Identification of Tctex2β, a Novel Dynein Light Chain Family Member That Interacts with Different Transforming Growth Factor-β Receptors*

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Endoglin is a membrane-inserted protein that is preferentially synthesized in angiogenic vascular endothelial and smooth muscle cells. Endoglin associates with members of the transforming growth factor-β (TGF-β) receptor family and has been identified as the gene involved in hereditary hemorrhagic telangiectasia. Although endoglin is known to affect cell responses to TGF-β, its mode of action is largely unknown. We performed yeast two-hybrid screening of a human placental cDNA library and isolated a new endoglin-binding partner, a novel 221-amino acid member of the Tctex1/2 family of cytoplasmic dynein light chains named Tctex2β, as the founder of a new Tctex1/2 subfamily. The interaction was localized exclusively to the cytoplasmic domain of endoglin. Reverse transcription-PCR showed expression of Tctex2β in a wide range of tissues, including vascular endothelial and smooth muscle cells, placenta, and testis, as well as in several tumor cell lines. High expression levels were found in human umbilical vein endothelial cells and the large cell lung cancer cell line. Forced expression of Tctex2β had a profound inhibitory effect on TGF-β signaling. Additional Tctex2β-interacting receptors were identified to be the TGF-β type II receptor and most likely betaglycan, but not ALK5, ALK1, or the bone morphogenetic protein type II receptor. Upon fluorescence tagging, co-localization of Tctex2β and endoglin, as well as Tctex2β, endoglin, and the TGF-β type II receptor, was observed by different microscopy techniques. Our findings link endoglin for the first time to the vascular disorder hereditary hemorrhagic telangiectasia type 1, an autosomal dominant inherited vascular disorder characterized by multi-systemic vascular dysplasia and recurrent hemorrhage (13). Mice embryos homozygoously deleted of endoglin are unable to progress beyond embryonic days 10.5–11.5, with failure to form mature blood vessels in the yolk sac, poor vascular smooth muscle development, and arrested endothelial remodeling (14–16).

TGF-β is involved in vascular development and homeostasis with both pro- and anti-angiogenic effects (17, 18). Upon ligand binding, TBR II recruits, phosphorylates, and activates the ALK5 (activin receptor-like kinase-5) type I receptor. Activated ALK5 phosphorylates the receptor Smads (Smad2/Smad3), which are then translocated to the nucleus, where they act as co-transcription factors on specific genes (19). In endothelial cells, a second type I receptor exists, ALK1. ALK5 and ALK1 mediate, in a concentration-dependent manner, TGF-β signaling with opposing effects (20, 21). Mutations in ALK1 also lead to the vascular disorder hereditary hemorrhagic telangiectasia type 2 (13). ALK1 signaling is propagated through Smad1/Smad5, promoting vascular endothelial cell proliferation and migration (“activation phase”), whereas ALK5 signaling suppresses endothelial cell proliferation and induces smooth muscle differentiation (“resolution phase”) (18). Thus, angiogenesis

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9 The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) DQ132241.

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5 The abbreviations used are: TGF-β, transforming growth factor-β; TβRII, transforming growth factor-β type II receptor; DCL, dynein light chain; HA, hemagglutinin; wt, wild-type; ECFP, enhanced cyan fluorescent protein; EGFP, enhanced green fluorescent protein; X-gal, 5-bromo-4-chloro-3-indolylβ-D-galactopyranoside; RACE, rapid amplification of cDNA ends; RT, reverse transcription; HUVECs, human umbilical vein endothelial cells; LCLC, large cell lung cancer; TRITC, tetramethylrhodamine isothiocyanate; EST, expressed sequence tag; BMPRII, bone morphogenetic protein type II receptor; ActRIIA, activin type IIA receptor.
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seems to be regulated in part by an ALK1/ALK5 signaling balance.

Recent findings suggest that endoglin is involved in the fine-tuning of this balance between TGF-β/ALK1 and TGF-β/ALK5 signaling. Endoglin interacts with TβRII, ALK5, and ALK1 both in vitro and in vivo (11, 22, 23). Ectopic expression of endoglin in several cell types inhibits the TGF-β/ALK5 signaling pathway and counteracts TGF-β-induced growth inhibition, but promotes TGF-β/ALK1 signaling (24–27). Of note is the discrepancy that, although the extracellular domain of endoglin is involved in and required for both pathways, the relatively short cytoplasmic domain of 47 amino acids is required only for TGF-β/ALK5 (but not TGF-β/ALK1) signaling (24). Thus, this domain appears to have a key role in regulating the signaling balance in endothelial cells.

The cytoplasmic domain of endoglin is highly conserved in human, rat, and mouse with 99% identity, and there is 71% homology between the corresponding domains in human endoglin and betaglycan. Recent findings have identified the LIM domain proteins ZRP-1 (zyxin-related protein-1) and zyxin as intracellular partners for the cytoplasmic domain of endoglin (28, 29). Interaction of ZRP-1 or zyxin with endoglin influences the intracellular distributions of proteins involved in the organization of the actin cytoskeleton or the assembly of focal adhesions, respectively. However, the mechanisms by which endoglin achieves these functions remain unknown.

To gain more understanding of the role of endoglin in TGF-β signaling and angiogenesis, we performed yeast two-hybrid screening to identify cellular binding partners for this protein. We report here the interaction between a novel Tctex1/2 (t-complex testis-expressed protein-1/2)-like protein, designated Tctex2β, and the cytoplasmic domain of endoglin as well as TβRII. To the best of our knowledge, this is the first evidence that endoglin can interact with a member of the dynein light chain (DLC) protein family.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Cell media and fetal bovine serum were purchased from Invitrogen and PAA Laboratories. HEK293, NIH3T3, HeLa, COS-1, HepG2, and mink lung epithelial cells (Mv1Lu) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, penicillin/streptomycin, and l-glutamine. Primary human dermal microvascular endothelial cells were obtained from Promocell (Heidelberg, Germany) and cultured in endothelial cell basal medium MV with SupplementPack MV. All cell lines were cultured at 37 °C in a humidified 5% CO₂ environment.

**Antibodies and Cytokine**—His-tagged proteins were detected in immunoblots or precipitated with anti-His monoclonal antibody (Serotec, GmbH, Germany). Hemagglutinin (HA)-tagged proteins were detected in immunoblots with anti-HA monoclonal antibody 12CA5 (22). Endoglin was immunodetected or precipitated with anti-endoglin polyclonal antibody (22), TGF-β1 and TGF-β3 were purchased from R&D Systems (Wiesbaden, Germany).

