A Novel Fusion Gene, SYT–SSX4, in Synovial Sarcoma

Cloning of the translocation t(X;18)(p11.2;q11.2) from human synovial sarcoma revealed a fusion between the SYT gene, also known as SSXT, located on chromosome 18 and the SSX gene located on the X chromosome *(1)*. Five variants of the SSX gene (SSX1, SSX2, SSX3, SSX4, and SSX5) have been identified *(2)* but, to date, only SSX1 and SSX2 have been shown to fuse with the SYT gene in the translocation t(X;18) in synovial sarcoma *(3,4)*.

We analyzed the type of SYT–SSX fusion messenger RNA in biopsy specimens from three synovial sarcomas by nested reverse transcription–polymerase chain reaction (RT–PCR) amplification. Two of the tumors were positive with either the SSX1 or the SSX2 primer, and one was positive with both RT–PCR assays (Fig. 1, A–C). Sequence analysis showed that the 5′ end of the 581-base-pair (bp) amplicon of the biopsy specimen in question contained a 187-bp fragment with 100% homology to the SYT gene linked to a 246-bp fragment with 100% homology to the long splice variant of SSX4. The breakpoint on SSX4 was identical to that observed for SSX1 and SSX2, in that all of the SSX genes involved in the SYT–SSX fusion genes split between the fourth and the fifth exons, leaving the fifth and sixth exons of the SSX variants to fuse with the 3′ region of SYT.

The major differences in sequences among the three SSX variants are found in exon 5. The primers used for discriminating SSX1 from SSX2 recognize two different 21-bp sequences in this exon (Fig. 1, D). Analysis of the binding sites for the primers revealed a difference of 4 bp for the SSX4 sequence compared with 5 bp for SSX2 by use of an SSX1-reverse primer. With an SSX2-reverse primer, there was a mismatch of 4 bp for primer binding to SSX4 and 5 bp for binding to SSX1. Since both SSX1 and SSX2 primers recognized the SSX4 complementary DNA sequence, this recognition indicates that the difference at the four positions is not enough to discriminate SSX4 from SSX1 or SSX2. Template mismatch with T as the 3′ terminal base has been reported previously *(5)*. In a majority of specimens examined in previous studies *(3,6,7)*, the type of fusion gene analysis has been based on PCR without sequence analysis, suggesting that the detection of the SYT–SSX4 fusion variant may have been underestimated.

Recent evidence *(6)* indicates the prognostic importance of the two fusion gene variants SYT–SSX1 and SYT–SSX2 in synovial sarcoma. This evidence suggests that the base-pair differences between the SSX transcripts may have biologic significance. It is tempting to speculate that these deviations may be localized to exon 5. In all three SYT–

