

Tensin1 and a previously undocumented family member, tensin2, positively regulate cell migration

Huaiyang Chen, Ian C. Duncan, Hormozd Bozorgchami, and Su Hao Lo*

Center for Tissue Regeneration and Repair, Department of Orthopaedic Surgery, University of California at Davis, Sacramento, CA 95817

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Tensin is a focal adhesion molecule that binds to actin filaments and participates in signaling pathways. In this study, we have characterized a previously undocumented tensin family member, tensin2/KIAA 1075. Human tensin2 cDNA encodes a 1,285-aa sequence that shares extensive homology with tensin1 at its amino- and carboxyl-terminal ends, which include the actin-binding domain, the Src homology 2 (SH2) domain, and the phosphotyrosine binding (PTB) domain. Analysis of the genomic structures of tensin1 and tensin2 further confirmed that they represent a single gene family. Examination of tensin2 mRNA distribution revealed that heart, kidney, skeletal muscle, and liver were tissues of high expression. The endogenous and recombinant tensin2 were expressed as a 170-kDa protein in NIH 3T3 cells. The subcellular localization of tensin2 was determined by transfection of green fluorescence protein (GFP)–tensin2 fusion construct. The results indicated that tensin2 is also localized to focal adhesions. Finally, functional analysis of tensin genes has demonstrated that expression of tensin genes is able to promote cell migration on fibronectin, indicating that the tensin family plays a role in regulating cell motility.

focal adhesion | KIAA 1075 | cell motility

Tensin1 is a 220-kDa protein localized to focal adhesions (1–3), the transmembrane junctions between the extracellular matrix, and the cytoskeleton. On integrin ligation, tensin1 is rapidly translocated to the assembling focal adhesion sites (4). Northern blot analysis revealed two transcripts in all mouse tissues examined, whereas most human tissues express one transcript, except in the heart and skeletal muscle where two transcripts are found (3, 5). The amino terminal of tensin1 is able to interact with actin filaments (6). Within this region, it also shares sequence homology with auxilin (a coat protein of brain clathrin coated vesicles; ref. 7), GAK (a cyclin G associated kinase; ref. 8), and PTEN/MMAC 1 (phosphatase and tensin1 homologue on chromosome 10/mutated in multiple advanced cancers 1: a tumor suppressor at human chromosome 10q23; refs. 9 and 10). The significance of this sequence similarity is not clear, because none of these molecules binds to F-actin and only GAK has been found at focal adhesions (11). The center part of tensin1 is able to retard the G-actin polymerization rate by interacting with the barbed end of actin (6). The carboxyl terminal of tensin1 contains two phosphotyrosine-binding motifs, the Src homology domain 2 (SH2; ref. 12), and the phosphotyrosine-binding domain (PTB). In addition to interacting with phosphotyrosine, tensin1 is also a phosphoprotein. Several factors including the extracellular matrix (13), platelet-derived growth factor (PDGF) (14), thrombin, angiotensin (15), and oncogenes such as v-src or bcr/abl (12, 16) are known to induce the tyrosine phosphorylation of tensin1. In addition, tensin1 is a substrate of calpain II (3), a focal adhesion protease involved in dis/assembly of focal adhesions. These findings suggest that tensin1 acts as a bridge linking the extracellular matrix (ECM), the actin cytoskeleton, and signal transduction (17).

In addition to focal adhesions, tensin1 is also concentrated at fibrillar adhesions (or ECM contacts; ref. 18). This structure contains integrin $\alpha 5 \beta 1$ and binds fibrils of fibronectin parallel to actin bundles and tensin1, but does not contain other focal adhesion molecules, such as vinculin and paxillin in human primary fibroblast

system (18). Studies using the same system have demonstrated that tensin1 is critical for integrin translocation away from focal adhesions to fibrillar adhesions and for fibronectin fibrillogenesis, which converts soluble fibronectin molecules into elaborate fibrillar matrices (19).

The role of tensin1 in mice has been explored through gene targeting (5). Even though tensin1 is broadly expressed in mouse embryos and various adult tissues, mice that lacked tensin1 developed normally and appeared healthy for several months. Whereas most tissues seemed to be normal in older mice, multiple cysts were detected in the kidney. The progressive cyst formation led to kidney degeneration, and the mice subsequently died from renal failure. These results demonstrated that tensin1 is not necessary for mouse embryogenesis, but is required for the maintenance of normal renal function (5). Furthermore, we have found the skeletal muscle regeneration process is delayed in these mutant mice, indicating a role of tensin1 in wound healing (20). Given the widespread importance of focal adhesions in development and differentiation, and the fact that genetic ablation of many focal adhesion molecules, including integrin $\alpha 5 \beta 1$, focal adhesion kinase, and vinculin (21–24), leads to early embryonic lethality, the relatively mild phenotypes of tensin1 null mice are somewhat surprising. It is possible that a tensin1 related protein(s) might compensate for the loss of tensin1 in tissues other than the kidney. Alternatively, they may have a redundant role in most organs.

