Development and Characterization of Antibodies Specific for the SS18-SSX Fusion Protein in Synovial Sarcoma

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ABSTRACT

Synovial sarcoma (SS) is a soft tissue cancer characterized by the fusion of the SS18 gene, which encodes for a subunit of the BAF ATP-dependent chromatin remodeling complex, with either SSX1, SSX2, or SSX4 genes (1,2). The resulting SS18-SSX fusion protein is incorporated into the BAF complex, where it evicts wild-type SMARCB1 (BAF47) and retargets the complex to polycomb-repressed chromatin domains, thus activating oncogenic gene expression to drive tumor progression (3).

Traditional means of diagnosis of SS include immunohistochemical staining, including that for the biomarker TLE1, but TLE1 is not overexpressed in all tumors. As a result, SS cases ultimately need to be confirmed by either FISH or PCR assays to detect the SS18-SSX fusion (4,5).

We have developed the first highly specific, recombinant monoclonal antibodies for the detection of SS18-SSX fusion proteins by immunohistochemistry and for the capture of SS18-SSX-containing BAF complexes in cells, with downstream utility in biochemistry and genomics-centered approaches such as ChIP-seq. SS18-SSX (E9X9V) XP® Rabbit mAb #72364 directly targets the highly conserved fusion breakpoint, while SSX (E5A2C) Rabbit mAb #23855 detects the C-terminus of the SSX protein family and is capable of recognizing alternative breakpoints. Taken together, our data show that these antibodies can accurately and reproducibly detect the SS18-SSX fusion protein in SS cells and tissues.

REFERENCES

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3. Kadoch, C. and Crabtree, G.R. (2013) Cell, 151(1) 71-85.
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Antigen Design Overview

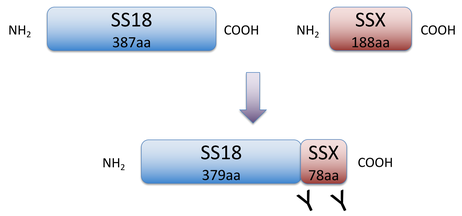

Figure 1: Antigen design on the fusion protein. Rabbits were injected with peptides surrounding the breakpoint of SS18-SSX fusion protein or peptides from the C-terminus of the SSX protein family. Sequences are conserved among SSX1, SSX2, and SSX4 proteins, which are all potential fusion partners of SS18 in synovial sarcoma.

Detection of SS18-SSX by Western Blotting

SS18-SSX (E9X9V) #72364 SSX (E5A2C) #23855

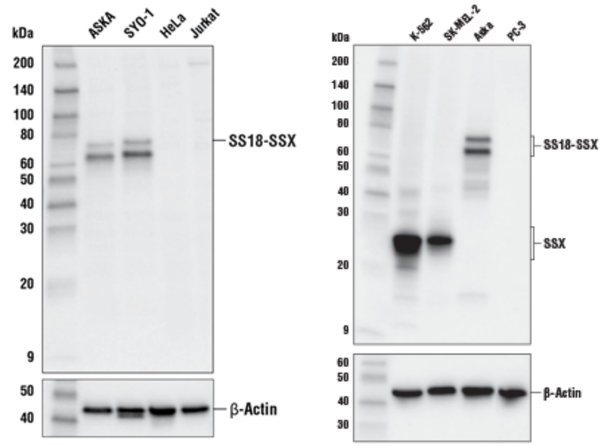

Figure 2: Western blot analysis of extracts from various cell lines using SS18-SSX (E9X9V) XP® Rabbit mAb #72364 (left panel) or SSX (E5A2C) Rabbit mAb (Carboxy-terminal Antigen) #23855 (right panel). ASKA and SYO-1 are SS18-SSX1 and SS18-SSX2 fusion cell lines. PC-3 cells do not express SSX protein.

