IDENTIFICATION AND CHARACTERIZATION OF SYT-SSX FUSION PROTEIN IN SYNOVIAL SARCOMA

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INTRODUCTION
SYT-SSX fusion proteins are chimeric gene products induced by tumor-specific translocation t(X;18)(p11.2;q11.2) involved in synovial sarcomas. This fusion gene consists of two subtypes, SYT-SSX1 and SYT-SSX2, the difference being a breakpoint at Xp11.2. Previous analyses of synovial sarcomas using reverse transcription-polymerase chain reaction (RT-PCR) demonstrated that the SYT-SSX fusion gene is detected in this tumor with high specificity and sensitivity. These observations strongly suggest that the SYT-SSX fusion genes are translated into proteins which may correlate with the tumorigenesis of this sarcoma. However, the SYT-SSX proteins have not been analyzed with intact human synovial sarcoma tissues, and specific antibodies for routine immunohistochemical analysis have not been available. In this study, we identified the molecular weight of the SYT-SSX fusion gene products of synovial sarcomas and detected their immunolocalization in tumor tissue samples, using newly developed polyclonal antibodies.

METHODS
Production of polyclonal antibodies against SYT-SSX

The first 114 amino acids and the C-terminal 96 amino acids were selected as immunogens to prepare anti-SYT and anti-SSX antibodies.

Each recombinant protein was injected subcutaneously into New Zealand White rabbits, then the whole blood was withdrawn after three booster injections. Purification of the serum proteins was performed by affinity chromatography. The specificity of the polyclonal antibodies was tested by immunoprecipitation of the transiently transfected COS-7 cells with the SYT-SSX1 or SYT-SSX2 fusion gene.

1. Identification of SYT-SSX protein
The total cell lysate of a human synovial sarcoma cell line HS-SY-II was separated on a 10% SDS-PAGE and electrically transferred to a PVDF membrane. After blocking, the membrane was incubated with the two polyclonal antibodies, and then with an anti-rabbit secondary antibody. The blots were then visualized by autoradiography.

2. Detection of SYT-SSX protein in synovial sarcoma tissues
Fresh frozen tissues of two synovial sarcomas and a malignant fibrous histiocytoma (MFH) were homogenized and then boiled with SDS for 5 min. The tumor extracts obtained were separated on SDS-PAGE, and western blot analysis was performed as described above. Immunohistochemistry was also performed by anti-SSX polyclonal antibody using frozen sections of a synovial sarcoma and a MFH.

RESULTS and DISCUSSION
The western blot analysis using the HS-SY-II cell lysates demonstrated that a 61kDa band was recognized by both anti-SYT and anti-SSX antibodies (Figure 1). This result indicates that SYT-SSX fusion gene is translated into a 61kDa protein in synovial sarcoma.

In the western blotting using the tumor extracts, this 61kDa protein was detected in synovial sarcomas but not in MFH (Figure 2). This data shows that SYT-SSX fusion genes are expressed in intact synovial sarcoma tissues. Immunohistochemical analysis with the anti-SSX antibody revealed nuclear stainings, indicating that SYT-SSX protein is localized in the nucleus of the tumor cells.

SUMMARY
1. SYT-SSX fusion gene is translated into a 61kDa protein in synovial sarcomas.
2. SYT-SSX protein was suggested to be a nuclear protein.
3. The anti-SYT and anti-SSX polyclonal antibodies were useful for detection of SYT-SSX protein by western blot analysis and immunohistochemistry.

Figure 1 Western blot analyses of a human synovial sarcoma cell line HS-SY-II (A) and tumor tissues (B).

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