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Deletion of the Tensin2 SH2-PTB domain, but not the loss of its PTPase activity, induces podocyte injury in FVB/N mouse strain

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Abstract: Tensin2 (TNS2) is a focal adhesion-localized protein possessing N-terminal tandem protein tyrosine phosphatase (PTPase) and C2 domains, and C-terminal tandem Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains. Genetic deletion of *Tns2* in a susceptible murine strain leads to podocyte alterations after birth. To clarify the domain contributions to podocyte maintenance, we generated two *Tns2*-mutant mice with the genetic background of the susceptible FVB/NJ strain, $Tns2^{\Delta C}$ and $Tns2^{CS}$ mice, carrying a SH2-PTB domain deletion and a PTPase domain inactivation, respectively. The $Tns2^{\Delta C}$ mice developed massive albuminuria, severe glomerular injury and podocyte alterations similarly to those in *Tns2*-deficient mice. In contrast, the $Tns2^{CS}$ mice showed no obvious phenotypic abnormalities. These results indicate that the TNS2 SH2-PTB domain, but not its PTPase activity, plays a role in podocyte maintenance. Furthermore, in a podocyte cell line, the truncated TNS2 mutant lacking the SH2-PTB domain lost the ability to localize to focal adhesion. Taken together, these data suggest that TNS2 recruitment to focal adhesion is required to maintain postnatal podocytes on a susceptible genetic background.
Key words: chronic kidney disease, focal adhesion, nephropathy, podocyte, tensin

Introduction

Tensin2 (TNS2, also known as TENC1 or C1-TEN) is a member of the tensin family that includes Tensin1 (TNS1), Tensin3 (TNS3) and Tensin4 (TNS4) in mammals. All tensins possess C-terminal tandem Src homology 2 (SH2)-phosphotyrosine binding (PTB) domains, which bind short peptide motifs containing phosphorylated tyrosines and the cytoplasmic tails of cell surface receptors (including receptor tyrosine kinases and integrins) through NPXY motifs [8, 24, 26]. An additional characteristic of tensins, except for TNS4, is the presence of a protein tyrosine phosphatase (PTPase) domain paired with the C2 domain, resembling that of PTEN [6, 8]. However, TNS1 lacks an essential catalytic cysteine residue for PTPase enzymatic activity, which catalyzes the dephosphorylation of phosphotyrosyl proteins [6]. Moreover, structural analyses suggest that the TNS3 catalytic pocket is structurally different from those of PTEN and TNS2 [7]. Indeed, only TNS2 appears to have PTEN-like PTPase activity [7]. It is easy to imagine that these proteins, which possess SH2-PTB and PTPase domains, interact with tyrosine phosphorylation signaling pathways.

Tensin functions have been well investigated in cancer cells with a focus on focal adhesion, migration, prolif-

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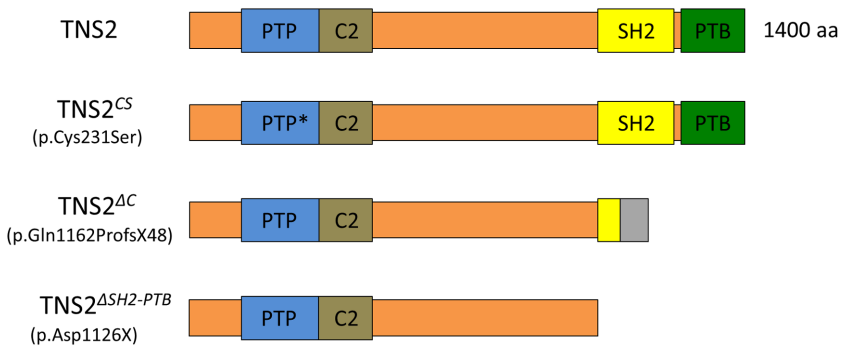

Fig. 1. Schematic illustrations of the mouse TNS2 protein and the mutant proteins. Asterisk indicates catalytic inactivity.

