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Structure, Expression, and Chromosome Mapping of *LATS2*, a Mammalian Homologue of the *Drosophila* Tumor Suppressor Gene *lats/warts*

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We have cloned and characterized *LATS2*, a novel mammalian homologue of the *Drosophila* tumor suppressor gene *lats/warts*. Northern blot analysis showed ubiquitous expression of mouse *LATS2 (MmLATS2)* mRNA, whereas expression of human *LATS2 (HsLATS2)* mRNA was enhanced in skeletal muscle and heart. Immunoblotting analysis of fractionated cell lysates showed HsLats2 to be a nuclear protein. We mapped the *MmLATS2* gene to mouse chromosome 14 by interspecific backcross analysis. We also mapped the *HsLATS2* gene (by fluorescence *in situ* hybridization) to the 13q11–q12 region, in which a loss of heterozygosity has been frequently observed in many primary cancers and to which the tumor suppressor genes *RB* and *BRCA2* have also been mapped.

INTRODUCTION

Tumorigenesis in human cancers is a complex process involving a series of genetic changes such as activation of oncogenes or inactivation or loss of tumor suppressor genes. The *Drosophila* tumor suppressor gene *warts* was first identified in a mutant that showed a superficial wart-like phenotype due to homozygous loss of this gene (Justice *et al.*, 1995). The same gene, named *lats* (large tumor suppressor), was also isolated as a recessive overproliferation mutant by screening and examining clones of mutant cells in *Drosophila* genetic mosaics (Xu *et al.*, 1995). It encodes a putative Ser/Thr protein kinase that shares homology with the budding yeast kinases Dbf2 and Dbf20, which are involved in cell cycle regulation (Toyn and Johnston, 1994).

In the course of comprehensive isolation of mouse genes whose expression was induced during spermiogenesis (Fujii *et al.*, 1999) using an improved cDNA library subtraction method (Kobori *et al.*, 1998), we isolated a clone encoding a putative protein kinase with a structure homologous to that of the *warts/lats* protein kinase. The clone encoded a predicted protein of 1043 amino acids, which was homologous to the *warts/lats* kinase not only in the kinase domain but also in the surrounding regions. Very recently, a human homologue of the *lats* gene was isolated and named *LATS1* (Tao *et al.*, 1999). For consistency, since the *lats* homologue we isolated has a very similar structure to *LATS1*, we have named it *LATS2*. Here we report the cDNA cloning, structure, expression, and chromosomal localization of mouse and human *LATS2*.

MATERIALS AND METHODS

Isolation of LATS2 cDNA. A subtracted cDNA library was prepared from mouse testis as described previously (Fujii *et al.*, 1999). From this library, we have isolated to date more than 150 genes whose transcription is up-regulated in sperm-producing testis. One of these cDNA clones encoded a protein similar to the *Drosophila* tumor suppressor *lats*, which we named mouse *LATS2*. The identity of the *MmLATS2* gene was confirmed by sequencing the inserts of five independent clones, and a clone matching the consensus sequence was selected for further analysis. Using the cDNA insert from the mouse *LATS2* clone, we isolated a human *LATS2* cDNA from a human testis cDNA library by colony hybridization. DNA sequences of the cDNA inserts were determined by the dideoxy chain termination method using T7 promoter-specific fluorescent dye-labeled primers and a SequiTherm Cycle Sequencing Kit (Epicenter Technologies, Madison, WI). The reaction products were analyzed on a LI-COR Model 4000L DNA sequencer (LI-COR Inc., Lincoln, NE). DNA database searches were carried out against all entries of the DDBJ/

Sequence data reported in this article have been deposited with the DDBJ/GenBank/EMBL DataLibraries under Accession Nos. AB023958 (*MmLATS2* cDNA) and AB028019 (*HsLATS2* cDNA).

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GenBank/EMBL Data Libraries using the BLAST family of algorithms (http://www.ddbj.nig.ac.jp/E-mail/homology-j.html).

Northern blotting. Preparation of RNA from various tissues and fractionation of germ, Leydig, and Sertoli cells from adult mice were performed as described previously (Koga *et al.*, 1998). RNA samples were subjected to electrophoresis in agarose gels and transferred to a nylon membrane (Biodyne A, PALL, Port Washington, NY). cDNA inserts were excised from plasmid clones by *Sma*I/*Not*I digestion, radiolabeled with [α-^{32}P]dCTP using a Random Primer DNA Labeling Kit (TaKaRa, Tokyo, Japan), and used as hybridization probes.

