Tetraspanin12 regulates ADAM10-dependent cleavage of amyloid precursor protein

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ABSTRACT **Using mass spectrometry, we identified ADAM10 (a membrane-associated metalloproteinase) as a partner for TSPAN12, a tetraspanin protein. TSPAN12-ADAM10 interaction was confirmed by reciprocal coimmunoprecipitation in multiple tumor cell lines. TSPAN12, to a greater extent than other tetraspanins (CD81, CD151, CD9, and CD82), associated with ADAM10 but not with ADAM17. Overexpression of TSPAN12 enhanced ADAM10-dependent shedding of amyloid precursor protein (APP) in MCF7 (breast cancer) and SH-SY5Y (neuroblastoma) cell lines. Conversely, siRNA ablation of endogenous TSPAN12 markedly diminished APP proteolysis in both cell lines. Furthermore, TSPAN12 overexpression enhanced ADAM10 prodomain maturation, whereas TSPAN12 ablation diminished ADAM10 maturation. A palmitoylation-deficient TSPAN12 mutant failed to associate with ADAM10, inhibited ADAM10-dependent proteolysis of APP, and inhibited ADAM10 maturation, most likely by interfering with endogenous wild-type TSPAN12. In conclusion, TSPAN12 serves as a novel and robust partner for ADAM10 and promotes ADAM10 maturation, thereby facilitating ADAM10-dependent proteolysis of APP. This novel mode of regulating APP cleavage is of relevance to Alzheimer's disease therapy.—Xu, D., Sharma, C., Hemler, M. E. Tetraspanin12 regulates ADAM10-dependent cleavage of amyloid precursor protein. *FASEB J.* 23, 3674–3681 (2009). www.fasebj.org**

Key Words: Alzheimer's disease · α-secretase · pericellular proteolysis

ADAM10 (kuzbanian; kuz) is a member of the ADAM (a disintegrin and metalloproteinase) family of membrane-bound zinc-dependent metalloproteinases (1). ADAM10 causes shedding of amyloid precursor protein (APP) and more than 20 other membrane-bound proteins, including pro EGF, ephrin A2, E-cadherin, CD44, and Notch (2, 3). ADAM10-deficient mice die early in embryogenesis due to deficient Notch signaling (4). During maturation, proprotein convertases (*e.g.*, PC7 and furin) cleave the N-terminal prodomain to yield active ADAM10 (5). ADAM10 association with tetraspanin proteins (CD9, CD81, and CD82) was suggested to inhibit ADAM10 proteolysis of epidermal growth factor (EGF) and/or TNF-α (6).

APP is a type I transmembrane protein, which can be processed to yield Aβ peptide, a triggering factor in Alzheimer's disease (7). To produce Aβ peptide, full-length APP is first cut by β-secretase, yielding a fragment, then further cut by γ-secretase (8). Alternatively, full-length APP is cleaved by α-secretase, releasing a soluble fragment (sAPPα) with neuroprotective properties (9). Major goals of Alzheimer's disease research are to inhibit β- and γ-secretase processing of APP (10, 11) and to enhance α-secretase activity (12). Among ADAM proteinases with α-secretase activity, ADAM10 may have the most neuroprotective potential, and strategies to enhance ADAM10 activity are being developed (9, 12, 13). Moderate neuronal overexpression of ADAM10 in transgenic mice yielded a therapeutically beneficial increase in α-secretase activity (13).

The tetraspanin protein family, containing 33 mammalian members, is defined by characteristic conserved amino acids and specific structural features, including 4 transmembrane domains (14, 15). Widely expressed tetraspanin proteins regulate immune-cell and platelet functions, fertilization, and development in brain, skin, kidney, and nervous system (15–18). Tetraspanins and protein partners function within complexes called tetraspanin-enriched microdomains (TEMs) (14, 15, 19). Tetraspanin TSPAN12 (NET2) has an atypically long C-terminal cytoplasmic tail (~60 aa) and is widely expressed on many cell and tissue types (20). TSPAN12, acting together with other tetraspanin proteins, has an incremental positive effect on the cell-surface expression and function of MT1-MMP (21). Nothing else is known regarding TSPAN12 functions. To provide insight into TSPAN12 functions, we first sought to identify its transmembrane protein partners. Using mass spectrometry, we identified ADAM10 among several putative TSPAN12 partners. Then we showed that TSPAN12 associates with mature ADAM10, promotes ADAM10 maturation, and enhances ADAM10 dependent cleavage of APP.