**Expression Constructs**—For the yeast two-hybrid screening and the following growth assay, the coding sequences of the cytoplasmic domain of human endoglin (amino acids 612–658, Endo<sup>cyto</sup>), a truncated form of endoglin lacking the cytoplasmic domain (amino acids 1–611, Endo<sup>Pcyto</sup>), and full-length wild-type (wt) endoglin (amino acids 1–658, Endo<sup>wt</sup>) were PCR-amplified from pCMV5-endoglin (30) and cloned in-frame into pGPT9-2 (Clontech), resulting in the pGPT9-Endo<sup>cyto</sup>, pGPT9-Endo<sup>Pcyto</sup>, and pGPT9-Endo<sup>wt</sup> constructs, respectively. The cytoplasmic domains of different TGF-β type II (pGPT9-TβRII<sup>cyto</sup>), amino acids 186–567; pGPT9-ActRIIA<sup>cyto</sup>, amino acids 162–513; and pGPT9-BMPRII<sup>cyto</sup>, amino acids 530–1038) and type I (pGPT9-ALK5<sup>cyto</sup>), amino acids 148–503; pGPT9-ALK1<sup>cyto</sup>, amino acids 142–503; and pGPT9-caALK1<sup>cyto</sup> (where “ca” is constitutively active), amino acids 142–503 with mutation Q201D, respectively) receptors were as described previously (31). The cytoplasmic domain of betaglycan was PCR-amplified from pGEX4T3-betaglycan (kindly provided by Dr. Calvin Vary) (28) and cloned into pGPT9, yielding pGPT9-betaglycan<sup>cyto</sup> (amino acids 805–849). The complete coding sequence of Tctex2β was PCR-amplified and cloned into pACT2 (Clontech), generating pACT2-Tctex2β.

For the mammalian two-hybrid assay (Clontech), the coding sequence of the endoglin cytoplasmic domain was cloned into vector pM, resulting in pM-Endo. The whole coding sequence of Tctex2β cloned into pVP16, yielding pVP16-Tctex2β. For expression in mammalian cells and immunoprecipitation assays, the full coding sequence of Tctex2β was fused to the N-terminal His<sub>6</sub> tag of the pcDNA4/HisMax vector (Invitrogen), resulting in His-Tctex2β. Human endoglin and HA-tagged TβRII (TβRII<sup>HA</sup>) are in the pCMV5 vector (22, 30). For fluorescence microscopy studies, human endoglin was fused to the C-terminal enhanced green fluorescent protein (EGFP) sequence of the pEGFP-C1 vector (Clontech), resulting in EGFP-Tctex2β. The correct sequences and reading frames of all constructs derived from PCR products were verified by DNA sequencing.

**Yeast Two-hybrid Screening and Growth Assay**—A yeast strain (P69-4A) based on the Gal4 system was used (32). Yeast media were purchased from Clontech. Yeast transformations were performed using a standard lithium acetate method as recommended in the Matchmaker yeast protocol handbook (Clontech). For library screening, a sequential transformation was performed with first the bait plasmid (pGPT9-Endo<sup>cyto</sup>) and then with 50 μg of human placental library cDNA (cloned into the pACT2 vector). The co-transformants were streaked onto appropriate selective media plates. To determine the strength of interactions, high stringency synthetic medium lacking histidine and adenine but containing X-gal was also used. Interacting clones were selected by their abilities to grow on or turn blue on appropriate selective plates. Plasmid DNA from positive yeast clones was rescued into bacterial strain KC8 (Clontech) and sequenced using a poly(T) sequencing primer. The resultant sequences were checked for similarity to known transcripts in the nucleotide sequence data bases using the BLAST algorithm. For yeast two-hybrid growth assays, yeast cells were sequentially transformed first with endoglin, beta-
glycan, or different type I/II receptor constructs and then with pACT2-Tctex2β. Phenotypes of yeast co-transformants on selective media were tested.

**Mammalian Two-hybrid Assay**—HEK293 cells were seeded 1 night before transfection at an initial density of 1.5 × 10⁵ cells/well in 6-well plates in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum. Prior to transfection, cells were switched to medium containing 5% fetal bovine serum to reduce the endogenous level of alkaline phosphatase in the serum. Upon reaching 50–60% confluence, cells were transfected with the pG5SEAP reporter construct (0.3 µg) and a specific combination of pM-Endo (1.5 µg)/pVP16-Tctex2β (1.5 µg) or pM (1.5 µg)/pVP16 (1.5 µg) as a negative control using the CalPhos™ mammalian transfection kit (Clontech). 48 h after transfection, the cell culture medium was collected and subjected to secreted alkaline phosphatase activity assay using the Great EscAPe kit (Clontech). Relative light units were measured using a Berthold Lumat LB 9507 luminometer and expressed as the means ± S.E. Values from mock-transfected samples served as the basal level of alkaline phosphatase activity in the culture medium. Transfection experiments were carried out in triplicate and repeated three times.

**Immunoprecipitation and Western Blotting**—HEK293 cells were transfected with appropriate combinations of pCMV5, H₄T-Tctex2β, endoglin, and TβRIIHA as indicated. 48 h later, cells were lysed with lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and Sigma protease inhibitor mixture) at 4 °C for 30 min. The cell lysates were precleared with 20 µl of protein G-Sepharose (Sigma) for 1 h before they were immunoprecipitated overnight at 4 °C with 50 µl of protein G-Sepharose and the indicated antibodies. The immunocomplexes were then washed three times with 500 µl of lysis buffer and resolved in 20 µl of Laemmli buffer. The resulting immunoprecipitated sample and 20 µl of each total cell lysate were separated on SDS-8% (for endoglin and TβRIIHA) and 12% (for H₄T-Tctex2β) polyacrylamide gels and blotted onto nitrocellulose membranes (Bio-Rad) for immunoblotting with the indicated antibodies. Immunodetection was performed with the ECL Western blotting detection kit (Amersham Biosciences) or with the Odyssey infrared imaging system (LI-COR Biosciences).