Preparation of antibody and immunoblotting. To establish a mouse hybridoma producing anti-human Lats2 antibody (3D10), the N-terminal portion of human Lats2 (amino acids 78–256) was produced in *Escherichia coli* as a GST (glutathione *S*-transferase)–fusion protein, and the purified fusion protein was used to immunize mice. Mice were immunized subcutaneously by standard protocol with ~60 μg of fusion protein in Freund's complete adjuvant five times at biweekly intervals and boosted with fusion proteins in Freund's incomplete adjuvant. Preparation of hybridomas producing monoclonal antibody and antibody purification were carried out according to standard procedures (Harlow and Lane, 1988). Anti-14-3-3β monoclonal antibody and anti-HsOrc2 polyclonal antibody were obtained from MBL Co. Ltd. (Nagoya, Japan).

For immunoblotting analysis, fractionated samples were mixed with an equal volume of 2× Laemmli's sample buffer and then heated at 100°C for 5 min. For preparation of whole-cell extracts, cells were directly lysed in 1× Laemmli's sample buffer and subsequently heated at 100°C for 5 min. Proteins were resolved by SDS–PAGE on 7.5% gels and then transferred to PVDF membranes (Millipore). Membranes were probed with monoclonal antibody against HsLats2. After being washed, blots were incubated with HRP-conjugated anti-mouse IgG (MBL Co. Ltd., Nagoya, Japan). Immunoreactive bands were detected by enhanced chemiluminescence (Amersham).

Cell culture and subcellular fractionation. All cell lines were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum (Hyclone Laboratory, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin. Nuclear and cytoplasmic fractions were prepared by the method of Schreiber *et al.* (1989). Asynchronously growing HeLa–S3 cells were washed twice with ice-cold phosphate-buffered saline and collected in Tris-buffered saline. After centrifugation, the pellet was resuspended in buffer A [10 mM Hepes (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF] and allowed to swell on ice for 15 min, after which 0.6% (final concentration) Nonidet-P40 was added, and the tube was vortexed for 10 s. The homogenate was centrifuged (1500*g*, 30 s) and the supernatant collected as the cytoplasmic fraction. The nuclear pellet was resuspended in buffer C [20 mM Hepes (pH 7.9) 400 mM NaCl, 1 mM EGTA, 1 mM DTT, 1 mM PMSF], and the tube was vigorously rocked at 4°C for 15 min on a shaking platform. The nuclear extracts were clarified by centrifugation at 15,000*g* for 5 min.

Interspecific mouse backcross mapping. Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as described by Copeland and Jenkins (1991). A total of 205 N_2 mice were used to map the *Lats2* locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described by Jenkins *et al.* (1982). All blots were prepared with Hybond-N$^+$ nylon membrane (Amersham). The probe, an *Eco*RI/*Not*I fragment of mouse cDNA approximately 670 bp in length, was labeled with [α-^{32}P]dCTP using a random primed labeling kit (Stratagene), and blots were washed to a final stringency of 1× SSCP, 0.1% SDS, at 65°C. A fragment of 17.0 kb was detected in *Pst*I-digested C57BL/6J DNA, and a fragment of 3.6 kb was detected in *Pst*I-digested *M. spretus* DNA. The presence or absence of the 3.6-kb *Pst*I *M. spretus*-specific fragment was followed in backcross mice.

A description of probes and RFLPs for analysis of loci linked to *Lats2*, including *Gzmb*, *Gjb2*, and *Nfl*, has been reported previously (Wang *et al.*, 1996). Recombination distances were calculated using Map Manager, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Fluorescence in situ hybridization. FISH (fluorescence *in situ* hybridization) analysis of R-banded human chromosomes was performed as described previously (Endo *et al.*, 1996) using human *LATS2* cDNA (5-kb insert in pAP3neo vector; Kobori *et al.*, 1999) as a probe. Briefly, 150 ng of biotin-labeled cDNA probe was hybridized to metaphase chromosome spreads, which were prepared as described by Takahashi *et al.* (1990). For fluorochrome detection, the slides were incubated with fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, Burlingame, CA) and then washed. The FITC signals were amplified by incubation with biotin-conjugated goat anti-avidin Ab (Vector Laboratories) followed by incubation with FITC-conjugated avidin. Preparations were counterstained with propidium iodide and examined with a laser scanning microscope (Zeiss LSM).

RESULTS

Isolation of Mouse and Human LATS2 cDNAs

To isolate systematically mouse genes whose transcription is specifically induced during spermiogenesis, we prepared an adult mouse testis cDNA library subtracted against biotin-labeled mRNA of prepubertal mouse testis (Fujii *et al.*, 1999) using an improved subtraction method (Kobori *et al.*, 1998). Since mice produce no sperm prior to puberty, this strategy can be expected to yield sperm- or haploid cell-specific cDNA species. In addition to 84 species whose expression was dramatically induced during spermiogenesis (as judged by Northern analysis), we identified 56 species whose transcription was moderately induced. Nucleotide sequence analysis revealed that one of the latter group of cDNAs encoded a predicted protein of 1043 amino acids that was homologous to the *Drosophila warts/lats* kinase (Justice *et al.*, 1995; Xu *et al.*, 1995). We named this gene *LATS2*, since another mammalian homologue of the *warts/lats* gene was recently isolated and named *LATS1* (Tao *et al.*, 1999). By screening a human testis cDNA library by colony hybridization with mouse *LATS2* cDNA as a probe, we also isolated human *LATS2* cDNA.