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doi: 10.1096/fj.09-133462

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MATERIALS AND METHODS

Cells and antibodies

Cell lines from American Type Culture Collection (Manassas, VA, USA) were maintained as described previously (22). Antibodies to ADAM10 (mAb MAB1427; R&D Systems, Minneapolis, MN, USA; polyclonal Ab1997; Abcam, Cambridge, MA, USA) and ADAM17 (mAb 111633; R&D) recognize both pro and active forms. Anti-FLAG antibodies were mAb M2, M2-agarose, and pAb F7425 (Sigma, St. Louis, MO, USA). Other antibodies were mAbs 6E10 and 22C11 (Chemicon, Temecula, CA, USA) to APP; mAb Tub2.1 (Sigma) to β-tubulin; pAb 632460 (Clontech, Palo Alto, CA, USA) to GFP; mAb 5C11 (23) to CD151, and mAb 8G6 (22) to CD147. Antibodies to tetraspanins CD9, CD81, and CD82 were as described previously (23). Despite several attempts, no useful anti-TSPAN12 antibodies have been produced.

cDNAs

cDNA for human TSPAN12 was amplified using reverse transcriptase (Promega, Madison, WI, USA) and Pfu polymerase (Stratagene, La Jolla, CA, USA). Total RNA was purified from HT1080 cells (RNeasy kit; Qiagen, Valencia, CA, USA). RT primers were sense: 5′-GGG AGT AGG ATG TGG TGA AAG GA-3′; antisense: GTA CAT ACT ATG TGT TTCAGA AAT ATG. PCR primers were sense: 5′-CCG CTC GAG GGC ACC ATG GCC AGA GAA GAT TCC GTG AAG (with *Xho*I at the 5′ end); antisense: 5′-CCG GAT CCT CAC TTG TCA TCG TCA TCC TTG TAA TC*G CCC CC*G CCT AAC TCC TCC ATC TCA AAG TGT GTA-3′ (with *Bam*H I at 5′ end; one FLAG-tag at C-terminus). The PCR fragment for TSPAN12 and cDNAs for CD81, CD9, CD151, CD82, SDFR1, and CD147 were inserted into retrovirus vector pLXIZ (*Xho*I/*Bam*H I), and some also were inserted into plasmid AcGFP1 (Clontech) with C-terminal GFP. Cysteines 9, 12, and 83 [in TSPAN12(Pal$^-$)] and 9, 227 and 228 [in CD81(Pal$^-$)] were changed to serine. Stable cell lines were established as described previously (22).

LC-MS/MS

HT1080 cells stably expressing human TSPAN12-FLAG and CD81-FLAG (FLAGs at C terminus) were lysed. Proteins were purified using anti-FLAG beads and resolved by SDS-PAGE (0.5 cm in length). Then the entire area was in-gel digested, and proteins were identified using LC-MS/MS as described previously (22), at the Taplin Biological Mass Spectrometry Facility (Harvard Medical School).

Immunoprecipitation, Western blotting and [^{3}H]palmitate labeling

Cultured cells were lysed at 0°C (25 mM HEPES, pH 7.5; 150 mM NaCl; and 2.5 mM EDTA) with either 1% Brij 96 (Fluka AG, Buchs, Switzerland), or 0.5 or 1% Brij 97 (Sigma) or 1% Triton X-100 (Roche Applied Science, Indianapolis, IN, USA) plus proteinase inhibitor cocktail (Roche Applied Science). Immunoprecipitation, Western blotting, and DSP cross-linking were as described previously (22). [^{3}H]palmitate labeling and detection was as described previously (24). Relative band densities were quantitated using ImageQuant TL software (GE Healthcare, Piscataway, NJ, USA).

SiRNA-mediated gene silencing

SiRNA duplexes, transfected using Lipofactamine 2000 (Invitrogen, Carlsbad, CA, USA), were used to knock down human TSPAN12 (Dharmacon Inc., Chicago, IL, USA; catalog no. M-012466-00 for NM_012338) and ADAM10 (Dharmacon; M-004503-01 for NM_001110). Tetraspanins CD151, CD81, and CD9 were depleted as described previously (21, 25). For TSPAN12, siRNA transfection (of 30–40% confluent cells) was at d 0 and again at d 3, and experiments were done at d 5 and 6. For ADAM10, experiments were performed 3 d after transfection. Silencing efficiency was evaluated using RT-PCR and/or Western blotting.

Immunofluorescence localization of TSPAN12-GFP

After culture for 24–48 h on slide chambers (BD Falcon; BD Biosciences, Franklin Lakes, NJ, USA), subconfluent cells were washed with PBS and fixed (3.7% PFA, 15 min). After blocking (1 h in PBS containing 1% BSA and 2% goat serum at 25°C) and washing (3 times with PBS), primary antibody (1:50–100 dilution in 1% BSA in PBS) was added for 1 h at room temperature. Then, after 3 washes, secondary antibodies were added at 1:100 (goat anti-rabbit IgG Alexa 594 or Alexa Fluor 488 goat anti-mouse in 1% BSA in PBS) for 1 h at 25°C. After 3 PBS washes, coverslips were air-dried and mounted (in solution containing DAPI; Vector Laboratories, Burlingame, CA, USA). Fluorescence images were acquired at 1-μm depth intervals, by confocal microscopy (Zeiss LMS510; Carl Zeiss, Oberkochen, Germany) at Harvard NeuroDiscovery Center. Staining was repeated ≥3 times.