**5’-Rapid Amplification of cDNA Ends (RACE) and Reverse Transcription (RT)-PCR**—For cloning of Tctex2β cDNA, 5’-RACE was performed with the SMART™ RACE kit (Clontech) using human placental and testis total RNAs (Clontech). RACE-PCR products were cloned into the pCR2.1-TA cloning vector (Invitrogen) and then sequenced using forward and reverse M13 sequencing primers. The Tctex2β gene-specific primer used for 5’-RACE was 5’-TTGTAGCGTGCCGCGGCTGAGCTCG-3’. For RT-PCR, total RNAs from primary human umbilical vein endothelial cells (HUVECs) and HMEC-1, HepG2, large cell lung cancer (LCLC), and T47D cells were isolated as described previously (31). Total RNA from smooth muscle cells was generously supplied by Dr. Judith Gillard (Department of Biophysics, University of Manchester). To exclude the possibility of genomic DNA contamination, total RNA was digested with RNase-free DNase I (Roche Applied Science, Mannheim, Germany) according to the manufacturer’s instructions. Subsequently, 1 µg of each total RNA was reverse-transcribed in a 50-µl volume with a mixture of oligo(dT) oligonucleotides and (dN)₆ random hexamers following the Fermentas RevertAid H Minus Moloney murine leukemia virus protocol. In parallel, negative control samples lacked Moloney murine leukemia virus. 2 µl of the resulting first-strand cDNA was PCR-amplified with appropriate primer pairs using a ReadyMix™ PCR kit (Sigma). Samples were first held at 96 °C for 5 min and then cycled 30 times at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, followed by an additional 7 min at 72 °C. PCR products were visualized by electrophoresis on 1.5% agarose gel containing ethidium bromide. Primer pairs were based on human sequences (given here from 5’ to 3’): Tctex2β, ATGGCCACAG-GCCCTCTGCC (forward) and TCACCTGCGATAGAGCCCGT (reverse); and glyceraldehyde-3-phosphate dehydrogenase, TCA-ACGGATTGGGTGTAT (forward) and ATGAGTCTCTTCCACGATAC (reverse).

**Fluorescence Microscopy and Immunofluorescence**—For fluorescence microscopy, HeLa cells were seeded on coverslips and transfected as indicated. 1 day after transfection, cells were fixed with 4% paraformaldehyde on ice, mounted on Mowiol (Dabco), and used for digital imaging.

Two-photon microscope images were obtained with the TriMscope 2-photon system for multiphoton microscopy (LaVision BioTec, Bielefeld, Germany) with a Zeiss Axiovert 200 fluorescence microscope equipped with a 63× oil objective. This system was upgraded for two-photon live-time imaging with a Chameleon XR laser (Coherent Inc.). Two-photon excitation was at 800 nm, and emission for GFP was at 530 nm and for CFP at 480 nm. Images were analyzed using ImSpector Version 3.0 software. Images obtained with the Zeiss ApoTome system were acquired with an inverted wide-field microscope (Zeiss Axiovert 200 M and Axiovision Version 05.2005 software) equipped with a 75-watt xenon lamp (Hamamatsu Photoconics), a 12-bit CCD camera (Zeiss AxioCam HRm), a 63× oil objective (Plan Neofluar, 1.25 numerical aperture), and appropriate filter sets for the different fluorophores (CFP, excitation at 436/20 nm, beam splitter at 455 nm, and emission at 480/40 nm; yellow fluorescent protein, excitation at 500/20 nm, beam splitter at 515 nm, and emission at 535/30 nm; and DsRed, excitation at 565/30 nm, beam splitter 585 nm, and emission at 620/60 nm).

Immunofluorescence was done as described (31). In brief, HeLa cells transfected with endoglin were seeded on coverslips, fixed, permeabilized, and incubated with a 1:100 dilution of the endoglin-specific monoclonal antibody SN6 (Serotec). Subsequently, cells were incubated with a TRITC-labeled anti-mouse secondary antibody (Molecular Probes). After several washing steps, cells were mounted on Mowiol and used for fluorescence microscopy.

For confocal microscopy, 1 night before transfection, NIH3T3 or HEK293 cells were seeded on polylysine-coated coverslips in 6-well plates at a density of 0.8 × 10⁵ cells/well. Cells were transiently transfected with combinations of different constructs (ECFP-Tctex2β, EndoHeRed, or vectors alone as controls). After an additional 48 h, cells were fixed with 4% paraformaldehyde on ice. Coverslips were mounted on Mowiol...
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and subjected to fluorescence analysis using a Zeiss confocal microscope. Cells were imaged using LSM 510 software, and the Multi-track function was chosen to collect potentially overlapping emissions separately. Green and red fluorophores were excited using the argon (488 nm) and helium/neon (543 nm) visible lasers, respectively.

Luciferase Reporter Assay—The luciferase assay was performed as described previously (31). In brief, Mv1Lu or HepG2 cells were transiently transfected with (CAGA)12-luciferase (an ALK5 signaling-responsive reporter construct) (33) together with HisTctex2β or endoglin constructs as indicated. The pEYFP-N1 vector (Clontech) was always included to serve as an internal control for transfection efficiency. Luciferase activity was assayed 16 h after incubation with 4 ng/ml TGF-β3 or TGF-β1. All experiments were performed in triplicate and repeated at least three times.

Proteasome Inhibitor Assay—The proteasome inhibitors MG132, lactacystin, and proteasome inhibitor I (proteasome inhibitor set I, catalog no. 539164, Calbiochem) were used. Inhibitors were suspended in Me2SO at 5 mM. HEK293 or COS-1 cells were seeded in 6-well plates. Cells were mock-transfected; transfected with HisTctex2β, endoglin, or TβRIIHA alone; or cotransfected with HisTctex2β and endoglin or with HisTctex2β and TβRIIHA using the cationic transfection reagent jetPEITM (Polyplus Transfection) according to the manufacturer’s instructions. The next day, cells were either left untreated or were incubated overnight for 16 h with a combination of 1 μl/ml each inhibitor or equal amounts of Me2SO. After incubation, cells were lysed in 400 μl, and the protein concentration of each lysate was determined using the BCA™ protein assay (Pierce) according to the manufacturer’s instructions. Subsequently, equal amounts of protein (30 μg) were separated by SDS-PAGE and blotted onto a nitrocellulose membrane (Macherey-Nagel). After incubation of the membrane separated by SDS-PAGE and blotted onto a nitrocellulose membrane (Macherey-Nagel). After incubation of the membrane subjected to fluorescence analysis using a Zeiss confocal imaging system.