Here, we report the identification of a previously undocumented member of the tensin family, tensin2, its gene structure, expression pattern, molecular mass, and subcellular distribution. In addition, functional analysis has demonstrated that tensin genes positively regulate cell motility.

Materials and Methods

Computer Analysis. Human tensin1 cDNA sequence (3) was used as a probe to search for similar sequences in the available databases by using the advanced BLAST search at the National Center for Biotechnology Information.

Northern Blot Analysis. Human multiple tissue Northern blot (CLONTECH) was hybridized under high stringency conditions (5) with a ^{32}P -labeled cDNA probe of tensin2 coding sequence.

Recombinant Green Fluorescence Protein (GFP) Fused Tensin2. The full-length coding region of human tensin2 cDNA (KIAA 1075, kindly provided by O. Ohara at the Kazusa DNA Research Institute, Chiba, Japan) was subcloned into pEGFP (CLONTECH), or pcDNA-His (Invitrogen) vectors for the expression of GFP–tensin2 fusion protein, or 6 \times His-tagged tensin2, respectively. These constructs were transfected into indicated cell lines by using SuperFect reagents (Qiagen, Chatsworth, CA) ac-

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Abbreviations: SH2, Src homology 2; PTB domain, phosphotyrosine binding domain; GFP, green fluorescence protein; ECM, extracellular matrix.

*To whom reprint requests should be addressed at: University of California at Davis, 4635 Second Avenue, Room 2000, Sacramento, CA 95817. E-mail: shlo@ucdavis.edu.

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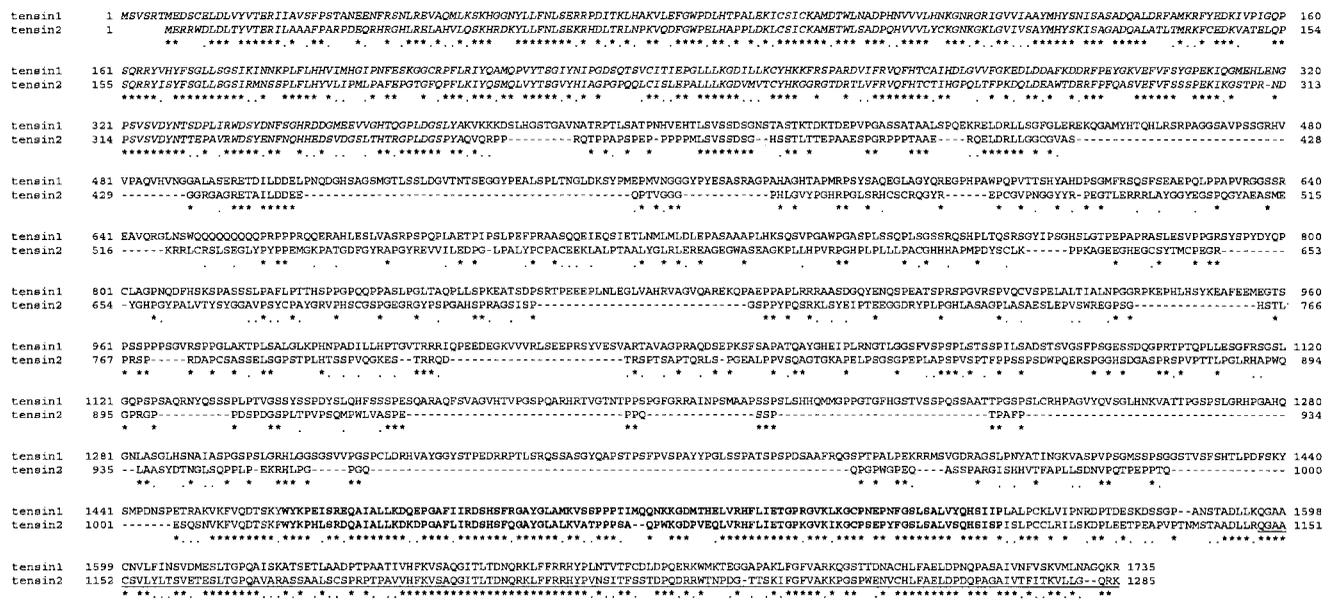


Fig. 1. Alignment of human tensin1 and tensin2 aa sequences. Asterisks indicate identical residues, and similar residues are labeled with a dot. The actin-binding regions are in italics. The SH2 domains are in bold. The PTB domains are underlined.

coding to the manufacturer's instructions. For stable transfectants, cells were selected against 800 $\mu\text{g/ml}$ G418 in the medium for 10 days, and the surviving colonies were picked up by a cloning filter pretreated with trypsin and transferred to 24-well plates. The positive clones were verified by GFP labeling and Western blot analysis.