APP detection

Cells were washed 3 times in PBS and incubated in serum-free medium (3–4 h). Then medium was concentrated 2-fold (Microcon filter; Millipore, Bedford, MA, USA; 10-kD cutoff). Samples were run on 4–20% precast gradient gel (Invitrogen) before APP blotting. To confirm cell equivalency, cells were lysed (1% Triton X-100 buffer), and proteins were quantified using a DC Protein Assay kit (Bio-Rad, Hercules, CA, USA).

RESULTS

TSPAN12 interacts with ADAM10 and other transmembrane proteins

Putative TSPAN12 partners (**Table 1**; identified by LC-MS/MS) include TSPAN12 itself and ADAM10. MMP14 (MT1-MMP) association is consistent with prior results (21). Other possible TSPAN12 partners (CD44, CD29, and CD49c) were shown previously to associate with other tetraspanin proteins (26–29), but TSPAN12 association still needs to be confirmed. Coimmunoprecipitation experiments did not confirm TSPAN12 associations with CD13, CD71, ADAM17, and EGFR. Associations with EphA2, CA08, LRP10, CAV1, and TR10B remain to be confirmed. TSPAN12 and CD81 partners (Table 1) are almost completely nonoverlapping, indicating specificity.

Confirming TSPAN12-ADAM10 association, TSPAN12 immunoprecipitation from HT1080 and MDA231 cells readily yielded endogenous ADAM10 (**Fig. 1*A*, *B***; lane 3).

TABLE 1. *Membrane protein complexes with TSPAN12 and CD81*

Name (protein ID)	TSPAN12	CD81
TSPAN12 (O95859)[a]	5	0
MMP14 (P50281)[b]	10	0
CD29 (P05556) (integrin β1)[c]	6	0
CD49c (P26006) (integrin α3)[c]	3	0
ADAM10 (O14672)[c]	4	0
CD44 (P16070)[d]	9	0
CD13 (P15144)[e,g]	3	1
CD71 (P02786)[e]	5	0
ADAM17 (P78536)[e]	3	0
EGFR (P00533)[e]	2	0
EphA2 (P29317)[f]	6	0
CA08 (Q9bxs4)[f]	3	0
LRP 10 (Q7z4f1)[f]	3	0
CAV1 (Q03135) (caveolin 1)[f]	2	0
TR10B (O14763)[f]	2	0
CD81 (P60033)[a]	0	1
EWIF/FPRP (Q9P2B2)[h]	0	12

Values represent number of distinct peptides. Complete peptide information is given in Supplemental Table S1. [a]As expected, TSPAN12 and CD81 yield TSPAN12 and CD81 peptides. [b]Association with MMP14 (MT1-MMP) has been seen elsewhere (21). [c]Association has been confirmed by coimmunoprecipitation. [d]TSPAN12-CD44 association is consistent with CD44 associating with other tetraspanins (47) and being a substrate for ADAM10 (48). [e]Coimmunoprecipitation experiments did not confirm interactions with TSPAN12. [f]These TSPAN12 interactions have not been tested further. [g]Interactions of CD13 with CD81 have been confirmed by coimmunoprecipitation. [h]EWIF/FPRP interaction with CD81 has been seen previously (49, 50).

Despite comparable expression (bottom panels), tetraspanin CD81 yielded much less ADAM10 (Fig. 1*A*, lane 6; *B*, lane 4), while tetraspanin CD82 (Fig. 1*B*, lane 5) and transmembrane protein SDFR1 (Fig. 1*A*, lane 5) yielded no detectable ADAM10. Tetraspanins CD9 and CD151 also did not associate with ADAM10 (not shown). Pro-ADAM10 (~98 kDa) was abundant in whole-cell lysates (Fig. 1*A*, *B*; lane 1), and both pro- and mature ADAM10 could be readily immunoprecipitated using anti-ADAM10 antibody (Fig. 1*C*). However, only mature ADAM10 (~68 kDa) associated with TSPAN12 (Fig. 1*A*, *B*; top panels). In reciprocal experiments, immunoprecipitation of ADAM10 endogenously expressed in HT1080 cells yielded abundant TSPAN12, lesser CD81, and no SDFR1 (Fig. 1*D*). By RT-PCR, overexpressed TSPAN12 was only ~2-fold greater than endogenous TSPAN12 in HT1080 cells (Supplemental Fig. S1) and in other cell lines (not shown).