Cell Migration in the Wound Assay—HeLa cells were seeded in 6-well plates and either singly transfected with 3 μg of mock DNA or 1.5 μg of HisTctex2β, endoglin, or TβRII supplemented with 1.5 μg of mock DNA or doubly transfected with 1.5 μg of HisTctex2β and 1.5 μg of endoglin or TβRII using jetPEITM. The next day, cells were wounded with a 20–200-μl tip. Cells were washed once with phosphate-buffered saline, and fresh growth medium was added. Subsequently, the wounded cell layers were photographed at four different positions. After 48 h, the same positions were photographed again to document cell migration.

Cell Proliferation Assay—To assess cell proliferation under fixed cell culture conditions, the increase in cell numbers was measured after different time points by staining the cell nuclei with crystal violet as described previously (34). In brief, 5 × 10^5 primary human dermal microvascular endothelial cells were transfected with 3 μg of mock DNA or HisTctex2β by nucleofection with a Nucleofector® (Amazex Biosystems, Cologne, Germany) according to the manufacturer’s instructions using the S-005 nucleofection program. After nucleofection, cells were plated in 6-well plates to recover for 1 day. Subsequently, cells were trypsinized and seeded in a 96-well plate at a density of 2000 cells/well. After 4 h of adhesion, cells were incubated for 24 and 48 h with 0.5 ng/ml TGF-β1 or 4 ng/ml TGF-β1 or were left untreated. After different time points, cells were fixed with 5.5% glutaraldehyde and subsequently washed three times with double-distilled H2O and then air-dried for 1 h. A 0.1% crystal violet solution (100 μl/well; pH 4.5) was added for 20 min. Cells were washed three times with double-distilled H2O and air-dried again. Finally, 10% acidic acid (100 μl/well) was added, and extinction was measured at 595 nm. An aliquot of cells assayed after 4 h of adhesion served as a reference at time 0.

RESULTS

Identification of Tctex2β, a Novel Tctex1/2 Family Protein, as an Interacting Partner for the Cytoplasmic Domain of Endoglin—To identify new intracellular binding partners for endoglin, the yeast Gal4 two-hybrid system was used (32). A human placental cDNA library was screened with the cytoplasmic domain of endoglin (Endocyto) as bait and cloned into the Gal4 DNA-binding domain vector pGBT9. A number of clones strongly and reproducibly interacting with endoglin were identified as determined by their abilities to grow in the absence of adenine and histidine and to activate the lacZ reporter. Among the identified clones was ZRP-1, confirming a previous report (29). However, the strongest interaction was identified in a clone provisionally named N22.1 with an ~650-bp insert. When sequenced, it was found to correspond to a hypothetical protein (GenBank™ accession number XM_291623) with homology to mouse Tctex1. The N22.1 clone is only a partial sequence of this predicted protein, corresponding to the C-terminal 182-amino acid region (residues 40–221).

A computational expression sequence tag (EST) “walk” was performed in the EST data base. The only two EST entries overlapping with N22.1 correspond to its 3’- and 5’-ends, respectively. The 3’-EST, from human lung epithelial cells (699 bp; GenBank™ accession number CA314455), covered the whole N22.1 insert sequence, whereas the 5’-EST, from human placenta (726 bp; GenBank™ accession number CF994778) extended an additional 626 bp upstream of the insert sequence. To determine and isolate the full-length N22.1 cDNA, 5’-RACE with human placental and testis RNAs was performed. The cloned and sequenced RACE products confirmed the 5’-EST sequence from our computational analysis. Taken together, the complete N22.1 cDNA was determined to be 1276 bp long, consisting of a single open reading frame of 666 bp, a 5’-untranslated region of 509 bp, and a 3’-untranslated region of 101 bp containing a consensus polyadenylation signal (aaat) located 15 bases upstream of the poly(A) tail.

Because of the homology to the Tctex1/2 protein family and additional analyses (see below), N22.1 was renamed and is henceforth referred to as Tctex2β. The complete nucleotide sequence and its deduced amino acid sequence are shown in Fig. 1A and have been deposited in the GenBank™ Data Bank with accession number DQ132441. An additional BLASTn search with the whole cDNA mapped this gene to human chromosome 1p39.1, with two exons and one intron of 108 bp. The existence of the intron was confirmed by RT-PCR with primer
pairs located in exons 1 and 2 flanking the intron (data not shown).

Bioinformatic Analysis of the Tctex2β Protein—A series of software-based analyses were performed to provide clues about the structural features of the Tctex2β protein. The Compute pi/Mw program showed that Tctex2β has a theoretical pi of 9.87 and an expected molecular mass of 23 kDa. In amino acid composition, it is ~10% serine and has a proline-rich N terminus (20% prolines of the N-terminal 114 amino acids). Motif searching by PROSITE (35) showed four consensus protein kinase C phosphorylation sites and two casein kinase II phosphorylation sites. Secondary structure prediction by PSIPRED (36) showed the layout of the helix-amino acids). Motif searching by PROSITE (35) showed four consensus protein kinase C phosphorylation sites and two casein kinase II phosphorylation sites. Secondary structure prediction by PSIPRED (36) showed the layout of the helix-strand structure, in which the N-terminal section formed several α-helices, whereas in the C terminus, four β-strands were predicted. This predicted structure agrees very well with the known secondary structure of the Tctex1 protein (37, 38).

By BLASTp and EST data base searches, Tctex2β homologs have been identified in several different mammalian species, including mouse (70% identity; GenBank™ accession number BC092499), pig (80% identity; GenBank™ accession number AJ973122), rat (74% identity; GenBank™ accession number AJ973123), and chimpanzee (98% identity; GenBank™ accession number XM_513130). An alignment of amino acid sequences using ClustalW (39) revealed the homology between Tctex2β and members of the Tctex1/2 protein family, especially in the C-terminal regions (Fig. 1B). For example, Tctex2β shares 27% identity with mouse Tctex2, 18% identity with human Tctex1, and 26% identity with a human Tctex2b homolog (GenBank™ accession number BC021177). The unrooted tree generated by PhyloDraw (40) shows the phylogeny of Tctex2β (Fig. 1C). Of the 15 proteins we aligned, human Tctex2β and its several close homologs clearly form a distinct subdivision (the Tctex2β group) different from the other three subfamilies, i.e. the Tctex1 group (including mouse, rat, human, and Chlamydomonas Tctex1 as well as human RP3), Tctex2 group (including mouse, rat, and human Tctex2) and Tctex2b group (including Chlamydomonas Tctex2b and a human testis Tctex2b homolog).

