

Inactivation of *tensin3* in mice results in growth retardation and postnatal lethality

Ming-Ko Chiang, Yi-Chun Liao, Yasuko Kuwabara, Su Hao Lo*

Center for Tissue Regeneration and Repair, Department of Orthopaedic Surgery, University of California-Davis, 4635 Second Avenue, Room 2000 Sacramento, CA 95817, USA

Received for publication 12 July 2004, revised 15 December 2004, accepted 21 December 2004
Available online 19 January 2005

Abstract

Tensin family is a group of focal adhesion proteins that interact with integrins, actin, and phosphotyrosine-containing proteins. To explore the *in vivo* functions of a new member of the family, *tensin3*, we have generated mutant mice with a disrupted *tensin3* gene. Inactivation of *tensin3* resulted in growth retardation and postnatal lethality in one third of the homozygous mutants. Histological analysis of those mutants showed incomplete development of the small intestine, lung, and bone. Villus formation in the small intestine was affected and cells migrated slower in the runt mutants. Their lungs also displayed enlarged air space suggesting defects in alveogenesis. In addition, the resting zone was thicker and fewer proliferating cells were present in the growth plates of *tensin3*^{-/-} tibiae. These observations indicate that *tensin3* is essential for normal development and functions of the small intestine, lung, and bone. These phenotypes of the runt *tensin3*^{-/-} mice are similar to some clinical features of Silver–Russell syndrome (SRS) which is a genetically inherited defect. About 10% of SRS cases have been linked to abnormality in chromosome 7p11.2–13, where human *tensin3* gene is located, suggesting a potential link between *tensin3* and SRS.

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Keywords: Tensin; Focal adhesion; Growth retardation; Postnatal lethality; Alveogenesis; Growth plates; Silver–Russell syndrome

Introduction

Focal adhesions play important roles in diverse biological processes such as cell adhesion, migration, proliferation, differentiation, and tissue development (Burridge et al., 1988; Hynes, 1992; Jockusch et al., 1995; Schwartz et al., 1995). At these sites, cells establish transmembrane linkages between the extracellular matrix (ECM) and the actin cytoskeleton. These transmembrane linkages are mediated mainly by integrin receptors (Hynes, 1992). Whereas the extracellular domain of integrin interacts with ECM, the cytoplasmic tail anchors the actin cytoskeleton to the plasma membrane through a protein complex. Some of the constituents of focal adhesions participate in the

structural link between receptors and the actin cytoskeleton, while others are signaling molecules, including protein kinases and phosphatases, their substrates, and various adaptor proteins (Jockusch et al., 1995; Yamada and Geiger, 1997).

Tensin1 is a focal adhesion protein capable of binding to several structural and signaling molecules. It interacts with actin filaments at multiple sites, enabling tensin1 both to cap the barbed ends and to cross-link actin filaments (Lo et al., 1994). The Src Homology 2 (SH2) domain of tensin1 mediates its interaction with tyrosine-phosphorylated proteins including phosphatidylinositol 3 kinase, p130Cas, and focal adhesion kinase (Auger et al., 1996). We have recently identified three molecules, *tensin2*, *tensin3*, and *cten* (C-terminal tensin-like protein), with extensive homology to tensin1, indicating that tensin is a gene family (Chen et al., 2002; Cui et al., 2004; Lo and Lo, 2002). All tensin members are associated with focal adhesion sites and their functional domains, such as the actin-binding, SH2, and phosphotyrosine-binding (PTB) domains, are extremely

Abbreviations: ECM, extracellular matrix; EGFR, epidermal growth factor receptor; FAB, focal adhesion binding; SH2, Src homology 2; PTB, phosphotyrosine-binding; SRS, Silver–Russell syndrome.

* Corresponding author. Fax: +1 916 734 5750.

E-mail address: shlo@ucdavis.edu (S.H. Lo).

conserved (Fig. 1). Because *cten* is a shorter polypeptide and lacks the N-terminal regions found in other tensins, homology is limited to the C-terminus, which contains the SH2 and PTB domains. Tensin1, tensin2, and tensin3 are broadly expressed in various tissues, and their expression patterns are very similar with each other. In contrast, *cten* expression is more restricted in tissues such as the placenta and prostate (Lo and Lo, 2002).

Recently, it has been demonstrated that the fly gene *blistry* encodes *Drosophila* ortholog of tensin and is important for wing development (Lee et al., 2003; Torgler et al., 2004). The *Drosophila* tensin loss-of-function mutations resulted in the formation of blisters in the wings, which was due to a defective wing unfolding process. Using a genetic approach, tensin was shown to functionally interact with integrin and the components of the JNK signaling pathway (Lee et al., 2003). In addition, the localization of tensin to integrin adhesive sites in flies requires integrins, talin, and integrin-linked kinase (Torgler et al., 2004).

Previous gene targeting studies in mice have demonstrated that many focal adhesion components play essential roles in embryonic development. Inactivation of these genes, such as integrins, focal adhesion kinase, talin, vinculin, paxillin, and p130Cas, leads to early embryonic lethality (Fassler and Meyer, 1995; Furuta et al., 1995; Hagel et al., 2002; Honda et al., 1998; Monkley et al., 2000; Stephens et al., 1995; Wade et al., 2002; Xu et al., 1998). In contrast, ablation of tensin1 expression in mice did not result in embryonic lethality. Instead, defects were found in adult kidneys and mutant mice eventually died from renal failure several months later (Lo et al., 1997). In addition, the muscle regeneration process was delayed in tensin1 null mice (Ishii and Lo, 2001). The relatively “mild” phenotypes of tensin1 null mice might be explained by functional redundancy. Since tensin is a gene family, it is natural to expect that many of their common roles in regulating the development and differentiation of various tissues are redundant. On the other hand, they appear to have

individual roles critical for specific tissues. For example, tensin1 is crucial for normal renal function. Furthermore, despite the high sequence similarities of tensins at the N- and C-termini, the middle regions are highly divergent, suggesting that each tensin member may have acquired unique biological functions.