eration, cytoskeleton, integrin-mediated signal transduction, and so on. Structurally resembling PTEN and binding to Rho GTPase-activating protein DLC1 [8], both tumor suppressors, makes tensins an appealing target for cancer research. However, their biological functions remain poorly understood. *Tns2*-null mutant (called *Tns2*^{nph}) mice develop renal failure caused by postnatal alterations in podocytes, although this occurs in a strain-dependent manner. *Tns2*^{nph} induces podocyte foot process effacement accompanied by glomerular basement membrane (GBM) thickening, and subsequent glomerular and tubulointerstitial injuries in ICGN, which is the original strain possessing the *nph* mutation, FVB/NJ (FVB) and DBA/2J strains, but only modest GBM thickening in C57BL/6J and 129^{+Ter}/SvJcl strains [15, 16, 22, 27, 28]. Recently, to clarify the domain contributions, we have generated TNS2 C-terminal deletion mutant (designated *Tns2*$^{\Delta C}$, Fig. 1) mice with a frameshift mutation in exon 22, resulting in truncation of the SH2 domain and deletion of the PTB domain [13]. These mice showed podocyte abnormalities, GBM thickening, glomerular injury and proteinuria, suggesting that the TNS2 SH2-PTB domains are involved in TNS2 function in podocytes [13]. However, we cannot conclude that the results obtained from *Tns2*$^{\Delta C}$ in mice are equal to those from *Tns2*^{nph}; i.e., that TNS2 SH2-PTB domains play a critical role in maintaining podocytes, as it was impossible to compare the *Tns2*$^{\Delta C}$ mice, which were F1 progeny from an N4 backcross (BDF1 × FVB), with the *Tns2*^{nph} mice in an FVB genetic background due to differences in genetic background. We also cannot exclude the possible contributions of other functional domains, such as the PTPase domain.

In this study, we generated a novel mutant mouse carrying a missense mutation (p.Cys231Ser) in TNS2 (designated *Tns2*^{CS}, Fig. 1), resulting in the catalytic inactivation of the PTPase domain [6, 11]. We then assessed the effects of these *Tns2* mutations (*Tns2*^{nph}, *Tns2*$^{\Delta C}$ and *Tns2*^{CS}) on podocytes in mice with an FVB genetic background to investigate the contribution of each of the SH2-PTB and PTPase domains to podocyte maintenance.

Materials and Methods

Ethical statement

All research was conducted according to the Regulations for the Care and Use of Laboratory Animals of Kitasato University. The animal experimentation protocol was approved by the President of Kitasato University based on the judgment made by the Institutional Animal Care and Use Committee of Kitasato University (Approval ID: 18-072, 17-100, 16-074, 15-049).

Animals

Tns2^{nph} mice with an FVB genetic background (hereafter simply, *Tns2*^{nph} mice) were generated as described previously [22]. *Tns2*$^{\Delta C}$ mice with an FVB genetic background (hereafter simply, *Tns2*$^{\Delta C}$ mice) were generated by further backcrossing the previously described *Tns2*$^{\Delta C}$ mice [13] 7 times onto FVB for 11 generations in total. *Tns2*^{CS} mice with an FVB genetic background (hereafter simply, *Tns2*^{CS} mice) were generated by CRISPR/Cas9-mediated genome editing as described previously [17]. Briefly, the guide RNA (gRNA) guiding sequence for the catalytic site of the PTPase domain (NCBI accession numbers NM_001355636 and NM_153533) was designed as follows: 5'-CGTGGTTGTGTTGTACTGCA-3' (where the underlined residues correspond to an essential catalytic residue Cys231 [6, 11]). From the DNA oligonucleotides consisting of tandemly arranged T7 promoter and gRNA sequences, gRNA was transcribed *in vitro* using the MEGAshortscript T7 kit (Thermo Fisher Scientific, Waltham, MA, USA). A single-stranded oligodeoxynucleotide (ssODN) for the targeted insertion (5'-GCCGACCCTCAGCACGTGGTTGTGTTGTACAGCAAGGTGAGCTGGGACCTT-

GGGGTCACAG-3', where underlined residue indicates a point mutation in the reference genome sequence, NCBI accession number NC_000081, resulting in a single amino acid alteration Cys231Ser) was synthesized artificially (Eurofins Genomics, Brussels, Belgium). Cas9 mRNA was synthesized by mMESSAGE mMACHINE T7 Ultra transcription kit (Thermo Fisher Scientific). The Cas9 mRNA (20 ng/μl), gRNA (10 ng/μl) and ssODN (50 ng/μl) were delivered into fertilized eggs from FVB mice by microinjection. After overnight culture, two-cell embryos were transferred into pseudopregnant female mice. The TNS2 PTPase domain-coding region of the genome from the offspring was amplified by PCR and sequenced with the following primers: *Tns2*^{CS} forward, 5'-GCAAGACTTTGGTTGGCCTG-3' and *Tns2*^{CS} reverse, 5'-GGGACAGATGAGGAAAGGCC-3'. All mutant strains were maintained by backcrossing to FVB mice. Homozygous mutant mice were generated by crossing between the heterozygous mutant mice themselves and used for experiments.

The animal facility was air-conditioned at 22 ± 2°C, maintained at 40–60% relative humidity, and mice were maintained under a 12 h light-dark cycle. A standard laboratory diet, CE-2 (Clea Japan), and tap water were available *ad libitum*. The animals' microbiological status was monitored periodically according to the Japanese Association of Laboratory Animal Facilities of Public and Private Universities guidelines. A humane endpoint was applied when the mice with severe anemia became moribund.

