The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine

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INTRODUCTION

Mucins are found on epithelial surfaces where they are the dominating protein components of the mucus that lines and protects the surface of luminal organs [1]. Mucins are characterized by the mucin or PTS domains, which comprise serine-, threonine- and proline-rich, often repeated sequences that carry a high number of O-linked glycans. The glycan content often reaches 80% of the molecular mass and contributes to the gel-like properties of the mucus. Based on their structure, mucins are subdivided into membrane-bound and secreted, gel-forming mucins. While the gel-forming mucins constitute the free-floating mucus network, the transmembrane mucins with their membrane domain are tightly anchored in the epithelial cells [2]. Typical of the transmembrane mucins is their heterodimeric nature with a large and heavily O-glycosylated extracellular domain that extends far from the cell surface. The transmembrane mucins contain either a SEA (sea-urchin sperm protein, enterokinase and agrin) or a von Willebrand D domain (only MUC4) close to the extracellular membrane surface. Both these domains are cleaved in the endoplasmic reticulum, the SEA domain by an autocatalytic cleavage during folding [3]. After cleavage, the two parts remain tightly associated by non-covalent bonds. Although not proven, it has been suggested that the SEA domain can act as a sensor for mechanical forces [3]. The cytoplasmic tails of the transmembrane mucins are usually short, but specific interactions precipitates with the scaffold protein PDZK1 as identified by MS. This was mediated through the C-terminal PDZ-interaction site in MUC17, which was capable of binding to three of the four PDZ domains in PDZK1. Immunostaining of wild-type or Pdzk1−/− mouse jejunum with an antiserum against Muc3(17), the mouse orthologue of human MUC17, revealed strong brush-border membrane staining in the wild-type mice compared with an intracellular Muc3(17) staining in the Pdzk1−/− mice. This suggests that Pdzk1 plays a specific role in stabilizing Muc3(17) in the apical membrane of small intestinal enterocytes.

Key words: cystic fibrosis transmembrane conductance regulator (CFTR), Muc3, MS, Na+/H+ -exchanger regulatory factor 1 (NHERF1), PDZ domain protein, transmembrane mucin.
dependent on palmitoylation of the cysteine residues in the MUC1 transmembrane domain [10]. Such cysteine residues are absent from the MUC3, 12 and 17 mucin sequences and this suggests other mechanisms for apical targeting and potential recycling.

Common moderators of polarized expression and function of membrane proteins in epithelial cells are the PDZ domain proteins (commonly called PDZ proteins). PDZ domains are the most abundant protein–protein interaction domains in metazoans. By specific binding to short C-terminal sequences of their ligand proteins, they assemble protein networks and signalling complexes and assist in targeting and retention of interacting proteins at specialized compartments of the cell. To help coordinate this, PDZ proteins often contain multiple PDZ domains in tandem and, sometimes, a range of other protein interaction domains [e.g. SH3 (Src homology 3), PH (pleckstrin homology), LIM and GUK (guanylate kinase-like) domains] [11,12].

The NHERF (Na+/H+-exchanger regulatory factor) family of PDZ proteins consists of four proteins, NHERF1, NHERF2, PDZK1 (PDZ domain containing 1) and PDZK2 [IKEPP (intestinal and kidney-enriched PDZ protein)]. They are present at the brush border of, for instance, mammalian intestine and renal proximal tubules [13]. These proteins have two or four PDZ domains and the NHERF1 and NHERF2 also comprise ERM (ezrin/radixin/moesin) domains with which they attach to the cytoskeleton. PDZK1 [also called CAP70 (CFTR-associated protein 70), NaPi2-Cap1 (Na/Pi co-transporter C-terminal associated protein 1) or CLAMP (C-terminal Linking and Modulating Protein)] is expressed at the apical border of epithelial cells in the kidney, liver, gastrointestinal tract and pancreas [14]. Due to its multiple PDZ domains, it can simultaneously bind to several ligand proteins as shown for transporters and ion channels of the apical membrane. These include the CFTR (CF cystic fibrosis) transmembrane conductance regulator, MR2 (multidrug resistance-associated protein 2), SR-B1 (scavenger receptor B, type I) and the CIC-3B chloride channel [15–18]. From studies of the apical membrane of epithelial cells in the kidney, it has been established that PDZK1, together with NHERF1, creates an extended network beneath the apical membrane to which membrane proteins and regulatory elements can attach [19,20].

