SHORT COMMUNICATION

Limatin (*LIMAB1*), an Actin-Binding LIM Protein, Maps to Mouse Chromosome 19 and Human Chromosome 10q25, a Region Frequently Deleted in Human Cancers

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LIM domains, found in over 60 proteins, play key roles in the regulation of developmental pathways. They were first identified as cysteine-rich motifs found in the three proteins Lin-11, Isl-1, and Mec-3. LIM proteins frequently contain DNA-binding homeodomains, allowing these proteins to activate transcription. LIM domains also function as protein-binding interfaces, mediating specific protein–protein interactions. Limatin is a novel LIM protein that binds to actin filaments via a domain that is homologous to erythrocyte dematin. Here we report the murine and human chromosomal localizations of limatin (*LIMAB1*). Limatin was mapped to mouse Chromosome 19 by restriction fragment length polymorphism analysis and to human chromosome region 10q25 by fluorescence *in situ* hybridization. Radiation hybrid mapping placed *LIMAB1* in a 37-cR interval between markers D10S554 and D10S2390. Interestingly, 10q25 is a region of frequent loss of heterozygosity in human tumors, thus identifying limatin as a candidate tumor suppressor gene.

Each of the original three LIM proteins, Lin-11, Isl-1, and Mec-3, is involved in cellular differentiation and contains a DNA-binding homeodomain in addition to LIM domains (15). Many LIM proteins are required for cell fate determination during development (16, 18). Limatin (LIM + dematin), also known as ab/LIM (actin-binding LIM), is a cytoskeletal LIM protein whose carboxyl-terminal headpiece domain is 50% identical to dematin, an actin-bundling protein of the erythroid cytoskeleton (1, 11, 13). Interestingly, limatin is the first protein known to share homology with the amino-terminal domain of dematin (36% identity) (Fig. 1). The function of the N-terminal domain of dematin (amino acids 1–308) is not yet known. The headpiece domain of limatin is homologous to the headpiece domains found in dematin (55%) and villin (31%) (Fig. 1). Limatin exists in three isoforms, all varying in the number of LIM domains, that are alternatively spliced from a single gene (13). Like dematin, limatin also binds to actin filaments *in vitro* (11, 13). The function of limatin may be to bind to the cytoskeleton and interact with other LIM proteins to modulate cytoskeleton–membrane interactions, in as yet unknown signaling pathways. It may also serve to link the cytoskeleton to the regulation of gene expression, a function shared by many LIM proteins (15).

Here we report the mapping of the mouse limatin gene (*Limab1*) to Chromosome 19 by restriction fragment length polymorphism (RFLP) analysis and the human limatin gene (*LIMAB1*) to chromosome region 10q25 by fluorescence *in situ* hybridization (FISH) and radiation hybrid (RH) mapping. Primer pair A6/A4 (A6, 5′-TGC CAT GGC TGT GGG GAG TTC; A4, 5′-CAG AGT TGA CAA AGG CAG TCT CTC), derived from the second LIM domain of the limatin human cDNA sequence, amplifies a 2.9-kb product from murine genomic DNA, invariably due to the presence of an intron. The PCR product was subcloned into pCRII (Invitrogen), and the ends were sequenced to confirm specificity for limatin. The PCR product was used as a probe to map *Limab1* in the mouse using 94 progeny from The Jackson Laboratory BSS interspecific backcross [(C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei] (14). A *Hin*dIII RFLP between the parental strains was detected by Southern blotting of genomic DNA (*Hin*dIII band sizes were 5.1 and 1.9 kb in C57BL/6J and 6.7, 4.1, and 2.3 kb in *Mus spretus*). The typing data obtained have been deposited in the Mouse Genome Database (Accession No. MGD-JNUM-40509) and can be accessed through the World Wide Web (http://www.jax.org). One recombination occurred between *Limab1* and *Emx2*/*Xmv18*, placing *Limab1* on Chromosome 19, approximately 54 cM distal to the centromere (Fig. 2A). This region is conserved with human 10q24–q26 (2).