Here, we report the possible roles of tensin3 in various tissues by analyzing tensin3^{-/-} mutant mice.

Materials and methods

Embryonic stem (ES) cells and mice

Tensin3ⁱⁿ gene-trap SV129ES cells (XK294) were obtained from BayGenomics (UCSF) and injected into the C57BL/6 blastocysts. The resulting male chimeras were mated with C57BL/6 females, and the tensin3ⁱⁿ line was subsequently maintained on a mixed C57BL/6:SV129 genetic background. All breeding were carried out under standard animal housing conditions in the UCD animal facility.

Mapping of the tensin3ⁱⁿ allele

The 5'-RACE PCR performed previously by BayGenomics had indicated that the integration site of the construct was at the intron 4 of tensin3. Eleven primers (T3-1F to T3-11F) that evenly distribute throughout the intron 4 (approximately 30 kb) were used separately in combination with the βGeo specific primer (βGeoR: 5'-AATTCAGACGGCAAACGACTGTC-3') to amplify the flanking sequence of the integration site. A 1109-bp DNA fragment was amplified with the use of T3-9F and βGeoR as primers. This fragment was subcloned into the TopoII vector (Invitrogen) and the integration site was further identified by DNA sequencing. Later, animals were genotyped by PCR using primers that identified the disrupted intron 4 of the tensin3 gene (T3-9F: 5'-AGGGA-GATCAGGCACCTCTTTGTT-3' and βGeoR) and primers that identified the wild-type intron 4 of the tensin3 gene (T3-9F and T3-9R: 5'-GTGCCTCAGGCTCCTTGTC-CATA-3').

BrdU incorporation and histological staining

To visualize proliferating cells *in vivo*, mice were injected intraperitoneally with 120 mg/kg BrdU (Sigma), 12 mg/kg 5-fluoro-2'-deoxyuridine (Sigma) in phosphate-buffered saline (PBS) 2 or 48 h prior to killing. Isolated tissues were fixed in 4% paraformaldehyde/PBS at 4°C overnight, dehydrated with increasing concentration of ethanol, embedded in paraffin and sectioned at 7 μm. BrdU incorporation was detected by using anti-BrdU mouse monoclonal antibody (Oncogene) and fluorescein-conjugated anti-mouse IgG antibody (Zymed). Staining sections

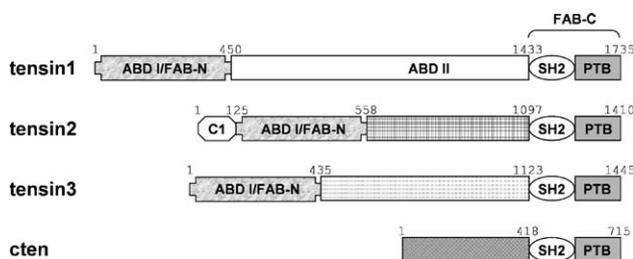


Fig. 1. Functional and structural domains of the tensin family. Human tensin members are highly conserved at N- and C-terminal regions. The N-terminus contains the actin-binding domain (ABD) I that interacts with actin filaments. This region also contains a focal adhesion-binding (FAB) activity and the PTEN homologous sequence. ABD II in the center region of tensin1 retards the actin polymerization rate. The C-terminus contains a SH2 domain, PTB domain, and another FAB site. C1 indicates a protein kinase C conserved region 1. The center regions of tensin members show no sequence homology.

with Alcian Blue, Methyl Green, and Neutral Red was performed using standard protocols.

In situ hybridization and immunohistochemistry

In situ hybridization was performed as described previously using digoxigenin-labeled antisense riboprobes (Wilkinson, 1992; Wilkinson and Nieto, 1993). Stringent washing for in situ hybridization was performed in 50% formamide/2× SSC once at 60°C for 30 min, followed by a wash at 65°C for 30 min. Slides were then washed three times with wash buffer (10 mM Tris-HCl, pH 8.0; 0.5 M NaCl, 5 mM EDTA) at room temperature for 10 min. After treatment with RNase A (25 µg/ml) and RNase T1 (100 U/ml) at 37°C for 45 min, slides were washed twice in 50% formamide/2× SSC at 65°C for 30 min.

For antibody staining, tissue sections were de-waxed and rehydrated, washed three times for 10 min each in PBS. After treatment with 3% H₂O₂ for 10 min, sections were pre-blocked in PBS containing 10% goat serum for 30 min and then incubated with diluted primary antibodies in 5% goat serum overnight at 4°C. The following primary antibodies were used in the analysis: rabbit anti-L-FABP (Novus Biologicals), rabbit anti-smooth muscle actin (Sigma), and mouse anti-β III tubulin (Sigma). The bound antibodies on the sections were detected by using NBA staining kit (Zymed) as instructed by the manufacture.

