Hippo Pathway Gene Mutations in Malignant Mesothelioma

Revealed by RNA and Targeted Exon Sequencing

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Introduction: Malignant mesothelioma (MM) is an aggressive neoplasm causatively associated with exposure to asbestos. MM is rarely responsive to conventional cytotoxic drugs, and the outcome remains dismal. It is, therefore, necessary to identify the signaling pathways that drive MM and to develop new therapeutics specifically targeting the molecules involved.

Methods: We performed comprehensive RNA sequencing of 12 MM cell lines and four clinical samples using so-called next-generation sequencers.

Results: We found 15 novel fusion transcripts including one derived from chromosomal translocation between the *large tumor suppressor 1* (*LATS1*) and *presenilin-1* (*PSEN1*) genes. LATS1 is one of the central players of the emerging Hippo signaling pathway. The *LATS1–PSEN1* fusion gene product lacked the ability to phosphorylate yes-associated protein and to suppress the growth of a MM cell line. The wild-type *LATS1* allele was undetectable in this cell line, indicating two-hit genetic inactivation of its tumor suppressor function. Using pathway-targeted exon sequencing, we further identified a total of 11 somatic mutations in four Hippo pathway genes (neurofibromatosis type 2 [*NF2*], *LATS2*, *RASSF1*, and *SAV1*) in 35% (8 of 23) of clinical samples. Nuclear staining of yes-associated protein was detected in 55% (24 of 44) of the clinical samples. Expression and/or phosphorylation of the Hippo signaling proteins, RASSF1, Merlin (NF2), LATS1, and LATS2, was frequently absent.

Conclusions: The frequent alterations of Hippo pathway molecules found in this study indicate the therapeutic feasibility of targeting this pathway in patients with MM.

Key Words: Malignant mesothelioma, Next-generation sequencer, Hippo pathway, LATS1, Fusion gene.

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Malignant mesothelioma (MM) is a highly aggressive neoplasm that arises from mesothelial cells covering the surfaces of the pleura, peritoneum, and pericardium. Etiologically, MM is associated with exposure to asbestos and other fibrous minerals.[1] Surgical resection is theoretically the most effective treatment for any solid malignant tumors, but complete removal of MM is rarely attainable because of its disseminative and invasive growth.[2] Although there has been significant progress in the development of combination chemotherapies, MM rarely responds to conventional cytotoxic drugs, and the outcome remains dismal.[3] It is, therefore, necessary to develop new therapeutics specifically targeting molecules essential for the development and/or progression of MM.

The Hippo signaling pathway is a regulator of organ size and tissue regeneration.[4] Somatic mutations of two genes in the Hippo signaling pathway have been reported in MM cells.[5] The *NF2* gene,[6,7] which encodes the merlin tumor suppressor protein, shows the highest frequency of mutation. Deletion and mutation of *large tumor suppressor 2* (*LATS2*) have been identified in several MM cell lines and clinical samples, and its tumor suppressive role has been demonstrated in vitro.[5] However, many other signaling molecules are known to regulate the Hippo pathway,[8] and their spectrum of aberrations and clinical relevance in MM remain largely unknown.

The development of high-throughput sequencers (or so-called next-generation sequencers [NGS]) has made it possible to rapidly survey oncogenic and tumor-suppressive signaling molecules in various cancers on a genome-wide basis.[9] In the present study, we investigated the MM genome using unbiased whole-exon (exome) and RNA (transcriptome) sequencing and found a novel gene fusion between the *LATS1* and presenilin-1 genes (*LATS1–PSEN1*). The fusion gene product lacked the kinase activity of LATS1, and the *LATS1* locus was

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inactivated by a two-hit genetic event. We then focused on the Hippo signaling pathway and sequenced all exons of the 40 genes known to constitute the pathway.

MATERIALS AND METHODS

Cell Lines and Clinical Samples

The 19 MM cell lines used in this study and their suppliers are listed in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A805). An immortalized mesothelial cell line, MeT-5A, was purchased from the American Type Culture Collection (Manassas, VA). The human embryonic kidney cell line HEK293 was obtained from the Health Science Research Resources Bank (Osaka, Japan). The cell lines were maintained in accordance with the suppliers' recommendations.

The clinical and pathological characteristics of 23 patients whose fresh frozen tissue samples were analyzed by NGS are summarized in Supplementary Table 2 (Supplemental Digital Content 1, http://links.lww.com/JTO/A805). Formalin-fixed paraffin-embedded tissue sections were collected from 44 MM patients (including the aforementioned 23 patients) who underwent panpleuropneumonectomy or pleural biopsy at the National Cancer Center (NCC) Hospital (Tokyo, Japan) between 1995 and 2012 with written informed consent. This study was conducted with approval from the Institutional Review Board of the National Cancer Center.