Genomic Occupancy of SS18-SSX Fusion

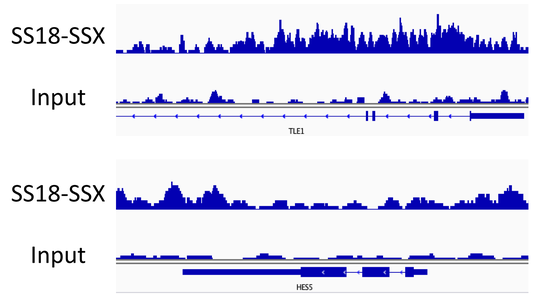

Figure 3: Detection of SS18-SSX on ASKA chromatin using SS18-SSX (E9X9V) XP® Rabbit mAb #72364. Tracks are shown for SS18-SSX (upper track) and input (lower track) over the TLE1 (top) and HES5 gene loci (bottom). This utility allows for the genomic mapping of fusion-containing BAF complexes.

IHC Detection of Fusion Protein In Cells

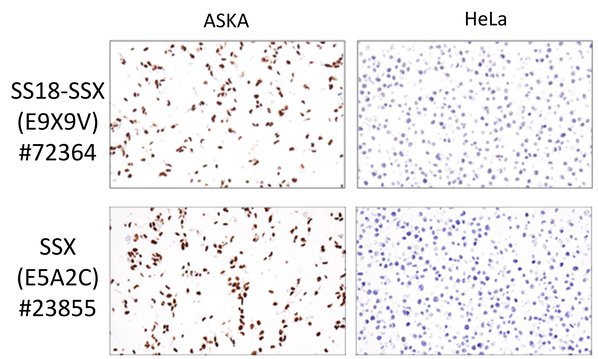

Figure 4: Immunohistochemical staining of a synovial sarcoma cell line containing an SS18-SSX fusion (ASKA, left) versus a cell line lacking the fusion protein (HeLa, right) using SS18-SSX (E9X9V) XP® Rabbit mAb #72364 or SSX (E5A2C) Rabbit mAb (Carboxy-terminal Antigen) #23855.

IHC Detection of Fusion Protein In Tissues

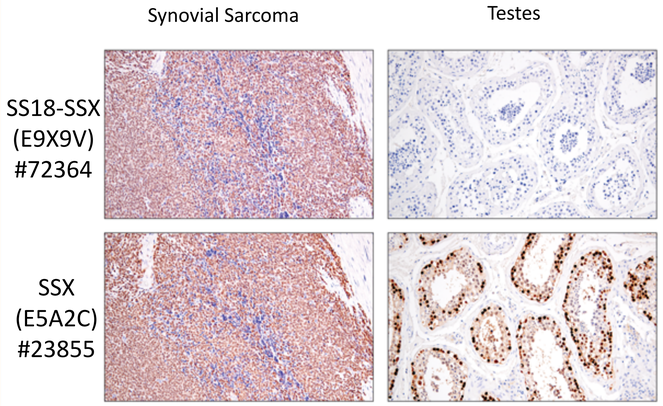

Figure 5: Immunohistochemical staining of synovial sarcoma and testes tissue using SS18-SSX (E9X9V) XP® Rabbit mAb #72364 (upper) or SSX (E5A2C) Rabbit mAb (Carboxy-terminal Antigen) #23855 (lower). SSX protein is expressed in the testes and detected by the SSX E5A2C antibody, while the fusion-specific SS18-SSX E9X9V antibody only stains the synovial sarcoma tissue.

Discussion

SS18-SSX (E9X9V) XP® Rabbit mAb #72364 and SSX (E5A2C) Rabbit mAb #23855 are two recombinant rabbit monoclonal antibodies that can be used to detect the synovial sarcoma SS18-SSX fusion protein in multiple applications, including western blot, IP, IHC, and ChIP-Seq. These antibodies will allow for the specific mapping of SS18-SSX-containing BAF complexes on chromatin and can differentiate synovial sarcomas from other sarcoma subtypes by IHC. Detection of the SS18-SSX fusion in tumor samples by IHC is simple and fast, circumventing the need for FISH and other molecular techniques. Furthermore, while the SS18-SSX fusion breakpoint is highly conserved (~96%) and is detected by the SS18-SSX (E9X9V) XP® Rabbit mAb #72364, non-canonical breakpoints are detected with SSX (E5A2C) Rabbit mAb #23855. Thus combinatorial use of both antibodies will detect close to 100% of all synovial sarcoma fusion events.

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