Western blot analysis of tissue lysates

Selected tissues were homogenized in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.4; 1 mM EDTA, 1 mM PMSF, 1% Triton X-100, 1% sodium deoxycholic acid, 0.1% SDS, 5 µg/ml of aprotinin, 5 µg/ml of leupeptin). Following centrifugation, lysates containing equal amounts of protein (50 µg) were subjected to SDS/PAGE analysis on 8% gels before being transferred onto nitrocellulose membranes. Membranes were blocked at room temperature for 1 h with 5% nonfat dry milk in TBST (10 mM Tris-HCl, pH 8.0; 150 mM NaCl, 0.1% Tween 20) followed by incubation with primary antibody for 1 h at room temperature. After three washes with TBST for 5 min each, the membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG for 1 h at room temperature. After three more washes, the bound antibodies were visualized by using the SuperSignal West Pico Chemiluminescent Substrate (Pierce).

Results

Expression of tensin3 in developing embryos

Like its human counterpart (Cui et al., 2004), mouse *tensin3* is broadly expressed. As shown in Fig. 2A, *tensin3*

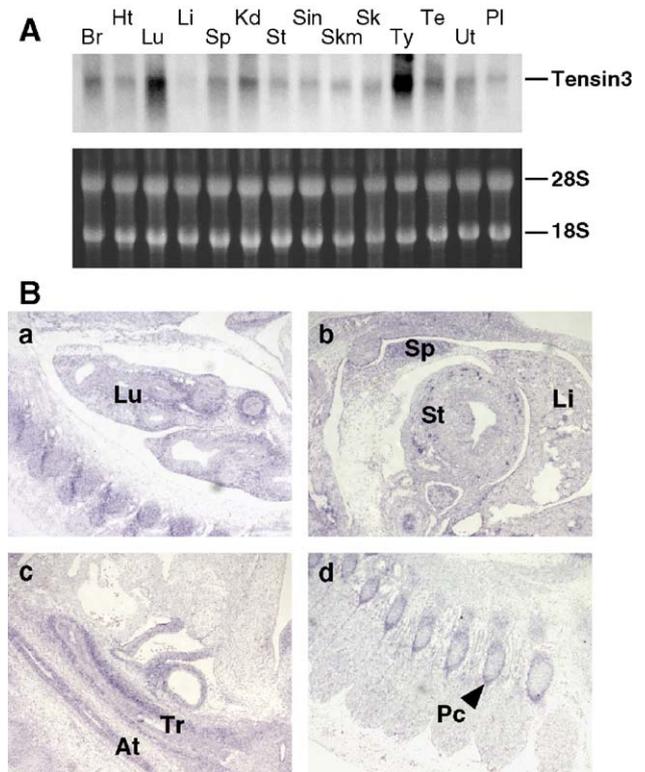


Fig. 2. Expression of *tensin3* in mouse. (A) Northern analysis of *tensin3* transcripts in various adult mouse tissues. The *tensin3* probe detected a band of approximate 8 kb. (B) In situ hybridization of *tensin3*. Paraffin sections of E13.5 embryos were hybridized with a probe complementary to the 3'-untranslated region of *tensin3* message. Abbreviations: Br, brain; Ht, heart; Lu, lung; Li, liver; Sp, spleen; Kd, kidney; St, stomach; Sin, small intestine; Skm, skeletal muscle; Sk, skin; Ty, thymus; Te, testis; Ut, uterus; Pl, placenta; At, aorta; Tr, trachea; Pc, perichondrium.

was detected in most of the adult tissues. Similar broad expression patterns of *tensin3* were also observed in developing embryos. At 13.5 day post-coitum (dpc), strong expression of *tensin3* was detected in lung (including both mesenchyme and epithelium), aorta, stomach, and perichondrium (Fig. 2B). The expression patterns of the closely related *tensin1* and *tensin2* overlap with that of *tensin3* in many tissues, and may therefore be redundant in the development of those tissues (Chen et al., 2002; Lo et al., 1997).

Disruption of the tensin3 gene in mice

An embryonic stem cell line with a disrupted *tensin3* gene was generated by using a gene-trap approach (Skarnes, 2000). The gene-trap construct which contained a strong splice acceptor was introduced into ES cells, and resulted in an insertional mutation in the *tensin3* gene. From the sequence of the resulting mRNA, the gene-trap construct was found inserted in the intron between exon 4 and exon 5 of *tensin3*, and we named the allele *tensin3*ⁱⁿ (for insertion mutant). The precise insertion site was further mapped by PCR analysis and DNA sequencing, and three primers (T3-

9F, T3-9R, and β GeoR) were designed to distinguish the wild-type and the mutant alleles (Fig. 3). The ES cells carrying the mutated allele were injected into blastocysts and resulted in germ line chimeras that were used to generate heterozygous mutant mice. Crossing between heterozygous mice produced pups with expected Mendelian genotype ratio, indicating that inactivation of *tensin3* expression did not lead to embryonic lethality. Heterozygous mutant mice were virtually indistinguishable from wild-type littermates in overall morphology and growth behavior. In contrast, approximately 33% (17 out of 51) of *tensin3ⁱⁿ* homozygous (*tensin3^{-/-}*) mutants were significantly small in sizes (*tensin3^{-/-}* vs. *tensin3^{+/+}* = 4.8 vs. 8.4g at day 14) and died within 20 days after birth (Figs. 4A–C). Two third of the *tensin3^{-/-}* mice, however, appeared to be normal in sizes.

To confirm the insertional mutation has inactivated *tensin3* expression, its expression was analyzed by western blotting (Fig. 4D). No *tensin3* protein was detected in *tensin3^{-/-}* mice either with or without runt phenotypes. This result demonstrates that *tensin3ⁱⁿ* allele was a null allele of the gene and the mutant mice showing no phenotypes were not due to unexpectedly leaking expression of *tensin3*. We further tested whether the lack of phenotypes in these *tensin3^{-/-}* mice were rescued by up-regulation of *tensin1*. However, no significant difference in *tensin1* protein expressions was detected between *tensin3^{-/-}* and wild-type mouse tissues (Fig. 4D). We could not detect *tensin2* proteins in these tissues by our anti-*tensin2* antibodies (not

shown). Most likely, the *tensin3^{-/-}* mice showing no defect is due to the variation of mixed genetic background (C57BL/6:SV129). We are currently backcrossing the mice to test this possibility.

