From the Departments of Orthopedics, Söder Hospital and Karolinska Hospital and Department of Cellular and Molecular Tumor Pathology, Karolinska Institute, Stockholm, Sweden

Synovial sarcoma

A Scandinavian Sarcoma Group project

Björn Skytting

ACTA ORTHOPAEDICA SCANDINAVICA SUPPLEMENTUM NO. 291, VOL. 71, 2000

SCANDINAVIAN UNIVERSITY PRESS

Oslo - Copenhagen - Stockholm - Boston

Appendix

This presentation is based on the following papers:

I. Clinical course in synovial sarcoma—a Scandinavian Sarcoma Group study of 104 patients. Skytting B T, Bauer H C F, Perfekt R, Huuhtanen R, Alvegård T, Berlin Ö, Gustafson P, Klepp R, Löfvenborg R, Sæter G, Trovik C, Wahlström O. Acta Orthop Scand 1999; 70 (6): 536-42.

II. Synovial sarcoma—identification of favorable and unfavorable histologic types—a Scandinavian Sarcoma Group study of 104 cases. Skytting B T, Meis-Kindblom J M, Larsson O, Virolainen M, Perfekt R, Åkerman M, Kindblom L-G. Acta Orthop Scand 1999; 70 (6): 543-54.

III. Ki-67 is strongly prognostic in synovial sarcoma—analysis based on 86 patients from the Scandinavian Sarcoma Group Register. Skytting B T, Bauer H C F, Perfekt R, Nilsson G, Larsson O. Br J Cancer 1999; 80:1809-14.

IV. The SYT-SSX1 variant of synovial sarcoma is associated with high rate of tumor cell proliferation and poor clinical outcome. Nilsson G, Skytting B, Xie Y, Brodin B, Perfekt R, Mandahl N, Lundeberg J, Uhlén M, Larsson O. Cancer Res 1999; 59: 3180-4.

V. Genetic imbalances in 67 synovial sarcomas evaluated by comparative genomic hybridization. Szymanska J, Serra M, Skytting B, Larsson O, Virolainen M, Åkerman M, Tarkkanen M, Huuhtanen, Picci P, Bacchini P, Asko-Seljavaara S, Eloma I, Knuutila S. Genes, Chromosomes & Cancer 1998; 23: 213-9.

VI. Clinical importance of secondary aberrations in synovial sarcoma evaluated by comparative genomic hybridization. Skytting B T, Szymanska J, Aalto Y, Lushnikova T, Blomqvist C, Elomaa I, Larsson O, Knuutila S. Cancer Genet Cytogenet 1999; 115: 39-46.

Summary

Synovial sarcoma accounts for 5–10% of all soft tissue sarcomas. More than 90% are found in the extremities or trunk wall. Characteristic for synovial sarcoma is the translocation t(X;18) (p11.2;q11.2). Cloning of the breakpoints of this translocation revealed fusion of two novel genes, *SYT* and *SSX*. The *SYT* gene, located on chromosome 18, is fused with one of three closely related genes; *SSX1*, *SSX2* or *SSX4* located on the X chromosome.

The long term survival rates have continuously improved and have at best been reported to around 50%. However, since almost no population based studies on synovial sarcoma have been reported, these improvements may be due to differences in patient selection due to a changes in referral practice. This project was based on a consecutive series of synovial sarcoma patients from the Scandinavian Sarcoma Group Register acquired during a 9-year period. Only surgically treated patients without metastases at diagnosis were included in the prognostic analyses. The tumors were defined clinically, histopathologically, molecular and cytogenetically and these features were related to clinical course.

Epidemiology

34 of 104 patients developed metastases. The overall 5 and 7 years survival rates were 0.76 (95% CI 0.66–0.83) and 0.69 (0.58–0.78), respectively. Large tumor size and amputation were significantly associated with impaired metastasis free survival. In addition, patients with local recurrence had a higher risk for metastases following the local event.

Histology

All were high grade lesions, 74 Grade III and 30 IV. Kaplan-Meier estimates of metastasis-free survival at 5 years were 83% (95% CI 72–92%) for patients with Grade III tumors *versus* 31% (95% CI 13–51%) for Grade IV. Histologic grading conveyed more prognostic information than any single histologic factor.

Immunostaining with anti-Ki-67 antibodies (MIB1) and p53 based on formalin-fixed paraffin-embedded material from 86 patients revealed that MIB-1 ≥ 10% was associated with poorer metastasis-free survival but p53 was not.

Genetics

Type of fusion transcripts (SYT-SSX1 or SYT-SSX2) and Ki-67 were assessed in fresh frozen tissue from 33 patients. The 5-year metastasis-free survival for patients with SYT-SSX1 was 42% *versus* 89% for those with SYT-SSX2. The hazard ratio for metastasis associated with the SYT-SSX1 fusion transcripts was 7 (95% CI 1.5–36, log-rank $p = 0.004$). There was a significant association between SYT-SSX1 and high tumor proliferation rate.

Comparative Genomic Hybridization revealed DNA sequence copy number changes in 35 of 69 tumor specimens. The frequency of aberrations/tumor were higher in monophasic tumors than in biphasic. Gains of chromosome 8 were associated with large tumors (>5 cm). There was no obvious association between secondary aberrations and clinical outcome.

Conclusions

Large tumor size, local recurrence, histologic Grade IV, MIB1 index ≥ 10 and possibly SYT-SSX1 fusion transcript were associated with impaired clinical outcome.

Introduction

Synovial sarcoma is a rare soft tissue tumor, accounting for 5–10% (Gustafson 1994, Enzinger and Weiss 1995) of all soft tissue sarcomas. Synovial sarcoma is mostly located in the extremities and can occur at any age including childhood but most commonly in young adults (Cadman et al. 1965, Moberger et al. 1968, Wright et al. 1982, Brodsky et al. 1992, Mullen and Zagars 1994, Choong et al. 1995). More than 90% are found in the extremities or trunk wall (Moberger et al. 1968, Wright et al. 1985). The earliest convincing morphological description of synovial sarcoma is attributed to Lejars and Rubens-Duval in 1910, who described a biphasic synovial sarcoma and also suggested the existence of a monophasic subtype, composed exclusively of spindle cells. The term synovial sarcoma was coined by Knox in 1934 who stressed the morphologic biphasic appearance for the diagnosis. The morphologic appearance and the common proximity to joints or tendon sheaths gave rise to the term but there is now no evidence that synovial sarcoma cells have a synovial origin. Immunohistochemistry, and to a certain extent, electron microscopy, have made it possible to recognize epithelial-like components in sarcomas of predominantly spindle cell type, permitting recognition of monophasic synovial sarcoma. With the discovery of the translocation t(X;18)(p11.2;q11.2) (Turc-Carel et al. 1986, Limon et al. 1986), so far specific for synovial sarcoma, the entity of synovial sarcoma could be considered distinct. Cloning of the breakpoints of this translocation revealed fusion of two novel genes, SYT and SSX (Clark et al. 1994). The SYT gene, located on chromosome 18, is fused with one of 3 closely related genes; SSX1, SSX2 or recently SSX4 (Skytting et al, 1999) located on the X chromosome (Crew et al, 1995; de Leeuwe et al, 1995).

Surgery is the main treatment for synovial sarcoma often supplemented with radiotherapy. Amputation was earlier common, but with improved imaging techniques, CT and MRI, limb sparing surgery is performed in most cases. An excellent local control (Choong et al 1995) has been reported from specialized centers. In contrast local recurrence rates of up to 80% have been recorded (Menendez et al. 1992) after inadequate surgery without supplementary radiotherapy. Adjuvant chemotherapy, mostly based on Ifosfamide is frequently administered to children (Ladenstein et al. 1993) but its benefit remains unproven. There are no randomized trials and the number of patients included in trials are small (Kampe et al., 1993; Rosen et al., 1994). Synovial sarcomas primarily metasta-size to the lungs. Both thoracic surgery and chemotherapy has been shown to be of limited value.

The long-term survival rates have continuously improved and have at best been reported to around 50% (Choong, 1995). However, since almost no population based studies on synovial sarcoma have been reported, these improvements may be due to differences in patient selection because of a changes in referral practice.

There are numerous publications on synovial sarcoma, but there are only a few reports based on series comprising more than 100 patients and complete long-term follow-up to permit reliable clinicopathological conclusions. Prognostic factors have not been well established and the relationship between clinicopathogenetic features and clinical course remains controversial.

Major developments have been made in immunohistochemistry, cytogenetics and molecular genetics, permitting the application of these methods not only for diagnostic but also for prognostic purposes. Comparative Genomic Hybridization (CGH) have made it possible to screen tumor cells for genetic aberrations. The basic principle of CGH is that underrepresented areas might express tumor suppressor genes and over-represented areas, oncogenes. With PCR and DNA sequencing analysis, the distinction between tumors containing SYT-SSX1 and SYT-SSX2 fusion transcript is possible, recently claimed to be of prognostic importance (Kawai et al. 1998).

This project was based on a large consecutive series of synovial sarcoma patients from the Scandinavian Sarcoma Group Register acquired during an 9-year period. The tumors were defined clinically, histopathologically and genetically. These features were related to clinical course.

Patients

The project was based on patients with synovial sarcoma reported to the Scandinavian Sarcoma Group (SSG) Register (Figure 1). In addition synovial sarcoma patients were identified in the National Swedish Cancer Register (NSCR) and archival tumor tissue from Italian patients was used for analysis in Paper **V** (Figure 2). The material in Papers **I, II**, and **III** was based on148 patients, *i.e*. 136 patients from the SSG Register and 12 patients from the NSCR (Figure 2). Consultation with the Swedish Solid Childhood Tumor Registry verified that children with synovial sarcoma were reported to the SSG Register. The inclusion criteria were: diagnosis between March, 1, 1986 and December, 31, 1994, histopathologically reviewed as synovial sarcoma, no clinically detectable metastases at the time of diagnosis, tumor location in the extremities or trunk wall and patients operated in a curative attempt.

18 patients were excluded because the histological review (Paper **II**) did not support the diagnosis of synovial sarcoma. 16 additional patients were excluded because of metastatic disease at the time of primary diagnosis (7), tumor location outside the extremity or trunk wall (8), or because no curative surgical treatment was given (1). 10 Nor-

Figure 1. Number of patients entering the study from different SSG centers (Papers I and II).

wegian or Finnish patients reported to the SSG Register who received all treatment for primary tumor outside an SSG center were excluded due to risk of systemic bias regarding clinical characteristics between patients primarily treated at an SSG center versus outside. However, all Swedish patients were included since they may be regarded as a population based cohort.

104 patients constituted the basis for the studies in Papers **I** (clinical) and **II** (histopathological). The same patients formed the basis for Paper **III** (immunohistochemical), but archival tissue was only available in 84 patients for analysis of Ki-67 and in 70 patients for analysis of p 53. This applied also for Paper **VI** (CGH II) except that 4 patients with metastases at diagnosis were added leaving 71 patients for analysis. Paper **V** (CGH I) was based on a heterogeneous material of 20 cases from Instituto Ortopedici Rizzoli (Bologna, Italy) and 47 from the SSG Register. Paper **IV** (primary aberration) was based on 20 patients from the SSG Register where fresh frozen tissue were available. To expand the series 13 patients, diagnosed after December 31, 1994, were included.

Medical records were reviewed in all cases to verify and complete reported clinical data. This was accomplished by visiting the different SSG centers or by requesting medical charts. The follow-up period was calculated from the day of diagnosis. No patient was lost to follow-up. Two patients who died of non-tumor related causes were censored at the time of death in the analysis of metastasis-free survival. The median follow-up for survivors was 6 (3–11) years.