Utilizing PDZ domain arrays, we have screened PDZ domains for binding to the cytoplasmic tail of human MUC3, MUC12 and MUC17, identifying a major interaction between MUC17 and PDZK1. Pull-down experiments using the cytoplasmic tails of the MUC3, MUC12 and MUC17 mucins affirmed PDZK1 as a binding partner for the MUC17 mucin. The absence of Pdzk1 in mouse jejunum relocalized Muc3(17) from the apical membrane to subapical complexes and assist in targeting and retention of interacting proteins at specialized compartments of the cell. To help coordinate this, PDZ proteins often contain multiple PDZ domains in tandem and, sometimes, a range of other protein interaction domains [e.g. SH3 (Src homology 3), PH (pleckstrin homology), LIM and GUK (guanylate kinase-like) domains] [11,12].

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**MATERIALS AND METHODS**

**Plasmids**

Plasmids (pGST-MUC3-CT, pGST-MUC12-CT and pGST-MUC17-CT; CT indicates cytoplasmic) encoding the complete cytoplasmic parts of the human MUC3, MUC12 and MUC17 mucins fused with GST (glutathione transferase) were generated by RT–PCR (reverse transcription–PCR) (Taq polymerase; Fermentas) by using the primer pairs 5′-GGGAATTCGGCTGCAGCTCCGGATGGTG, and 5′-CCAAGAGAGAGGTGAAACGGC and 5′-CCCCGCAGGGCTCACACTGAGG; 5′-GGGAATTCGGCTGCAGCTCCGGATGGTG, and 5′-CCAAGAGAGAGGTGAAACGGC and 5′-CCCCGCAGGGCTCACACTGAGG; 5′-GGGAATTCGGCTGCAGCTCCGGATGGTG, and 5′-CCAAGAGAGAGGTGAAACGGC and 5′-CCCCGCAGGGCTCACACTGAGG; the pGEX-5X3 vector respectively, and cloned into the pGEX-4T-1 vector (GE Healthcare). A human small intestinal cDNA library was used as a template (Invitrogen). The GST–MUC17CT-ΔSF was generated by site-directed mutagenesis (QuikChange®; Stratagene) introducing a premature stop codon in pGST-MUC17-CT. The plasmids encoding FLAG-tagged PDZK1 and PDZK1 PDZ domains in the pGEX-5X3 vector were described previously [18]. The YFP (yellow fluorescent protein)-tagged YFP–MUC17-CT plasmid was generated by removing the EcoRI site by mutagenesis and subcloning the MUC17-CT fragment into a pEYFP vector (Clontech) where Cys606 had been deleted by mutagenesis to prevent dimerization of YFP. The pGST-Muc3(17)-CT plasmid, encoding the complete cytoplasmic tail of Muc3(17), was generated by cloning an annealed oligonucleotide 5′-GATCCGTGTAACACCCCTCCAGCTTTCCTAAACACCACATAAAACCAGAAAAGATC-CAGATTACAGGCCCAGGTAGTGCATGCATGTTGAAG-AG-5′-AAATTCCTAAACAGGTATGTCACTACCTGCGGGCCGCTCGAATCTGGATCTTTC TTTCTGGGTTTATGTGGTTTA-AGGGAAGCTGGAGGTGTGTGTACTACAG (MWG Biotech) with BamHI and EcoRI overhangs into the pGEX-4T-1 vector.