Structure of Mouse and Human Lats2

The predicted mouse Lats2 gene product contained 1043 amino acid residues and was highly homologous to mouse Lats1 at the primary sequence level (Fig. 1A). Sequence similarity was highest in the putative Ser/Thr kinase domain (80% identity) at the C-terminal end of the protein. Mouse Lats2 and fruit-fly Lats/Warts shared 75% sequence identity in the kinase domain (Fig. 1B). Sequence similarity between mouse Lats2 and fruit-fly Lats extended to the N-terminus, although the similarity in this region was much lower than in the kinase domain. Structural comparisons between mouse Lats1 and Lats2 revealed a similar pattern of high similarity in the C-terminal half of the molecule including the kinase domain and low similarity in the N-terminal half. Mouse and human Lats1 and fruit-fly and *Caenorhabditis elegans* Lats all con-

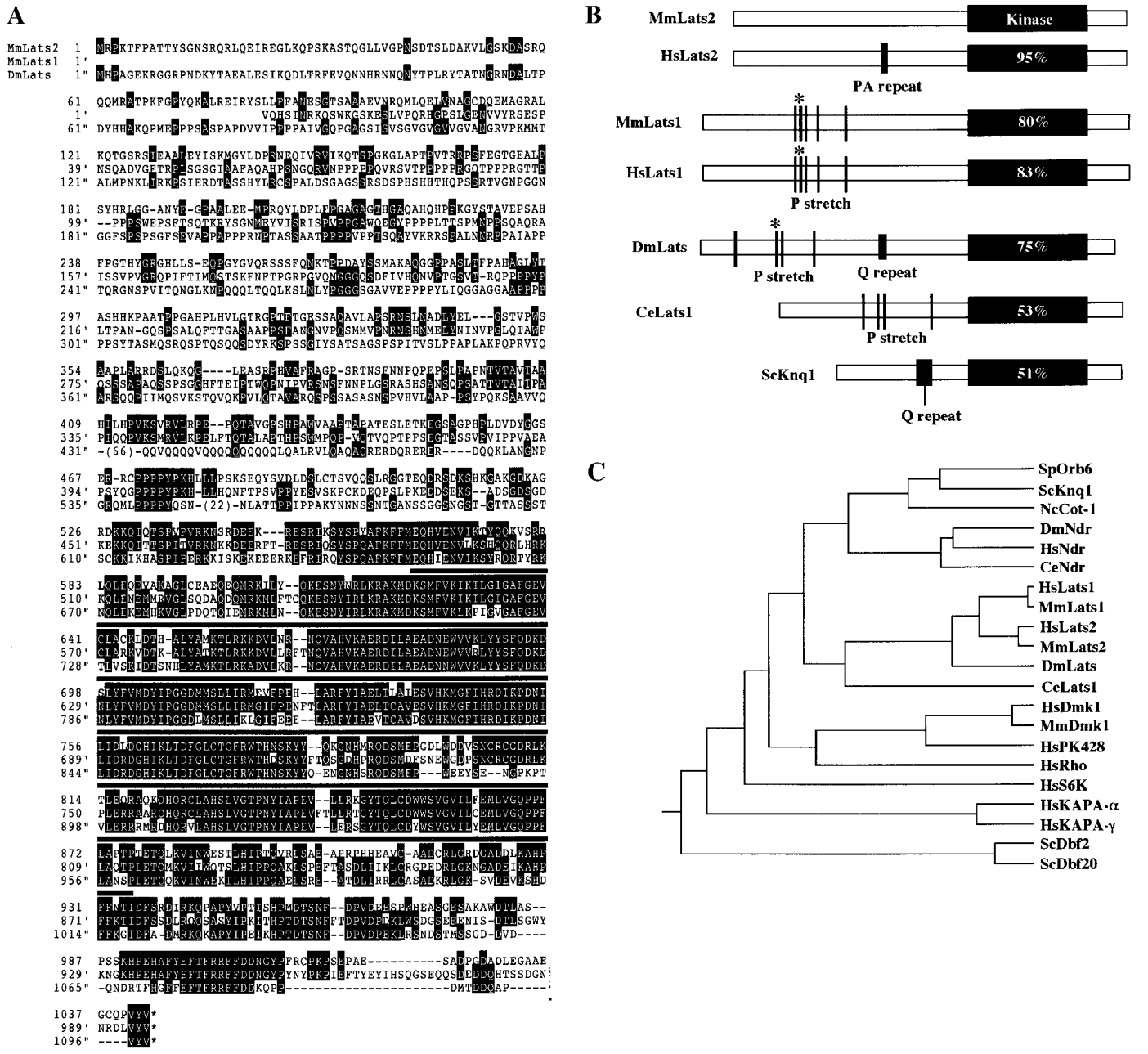

FIG. 1. (**A**) Amino acid sequence comparisons between mouse Lats2, mouse Lats1, and *Drosophila* Lats. Identical amino acids are indicated by shaded backgrounds. The Ser/Thr kinase domain is indicated by thick horizontal bars drawn above the amino acid sequence. (**B**) Schematic drawings of Lats2 proteins of mouse (MmLats2) and human (HsLats2) together with Lats1 proteins of mouse (MmLats1) and human (HsLats1), fruit-fly Lats (DmLats), nematode Lats1 (CeLats1), and budding yeast Lats1-like protein (Cbk1; P53894). The locations of the alternating proline–alanine sequence (PAPA repeat), polyproline sequence (P stretch), and polyglutamine repeat (Q repeat) are shown. Asterisks indicate the locations of consensus sequences (PXXPXR) for potential SH3-binding sites. (**C**) Dendrogram of the kinase domains of Lats proteins and other Ser/Thr kinases clustered by the PileUp program. The dendrogram indicates similarity on the horizontal axis, proportional to the length of the branch line before bifurcation. Hs, *Homo sapiens;* Mm, *Mus musculus;* Dm, *Drosophila melanogaster;* Nc, *Neurospora crassa;* Sc, *Saccharomyces cerevisiae;* Sp, *Schizosaccharomyces pombe.*

tain four or five stretches of several proline residues in the N-terminal region, one of which matches the consensus sequence (PXXPXR) for a potential SH3 (Src homology 3) binding site (Feng *et al.*, 1994). However, no such sequences were found in Lats2, suggesting a functional distinction between the mammalian Lats homologues. Human Lats2, but neither mouse Lats2 nor any other Lats homologue, possesses seven repeats of alternating proline–alanine residues (PAPA repeat). Such a sequence was also found in human $p57^{KIP2}$ but not in mouse $p57^{KIP2}$ (Matsuoka *et al.*, 1995) and may be involved in protein–protein interactions. The polyglutamine repeat found in fruit-fly Lats and yeast Lats-like protein is not found in Lats proteins of other species.