Comments

This patient series is unique in several ways. Although there were more than 100 patients these were collected during less than 10 years as opposed to many previous studies (Wright et al. 1982, Mullen and Zagars 1994). The only previous large population based series was published by Moberger and Nilsonne in 1968. The series was multicenter and multinational but nevertheless partly population based. Unfortunately, the immunohistochemical and cytogenetic studies could not be applied to all cases due to lack of sufficient archival tissue. Most importantly, none of the patients have been lost to follow-up.

Assessment of clinical data

The following patient and tumor characteristics were assessed: age, sex, tumor location, depth, compartmentalization, size, surgical procedure and margin, radiotherapy and treatment center. Age was dichotomized at 20 to facilitate comparison with other studies (Brodsky et al. 1991, Oda et al. 1993, Mullen and Zagars 1994, Choong et al. 1995). Location of primary tumor was characterized as *proximal* in knee, thigh, groin, gluteal, trunk, shoulder, upper arm, elbow and *distal* in lower leg, foot, lower arm and hand. Superficially located tumors without involvement of the deep fascia were *subcutaneous* , all others were considered *deep-seated* . Tumors violating the boundary of the compartment (Enneking et al. 1980) were *extracompartmental* , tumors within the compartment were referred to as *intracom-partmental* . Tumor size (cm), was defined as the largest diameter assessed from the surgical specimen or from MRI/CT examinations. The surgical procedure was classified as *local excision or amputation*. The surgical margins; intralesional, marginal, wide and compartmental were classified according to Enneking et al (1980).

Patients with a marginal surgical margin and adjuvant radiotherapy or with a wide/compartmental margin were considered *adequately treated* as opposed to *inadequate treatment; i.e.* intralesional or marginal margin without radiotherapy. Patients receiving all or final surgery for primary tumor at an SSG institution were considered to be treated at an SSG center.

Statistics

Metastases-free and overall survival were analyzed multi- and univariately according to Cox's proportional hazards model supplemented with univariate comparisons using the log-rank test and Kaplan-Meier survival estimates. Two-sided p-values from Fisher's exact test were used to assess associations between categorical variables. A p-value < 0.05 was considered significant. The statistical analyses were performed by Roland Perfekt Ph.D., Southern Swedish Regional Tumour Registry, University Hospital in Lund, Sweden.

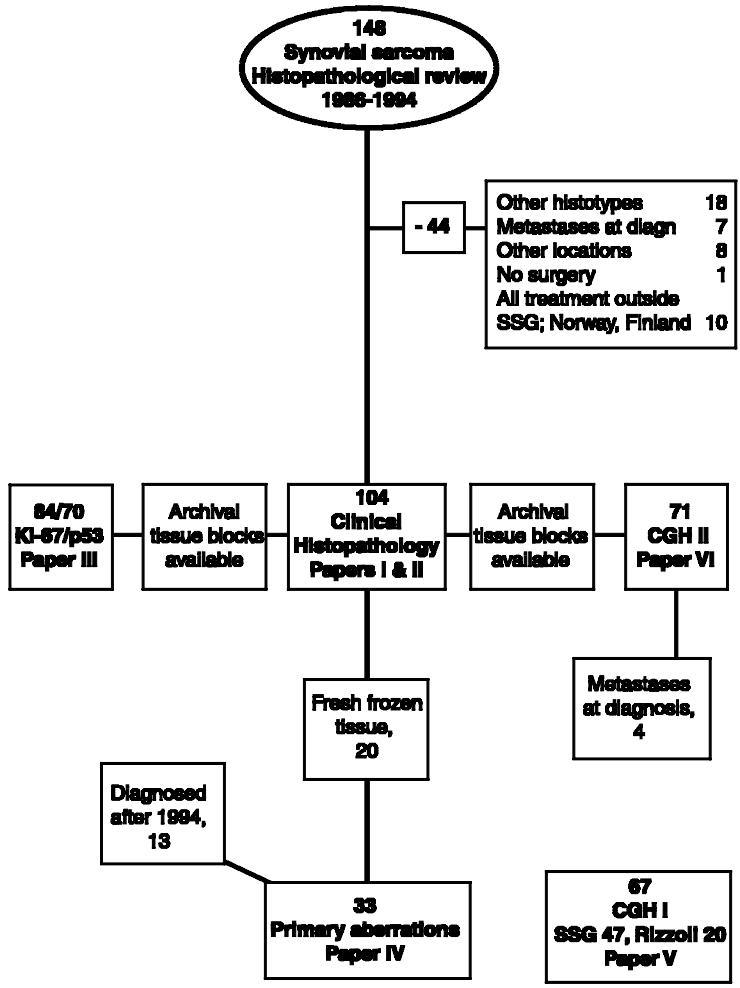

Figure 2. Patient material and selection of patients in the various studies.

Histopathogenetic methods and findings

Histopathological assessment

Archival material, including original slides, paraffin blocks and histologic reports were collected. All slides were re-examined by the pathologists of the SSG's peer-review group without access to clinical data. In selected cases immunohistochemical stains were performed. The tumors were all subclassified as monophasic fibrous or biphasic synovial sarcoma, and evaluated regarding the presence of poorly differentiated areas, tumor necrosis, vascular invasion, type of tumor interface with normal tissue, mitotic activity, and tumor grade.

Subtype: The lesions were subclassified as biphasic or monophasic fibrous type based on light microscopic features.

Poorly differentiated: Beside the subtypes biphasic or monophasic fibrous types, lesions with poorly differentiated areas were identified as previously described (Meis-Kindblom et al. 1996, Folpe et al. 1998, van de Rijn et al. 1999).

Tumor grade: According to SSG protocols, all soft tissue sarcomas were graded using a IV-tiered scale modified from Broders et al. 1939. All 104 synovial sarcomas were regarded as high grade (III–IV). Tumors containing poorly differentiated areas were Grade IV, as were lesions with unusually prominent nuclear atypia, high cellularity and nuclear crowding, high mitotic activity, or necrosis. All other tumors were Grade III.

Tumor necrosis: Lesions with a microscopic focus of necrosis regardless of size, evaluated in 103 cases.

Vascular invasion: Tumor floating freely within a vessel lumen as well as tumor invading through the vessel wall into the lumen without covering

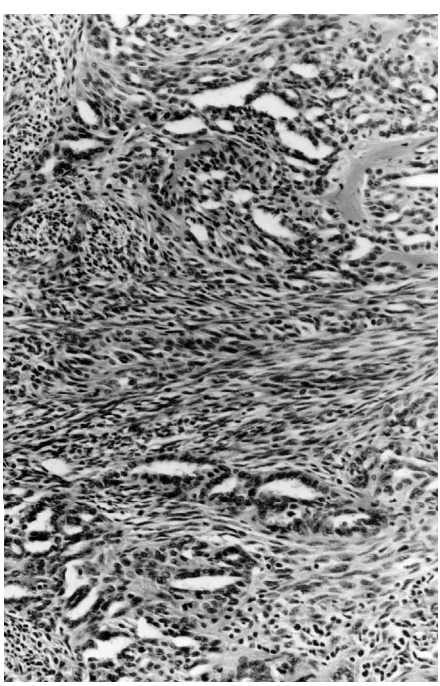

Figure 3. Grade III, biphasic synovial sarcoma showing distinct glands and a spindle cell component that is characterized by uniform cells with relatively small, bland, oval nuclei.

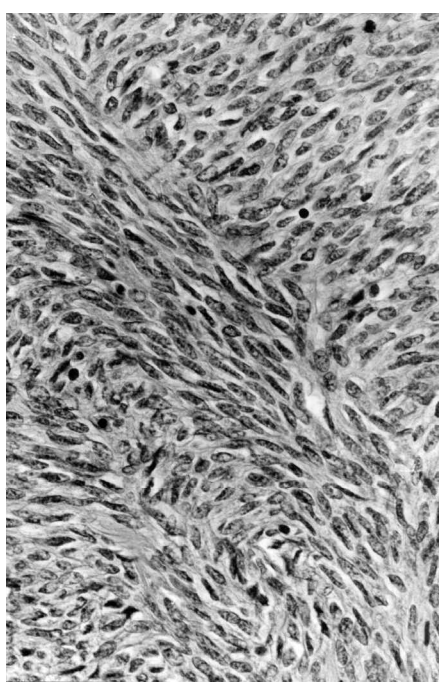

Figure 4. Grade III, monophasic fibrous synovial sarcoma showing oval and elongated nuclei that are uniform with an evenly distributed chromatin pattern and inconspicuous nucleoli.

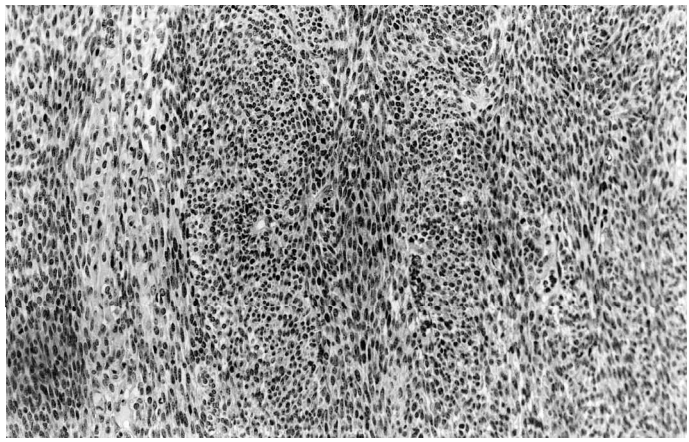

Figure 5. Grade IV, monophasic fibrous synovial sarcoma with a poorly differentiated area resembling a malignant peripheral nerve sheath tumor and characterized by increased cellularity, nuclear crowding and atypia, nucleolar prominence, and high mitotic activity.

endothelium, evaluated in 103 cases.

Growth pattern: The interface between the tumor and the surrounding tissue was assessed as "pushing" or "infiltrative", evaluated in 93 cases. In 10 cases there was no surrounding normal tissue.

Mitotic activity: Mitotic activity was evaluated by counting the number of mitotic figures per mm^2 and registered as low (0–2), moderate (3–9), and high (10 or more).

Findings

33 tumors were classified as biphasic synovial sarcoma (Figure 3) and 71 as mono-phasic, fibrous type (Figure 4). Poorly differentiated areas (Figure 5) were seen in 22 cases; only 2 of these were biphasic. 74 tumors were Grade III and 30 Grade IV. 8 of the 30 grade IV tumors had no "poorly differentiated" areas; 3 of these were biphasic and five monophasic.

Immunohistochemichal examinations

The monoclonal antibody Ki-67 has proven to be a reliable marker for measuring cell proliferation in human neoplasms (Brown et al. 1990). Anti-Ki-67 exclusively reacts with a protein (Ki-67) expressed only during the proliferating phase of the cell cycle (late G_1, S, G_2 and mitosis) (Gerdes et al. 1984). The p53 gene, a tumor suppressor gene, is believed to play an active role in cell growth control (Raycroft et al. 1990). It acts as a regulatory check point in the cell cycle, arresting the cell in G_1 phase if DNA damage has occurred (Yin et al., 1992). In contrast, mutated p53, found in neoplastic cells, fails to block cell cycle progression (Nigro et al, 1989). Therefore, p53 mutations may contribute to uncontrolled cell growth.

All original hematoxylin and eosin-stained slides were re-examined and matched to the corresponding paraffin-embedded tissue blocks (Paper **III**). One representative block per tumor was selected for immunostaining which was performed according to the standard ABC-technique (Elite Standard Kit. Cat. PK-6100; Vector, Burlingame, Ca). Antigen retrieval was performed by immersing the specimens for 10 minutes in a citrate buffer at pH 6 and heating in a microwave oven. The primary antibodies used were: anti-Ki-67 (MIB-1; Immunotech, Marseille, France) 1:50 and anti p53 (DO-1, SDS; Santa Cruz Biotechnology, Santa Cruz, Ca) 1:100. A biotinylated antimouse immunoglobulin G was used as a secondary antibody. The peroxidase reaction was developed using 3,3 - diaminobenzidine (diaminobenzidine tetrahydrochloride, 0.6 mg/ mL with 0.03% hydrogen peroxide) for 6 minutes. Hematoxylin was used as the nuclear counterstain.