Confirming the Specific Interaction between Endoglin and Tctex2β—The interaction between endoglin and full-length Tctex2β was reconfirmed in yeast by sequential transformation, first with different endoglin constructs as indicated in Table 1 or the pGBT9 empty vector and subsequently with pACT2-Tctex2β. For this purpose, full-length wild-type endoglin (Endowt) and the endoglin-coding sequence lacking the cytoplasmic part (EndoΔCT) were cloned into pGBT9. As summarized in Table 1 (first four rows), yeast cells co-transformed with either Endowt/Tctex2β or EndoΔCT/Tctex2β not only were able to grow on His/Adeficient selection medium, but also could turn blue because of activation of the lacZ reporter. However, neither the Tctex2β/pGBT9 nor EndoΔCT/Tctex2β co-transformant was able to grow on selective medium. These results strongly suggest that Tctex2β specifically interacts with the cytoplasmic domain of endoglin.

The interaction between endoglin and Tctex2β was also confirmed using a mammalian two-hybrid assay in HEK293 cells in which the cytoplasmic domain of endoglin was fused to the DNA-binding domain of the pM vector, whereas Tctex2β was fused to the activation domain of the pVP16 vector. A third vector, pG5SEAP, encoding the secreted alkaline phosphatase gene, served as the reporter, the induction of which is caused by interaction of pM and pVP16 fusion proteins. Cotransfection of the three constructs, pM-endoglin, pVP16-Tctex2β, and pG5SEAP, resulted in a 4.1-fold induction of secreted alkaline phosphatase activity compared with the negative control samples (p < 0.01). This indicates that the endoglin/Tctex2β interaction also happens in mammalian cells.

HEK293 cells were transiently transfected with the pCMV5 vector (mock), HisTctex2β, or Endowt alone or with HisTctex2β/Endowt together as indicated in Fig. 2. Western blotting of the total cell lysate using anti-His antibody showed a single specific band (~28 kDa), which corresponded to the 221-amino acid Tctex2β polypeptide. Endoglin showed two bands, with the lower molecular mass band corresponding to the non-fully processed form. As shown in Fig. 2A, Tctex2β was coprecipitated by anti-endoglin antibody from the lysates of HisTctex2β/Endowt cotransfectants. Furthermore, we observed that HEK293 cells expressed low amounts of endogenous endoglin. Thus, we transfected HEK293 cells with HisTctex2β alone and precipitated the endogenous endoglin protein from cell lysates. Subsequent Western blotting demonstrated that the His-tagged Tctex2β protein was coprecipitated (Fig. 2B). These experiments further confirm the specific in vivo interaction between endoglin and Tctex2β.

Expression Profiling of Tctex2β—To investigate the potential physiological relevance of the novel Tctex2β protein, the expression pattern of Tctex2β was studied by RT-PCR analysis with glyceraldehyde-3-phosphate dehydrogenase as an internal control. Given that the whole coding sequence of Tctex2β is within one exon, genome-free cDNAs were prepared. This was verified by the fact that non-reverse transcriptase-treated samples gave negative PCR results (Fig. 3, lanes 2). Tctex2β gene expression could be seen in primary vascular endothelial cells (HUVECs), an endothelial cell line (HMEC-1) and smooth muscle cells, all of which are supposed to be key cell types of endoglin function. Expression was also detected in other tissues and cell types, including human placenta and testis, as well as in several tumor cell lines such as LCLC, HepG2 (hepatocellular carcinoma cells), and T47D (breast cancer cells). Different levels of expression were observed, with the highest expression in LCLC cells and HUVECs; lower expression in HepG2, HMEC-1, and smooth muscle cells; and weakest expression in T47D cells, placenta, and testis.

Tctex2β Inhibits TGF-β-induced ALK5 Signaling—Considering the role of endoglin in modulating cell responses to TGF-β, we next investigated whether Tctex2β is also involved in the TGF-β signaling pathway and what the functional implications of the interaction between endoglin and Tctex2β are. The (CAGA)17-luciferase reporter has been widely used as a specific reporter for TGF-β/ALK5 signaling specifically responding to Smad3/Smad4 activity (33). MvL1u cells were used to test the role of Tctex2β and endoglin in TGF-β signal-
ing. Cells transfected with CAGA \(_2\) -luciferase responded with a \(\geq 11\)-fold increase in reporter activity after TGF-\(\beta3\) treatment (Fig. 4A). Overexpression of endoglin decreased ligand-induced reporter activity by 33% as expected. In contrast, with a (activin type IIA receptor (ActRIIA), T\(_{\text{ALK1}}\), and constitutively active ALK1), type II receptors mic domains of different type I receptors (ALK5, wild-type endoglin and Tctex2 overexpression caused an even stronger down-regulation of signaling compared with endoglin, with 45% inhibition of ligand-induced reporter activity. However, cotransfection of endoglin and Tctex2 did not alter the level of inhibition as for Tctex2 alone, suggesting that there is no synergistic inhibitory effect for endoglin and Tctex2. Similar results were obtained with HepG2 cells. Here again, overexpression of Tctex2 inhibited TGF-\(\beta1\)-induced signaling (Fig. 4B). This suggests that the inhibitory activity of Tctex2 on signaling is less likely cell-type specific, but a more general feature of Tctex2 function.

**Analysis of the Interaction between Tctex2 and Other Members of the TGF-\(\beta\) Receptor Family**—We were greatly interested in the possibility that Tctex2 might interact with other members of the TGF-\(\beta\) receptor family. In view of this, the cytoplasmic domains of different type I receptors (ALK5, wild-type ALK1, and constitutively active ALK1), type II receptors (activin type IIA receptor (ActRIIA), T\(_{\text{BRII}}\), and bone morphogenetic protein type II receptor (BMPRII)), and the betaglycan type III receptor were cloned into the pGBT9 vector and tested for their ability to interact with pACT2-Tctex2 in a yeast two-hybrid system. Of the different receptors, T\(_{\text{BRII}}\), betaglycan, and ActRIIA could also associate with Tctex2, as inferred by colony growth on His/Ade-deficient plates, whereas BMPRII, ALK5, ALK1, and constitutively active ALK1 failed to interact.