Further analysis of kinase domain amino acid se-

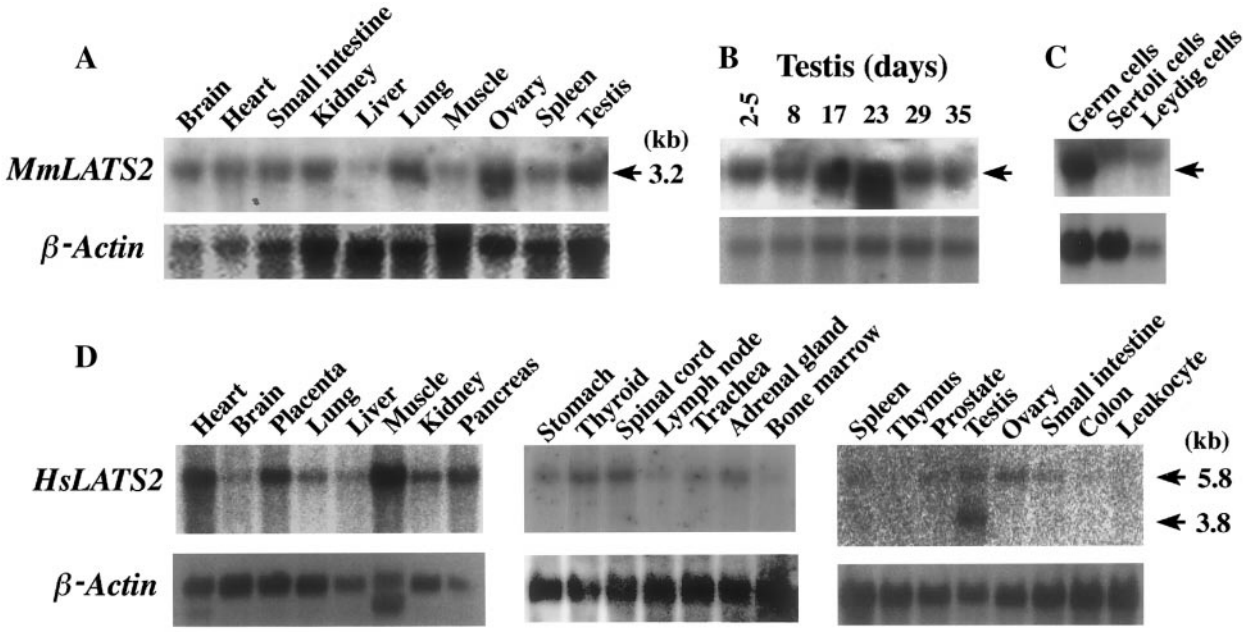

FIG. 2. Northern blot analysis of mouse and human *LATS2*. RNA was prepared from various tissues of an adult mouse (**A**), from the testis at various developmental stages (**B**), and from fractionated testicular cells (**C**). Northern blots of RNA from various human tissues were obtained from Clontech (**D**). Mouse or human *LATS2* cDNA inserts were radiolabeled and used as hybridization probes. After autoradiography, the first probe was stripped from the filter, which was rehybridized with β-actin cDNA as a loading control.

quences was performed to determine the relationship between Lats1, Lats2, and fruit-fly Lats. We conducted BLAST searches using the Lats2 kinase domain against all entries of the DDBJ/GenBank/EMBL DataLibraries, collected the amino acid sequences of the kinase domains of 20 Ser/Thr kinases, and constructed a dendrogram showing the sequence relationships among these kinase domains using the "PileUp" program of the GCG software package (Genetics Computer Group, Madison, WI). As shown in Fig. 1C, the result clearly indicates that Lats2 is most closely related to Lats1, followed by fruit-fly and nematode Lats.

Expression of the LATS2 Gene

To characterize the *LATS2* gene further, we examined its transcription patterns in various mouse tissues. Northern analysis of RNA extracted from various adult mouse tissues using *LATS2* cDNA as a probe indicated that expression of the *LATS2* gene was ubiquitous, although mRNA levels were comparatively high in ovary and testis (Fig. 2A). We therefore examined the expression pattern of *LATS2* in the testis in more detail. Total RNA extracted from testis of mice of varying ages was subjected to Northern blot analysis (Fig. 2B). *LATS2* mRNA could be detected in even the earliest prepubertal testis (2–5, 8, and 17 days old); *LATS2* transcription increased in postpubertal testis (23 days old) and declined in adult mouse testis (29 and 35 days old). We also examined the detailed localization of *LATS2* mRNA using total RNA prepared from fractionated mouse testicular cells, i.e., germ, Leydig, and Sertoli cells (Fig. 2C); the quality of this RNA was verified in a previous report (Koga *et al.*, 1998). Most of the *LATS2* mRNA was detected in the germ cell fraction.

We also analyzed the expression of human *LATS2* using multiple-tissue Northern blots obtained from Clontech (Palo Alto, CA). As shown in Fig. 2D, the expression pattern is distinct from that of the mouse homologue. Human *LATS2* is most strongly expressed in skeletal muscle and heart, whereas *LATS2* expression in these tissues is moderate in mouse. Of further note is a smaller (3.8 kb) transcript specific to human testis. Comparison of the DNA sequences of several cDNA clones isolated from the human cDNA library suggested that this 3.8-kb transcript has the same open reading frame as the 5.8-kb transcript, but a shorter 3′ untranslated region (data not shown).

Preparation of Antibody and Subcellular Distribution of Lats2

To examine the subcellular distribution of endogenous Lats2 protein, we raised a monoclonal mouse antibody (3D10) against a recombinant GST–fusion protein containing the N-terminal portion (amino acids 78–256) of HsLats2. As shown in Fig. 3A, the anti-HsLats2 monoclonal antibody (3D10) specifically recognized a band of approximately the same size as the 6Myc-tagged HsLats2 protein expressed in 293T human embryonic kidney cells (2 μg/lane of the whole extract). The 3D10 antibody also recognized a C-terminally truncated form of 6Myc-tagged HsLats2, which contained the epitope for 3D10, but not the C-terminal portion of Myc-tagged HsLats2 protein from which the epitope was absent. Although the endogenous HsLats2 cannot be detected by the 3D10 antibody in the whole