A semiquantitative score was employed to assess the percentage of cells that were positively stained regardless of staining intensity. The per-

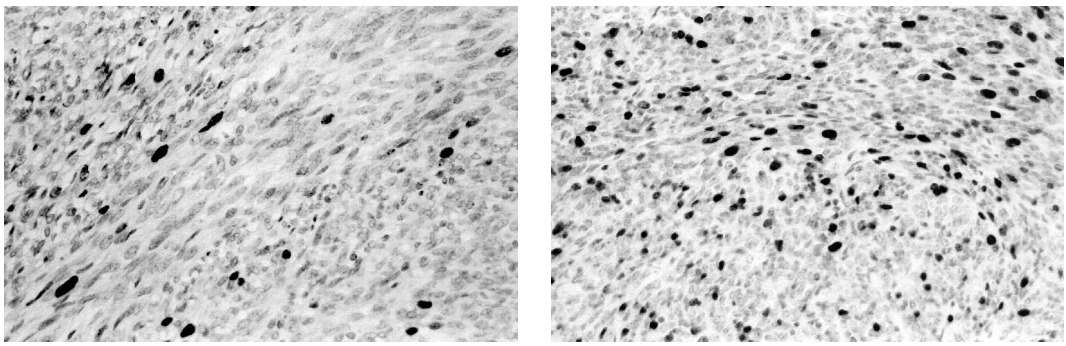

Figure 6. Left: Low proliferating activity: MIB-1 index = 0–9%. Right: High proliferating activity: MIB-1 index ≥ 10%.

centage of MIB-1 and p53 positive cells in 10 high power fields (x 250) were graded: 0–1%, 2–9%, 10–24%, 25–49%, 50–74% and 75–100%. A MIB-1 index of 10% or more was considered *highly proliferative* (Figure 6). Specimens where at least 25% of the nuclei stained positive for p53 were regarded as carrying p53 mutations similar to a previous study (Drobnjak et al. 1994).

All the immunohistochemically stained slides (which were coded) were analyzed microscopically independently by me and Olle Larsson without knowledge of clinical data. In each case we analyzed more than 1000 cells.

Findings

Proliferation index was assessed by the MIB-1 antibody in 84 patients. Nuclear over-expression of p53 was determined in 70 patients. The distribution of MIB-1-labled nuclei among the 84 cases was: *0–1%*, 13; *2–9%*, 33; *10–24%*, 22; *25–49%*, 14; *50–74%*, 1; *75–100%*, 1. In 38 of 84 patients the MIB-1 index was ≥ 10%. The p53 distribution was: *0–1%*, 41; *2–9%*, 6; *10–24%*, 4; *25–49%*, 11; *50–74%*, 8.

Comments

Initially, Ki-67 could only be applied on fresh tissue since the antigen which it detects is denaturated by tissue fixation. With the introduction of the MIB-1 antibody, a true Ki-67 equivalent, and a new antigen retrieval technique with the use of microwaves (Shi et al. 1991), immunostaining of formalin-fixed paraffin-embedded material was possible with a high reproducibility, equaling results obtained in fresh material (Gerdes et al. 1992, Cattoretti et al. 1992).

The cut off values for MIB-1 index varies throughout the literature, probably depending on the tissue material observed and possibly by the evaluation (median value/ area of greatest density of staining). For sacomas a cut-off value in the range of 10% (Choong et al. 1995) to 40% (Levine et al. 1997) has been utilized. Following Choong and coworkers, we divided the material into two groups of approximately equal sizes, based on the employed semiquantitative score.

Image analysis technology has been used for the assessment of Ki-67 staining to reduce intraobserver variability (Zehr et al. 1990). With a clear cut staining pattern for positive nuclei, typically for MIB-1, we did not experience difficulties in scoring the specimens visually.

The significance of p53 immunostaining is controversial since false negative detection due to *e.g.* nonsense, frame-shift, or splize mutations, or gross deletions (Wadayama et al. 1993) and false positive detection due to stabilization of wild-type p53 by e.g. Mdm2 or DNA damage in cells (Cordon-Cardo et al. 1994), exists. Recently, the mdm2 gene, responsible for producing a protein that binds to p53 and eliminates its ability to function as a transcription factor, has been shown to be amplified in soft tissue sarcoma, resulting in an inactivation of wild-type p53. High levels of Mdm2 has also been associated with poor survival especially with an over-expression of p53 in the same tumor (Cordon-Cardo et al. 1994). The Mdm2 protein was not assessed in Paper **III**. Evaluation regarding prognostic relevance in soft tissue sarcomas of different p53 antibodies revealed a positive marker frequency of 36–63% (Wurl et al. 1997). Nonetheless, an association be-

tween positive p53 immuno-staining and impaired clinical course has been reported (Drobnjak et al. 1994, Kawai et al. 1994, Wurl et al. 1997).

RT-PCR

RT-PCR is an enzymatic reaction based on RNA, with the aim to multiply a specific genetic material. RT-PCR can be used to identify the translocation between the chromosomes X and 18 (t(X;18)) diagnostic for synovial sarcoma. The method demands fresh or fresh frozen material. This study was based on 33 patients with histologically verified synovial sarcoma and fresh-frozen tissue available (Paper **IV**). The mean tumor size was 7 (3–23) cm. The tumor specimen used for analysis came from primary lesions in all 33 patients. All histologic material was reviewed by Olle Larsson and classified according to histologic type, subtype and Ki-67 index.

RNA extraction: Total RNA was isolated using Qiaquick Rneasy (Qiagen). Approximately 30 mg of frozen tumor tissue were disrupted in lysis buffer containing denaturing guanidinium isothiocyanate (GITC), which immediately inactivates Rnases to ensure intact RNA and then homogenized. Ethanol was added to promote selective binding of RNA to a silica-gel membrane in a spin column. RNA was then eluted in 30 ml of water.

RT-step: RNA (1 mg) was reversed transcribed using the primer SSX-A 5'-CACTTGCTATG-CACCTGATG-3' (Fligman et al. 1995). The RT solution consisted of 2mM of a dNTP DNA polymerization mix (Pharmacia Biotech) , 20 U RNase inhibitor (Boeringer Mannheim), 10 mg bovine serum albumin, (MBI Fermentas), 4.0 mL 5 x first strand buffer (Gibco BRL Life Technologies), 2.0 mmol DTT (Gibco), 200 U Superscript RT (Gibco) in a final volume of 20 mL; 3.30 mM of primer was added to the suspension. The reaction was performed at 42 °C for 1 hour. For each tumor sample tested, four negative controls were included; A) RNA and SSX-primer but no reverse transcriptase, B) RNA and Actin-primer but no reverse transcriptase, C) no RNA but SSX-primer and reverse transcriptase and D) no RNA but Actin-primer and reverse transcriptase. This was to make sure that the generated PCR-products were only generated from RNA and did not represent DNA contamination. One positive control to confirm that the samples could generate a PCR product was also included. In all RT-PCR experiments strict precautions were taken to avoid cross contamination or product carry over.

PCR: The resulting cDNA was amplified by PCR using SYT primer 5'-AGACCAACA-CAGCCTGGACCA-3' (Fligman et al. 1995). and SSX primer 5'-TGCTATGCACCTGATGACGA-3' (Crew et al. 1995). The SSX primer is a consensus primer for both SYT-SSX1 and SYT-SXX2. The 50 mL PCR reaction solution consisted of 200 mM dNTPs DNA polymerization mix (Phamacia Biotech), 10 mM 10 x PCR buffer (Perkin Elmer), 2.5 mM $MgCl_2$ (Perkin Elmer), 5 mL cDNA from RT and 1 U AmpliTaq DNA polymerase (Perkin Elmer). Of each primer 0.54–0.66 mM was used. Amplification was performed at 94 °C (separation) for 30 sec, 64 °C (annealing of primers) for 30 sec and 72 °C (elongation) for 30 sec with a final elongation for 10 min for a total of 35 cycles.

In order to discriminate between the two fusion types a heminested reamplification was performed using the SSX1 specific primer 5'-GGTG-CAGTTGTTTCCCATCG-3' and the SSX2 specific primer 5'-TCTCGTGAATCTTCTCAGAG-G-3' with 5 % of the first round PCR product for 25 cycles. The re-amplification was otherwise performed under the same conditions. The pre- and post-amplification steps were separated from each other. The PCR products were detected by ethidium bromide staining on a 2% agarose gel.

Sequence analysis according to Sanger: In order to analyze the breakpoint sequences PCR products were directly sequenced by cycle sequencing with dye labelled terminators (BigDye Terminators, Perkin Elmer, Norwalk, CO, USA), and analyzed on a DNA sequencer ABI PRISM 377XL (PE Applied Biosystems, Foster City, California, USA). For each reaction a mixture of 8 mL Terminator Ready Reaction Mix (Perkin Elmer, Norwalk, CO, USA), approximately 50 ng of DNA from the first round PCR amplification and 3.2 pmol primer (the same primers used in the PCR) was prepared. The cycle sequence was performed at 96 °C for 10 sec., 50 °C for 5 sec. and 60 °C for 4 min. repeated for 25 cycles.

Findings

Sequence analysis showed transcripts identical or near identical to *SYT-SSX1* in 13 cases and to *SYT-SSX2* in 19 cases. All *SYT-SSX2* transcripts except a 57 base pairs (bp) longer variant corresponded exactly to previously described *SYT-SSX2* (Crew et al. 1995). The extra 57 bp represents an insertion between the ordinary *SYT* and *SSX2* fusion. Due to this difference, we excluded this case from the prognostic analysis. 4 cases were biphasic and 28 monophasic (case 28 missing due to pre-operative treatment). All four biphasic tumors had fusion transcript *SYT-SSX 1*. No difference was seen between patients with *SYT-SSX1* or *SYT-SSX2* fusion transcript regarding clinical characteristics or treatment. There was a significant association between anti-Ki-67 and SYT-SSX1.

Comments

In our study, although only 4 tumors were biphasic, we found an association between the gene involved in the fusion transcript and the histologic subtype. This has been confirmed in other studies, where biphasic tumors had SYT-SSX1 fusion transcript (de Leeuw et al. 1994, Janz et al, 1995, Kawai et al. 1998). However, one group has reported on two biphasic tumors with SYT-SSX2 fusion transcript (Crew et al. 1995). Since approximately half of all tumors with SYT-SSX1 have been considered to be of the monophasic subtype, the relationship between fusion transcript and morphologic appearance is complex.

Comparative genomic hybridization (CGH)

CGH is a screening method for detecting DNA sequence copy number changes of the entire genome (Kallioniemi et al. 1994). The purpose of CGH is to detect non-random genetic changes. Unlike conventional cytogenetics, CGH does not require culture of tumor cells, instead CGH can be performed on DNA isolated from archival paraffin embedded tumor specimens.

CGH using direct fluorochrome-conjugated nucleotides was performed according to protocol first described by Kallioniemi et al. (1994), and modified by El-Rifai et al. (1997). Genomic DNA from paraffin sections and from peripheral blood of healthy male and female (normal reference DNA) were extracted by standard methods (Papers **V** and **VI**).

The hybridization was studied using a Leitz fluoroscence microscope and the ISIS digital image analysis system (MetaSystems GmbH). Three single color images, red for the tumor DNA, green for the normal reference DNA, and blue for the DNA counterstain were obtained from 7–10 metaphases per specimen. Only high-quality metaphases showing smooth and high-intensity hybridization and a similar degree of chromosome condensation were analyzed. The chromosomes were identified with references to the DAPI banding pattern and the centromere positions. The green and red fluorescence intensities were calculated and the red-to-green ratio profiles along the chromosome axis were displayed. To normalize the ratio profiles, the average green to red intensity was set to 1.0 for the entire metaphase.

