td>4.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Loss of 18p</td>
<td>8.3</td>
<td>&lt;0.001</td>
<td>Loss of 14p</td>
<td>3.3</td>
<td>0.025</td>
</tr>
<tr>
<td>Loss of 10q1</td>
<td>5.9</td>
<td>0.005</td>
<td>Loss of 22p</td>
<td>2.2</td>
<td>0.031</td>
</tr>
<tr>
<td>Loss of 10q2</td>
<td>4.2</td>
<td>0.014</td>
<td>Loss of 18q2</td>
<td>4.2</td>
<td>0.037</td>
</tr>
<tr>
<td>Loss of 2q2</td>
<td>4.5</td>
<td>0.015</td>
<td>Gain of 1q2</td>
<td>5.9</td>
<td>0.048</td>
</tr>
<tr>
<td>Loss of 2q3</td>
<td>4.5</td>
<td>0.015</td>
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<tr>
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<td>4.5</td>
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</table>
Cytogenetic analysis has demonstrated that both benign and malignant tumors often have characteristic chromosomal aberrations and that the karyotype may be important in diagnosis and treatment [5,7,16,17]. STS often reveal a very complex karyotype due to a large number of chromosomal abnormalities [13]. As all these chromosomal changes, either alone or in combination, might be responsible for certain steps in oncogenesis, it is very difficult to relate these changes to the oncogenic process. Another problem is the difficulty to interpret and compare different studies, as most of them have presented karyotypes in such a way that comparison and statistical analysis is hardly possible. To overcome this problem, we started a database in which all karyotypes are interpreted and entered in a uniform fashion, which makes a computer-assisted analysis of large groups of karyotypes possible, as described by Plaat et al. [13]. Data from the database were linked to clinical outcome to determine the prognostic importance of shared cytogenetic abnormalities.

In the present series, overrepresentation of STS with balanced translocations (especially the myxoid and round-cell liposarcoma subtype, characterized by the t(12;16) (q13;p11)
translocation) seems to be of minor importance, as such translocations do not result in net gain or loss of chromosomal material. Correlation between cytogenetic alterations and prognosis has been documented in other human malignancies [18-22]. For STS, however, hardly any data exist on the prognostic relevance of specific chromosomal aberrations [23-25]. In the present study, univariate analysis of involved chromosomal changes revealed many regions with chromosomal alterations as negative prognostic factors. Several studies have identified cytogenetic bands in these regions involved in human tumor development and progression. Specific information on STS, on the other hand, is very scanty.

In the present study, chromosomal gain in 1q1 was the most important negative prognostic factor regarding survival. A relation between alterations at the long arm of chromosome 1 and human malignancies has been demonstrated in various tumor types, suggesting the existence of oncogenes (breast cancer [26], prostate cancer [27], and endometrium cancer [28]), as well as tumor suppressor genes (breast cancer [26]) and medulloblastoma [29]). In liposarcomas, there are indications that amplification of genes located at 1q21-24, often with concomitant gain in 12q14-21, plays a significant role in development and progression [30]. Furthermore, the long arm of chromosome 1 is a region of particular interest with regard to metastasis, as it harbors the KiSS-1 metastasis-suppressor gene at 1q32, which has been identified as a metastasis-suppressor gene in malignant melanoma and breast cancer [31]. However, as gain of chromosomal material at the long arm of chromosome 1 was a negative prognosticator for metastasis-free survival, it seems very unlikely that this metastasis-suppressor gene is involved in the metastatic process of STS.

The other negative prognostic factor regarding survival, loss in 4p, was surprising, as there is only very limited information on its role in human malignancies [32,33]. In their search for the human homologue of the SH3BP2 protein in bladder cancer, Bell et al. identified an interesting gene at 4p16.3 that is a potential negative regulator of the Abl gene [34], a proto-oncogene that has been related to differentiation and apoptosis inhibition in chondrosarcoma [35]. The association between high malignancy grade, a well-known negative prognostic factor regarding disease-specific survival, and low amounts of apoptosis in STS [36], might further support a potential role of SH3BP2 and Abl genes in STS prognosis.

