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SAS Amplification in Soft Tissue Sarcomas[1]

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ABSTRACT

Gene amplification is an important mechanism of increased gene expression in a number of human solid tumors. We have recently identified and cloned sequences from a novel DNA amplification unit in malignant fibrous histiocytoma. The amplified sequences are derived from chromosome 12q13–14 and encode a gene designated *SAS* (sarcoma amplified sequence). In the present study, a series of soft tissue sarcomas was studied to characterize further the phenomenon of *SAS* amplification. Seven of 22 (32%) malignant fibrous histiocytomas and three liposarcomas contained *SAS* amplification. Strikingly, all of the tumors with *SAS* amplification occurred in central sites (*i.e.*, in the abdominal or inguinal regions) rather than in the extremities (*i.e.*, in the arms or legs). These observations demonstrate that *SAS* amplification occurs with a significant frequency in mesenchymal tumors and is particularly associated with abdominal disease.

INTRODUCTION

Evidence in model systems suggests that gene amplification is a manifestation of genomic instability in transformed cells (1). In naturally occurring tumors, clones which gain a proliferative advantage through oncogene amplification may become predominant. This phenomenon occurs frequently in certain human cancers. Among the most intensively studied examples are breast cancer [HER-2] (2), neuroblastoma [N-*myc*] (3), small cell lung cancer [c-*myc*, N-*myc* (4), and L-*myc* (5)], and brain tumors [epidermal growth factor receptor (6), c-*myc* (7), N-*myc* (8), and *GLI* (9)]. In neuroblastoma, N-*myc* amplification has become a clinically important indicator of poor prognosis independent of advanced stage (10). New instances of gene amplification continue to be encountered, such as the amplification of the cyclin related gene *PRAD1* in breast cancer (11).

Although MFH[3] is the most frequent soft tissue sarcoma of adults, genetic alterations which contribute to its pathogenesis are poorly understood. Recently we cloned a novel amplified DNA sequence from a MFH tissue specimen using a combination of in-gel renaturation and the polymerase chain reaction (12). This amplification unit is derived from chromosome 12q13–14, a region frequently rearranged in soft tissue tumors (13, 14). It contains at least one gene, which has been designated *SAS*. In the present study, we examined a series of MFH tumors to determine the frequency and magnitude of *SAS* amplification.

MATERIALS AND METHODS

Tumor Material. Tumor material was obtained at routine biopsy in accordance with University of Michigan Human Subjects guidelines. The tumors were stored at −80°C prior to extraction.

High Molecular Weight DNA Extraction. Frozen tissue in plastic bags was immersed in liquid nitrogen and pulverized. DNA was isolated by overnight incubation of the pulverized tissue with sodium dodecyl sulfate-proteinase K and subsequent phenol/chloroform extraction (15). DNA was quantitated by a 4′,6-diamidino-2-phenylindole fluorimetric assay (16).

Southern Blotting. *Eco*RI digested genomic DNA (7.5 μg) was electrophoresed in 0.8% agarose gels (17) and transferred to Zeta-probe membranes (Bio-Rad) by capillary blotting overnight as previously described (12).

Probes. Probes used were pSJB2, a probe for *SAS* (12); pAL-1, a probe for *int-1* (obtained from American Type Culture Collection); and the 702-base pair *Bam*HI fragment from the 5′ end of the *GLI* complementary DNA pcGLI (18) (kindly provided by Bert Vogelstein, The Johns Hopkins University).

Probe Labeling and Southern Blot Hybridization. Probes were radiolabeled with [^{32}P]dCTP (ICN/Biomedicals, Inc., Lisle, IL) by the oligo labeling method (19). Blots were hybridized, washed, and autoradiographed as previously described (12).

RESULTS

Since *SAS* was cloned from a MFH tumor biopsy, we focused our study on MFH, screening 22 specimens for *SAS* amplification by Southern blot analysis (Table 1). To ensure consistency in the pathological classification of the tumors which exhibited *SAS* amplification, specimens were reviewed by one of us (S. W. W.). Cases of MFH conformed to the accepted histological criteria (20). All were high grade pleomorphic sarcomas composed of plump spindled and giant cells arranged haphazardly or in a focal storiform pattern. No areas of specific differentiation were documented by light microscopy (Fig. 1). We also observed *SAS* amplification in 3 specimens (2 of which were derived from the same patient) which on pathological review were classified as low grade liposarcomas (Table 2). These tumors were composed of adipocytes separated from one another by a myxoid or fibrous stroma containing isolated pleomorphic cells and occasional lipoblasts (Fig. 2).