For use in functional studies, we sought to identify minimally mutated TSPAN12, no longer associating with ADAM10. TSPAN12 C-terminal deletion mutants (Δ267 and Δ261), and CD81(N′-LEL)/TSPAN12 lost ADAM10 association (**Fig. 2*A***, lanes 4, 5, 8). Other TSPAN12 mutants [CD81(N′-SEL/TSPAN12) and TSPAN12(R88E)], partially lost ADAM10 association (Fig. 2*A*, lanes 7, 9). Palmitoylation-deficient [TSPAN12(Pal$^-$)] was prepared by mutating membrane-proximal cysteines 9, 12, and 83 to serine. TSPAN12(Pal$^-$) showed minimal ADAM10 association (Figs. 1*A*, lane 4, and 2*A*, lane 6). Anti-FLAG immunoblotting confirmed the presence of mutants in HT1080 lysate (Fig. 2*A*, bottom panel). ADAM10 association results are summarized in Fig. 2*B*. Also, all mutants are indicated schematically in Fig. 2*B* and described in more detail in Supplemental Table S2. Because TSPAN12(Pal$^-$) showed maximal loss of ADAM10 association with minimal change to the native tetraspanin protein, it was chosen for further study. TSPAN12, but not TSPAN12(Pal$^-$), incorporated [^{3}H]palmitate (Supplemental Fig. S2*A*, lanes 1, 4). Following biotinylation of intact cells, we determined that TSPAN12(Pal$^-$) expression on the cell surface was ~64% reduced, compared to TSPAN12 (Supplemental Fig. S2*B*, lanes 1, 2), with both abundantly expressed in total cell lysate (Supplemental Fig. S2*C*, lanes 1, 2).

TSPAN12 affects ADAM10 function

To assess function, we focused on ADAM10-dependent APP shedding, which releases a fragment of ~110–120

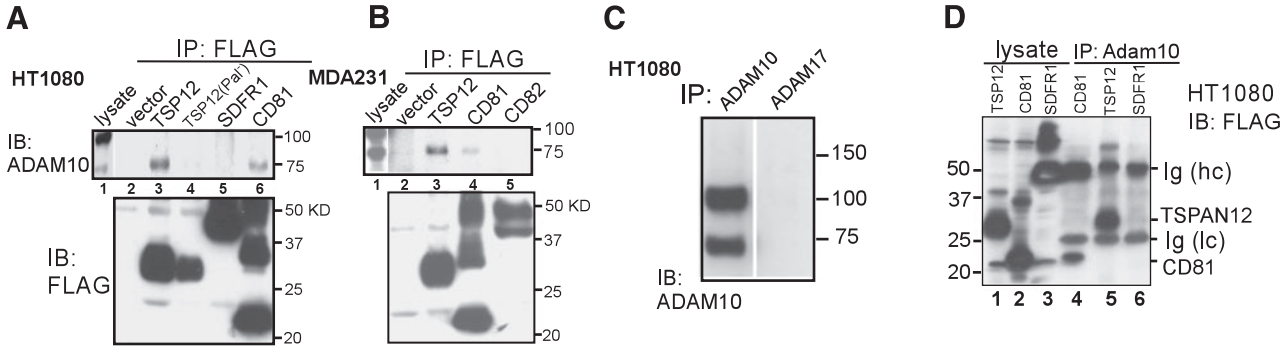
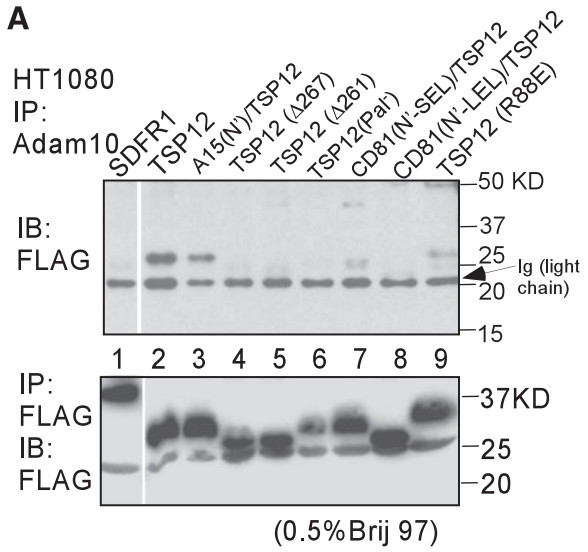


Figure 1. Confirmation of ADAM10-TSPAN12 association. *A*, *B*) Indicated FLAG-tagged proteins were immunoprecipitated from HT1080 (*A*) and MDA231 cells (*B*). ADAM10 (top panel) and FLAG-tagged proteins (bottom panel) were detected by immunoblotting. Pro-ADAM10, 95 kDa; mature ADAM10, ~68 kDa. *C*) Endogenously expressed ADAM10 (MAB1427) and ADAM17 were immunoprecipitated from HT1080 cells, after cells were lysed in 0.5% Brij 97. Resulting proteins were detected using anti-ADAM10 polyclonal antibody. Presence of ADAM17 was confirmed using anti-ADAM17 polyclonal antibody (not shown). *D*) HT1080 cells stably expressing the indicated FLAG-tagged proteins were lysed in 0.5% Brij 97. Anti-FLAG immunoblotting was used to detect proteins in lysates (lanes 1–3) and in complexes after ADAM10 was immunoprecipitated using mAb MAB1427 (lanes 4–6). CD81 appears as dimer and monomer, as described previously (32). Ig(hc), immunoglobulin heavy chain; Ig(lc), immunoglobulin light chain.