Immunoblot. Cell cultures were treated with lysis buffer (50 mM Tris, pH 7.5/150 mM NaCl/1 mM EDTA/1% Nonidet P-40), including protease inhibitors (1 mM PMSF/10 $\mu\text{g/ml}$ leupeptin/10 $\mu\text{g/ml}$ pepstatin). Samples were centrifuged at 13,000 rpm in a Microfuge (Beckman) at 4°C for 15 min. Protein concentration was measured by using the Bio-Rad protein assay reagent. Thirty micrograms of protein was subjected to SDS/PAGE on a 10% gel. Proteins were then transferred onto nitrocellulose membranes, which were stained with Ponceau S to verify equal loading and transfer. Membranes were incubated with blocking solution containing 5% skim milk in TBST (50 mM Tris, pH 7.5/150 mM NaCl/0.1% Tween 20) for 1 h at room temperature. Membranes were incubated with the primary antibody in blocking solution at room temperature for 1 h. After washing three times with TBST for 10 min each, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-mouse or -rabbit IgG for 1 h at room temperature. After washing three times, the bound antibody was visualized by using the ECL Western blotting reagent (Amersham Pharmacia).

Generation of Antibodies Against Tensin2. Anti-peptide antiserum was raised against a tensin2 peptide (residues 24–34): cgggR²⁴PDEQRHRGHL³⁴. Three glycine residues were assigned to the N-terminal end of the peptide to allow more prominent presentation of this short peptide. In addition, a cysteine was assigned so the peptide could be coupled to a carrier, keyhole limpet hemocyanin (KLH). The serum was purified by using peptide affinity matrix. A second antiserum was raised against glutathione S-transferase (GST)-tensin2 (residues 276–635) fusion proteins. The serum was also purified by using the recombinant protein (after removing the GST portion by factor Xa) affinity matrix.

Fluorescence Microscopy. NIH 3T3 cells grown on glass coverslips were transfected with human tensin2 in pEGFP vector by using

SuperFect transfection reagent (Qiagen). After 24 h, cells were fixed with 3.7% paraformaldehyde for 15 min at room temperature and were permeabilized with 0.5% Triton X-100 for 15 min. After rinsing with PBS, cells were incubated with primary antibodies for 1 h. Samples were then incubated with the Texas Red-conjugated secondary antibody for 1 h and visualized with a Zeiss LSM 510 confocal microscope.

Cell Migration Assay. Cell migration assays were performed by using modified Boyden chambers. The lower surface of the membrane was coated with 10 $\mu\text{g/ml}$ fibronectin for 2 h at 37°C. The lower chamber was filled with 0.6 ml of DMEM with 10% FBS. Cells were harvested with trypsin/EDTA and washed once with serum-free DMEM containing 20 $\mu\text{g/ml}$ trypsin inhibitor and resuspended to 1×10^6 cells per milliliter. One hundred microliters was added to the upper chamber, and the cells were allowed to migrate at 37°C, 5% CO₂, for 15 h (for 293 cells) or 5 h (for fibroblasts). The upper surface of the membrane was wiped with a cotton tip to mechanically remove nonmigratory cells. The migrant cells attached to the lower surface were fixed in 10% formalin at room temperature for 30 min, and then stained for 20 min with a solution containing 1% crystal violet and 2% ethanol in 100 mM borate buffer (pH 9.0). The stained cells per field were photographed with a charge-coupled device (CCD) camera attached to the inverted phase-contrast microscope. Four fields per chamber were photographed. The acquired images were then processed in PHOTOSHOP (Adobe Systems, Mountain View, CA), and cell numbers were counted. The migration rates were indicated by the total number of cells per field. Each cell line was analyzed in duplicate wells in three separate experiments.

Results

Identification of cDNA Sequences Related to Tensin1. To examine whether there are other tensin family members, we routinely search the available database by using the human tensin1 cDNA sequence. Recently, we found the KIAA 1075 cDNA sequence (25), which contains an open-reading-frame encoding 1285-aa residues and which shares extensive sequence homologies with tensin1. The most conserved regions between these two proteins are at the N- and C-terminal ends ($\approx 60\%$ and 67% identities; Fig. 1). The center region shares very limited similarity to tensin1 and is very rich in