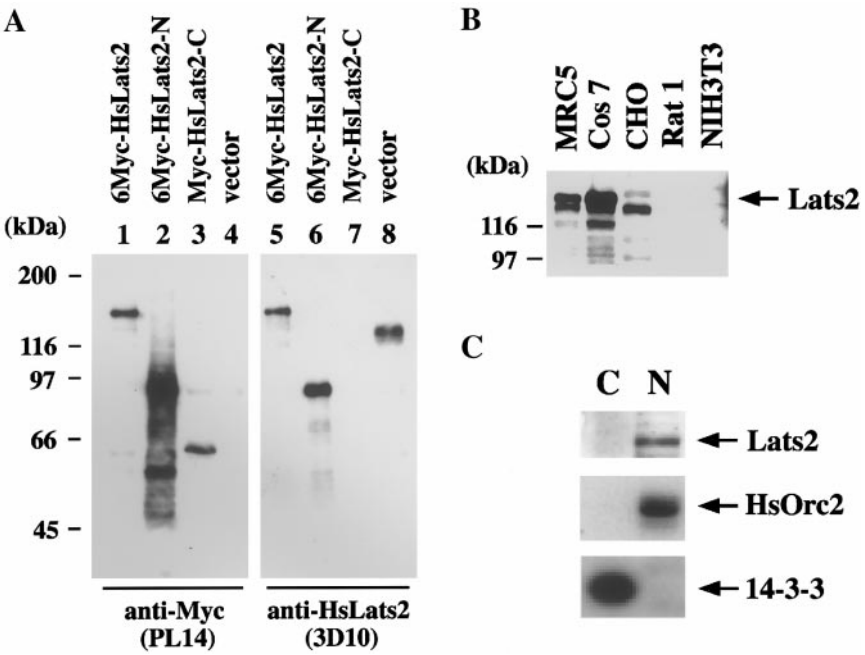

FIG. 3. Subcellular distribution of endogenous human Lats2. (**A**) Specificity of the affinity-purified anti-HsLats2 monoclonal antibody (3D10). Whole extracts of 293T cells expressing 6Myc-HsLats2, 6Myc-HsLats2-N-terminal region, or Myc-HsLats2-C-terminal region (lanes **1–3,** and **5–7;** 2 μg/lane), and the nuclear extracts (by 300 mM NaCl) of 293T cells expressing empty vector alone (lanes **4** and **8;** 5 μg/lane) were subjected to 7.5% SDS–polyacrylamide gel electrophoresis and then immunoblotted with 1 μg/ml of anti-Myc monoclonal antibody (PL14; lanes **1–4**) or with 5 μg/ml of anti-HsLats2 monoclonal antibody (3D10; lanes **5–8**). (**B**) Immunoblotting of whole-cell extracts from various cell lines with 3D10 antibody. 3D10 antibody does not appear to cross-react with rat or mouse Lats2. (**C**) Subcellular localization of HsLats2. Asynchronous HeLa cells were fractionated into cytoplasmic (**C**) and nuclear (**N**) fractions, and each fraction was immunoblotted with 3D10 antibody, anti-14-3-3β monoclonal antibody, or anti-HsOrc2 polyclonal antibody (MBL Co., Ltd.). HsLats2 was found to be localized in the nucleus of HeLa cells.

extract of 293T cells, it can be recognized as a single band of 125 kDa (lane 8), which is consistent with the predicted size of HsLats2, when the sample was concentrated by separation of the nucleus from the cytoplasm and subsequent extraction by 300 mM NaCl (5 μg/lane of the concentrated nuclear extract). Thus, immunoblotting with anti-Myc monoclonal antibody (PL14) indicated that the bands detected by 3D10 represented bona fide HsLats2 protein. Together with Lats2 in human (MRC5) cells, 3D10 antibody also recognized the Lats2 proteins of other mammalian species including monkey (COS7) and Chinese hamster (CHO), but not those of rat (Rat1) or mouse (NIH3T3), as shown in Fig. 3B. One of the two major bands that were detected at around 125 kDa in MRC5 and COS7 cells may represent a phosphorylated form of Lats2. The lower band at 116 kDa in COS7 cell extracts may be due to protein degradation, since we often observed many bands of lower molecular mass in cell extracts that were not prepared with rigorous care for the integrity of the proteins (data not shown).

We next examined subcellular fractions for the presence of HsLats2 by immunoblotting. Immunoblot analysis of separated cytoplasmic and nuclear fractions of HeLa–S3 cells showed that almost all of the endogenous HsLats2 protein was localized in the nuclear fraction (*N*) (Fig. 3C, top). We confirmed that another nuclear protein, HsOrc2, was also localized in the nuclear fraction (Fig. 3C, middle). In contrast, a typical cytoplasmic protein, 14-3-3β, which was also used as a control for successful fractionation, was found exclusively in the cytoplasmic fraction (Fig. 3C, bottom). The result indicates that HsLats2 is a nuclear protein.

Chromosomal Location of the Mouse LATS2 Gene

The chromosomal location of mouse *LATS2* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J $\times$ *M. spretus*)F_1 $\times$ C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 2700 loci that are well distributed among all the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using a mouse cDNA probe. A 3.6-kb *Pst*I *M. spretus* RFLP was used to follow the segregation of the *LATS2* locus in backcross mice. The mapping results indicated that *LATS2* is located in the central region of mouse chromosome 14 linked to *Gzmb*, *Gjb2*, and *Nfl*. Although 176 mice were analyzed for every marker in the segregation analysis (Fig. 4), up to 189 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies. The ratios of