*Small intestine development was disturbed and the migration of the intestinal epithelial cells was defected in *tensin3^{-/-}* mice*

Because of the growth retardation phenotype of *tensin3^{-/-}* mutants and the expression of *tensin3* in normal gastrointestinal tract, we hypothesized that gut development might be affected and resulted in digestive problems in these mice. As shown in Fig. 5, the digestive tract of the homozygous mutant was approximately half the length of its wild-type littermate's gut. Stomach and spleen were also considerably smaller. Although little, we have found residual milk in mutant stomachs, indicating that mutant pups could suck milk. Histological examinations revealed abnormal morphology of the villi in the small intestine (Fig. 6). The alignment of the epithelial cells in the villi was irregular and the cytoplasm of individual cells was smaller in *tensin3^{-/-}* mutants. Moreover, when stained with an anti-L-FABP (Liver-Fatty Acid Binding Protein) antibody, a marker for differentiated enterocytes, the villi of *tensin3^{-/-}* animal displayed little staining compared to the wild-type controls (Figs. 6A–D). This indicates a defect in enterocyte differentiation. In contrast, other cell types in the intestine did not appear to be affected in *tensin3^{-/-}* mutants. Mucus-producing goblet cells, stained by Alcian Blue, were detected with similar distributions both in *tensin3^{-/-}* mutant and control littermate (Figs. 6E, F). Also, no difference was observed regarding the presence of the smooth muscle and the enteric nervous system in the intestine of the mutants (Figs. 6G–J).

To investigate whether the proliferation and migration of epithelial cells were compromised in mutant small intestine, we have injected BrdU into mice and monitored its incorporation 2 h after administration. The BrdU-labeled cells were limited in crypt epithelia and the populations were similar between *tensin3^{-/-}* and control small intestines (Figs. 7A, B). However, if we allowed these cells to migrate for 48 h, BrdU-labeled wild-type cells were found further away from crypts (Figs. 7C, D), indicating that *tensin3* is critical for cell migration in intestinal epithelia. Interestingly, we also detected a migratory defect in *tensin1* null cells and overexpressing either *tensin1* or *tensin2* were able to promote cell migration (Chen and Lo, 2003; Chen et al., 2002), suggesting that regulation of cell migration is a common role of *tensin* family members.

*The lungs of *tensin3^{-/-}* mutants display enlarged distal air space*

In addition to the digestive tract, other organs of *tensin3^{-/-}* mutants were also examined and no difference

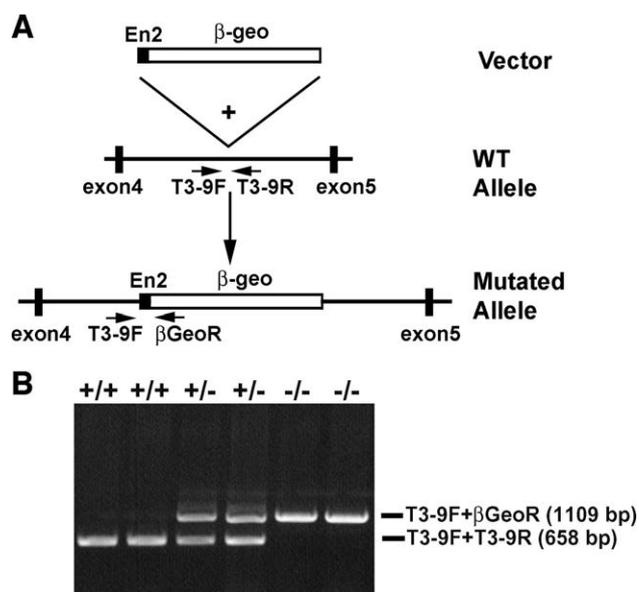


Fig. 3. Gene-trap disruption of *tensin3* gene locus. (A) Schematic representation of the partial genomic structure of the mouse *tensin3* gene before and after the gene-trap integration. T3-9F, T3-9R, and β GeoR primers were designed to distinguish the wild-type allele from the mutated allele. (B) PCR analysis of DNA from siblings obtained in crosses of *tensin3* heterozygous parents. T3-9F, T3-9R, and β GeoR primers were used to amplify a 658-bp fragment (wild-type allele) or an 1109-bp fragment (mutated allele).