Table 1. Organization of human tensin genes

Exon no.	Codon phase	3' Splice acceptor	Exon size, bp	5' Splice donor	Intron size, bp
Human tensin1					
E1			≈174	TGCAGGAAAGgtgagtgaat	23,628
E2	N	ccttccacagGTGGCTGCC	42	TGAGCTGGTggtgagtgaca	12,994
E3	N	ctgtccacagCCCATCACCA	42	AGTGGACAAGgtgagtgatg	824
E4	N	ctgtctgcagGGAGAAGGAG	51	CGAGGACAAGgtgagtgtgg	5,822
E5	N	tccttccacagGGCTCCACCA	50	AGCCCATCAGgtatgcctgg	2,493
E6	N	gtctccctagAAACATGAGT	172	CAACTACCTGgtgaggatgg	2,741
E7	O	ctccttctagCTGTTCAACC	51	CCATGCCAAGgtgagccagg	1,444
E8	O	tgggcccacagGTAAGTGAAT	123	ACACAACAAGgtcagagctg	426
E9	O	gctcttccagGGAACCGGAG	65	TTTCTGCCAGgttaagagggg	1,902
E10	II	ctccctgcagTGC GGACCAG	84	CCCAAGAAGgtgggtctct	4,315
E11	II	accactacagGTACGTGCAT	113	TCTAAGGAGgtacctgcct	429
E12	I	acttttacagGATGTCGGCC	61	CTGGCATCTAgttaagtatta	224
E13	II	tctcccttagCAACATCCCA	76	AGACATCTTGTgtgagtgtg	579
E14	O	tcccacccagCTGAAGTGCT	130	GCTTTCAAAgtgctgctcct	2,623
E15	I	ctttttccagATGATCGATT	66	AAAATTCAAgtataagccg	1,331
E16	I	tcccacttagGCATGGAGCA	117	GGCATGGAGgttaggtggga	31,810
E17	I	tcccttgcagAGGTGGTGGG	1,578	AGGGTAGCAGgttaagaggtt	11,298
E18	I	ttccctccagGGGTACAGGC	197	GCCCAAGAGgtgagtctct	848
E19	O	tctccacacagCCCCATTGC	76	CCCATGGGGgttaagaattc	3,565
E20	I	cctggcacagTGC GGTCGCC	93	CCCACAGGAGgtgagttggg	1,064
E21	I	ttttccttagTTACCAGAAG	24	ATCCAGCCAGgttaggtgggc	484
E22	I	cttccctcagAGGAAGATGA	39	CTGTGAGAAGgttaagttaga	7,905
E23	I	ccccttccagAGCCCGGAG	199	GTGCTGACAGgttaagccggg	2,980
E24	II	ctctctgcagCACTTCAGTG	1,070	TCCATGCCAGgtgaggtctct	2,691
E25	I	cctgctttagACAACAGCCC	86	AGGGAGCAGgtgagtgtctg	1,095
E26	O	ccccatgcagCCATCGCGCT	133	AATAAAAAAGgttaacctcat	377
E27	I	tttgtcccagGAGACATGAC	93	CCAACTTCGgtgagctgtc	769
E28	I	ctccctacagGATCGCTGTC	78	CCAAACCGAGgttagaacctc	2,048
E29	I	tttgtccttagACCCACAGA	72	CAAGGGGCAGgtgagtggtc	256
E30	I	tttcttccagCCTGCAATGT	169	ACCAGAGAAgttaagactcc	1,151
E31	II	tgtgctgcagGCTCTTTTTC	69	AGGAAAGAAgttaagctctc	495
E32	II	ctctccacagGTGGATGAAA	33	CCCCTGCTAgttaagtgtag	3,471
E33	II	ccgcctgcagGCTCTTCGGC	≈701		
Human tensin2					
E1		ccaccttcagCCTAGGAAAG	109	TCGTGCAGAGgtgaggctct	491
E2	N	ccttccctagTCTGCAAGGT	38	TGAAGCAAAGgtgggtatct	918
E3	N	ccccttccagGTGACTTCAG	39	CGTGGAGTTGgtgagtgcgc	324
E4	N	ctccctctagCGGCGAAACA	39	AGAGCACCTGgttaaggtgat	152
E5	N	taaccaccagGGATCCAAAT	50	CTCTGCCCAGgttaagttagg	255
E6	N	tcctcccacagGAGCTTCAGC	172	CAAGTACCTGgtgagggggcg	744
E7	O	tttatctcagCTCTCAACC	51	AAACCCCAAGgtatgaagga	331
E8	O	ttctccccagGTTCAAGACT	123	ATACTGCAAGgtgggccagg	90
E9	O	tgtctgtcagGGAACAAGC	65	TCTCTGCAGgtgaggtctcc	1,167
E10	II	ctatcccacagGGCGGACCAG	84	CCCAGCGTCGgtgagcagcc	470
E11	II	cctccaccagATATATCAGC	113	CCTGGCACAGgtgagtctgc	93
E12	I	gcccctccagGCTTCCAGCC	61	CTGGAGTCTAgtgagtgtctc	193
E13	II	ctccccacagTCACATTGCA	76	CGATGTCATGgtgagggggg	213
E14	O	tttctcccagGTAACATGTT	130	GCTTGACTGgtgagtctga	125
E15	I	ctccccatagATGAGAGGTT	66	AAGATCAAAGgttaagagcag	76
E16	I	ccttccacagGCAGCACTCC	114	AGTGTGGATGgtgagcagggc	219
E17	I	ccccactcagGCTCCTTGAC	1,212	GCATCTGCAGgtgaggggca	146
E18	I	ttcactgcagAGTCGCTGGG	151	GCAAGGAAAGgtatgcagag	119
E19	II	caccctacagCACCCGGCAG	590	CAAACCCCAAGgtataaaggc	115
E20	I	cctgtcctagAGCCTCCTAC	90	CGTGACCAAGgtgagaagcc	345
E21	I	caccctgcagCCATTGCCCT	125	CCCTGGAAGgtacagaaca	213
E22	I	ctcctctcagGGGACCCCGT	93	CCCTACTTTGgtgagaagca	130
E23	I	tctcccacagGCAGCTGTC	78	CCCAGCAAAGgtgagtgtct	166
E24	I	ttgccctcagATCCTCTGGA	78	CAGGGTGCTGgttagagactt	391
E25	I	gcccctgtagCCTGCAGCGT	169	ACCAAGGAAgtatgtatac	91
E26	II	ctccttgcagGCTCTTTTTC	69	AAGACCGGAGgtgactctcc	145
E27	II	ccttctgcagATGGACCAAC	30	CCACCTCAAgttaagctccc	151
E28	II	atccccttagGATCTTTGGT	628		