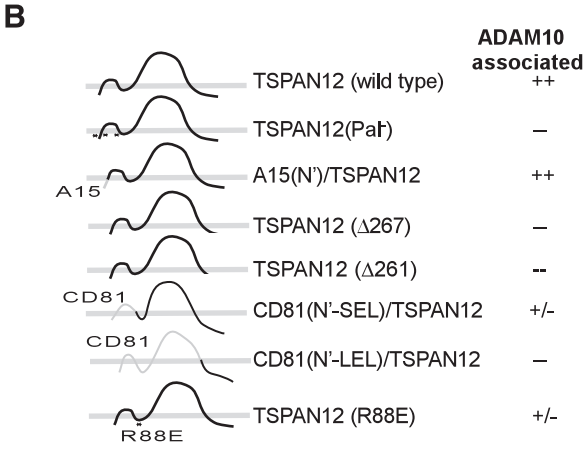


Figure 2. TSPAN12 mutations variably disrupt ADAM10 association. *A*) HT1080 cells stably expressing FLAG-tagged proteins were lysed (0.5% Brij 97), and then ADAM10 (top panel) and FLAG-tagged proteins (bottom panel) were immunoprecipitated. Proteins were then detected by anti-FLAG immunoblotting (both panels). *B*) ADAM10 association is summarized for TSPAN12 mutants. SEL, small extracellular loop; LEL, large extracellular loop. Additional mutant information is in Supplemental Table S2.

kDa (30, 31). Anti-FLAG immunoblotting confirmed stable expression of TSPAN12-FLAG and control FLAG in MCF7 (breast cancer) and SH-SY5Y (neuroblastoma) cell lines (not shown). TSPAN12 overexpression stimulated, by 120–160%, release of APP fragments of ~110 kDa (**Fig. 3*A***, lane 2). By contrast, TSPAN12(Pal$^-$) inhibited APP shedding (32–36% of control cells) in both MCF7 and SH-SY5Ycell lines (Fig. 3*A*, lane 3, both panels). Palmitoylation-deficient CD81 [CD81(Pal$^-$)], CD81, and CD9 had only marginal effects on APP shedding (Fig. 3*A*).

Ablation of endogenous ADAM10 (by>95%; Supplemental Fig. S3*A*) from SH-SY5Y and MCF7 cells reduced APP shedding to 13–21% of control levels (Fig. 3*B*). Knockdown of endogenous TSPAN12 (Supplemental Fig. S4) also reduced APP shedding (Fig. 3*B*; 32–43% of control), whereas knockdown of tetraspanins CD81 and CD82 (by 90–95%; refs. 21, 25) had less of an inhibitory effect (Fig. 3*B*). Reduced APP shedding due to ADAM10 and TSPAN12 knockdown was confirmed (Supplemental Fig. S5) using siRNAs with sequences distinct from those used in Fig. 3*B*). In summary, on multiple cell lines, ADAM10-dependent APP shedding is markedly affected by TSPAN12, to a greater extent than by other tetraspanin proteins.

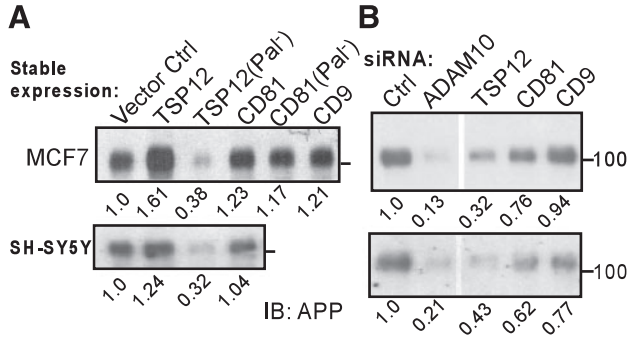


Figure 3. TSPAN12 expression influences ADAM10-dependent proteolysis of APP. *A*) Indicated FLAG-tagged proteins were expressed stably in MCF7 and SH-SY5Y cells; shed APP was detected by immunoblotting. *B*) Endogenous ADAM10 (siRNA = ADAM10–3), TSPAN12 (siRNA-1), CD81 (21) and CD9 (25) were knocked down, and shed APP was detected. Numbers below panels represent protein density scanning results.