**TABLE 1**  
Summary of the interactions between Tctex2\(\beta\) and different members of the TGF-\(\beta\) receptor family

Yeast two-hybrid growth assays were performed by sequential transformation of yeast cells, first with appropriate domains of different TGF-\(\beta\) receptors fused to the DNA-binding domain (BD) of pGBT9 and then with full-length Tctex2 fused to the activation domain (AD) of pACT2. The pGBT9 or pACT2 vector was used as an appropriate negative control. The presence and strength of interactions between the indicated proteins were judged by the abilities of yeast co-transformants to grow on His/Ade-deficient plates or to turn blue on His/Ade-deficient X-gal plates. −, no interaction; +, interaction; blue, strong interaction.

| BD construct                  | AD construct          | pACT2-Tctex2\(\beta\) | pACT2 |
|-------------------------------|-----------------------|-----------------------|-------|
| pGBT9 alone                   |                       |                       |       |
| pGBT9-Endo\(^\text{Alk5}\)    |                       | +/blue                |       |
| pGBT9-Endo\(^\text{ActRIIA}\)|                       | +/blue                |       |
| pGBT9-Endo\(^{\text{BMPRII}}\)|                       | +/blue                |       |
| pGBT9-ALK5\(^{\text{Alk1}}\) |                       | −                     |       |
| pGBT9-ALK1\(^{\text{Alk1}}\) |                       | −                     |       |

**FIGURE 2.** Endoglin associates with Tctex2\(\beta\) in co-immunoprecipitation studies. A, HEK293 cells were cotransfected with \(\text{\^H}\)Tctex2\(\beta\), endoglin, or vector alone as indicated. Cell lysates were subjected to immunoprecipitations (IP) using anti-endoglin (Eng) polyclonal antibody, and precipitated \(\text{\^H}\)Tctex2\(\beta\) was detected by Western blotting (WB) using anti-His antibody (upper panel). Expression of the recombinant proteins was confirmed by Western blotting of total cell lysates with either anti-His (middle panel) and anti-endoglin (lower panel) antibody. B, HEK293 cells were transfected with \(\text{\^H}\)Tctex2\(\beta\) or vector alone as indicated. Cell lysates were subjected to immunoprecipitations using anti-endoglin polyclonal antibody, and precipitated \(\text{\^H}\)Tctex2\(\beta\) was detected by Western blotting using anti-His antibody. Expression of endogenous endoglin was shown by Western blotting of total cell lysates (TCL) with the anti-endoglin polyclonal antibody also used for immunoprecipitation.

**FIGURE 3.** Expression profiling of Tctex2\(\beta\) in different human tissues and cell types. Representative RT-PCR results from human placenta, testis, smooth muscle cells (SMC), HUVECs, HMEC-1 cells, LCLC cells, HepG2 cells, and T47D cells with Tctex2\(\beta\)-specific primers are shown. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific RT-PCR served as a control. Lanes 1, first-strand cDNA samples; lanes 2, negative control samples without reverse transcriptase. A tissue-specific expression pattern is discernible, with the highest expression in LCLC cells and HUVECs. The identities of the PCR products were confirmed by sequencing.

with Tctex2\(\beta\) (Table 1, fifth through eleventh rows). Nevertheless, it is worth noting that, as judged by the inability of T\(_{\text{BRII}}\)/Tctex2\(\beta\) or ActRIIA/Tctex2\(\beta\) co-transformants to turn blue on

**FIGURE 1.** Molecular cloning and phylogenetic analysis of Tctex2\(\beta\). A, nucleotide and deduced amino acid sequences of human Tctex2\(\beta\). Note that the 3′-untranslated region contains a perfect copy of the polyadenylation signal (\_<u>_underlined_</u>_). B, alignment of the Tctex1/2 family proteins generated by ClustalW and shaded with BoxShade. The aligned sequences are as follows: human Tctex2\(\beta\) (GenBank\(\text{TM}\) accession number DQ132441), chimpanzee mRNA (GenBank\(\text{TM}\) accession number XM_513130), pig cDNA (GenBank\(\text{TM}\) accession number A973122), mouse cDNA (GenBank\(\text{TM}\) accession number AK029345), rat EST (GenBank\(\text{TM}\) accession number A973123), Chlamydomonas (Chlamyd) Tctex2b (GenBank\(\text{TM}\) accession number BC087868), human Tctex2b homolog (GenBank\(\text{TM}\) accession number BC021177), mouse Tctex2 (GenBank\(\text{TM}\) accession number U21674), rat Tctex2 (GenBank\(\text{TM}\) accession number XM_344846), human Tctex2 (GenBank\(\text{TM}\) accession number AF519569), human Tctex1 (GenBank\(\text{TM}\) accession number NM_006519), rat Tctex1 (GenBank\(\text{TM}\) accession number AB010119), mouse Tctex1 (GenBank\(\text{TM}\) accession number BC087868), Chlamydomonas Tctex1 (GenBank\(\text{TM}\) accession number AF039437), and human RP3 (GenBank\(\text{TM}\) accession number U025566). C, phylogenetic tree of Tctex1/2 family proteins. The unrooted tree generated by PhyloDraw reveals four subdivisions, Tctex1, Tctex2, Tctex2b, and Tctex2\(\beta\).
high stringency plates, it seemed that these interactions were less strong than the endoglin/Tctex2β or betaglycan/Tctex2β interaction. Association between TβRII and Tctex2β was further confirmed by co-immunoprecipitation studies in HEK293 cells (Fig. 5), whereas interaction between ActRIIA and Tctex2β could not be detected by co-immunoprecipitation (data not shown). Subcellular Distribution of Tctex2β and Co-localization Studies with Endoglin and TβRII—HeLa, HEK293, and NIH3T3 cells were transiently transfected with EGFPTctex2β, endoglin, or a combination of the two. Cells were incubated with or without TGF-β3 (4 ng/ml) for 16 h, after which luciferase activities were measured. HepG2 cells were transfected as described for Mv1Lu cells and incubated with or without TGF-β1 (4 ng/ml) for 16 h, after which luciferase activities were measured. All transfection experiments were performed in triplicate and repeated at least three times, and the results shown are the average fold changes. S.D. values are indicated by error bars, and all experiments were normalized for enhanced yellow fluorescent protein fluorescence intensity. *, p < 0.05 compared with the mock transfection group; **, p < 0.01. results were also found in HEK293 and NIH3T3 cells (data not shown). HeLa cells transfected with EGFPTctex2β alone clearly showed that Tctex2β localized to vesicles and microtubules and was highly abundant in the nucleus, but not in the nucleoli (Fig. 6, A–C). Cells cotransfected with endoglin and Tctex2β showed clear co-localization of Tctex2β and endoglin (Fig. 6A). Similar