Measurement of urinary albumin excretion

Urine samples were collected from 8-week-old mice by gentle manual compression of the abdomen. To measure albumin, urine samples were diluted with sample buffer containing 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, 60 mM Tris-HCL (pH 6.8) and bromophenol blue, and heated at 95°C for 5 min. Samples containing 1 μl of urine were applied to 10% SDS-polyacrylamide gel electrophoresis. As a positive control, 5 μg of bovine serum albumin was loaded simultaneously. The gel was fixed and stained with Coomassie brilliant blue (CBB; Nacalai Tesque, Kyoto, Japan) according to manufacturer's instructions, and scanned using a standard commercial scanner. CBB-stained band corresponding to urinary albumin was quantified using the ImageJ gel analysis program (http://imagej.net/). Urinary creatinine was measured using a creatinine colorimetric assay kit (Cayman chemical, Ann Arbor, MI, USA) according to manufacturer's instructions. The urinary albumin excretion was normalized against the urinary creatinine. Urine collection was performed twice over a three-day period, and the measured urinary albumin excretion was averaged for each mouse.

Histology

Kidneys from 8-week-old mice were fixed with 4% paraformaldehyde (PFA) at 4°C overnight. The PFA-fixed paraffin sections (2 μm in thickness) were subjected to normal histological processes and stained with periodic acid-Schiff (PAS) solution. To quantify the severity of glomerular damage, we used a histological injury score as previously described [22], with a slight modification. We subdivided the severity of glomerular histopathology into seven stages characterized by the following observations: 0, no abnormality; 1, mild expansion of the mesangial matrix; 2, partial thickening of the GBM; 3, vascular stenosis (partial expansion of the mesangial matrix); 4, entire expansion of the mesangial matrix; 5, abnormal dilation of the capillary lumen; and 6, retraction and collapse of the glomerular tuft. Glomerular histopathology was scored blindly and independently by 2 observers. The glomerular injury score was calculated as a mean of these ratings for twenty-five randomly selected cortical glomeruli averaged for the 2 independent observers.

Transmission electron microscopy

Kidneys from 8-week-old mice were cut into small pieces (1 mm^3) and prefixed in Karnovsky solution (2% glutaraldehyde, 2% PFA in 50 mM cacodylate buffer, pH 7.4), and then fixed in buffered 1% osmium tetroxide for 2 h. The fixed tissue was dehydrated by graded alcohol, and embedded in epoxy resin. The epoxy resin-embedded specimens were sectioned at a thickness of 70 nm and stained with uranyl acetate and lead citrate, and observed under an H-7650 transmission electron microscope (Hitachi High-Technologies, Tokyo, Japan).

Plasmid construction

The full-length cDNA fragment encoding TNS2 (1400 amino acid, NCBI accession number NM_153533) from FVB mice was subcloned into pCMV-SPORT6 (Invitrogen). The initiation codon and FLAG-encoding sequence were then attached at the 5'-end of the open reading frame of *Tns2*. To generate a truncated TNS2 mutant lacking the SH2-PTB domain (Fig. 1), we inserted tandem stop codons after Gln1125, which is the first residue of the TNS2 SH2 domain [3]. The mutant DNA fragment was synthesized from the wild-type sequence by fusion PCR using the following primers: outside forward, 5'-ACTTCAGATAGCCCAGATGGCTC-3', outside reverse, 5'-AAATAGGGCTCAGTGGGACAAC-3', inside forward, 5'-TAATAGGAATTC-

GATACATCAAAGTTCTGGTACAA-3' and inside reverse, 5'-GAATTCCTATTACTGGACAAACTTGACATTGCT-3' (where the underlined residues correspond to overlap mutant sequence including the tandem stop codons). The *SacI-BamHI*-digested mutant fragment was substituted for the *Sac*I-*Bam*HI-digested wild-type fragment in the FLAG-tagged TNS2 in the expression plasmid by DNA ligation.

Lipofection and western blotting

Human embryo kidney cell line 293FT (Thermo Fisher Scientific) was maintained in DMEM containing 10% fetal bovine serum. Each of the *Tns2* expression plasmids was introduced into 293FT cells using Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. At 48 h after transfection, cells were lysed in RIPA buffer and boiled with 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol. Lysates were electrophoresed in a SDS-polyacrylamide gel and blotted on polyvinylidene difluoride membranes (GE Healthcare, Chicago, IL, USA). For blocking non-specific binding, the membranes were incubated in Blocking One reagent (Nacalai Tesque) for 1 h at room temperature. The membranes were incubated with mouse monoclonal antibody against the N-terminal DYKDDDDK peptide (1:10,000, Clone number 2H8, Trans Genic, Kobe, Japan) or rabbit polyclonal antibody targeting the phosphorylated Tyr483 of TNS2 (1:1,000, Aviva Systems Biology, San Diego, CA, USA) for 1 h at room temperature. Concomitantly, rabbit polyclonal antibody against GAPDH (1:10,000, Abcam, Cambridge, UK) was used for a loading control. Thereafter, the membranes were incubated with secondary horseradish peroxidase (HRP)-conjugated antibodies against mouse IgG (1:30,000, Fujifilm Wako Pure Chemical, Osaka, Japan) or rabbit IgG (1:5,000, Immuno Reagents, Raleigh, NC, USA) for 1 h at room temperature. After incubation with ECL Prime detection reagent (GE Healthcare), the blots were imaged using an Omega Lum C imaging system (Gel Co., San Francisco, CA, USA).