A representative Southern blot analysis for *SAS* amplification is shown in Fig. 3. In contrast to the single copy control, an intense hybridization signal indicative of *SAS* amplification is apparent in lanes derived from MFH tumors 6, 7, 20, and 21 and liposarcomas 1 and 2. To verify comparable loading, the blots were also reprobed with two probes, *int-1* and *GLI*, which also map to the long arm of human chromosome 12. In order to estimate *SAS* copy number, quantitative Southern analysis was performed on those *SAS* amplified tumors from which sufficient DNA was available (Fig. 4). *SAS* copy number in tumors exhibiting amplification was increased from 5- to 50-fold relative to diploid DNA. Because surgical specimens often contain some stromal elements, this method generates a minimum estimate of *SAS* copy number. Additionally, any analysis of tissue DNA does not account for tumor cell heterogeneity.

Overall, 7 of 22 (32%) malignant fibrous histiocytomas exhibited *SAS* amplification. Of interest, specimens with *SAS* amplification were more likely to be large and intraabdominal rather than originating in the extremities. All five of the intraabdominal MFH tumors studied (MFHs 7, 10, 11, 13, and 20) exhibited SAS amplification while the sixth amplified tumor

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[3] The abbreviations used are: MFH, malignant fibrous histiocytoma; SAS, sarcoma amplified sequence.

Table 1 *SAS amplification in MFH tumors*

Specimen	Age (yr)	Sex	Site	Amplification (copy no.)
MFH 1	51	F	Recurrent leg	–
MFH 2	73	F	Thigh	–
MFH 3	42	M	Thigh	–
MFH 4	71	F	Thigh	–
MFH 5	72	F	Recurrent arm	–
MFH 6	60	M	Recurrent inguinal	+ (10)
MFH 7	58	M	Retroperitoneum	+ (10)
MFH 8	69	F	Thigh	–
MFH 9	61	F	Thigh	–
MFH 10[a]	70	M	Retroperitoneum	+
MFH 11[a]	65	M	Retroperitoneum	+ (10)
MFH 12	75	M	Forearm	–
MFH 13[a]	74	M	Retroperitoneum	+
MFH 14	37	M	Forearm	–
MFH 15	48	F	Thigh	–
MFH 16	76	F	Hand	–
MFH 17	62	M	Forearm	–
MFH 18	62	F	Thigh	–
MFH 19	75	M	Forearm	–
MFH 20	50	M	Abdominal	+ (10)
MFH 21[b]	62	F	Chest wall	+ (20)
MFH 22	90	F	Recurrent chest wall	–

[a] MFH 13 was identified as ST-23493, MFH 11 as ST-24069, and MFH 10 as ST-23987 in Ref. 12.
[b] MFH 21 was a chest wall lesion metastatic from a tumor located in the retroperitoneum.

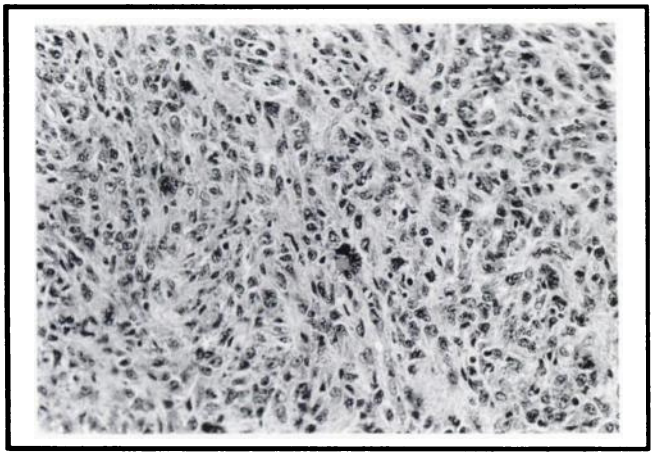

Fig. 1. Histological appearance of case 13 showing malignant fibrous histiocytoma with plump spindled cells and pleomorphic giant cells arranged in a vague storiform pattern.

Table 2 *SAS amplification in liposarcomas*

Specimen	Age (yr)	Sex	Site	Amplification (copy no.)
Lipo 1	73	F	Abdomen	+ (5)
Lipo 2[a]	79	M	Retroperitoneum	+ (50)
Lipo 3[a]	78	M	Retroperitoneum	+

[a] Liposarcoma specimens 2 and 3 originated from the same patient. Specimen 2 was obtained at recurrence 1 year subsequent to specimen 3.

(MFH 22) was a metastasis from an abdominal primary. The seventh case (MFH 6) was an inguinal recurrence of a paratesticular tumor. No extremity MFH specimen demonstrated *SAS* amplification. All three of the *SAS* amplified liposarcoma specimens were also derived from intraabdominal tumors.