Exon/intron boundaries were determined by comparison of sequences of genomic DNA and cDNA. In the splice site, exon sequences are indicated by uppercase letters, and intron sequences are indicated by lowercase letters. Codon phase refers to the codon split at the splice acceptor. Introns that do not split codon triplets are indicated by phase O, interruption after the first nucleotide is indicated by I, and interruption after the second nucleotide is indicated by II. N indicates noncoding region.

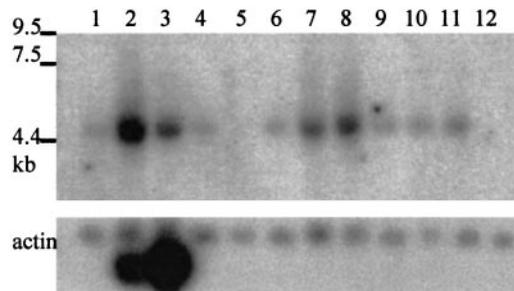


Fig. 2. Tissue distribution of tensin2 mRNA. Northern blot analysis of tensin2 (Upper) in human tissues: brain (lane 1), heart (lane 2), skeletal muscle (lane 3), colon (lane 4), thymus (lane 5), spleen (lane 6), kidney (lane 7), liver (lane 8), small intestine (lane 9), placenta (lane 10), lung (lane 11), and peripheral blood leukocyte (lane 12). The blot (from CLONTECH) had 2 μ g of mRNA loaded per lane. The same blot was striped and probed with β -actin cDNA probe (Lower). The smaller sized band appearing in lanes 2 and 3 corresponds to the expected size of α -actin mRNA.

proline residues. The N-terminal region of tensin1 contains the actin-binding domain and the PTEN (phosphatase and tensin1 homologue on chromosome 10) homology region, whereas the C-terminal region includes the SH2 domain and the PTB domain. Therefore, this new gene product is likely to have actin-binding and phosphotyrosine-interacting activities similar to tensin1. In addition, it is, like tensin1, localized to focal adhesions (see below). Because of these similarities, we named this molecule tensin2.

Genomic Structures of Tensin Genes. Human tensin1 and tensin2 cDNA sequences were used to search against available human genomic sequences, leading us to discover that tensin1 and tensin2 genes are localized on chromosome 2 and 12, respectively. Interestingly, tensin1 gene is about 150 kb, whereas tensin2 gene is only 12.5 kb. The intron/exon borders of human tensin genes were defined by comparing the cDNA and human genomic sequences, revealing 33 exons within the tensin1 gene and 28 exons within the tensin2 gene (Table 1). All splice acceptor/donor sites for these exons conform to the GT-AG rule. The putative start codons are in exon 6 of tensin1 and tensin2, whereas stop codons are in exon 33 of tensin1 and exon 28 of tensin2. Analysis of the intron/exon boundaries revealed an interesting evolutionary relationship between tensin1 and tensin2 genes. Eleven exons starting from exon 5 in tensin2 are identical in size to the same exons in tensin1. These exons encode the actin-binding domain. In addition, the last eight exons, which encode the SH2 and PTB domains, are almost identical (Table 1). These conserved genomic structures of human tensin1 and tensin2 confirm that they represent a single gene family.

Expression of Tensin2 in Human Tissues. The expression of tensin2 in human tissues was examined by Northern blot analysis. It revealed a 5-kb message in most of the tissues examined, with higher levels in the human heart, skeletal muscle, kidney, and liver (Fig. 2). Expression in the brain, colon, thymus, and peripheral blood leukocyte was extremely low. This expression pattern is very similar to that of tensin1 (3). The observed parallel in tissue expression of tensin1 and tensin2 suggests a potential functional overlap. However, targeted disruption of tensin1 results in kidney defects regardless of the abundant expression of tensin2, strongly suggesting that the two genes play some nonredundant roles in kidney.