Electroporation and immunofluorescent staining

A podocyte cell line [23] derived from mice carrying a temperature-sensitive mutant of the immortalizing SV40 large T antigen under control of the interferon-γ (IFN-γ)-inducible *H-2K*^{b} promoter was kindly provided by Dr. Karlhans Endlich. Cells were cultured in RPMI1640 medium containing 10% fetal bovine serum and 100 U/ml recombinant mouse IFN-γ (Merck, Darmstadt, Germany) in a 33°C incubator supplied with 5% CO_2. For transfection, cells were collected by TrypLE Express dissociation reagent (Thermo Fisher Scientific), centrifuged and resuspended in reduced serum medium Opti-MEM (Thermo Fisher Scientific). Then, 100 μl of the cell suspension containing 2 × 10^5 cells and 10 μg of the *Tns2* expression plasmid was placed into an electroporation cuvette with a 2-mm gap. Electric pulses were generated using an electroporator NEPA21 (Nepa Gene, Ichikawa, Japan). Parameters for the poring pulse were 180 V × 2 times with a 7.5 ms pulse length, a 50 ms pulse interval, and a 10% decay rate. Those for the transfer pulse were 20 V × 10 times with a 50 ms pulse length, a 50 ms pulse interval, polarity-exchanged, and a 40% decay rate. After electroporation, the cell suspension was transferred into growth medium, seeded in a chamber slide (Nunc Lab-Tek II, Product number 154534, Thermo Fisher Scientific) and cultured in growth condition for 48 h. Cells were washed three times in PBS, fixed in 4% PFA for 20 min at room temperature, and incubated with PBS and 0.3% Tween 20 (PBST) for 20 min at room temperature. For blocking non-specific binding, cells were incubated with 5% goat serum in 0.3% PBST for 1 h at room temperature. After blocking, cells were incubated with mouse primary antibody against the N-terminal DYKDDDDK peptide (1:400) at 4°C overnight, followed by incubation with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Cell Signaling Technology, Danvers, MA, USA) for 30 min at room temperature. Cells were mounted in ProLong Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific). The sample was imaged using an LSM 710 confocal microscope (Carl Zeiss, Oberkochen, Germany).

Statistics

Data are expressed as means ± standard deviation. Bonferroni's multiple comparison test was used for calculating statistical significance and carried out using GraphPad Prism 5 software (MDF, Tokyo, Japan). A P value <0.05 was considered to be statistically significant.

Results

Tensin2 SH2-PTB domain deletion induces albuminuria and podocyte injury in mice

Tns2^{CS} mutant mice were born according to Mendelian rules without apparent defects (e.g., body size, behavior and fertility). We evaluated the severity of podocyte injury in three different *Tns2* mutant strains, *Tns2*^{nph}, *Tns2*$^{\Delta C}$ and *Tns2*^{CS}, on an FVB genetic background at 8 weeks old. *Tns2*^{nph} and *Tns2*$^{\Delta C}$ mice, but not *Tns2*^{CS} mice, developed massive albuminuria (Figs. 2A and B). Urinary albumin excretion in *Tns2*$^{\Delta C}$ mice was equal to