**Antibodies**

The Muc3(17) protein was detected by using the pAb (polyclonal antibody) anti-Muc3(17)-S2 [8], the FLAG tag by using the mAb (monoclonal antibody) anti-FLAG M2 (Sigma) and YFP by using the mAb anti-GFP (green fluorescent protein) (BD Biosciences). Antibody against Pdzk1 was raised by immunizing rabbits with the peptides CLRAGPEQKGQIIKDI and CQSQELPNGSVKEGPA, coupled with keyhole-limpet (Diodostra aspera) haemocyanin conjugate via the heterobifunctional linker MBS (m-maleimido-benzoyl-N-hydroxysuccinimidyl ester). Affinity-purified antibody was made solely using the second peptide sequence.

**Cell culture**

Syrian hamster kidney BHK (baby-hamster kidney)-21 and human colon adenocarcinoma HT-29 cell lines (A.T.C.C.) were cultured at 37°C in 5% CO2. The BHK-21 cell lines were transiently transfected by using Lipofectamine™ PLUS or Lipofectamine™ 2000 reagents (Invitrogen).

**PDZ domain array**

Cytoplasmic tail of MUC17 tagged with YFP was expressed in BHK-21 cells. TranSignal™ PDZ Domain Array II and IV (Panomics, Redwood City, CA, U.S.A.) were used as instructed by the manufacturer. Bound cytoplasmic tail of MUC17 was detected with the mAb anti-GFP (Clontech).

**Immunoprecipitation and pull-downs**

Immunoprecipitations and GST pull-down experiments were performed as described previously [21]. Briefly, binding was performed in 0.09 or 1% Nonidet P40 lysis buffer (Nonidet P40, 150 mM NaCl, 50 mM Tris/HCl, pH 7.4, and 20 mM NaMoO4) containing Complete™ protease inhibitor cocktail (Roche) overnight at 4°C. Glutathione–Sepharose™ 4B beads (GE Healthcare) were washed four times with 0.09% Nonidet P40 lysis buffer and bound proteins were eluted for 30 min with SDS/PAGE sample buffer containing 1,4-dithiothreitol and loaded on to SDS/PAGE gels.

**In-gel trypsin digestion and MS**

The gels were stained with Coomassie Brilliant Blue Protein Stain (Pierce). The bands to be analysed were cut out from the gel and
digested with trypsin as described in [21]. The peptides were analysed by nano-LC (liquid chromatography)-MS and MS/MS (tandem MS) by using a Thermo LTQ-FT instrument and a 50 μm × 20 cm Kromasil C18 column eluted with an acetonitrile gradient in water at a flow rate of 200 nl/min. The obtained MS/MS spectra and accurate molecular masses of the peptides were searched against the Swiss-Prot protein database using Mascot software.

**Breeding of knockout mice**

The Pdzk1-deficient mouse strain was generated in the Department of Pathology, Beth Israel Deaconess Medical Center as described before [22]. These mice have normal body electrolyte concentration and normal intestinal mucosal histology. Pdzk1+/− and Pdzk1+/- mice were bred on a 129SvEv background in the animal facility of the Hanover Medical School under standardized light and climate conditions and had access to water and chow ad libitum. For the experiments in the present study, age- and sex-matched littermates of 2–3 months of age were used. NHERF1−/− mice backcrossed for 8 generations into the FVB/n background were produced from heterozygotes that were generated at the Duke University Medical Center as described before [23]. NHERF2−/− mice showing complete absence of NHERF2 protein in all tissues studied were produced from heterozygotes generated at the Erasmus University Medical Center and were backcrossed for 14 generations into the FVB/n background. C57/BL6 mice were used as wild-type controls. Experiments followed protocols at the Hanover Medical School and at the Erasmus University Medical Center Rotterdam, approved by the local authorities for the regulation of animal welfare (Regierungspräsidium) and by the Dutch Animal Welfare Committee (DEC) respectively.