The ratio of red-to-green fluorescence intensities measured along each chromosome reflects the relative copy number of the homologous sequences in tumor and reference DNA. DNA copy number changes in tumor genome can be determined from these ratios. Chromosomal regions were considered to be over-represented when the green-to-red ratio was above 1.17 (gains) or 1.5 (high-level amplifications), and under-represented when the ratio was below 0.85 (losses). The cut-off values were deduced from the analysis of negative control experiments in which two differentially labeled normal DNAs were hybridized against one another. Only ratio changes that exceeded the fluctuation observed in negative control experiments were interpreted as true gains or losses in tumor DNA. To confirm the interpretation, statistical probability limits (1% error probability) were used.

Two controls, negative (hybridization of differentially labeled normal female DNA against normal male DNA), and positive (tumor specimen with known copy number changes) were included in each CGH experiment.

Findings

CGH was successfully performed in 67 tumors, 37 had copy number changes, affecting most often entire chromosomes or chromosome arms (Paper

V). Among the aberrant cases the mean value of changes per tumor was 5 (1–17). Gains and losses were equally distributed. Gains affecting chromosomes 8 and 12, and losses affecting chromosome 13 were the most common copy number changes. No aberrations specific to histological subtype were identified. However, the frequency of aberrations varied with respect to histologic subtype. Aberrations were twice as common in mono-phasic tumors compared to biphasic. High-level amplification was uncommon.

In the prognostic CGH study (Paper **VI**), 35 of 69 synovial sarcomas showed DNA sequence copy number changes. The median value of aberrations/ tumor was 2.2 (0–17). The most frequent aberrations were gains of chromosome 2, 8 and 12 q (12q14-15), and losses of 3p, 13q (13q21-31). High-level amplifications were only seen in 6 lesions; in three of which more than one amplification were present simultaneously. Under-representation of 3p sequences was the most frequently observed individual copy-number change, 13/69. Two-thirds of the monophasic tumors showed genetic aberrations as opposed to one-third among the biphasic. The frequency of aberrations/ tumor for monophasic lesions were higher (mean 4.7) than for biphasic tumors (mean 2.1).

A trend for large tumor size (> 5 cm) and increased number of aberrations (>2/specimen) was noted. Aberrations with gains on chromosome 8 was significantly over-represented in patients with large tumors (>5cm).

Comments

Though CGH is a screening method for DNA sequence copy number changes of the entire genome it only detects "average" genetic abnormalities of the tumor specimen. Furthermore, only aberrations involving losses or gains of DNA sequences can be detected, whereas balanced chromosome abnormalities *e.g.* reciprocal translocations, inversions and point mutations are not detectable (Kallioniemi et al. 1994). Neither can CGH recognize changes if the fraction of normal cells is high or if cells are polyploid. Therefore normal findings may be false negative. The samples in our material contained a high fraction of tumor cells, and only in 3 cases was the tumor cell concentration was lower than 50%.

Epidemiology

The following observations were based on the 104 patients in Papers **I** and **II**. There were 53 men and 51 women with a median age of 38 (6–81) years (Figure 7).

The annual incidence in Sweden was 1 per million inhabitants. The tumor frequency was fairly evenly distributed between 10 and 70 years of age, with a peak in adolescence. The relative age specific incidence based on the Swedish material showed surprisingly a second peak incidence in the 50–60 age group (Figure 8).

Among the primary tumors 66% were located in the lower extremities, 25% in the upper extremities and 9% in the trunk wall (Table 1).

The thigh was the most common site followed in order of decreasing frequency by the foot, lower leg and knee. No tumors were located intra-articularly. The median tumor size was 5 (1–20) cm. Proximally located tumors were generally larger than distally. Only 10% of the tumors were subcutaneous. Approximately half of the patients were referred to an SSG center without prior surgery. During this nine year period, 16 of the 60 Swedish patients received all treatment for primary tumor outside an SSG center. There was no apparent systematic bias regarding clinical characteristics between patients primarily treated at an SSG center versus outside.

Table 1. Location of 104 synovial sarcomas

Location		n
Trunk	Lower trunk	5
	Upper trunk	4
Upper extremities	Lower arm-wrist	11
	Hand	7
	Shoulder	5
	Elbow-upper arm	3
Lower extremities	Thigh	19
	Foot	15
	Low leg	15
	Knee	13
	Groin-gluteal	7

Comments

Compared to previous studies, more patients were referred before surgery, the lesions were smaller, and the patients were also older. These differences reflect the better referral practices in the Scandinavian countries than in the U. S. A. (Mankin et al. 1996).

Interestingly, the age specific incidence of synovial sarcoma does not decline after the adolscence period as previously has been reported. There was even a tendency for an increased incidence up to the 6th decade but the patient series was too small for definite conclusion. Due to an

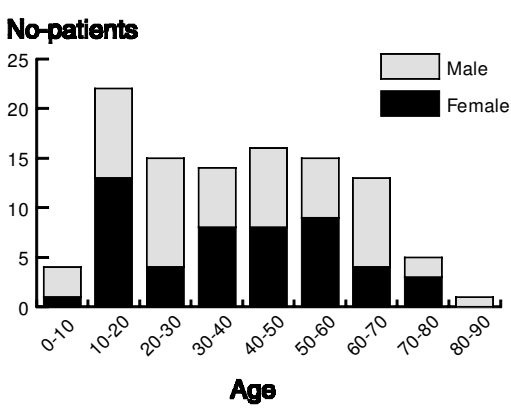

Figure 7. Age and sex distribution of 104 patients with synovial sarcoma (Papers **I** and **II**).

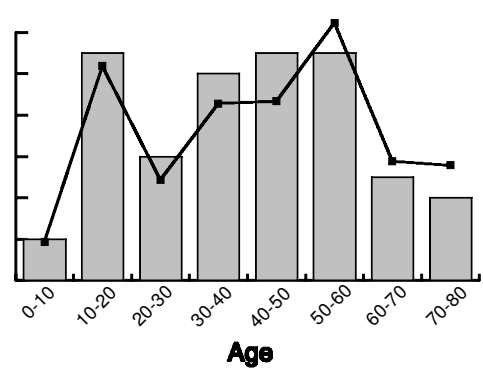

Figure 8. Relative age (bars) and population adjusted age distribution (graph), based on the Swedish patients (60).

increasing use of molecular and cytogenetic techniques *e.g.* RT-PCR and FISH where the translocation t(X;18) can be confirmed, the ratio of synovial sarcoma among the elderly population will most certainly rise. No difference in sex distribution was seen in this study in contrast to some earlier studies showing a male predominance (Haagensen and Staut 1944, Golouh et al. 1990). Enzinger and Weiss (1995) has reported a male to female ratio of 1.2:1. Since this series can be regarded as population based the true median age of synovial sarcoma patients at diagnosis is most likely higher and the median tumor size smaller than earlier reported (Mullen et al. 1994, Cameron et al. 1974, Chong et al. 1995, Singer et al. 1996 Wright et al. 1985, Golouh et al. 1990). Synovial sarcoma seems to be rare in early childhood. Furthermore, superficially located synovial sarcomas are uncommon. In comparison to other soft tissue sarcomas (MFH and liposarcoma), synovial sarcomas are generally smaller in size, occur in younger individuals and are more peripherally located. Foot and hand are common sites, but almost none were located in toes or fingers.

Clinical course

Treatment

54 patients had all surgery and 34 had re-excisions performed at an SSG center while 16 had all surgery for the primary tumor outside (Paper **I**). Local excision was performed in 77 patients and amputation in 27. The amputation rate was low compared to what has been reported, *e. g.* from the Mayo Clinic; 51% (Wright et al. 1982) and 56% (Choong et al. 1995), reflecting changes in treatment policy and differences in referral. The final surgical margin was intralesional or marginal in 41 patients and wide or compartmental in 63. None had preoperative radiotherapy but 21 were treated postoperatively due to an intralesional or marginal surgical margin. 7 patients, all children or adolescence, had adjuvant chemotherapy, most often ifosfamide based.

Local recurrence and metastasis

73 of 104 patients had no evidence of disease at a median interval of 6 (3–11) years; 26 patients died of disease at a median interval of 3 (0.4–6) years; and 5 patients were alive with either local recurrence or metastasis at a median interval of 4.5 (3–10) years.

16 patients developed local recurrences. The median interval to local recurrence was 2 (0.3–4) years. The crude local recurrence rate was 0.08 among patients treated at an SSG center and 0.6 among patients treated outside.

34 patients developed metastases. The metastases first appeared in the lungs in 28 patients and in the lymph nodes or multiple other soft tissue sites in 6 patients. The median interval to metastases was 1.5 (0.2–6) years. The median survival (Kaplan-Meier) after metastasis was 1.8 years. The metastasis free survival rate for the whole series of 104 patients was 0.68 (95% CI 0.58–0.76) at 5 years and 0.65 (0.54–0.74) at 7 years (Figure 9). The corresponding overall 5 years survival rates were 0.76 (0.66–0.83) and 0.69 (0.58–0.78), respectively (Figure 10).

The adequacy of the surgical treatment was closely related to where the patient was treated. Only 5 of 16 patients treated outside an SSG center had adequate surgical treatment. The corresponding figure for SSG treated patients was 74 out of 88. Inadequate treatment, significantly associated with local recurrence, had no impact on metastasis-free survival.

Among the 34 patients who developed metastases, 11 had lung surgery plus chemotherapy and/or radiotherapy, 7 had lung surgery only and 5 chemotherapy only. 11 patients were not treated. At last follow-up, 26 of 34 patients with metastasis had died from tumor. 5 patients were alive with persistent disease. 3 patients had no evidence of disease at 6, 7 and 11 years respectively, after treatment for metastases.

Comments

Our results compare favorably to previous series of synovial sarcoma, and, in fact, the reported survival has continuously improved since the entity was first described. This improvement has been explained by more adequate surgery (Wright et al. 1985) and more aggressive treatment (Choong et al. 1995) but it can also be ascribed to differences in patient selection due to changing referral practices. Unfortunately, most published series lack such essential data as treatment before referral, tumor size and location. A uniformly applied system of classification of surgical margin has also been lacking. Hence, it is difficult to compare different patient series of synovial sarcoma.

Many studies have shown that the incidence of local recurrence is largely dependent on the achieved surgical margin and radiotherapy. Local recurrence rates of 0.8 (Menendez et al.1992) has been reported after inadequate treatment. In contrast, the local recurrence rate at SSG centers was 0.08, even though only 25% of the patients received adjuvant radiotherapy.

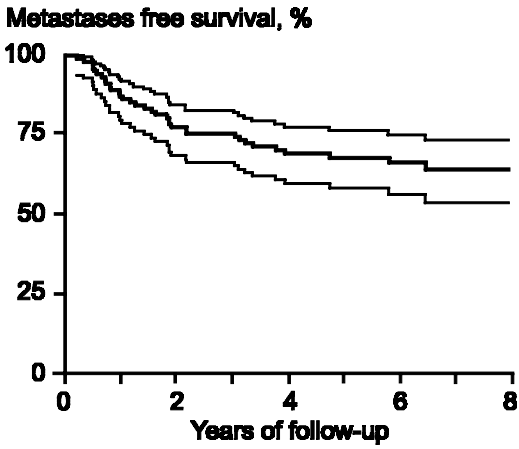

Figure 9. Kaplan-Meier survival estimate for metastasis free survival in 104 patients with synovial sarcomas. 95% pointwise confidence band shown.