DISCUSSION

This study demonstrates that *SAS* amplification occurs in a significant subset of human soft tissue sarcomas at a level of 5- to 50-fold. Although originally identified in MFH, SAS amplification also was observed in liposarcoma. Prior to this report the only molecular genetic changes reported in MFH were alterations in the retinoblastoma susceptibility gene in two cases reported by Andrulis (21) and one by Friend (22).

Although we cannot yet fully define the relationship between *SAS* amplification and the clinical behavior of these tumors, it may be significant that we observed *SAS* amplification primarily in tumors of the abdomen and retroperitoneum and not in tumors of extremity sites. One explanation is that deeply situated lesions of the retroperitoneum have been present for prolonged periods and are therefore more likely to acquire additional genetic alterations such as *SAS* amplification prior to diagnosis. Alternatively, various anatomic sites may be predisposed to different genetic abnormalities. It is of particular interest that *SAS* amplification was noted in MFH. This tumor is the most common sarcoma of adults and serves as a model or prototype of adult high grade sarcomas in general (23, 24).

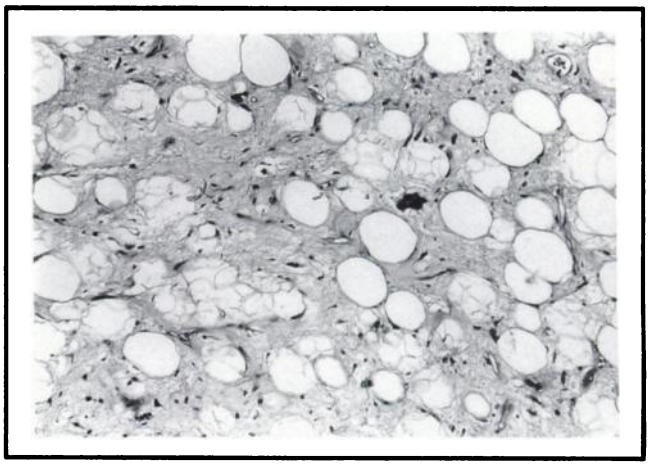

Fig. 2. Low grade liposarcoma (Table 2, case 3) showing adipocytes separated by myxoid matrix containing an occasional bizarre cell.

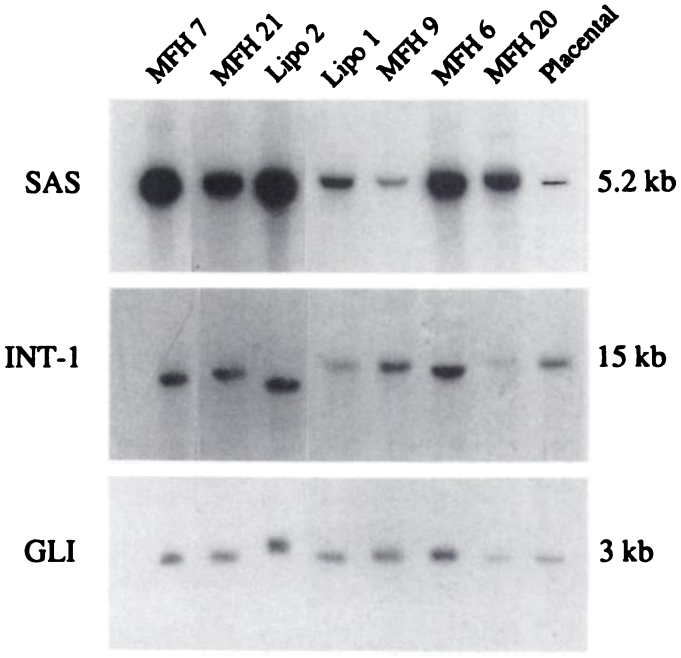

Fig. 3. Southern blot demonstrating *SAS* amplification in tumors MFH 7, MFH 21, liposarcoma 2, liposarcoma 1, MFH 6, and MFH 20. Two control probes, *GLI* and *int-1*, do not show amplification. Tumor DNA (7.5 μg) was digested with *Eco*RI, separated on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized sequentially with probes for *SAS*, *int-1*, and *GLI*.