**Mouse tissue preparation and lysis**

Small intestine of wild-type mice (C57/BL6) was excised and washed gently with ice-cold PBS containing Complete™ EDTA-free protease inhibitor cocktail (20 times) (Roche), 10 mM EDTA and 1 mM PMSF. The small intestine was cut into smaller pieces and homogenized in a Dounce homogenizer (pestle B) and phenylalanine failed to interact with PDZK1 (Figure 2B), as PDZ proteins and further explored (results not shown).

**Immunohistochemistry**

Intestinal tissue from mouse jejunum and colon of wild-type, Pdzk1−/− [22], Nherf1−/−, Nherf2−/− and Nherf1+2/− mice were fixed, paraffin-embedded and sectioned (10 μm). The tissue sections were deparaffinized and rehydrated in xylene (6 min), 100% ethanol (4 min) and 70% (v/v) ethanol (2 min), and washed with water. Antigen retrieval was performed by boiling twice for 2 and 7 min in 0.01 M citric acid (pH 6.0) and washed with PBS. Tissues were encosed with a PAP pen and blocked with 1% BSA in PBS. Primary and secondary antibodies were added in dilution buffer (0.1% BSA and 0.05% Triton X-100). Coverslips were mounted using Prolong Gold antifade (Invitrogen) and polymerized for 24 h. Samples were examined using an immunofluorescence microscope (Axiolab; Zeiss) or an LSM Meta 510 confocal microscope (Zeiss).

**RESULTS**

**MUC17 is a PDZ-domain-binding protein**

Analysis of the cytoplasmic sequences of the MUC3, MUC12 and MUC17 mucins revealed that all had sequences in their extreme C-termini resembling PDZ-domain-binding ligands of class I (X-S/T-X-Φ, where X represents any amino acid and Φ represents a hydrophobic amino acid) (Figure 1A). In order to investigate whether MUC3, MUC12 and MUC17 C-terminal tails were able to bind scaffolding PDZ proteins, we expressed the YFP-tagged cytoplasmic tails of the mentioned membrane-associated mucins in BHK-21 cells and assessed their ability to bind to PDZ domains immobilized on four protein PDZ domain arrays. Interestingly, we observed a strong binding of YFP–MUC17-CT to the second PDZ domain of PDZK1, the third member of the NHERF family (Figure 1B). YFP–MUC17-CT also displayed weak binding to PDZK1-D1 and NHERF1-D1. Faint interactions were also observed between YFP–MUC12-CT and NHERF1-D1, NHERF2-D1 and PDZK1-D2.

The cytoplasmic tails of the MUC3, MUC12 and MUC17 mucins were fused with GST and the fusion proteins were used for pull-down experiments in lysates from the intestinal epithelial colon cancer cell line HT-29. Nine bands were excised and analysed using MS after in-gel trypsinization. A unique 70 kDa Cooamassie Brilliant Blue-stained band was present in the co-precipitate with the MUC17 C-terminal tail, whereas the band was absent from co-precipitates with GST and MUC3, and MUC12 C-terminal tails (Figure 1C). MS identified 20 peptides covering 37% of the human PDZK1 protein sequence (Figure 1D). No other precipitated bands for the three mucin tails were identified as PDZ proteins and further explored (results not shown).

**PDZK1 interacts with MUC17**

To further prove the interaction between MUC17 and PDZK1, FLAG-tagged PDZK1 was transiently expressed in BHK-21 cells. Lysates from these cells were exposed to immobilized GST–MUC3, GST–MUC12 and GST–MUC17 C-terminal fusion proteins. The bound proteins were separated on SDS/PAGE and analysed by Western blotting using the mAb anti-FLAG M2. As expected, the precipitate from the GST–MUC17-CT pull-down contained PDZK1 (Figure 2A). GST alone and both of the other two mucins were negative for PDZK1 binding. This suggests high binding specificity between MUC17 and PDZK1, as the MUC3 and MUC12 mucins were negative despite their typical PDZ ligand sequences of the same subclass. Consequently, the novel PDZK1 binding to MUC17-CT became our main point of interest.