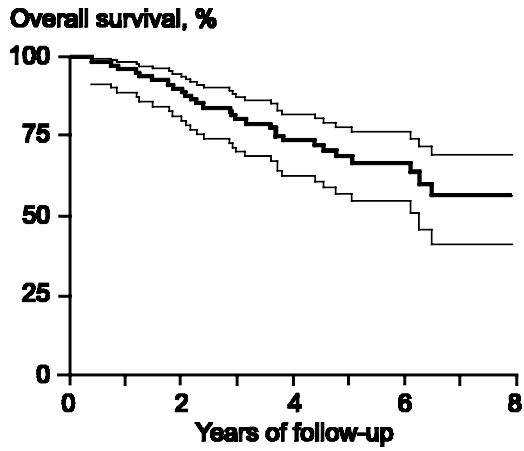

Figure 10. Kaplan-Meier survival estimate for over-all survival in 104 patients with synovial sarcomas.

The poor results of surgery among Swedish patients treated outside of SSG centers, reflected by the high percentage of local recurrence, highlight the importance of referral of patients with soft tissue tumors to sarcoma centers for diagnosis and treatment (Gustafson et al. 1994). Inadequate local treatment was mostly seen outside of sarcoma centers.

In soft tissue sarcoma local recurrence usually occurs within the first 2 years. It has been reported that late occurring local recurrence is characteristic for synovial sarcoma. In our study, 75% of all local recurrences were diagnosed within the first 2 years. None have appeared after 4 years after initial surgery. Therefore we think late recurrences are a rare feature and may reflect deposition of tumor cells of lower malignancy grade (Moberger et al. 1968).

Assessment of local recurrence rate is hazardous since "a competing risk situation" versus metastasis appears, meaning that local recurrences that appears concomitantly with, or after, metastasis may be ignored. The local recurrence rate is also dependent on the treatment modality; with a high amputation rate the local recurrence rate must be low. Furthermore, dead patients are lost to follow-up with respect to local recurrence.

The importance of local recurrence for survival is debated. Retrospective studies of soft tissue sarcoma show that local recurrence is associated with an increased rate of metastasis. In this study local recurrence was strongly associated with metastasis. However, no association was found between inadequate local treatment and reduced metastasis-free survival. Therefore it seems likely that distant spread in synovial sarcoma as well as in many other soft tissue sarcomas, takes place more or less regardless of local control. The association between local recurrence and metastasis may be due to unfavorable prognostic features for distant relapse among patients with local relapse.

Prognostic factors

Clinical factors

By univariate analyses, large tumor size, amputation, deep tumor site and extracompart-mental location were associated with impaired metastases free survival, (Table 2). The risk factors were associated to a varying extent *e. g.* compartmentalization and tumor depth were both related to tumor size and surgical procedure. Local excision was used for all intracompartmentally located tumors, whereas amputation was chosen for 40 % of extracompartmental tumors.

In a Cox's proportional hazards model, tumor size, amputation and local recurrence were associated with reduced metastasis-free survival (Table 3).

Table 3. Metastasis-free survival analyzed multivariately according to Cox's proportional hazards model. Local recurrence is entered as a time-dependent covariable

	Hazard ratio	95% CI	P-value
Tumor size, cm			
1–3 (reference)	1	–	–
4–5	4.3	1.2–16	0.03
> 5–20	7.2	2.1–24	0.001
Amputation	2.9	1.4–6.3	0.005
Local recurrence	9.8	3.6–26	< 0.001

Based on 100 patients

Table 2. Prognostic factors related to metastasis-free survival in 104 patients with synovial sarcoma and no metastasis at time of diagnosis, including estimates from univariate Cox proportional hazards analyses

Variable	Criteria	No.	5-year MFSR [a]	95% CI	Hazard ratio	95% CI	P-value log-rank
All patients		104	0.68	0.58–0.76			
Age	< = 20	26	0.80	0.59–0.91	1		0.21
	> 20	78	0.64	0.52–0.74	1.7	0.72–4.2	
Sex	Male	53	0.62	0.48–0.74	1		0.32
	Female	51	0.74	0.59–0.84	0.71	0.36–1.4	
Localization [b]	Proximal	56	0.68	0.54–0.79	1		0.99
	Distal	48	0.68	0.52–0.79	0.99	0.51–2.0	
Depth [c]	Subcutaneous [d]	10	1.00	–			0.034
	Deep	88	0.64	0.53–0.73	–	–	
Compartment [c]	Extra	69	0.62	0.49–0.72	1		0.037
	Intra	29	0.81	0.55–0.88	0.38	0.15–0.98	
Tumor size, cm [e]	1–3	31	0.89	0.70–0.96	1		0.0005
	4–5	31	0.70	0.50–0.83	3.6	0.96–13	
	> 5–20	38	0.54	0.36–0.68	7.5	2.2–25	
Surgical procedure	Local excision	77	0.8	0.61–0.92	1		0.010
	Amputation	27	0.62	0.49–0.72	2.4	1.2–4.8	
Local treatment	Adequate [f]	79	0.69	0.57–0.78	1		0.98
	Inadequate [g]	25	0.66	0.43–0.81	1.0	0.46–2.2	
Treatment center	SSG center	88	0.71	0.60–0.79	1		0.38
	Outside	16	0.54	0.27–0.75	1.45	0.63–3.3	

[a] MFSR metastasis-free survival rate
[b] Location distal to elbow or knee
[c] Compartmentalization and depth determined in 98 patients
[d] Subcutaneous –no penetration of the subcutaneous fascia
[e] Tumor size determined in 100 patients
[f] Adequate –at least marginal margin and radiotherapy or wide/compartmental
[g] Inadequate–intralesional margin or marginal margin without radiotherapy

Histopathological factors

Among the factors initially examined in univariate analyses tumor size (Figure 11), poorly differentiated areas (Figure 12), microscopic tumor necrosis, mitotic activity, vascular invasion and malignancy grade (Figure 13) were all significantly associated with metastasis-free survival (Table 4). Age > 20 years, monophasic subtype and infiltrative growth pattern were also associated but to a lesser and not significant extent.

It has previously been reported that tumor size and age at diagnosis are important prognostic factors for metastasis-free survival in synovial sarcoma. The effects of histologic variables were adjusted for the impact of age and tumor size in separate Cox regression hazard models. The inclusion of age at diagnosis, dichotomized at 20 years, left the hazard ratios essentially unchanged. This was not an effect of dichotomization since the same results where seen after entering age as a continuous covariate. The analyses suggest that grading of tumors includes more information than any *single* histologic factor. Only little additional prognostic information was gained by combining tumor size with grade (Figure 14).

Interpretation of the multivariate effect of the histologic factors is complicated since the variables are more or less associated with each other and with tumor size. The analysis is further limited by the relatively small size of the series.

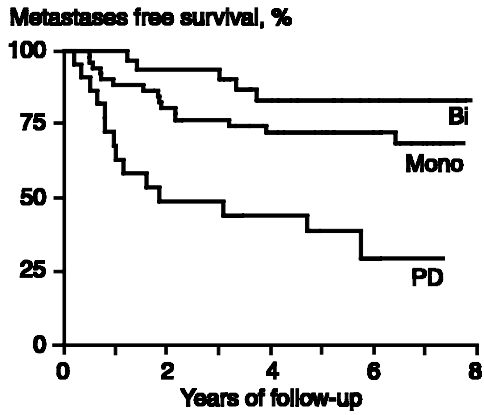

Figure 12. Kaplan-Meier estimate of survival of patients with synovial sarcomas comparing those with poorly differentiated areas and those without.

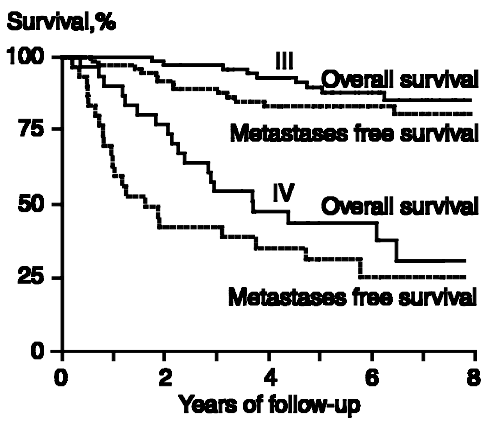

Figure 13. Kaplan-Meier estimate of survival of patients with grade III and grade IV synovial sarcomas.

Figure 11. Kaplan-Meier estimate of survival of patients with synovial sarcoma < or > 5 cm in size.

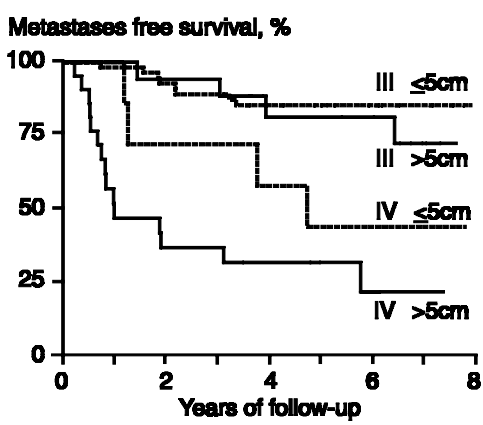

Figure 14. Kaplan-Meier estimate of survival illustrating the combined impact of tumor grade and tumor size for grade III and grade IV synovial sarcomas that are < and > 5 cm in size.

Table 4. Univariate analysis of prognostic factors related to metastasis-free survival

Variable	Criteria	No.	5-yr MFSR [a]	Hazard ratio	P-value [b]
All patients		104	0.68		
Tumor size, cm	1–3	31	0.89	1	0.0005
	4–5	31	0.70	3.6	
	> 5–20	38	0.54	7.5	
Age, years	≤ 20	26	0.80	1	0.21
	> 20	78	0.64	1.7	
Grade	III-favorable	74	0.83	1	<0.0001
	IV-unfavorable	30	0.31	7.0	
Histologic subtype	Biphasic	33	0.78	1	0.08
	Monophasic	71	0.64	2.1	
Poorly differentiated areas	Yes	22	0.38	4.0	<0.0001
	No	82	0.76		
Microscopic necrosis	Yes	32	0.45	3.0	0.0009
	No	71	0.82	1	
Tumor growth pattern	Diffusely infiltrating	70	0.64	1.7	0.3
	Pushing	23	0.83	1	
Vascular invasion	Yes	16	0.47	2.2	0.04
	No	87	0.71	1	
Mitotic activity	Low	39	0.74	1	0.002
	Moderate	32	0.82	0.56	
	High	33	0.48	2.5	

[a] Metastasis-free survival rate at 5 years according to Kaplan-Meier estimate
[b] P-values obtained by a log-rank test

Immunohistochemical factors

There was a large difference in survival rate for patients with a MIB-1 index ≥ 10% versus patients with a low index (Figure15). 28 of 38 patients developed metastases in the high proliferative group *versus* only 12 of 46 in the low proliferative group. No difference in metastasis-free survival was detected for patients staining highly positive for p53 versus those who did not.

In a multivariate analysis based on tumor size and MIB-1 index, both factors gave a significant contribution to the model. The 5-year survival rate among the 30 patients with small and low proliferative tumors was as high as 0.83 (95% CI 0.64–0.92), but only 0.31 (95% CI 0.11–0.53) among the 18 with large and high proliferative tumors (Figure 16).

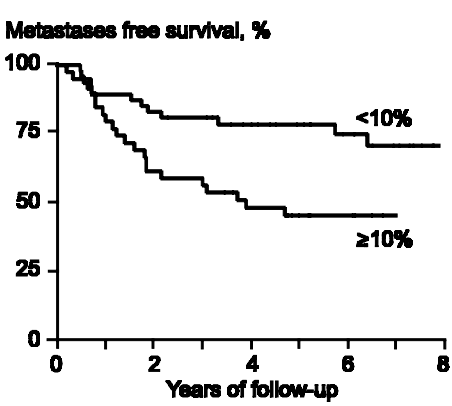

Figure 15. Kaplan-Meier estimates for metastasis-free survival among 84 synovial sarcoma patients based on low (0–9%; n = 46) and high (≥10%; n = 38) proliferative MIB-1 index, p = 0.007 (log-rank test).