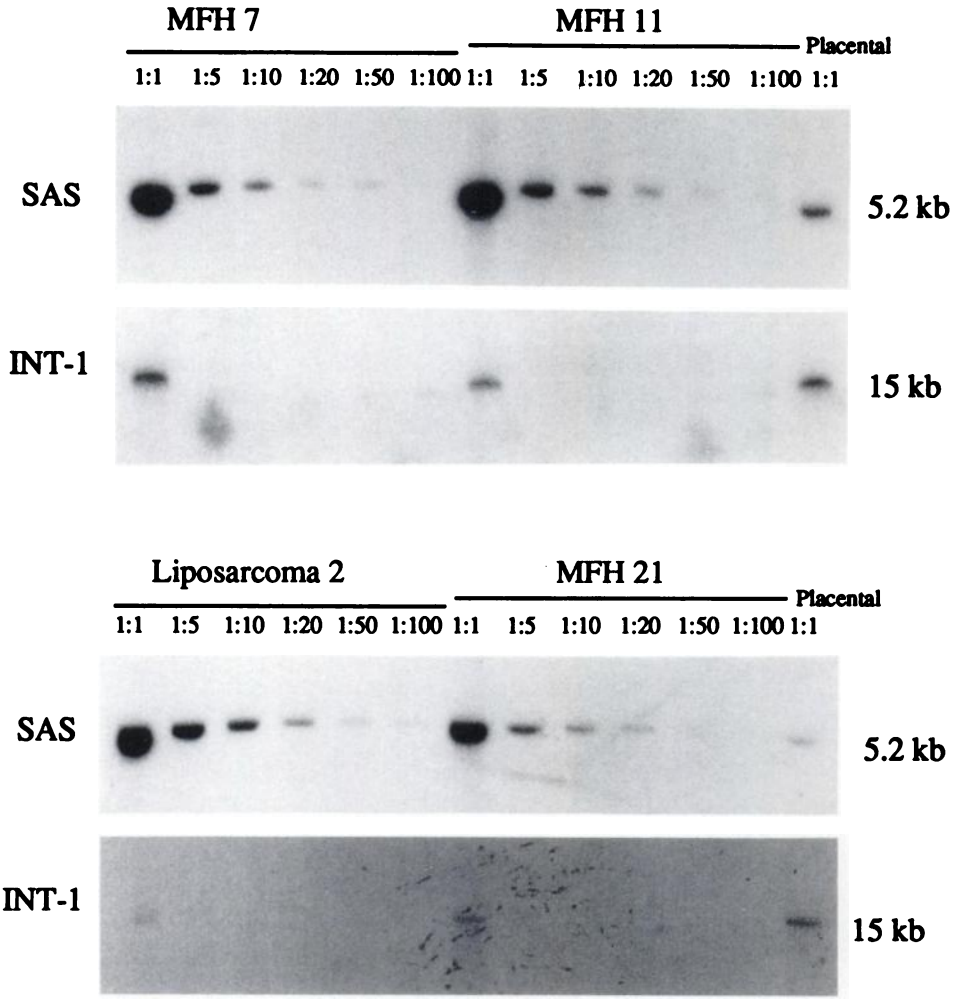

Fig. 4. Estimation of *SAS* copy number in four tumors with *SAS* amplification by Southern blot analysis. Intensity of signal was judged to be equivalent to that of placental DNA at a 1:10 dilution for MFH 7, a 1:10 dilution for MFH 11, a 1:50 dilution for liposarcoma 2, and a 1:20 dilution for MFH 21. Serial dilutions of EcoRI DNA digested (5.0, 1.0, 0.5, 0.25, 0.1, and 0.05 μg) were analyzed by sequential hybridization with probes for *SAS* and *int-1* as described in Fig. 3.

Despite its frequency there have been few parameters, apart from size and depth, that have served to predict outcome in this group of lesions. The genetic diversity which we have observed within these tumors introduces the possibility that *SAS* amplification may be of prognostic significance. The observation that two low grade liposarcomas also had evidence of *SAS* amplification is noteworthy. It is known that low grade liposarcomas, particularly of the retroperitoneum, can dedifferentiate or progress histologically with time to tumors indistinguishable from MFH (25). This progression seems to be a time dependent phenomenon and may well be accompanied by a series of genetic alterations. Additional clinical studies will be necessary to assess any link between *SAS* amplification, stage of disease, and prognosis.

We were unable to address the issue of *SAS* expression in this group of specimens since they were not collected and stored using techniques necessary for the preservation of intact RNA. Based on the experience with other systems, it is likely that *SAS* is expressed in those tumors with *SAS* amplification. In this connection, it is important to note that the genetic analysis of the *SAS* amplification unit is still incomplete. It is uncertain whether *SAS* or an as yet unidentified linked gene is the biological target of the amplification event which we have described. Nonetheless, *SAS* amplification is now the most frequently observed change in MFH at the DNA level. The high frequency of *SAS* amplification detected in abdominal tumors is a striking observation which leads us to hypothesize that *SAS* amplification is biologically related to the progression of these tumors.

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