TSPAN12 affects ADAM10 maturation

Not only did TSPAN12 associate preferentially with active ADAM10 (Figs. 1, 2), but also it promoted ADAM10 maturation (**Fig. 4*A***). Expression of additional TSPAN12 in MCF7 cells increased the ratio of mature/precursor ADAM10 from ~3.5 to ~5.4, as detected in cell lysates. Conversely, TSPAN12(Pal$^-$)

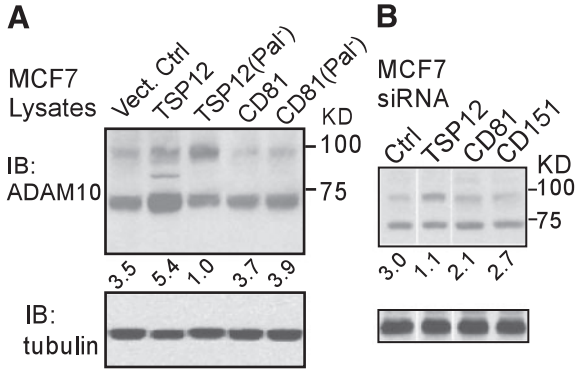


Figure 4. TSPAN12 expression influences ADAM10 maturation. *A*) MCF7 cells expressing the indicated FLAG-tagged proteins were lysed (0.5% Brij 97), and ADAM10 and tubulin were detected by immunoblotting. The ~85-kDa protein in the TSPAN12 lane likely represents an atypical form of ADAM10 that has been intermediately processed. *B*) MCF7 cells were treated with siRNAs (as in Fig. 3*B*), and then lysed and immunoblotted as in *A*. Numbers below panels represent ADAM10 active/prodomain ratios.

decreased the maturation ratio (from ~3.5 to ~0.96), while control proteins [CD81 and CD81(Pal$^-$)] had minimal effect on ADAM10 processing (Fig. 4*A*). Similar results were seen in 1% Brij 97 (Fig. 4*A*) and Triton X-114 (not shown) detergent lysates. Conversely, siRNA-mediated knockdown of endogenous TSPAN12 diminished ADAM10 maturation (from ~3.0 to ~1.1), while knockdown of tetraspanins CD81 and CD151 had much less of an effect (Fig. 4*B*). Overexpression, knockdown, or mutation of TSPAN12 did not affect the subcellular distribution of ADAM10 (Supplemental Fig. S6). Also, these manipulations of TSPAN12 minimally affected ADAM10 cell-surface levels (Supplemental Table S3 and Supplemental Fig. S3*B*).

TSPAN12(Pal$^-$) interferes with TSPAN12

Despite not associating with ADAM10, TSPAN12(Pal$^-$) markedly decreased ADAM10 function (APP shedding) and maturation. Since tetraspanins homooligomerize (32), we suspected that TSPAN12(Pal$^-$) might directly associate with endogenous TSPAN12, perturb its function, and thereby indirectly affect ADAM10. Indeed, we did observe direct association of TSPAN12(Pal)-GFP with TSPAN12-FLAG in MCF7 cells. Following DSP crosslinking, immunoprecipitation of TSPAN12-FLAG yielded TSPAN12(Pal$^-$)-GFP (~65 kD), under reducing conditions (**Fig. 5*A***, lane 8). The sharp band corresponding to TSPAN12(Pal$^-$)-GFP was not seen under the following conditions: DSP treatment was omitted (Fig. 5, lane 3); TSPAN12-FLAG was replaced with SDFR1-FLAG (Fig. 5, lanes 9, 4); TSPAN12-FLAG was replaced with vector alone (Fig. 5, lanes 10, 5); TSPAN12(Pal$^-$)-GFP was replaced with CD81(Pal$^-$)-GFP (Fig. 5, lanes 6, 11).

In addition, we showed that non-GFP TSPAN12(Pal$^-$) alters the distribution of TSPAN12-GFP. TSPAN12-GFP is mostly membrane-proximal in fixed MCF7 cells (Fig. 5*Ba*). However, expression of TSPAN12(Pal$^-$)-FLAG (Fig. 5*Bc*), but not control (SDFR1-FLAG; Fig. 5*Bb*), caused TSPAN12-GFP redistribution into a punctate pattern. When TSPAN12(Pal$^-$) was itself GFP tagged, it showed punctate distribution (Fig. 5*Bd*). Hence, TSPAN12(Pal$^-$) associates with wild-type TSPAN12 and dominantly affects its subcellular localization.

DISCUSSION

ADAM10, a cell surface metalloproteinase, cleaves >20 different membrane proteins, including growth factors, growth factor receptors, adhesion molecules, and various other signaling molecules (3). ADAM10 plays critical roles during human embryonic development (4), cancer (3), diabetes (33), and Alzheimer's disease (13). Here we show that ADAM10 associates preferentially with TSPAN12, a tetraspanin protein. In addition, we used three different molecular approaches (TSPAN12 overexpression, knockdown, and mutation), to show that TSPAN12 enhances ADAM10 maturation and promotes ADAM10-dependent cleavage of APP, a key contributor to Alzheimer's disease.