At univariate analysis, aberrations in many other chromosomal regions were related to survival. Because of the magnitude of genes, located at these regions, and the fact that we did not investigate specific gene products, it is impossible to predict which chromosomal bands and which genes will be involved, although some regions (9p, chromosome 17, and 18p) are very intriguing. The negative prognostic importance of loss in region 9p2 was not unexpected, as this region contains the p16 (CDKN2A/INK4A) and p15 (CDKN2B/INK4B) genes, which act as negative regulators of proliferation of normal cells. Deletions or mutations of these CDK (cyclin-dependent kinase)-4 and 6 inhibitors lead to unchecked cell growth, which has been demonstrated in many human cancer types [18,21,37]. In Ewing’s sarcoma, Wei et al. demonstrated that p16 deletion was a very strong negative prognostic factor (P<0.001) [37]. In Wilms’ tumors, loss of p16 also seems
to be of prognostic importance, as it correlates with advanced tumor stage [39]. In STS, however, the prognostic impact of both p16 and p15 alterations remains contradictory. Orlow et al. reported a significant relation between p16 deletions or alterations and poor survival in 46 STS (P=0.036 and 0.005, respectively), with alteration of the IKN4A/B gene being the only statistically significant predictor for poor survival when controlling for tumor grade and size (P=0.03) [40]. Yao and Meye investigated the role of the p16 gene status and expression in STS, and reported a low frequency of deletions and mutations of this gene in STS, in contrast to Simons et al., who demonstrated a loss of 9p21 in 55% of MFH [42-44]. Moreover, Yao et al. suggested that CDK4 might act as an oncogene in STS, which is in contrast to findings in other human malignancies where CDK4 acts as a tumor suppressor gene [41]. In the present study, however, loss of region 9p2 is a statistically significant negative prognostic factor (P=0.037), suggesting the location of a STS suppressor gene in this region.

The prognostic importance of chromosomal loss at the short arm of chromosome 17 is very interesting, as some important tumor suppressor genes are located there. TP53, located at 17p13.1, is the most common tumor suppressor gene, altered in many malignancies. A study from the Memorial Sloan Kettering Cancer Center demonstrated that 17p deletions and p53 mutations were common events in adult STS [44]. Dei Tos et al. demonstrated a 30% incidence of p53 aberrations in myxoid and round cell liposarcoma [45]. However, these studies did not provide any information on the prognostic importance of the p53 alteration. In a series of 113 bone and soft tissue sarcomas, Mousses et al. reported p53 alterations at different frequencies in various sarcomas; furthermore, these alterations were not associated with prognosis [46]. As p53 expression was not examined in the present study, we can not be sure whether this gene product is responsible for the poor prognosis in patients with loss of the short arm of chromosome 17, or whether other suppressor genes at 17p are involved, as has been reported in sporadic breast cancer [47].

Besides chromosomal loss at the short arm of chromosome 17, also loss at its long arm had prognostic importance. One of the genes that might contribute to this is the proto-oncogene c-erbB-2, at band 17q21.1. Although a correlation between loss of 17q and a high degree of amplification of c-erbB-2 has been demonstrated in human breast cancer [48], no reports on its prognostic importance in STS are available.

Loss of the short arm of chromosome 18 (18p1) was another important negative prognostic factor, both in metastasis-free and overall survival, suggesting the location of (a) putative suppressor gene(s). In the literature, there is some evidence for the presence of tumor suppressor genes on the short arm of chromosome 18, involved in breast carcinoma, NSCLC, and brain tumors [49]. Apart from that, information on the role of 18p in human malignancy is extremely scarce.

Except for retroperitoneal STS, survival in STS has been related directly to metastasis. In human malignancies in general, and in STS in particular, the cytogenetic base for metastasis remains obscure. As reports revealing possible clues are awaited, the finding that loss of 18p was the only statistically significant negative prognostic factor regarding metastasis-free survival is very interesting, as it suggests the location of a putative (sarcoma-) metastasis suppressor gene.
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The computer-assisted approach, used in the present study, is valuable in the analysis of large groups of complex karyotypes to detect common chromosomal alterations. The strong association between gain in the long arm of chromosome 1 and loss in the short arm of chromosome 18 in patients with the shortest survival has not been reported before, and its importance in STS prognosis remains unclear. In conclusion, the present study provides indications for correlations between cytogenetic changes and metastasis and prognosis in STS. Some of these findings confirm earlier reports, whereas many are novel in STS, and need to be confirmed in additional studies. The strong association between alterations in the long arm of chromosome 1 and the short arm of chromosome 18 in non-survivors is very challenging and may have prognostic value in STS.

References

14. Plaat BEC, Muntinghe FLH, Molenaar WM, et al. Clinical outcome of patients with previously