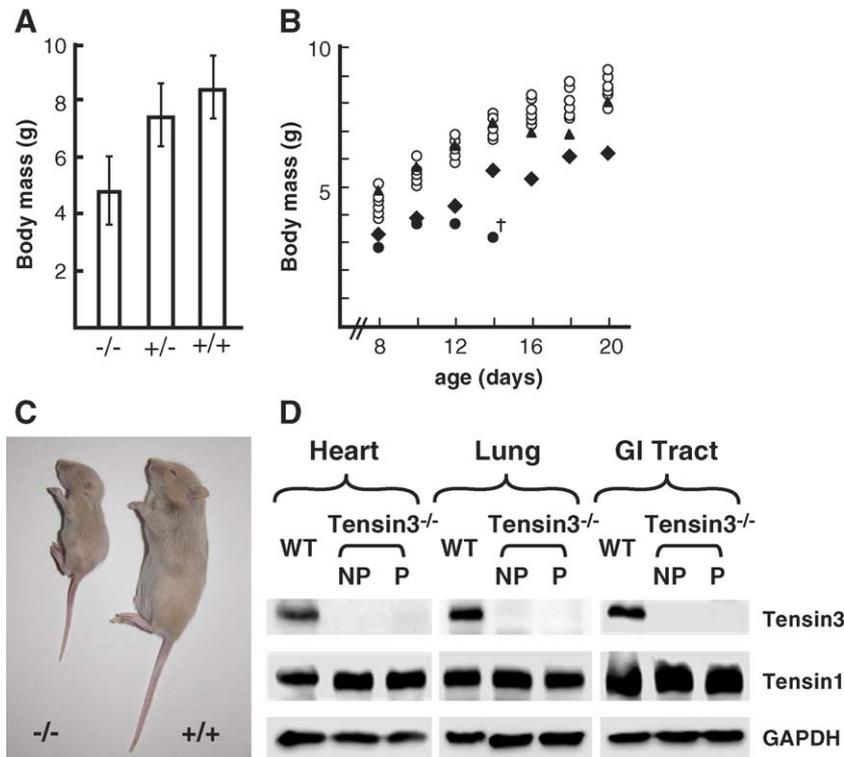


Fig. 4. *Tensin3*^{-/-} mice exhibit growth retardation. (A) Comparison of average body mass of the postnatal day 14 *tensin3*^{-/-} mutants (4.8 ± 1.2 g, $n = 9$) and their heterozygous (7.4 ± 1.1 g, $n = 22$) and wild-type (8.4 ± 1.1 g, $n = 7$) littermates. Error bar represents two standard deviations. (B) Body mass curves of two litters of new born mice, including wild-type and heterozygous pups (open circles), and three homozygous mutants (closed squares, triangles, and circles). A mouse that died during the analysis is indicated by a cross. (C) A 12-day postnatal *tensin3*^{-/-} mutant and its wild-type littermate. (D) Western analysis of tensin1 and tensin3 proteins in heart, lung and gastrointestinal (GI) tract of wild-type (WT), *tensin3*^{-/-} showing no phenotypes (NP) and *tensin3*^{-/-} mice with phenotypes (P). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein loading and transferred control.

in gross morphology was observed besides being smaller in size. When lung histology was examined, we consistently found fewer but larger terminal airways in the 5-day-old



Fig. 5. Gross morphologies of 12-day-old stomachs, intestines, and spleens from *tensin3*^{-/-} and wild-type mice. The digestive tract of *tensin3*^{-/-} mutant was approximately half the length of its wild-type littermate's gut. Stomach and spleen of the mutant were also smaller than those of the wild-type animal.

mutant lungs (Fig. 8). At this age, alveogenesis has been initiated and many septa structures can be seen growing out from the wall of the pre-alveolar saccules in wild-type lungs (Figs. 8A, C). On the other hand, much fewer septa structures were observed in mutant lungs (Figs. 8B, D). In addition, more blood cells were found in mutant lungs. While the 15-day-old wild-type lungs had elaborated many alveoli, the mutant lungs remained similar to those of younger animals (Figs. 8E, F). The walls of the mutant airway were relatively smooth, unlike those of the controls. These observations suggest that the lung defect seen in *tensin3*^{-/-} mutants are due to a failure of secondary septation.

Bone cell defects in *tensin3*^{-/-} mice

Since *tensin3*^{-/-} mice were significantly smaller than their wild-type littermates, histological analysis of the long bone was performed to examine any defects in postnatal skeletal development. We found the resting zones of the growth plate in 12-day-old mutants appear to be larger than controls, while the proliferating and hypertrophic regions of these mice remained similar (Figs. 9A–D). On the other hand, the proliferation activity of chondrocytes measured by BrdU labeling was lower in mutants than