It has been shown that the amino acids in the −0 and −2 positions from the C-terminus are critical for most PDZ interactions that are mediated through C-terminal ligands [11,12,24]. To examine whether this was true also for MUC17 and PDZK1, the two last amino acids of the extreme C-terminus of MUC17 in the GST–MUC17-CT fusion protein were deleted and GST pull-down experiments were performed. MUC17 lacking serine and phenylalanine failed to interact with PDZK1 (Figure 2B), corroborating that the interaction is PDZ-mediated.

To pinpoint the specific PDZ domain(s) in PDZK1 that mediates the interaction with MUC17, all four domains, separate or in tandem, were fused with GST [18]. These fusion proteins were used to perform pull-down experiments in lysates prepared from BHK-21 cells transiently expressing a YFP-tagged C-terminal tail of MUC17. SDS/PAGE separation of co-precipitated material and subsequent Western-blot analysis using an anti-GFP mAb revealed that PDZ domains 1, 2 and 4 of PDZK1 were able to interact with the C-terminus of MUC17 (Figure 2C). PDZ domain 3 potentially also binds to MUC17, but with lower affinity. Alignment of amino acid sequences for the four PDZ domains did not reveal any specific variations in the known conserved regions of the PDZ domain (results not shown) that correlated with the observed differences in affinity for the MUC17 cytoplasmic tail.
Pdzk1 stabilizes Muc3(17) at the enterocyte apical membrane in the mouse small intestine

To address whether PDZK1 had any influence on the localization of MUC17, we turned to mice with ablation of Pdzk1 [22]. The extreme C-terminal tail of the human MUC17 mucin has the sequence -TTSF and its mouse orthologue, Muc3(17), has the sequence -MTSL (Figure 1A). Thus the Muc3(17) sequence is different, but still represents a class I PDZ ligand. To determine whether the mouse Pdzk1 and Muc3(17) were able to interact, a GST fusion protein with the C-terminal tail of Muc3(17) was generated. This was used to perform GST pull-down experiments in lysates prepared from wild-type mouse jejunum. Both the human GST–MUC17-CT and the mouse GST–Muc3(17)-CT fusion proteins were able to interact with the mouse Pdzk1 (Figure 3). This suggests a conservation of the interaction between these two molecules despite the sequence differences.

Jejunum from wild-type or Pdzk1−/− mice was sectioned and studied by immunofluorescence using the pAb anti-Muc3(17)-S2 directed against a juxtamembrane sequence in the extracellular part of mouse Muc3(17) [8]. The confocal fluorescence microscope images revealed a strong apical brush-border staining in the wild-type animals with an increase in intensity towards the tip of the villi (Figure 4A). In contrast, the epithelial cells of the Pdzk1−/− mouse jejunum showed a prominent, particulate cytoplasmic staining with an accumulation in vesicular-like structures subapical as well as perinuclear (Figures 4B and 4C). The apical membrane was also stained, although relatively weakly as compared with the wild-type animals.

To investigate the role of Pdzk1 in Muc3(17) localization in the colon and whether absence of Pdzk1 caused the same relocalization of Muc3(17) as in the small intestine, colon sections from wild-type and Pdzk1−/− mice were stained with the pAb anti-Muc3(17)-S2. No difference could be observed in the subcellular localization of Muc3(17) in the colon of any of the knockout mice compared with wild-type controls (Figure 4D). The weak interaction between MUC17 and NHERF1, but not NHERF2, as found on the PDZ protein arrays (Figure 1) prompted us to analyse also the localization of Muc3(17) in Nherf1, Nherf2 or Nherf1+/− mice. Intestinal sections were stained with the pAb anti-Muc3(17)-S2. Immunostaining of jejunum (Figure 4E) and colon (Figure 4F) revealed apical membrane staining of Muc3(17) in enterocytes, and no alteration in the localization of Muc3(17) in the Nherf1, Nherf2 and combined knockout mice. Thus we could not verify any functional effect of Nherf1 and Nherf2 on the Muc3(17) localization.

