SHORT REPORT

Isolation and characterization of the mouse homolog of SYT, a gene implicated in the development of human synovial sarcomas

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In a previous study we reported the isolation of the human synovial sarcoma-associated t(X;18) breakpoint. As a result of this translocation, the SYT gene on chromosome 18 fuses to either the SSXI or the SSX2 gene on the X chromosome, depending on the exact location of the breakpoint within band Xp11.2. As yet, little is known about the modes of action of the SYT and SSX genes and their respective (fusion) products. Here we report the isolation of the mouse homolog of SYT, its full length cDNA sequence, its chromosomal localization, and its spatio-temporal expression patterns in adult and embryonic tissues. The SYT gene was found to be well conserved during evolution and is part of a region of synteny between the human and mouse chromosomes 18. In early embryogenesis, Syt is ubiquitously expressed. In later stages, the expression becomes confined to cartilage tissues, specific neuronal cells and some epithelial derived tissues. In mature testis, expression was specifically observed in primary spermatocytes.

Keywords: synovial sarcoma; mouse Syt gene/SH2-; SH3-binding domains; annexin-like repeats; chromosome 18

Synovial sarcoma is a soft tissue tumor mainly occurring in the extremities of children and young adults. At the cytogenetic level a characteristic t(X;18)(p11.2;q11.2) has been observed, sometimes as the sole cytogenetic anomaly. It has been assumed that this chromosomal anomaly represents the key molecular event in the pathogenesis of these tumors (Turc-Carel et al., 1987). We and others found that in different t(X;18)-bearing tumors the breakpoints may occur at two distinct sites within band Xp11.2 (de Leeuw et al., 1993b; Shipley et al., 1994). Interestingly, we noted that the occurrence of these different breakpoints correlates with the histologic phenotype displayed by the tumors, i.e., biphasic vs monophasic, respectively (de Leeuw et al., 1993b; Olde Weghuis et al., 1994; Janz et al., 1995). Recently, the t(X;18) chromosomal breakpoint could be isolated (de Leeuw et al., 1993a; 1994a,b). Subsequently, a chimaeric (X;18) cDNA clone was isolated (Clark et al., 1994), and the contributing genes were referred to as SYT (chromosome 18) and SSX (X chromosome). In accordance with the observed differences in X chromosomal breakpoints, two alternative fusion genes, SYT-SSX1 and SYT-SSX2, were defined (de Leeuw et al., 1995; Crew et al., 1995). In the SSX gene products, Krüppel-associated boxes (KRABs) could be identified (Crew et al., 1995). KRABs are often found in transcriptional regulators which resemble the Drosophila Krüppel gene product (Licht et al., 1993; Witzgal et al., 1994). The amino acid sequence of the human SYT gene is rich in glutamine, proline and glycine (16.2%, 16.2% and 14.7%, respectively). Within the C-terminal part three putative Src Homology (SH) binding domains could be distinguished (Clark et al., 1994). The presence of these putative SH binding domains suggests that SYT is involved in cellular signal transduction pathways (Schlessinger, 1994).

Based on the assumption that conservation between the mouse homolog and human SYT would be maximal within the open reading frame, two human cDNA-derived primers (SYT-UP 5'-GAAACAT-GATGTCGTGGCGCTTC-3' and SYT-REV 5'-TGGTAAACGCTTGTGCTTGTG-3'; de Leeuw et al., 1995) were selected and applied to a low stringency reverse transcription polymerase chain reaction (RT-PCR) on RNA extracted from mouse fetal brain tissue. Several fragments could be amplified, one of which was used as a probe to screen a mouse fetal brain cDNA library in lambda ZAP (Stratagene), applying standard procedures as described before (de Leeuw et al., 1993b; de Bruijn et al., 1994). One positive clone, with an insert of approximately 3.1 Kilobases (kb), appeared to contain a full length mouse Syt cDNA. This fragment was subcloned and completely sequenced (Figure 1). The total length of this mouse Syt cDNA clone exceeds the one published for human SYT (Clark et al., 1994), which can be attributed to a few distinct differences. Firstly, both the 5' and the 3' untranslated regions of mouse Syt are longer than those of human SYT and, secondly, in the open reading frame from position 1059 (in the mouse sequence; Figure 1) an addition of 93 basepairs is found. This insertion does not disturb the open reading frame nor does it have any overt effect on the overall amino acid constitution of the predicted mouse Syt protein. The sequence of this insert suggests that it is an extra exon spliced into this cDNA, since the ends resemble exon donor and acceptor sites. The homology between mouse and human sequences was found to be high, 90% identity at the DNA level in the open reading frame and 95% identity at the amino acid level, excluding the aforementioned insert. Comparison