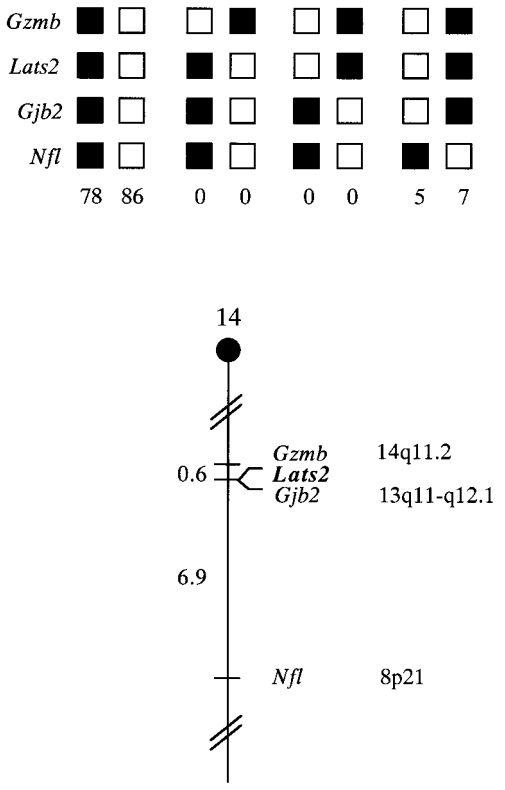

FIG. 4. *LATS2* maps to the central region of mouse chromosome 14. *LATS2* was located on mouse chromosome 14 by interspecific backcross analysis. The segregation patterns of *LATS2* and flanking genes in 176 backcross animals that were typed for all loci are shown at the top of the figure. For individual pairs of loci, more than 176 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The black boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of an *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 14 linkage map showing the location of *LATS2* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci are shown in centimorgans at the left of the chromosome, and the positions of loci in human chromosomes, where known, are shown at the right. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are centromere–*Gzmb*–1/180–*LATS2*–0/185–*Gjb2*–13/189–*Nfl*. The recombination frequencies (expressed as genetic distances in centimorgans ± standard error) are *Gzmb*–0.6 ± 0.6–(*Lats2*, *Gjb2*)–6.9 ± 1.8. No recombinants were detected between *LATS2* and *Gjb2* in 185 animals typed in common, suggesting that the two loci are within 1.6 cM of each other (upper 95% confidence limit). The proximal region of mouse chromosome 14 shares regions of homology with human chromosomes 14q, 13q, and 8p (summarized in Fig. 4). In particular, *Gjb2* has been mapped to 13q11–q12.1.

Chromosomal Location of the Human LATS2 Gene

The close linkage between *Gjb2* and *LATS2* in mouse suggested that the human homologue of *LATS2* might also map to 13q. We performed FISH analysis using the cDNA insert of our human *LATS2* clone as a probe. As shown in Fig. 5, specific FISH signals generated from biotinylated human *LATS2* cDNA were observed at the centromeric region of the long arm of human chromosome 13. No other reproducible signal was observed on metaphase chromosomes. Thus, FISH analysis indicated that the human *LATS2* gene maps to the 13q11–q12 region.

DISCUSSION

In this study, we report the cloning and characterization of mouse and human cDNAs encoding Lats2. We showed that Lats2 is a bona fide mammalian homologue of the *Drosophila* tumor suppressor Lats/Warts by amino acid sequence comparison (Fig. 1). *C. elegans* also has a gene encoding a Lats-like protein. Together, these proteins define a new subfamily of protein kinases that are closely related to but distinct from the Ndr protein kinases and the cyclic AMP-dependent kinases. The C-terminal regions of mammalian Lats1 and Lats2 contain the putative Ser/Thr protein kinase domain and closely resemble each other, whereas sequence similarity in the N-terminal region of the molecule is very low. The absence in mammalian Lats2 of the putative SH3-binding motifs found in mammalian Lats1 and *Drosophila* Lats suggests that the role of Lats2 in the potential signal transduction pathway may be distinct from those of Lats1 and *Drosophila* Lats. Homology searches for Lats-like proteins in lower eukaryotes identified fission yeast Orb6 and budding yeast Dbf2, Dbf20, and Cbk1/Knq1 (P53894). However, sequence analysis suggests that Dbf2 and Dbf20 are distant relatives and that Cbk1/Knq1 and Orb6 are more closely related to Ndr protein kinases (Millward *et al.*, 1995). It is noteworthy that disruption of the *CBK1* gene showed no alteration of phenotype (http://genome-www.stanford.edu/Saccharomyces/), while Orb6 is required to maintain cell polarity throughout the interphase period of the cell cycle and to promote actin reorganization during morphological transitions (Verde *et al.*, 1998).