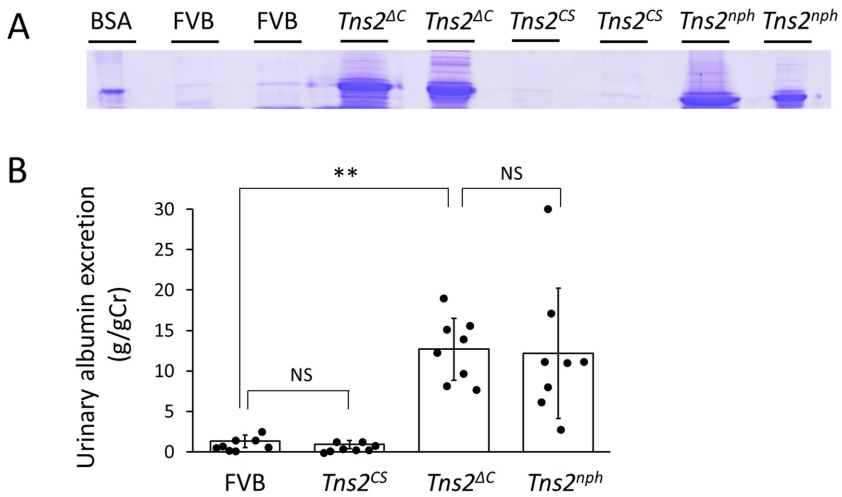

Fig. 2. Urinary albumin excretion in the *Tns2*^{nph}, *Tns2*^{CS}, *Tns2*$^{\Delta C}$ and wild-type FVB mice. Mice were analyzed at 8 weeks old (n=8). (A) SDS-polyacrylamide gel electrophoresis analysis of urinary albumin. BSA, bovine serum albumin. (B) Urinary albumin excretion. There were no significant differences in urinary creatinine among strains. Asterisk, $P<0.0001$. NS, not significant.

that in *Tns2*^{nph} mice (Fig. 2B). Urinary albumin excretion in *Tns2*^{CS} mice was in the normal range (Fig. 2B). Histological analysis revealed that almost all glomeruli in the *Tns2*^{nph} and *Tns2*$^{\Delta C}$ mice showed entire expansion of the mesangial matrix, abnormal dilation of the capillary lumen or retraction and collapse of the glomerular tuft, while almost no glomeruli in the *Tns2*^{CS} mice showed any abnormalities (Fig. 3A). The retraction and collapse of the glomerular tuft was more frequently observed in the *Tns2*^{nph} than in the *Tns2*$^{\Delta C}$ mice, resulting in a significant difference in glomerular injury score between the *Tns2*^{nph} and *Tns2*$^{\Delta C}$ mice (Fig. 3B). Ultrastructural analysis showed podocyte foot process effacement and GBM thickening in the *Tns2*^{nph} and *Tns2*$^{\Delta C}$ mice. Furthermore, the glomerular structures appeared more extraordinary in the *Tns2*^{nph} than in the *Tns2*$^{\Delta C}$ mice with respect to the thickness of GBM, the number of GBM protrusion and the morphology of foot process (Fig. 4). In the *Tns2*^{CS} mice, no abnormalities in glomerular morphology were detected by transmission electron microscopy (Fig. 4).

SH2-PTB domain deletion impairs the ability of tensin2 to localize to focal adhesion

To understand the molecular mechanism by which deletion of the TNS2 SH2-PTB domain induces podocyte injury, we generated plasmids encoding FLAG-tagged TNS2 or FLAG-tagged truncated TNS2 lacking the C-terminal SH2-PTB domain (TNS2$^{\Delta SH2\text{-}PTB}$). The recombinant protein expression was verified by western blot assay (Fig. 5A). When expressed in the podocyte cell line, full-length TNS2 formed punctate cytoplasmic foci and tended to localize to the cell periphery, while TNS2$^{\Delta SH2\text{-}PTB}$ formed numerous tiny foci and diffused in the cytoplasm (Fig. 5B). The SH2-PTB domain deletion underwent changes in the localization of TNS2 in the podocyte cell line.

Discussion

Tns2 deficiency leads to podocyte foot process effacement accompanied by GBM thickening, and subsequent glomerular and tubulointerstitial injuries in susceptible murine strains including FVB. In this study, to investigate whether each of the SH2-PTB and PTPase domains in TNS2 plays a role in podocyte maintenance, we compared the severity of glomerular injury in two different *Tns2*-mutant mice with an FVB genetic background, *Tns2*$^{\Delta C}$ and *Tns2*^{CS} mice, carrying a SH2-PTB domain deletion and PTPase domain inactivation, respectively, to that in *Tns2* null mutant mice with an FVB genetic background, *Tns2*^{nph} mice, or wild-type FVB mice. Our results revealed that the *Tns2*$^{\Delta C}$ mice developed massive alubuminuria, severe glomerular injury and podocyte alterations similarly to those in *Tns2*^{nph} mice. In contrast, there were no significant differences in urinary albumin excretion or glomerular injury score between *Tns2*^{CS} and wild-type FVB mice. Furthermore, electron microscopy showed that there were no abnormalities in the glomeruli from *Tns2*^{CS} mice. Our results clearly suggest that deletion of the TNS2 SH2-PTB domain, but not the loss of its PTPase activity, induces podocyte injury.