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This is a continuation of the analysis of the mouse Syt cDNA, focusing on nucleotide and amino acid sequences. The sequence described is underlined and corresponds to positions 376, 413, and 392 in the cDNA. Nucleotides were analyzed using an automated DNA sequencer. Two putative polyadenylation signals at positions 3041 and 3070 are also highlighted.

**Figure 1** Nucleotide sequence of the mouse Syt cDNA and the derived amino acid sequence with start- and stopcodons printed in bold. Two putative polyadenylation signals at positions 3041 and 3070 are also printed in bold. The arrows indicate two imperfect ribosome binding sites.

**Figure 2** Multiple tissue Northern blot, hybridized with the mouse Syt cDNA (a). Stronger signals can be observed in the lanes containing heart, kidney and testis RNA. As an indication for the total amount of RNA loaded, (b) shows the corresponding ribosomal 18S band, stained with Ethidium Bromide. Probe labeling and hybridization procedures were carried out as described before (de Bruijn et al., 1994).
of the human and mouse cDNA sequences shows that the start codon suggested for the human SYT gene (Clark et al., 1994) exactly coincides with the first ATG present in mouse Syt at position 179 (Figure 1). Further confirmation that this is the actual translation initiation site comes from the observation that the homology between mouse and human Syt cDNA drops to about 70% before this ATG. Computer analysis of the total mouse cDNA insert using the TESTCODE program shows that triplets in the open reading frame

Figure 3 Sagittal sections through a day 12.5 p.c. (a and b) and a day 14.5 p.c. (c and d) mouse embryo hybridized with a Syt RNA probe. Left brightfield and right darkfield illumination. At 12.5 d.p.c. expression can be observed in Meckel's cartilage (Mc), vertebral bodies (vb), spinal root ganglia (sgr), the ependymal lining of the ventricles (el) and the mantle layer of the central nervous system (ma), with clear absence of signal in the marginal layer (ml). At 14.5 d.p.c. expression can be observed in the ribs (r), olfactory epithelium (ole), turbinate bone (tb), tooth bud (to) and the petrous part of the temporal bone (ptb). Expression continues in Meckel's cartilage and the ependymal lining of the ventricles. Bar = 1 mm.
have a non-random composition of every third base (data not shown). This non-randomness can be attributed to the presence of an expressed sequence (Fickett et al., 1982). Analysis of the amino acid sequence shows that many of the features described for human Syt can also be found in the mouse homolog. Searches of different databases using the FASTA and BLAST search algorithms did not yield strong homologies to any other known gene. Mouse Syt is rich in glutamine, proline and glycine (18.6%, 15.8% and 13.8%, respectively). Glutamine/proline rich stretches have previously been found to be present in transcriptional activators. Interestingly, within the mouse Syt glutamine/proline rich stretch, we found an imperfect direct repeat of 13 amino acids (Figure 1, positions 344–356 and 357–369). Repeat units, reminiscent of this one have also been found in Calcium binding proteins (Annexin VII, Greenwood et al., 1991) and cyclic AMP binding proteins (CABP, Grant et al., 1990) of Dictyostelium discoideum. These repeat units may vary in length and number of repeats, but they are also invariably rich in tyrosine residues. It has been suggested that these structures form beta sheets which may be involved in protein-protein interactions (Zhang-Keck et al., 1993).

The total mouse Syt cDNA clone was used as a probe on Northern blots containing total RNAs extracted from adult mouse muscle, heart, brain, kidney, liver and testis (Figure 2). Overall, ubiquitous expression was observed with relatively higher mRNA levels in heart, kidney and testis. In order to extend these analyses and to reveal spatio-temporal expression patterns during mouse embryogenesis, mRNA in situ hybridizations were carried out as described before (Schmidt et al., 1989) on serial sections obtained from mouse embryos at different stages of development using Syt 'anti-sense' RNA as a probe. Figure 3b shows the expression pattern in a 12.5 day post coitum (d.p.c.) mouse embryo. At this developmental stage, again Syt appears to be ubiquitously expressed. Relatively strong expression is detected in cartilage and specific neuronal cells. Expression in cartilage is evident in the primordia of the vertebral bodies and in Meckel's cartilage. In neuronal cells, elevated expression marks the ependymal lining of the ventricles, the spinal root ganglia, and – with less intensity – the mantle layer of the spinal cord with apparent absence of signals in the adjacent marginal layer. At stage 14.5 d.p.c. the pattern of expression is more confined. In Figure 3d, a more parasagittal section is shown.