FIGURE 4. Tctex2β inhibits TGF-β signaling. The effect of Tctex2β on ALK5 signaling was investigated in the epithelial mink lung cell line Mv1Lu and the hepatoma cell line HepG2. A, Mv1Lu cells were transfected with the (CAGA)12-luciferase reporter construct together with HisTctex2β, endoglin, or a combination of the two. Cells were incubated with or without TGF-β3 (4 ng/ml) for 16 h, after which luciferase activities were measured. B, HepG2 cells were transfected as described for Mv1Lu cells and incubated with or without TGF-β1 (4 ng/ml) for 16 h, after which luciferase activities were measured. All transfection experiments were performed in triplicate and repeated at least three times, and the results shown are the average fold changes. S.D. values are indicated by error bars, and all experiments were normalized for enhanced yellow fluorescent protein fluorescence intensity. *, p < 0.05 compared with the mock transfection group; **, p < 0.01.

FIGURE 5. Verification of the interaction between Tctex2β and TβRII. HEK293 cells were mock-transfected or transfected with HisTctex2β, TβRIIHA, or HisTctex2β/TβRIIHA as indicated. Total cell lysates were subjected to immunoprecipitation (IP) using anti-His antibody, and precipitated TβRIIHA was detected by Western blotting (WB) using anti-HA antibody (upper panel). Expression of the recombinant proteins was confirmed by SDS-PAGE and Western blotting of total cell lysates with either anti-HA (middle panel) or anti-His (lower panel) antibody.

FIGURE 6. Subcellular distribution of Tctex2β and co-localization with endoglin. A, HeLa cells were cotransfected with EGFPTctex2β and EndoECFP. Subcellular localization of EGFPTctex2β and EndoECFP is shown in green and red (false-colored), respectively. Co-localization of Tctex2β and endoglin is represented by yellow in the overlaid images. Images were taken by two-photon microscopy with the TriMScope 2-photon system for multiphoton microscopy. B, shown is a series of Z-stack images of HeLa cells transfected with EGFPTctex2β. Images were taken with the TriMScope 2-photon system as described for A. C, shown is a composite image of Z-stack images from B.
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Endoglin and TβRII both localized to vesicles and the plasma membrane and clearly co-localized, although there was not a complete vesicular overlap (Fig. 7). Tctex2β was, as seen before, distributed throughout the cytoplasm as well as in the nucleus and was associated with vesicles and what we think are microtubule structures. Co-localization of Tctex2β, endoglin, and TβRII was seen at the cell membrane forming the junction (Fig. 7, white arrowheads) between two cells and in cellular protrusions and therefore maybe areas of cell adhesion. Surprisingly, there was almost no co-localization of Tctex2β, endoglin, and TβRII in vesicles. Vesicles containing endoglin and TβRII were in general larger than and distinct from those associated with Tctex2β, although these data at least suggest that Tctex2β, endoglin, and TβRII can be present in one protein complex.

No Detectable Influence of Tctex2β on Cell Proliferation and Migration—To analyze what effects Tctex2β might have on cellular functions, we investigated its influence on cell proliferation. Given that Tctex2β interacts with endoglin and that endoglin is highly expressed in endothelial cells, primary human dermal microvascular endothelial cells were used. Cells were mock-transfected or transfected with Tctex2β and treated with low (0.5 ng/ml) and high (4 ng/ml) amounts of TGF-β1 for 24 and 48 h. This experiment was repeated three times, but there was no significant difference in the proliferation rate of Tctex2β- and mock-transfected cells (data not shown).

Next, we tested the effect of Tctex2β on cell migration. For this purpose, HeLa cells were mock-transfected or transfected with Tctex2β alone or in combination with endoglin or TβRII or with all three together. Subsequently, cells were wounded, and cell migration was analyzed after 48 h. No obvious difference could be observed between Tctex2β- and non-Tctex2β-transfected cells.

Ectopic Tctex2β Expression Increases Endoglin and TβRII Protein Amounts—Over the course of our analyses, we observed that the amounts of endoglin or TβRII from Tctex2β cotransfections detected by Western blotting varied compared with single transfections. Thus, we speculated that Tctex2β might influence the proteasomal degradation of endoglin and TβRII. To test this hypothesis, HEK293 cells were transfected with either the different expression constructs alone or with endoglin or TβRII in combination with Tctex2β. Transfected cells were then treated for 16 h with either proteasome inhibitors or Me2SO or were left untreated. After cell lysis, equal amounts of proteins were analyzed by SDS-PAGE and Western blotting for the amounts of endoglin, TβRII, and Tctex2β. Coexpression of endoglin or TβRII with Tctex2β clearly resulted in an increased amount of endoglin as well as TβRII (Fig. 8A). This stabilizing effect in the presence of Tctex2β was equal to or even higher than that in the proteasome inhibitor-treated single transfectants. The same result was also seen in COS-1 cells coexpressing endoglin and Tctex2β (Fig. 8B).

DISCUSSION

In this study, we have reported a new DLC protein named Tctex2β that interacts with the TGF-β type III receptor endoglin as well as TβRII. Sequence alignment, phylogenetic analysis, and secondary structure prediction clearly placed Tctex2β in the Tctex1/2 family of DLCs, a family that was first identified in mouse testis and later found in the cytoplasmic dyneins of many tissues (42, 43). On the basis of our bioinformatic analyses, we are confident of the existence of a new Tctex subfamily that interacts with endoglin and that Tctex2β is the first member of this family.
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A

B

FIGURE 8. Expression of Tctex2β might protect endoglin and TβRII from proteasomal degradation. We tested whether Tctex2β influences the stability and proteasomal degradation of endoglin or TβRII by transient transfection. HEK293 cells (A) or COS-1 cells (B) were transfected with the different expression constructs as indicated. 24 h after transfection, cells were treated with a combination of proteasome inhibitor I, MG132, and lactacystin. After 16 h, cells were lysed, and equal amounts of protein were analyzed by SDS-PAGE and Western blotting (WB) for the amounts of endoglin (Eng), TβRII, and Tctex2β with the indicated antibodies. DMSO, Me₃SO.

lesser particles. These movements are required for the spatial organization of the cytoplasm and, as a consequence, are crucial for many processes such as cell division, embryonic development, and regulation of signaling (44, 45). DLCs bind to the cargo and therefore determine cargo specificity. Three distinct families of cytoplasmic DLCs have been identified and designated LC8, LC7/Roadblock, and Tctex1/2. Accumulating evidence shows that DLCs can interact with different, functionally unrelated proteins, facilitating dynein association with specific cargoes. For example, known binding partners for Tctex1 include rhodopsin, Doc2, Fyn kinase, the Trk receptor, CD5, and CD155 (46, 47).