DNA and RNA Preparation

Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) or RNeasy Mini Kit columns (Qiagen) using the manufacturer's protocol. The quality of RNA was assessed by an Agilent 2100 Bioanalyzer

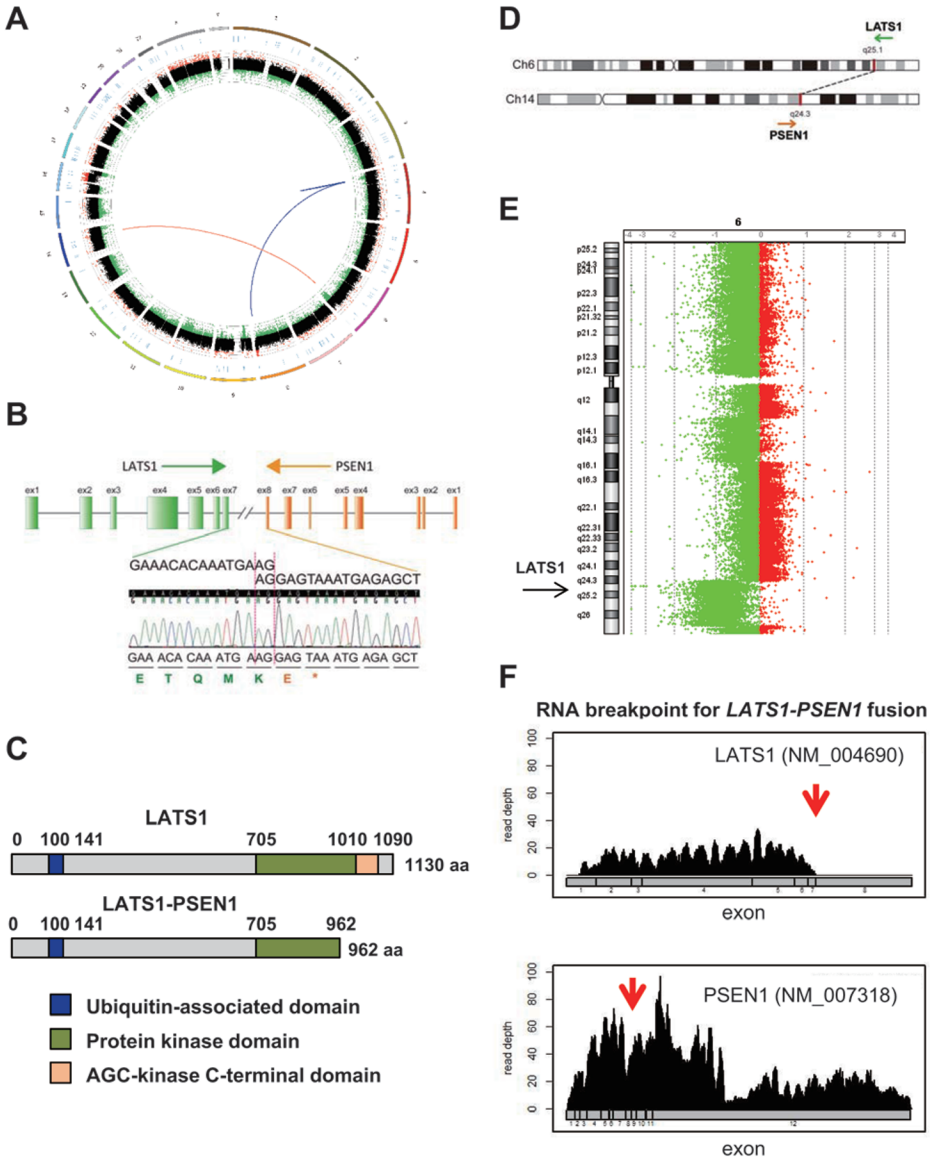

FIGURE 1. Identification of the *LATS1–PSEN1* fusion gene. *A*, Graphic representation of RNA sequencing and comparative genomic hybridization data for MSTO-211H cells. Chromosome ideograms and copy number variation are shown in the outer and middle layers, respectively. Chromosomal translocation is shown in the central layer. The *LATS1–PSEN1* fusion is shown in red, and the *SLIT2-AC006052.1* and *TRAPPC9-GBA3* fusions are shown in blue. *B*, Nucleotide and deduced amino acid sequences at the break/fusion point of the *LATS1–PSEN1* transcript. *C*, Schematic representation of the deduced domain structure of wild-type LATS1 and LATS1–PSEN1 proteins. Wild-type LATS1 contains the ubiquitin-associated domain (amino acids 100–141), protein kinase domain [705–1010], and AGC-kinase C-terminal domain [1010–1090]. *D*, Ideogram showing the chromosomal translocation of *LATS1* (chromosomes 6 at q25.1) and *PSEN1* (chromosome 14 at q24.3). *E*, Comparative genomic hybridization analysis of chromosome 6 of MSTO-211H cells. *F*, Expression level of each exon of the *LATS1* and *PSEN1* genes determined by counting the number of reads in RNA sequencing.