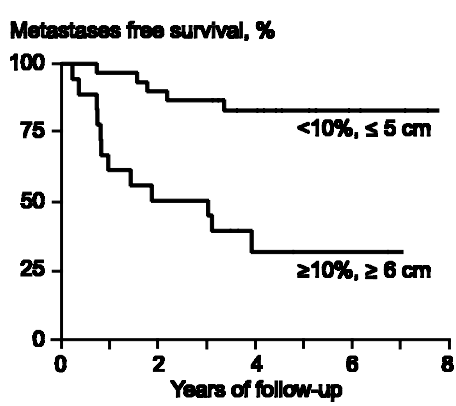

Figure 16. Kaplan-Meier estimates for metastasis-free survival in a low risk group (n = 30; tumor size ≤ 5 cm and MIB-1 < 10) versus a high risk group (n=18; tumor size > 5 and MIB-1 ≥ 10), p = 0.007 (log-rank test).

Molecular and cytogenetics

SYT-SSX1 and SYT-SSX2

Patients with the *SYT-SSX1* had significantly reduced metastasis-free survival, which corroborates the findings of Kawai et al. (1998) (Paper **IV**). However, in contrast to the previous study we did not find any increase of late occurring metastases among patients with *SYT-SSX2*. The 5-year metastasis-free survival for patients with *SYT-SSX1* was 42% compared to 89% for patients with *SYT-SSX2* (Figure 17). The hazard ratio with respect to metastases-free survival for patients with *SYT-SSX1* was 7 (95% CI 1.5–36, log-rank p = 0.004).

Comparative genomic hybridization (secondary aberrations)

No difference regarding metastasis-free or overall survival was seen between patients with or without tumors containing secondary copy number changes (Paper **VI**). No specific copy number change was linked to a significantly improved or impaired metastasis-free survival. However, patients with more than 2 secondary aberrations had worse metastasis-free survival than patients with 1–2 aberrations (Figure 18).

Comments

A number of features have been claimed to be of prognostic importance for metastasis-free and overall survival in synovial sarcoma. Most studies agree that tumor size is a key prognostic factor, also confirmed in this study. This relationship of tumor size has mostly been shown by employing a cut-off level of 5 cm (Zito et al. 1984, Goulouh et al 1990, Brodsky et al. 1992, Ladenstein et al. 1993, Choong et al. 1995, Singer et al. 1996, Kawai et al. 1998). However, the best clinical outcome appears to be in very small tumors (Mullen and Zagars, 1994), also apparent in our study. Therefore we believe that subdivision of tumor size, including small tumors *e.g.* ≤ 3 cm, provides further discrimination of the metastatic propensity.

Amputation was associated with an impaired metastasis-free survival also noted by others (Moberger et al. 1968). This group represents a selection of patients with poor prognosis.

Our study failed to show prognostic significance of several clinical features such as site (Hajdu et al. 1977, Oda et al. 1993) or sex (Moberger et al. 1968, Mullen and Zagars 1994) previously claimed to be of importance. There is no consensus that these features have any strong prognostic value. Young age has been associated with better survival (Hajdu et al. 1977, Wright et al. 1982, Choong et al. 1995). In our study, only a slight trend for higher survival rate was noted for patients £ 20 years old. This trend vanished in the multivariate analysis similarly to what Brodsky et al. (1992) reported in their series of 95 patients from MSKCC. One explanation for the discrepancies between the studies may be differences in the age distribution among the series. The median age was 30 years in both Chong's and Wright's material versus 34 years in Brodsky's and 38 years in our series. This discussion illustrates the difficulties in comparing prognostic studies among centers with

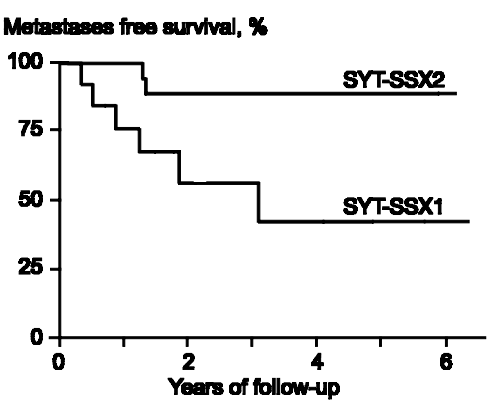

Figure 17. Metastasis-free survival in 19 patients with SYT-SSX2 and in 13 patients with SYT-SSX1 (log-rank p = 0.004).

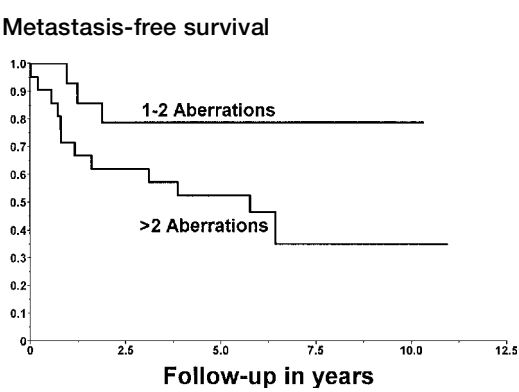

Figure 18. Metastasis-free survival for patients with 1–2 aberrations per tumor versus those with more than 2 aberrations as assessed by CGH (log-rank p = 0.05).

different referral practices.

The monophasic fibrous type is generally considered more ominous than the biphasic. This could be explained by the fact that most poorly differentiated tumors are of the monophasic fibrous type, in this study 20 of 22.

Grade IV tumors were associated with considerably worse prognosis than Grade III. Among the Grade IV tumors, were all poorly differentiated lesions and those characterized by high mitotic activity, tumor necrosis, nuclear atypia or high cellularity with nuclear crowding. Distinction between the two types of Grade IV tumors was subjective and of less importance since they both carry a poor clinical course.

In studies of soft tissue sarcoma from Lund, Sweden, vascular invasion was an adverse prognostic factor (Rööser et al. 1989, Gustafson et al. 1994). Its value as a predictor of poor outcome is limited in synovial sarcoma, because of the rarity with which it is observed. The histologic assessment of vascular invasion is problematic, even when immunostains are used. In addition, assessment of vascular invasion is dependent upon the extent of sampling, particularly around the periphery of the tumor. In only 16 of 103 tumors was vascular invasion seen. Hence, most patients who developed metastases had no observed vascular invasion.

Both mitotic activity and Ki-67 reflect tumor proliferation. Ki-67 is preferable, especially in archival material from different institutions, because of higher sensitivity. Furthermore, in estimating Ki-67 we employed a semi-quantitative method (% positively stained cells of the total number of cells counted). Hence, the problem of the size of the area inspected, obvious in mitotic counting is circumvented (Ellis and Whitehead 1981). A cut off value of MIB <10% defined a group with better survival compared to the group with MIB ≥ 10%. Similar results were recently reported (Bergh et al. 1999).

The prognostic value of Ki-67 in soft tissue sarcoma remains unclear. In angiosarcoma, high Ki-67 was associated with poor prognosis (Meis-Kindblom et al. 1998) but not in MFH (Zehr et al. 1990). Ki-67 has not been prognostic in multivariate analyses of mixed series of soft tissue sarcoma, except in Heslin's et al. study (1998) of high grade soft tissue sarcoma. The expression level of Ki-67 probably differs among histotypes, accounting for the varying prognostic significance.

Among high grade soft tissue sarcomas p-53 overexpression was not associated with poor prognosis (Drobnjak et al. 1994). Similarly, p-53 was not prognostic in this series of synovial sarcoma.

Over the last years several groups have reported associations between histopathological features and the two different fusion types, *SYT-SSX1* and *SYT-SSX2*, but none except Kawai et al. (1998) have compared type of fusion transcript and clinical outcome. Our findings show that patients with the *SYT-SSX1* have a significantly reduced metastasis-free survival, which corroborates the findings of Kawai et al. In contrast to their study, late occurring metastases were not common among patients with *SYT-SSX2*. The association with Ki-67 and SYT-SSX1 indicates a possibly higher proliferation rate in this fusion type. As discussed earlier, the monophasic subtype is known to be associated with a worse prognosis. However, since all biphasic tumors were found to carry SYT-SSX1 this is puzzling. Since the number of patients in both studies was small it is too early to determine the importance of the variant fusion transcripts.

Secondary genetic aberrations, as assessed by CGH, were found in 55% of synovial sarcoma, but no difference was seen regarding metastasis-free or overall survival among patients with or without secondary aberrations.

DNA flow cytometry based on 79 patients (from paper **I**) with formalin-fixed paraffin-embedded tumor tissue, revealed that only 9 (11%) were aneuploid (unpublished data Skytting, Larsson, Tribukait). There was a trend for impaired metastasis-free survival among aneuploid lesions. This contrasts to El-Naggars (1990) results, based on fresh-frozen tissue, where the non-diploid tumors accounted for one third, with a worse clinical outcome. It is possible that paraffin-embedded tissue is less optimal for DNA flow cytometry than fresh-frozen tissue, but may also be due to different means of evaluating the results and different methods. Cytogenetic analysis has estimated that approximately one third of synovial sarcomas carry no chromosome aberrations other than the t(X;18) (Mandahl et al. 1996). It seems that flow-cytometry based on formalin-fixed paraffin-embedded tumor tissue has a low sensitivity and is therefore of limited value in evaluating prognosis in synovial sarcoma

Biological aspects

An increasing number of sarcoma entities are characterized by specific translocations. Since this often times is the sole chromosomal aberration present, it is likely to be etiologic. Tumor specific translocations have been reported for Ewing sarcoma/PNET, clear cell sarcoma, myxoid liposarcoma, desmoplastic small round cell tumor, alveolar rhabdomyosarcoma and synovial sarcoma. The translocation t(X;18)(p11.2;q11.2) (Limon et al. 1986, Turc-Carel et al. 1986) is believed to play a causative role in tumor development of synovial sarcoma and is represented in >90% of the cases (Fligman et al. 1995, Kawai et al. 1998). Cloning of the translocation-breakpoint revealed a fusion between the SYT gene located on chromosome 18 and the SSX gene located on the X chromosome (Clark et al. 1994).

The germline SYT gene seems to be ubiquitously expressed in early embryogenesis (de Bruijn et al. 1996). However, in adult human tissues SYT is predominantly expressed in cartilage and neural cells (de Bruijn et al. 1996). In contrast, the SSX transcripts have only been detected in the testis and the thyroid (Crew et al. 1995, Tureci et al. 1996, Gure et al., 1997).

From screening of testicular cDNA libraries five variants of the SSX gene, *SSX1*, *SSX2, SSX3, SSX4* and *SSX5* have been identified (Crew et al. 1995, Tureci et al. 1996, Gure et al. 1997). *SSX1, SSX2* and *SSX3* have previously been described (Crew et al. 1995, de Leeuw et al. 1996) but *SSX4* and *SSX5* (de Leeuw et al.1996; Gure et al.1997) are genes recently detected in this family. Two sequences of *SSX4* are known, a long variant and a short lacking 136 bp. The missing sequence of the short clone represents the 5th exon of a total of 6 exons in an otherwise identical *SSX4* (Gure et al. 1997). The SSX-genes show strong homology especially in the C- and N-terminals (Gure et al. 1997). All except the *SSX3* are expressed in melanoma cells (Gure et al. 1997). However, *SSX4* is also expressed in fibrosarcoma (de Leeuw et al. 1996). SSX-gene products belong to a group referred to as cancer-testes (CT) antigens and are almost exclusively expressed in testes but also in certain types of cancer (Tureci et al. 1996). MAGE (van der Bruggen et al. 1991) and BAGE (Böel et al. 1995), two antigen families in the CT-group located on the X chromosome, have been found to be expressed in a higher frequency in metastatic melanomas compared to primary melanoma (Böel et al. 1995). However, the significance of selective CT activation is still unknown.