Figure 4 Parasagittal section through a day 18.5 p.c. mouse embryo. Expression has become more restricted. Pronounced expression can be observed in the olfactory epithelium (ole), incisor (in), glandula submandibularis (gs), thymus (thy) and lung (lu). Expression in the ribs (r) has disappeared. Bar = 1 mm.
Figure 5 Sections through a testis taken from an 80 day old adult mouse hybridized with a Syt RNA probe. Left brightfield and right darkfield illumination. Control hybridizations were performed with a sense riboprobe (c and d). (e and f) show higher magnification images of two positively hybridizing tubuli seminiferi from b. Syt expression can be observed in a broad band of primary spermatocytes (ps). Other, non-hybridizing, cell types are mature sperm (sp) and spermatids (st). Bar = 300 μm in a–d and 30 μm in e–f

Highest expression is seen in cartilage primordia of the ribs, of the petrous part of the temporal bone, which surrounds the stato-acoustic organ, and of the turbinate part of the maxilla, which surrounds the olfactory apparatus. Prominent expression is also seen in Meckel's cartilage and in the tooth bud of the first molar. In neuronal tissues, the expression is reduced as compared to stage 12.5 d.p.c. but is evident in the ependymal cells of the central ventricles and in the neurosensory olfactory epithelium. In further sections, enhanced expression was noted in both cell layers of the retina, kidney and lung (not shown). At stage 18.5 d.p.c. when ossification has replaced chondrification in most bones, Syt expression is reduced to background levels in bones (Figure 4b). This is most evident in the olfactory apparatus where a prominent signal is present only in the sensory epithelium. At this stage, high expression is observed in the primordia of the upper incisor, the glandula submandibularis and, somewhat less, in the thymus. Compared to earlier stages, the expression in lung and kidney is reduced.

In accordance with the abovementioned Northern blot analyses, higher levels of expression were observed in adult mouse testis and kidney. In kidney, an overall elevated level without any specification to certain structures was observed (not shown). In the testis of an 80 days old mouse, expression is prominent in certain tubuli seminiferi (Figure 5b). At enhanced magnification it is evident that expression is found only in primary spermatocytes which pass through the late prophase stages of the first meiotic division. In those tubuli with an elevated level of expression, a higher proportion of cells is represented by primary spermatocytes. According to the spermiogenic stages as defined by Oikberg (1956) expression is highest in tubuli from stage IX to XI. During these stages, a
broad layer of primary spermatocytes passes from pachytene to diplotene. Syt expression appears to be confined to these cells (Figure 5f).

To elucidate the genomic structure and to facilitate the chromosomal localization of the mouse Syt gene, we screened a mouse genomic library in EMBL3 using the total mouse Syt cDNA as a probe. Several overlapping phages were recovered and a preliminary intron-exon map of the gene was constructed. Partial sequence analysis of these phages confirmed the actual presence of the mouse Syt gene. The chromosomal localization of the gene was determined by fluorescence in situ hybridization (FISH) using one of the Syt-positive phages as a probe, essentially as described by de Leeuw et al. (1993a). In total, 30 mouse metaphase spreads were analysed and, in all cases, positive signals were observed on chromosome 18, region q11-q12, also harboring the mouse genomic region (18B1) is syntenic with human chromosome 18, region q11-q12, also harboring the SYT gene (Overhauser et al., 1995).

In conclusion, the mouse homolog of human SYT appears to have been well conserved over the course of evolution, both in chromosomal location and in constitution. We have shown that the expression of mouse Syt is restricted to cartilage forming cells, specific neuronal cells and some epithelial derived tissues. A highly specific expression is observed in mature testis. Particularly interesting is the notion that several parts of the Syt protein may be implicated in protein-protein interactions, either via SH2/SH3-binding domains or via annexin-like domains.

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