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mAb. Lysate input lane accounts for 10% of the protein amount used in the pull-down reactions.

incubated together with lysates from BHK-21 cells transiently expressing YFP–MUC17. Bound fusion proteins with each of the four PDZ domains of PDZK1, either separately or in tandem, were input lane accounts for 5% of the protein amount used in the pull-down reactions. (analysed by SDS/PAGE with subsequent Western blotting using anti-FLAG M2 mAb. Lysate

reactions. (Figure 2 The MUC17 mucin binds specific PDZ domains of PDZK1

immobilized GST fusion proteins with the C-terminal tails of MUC3, MUC12 and MUC17 were incubated together with lysates from BHK-21 cells transiently expressing FLAG-tagged PDZK1. Bound products were separated by SDS/PAGE followed by Western blotting using anti-FLAG M2 mAb. Lysate input lane accounts for 5% of the protein amount used in the pull-down reactions. (B) FLAG-tagged PDZK1 was transiently expressed in BHK-21 cells and lysates prepared from these cells were incubated with glutathione–Sepharose beads with immobilized GST–MUC17-CT, GST–MUC17-ΔSF fusion proteins or GST alone. Bound products were analysed by SDS/PAGE with subsequent Western blotting using anti-FLAG M2 mAb. Lysate input lane accounts for 5% of the protein amount used in the pull-down reactions. (C) GST fusion proteins with each of the four PDZ domains of PDZK1, either separately or in tandem, were incubated together with lysates from BHK-21 cells transiently expressing YFP–MUC17. Bound products were separated by SDS/PAGE and analysed by Western blotting using an anti-GFP mAb. Lysate input lane accounts for 10% of the protein amount used in the pull-down reactions.

DISCUSSION

To maintain an intact barrier of mucus on epithelial surfaces exposed to detrimental environments, in for instance the respiratory and gastrointestinal tracts, it is crucial that the mucus favours this. The highly co-ordinated regulatory mechanisms involved in the regulation of mucus are poorly understood and important to elucidate. Emerging knowledge points at multiple pathological conditions, such as various types of cancers and inflammatory diseases as associated with mucin aberrations. To further understand this, it is important to comprehend how the transmembrane mucins are involved in regulatory networks at mucous surfaces. Numerous ion channels bind to PDZ proteins, something that is known to regulate their activity [25]. The present study for the first time suggests a link between ion channels and the apical molecules that reaches furthest away from the epithelial cell membrane, the mucins. This supports the idea that the transmembrane mucins can act as apical sensors that can play a role in the regulation of the epithelial-cell apical surface liquid and ion homeostasis.

Transmembrane mucins are likely to play a pivotal role in maintaining homeostasis on mucosal surfaces, but apart from MUC1, not much is known about the mechanisms that regulate the localization and expression of other transmembrane mucins. Here, we demonstrate that the three gastrointestinal membrane-bound mucins, MUC3, MUC12 and MUC17, all have C-terminal sequences resembling PDZ ligands of class I. MUC17 was shown to interact with the multi-PDZ domain protein PDZK1 and this interaction appears important for proper localization of this mucin.