Recently, TNS2 has been identified as activating the mammalian target of rapamycin complex 1 (mTORC1)

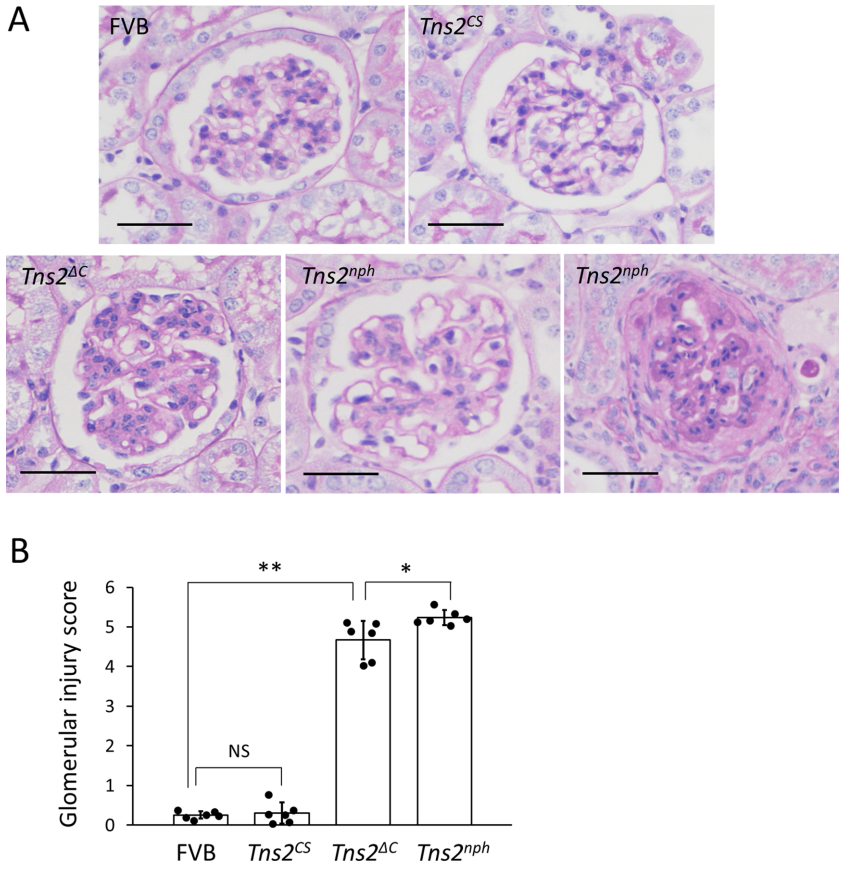

Fig. 3. Glomerular histology in the *Tns2*^{nph}, *Tns2*^{CS}, *Tns2*$^{\Delta C}$ and wild-type FVB mice. Mice were analyzed at 8 weeks old (n=6). (A) Representative glomerular structures under PAS staining. Most glomeruli in *Tns2*$^{\Delta C}$ and wild-type FVB mice were normal, and received a glomerular injury score of 0. In contrast, most glomeruli in *Tns2*^{nph} and *Tns2*$^{\Delta C}$ mice exhibited glomerular injury scores of 5. Glomeruli with a score of 6, which showed retraction and collapse of the glomerular tuft, were observed mostly in *Tns2*^{nph} mice. (B) Glomerular injury scores. Asterisks indicate *P* values, **<0.0001 and *<0.05. NS, not significant. Scale bars, 50 μm.