The function of wild type SYT and SSX proteins, as well as the SYT/SSX fusion proteins, is still largely unknown. The SYT protein, containing several proline, gluta-mine and glycine residues, is believed to play a role in transcription activation (Clark et al. 1994; Brett et al. 1997), since this resembles the domains of transcriptional co-activators (Mitchell et al. 1989). The SSX proteins contain a region located near the N-terminal, which is related to the Kruppel-associated box (KRAB-A) domain (Crew et al. 1995). This domain has previously been described in zinc finger proteins, where it represses transcription (Licht et al. 1993, Brett et al. 1997). However, the DNA binding zinkfinger domain is lacking in all SSX genes. In the C-terminal region an acidic domain, common in nuclear proteins, has been recognized (Shirakawa et al. 1990). Just recently a highly conserved motif of 33 amino acids, located at the C-terminus, with repressing activity was described (Lim et al. 1998). Both these domains are included in the fusion gene.

Previously, only *SSX1* and *SSX2* genes have been shown to be involved in the translocation of synovial sarcoma (de Leeuwe et al. 1996, Kawai et al. 1998). We have recently reported a new fusion product, SYT-SSX4 in t(X;18) synovial sarcoma (Skytting et al. 1999). This new fusion transcript showed strong resemblance to SYT-SSX1 and SYT-SSX2. Both *SYT-SSX1* and *SYT-SSX2* encode fusion proteins in which eight amino acids in the C-terminal of the normal SYT protein have become replaced by 78 amino acids encoded by the C-terminal of SSX genes (Figure 19) (Crew et al. 1995, de Leeuwe et al. 1995), and thereby ex-

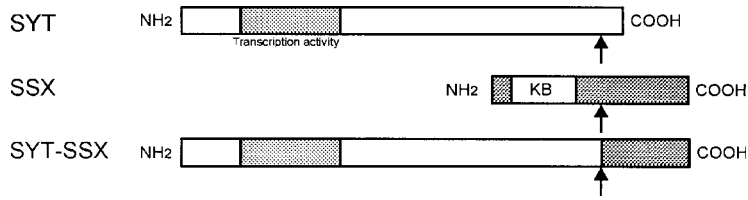

Figure 19. Schematic presentation of the SYT, SSX and SYT-SSX proteins. KB refers to KRAB associated box.

cluding the putative KRAB domain (Lim et al. 1998). With the fusion of the 3' sequence of SSX to the 3' of SYT, a modification of the function of SYT takes place, presumably resulting in a malignant phenotype.

Recently wild type SYT has been shown to be located to distinct nuclear bodies, previously unknown, in contrast to wild type SSX which showed a diffuse staining pattern throughout the nucleus. SYT-SSX fusion protein exhibits nuclear localization patterns similar to SYT indicating that SYT-SSX proteins are targeted to the nuclear domains where SYT normally resides, and by this way interfering or modifying the normal function of SYT (dos Santos et al. 1997, Brett et al. 1997). It is further speculated that SSX through a second domain located in the C-terminal part of SSX plays a role in gene expression by interaction with other DNA-binding proteins. The consequence of this translocation would be that genes that are normally repressed by SSX will instead be activated by the SYT activation domain (dos Santos et al. 1997).

It is well known that identical chromosomal translocations can produce heterogeneous fusion transcripts resulting in different disease phenotypes. In leukemia a translocation t(9;22) results in a new hybrid gene, BCR-ABL in which the precise location of the break point within the *BCR* (and in *ABL*), and thus the composition of the fusion BCR-ABL protein, may determine whether a patient develops acute lymphoblast leukemia or chronic myelogeneous leukemia (Melo 1996). Recently, in Ewing sarcoma it was reported that a group of patients with EWS-FLI 1 type 1 had a better prognosis than patients with other types of EWS-FLI1 (Zoubeck et al. 1996, de Alva et al. 1998). In alveolar rhabdomyosarcoma, two different gene fusions, PAX3-FHKR and PAX7-FHKR were associated with different phenotypes. Patients with PAX7-FHKR were younger and had more often tumors localized to extremities in comparison to PAX3-FHKR.

As mentioned earlier, we and Kawai et al. (1998) have presented evidence that SYT-SSX fusion type appears to be prog-nostically relevant in synovial sarcoma. Since the SYT sequence is identical in all known fusion types, this suggests that the base pair differences between the SSX transcripts, which overall are few in numbers, may be important for the biological function of the protein. It is tempting to speculate that these deviations may be localized to exon 5, since the largest differences between the fusion transcripts are found here. In this exon there is a 73 % homology of the amino acid sequence between SSX4 and SSX1, and 76 % of that between SSX4 and SSX2. Between SSX1 and SSX2 there is a 73 % amino acid homology. In all three SYT-SSX variants, the exon 5 domain contains comparably many residues for phosphorylation. In SSX4 there are five such residues (3 serines and 2 threonines),

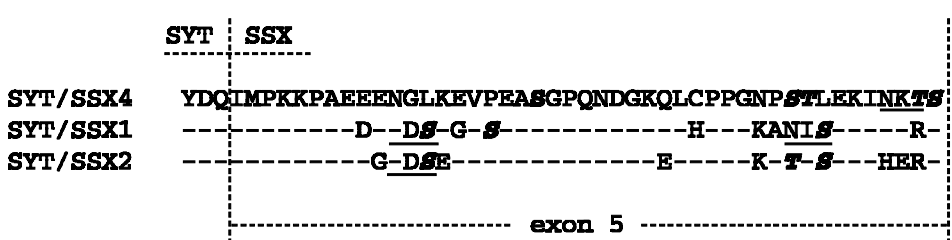

Figure 20. The predicted amino acid sequence within exon 5 of the SSX-genes in SYT-SSX4, SYT-SSX1 and SYT-SSX2. N-linked glycosylation sites are underlined and phosphorylation sites are marked in bold italics.

and in SSX1 and SSX2 there are 5 and 6 residues, respectively (Figure 20). Only two of these potential phosphorylation sites are common for the three variants. As is also shown in Figure 19 there is an SSX4-specific potential site for N-linked glycosylation at the C-terminal of the exon 5 domain. SSX1 contains two and SSX2 one N-linked glycosylation sites, one of which is common for these two variants. Thus, there are a lot of differences with regard to potential phosphorylation sites and N-linked glycosylation sites among the three variants of SYT-SSX. These deviations may underlie biological differences between them.

ACKNOWLEDGEMENTS

This study was based on a collaboration between the Scandinavian Sarcoma Group, Department of Medical Genetics, Haartman Institute, University of Helsinki and the Cellular and Molecular Tumor Pathology, CCK, at the Karolinska Hospital and Institute.

The study was supported by grants from the Cancer Society of Stockholm, IngaBritt and Arne Lundberg's Research Foundation in Gothenburg, The King Gustav V Jubilee Fund, The Swedish Cancer Society and Karolinska Institute's Funds.

References

de Alva E, Kawai A, Healey J H, Fligman I, Meyers P A, Huvos A G, Gerald W L, Jhanwar S C, Argani P, Antonescu C R, Pardo-Mindan F J, Ginsberg J, Womer R, Lawlar E R, Wunder J, Andrulis I, Sorensen P H B, Barr F G, Ladanyi M. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. J Clin Oncol 1998; 16: 1248-55.

Bergh P, Meis-Kindblom J M, Gherlinzoni F, Berlin Ö, Bacchini P, Bertoni F, Gunterberg B, Kindblom L-G. Synovial sarcoma. Identification of high and low risk groups. Submitted to Cancer.

Brett D, Whitehouse S, Antonson P, Shipley J, Cooper C, Goodwin G. The SYT protein involved in the t(X;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. Hum. Mol. Genet; 1997: 6: 1559-64.

Broders A C, Hargrave R, Meyerding H W. Pathological features of soft tissue fibrosarcoma with special reference to the grading of its malignancy. Surg Gynecol Obstet 1939; 69: 267-80.

Brodsky J T, Burt M E, Hajdu S I, Casper E S, Brennan M F. Tendosynovial sarcoma. Clinicopathologic features, treatment, and prognosis. Cancer 1992; 70: 484-9.

Brown D C, Gatter K C. Monoclonal antibody Ki-67: its use in histopathology. Histopathology 1990; 17: 489-503.

de Bruijn D H R, Baats E, Zechner U, De Leeuw B, Balemans M, Olde-Weghuis D, Hirning-Folz U, van Kessel A. Isolation and characterization of the mouse homolog of SYT, a gene implicated in the development of human synovial sarcoma. Oncogene 1996; 13: 643-8.

Boel P, Wildmann C, Sensi, M L, Brasseur R, Renauld J C, Coulie P, Boon T, van der Bruggen P. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 1995; 2: 167-75.

Cadman N L, Soule E H, Kelly P J. Synovial sarcoma. Cancer 1965; 18: 613-23.

Cagle L A, Mirra J M, Storm K, Roe D J, Eilber F R. Histologic Fetaures Relating to Prognosis in Synovial Sarcoma. Cancer 1987; 59: 1910-4.

Cameron H U, Kostuik J P. A long-term follow-up of synovial sarcoma. J Bone Joint Surg Am 1974; 56B: 613-7.

Cattoretti G, Becker M H G, Key G, Duchrow M, Schluter C, Galle J, Gerdes J. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB1 and MIB3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992; 168: 357-63.

Choong P F M, Pritchard D J, Sim F H, Rock M G, Nascimento A G. Long-term survival in high grade soft tissue sarcoma: prognostic factors in synovial sarcoma. Int J Oncol 1995; 7: 161-9.

Clark J, Rocques P J, Crew A J, Gill S, Shipley J, Chan A M. Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. Nat Genet 1994; 7: 502-8.

Cordon-Cardo C, Latres E, Drobnjak M, Oliva M R, Pollak D, Woodruff J M, Marechal V, Chen J, Brennan M F, Levine A J. Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. Cancer Res 1994; 54: 794-9.

Crew A J, Clark J, Fisher C, Gill S, Grimer R, Chand A. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. EMBO J 1995; 14: 2333-40.

Drobnjak M, Latres E, Pollak D, Karpeh M, Dudas M, Woodruff J M, Brennan M F, Cordon-Cardo C. Prognostic implications of p53 nuclear overexpression and high proliferation index of Ki-67 in adult soft-tissue sarcomas. J N C I 1994; 86: 549-54.

Ellis P S J, Whitehead R. Mitosis counting : a need for reappraisel. Hum Pathol 1981; 12: 3-4.

El-Naggar A K, Ayala A G, Abdul-Karim F W, McLemore D, Ballance W W, Garney L, Ro J Y, Batsakis J G. Synovial sarcoma. A DNA flow cytometric study. Cancer 1990; 65: 2295-300.

El-Rifai W, Larramendy M L, Björkvist A, Hemmer S, Knuutila S. Optimization of comparative genomic hybridization using fluorochrome conjugated to dCTP and dUTP. Lab Invest 1997; 77: 699-700.

Enneking W F, Spanier S S, Goodman M A. A system for the surgical staging of musculoskeletal sarcoma. Clin Orthop 1980; 153: 106-20.

Enzinger F M and Weiss S W. Soft tissue tumors. 3rd ed. C V Mosby Co, St Louis, Toronto, London 1995.

Fligman I, Lonardo F, Jhanwar S C, Gerald W L, Woodruff J, Ladanyi M. Molecular diagnosis of synovial sarcoma and characterization of a variant SYT-SSX2 fusion transcript. A J P 1995; 147: 1592-9.

Folpe A L, Schmidt R A, Chapman D, Gown A M. Poorly differentiated synovial sarcoma. Immunohistochemical distinction from primitive neuroectodermal tumors and high-grade malignant peripheral nerve sheath tumors. Am J Surg Pathol. 1998; 22: 673-82.

