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A novel Krüppel-associated box containing the SSX gene (SSX3) on the human X chromosome is not implicated in t(X;18)-positive synovial sarcomas

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Abstract. The human synovial sarcoma-specific translocation t(X;18) results in the fusion of the SYT gene on chromosome 18 with either one of the Krüppel-associated box (KRAB) containing SSX1 or SSX2 genes on the X chromosome, depending on the exact location of the breakpoint within band Xp11.2. Screening of a testis cDNA library yielded several SSX-positive clones. Subsequent sequence analysis revealed that one third of these clones represent an SSX gene that differs from both SSX1 and SSX2. This novel member of the family of KRAB containing SSX genes, which we designated SSX3, is 90% homologous to SSX1 and 95% homologous to SSX2 at the cDNA level. Somatic cell hybrid analysis indicated that SSX3 maps within Xp11.2→p11.1, the region that also harbors the SSX1 and SSX2 genes. However, we conclude from our RT-PCR data and from results reported in the literature that SSX3 does not act as a fusion partner to SYT in any of the 44 independent synovial sarcomas thus far tested.

Synovial sarcomas are soft-tissue tumors that occur mainly in adolescents and young adults. The chromosomal translocation t(X;18)(p11.2;q11.2) is found in the majority of these sarcomas and, as such, is thought to play a causative role in tumor formation (Turc-Carel et al., 1987). Two distinct chimeric products have been identified in different t(X;18)-positive synovial sarcomas, resulting from the fusion of the SYT gene on chromosome 18 to either the SSX1 or the SSX2 gene on the X chromosome (Clark et al., 1994; Leeuw et al., 1994a, 1995; Crew et al., 1995). These alternative fusion products have been correlated with different X-chromosomal breakpoints in fluorescence in situ hybridization experiments using Xp11.2-specific YACs as probes (Leeuw et al., 1993a, b, 1994b; Olde Weghuis et al., 1994; Shipley et al., 1994; Janz et al., 1995). Interestingly, there appears to be a positive relationship between the occurrence of these alternative X-chromosomal breakpoints and the most predominant histologic characteristics of the tumors, namely, whether they are monophasic or biphasic (Leeuw et al., 1994b; Janz et al., 1995). Here, we report the identification and chromosomal localization of a third member of the family of Krüppel-associated box containing SSX genes. This gene, however, is not implicated in t(X;18)-positive synovial sarcomas.

Materials and methods

Library screening and sequence analysis
The human fibrosarcoma HT1080 (Clonetech) and the human testis 5' stretch (Clonetech) cDNA libraries were used for screening, using essentially the same standard procedures described as before (Leeuw et al., 1993b, 1994a). DNA sequences were analyzed on an automated DNA sequencer (ABI 373A) using a Taq dye deoxy terminator cycle sequencing kit (Applied Biosystems).

Patient material and RT-PCR analysis
The patient material used in this study included nine synovial sarcomas that were extensively analyzed before (Leeuw et al., 1995) and six novel tumors. Diagnosis of these latter six tumors as synovial sarcomas was confirmed via a positive SYT-SSX RT-PCR score. RT-PCR and subsequent restriction enzyme analyses were performed as described previously (Leeuw et al., 1995).

Southern blot analysis
DNAs of hybrid and parental cell lines were isolated as described before (Leeuw et al., 1994a); digested to completion with a variety of restriction endonucleases (Life Technologies); and, after agarose gel electrophoresis,
Fig. 1. The SSX3 cDNA sequence compared to those of SSX1 and SSX2. Differences are printed in boldface type. The locations of the 
Bglll, EcoRV, SmaI, and Lspl restriction sites are indicated.

Fig. 2. Restriction digests of SSX3 testis cDNA clones (BamHI-XbaI inserts into pDR2) using BamHI/Bglll (lanes 1–5) or BamHI/EcoRV (lanes 6–10). Lanes 1 and 6: pDR2-30; lanes 2 and 7: pDR2-33; lanes 3 and 8: pDR2-34; lanes 4 and 9: pDR2-36; lanes 5 and 10: pDR2-42. Lane M: size marker (100-bp ladder, Life Technologies). The 650-bp marker band is indicated by an arrow. The asterisks indicate a 1.3-kb BamHI/EcoRV vector band.

Results and discussion

Screening of a human fibrosarcoma cDNA library with a synovial sarcoma-derived SYT-SSX RT-PCR product (Leeuw et al., 1995) yielded several partial SSX2 fragments. One of these fragments was used as a probe to screen a human testis cDNA library, which resulted in 15 positive clones. Five of these full-length clones (pDR2-30, pDR2-33, pDR2-34, pDR2-36, and pDR2-42) were found to lack a Bglll site that is normally present in both the SSX1 and SSX2 cDNAs (Leeuw et al., 1995). Sequencing of these five clones yielded identical results and revealed a novel SSX transcript that was significantly different from SSX1 and SSX2 (for comparison, see Fig. 1). Overall, this novel sequence, which we designated SSX3, is 90% homologous to SSX1 and 95% homologous to SSX2 at the cDNA level. At the position of the Bglll site in SSX1 and SSX2
Fig. 3. Restriction digest of SYT-SSX RT-PCR products from 12 different synovial sarcomas with EcoRV (A), BgII (B), Lspl (C), and Smal (D). The PCR was performed on 1 μl of RT material with SYT (5'-CAACACGCAAGATGCATACCA-3') and SSX (5'-CACTTGTATGACCTGTAG-3') primers (Leeuw et al., 1995). 1 min at 92 °C, 1 min at 48 °C, and 3 min at 72 °C for 35 cycles. Lanes 1–12 contain RT-PCR material from tumors 94-50418, 89-52115, 2374/90, CATC, 89-50654, 243090, H1ss, KN, PTN SS1, 4873/92, 950501XC, and 2214-287, respectively (Leeuw et al., 1993a, 1993b, 1994a, 1994b, 1995; Janz et al., 1995; and unpublished cases). Lane M: size marker (100-bp ladder, Life Technologies). The 600-bp marker band is indicated by an arrow.