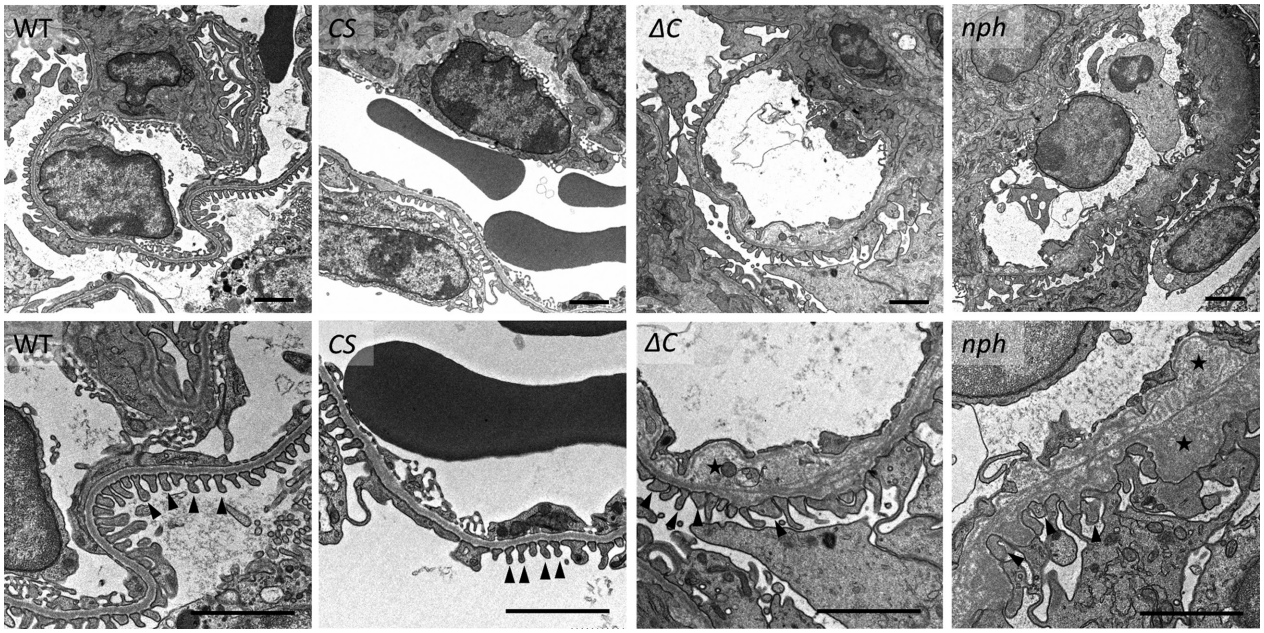

Fig. 4. Ultrastructure of the podocytes and GBM in the *Tns2*^{nph}, *Tns2*^{CS}, *Tns2*$^{\Delta C}$ and wild-type FVB mice. Mice were analyzed at 8 weeks old. Representative transmission electron microscopy images of glomeruli. Arrowheads, foot process. Stars, protrusion of the GBM. Scale bars, 2 μm.

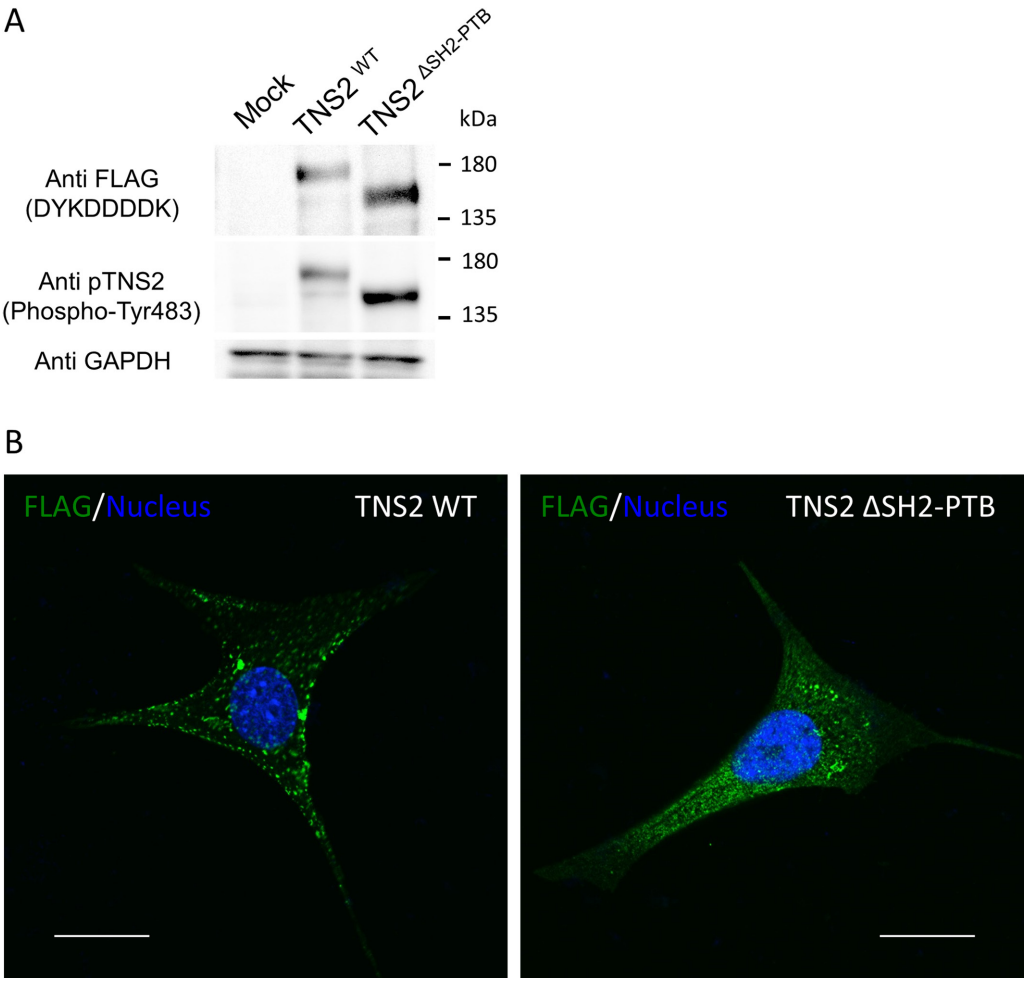

Fig. 5. Localization of full-length TNS2 and the truncated TNS2 mutant lacking the SH2-PTB domain in a podocyte cell line. (A) The FLAG-tagged recombinant protein expression was verified by western blot assay. (B) Immunofluorescent images of the podocyte cell line expressing recombinant TNS2. Scale bars, 20 μm.