Cell surface association

ADAM10-TSPAN12 association, first demonstrated in a mass spectrometry search for TSPAN12 partners, was confirmed in multiple cell lines, by reciprocal coimmunoprecipitation. ADAM10-TSPAN12 association was specific, as other tetraspanins (CD81, CD9, and CD151)

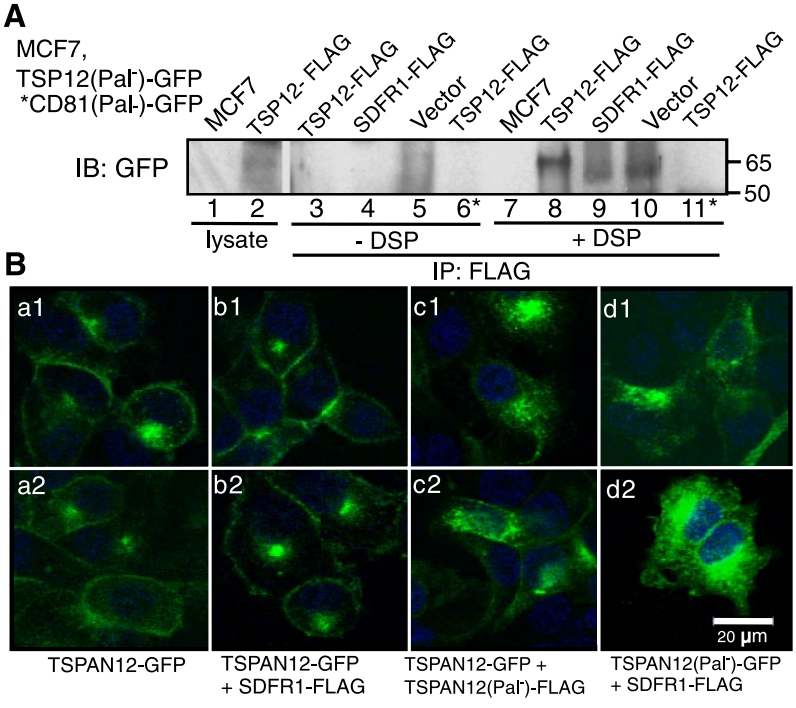


Figure 5. TSPAN12(Pal$^-$) mutant associates with wild-type TSPAN12 and perturbs its subcellular distribution. *A*) TSPAN12(Pal$^-$)-GFP (lanes 2–5, 7–10) or CD81(Pal$^-$)-GFP (lanes 6, 11) were expressed in MCF7 cells, together with vector-FLAG (lanes 5, 10) or other FLAG-tagged proteins (lanes 2–4, 6, 8, 9, 11). Lanes 1 and 7 are from untransfected MCF7 (no GFP or FLAG proteins). After lysis (1% Triton X-100) MCF7 cells were treated with (lanes 7–11) or without (lanes 1–6) covalent cross-linker DSP. After anti-FLAG immunoprecipitations, reduction of the dithiol cross-link, and SDS-PAGE, proteins were detected by GFP immunoblotting. Diffuse proteins of 50–60 kDa in lanes 5, 9, and 10 likely represent background proteins, immunoprecipitated by anti-FLAG antibody (even when no FLAG proteins are present, as in lanes 5, 10), which also weakly cross-react with anti-GFP antibody. *B*) Either TSPAN12-GFP (*a–c*) or TSPAN12(Pal$^-$)-GFP (*d*) was expressed in MCF7 cells, together with indicated FLAG-tagged proteins. FLAG- and GFP-tagged tetraspanins were expressed at comparable levels, as seen by immunoblotting (not shown).

did not associate to the same extent as TSPAN12. However, in Brij 97 lysates elsewhere (6), ADAM10 did associate with other tetraspanins (CD9, CD53, CD63, CD81, and CD151). This discrepancy may be explained by our lysates having minimal divalent cation levels, which can limit the range of tetraspanin protein associations (34). ADAM17, which is more structurally and functionally similar to ADAM10 than any other ADAM protein (1, 35), appeared in Table 1 as a possible TSPAN12 partner. However, further experiments by us (not shown) and by others (6) did not confirm ADAM17-tetraspanin association. TSPAN12 association with CD13 and CD71 was likewise not confirmed. TSPAN12 association with MMP14 (MT1-MMP) was confirmed elsewhere (21). Other TSPAN12 associations in Table 1 remain to be confirmed.

The TSPAN12 large extracellular loop, intracellular C-terminal tail, and palmitoylation sites all contributed to ADAM10 association. Large extracellular loop and C-terminal tail contributions are consistent with tetraspanin proteins having a rod-like structure (36), with extracellular and intracellular sites contacting partner proteins (18). Interactions occurring within TEMs are stabilized by tetraspanin palmitoylation (14, 19, 34, 37). The contribution of TSPAN12 palmitoylation to ADAM10 association indicates that TSPAN12-ADAM10 complexes likely occur within the context of TEMs.

TSPAN12 promotes ADAM10 function

APP, which is widely expressed on most cell and tissue types (http://www.genecards.org), undergoes ADAM10-dependent shedding (38), as confirmed here by siRNA knockdown of endogenous ADAM10. ADAM10-dependent APP proteolysis was promoted by TSPAN12 overexpression and inhibited by knockdown of endogenous TSPAN12, indicating that TSPAN12-ADAM10 complexes are functionally important. The TSPAN12(Pal$^-$) mutant, which does not associate with ADAM10, did not stimulate function. Instead, TSPAN12(Pal$^-$) inhibited function, through a dominant negative-like effect on endogenous TSPAN12.