Molecular Mass and Subcellular Localization of Tensin2. To examine the molecular mass of human tensin2, we have generated two constructs containing the tensin2 cDNA for expression in mammalian cells. The first construct contains the full-length tensin2 cDNA with a 6 \times His tag at the N terminus, whereas the second construct, tensin2 cDNA, was fused to the C terminus of the 28-kDa GFP. These constructs were transfected into NIH 3T3 cells. The

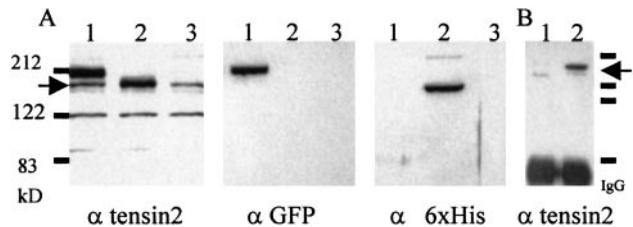


Fig. 3. The apparent molecular masses of recombinant and endogenous tensin2 in NIH 3T3 cells. (A) Cell lysates prepared from NIH 3T3 cells transfected with GFP-tensin2 (lane 1), 6 \times His-tagged tensin2 (lane 2), or nontransfected (lane 3) were analyzed by anti-tensin2 (K83), anti-GFP, and anti-6 \times His antibodies. (B) NIH 3T3 cell lysates were incubated with preimmune (lane 1) or anti-tensin2 (K12W) antibody (lane 2). The associated proteins were precipitated with protein G-Sepharose beads and immunoblotted by anti-tensin2 (K83) antibody. Arrow indicates the 170-kDa endogenous tensin2.

expression of recombinant and endogenous tensin2 in the cells was analyzed by immunoblotting. Two bands (170 and 125 kDa) were detected in cell lysate prepared from nontransfected NIH 3T3 cells by anti-tensin2 antibodies (Fig. 3). In addition to the endogenous protein, the 170-kDa band was expressed at significantly higher levels in cells transfected with 6 \times His-tagged tensin2, whereas an additional band of around 200 kDa was detected in cells transfected with GFP-tensin2. Detection with anti-6 \times His or anti-GFP antibodies confirmed the identities of the recombinant proteins (Fig. 3). Although the predicted molecular mass of tensin2 was 139 kDa, both recombinant and endogenous tensin2 migrated as a 170-kDa protein (GFP-tensin2 is about 200 kDa). We did not know the identity of the 125-kDa protein. It is unlikely to be an alternative spliced form, because we detected only one transcript by Northern blot analysis. It is possible that the anti-peptide antibody (K83) recognized a nonrelated protein. To test this possibility, we have raised another antibody against glutathione *S*-transferase (GST)-tensin2(276–635) fusion protein. Tensin2 was immunoprecipitated by this new antibody (K12W) from NIH 3T3 cell lysate and immunoblotted by using the anti-peptide antibody (K83) (Fig. 3B). We detected only the 170-kDa band in this experiment, indicating that the 170-kDa form is the authentic tensin2.

To determine protein subcellular localization of tensin2, we first tried both anti-tensin2 antibodies that had detected tensin2 on the immunoblot and immunoprecipitation assays for immunofluorescence staining studies. However, the antibodies did not detect any signal in NIH 3T3 cells (not shown). We then transiently transfected the GFP-tensin2 construct into NIH 3T3 cells and examined the cells by fluorescence microscopy. To compare the localization between GFP-tensin2 and other molecules, the samples were also labeled for tensin1, vinculin, and actin stress fibers. As shown in Fig. 4, GFP-tensin2 was localized at the end of the actin stress fibers and colocalized with vinculin and tensin1 at focal adhesions, whereas GFP alone was found in cytoplasm. However, GFP-tensin2 did not appear to target to ECM contacts as efficiently as tensin1 did.

Tensin Genes Regulate Cell Migration. Because focal adhesions are sites involved in regulating cell migration and tensin genes are primarily localized to these sites, we hypothesize that tensin genes are able to regulate cell migration. To test this hypothesis, we have established stable 293 cells expressing GFP-tensin1 or GFP-tensin2, and then tested their motilities on fibronectin by using the Boyden chamber migration assay (Fig. 5). The expression of GFP or GFP fusion proteins was confirmed by immunoblotting by using anti-GFP antibodies (Fig. 5). The migration results showed that cells expressing GFP-tensin1 or GFP-tensin2 migrated significantly faster than GFP control cells, suggesting that ectopic expression of tensin genes is able to promote cell migration. Because we have previously generated tensin1 null mice, we wanted to examine whether ablation of tensin1 in cells has the opposite effect. Fibro-