Gerdes J, Lemke H, Baisch H, Wacker H H, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984; 133: 1710-5.

Gerdes J, Becker M H G, Key G. Immunohistochemical detection of tumor growth fraction (Ki-67 antigen) in formalin-fixed and routinely processed tissues. J Pathol 1992; 168: 85-7.

Golouh R, Vuzevski V, Bracko M, van der Heul R O, Cervek J. Synovial sarcoma: a clinicopathological study of 36 cases. J Surg Oncol 1990; 45: 20-8.

Gure A O, Tureci Ö, Sahin U, Tsang S, Scanlan M J, Jäger E, Knuth A, Pfreundschuh M, Old L J, Chen Y-T. SSX: a multigene family with several members transcribed in normal testis and human cancer. Int J Cancer 1997; 72: 965-71.

Gustafson P, Dreinhöfer K E, Rydholm A. Soft tissue sarcoma should be treated at a tumor center. A comparison of quality of surgery in 375 patients. Acta Orthop Scand 1994; 1: 47-50.

Gustafson P. Soft tissue sarcoma. Acta Orthop Scand. (Suppl 259) 1994; 65: 1-31.

Haagensen C D, Staut A P. Synovial sarcoma. Ann Surg 1944; 120: 826-42.

Hajdu S I, Shui M H, Fortner J G. Tendosynovial sarcoma. Cancer 1977; 39: 1201-17.

Heslin M J, Cordon-Cardo C, Lewis J J, Woodruff J M, Brennan M F. Ki-67 detected by MIB-1 predicts distant metastasis and tumor mortality in primary, high grade extremity soft tissue sarcoma. Cancer 1998; 83: 490-7.

Janz M, De Leeuw B, Weghuis D O, Werner M, Nolte M, Geurts van Kessel A. Interphase cytogenetic analysis of distinct X-chromosomal translocation breakpoints in synovial sarcoma. J Pathol 1995; 175: 391-6.

Kallioniemi O P, Kallioniemi A, Piper J, Isola J, Waldman F M, Gray J W, Pinkel D. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosom Cancer 1994; 10: 231-43.

Kampe C E, Rosen G, Eilber F, Eckardt J, Lowenbraun S, Foster J, Forscher C, Selch M. Synovial sarcoma. A study of intensive chemotherapy in 14 patients with localized disease. Cancer 1993; 72: 2161-9.

Kawai A, Woodruff J, Healey J H, Brennan M, Antonescu C, Ladanyi M. SYT-SSX gene fusion as a determinant of morphology and prognosis in synovial sarcoma. N Engl J Med 1998; 338: 153-60.

Ladenstein R, Treuner J, Koscielniak E, d'Oleire F, Keim M. Synovial sarcoma of childhood and adolescence. Report of the German CWS-81 study. Cancer 1993; 71: 3647-55.

de Leeuw B, Suijkerbuijk R F, Olde Weghuis D, Meloni A M. Distinct Xp11.2 breakpoint regions in synovial sarcoma revealed by metaphase and interphase FISH: relationship to histologic subtypes. Cancer Genet Cytogenet 1994; 73: 89-94.

de Leeuw B, Balemans M, Olde Weghuis D, Geurts van Kessel A. Identification of two alternative fusion genes, SYT-SSX1 and SYT-SSX2, in t(X;18)(p11.2;q11.2)-positive synovial sarcomas. Hum Mol Genet 1995; 6: 1097-9.

de Leeuw B, Balemans M, Geurts van Kessel A. A novel Kr:uppel-associated box containing the SSX gene (SSX3) on the human X chromosome is not implicated in t(X;18)-positive synovial sarcomas. Cytogenet Cell Genet 1996; 73: 179-83.

Levine E A, Holzmayer T, Bacus S, Mechetner E, Mera R, Bolliger C, Roninson I B, Das Gupka T K. Evaluation of newer prognostic markers for adult soft tissue sarcoma. J Clin Oncol 1997; 15: 3249-57.

Licht J D, Ro M, English M A, Grossel M, Hansen U. Selective repression of transcriptional activators at a distance by the Drosphila Kruppel protein. Natl Acad Sci USA 1993; 90: 11361-5.

Lim F L, Soulez M, Koczan D, Thiesen H-J, Knight J C. A KRAB-related domain and a novel transcription repressor domain in proteins encoded by SSX genes that are disrupted in human sarcomas. Oncogene 1998; 17: 2013-8.

Limon J, Dal Cin P, Sandberg A A. Translocation involving the X chromosome in solid tumors : Presentation of two sarcomas with the t(X;18) (q13,p11). Cancer Genet Cytogenet 1986; 23: 87-91.

Mandahl N. Genetics of bone and soft tissue tumors. Adv cancer Res 1996; 69: 63-99.

Mankin H, Mankin C, Simon M. The hazards of biopsy revisited. For the members of the Musculoskeletal Tumor Society. J Bone Joint Surg 1996; 78-A: 656-63.

Meis-Kindblom J M, Stenman G, Kindblom L-G. Differential diagnosis of small round cell tumors. Semin Diagn Pathol. 1996;13: 213-41.

Meis-Kindblom J M, Kindblom L-G. Angiosarcoma in soft tissue: a study of 80 cases. Am J Surg Pathol 1998; 6: 683-97.

Melo J V. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 1996; 88: 2375-84.

Menendez L R, Brien E, Brien WW. Synovial sarcoma. A clinicopathologic study. Orthop Rev 1992; 21(4): 465-71.

Mitchell P J, Tijan R. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science 1989; 245: 371-8.

Moberger G, Nilsonne U, Friberg S. Synovial sarcoma. Acta Orthop Scand 1968; 111: 3-38.

Mullen J R, Zagars G K. Synovial sarcoma outcome following conservation surgery and radiotherapy. Radiother Oncol 1994; 33: 23-30.

Nilsson G, Skytting B, Xie Y, Brodin B, Perfekt R, Mandahl N, Lundeberg J, Uhlén M, Larsson O. The SYT-SSX1 Variant of Synovial Sarcoma is Associated with High Rate of Tumor Cell Proliferation and Poor Clinical Outcome. Cancer Res 1999; 59:3180-4.

Oda Y, Hashimoto H, Tsuneyoshi M, Takeshita S. Survival in synovial sarcoma. A multivariate study of prognostic factors with special emphasis on the comparison between early death and long-term survival. Am J Surg Pathol 1993; 17: 35-44.

Pack G T, Ariel I M. Synovial sarcoma (malignant synovioma). A report of 60 cases. Surgery 1950; 28: 1047-84.

Rosen G, Forscher C, Lowenbraun S, Eilber F, Eckardt J. Synovial sarcoma. Uniform response of metastases to high dose ifosfamide. Cancer 1994; 73: 2506-11.

Rööser B, Willén H, Hugosson A, Rydholm A. Prognostic factors in synovial sarcoma. Cancer 1989; 63: 2182-5.

dos Santos N R, de Bruijn D R H, Balemans M, Janssen B, Gärtner F, Lopes J M, de Leeuw B, Geurts van Kessel A. Nuclear localization of SYT, SSX and the synovial sarcoma- associated SYT-SSX fusion proteins. Hum Mol Genet 1997; 6: 1549-58.

Shi R-R, Key M E, Kalra K L. Antigen retrieval in formalin-fixed, paraffin-embedded tissues an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. Histochem Cytochem 1991; 39: 741-8.

Shirakawa H, Tsuda K, Yoshida M. Primary structure of non-histotone chromosomal protein HMG2 revealed by the nucleotide sequence. Biochemistry 1990; 29: 4419-23.

Singer S, Baldini E H, Demetri G D, Fletcher J A, Corson J M. Synovial sarcoma: prognostic significance of tumor size, margin of resection, and mitotic activity for survival. J Clin Oncol 1996; 14: 1201-8.

Skytting B T, Bauer H C F, Perfekt R, Huuhtanen R, Alvegård T, Berlin Ö, Gustafson P, Klepp R, Löfvenborg R, Sæter G, Trovik C, Wahlström O. Clinical Course in Synovial Sarcoma: a Scandinavian Sarcoma Group Study of 104 Patients. Acta Orthop Scand 1999; 70 (6): 536-42.

Skytting B T, Meis-Kindblom J M, Larsson O, Virolainen M, Perfekt R, Åkerman M, Kindblom L-G. Synovial Sarcoma: Identification of Favorable and Unfavorable Histologic Types – A Scandinavian Sarcoma Group Study of 104 Cases. Acta Orthop Scand 1999; 70 (6): 543-54.

Skytting B T, Bauer H C F, Perfekt R, Nilsson G, Larsson O. Ki-67 is Strongly Prognostic in Synovial Sarcoma. Analysis Based on 86 Patients from the Scandinavian Sarcoma Group Register. Br J Cancer 1999; 80:1809-14.

Skytting B T, Szymanska J, Aalto Y, Lushnikova T, Blomqvist C, Elomaa I, Larsson O, Knuutila S. Clinical Importance of Secondary Aberrations in Synovial Sarcoma Evaluated by Comparative Genomic Hybridization. Cancer Genet Cytogenet 1999; 115: 39-46.

Skytting B, Nilsson G, Brodin B, Xie Y, Lundborg J, Uhlen M, Larsson O. A novel fusion gene, SYT-SSX4, in synovial sacoma. J Natl Cancer Inst 1999; 91: 974-5.

Szymanska J, Serra M, Skytting B, Larsson O, Virolainen M, Åkerman M, Tarkkanen M, Huuhtanen, Picci P, Bacchini P, Asko-Seljavaara S, Eloma I, Knuutila S. Genetic Imbalances in 67 Synovial Sarcomas Evaluated by Comparative Genomic Hybridization. Genes, Chromosomes & Cancer 1998; 23: 213-9.

Turc-Carel C, Dal Cin P, Limon J, Li F, Sandberg A. Translocation X;18 in synovial sarcoma. Cancer Genet Cytogenet 1986; 23: 93.

Tureci Ö, Sahin U, Schobert I, Koslowski M, Schmitt H, Schild H-J, Stenner F, Seitz G, Rammensee H-G, Pfreundschuh M. The SSX-2 gene, which is involved in the t(X;18) translocation of synovial sarcomas, codes for the human tumor antigen HOM-MEL-40. Cancer Res 1996; 56: 4766-72.

Wadayama B, Toguchida J, Yamaguchi T, Sasaki M S, Kotoura Y, Yamamuro T. P53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. Br. J Cancer 1993; 68: 1134-9.

van de Rijn M, Barr F G, Xiong Q-B, Hedges M, Shipley J, Fisher C. Poorly differentiated synovial sarcoma. An analysis of clinical, pathologic, and molecular genetic features. Am J Surg Pathol 1999; 23: 106-12.

van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991; 254: 1643-7.

Varela-Duran J, Enzinger F M. Calcifying synovial sarcoma. Cancer 1982; 50: 345-52.

Wright P H, Sim F H, Soule E H, Taylor W F. Synovial sarcoma. J Bone Joint Surg Am 1982; 64: 112-22.

Wurl P, Taubert H, Meye A, Berger D, Lautenschläger C, Holzhausen H-J, Schmidt H, Kalthoff H, Rath F-W, Dralle H. Prognostic value of immunohistochemistry for p53 in primary soft tissue sarcomas: a multivariate analysis of five antibodies. J Cancer Res Oncol. 1987; 123: 502-8.

Zehr R J, Bauer T W, Marks K E, Weltevreden A. Ki-67 and grading of malignant fibrous histocytomas. Cancer 1990; 66: 1984-90.

Zito R A. Synovial sarcoma: an Australian series of 48 cases. Pathology 1984; 16: 45-52.

Zoubek A, Dockhorn-Dworniczak B, Delattre O, Christiansen H, Niggli F, Gatter-Menz I, Smith T L, Jürgens H, Gadner H, Kovar H. Does Expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? J Clin Oncol 1996; 14: 1245-51.