Most of the studies on PDZK1 function are performed in renal and hepatic tissues. There it plays a prominent role in the proper localization and function of a number of ion transporters in the plasma membrane. The Cl\(^-\)–anion exchanger SLC26A6 [solute carrier family 26 member 6; also known as CFEX (chloride/formate exchanger)] is expressed in the brush border of the proximal tubules. SLC26A6 was demonstrated to interact with PDZK1 via a C-terminal PDZ motif. In Pdzk1\(^{-/-}\) mice, both the protein levels and the ability of this transporter to mediate anion exchange across the plasma membrane were profoundly decreased [26]. Similarly, cell surface expression and transport activities of the urate–anion exchanger URAT1, the proton-coupled peptide transporter PEPT2 as well as OAT4 (organic anion transporter 4) were shown to depend on PDZK1 expression [27–29]. Our results show that Pdzk1 interacts with, and plays an essential role in maintaining, the mouse orthologue of MUC17, Muc3(17), at the apical membrane of enterocytes in the small intestine. Immunofluorescence staining of Pdzk1 in Pdzk1\(^{-/-}\) mice, it is more likely that Muc3(17) is properly targeted to the apical membrane, but fails to anchor there and is thereby readily internalized.
A disease where mucin synthesis and secretion is markedly deregulated is CF. It is characterized by heavy mucus accumulation in luminal organs, most prevalent in the airways, causing chronic bacterial infections followed by inflammation and severe lung tissue damage. No direct functional connection between mucus and CFTR is known so far. Although the airway symptoms are dominating the disease, the small intestine is commonly affected, with 10–15% of infants suffering from meconium ileus and a comparable number of CF adults from distal intestinal obstruction syndrome. These are both severe conditions where the distal small intestine becomes obstructed by dehydrated mucus [30]. We previously showed increased protein levels of the Muc3(17) mucin in mucus from the small intestine of Cftr−/− mice compared with wild-type mice [8]. NHERF1 is a well-known interactor and regulator of CFTR [31]. The first PDZ domain of NHERF1, PDZ1, binds CFTR with a greater affinity, enabling PDZ2 to interact with additional apical proteins [32]. Although NHERF1 could be a potential molecular connection between CFTR and the transmembrane mucin MUC17, we could not substantiate any interaction between NHERF1 and MUC17 in pull-downs. Neither were there any alternations in the apical expression of Muc3(17) in Nherf1, Nherf2 or Nherf1+2 knockouts, providing additional proof that NHERF1 or NHERF2 does not affect MUC17.

PDZK1 interacts with CFTR and was proposed to assemble two CFTR molecules in the plasma membrane, via simultaneous binding of two CFTR C-termini to domains 3 and 4, and thereby stimulate chloride channel activity [15]. The facts that PDZK1 is a well-known interacting partner of CFTR and that Pdzk1 binds Muc3(17) and stabilizes it at the apical membrane may suggest, for the first time, a direct connection between CFTR and mucins. It is an open question whether transmembrane mucins via PDZ proteins can influence the surface expression of CFTR or the opposite. However, recent studies in the small intestine of Pdzk1−/− mice reveal mild reduction in maximal Cftr activation and a large reduction in Na+ absorption, suggesting that Ctr is also affected in these mice [33]. This is in line with the suggestion, although still controversial, that the PDZ-interacting domain of CFTR has a role in its endocytic recycling. Deletion of this domain in CFTR did not affect the targeting to the apical membrane, but reduced the t1/2 of CFTR in the membrane [34]. Furthermore, Gage et al. [35] reported that type I PDZ ligands promote rapid recycling of G-protein-coupled receptors, such as the β2-adrenergic receptor. It has recently, without proof, been suggested that the fully glycosylated MUC17 undergoes an endocytic recycling cycle similar to the MUC1 mucin [36]. It can thus be postulated that PDZK1 is involved in post-endocytic sorting of MUC17 and that interaction between these proteins
facilitates endocytic recycling of the mucin back to the membrane. Absence of this interaction might then cause an accumulation of MUC17 in vesicles inside the cell, something that is consistent with our results.

In conclusion, PDZK1 was shown to interact with a classic PDZ-interaction motif in the extreme C-terminal tail of the mucin MUC17. Immunofluorescence microscopy in the small intestine of Pdzk1−/− mice revealed that Pdzk1 plays an essential role in stabilizing the mouse form of this mucin, Muc3(17), at the apical enterocyte membrane. Other mechanisms or another PDZ protein is probably responsible for the proper retention of this mucin in the apical membrane of the epithelial cells in the colon, as absence of Pdzk1 had no discernible effect on Muc3(17) localization. However, as Muc3(17) has a substantially lower expression in colon, the absence of Pdzk1 might have a less pronounced effect not as easy to observe.

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