Supplementary Materials and Methods

Primer sequences for *LATS2* mutation analysis

For *LATS2* mutation, primers used were as follows: LATS2ex1S, 5'-GTATGCGGCCGAGAGTGAC-3', and LATS2ex1AS, CCTCGGCACCACAGTAGG-3', for exon 1; LATS2ex2S, 5'-TGGAAGGAAAGAACATTTTCAA-3', and LATS2ex2AS, 5'-CTGTCCCATAGCTGCCTCTG-3', for exon 2; LATS2ex3S, 5'-GAGTGGGGATGGGAGTCTTT-3', and LATS2ex3AS, 5'-ACCATCTTTGCCCACTATGC-3', for exon 3; LATS2ex4S, 5'-CACACCCACTCTGATGCTGT-3', and LATS2ex4AS, 5'-AGGTGCTGCCCAATTCATAC-3', for exon 4; LATS2ex5S, 5'-ATGGGACTTCTCTAATGTGATTC-3', and LATS2ex5AS, 5'-CTACACATAGAAAGTGCATGTGG-3', for exon 5; LATS2ex6S, 5'-TCAGGAGCTGATTGGAATGAC-3', and LATS2ex6AS, 5'-GAAAGCTAGAGCCAGCGAGACT-3', for exon 6; LATS2ex7S, 5'-AACGCGCTTTCCCACTTAACAGG-3', and LATS2ex7AS, 5'-AATGCTGACCAAAGATTCATGG -3', for exon 7; and LATS2ex8S, 5'-CAGAACTGCCCAAGATGTGA-3', and LATS2ex8AS, 5'-CTTCCCTATTGGCCTGTGAG -3', for exon 8.

FISH analysis

BAC clones of RP11-23H13 (13q12) and RP11-1148G1(13q32) were obtained through BACPAC Resource center (Oakland, CA). RP11-23H13 probe was directly

labeled with Spectrum Green and RP11-1148G1 probe with Spectrum Orange using a standard nick-translation protocol. The probes were co-hybridized using standard FISH hybridization procedures. Blue 4,6′-diamidino-2-phenylindole-2 HCl (DAPI) was used for counterstaining. Digital images were recorded from an Olympus Fluorescence microscope with a cooled monochrome charged-coupled device (CCD) camera and MacProbe digital image system. Images were processed with MacProbe FISH analysis (PSI Scientific Systems, League City, TX) and Adobe Photoshop CS3.

Soft agar colony formation assay

Cells were transduced with lentiviral vectors for gene expression or shRNA-knockdown at the multiplicity of infection of 5 or 10, respectively. Forty-eight hours later, cells were trypsinized, and 2 x 10^4 cells were plated in 0.3% top agarose and cultured for 10 days. Colonies were counted under a light microscope.

Cell migration assay

The migration capacity was measured using Boyden chambers (BD Biosciences Discovery Labware, Bedford, MA) according to the manufacturer's protocol, as described previously (1).

Western blot analysis

Preparation of total cell lysates and Western blotting were carried out as described previously (1). In brief, cells growing confluently were rinsed twice with phosphate-buffered saline (PBS), lysed in sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris pH 6.8, 2% SDS, 2% 2-mercaptoethanol, and 10% glycerol) and

homogenized. Total cell lysate (30 μg) was subjected to SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore, Bedford, MA). Following blocking with 5% non-fat dry milk, the filters were incubated with the primary antibody, washed with PBS, reacted with the secondary antibody, and then detected with ECL (GE Healthcare, Buckinghamshire, UK). For knockdown experiments with sh-LATS2, intensities of each band of YAP and phospho-YAP were measured by Image analyzer Image Master VDS-L (GE Healthcare). Background intensity was also determined and subtracted from each signal.

Immunohistochemical analysis

Immunohistochemical (IHC) analysis was carried out on formalin-fixed, paraffin-embedded tissue sections of mesothelioma specimens. Sections (5-μm thick) were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. The antigens were retrieved by 45 min of heating at 98°C in 0.5% Immunosaver (Nisshin EM, Tokyo, Japan) in a waterbath. After blocking the endogenous peroxidase activity with 3% aqueous H_2O_2 solution for 15 min, the sections were reacted with a primary antibody (rabbit anti-LATS2 antibody at 2 μg/ml or rabbit anti-YAP antibody, 250x) for 1 h at room temperature, washed with PBS, treated with a biotinylated secondary antibody for 15 min at room temperature, and allowed to react for 15 min with the streptoavidin-peroxidase reagent using an Ultra-tech kit (Beckman Coulter, Marseille, France). The DAB (3, 3'-diaminobenzidine tetrahydrochloride) Liquid System (DakoCytomation, Glostrup, Denmark) was used to detect immunostaining. Normal rabbit serum or omission of primary antibodies served as negative controls. Immunoreactivity was evaluated independently by two investigators (H.M. and T. M.).

The intensity of staining was scored as strong (3+), moderate (2+), weak (1+), or negative (0), respectively.

References

1. Kawaguchi K, Murakami H, Taniguchi T, et al. Combined inhibition of MET and EGFR suppresses proliferation of malignant mesothelioma cells. Carcinogenesis 2009;30:1097-105.