The existence of a gene family often indicates indispensable but also differing functions of its members in different tissues. Expression profiling of human Tctex2β revealed that Tctex2β mRNA is present in primary vascular endothelial and smooth muscle cells, where endoglin is preferentially expressed. Thus, it is possible that Tctex2β might play a role in angiogenesis or other endoglin-related functions in the vasculature. Other than that, a range of Tctex2β expression levels were found in placenta, testis, and several tumor cell lines. Even though the Tctex2β expression levels varied among the different cell types tested, the data suggest a ubiquitous expression profile. The highest expression was seen in LCLC cells and HUVECs, whereas the Tctex2β mRNA level in testis was relatively low. In contrast, both Tctex1 and Tctex2 are enriched in testis, whereas Tctex1 expression in lung tissue is differentially regulated, with greatly decreased levels in adult lung. Tctex2 is more restricted to testis, liver, and fetal thymus, with only very low levels detectable in fetal and adult lungs (43). These first expression data for Tctex2β in comparison with Tctex1 and Tctex2 suggest that this family of DLCs might have overlapping but also diverging cell type-specific functions.

Endoglin is a new member of the TGF-β receptor family identified to interact with a DLC. BMPRII and the bone morphogenetic protein type IA (ALK3) and IB (ALK6) receptors have been reported to bind to Tctex1 (48). In vitro kinase assays demonstrated that BMPRII phosphorylates Tctex1, which is abolished by BMPRII mutations causing the human disorder primary pulmonary hypertension. TβRII has been shown to interact with and phosphorylate a member of the LC7/Roadblock group of DLCs, km23/mLc7-1 (41), upon TGF-β treatment. Overexpression of mLc7-1 induces specific TGF-β responses, including JNK (c-Jun N-terminal kinase) activation, c-Jun phosphorylation, and mink lung epithelial cell growth inhibition. Furthermore, TGF-β induces the recruitment of mLc7-1 to the dynein intermediate chain. In addition, it has been demonstrated that mLc7-1 is required for TGF-β-induced activation of the p3TP-Lux promoter reporter (49).

We also investigated the role of Tctex2β in TGF-β signaling and found that overexpression of Tctex2β in mink lung epithelial and HepG2 cells severely inhibited TGF-β-induced (CAGA)₁₁₂-luciferase reporter activity by >40% (Fig. 4, A and B). This inhibitory effect was even stronger than that seen for endoglin. However, coexpression of Tctex2β and endoglin demonstrated no synergistic effect of further reduced reporter activity. At the moment, we do not know whether Tctex2β is upstream or downstream of endoglin in the signaling cascade. However, because Tctex2β is a DLC, and DLCs are generally involved in signaling by mediating the transport of receptor signaling complexes in a retrograde fashion along the microtubules toward the nucleus, it is conceivable that Tctex2β operates downstream of endoglin. Our results seem to be contradictory to the up-regulation of TGF-β signaling by mLc7-1; however, the (CAGA)₁₁₂-luciferase reporter becomes activated by the Smad3/Smad4 pathway, whereas p3TP-Lux reporter induction depends on an intact MAPK (mitogen-activated protein kinase) pathway (49). Thus, it is possible that the functions of Tctex2β in Smad and non-Smad signaling pathways are different. In general, this raises the possibility that the function of DLCs might be pathway-specific.

Aside from endoglin, we have demonstrated that TβRII can associate with Tctex2β, too, and that the three proteins form a multimeric complex (Fig. 7). Fluorescence microscope images showed that the main co-localization of the multimeric Tctex2β-endoglin-TβRII complex occurred at the cell membrane, whereas in vesicles, only rarely did we observe the three proteins together or Tctex2β with endoglin or with TβRII. In contrast, vesicular co-localization of endoglin with TβRII was clearly visible. However, these vesicles appeared bloated com-
pared with the smaller sized vesicles associated with Tctex2β. Endoglin and TβRII are transmembrane proteins transported from the Golgi toward the cell membrane, an anterograde movement. Only during ligand-induced signaling or protein recycling/proteasomal degradation would we expect to see membrane receptors in retrograde moving vesicles. Tctex2β is a DLC protein family member. These proteins are thought to be involved in retrograde protein transport. This suggests that the observed endosomal co-localization of endoglin and TβRII is most likely in vesicles on their way to the cell membrane and that Tctex2β is, under the conditions used in this study, not involved in receptor internalization and hence receptor recycling/proteasomal degradation of endoglin and TβRII. The current hypothesis is that Tctex2β may block signaling by preventing receptor internalization, which could lead to an increased retention time of the receptors at the cell membrane. This is supported by our finding that coexpression of Tctex2β with endoglin or TβRII leads to a more or less equivalent increase in the two receptors comparable with that in endoglin- or TβRII-transfected cells treated with proteasome inhibitors. In addition, these data also suggest that membrane-localized endoglin and TβRII are constantly degraded by the proteasome system and replaced by newly synthesized endoglin and TβRII. Apart from that, because the cytoplasmic domain of TβRII, which interacts with Tctex2β, has kinase activity, it would be very interesting to test whether Tctex2β might be phosphorylated by the TβRII kinase domain and whether the phosphorylated Tctex2β is involved in determination of cargo specificity or other signaling events.

In summary, we have identified a new DLC protein (Tctex2β) as a novel binding partner for endoglin and TβRII. Tctex2β shows an unexpected role as a TGF-β signaling inhibitor. By fluorescence microscopy, co-localization of Tctex2β, endoglin, and TβRII could be detected at the cell membrane, but was hardly seen in vesicles. Furthermore, coexpression of Tctex2β with endoglin or TβRII appears to stabilize the two receptors, therefore preventing proteasomal degradation of endoglin and TβRII. This prompted us to speculate that Tctex2β might increase the retention time of endoglin and TβRII at the cell surface by blocking internalization, which would also lead to inhibition of ligand-induced signaling. Further studies are now necessary to investigate this hypothesis.

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