The *Drosophila lats/warts* gene has been identified as a tumor suppressor (Xu *et al.*, 1995; Justice *et al.*, 1995). Mutations in *lats* cause overgrowth phenotypes and a variety of developmental defects, and mosaic flies harboring *lats* mutant cells develop large tumors in various organs. *Lats1*$^{-/-}$ mice develop large soft-tissue sarcomas and ovarian stromal cell tumors with metastasis to the lungs and are susceptible to carcinogenic treatments, which cause them to develop soft-tissue sarcomas. The susceptibility to spontaneous and induced tumors of *Lats1*$^{-/-}$ mice strongly indicates that Lats1 acts as a tumor suppressor. The phenotypic dif-

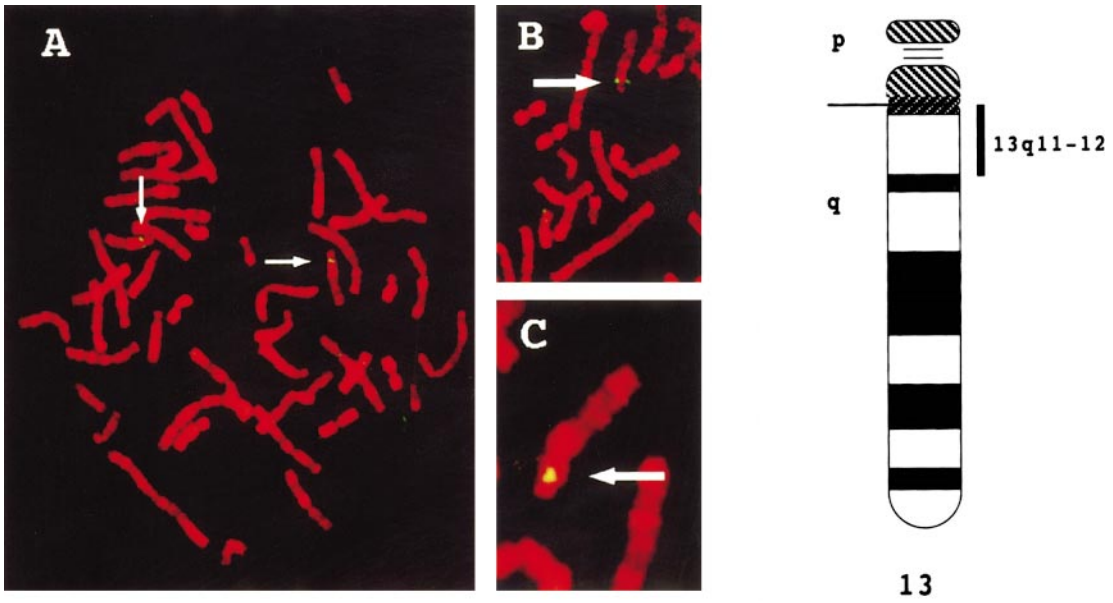

FIG. 5. Chromosomal localization of *HsLATS2* by FISH. FISH analysis of R-banded chromosomes was performed using biotinylated *HsLATS2* cDNA as a probe. Photographs of the entire metaphase (**A**) and partial metaphase (**B** and **C**) are shown. Separate images of FITC hybridization signals and propidium iodide-stained chromosomes were merged using image analysis software, and photographs were taken as digital images using a camera attached to a laser scanning microscope. Arrows indicate specific FITC signals on the long arm of chromosome 13. A schematic representation of human chromosome 13 and the location of the *LATS2* gene are shown on the right.

ference between fruit-fly *lats* and mouse *Lats1* mutants suggests the possibility of redundancy among *lats*-like genes in mammals. Our identification of *LATS2* as a new member of the *LATS* family may help to explain the phenotypic difference between fruit-fly *lats* and mouse *lats1*$^{-/-}$ mutants, in that Lats2 may partially complement the function of Lats1.

The similarity in structure and mRNA expression patterns between Lats1 and Lats2 suggests that the latter may also function as a tumor suppressor. We mapped in this report the human *LATS2* gene to chromosome 13q11–q12, in the vicinity of the well-documented tumor suppressor genes *RB* (at 13q14) and *BRCA2* (at 13q12–q13). Loss of heterozygosity (LOH) in this chromosome region has been frequently observed in primary cancers of the breast (Lee *et al.*, 1988), liver (Wang and Roger, 1988), lung (Weston *et al.*, 1989), ovary (Sato *et al.*, 1991), and bladder (Cairns *et al.*, 1991). The *RB* gene, mutations in which are responsible for retinoblastoma, is considered to be the most likely candidate gene involved in these tumors. *BRCA2*, the second gene responsible for familial breast cancer, is also a candidate gene in tumors showing LOH in 13q. However, *RB* and *BRCA2* have been identified as the targets of allelic deletions on chromosome 13q in only a few cases to date. We propose that the *LATS2* gene is another candidate tumor suppressor gene that may be responsible for tumors showing LOH at 13q.

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