signaling in podocytes through its action as a PTPase toward nephrin, which is a principal podocyte slit diaphragm component [11]. This mTORC1 activation is associated with podocyte dysfunction in diabetic nephropathy [5, 10] and, interestingly, TNS2 is upregulated in diabetic nephropathy model glomeruli [11]. Adenovirus-mediated *Tns2* overexpression induces mTORC1 activation and podocyte dysfunction in a PTPase-dependent manner [11]. On the other hand, decreased mTORC1 activity also induces podocyte dysfunction after birth [5]. These data indicate that balanced mTORC1 activity is required for podocyte homeostasis. Additionally, the effect of mTORC1 activity on podocytes seems to be modified by the age and genetic background of the mice [5]. Thus, we expected that decreased mTORC1 activity by the embryonic inactivation of the TNS2 PTPase domain might induce podocyte injury in mice with a diabetic nephropathy-susceptible FVB genetic background [30], while transient *Tns2*^{CS} overexpression did not induce podocyte injury in adult DBA/2 mice [11]. However, *Tns2*^{CS} mice developed neither podocyte dysfunction nor proteinuria. These results suggest that in normal podocytes, endogenous TNS2 PTPase activity is not involved in podocyte homeostasis.

Tensin family members are known as focal adhesion proteins, and are known to be localized to focal adhesion in at least in some cell types [8]. In a podocyte cell line, the transfected full-length TNS2 displayed a peripheral dotted distribution pattern typical of a focal adhesion protein as in other cell types [2, 9, 14, 31]. On the other hand, the distribution of the TNS2$^{\Delta SH2\text{-}PTB}$ in the podocyte cell line was distinct from that of the full-length TNS2; i.e., a focal adhesion protein. The results indicate that SH2-PTB domain deletion impairs the ability of TNS2 to localize to focal adhesion. In focal adhesion, integrin-based multiprotein complexes orchestrate the connection of actin bundles to the extracellular matrix through the cell membrane. These functions are essential for the formation of highly differentiated cells with an intricate cytoskeletal architecture; i.e., podocytes. Podo-

cytes have thick arms, major processes that extend toward the capillaries, and spread numerous projections, or foot processes, over the GBM. The foot processes connect to the matrix molecules of the GBM via transmembrane adhesion receptors (i.e., integrin α3β1), and interdigitate with the neighboring foot processes via intercellular junctions, known as the slit diaphragm, to form filtration slits. Dense actin bundles aligned longitudinally together with cortical thin actin filaments beneath the cell membrane shape the cytoskeleton of the foot processes [19]. Hence, failure of the actin bundles to connect with the GBM at the focal adhesions induces podocyte foot process effacement and detachment. These podocyte abnormalities have been observed in genetically modified mice lacking focal adhesion structural proteins, such as integrins α3/β1, talin1 and CD151 [20, 21, 25]. Conversely, inhibition of focal adhesion turnover by the deletion of focal adhesion regulatory proteins, such as FAK and CRK, attenuates foot process effacement in murine models [4, 12]. FAK phosphorylation at Tyr397, which activates the signaling pathway required for focal adhesion turnover, is increased in podocyte injury [12]. Such activation of FAK is also observed in the glomeruli of susceptible *Tns2*^{nph} mice [27, 28]. In focal adhesion, multiple reciprocal focal adhesion protein interactions, including those of FAK, are intricately accompanied by conformational changes related to function, which are caused by binding cofactors or ligands, phosphorylation and mechanical tension [18, 29].

Collectively, our results demonstrate that deletion of the TNS2 SH2-PTB domain induces podocyte alterations in the susceptible mice, most likely due to its impaired ability to localize to focal adhesion. The absence of TNS2 in focal adhesion is supposed to affect the conformational states of the binding partners in focal adhesion, and finally lead to FAK activation in the susceptible mice, while it was compensated for in the resistant mice. Recently, 6 novel homozygous or compound heterozygous missense mutations in *TNS2* have been detected in multiple families with nephrotic syndrome [1]. Interestingly, all the mutations are situated in the PTPase domain (1 of 6) or an unknown TNS2 unique region adjacent to the C2 and SH2-PTB domains (5 of 6), but not in the SH2-PTB domain [1]. Amino acid substitutions at these domains may not impair the ability of TNS2 to localize to focal adhesion, but change its conformational and, therefore, functional state. These molecular mechanisms need further study to clarify the TNS2-interacting proteins.

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