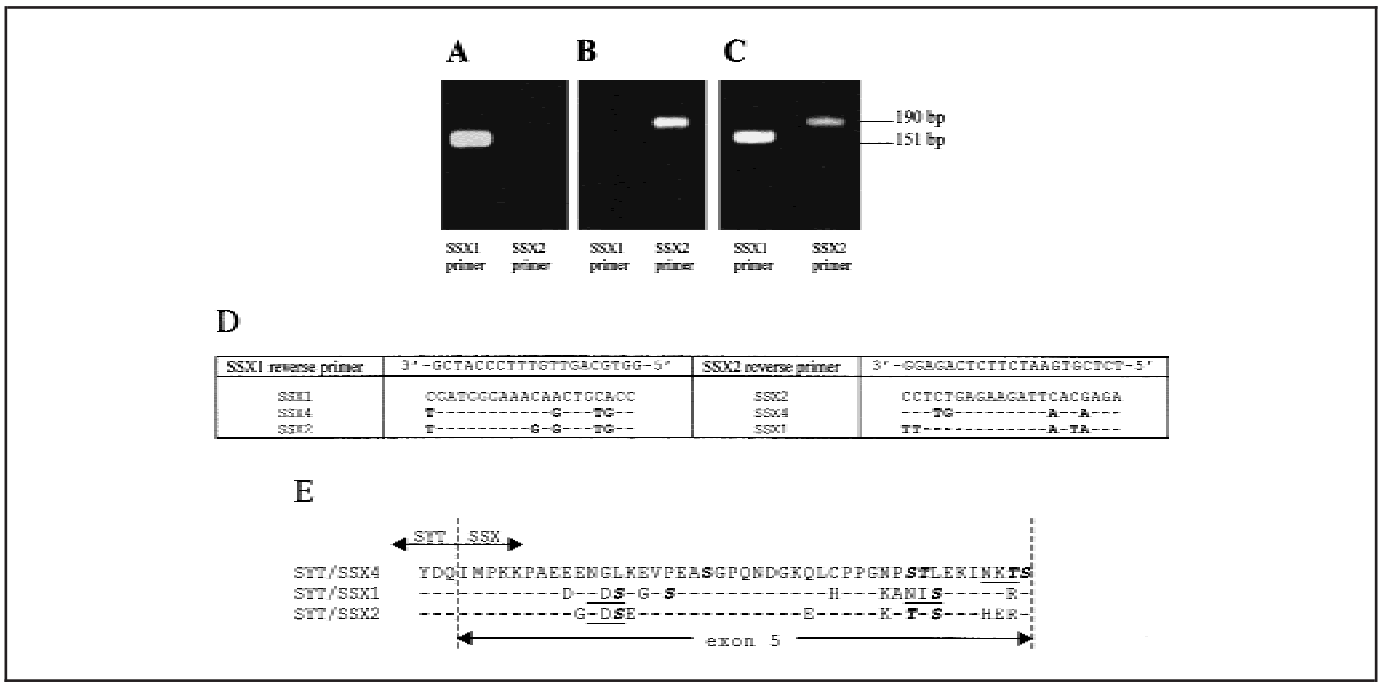

Fig. 1. Identification of a new transcript: SYT–SSX4 (GenBank accession no. AF114234). Total RNA (1 μg) from three different synovial sarcomas (**A, B, C**) was subjected to reverse transcription–polymerase chain reaction (RT–PCR) analysis with the use of the SSX primer 5′-CACTTGCTATGCACCTGATG-3′, which recognizes SSX transcripts in the complementary DNA (cDNA) synthesis step. The isolated cDNA (5 μL) was amplified by PCR with the SYT primer 5′-CAACAGCAAGATG-CATACCA-3′ and the SSX primer 5′-TGCTATGCACCTGATGACGA-3′. Reamplification with nested primers was then performed (**A, B, C**) by use of the SYT primer 5′-AGACCAACACAGCCTGGACCA-3′, the SSX1-specific primer 5′-GGTGCAGTTGTTTCCCATCG-3′, and the SSX2-specific primer 5′-TCTCGTGAATCTTCTCAGAGG-3′. Negative controls were included at every step of the RT–PCR preparations. A control without reverse transcriptase in the RT step was included. The PCR products were detected by ethidium bromide staining on a 2% agarose gel. **D**) Base-pair differences in exon 5 at target sites for SSX1, SSX2, and SSX4 with regard to the SSX1 and SSX2 reverse primers. **E**) The predicted amino acid sequences of the fusion points of exon 5 located within 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.***

SSX fusion variants, this domain contains several sites for phosphorylation. In SSX4, there are five such residues (three serines and two threonines); in SSX1 and SSX2, there are five and six residues, respectively (Fig. 1, E). Only two of these potential phosphorylation sites are common for the three variants. As is also shown in Fig. 1, E, there is a potential site for SSX4-specific, N-linked glycosylation at the C-terminus of the exon 5 domain. SSX1 contains two and SSX2 one N-linked glycosylation sites, one of which is common between these two variants. These deviations may underlie the biologic differences between them. Studies regarding the frequency of SYT–SSX4 and the potential biologic differences between the fusion transcripts are under way.

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