In a prior study, tetraspanins CD9, CD81, and CD82 were suggested to have a negative effect on ADAM10-dependent proteolysis of TNF-α and HB-EGF, which was overcome by anti-tetraspanin antibodies (6). In another case, CD9 suppressed proteolysis of transmembrane TNF-α, mediated by an unspecified protease (39). Here we observed only positive effects of TSPAN12 on ADAM10 function. Hence, compared to other tetraspanin proteins, TSPAN12 not only associates more proximally with ADAM10, but also exerts a different functional effect. We predict that TSPAN12 will promote ADAM10-dependent proteolysis of many, if not all, of its other membrane protein substrates (3). In this regard, another ADAM10 substrate, CD44 (40), may also associate with TSPAN12 (Table 1). Furthermore, shedding of ADAM10 substrate L1 (41), a cell-surface adhesion molecule, is supported by TSPAN12 (unpublished results). We confirmed that TSPAN12 is present at moderate levels in 7 of 7 different cell lines tested (by RT-PCR, not shown). The wide cell distributions of TSPAN12 and ADAM10 (see http://www.genecards.org) are consistent with functional interactions in multiple settings. TSPAN12 also supported functions of another protease (MMP14/MT1-MMP), although in that case, the incremental positive role of TSPAN12 depended on substantial augmentation by other tetraspanins (21).

Mechanistic insights

TSPAN12 manipulation (overexpression, knockdown, and mutation) did not affect total ADAM10 levels in the cell, or on the cell surface. However, TSPAN12 associated selectively with mature, processed ADAM10, and promoted maturation. ADAM10 matures in the trans-Golgi network, with maturation leading to increased APP shedding (5). Hence, TSPAN12 must promote ADAM10 function in a post-Golgi compartment. We suspect that TSPAN12 stabilizes active ADAM10 and/or accelerates ADAM10 activation by prodomain convertase. TSPAN12(Pal$^-$) inhibited both ADAM10 function and maturation, even though TSPAN12(Pal$^-$) failed to associate with ADAM10. Instead, TSPAN(Pal$^-$), which itself is mostly not at the cell surface, associated directly with wild-type TSPAN12, and partly diverted it from the plasma membrane. Hence, TSPAN(Pal$^-$) appears to impair ADAM10 maturation and function indirectly, by a dominant negative-like effect on endogenous TSPAN12. In the case of MT1-MMP, TSPAN12 and other tetraspanins protected newly synthesized protein (both pro and active) from degradation, thereby increasing total cell surface levels, without accelerating maturation (21). Although tetraspanins promote biosynthetic maturation of partner proteins, such as CD19 (42), and EWI-2 (43), we could find no other examples of tetraspanins promoting prodomain processing.

Therapeutic implications

ADAM10 is a major α-secretase, responsible for nonamyloidogenic processing of APP. Hence, amplification of ADAM10 function should lead to therapeutically desirable reduction in brain β-amyloid deposits (12). Strategies for amplifying ADAM10 α-secretase activity thus far include ADAM10 overexpression (13), overexpression of PC7 or furin to accelerate ADAM10 maturation (5), and retinoic acid induction of the ADAM10 promoter (31). Because TSPAN12 promotes ADAM10 α-secretase activity, agents stimulating TSPAN12 function or promoting TSPAN12-ADAM10 association should be therapeutically beneficial. As seen for other tetraspanins, soluble large loops and antitetraspanin antibodies can have agonistic effects (18). It remains to be seen whether TSPAN12 works together with other proteins supporting ADAM10 α-secretase activity, such as the intracellular neuronal protein SAP97 (44), and several G protein coupled receptors (31, 45).

In some cases, such as on tumor cells, it may be desirable to inhibit ADAM10 activity (3). This can be

achieved using soluble ADAM10 prodomain (30), dominant-negative ADAM10 (38), and hydroxamate-based metalloproteinase inhibitors (46). As shown here, targeting TSPAN12 should also diminish ADAM10 activity. This could be achieved by expression of an inhibitory TSPAN12 mutant [*e.g.*, TSPAN12(Pal$^-$)], or by RNAi knockdown, inhibitory antibodies, or inhibitory tetraspanin soluble large loop protein, such as seen for other tetraspanins (18).

CONCLUSIONS

We describe here an atypical tetraspanin protein, TSPAN12, which associates selectively with ADAM10, leading to accelerated ADAM10 activation and enhanced nonamyloidogenic shedding of APP. These results, together with others (6), indicate that ADAM10 functions in the context of TEMs. Promotion of TSPAN12-ADAM10-dependent functions should be therapeutically beneficial in Alzheimer's disease, whereas inhibition of TSPAN12-ADAM functions may be beneficial in cancer. FJ

This work was supported by National Institutes of Health grants GM38903 and CA102034 to M.E.H.

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Received for publication March 10, 2009.
Accepted for publication June 11, 2009.