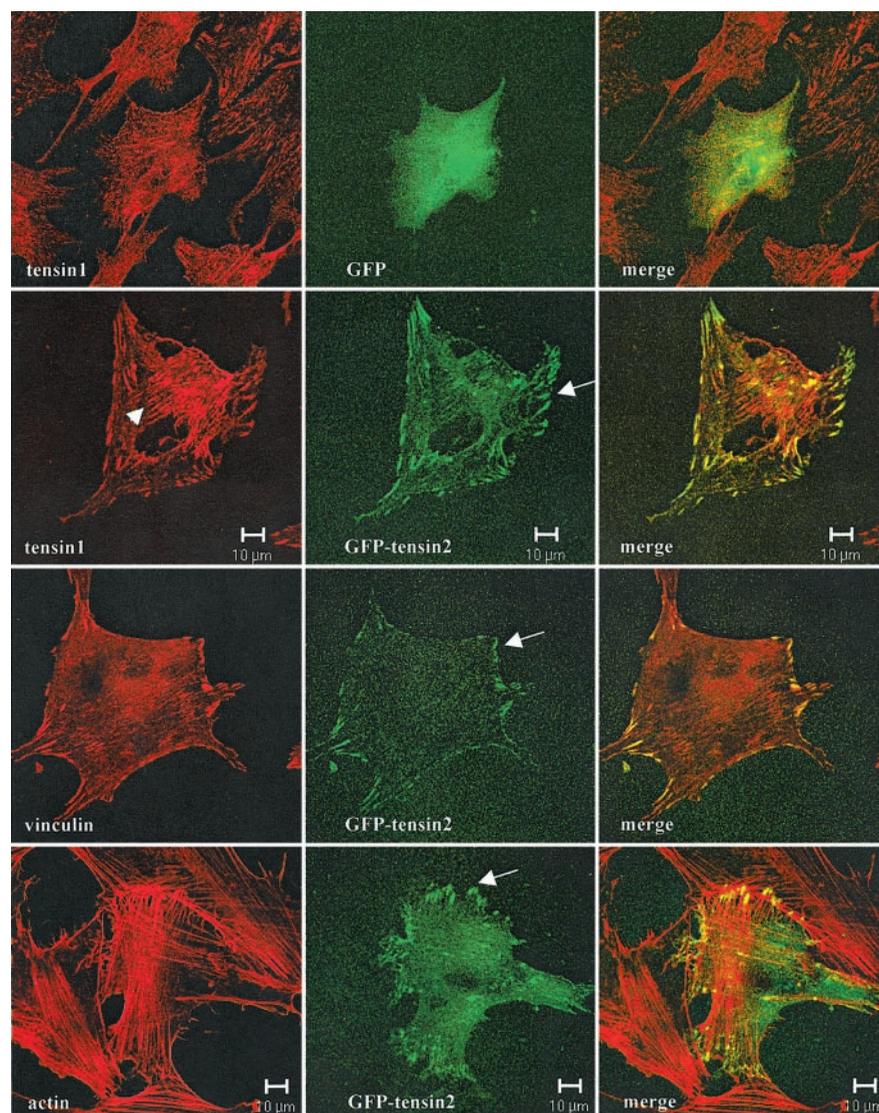


Fig. 4. Localization of GFP-tensin2 fusion proteins in NIH 3T3 cells. Cells grown on coverslips were transfected with GFP or GFP-tensin2 constructs. After 24 h, cells were fixed and labeled for tensin1, vinculin, and actin stress fibers. Cells were then visualized with a Zeiss LSM 510 laser scanning microscope. Arrows indicate focal adhesions. Arrowhead shows the ECM contacts.

blasts were isolated from 14-day tensin1 null as well as normal mouse embryos and were confirmed by the genotyping assay (not shown). The migration studies showed that cells without tensin1 migrated significantly slower than their normal counterpart cells. Taken together, these results indicate that tensin genes regulate cell migration. We further analyzed tensins' expressions in these cells. The immunoblot analysis of a series of dilution of cell lysates (Fig. 5C) showed that (i) the expressions of tensin2 in tensin1 +/− and −/− cells are the same, (ii) tensin2 is not detectable by anti-tensin2 antibody (83) in 10 μg of tensin1 +/− and −/− cells, and (iii) tensin1 is readily detected by anti-tensin1 antibody (UC70) in 10 μg tensin1 +/− cells. These results suggested that tensin2 did not up-regulate its expression to compensate tensin1's function in the mutant cells and tensin2's expression level seemed to be significantly lower than tensin1. These results might explain why tensin1 and tensin2 promote cell migration but tensin1 null cells still show slower migration.

Discussion

Although we have predicted that there may be additional tensin1-like molecules in the human genome, we recently were

able to provide solid evidence supporting this notion. The first clue suggesting the presence of a related molecule came during the course of cloning the human tensin1 cDNA, when similar DNA sequences were isolated along with the authentic sequence (3). In addition, many “human tensin1” EST clones in the databases show sequence diversities to our human tensin1 sequence (3). Finally, we have found that the KIAA 1075 cDNA sequence matches our tensin1-like sequence, which we have called tensin2. The amino acid sequence of tensin2 contains the actin-binding domain of tensin1, the PTEN (phosphatase and tensin1 homologue on chromosome 10) homology region, and the SH2 and PTB domains. These similarities are also reflected at the genomic levels. The sizes and arrangement of exons coding for these domains of tensin1 and tensin2 are almost identical, suggesting that these domain functions have been conserved during their evolution.