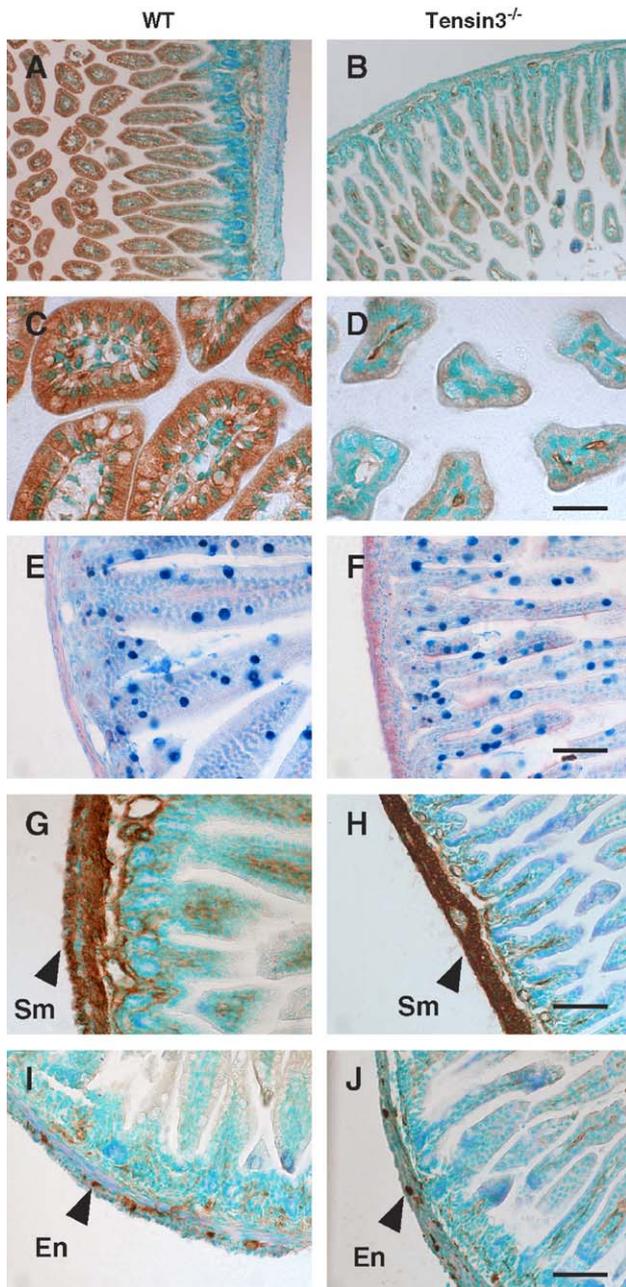


Fig. 6. Postnatal intestine defect in $\text{tensin3}^{-/-}$ mice. Paraffin sections of small intestine from day 10 postnatal wild-type mice (A, C, E, G, I) and $\text{tensin3}^{-/-}$ mutant mice (B, D, F, H, J) were stained with anti-L-FABP antibody (A–D), Alcian Blue (E, F), anti-smooth muscle actin antibody (G, H), and anti- β III tubulin antibody (I, J). The sections in A–D and G–J were counterstained with methyl green, and the sections in E and F were counterstained with fast red. Abbreviations: Sm, smooth muscle; En, enteric nervous system. Bar: 20 μm in D, or 50 μm in F, H, and J.

controls (7.2% vs. 11.2% of cells in the growth plates) (Figs. 9E, F). We also detected more blood cells in mutant bones, and the thickness of the mutant bones was significantly thinner than control bones (Figs. 9G, H). These results suggest that tensin3 may promote chondro-

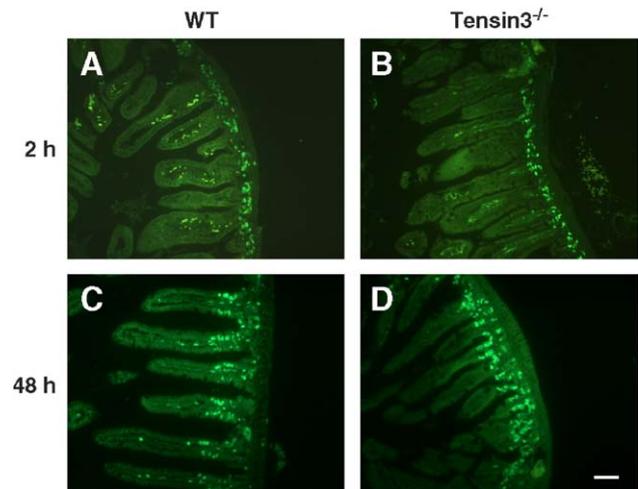


Fig. 7. Proliferation and migration of epithelial cells in the wild-type and $\text{tensin3}^{-/-}$ mice. Ten days postnatal wild-type (A, C) and $\text{tensin3}^{-/-}$ mutant mice (B, D) were killed 2 h (A, B) or 48 h (C, D) after BrdU injection. Paraffin sections from them were stained with anti-BrdU antibody. Scale bar: 100 μm .

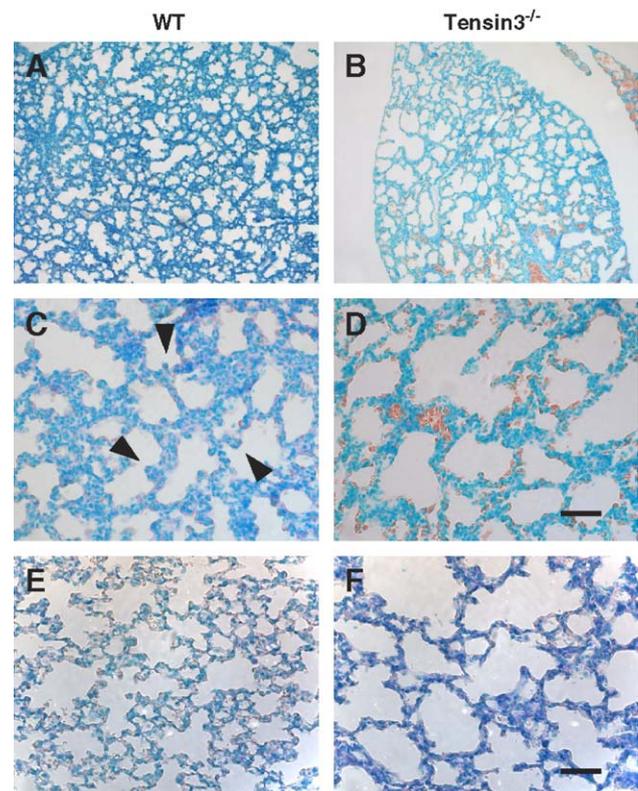


Fig. 8. Postnatal lung defect in $\text{tensin3}^{-/-}$ mice. (A–D) Methyl green stained paraffin sections of the lung from a 5-day postnatal $\text{tensin3}^{-/-}$ mouse (B, D) and its wild-type littermate (A, C). Note the enlarged terminal airspace, less alveolar septa, and more blood cells in the mutant lung. Arrowheads indicate the alveolar septa. (E–F) In 15-day-old samples, much more alveoli are observed in the wild-type lung (E) than in the lung of $\text{tensin3}^{-/-}$ mice (F). Scale bar: 100 μm .