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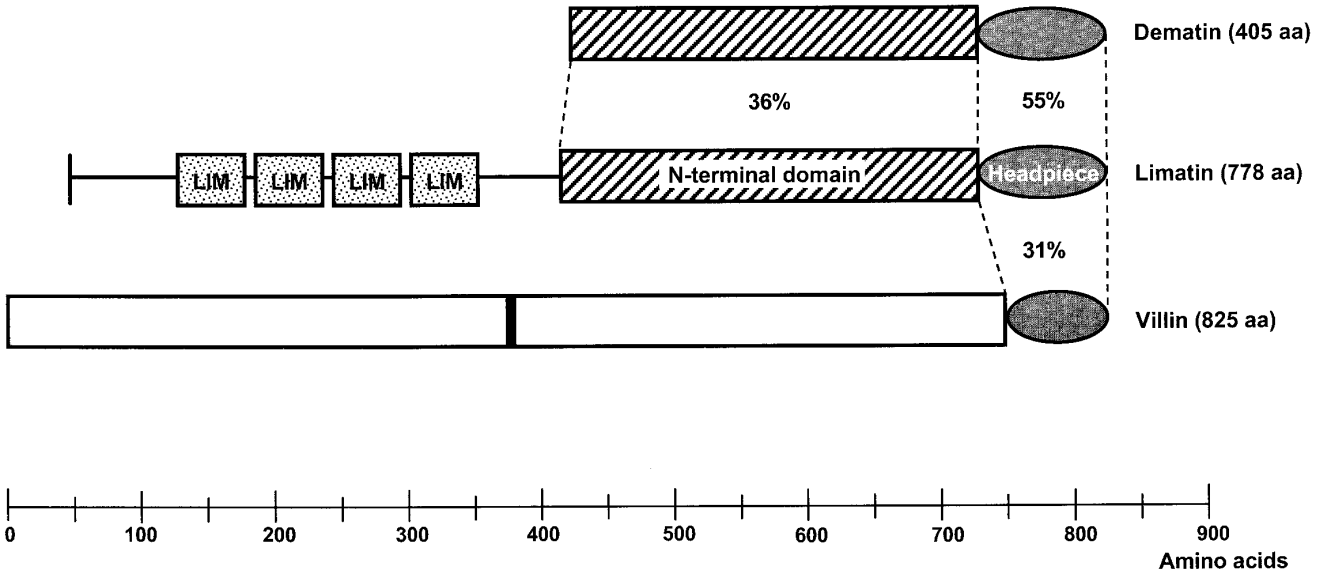

FIG. 1. Sequence comparison of limatin, dematin, and villin. Amino acid sequence similarities between N-terminal domains of limatin and dematin and headpiece domains of dematin and villin compared to the limatin headpiece using DNAStar. The villin headpiece does not contain the 22-amino-acid insertion found in limatin and dematin. The LIM domains of limatin and the N-terminal domain of villin were excluded in this comparison.

Regional localization of the human limatin gene (*LIMAB1*) was performed by FISH analysis. The primer pair A6/A4 amplifies a 4-kb product from human genomic DNA, again due to the presence of an intron. The PCR product was subcloned into pCRII and also sequenced to confirm specificity for limatin. The primers were then used to isolate a PAC clone from "Down to the Well" Human PAC DNA pools (Genome Systems Inc.). The PAC clone contained a portion of the limatin gene, as determined by PCR. Purified PAC DNA was labeled with digoxigenin-11–dUTP by nick-translation and hybridized to human metaphase chromosomes as previously described (7). Hybridizations were detected with rhodamine-conjugated antibody to digoxigenin (Boehringer Mannheim) and localized to 4′,6-diamidino-2-phenylindole (DAPI)-banded chromosomes. Rhodamine and DAPI were viewed through a triple-bandpass filter set (ChromaTech), and DAPI banding was viewed through a single bandpass filter set (Zeiss). Twelve hybridized metaphases were examined, and all revealed hybridization to chromosome region 10q25 (data reviewed but not shown).