Despite the high sequence similarities at both ends of tensin1 and tensin2 polypeptides, the center regions show very limited homology, if any. The center region of tensin2 is very rich in proline residues, which provides potential binding sites for interacting

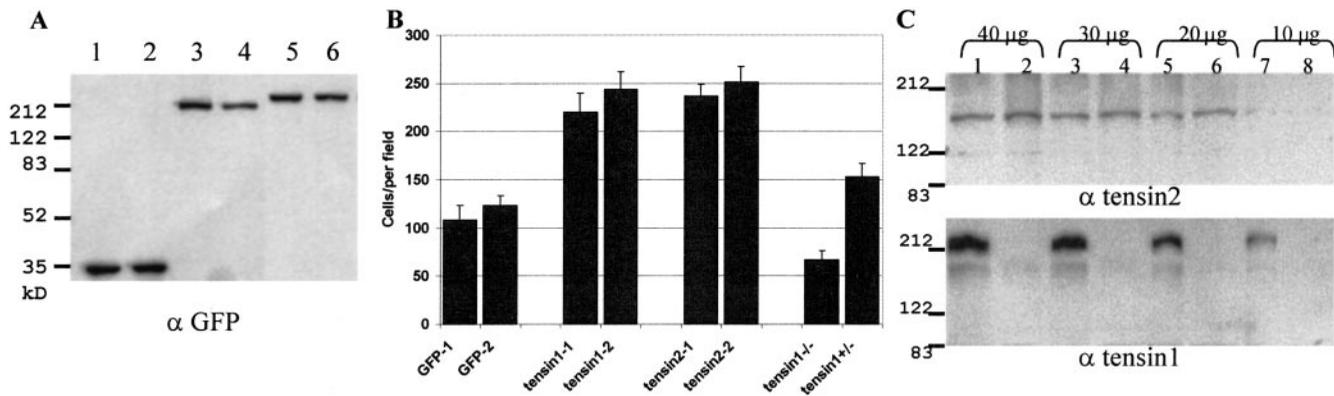


Fig. 5. Expression of tensin promotes cell migration. (A) HEK 293 cell lysates were prepared from two clones expressing GFP, GFP fused to wild-type tensin2, and GFP fused to wild-type tensin1. Total cell lysate proteins (30 μ g) were immunoblotted with anti-GFP antibodies to show the expression levels of GFP (lanes 1 and 2), GFP-tensin2 (lanes 3 and 4), and GFP-tensin1 (lanes 5 and 6). (B) Migration of these clones was analyzed by using the Boyden chamber. GFP-tensin1- and GFP-tensin2-expressing 293 cells migrated faster than the GFP-expressing cells. Tensin1^{-/-} fibroblasts migrated slower than tensin1^{+/-} cells. Two independently isolated tensin1^{-/-} and +/⁻ cells were used for the assays. Error bars are SD. (C) Expressions of tensin1 and tensin2 in tensin1^{+/-} (lanes 1, 3, 5, and 7) and -/- (lanes 2, 4, 6, and 8) cells were analyzed by immunoblotting with anti-tensin2 (83) (Upper) and anti-tensin1 (UC70) (Lower) antibodies. Amounts equal to 40 (lanes 1 and 2), 30 (lanes 3 and 4), 20 (lanes 5 and 6), and 10 (lanes 7 and 8) μ g of cell lysate proteins were loaded. Note that tensin2 was not detected in 10 μ g of cell lysates, and the 125-kDa band was not detected in these cell samples.

modules such as SH3 and WW domains (26). In addition, this portion of tensin2 does not contain the insertin (or actin-capping) domain present in tensin1 (6), suggesting that tensin2 probably is not able to regulate actin polymerization. These findings imply that the center portion of each protein was modified for a divergent function.

Tensin1-null mice develop cystic kidneys and have impaired healing processes of damaged skeletal muscle (5, 20). Because the kidneys were well developed at birth and degenerated with age, we think that the primary defect may lie in the regeneration process. Consistent with this observation is the fact that regeneration of damaged skeletal muscle in tensin1 null mice is delayed. Because localization of regenerating cells to the wounded site is a critical step in the healing process, we speculate that tensin1 is involved in regulating cell migration. In this study, we have demonstrated that both tensin1 and tensin2 are able to promote cell migration in HEK 293 cells stably expressing the protein, and that cells lacking tensin1 migrate slower than their normal counterpart cells, demonstrating that tensin genes are critical for normal cell migratory processes. Cell migration is a complex process that requires extensive rearrangements of cytoskeleton and signaling molecules. We speculate that tensin genes may serve as mediators that facilitate the recruitment of actin filaments and phosphotyrosine-containing proteins

involved in migratory processes to focal adhesions. Further research is needed to test this possibility. Several intracellular focal adhesion molecules including focal adhesion kinase (Fak), zyxin, vinculin, enabled/vasodilator-stimulated phosphoprotein (Ena/VASP), p130Cas, and small GTPases have been shown to be involved in regulating cell migration. Overexpression of Fak enhanced cell migration, whereas lack of Fak expression reduced cell migration (27, 28). In contrast, overexpression of vinculin suppressed cell migration (29), and vinculin-null cells migrated faster than the normal cells (21). Similarly, overexpression of Ena/VASP reduced cell motility, whereas depletion of Ena/VASP from the leading edges and focal adhesions promoted cell migration (30). Disruption of the interaction between zyxin and α -actinin suppressed migration (31). Finally, activation of Rac1, Cdc42, and RhoA is able to induce the formation of lamellipodia, membrane ruffles, filopodia, and focal adhesions, and to promote cell migration (32). Whether tensin genes participate in any of these pathways warrants further investigation.

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