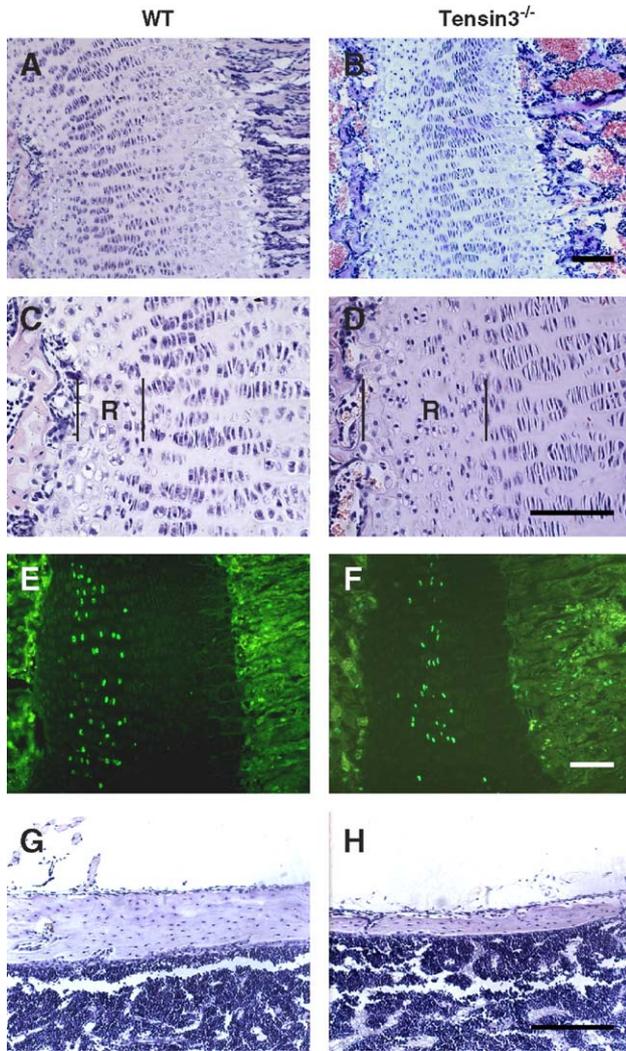


Fig. 9. Morphological changes of the growth plate and reduced numbers of proliferating chondrocytes in *tensin3*^{-/-} mice. Paraffin sections of 12-day-old proximal tibias from wild-type (A, C, E, G) and *tensin3*^{-/-} (B, D, F, H) mice were stained with Hematoxylin and Eosin (A, B, C, D, G, H), or detected for the BrdU-labeled cells (E, F). Abbreviation: R, resting zone. Scale bar: 100 μ m.

cytes to enter proliferating state, and thus contribute to bone growth.

Discussion

In this study, we have demonstrated that *tensin3* alone is important for proper growth of new born mice. One third of the mice lacking *tensin3* exhibited growth retardation and died within 3 weeks after birth. The cause of death is not completely understood but it might be due to a combination of defects in the intestine, lung, and bone. The common theme of these defects is related to incomplete development in these tissues (see below). However, two third of *tensin3*^{-/-} mice were normal in body weights. This variable penetrance is most likely due to the unknown regulator(s) in

the mixed genomic background as are in many other cases (Bennett et al., 2000; Kume et al., 2000; Parisi et al., 2003; Sakata et al., 2002; Sibilias and Wagner, 1995; Threadgill et al., 1995). In flies, ablation of *tensin* expression caused a defect in wing unfolding process and led to the formation of blisters in the wings (Lee et al., 2003; Torgler et al., 2004). Interestingly, this blistering phenotype could be rescued by fixed the back legs with glue, a method to reduce the mechanical stress generated from stroking the wings with their back legs during wing unfolding process (Torgler et al., 2004). This finding indicates a role of *tensin* in strengthening cell adhesion at a specific developmental stage and this role is not essential once the unfolding step is completed. Mouse *tensin3* might also have a similar role in strengthening cell adhesion at late-prenatal and/or early-postnatal development in tissues, such as the lung, small intestine, and bone. The yet to be identified genomic regulator(s) could be the crucial factor(s) deciding whether the *tensin3* mutant could survive or not.

The severity of the phenotypes found in EGF receptor (EGFR) null mice also depends on their genetic backgrounds. They range from early embryonic lethality to survival with growth retardation and die within 3 weeks after birth (Sibilias and Wagner, 1995; Threadgill et al., 1995). Interestingly, abnormalities were also detected in the intestine, lung, and bone of the *EGFR*^{-/-} mice. The overlapping phenotypes suggest that *tensin3* might be involved in the EGF signaling pathway. In fact, we have recently shown that EGF induced tyrosine phosphorylation of *tensin3* in cultured cells in a time- and dose-dependent manner (Cui et al., 2004). These data suggest that *tensin3* participates in EGF signaling pathway both in vitro and in vivo.

The intestinal epithelium establishes and maintains a precise spatial organization despite its continuous and rapid renewal. The epithelium is composed of four major differentiated cell types, enterocytes, Paneth cells, goblet cells, and enteroendocrine cells. These cells arise during a rapid, well organized, bipolar migration. Multipotent stem cells, anchored near the base of the crypts of Lieberkuhn, give rise to descendants that undergo commitment to differentiate along the enterocytic, enteroendocrine, and goblet cell lineages. Enterocytes (absorptive cells) account for ~85% of all the epithelial cells located along the intestine. The most important function of enterocytes is to absorb the nutrients molecules produced by the digestive process. In *tensin3*^{-/-} mutant mice, the enterocytes were not well differentiated as judged by lack of L-FABP staining (Figs. 6B, D) and showed a migratory defect (Fig. 7D). It is very likely that their function in absorbing nutrients was impaired and the animals were undernourished. The malnutrition might lead to growth retardation and result in death of the mutant mice.