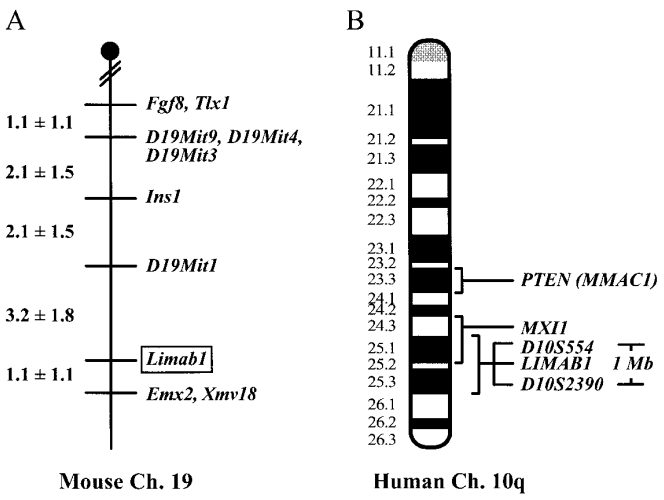

FIG. 2. Chromosomal localization of *LIMAB1* to mouse chromosome 19 and human chromosome 10q25. (**A**) Idiogram of mouse Chromosome 19 and location of *Limab1* relative to markers mapped in The Jackson Laboratory BSS panel. Genetic distances between markers (percentage recombination + SE) are indicated to the left, and gene symbols are shown to the right. Missing typings in nonrecombinant animals were inferred from flanking markers. The panel data and references for mapping the other loci are publicly available from The Jackson Laboratory Mapping Resource through http://www.jax.org/resources/documents/cmdata. (**B**) Idiogram of human chromosome 10q and location of *LIMAB1* relative to markers mapped in the Stanford RH panel. *LIMAB1* is distal to the locus of the *PTEN* and *MXI1* genes, in a 37-cR (~1 Mb) interval between markers D10S554 and D10S2390.

Finally, fine mapping of *LIMAB1* was determined by RH mapping. The primer pair A1/A3 (A1, 5′-ATG CCT GCC TTC CTT GGT CTA AAG; A3, 5′-ACG CCC ACG TGG GCA GTA GTC), derived from sequences upstream of the first LIM domain in the 5′ coding sequence, is specific for limatin and amplifies a product of 219 bp from human genomic DNA. A1/A3 was used to amplify the Stanford Human Genome Center G3 radiation hybrid panel (data not shown) (3). *LIMAB1* maps to 10q25 with very high probability (LOD > 13), 4.65 cR (~115 kb) from unidentified transcript SHGC-35224 (Fig. 2B). This transcript maps almost equidistant between markers D10S554 and D10S2390. Markers D10S554 and D10S2390 are approximately 37 cR apart on the Stanford RH framework, thus refining the *LIMAB1* locus to a 1-Mb interval on chromosome 10q (1 cR ~ 25 kb).

The long arm of chromosome 10 has been a region of intense study because of its propensity to undergo deletion in a variety of cancers, and it is thought to harbor several tumor suppressor genes (5, 6). *PTEN* (or *MMAC1*), a candidate tumor suppressor gene at 10q23.3, is mutated in cancers of the prostate, brain, kidney, and breast (9, 17). The *MXI1* gene, mapped to 10q24–q25, has been found to be mutated in some prostate cancers (4). There remains strong evidence of another tumor suppressor gene(s) on the telomeric end of 10q. LOH studies indicate that the region distal to the *MXI1* gene, at 10q25–q26, is lost in several cancers, including glioblastomas, prostate and endometrial carcinomas, and thyroid tumors (8, 10, 12, 19). The localization of *LIMAB1* to 10q25 identifies it as a candidate tumor suppressor gene. We speculate that inactivating mutations in limatin may disrupt a novel membrane-to-nucleus signaling pathway, causing a breakdown in cell integrity and leading to cellular transformation. Whether limatin is lost and/or mutated in cancers is an area of current study.

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