(Figs. 1 and 2) An EcoRV site is found in SSX3 that is not present in SSX1 and SSX2. Aside from these differences, SSX3 shows the same Smal site as SSX2 (absent from SSX1) and the same Lspl site as SSX1 (absent from SSX2). As a consequence, digestion of SYT-SSX RT-PCR products must reveal BgII and Lspl sites in case SSX1 is involved. BgII and Smal sites in case SSX2 is involved, and Smal, Lspl, and EcoRV sites in case SSX3 is involved. Previous sequencing and restriction enzyme digestion of SYT-SSX RT-PCR products from nine different synovial sarcomas revealed fusion products that contained only SSX1- or SSX2-derived sequences (Leeuw et al., 1995). Digestion of RT-PCR products from six additional independent synovial sarcomas revealed a BgII site and either an Smal or Lspl site, whereas none showed an EcoRV site or both Smal and Lspl sites (Fig. 3). From these results we conclude that the SSX3 gene is not fused to SYT in any of our 15 independent synovial sarcomas.

Crew et al. (1995) published a series of 29 synovial sarcomas which shared either a Smal site or Lspl site within the SYT-SSX RT-PCR products, but never both sites. They also mentioned that only SSX1 or SSX2 sequences were encountered. Therefore, we conclude that SSX3 is not included in the fusion with SYT in any of the 44 independent synovial sarcomas thus far tested. In addition, three synovial sarcomas without any evidence for the presence of SYT-SSX1 or SYT-SSX2 fusion products were reported (Crew et al., 1995). Whether in these cases SSX3 is involved cannot, at present, be excluded with certainty, but it should be expected that aberrant SSX transcripts would have been detected by the investigators using the highly homologous (95%) SSX2 as a probe.

Figure 4 compares the amino acid sequences of SSX1, SSX2, and SSX3 (marked 1, 2, and 3, respectively). Differences as compared to the SSX2 sequence are indicated by asterisks. The KRAB boxes are marked with the conserved amino acids in boldface type, and the semiconserved amino acids are underlined (modified after Crew et al., 1995).
have arisen by reversed transcription. In this respect, they are different from, for example, the interspersed OAT-like sequences, some of which have been reported to be processed pseudogenes (Lafreniere et al., 1991; Geraghty et al., 1993). It has been hypothesized before that the OATL1 and OATL2 clusters may have evolved via a duplication event of the entire region (Shipley et al., 1994). Our present results indicate that during the course of evolution, several of these duplication events must have occurred within this particular genomic segment.

In addition to the SSX3 gene reported here, we have evidence for the existence of at least two other SSX-like genes. The first one, designated SSX4, was found after RT-PCR with two SSX internal primers on RNA extracted from a primary human fibrosarcoma. Preliminary sequence data indicate that this gene may give rise to a protein truncated just after the KRAB A box. The second one, SSX5, was detected via the presence of an exon in one of our OATL1 YAC-derived cosmids (Leeuw et al., 1993b). This exon shows between 80% and 90% base-pair homology to the corresponding exons in SSX1, SSX2, SSX3, and SSX4. Whether SSX4 and SSX5 actually represent functional genes still remains to be established. The possible involvement of any of these novel SSX sequences in the development of other neoplastic disorders carrying X-autosome translocations, such as renal cell carcinomas (Sinke et al., 1993; Dijkhuizen et al., 1995), is currently under investigation.

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As such, the observed differences at the amino acid level between the KRAB B domains of SSX2 and SSX3 may give rise to functionally different interactions. The exact nature of these putative interactions, however, remains to be established.

The chromosome location of the SSX3 gene was established through the analysis of a panel of human x rodent somatic cell hybrids including the X-only line 578 (Sinke et al., 1993); its radiation-reduced derivative 578K17, which contains the Xp11.4→p11.1 segment as the only human constituent (Berger et al., 1992); and the synovial sarcoma-derived line H1synsarc containing Xp11.2→qter (Leeuw et al., 1993a). Since 578, 578K17, and H1synsarc exhibit restriction fragments similar to those present in control total human genomic DNA that hybridizes to our SSX3 cDNA probe (absent in the control hamster DNA under the stringency conditions applied [Fig. 5]), we conclude that the SSX3 gene must reside within Xp11.2→p11.1. This proximal Xp segment contains several low-copy repeats, among which are the OAT-like sequences (Sinke et al., 1993; Leeuw et al., 1994b). Interestingly, SSX1 is positioned precisely within the OATL1 cluster (Leeuw et al., 1993a). The identification of yet another SSX gene (SSX3) in Xp11.2→p11.1 is in full agreement with the repeated nature of this particular chromosomal segment (Lafreniere et al., 1991). Since SSX1 and SSX2 are functional, expressed genes containing intronic sequences (unpublished data), they do not seem to
References


References