The development of lung in mice involves five stages characterized by gross histological features. The first four stages, the embryonic, pseudoglandular, cannalicular, and

saccular stages, occur during gestation (Adamson, 1997). At the end of gestation, saccular stage lungs have formed alveolar ducts and air sacs. In mice, the final stage of lung development, alveogenesis, begins shortly after birth and is characterized by the development of the secondary septation and microvascular maturation. *Tensin3*^{-/-} mutant lungs contained relatively un-branching alveolar architecture and enlarged air spaces, as well as higher amounts of blood cells resulted from leakage of blood vessels, presumably, due to premature cell–cell and cell–matrix adhesions in endothelial cells. These phenomena were very similar to normal developing lungs prior to alveogenesis (Lindahl et al., 1997; Sun et al., 2000; Weinstein et al., 1998; Wendel et al., 2000). These data indicate that lack of *tensin3* expression led to a delay in lung development including terminal airway branching and microvascular maturation.

The exact mechanism of how *tensin3* regulates the development of small intestine and lung is still unclear. However, it is known that interactions between epithelial and surrounding mesenchymes are critical for the development of both organs (Kedinger et al., 1986; Minoo and King, 1994; Roberts et al., 1998). EGF has been shown to play a role in gastrointestinal epithelia restitution and mucosal repair. It does so by promoting migration of epithelial cells that requires modulation of cell–cell and cell–matrix interaction (Pignatelli, 1996; Playford and Shaw-Smith, 1996). It is possible that lacking *tensin3* impairs the ability of the epithelia to fully process environmental cues, such as EGF, which normally induces tyrosine phosphorylation of *tensin3*. In addition to EGF, many other factors have also been shown by genetics evidences to be critical for proper gut development. They include platelet-derived growth factor, Wnts, sonic hedgehog, bone morphogenetic proteins (Karlsson et al., 2000; Kuhnert et al., 2004; Narita et al., 2000; Ramalho-Santos et al., 2000; Smith and Tabin, 1999). Whether *tensin3* is involved in these signaling pathways remains to be tested in the future.

A number of growth factors and adhesion molecules have also been implicated in lung development. For example, disruption of EGFR results in a lung defect early in development (Miettinen et al., 1995), while loss of TGF- β also led to aberrant lung development. On the other hand, over-expression of sonic hedgehog and BMP can also disrupt branching morphogenesis (Bellusci et al., 1996, 1997). However, the lung phenotype of the *tensin3*^{-/-} mutants is more similar to that of the fibroblast growth factor 3 and 4 double null mice. The abnormality occurs at the late step of lung development, that is, alveogenesis. It is intriguing that the enlarged alveolar air space was also observed in $\alpha v\beta 6$ integrin null mutant (Morris et al., 2003). *Tensins* have been shown to bind integrin via their PTB domains (Calderwood et al., 2003) in cultured cells, and this interaction has also been demonstrated by genetics analysis in *Drosophila* (Lee et al., 2003; Torgler et al., 2004), suggesting that *tensin3* may cooperate with $\alpha v\beta 6$ integrin to maintain lung epithelial homeostasis.

Most of the bones in the vertebrate skeleton are formed by endochondral ossification. This process is initiated in the embryo by the differentiation of mesenchymal cells into chondrocytes and then the central part of chondrocytes becomes hypertrophic cartilage matrix, which in turn is invaded by blood vessels and gradually replaced by bone tissue. However, both ends of chondrocytes form the epiphyseal growth plates, which proliferate before undergoing hypertrophy. Enlarged resting regions and much fewer proliferating chondrocytes were observed in the mutant growth plates. In addition, higher amounts of blood cells and thinner bones were found in *tensin3*^{-/-} mutant bones. These phenotypes were very similar to premature normal bones (Colnot et al., 2004; Lanske et al., 1999; Schipani et al., 1997; Weir et al., 1996; Zelzer et al., 2004), suggesting that ablation of *tensin3* led to a delay in bone development. It is possible that lack of *tensin3* might arrest chondrocytes at the resting state, and then impair or delay the proliferation and terminal differentiation of chondrocytes into hypertrophic cells during endochondral ossification in postnatal longitudinal growth of bones.

Silver–Russell syndrome (SRS) was first described in 1953–1954 (Russell, 1954; Silver et al., 1953), and is a relatively common cause of growth failure in children presenting at pediatric clinics, with an incidence of approximately 1:10,000 births. The major phenotypic features are growth restriction, short limbs, and triangular face. The syndrome is genetically heterogeneous. However, abnormalities involving chromosomes 7 and 17 have been consistently associated with SRS patients. Maternally uniparental disomies of chromosome 7 is observed in approximately 10% of SRS cases and the duplicated regions has been defined to 7p11.2–p13 (Joyce et al., 1999; Kotzot et al., 1995; Monk et al., 2000; Preece et al., 1997). In addition, the orthologous region in the mouse (chromosome 11p12) is associated with growth phenotypes (Cattanach and Kirk, 1985). Interestingly, human *tensin3* is located at chromosome 7p12.3. The chromosome location of the *tensin3* gene and the similar phenotypes of *tensin3*^{-/-} mutant mice suggest that mutations in *tensin3* gene might link to a sub-population of the SRS patients.

Acknowledgments

We wish to acknowledge the BayGenomics for providing the gene trapped ES clone. We thank Nien-Tsu Chen for excellent technical supports. This study is supported in part by Shriner's Children Hospital grant (SHL).

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