Regulation of the SLC26A3 anion exchanger activity by the NHERF4 PDZ protein

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Directed by Professor Min Goo Lee

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기원합니다.

실험 때문에 바쁘다고 연락도 뜸한 못난 친구에게 잊지 않고 안부 전해주는 상진, 원용, 귀한, 병현, 지훈, 승근, 일규에게 감사하며, 군생활 동안 든든한 버팀목이 되어주신 Caddell 아저씨, 이젠 자신들도 대학생이라며 술자리를 독촉하는 혜원, 한슬이에게도 미안함과 고마움을 전합니다. 영원한 저의 실험조교님이자 인생멘토이신 능규 조교님과 오랜 시간 더욱 서로를 이해하고 닮아가고 사랑하는 미희에게 이 지면을 빌어 특별한 감사의 마음 전합니다.

다섯 조카녀석들 한진, 다현, 다정, 규한, 다민에게 다음엔 많이 놀아준다고 약속만하고 실천 못하는 거짓말쟁이 삼촌이 미안한 마음을 전하며, 저에게 이런 착하고 사랑스런 조카들을 선물해준 누이들과 매형 분들에게 감사하고 두 가정의 행복이 가득하길 기원합니다. 끝으로 우주와 같은 큰 사랑으로 보살펴주시는 아버지, 어머니께 가장 감사드리며 두 분의 건강과 사랑 영원하길 기원합니다. 서른 살이 넘도록 까지 학교다니는 외손자에게 따뜻한 아침밥 먹여 보내겠다고 당신의 새벽 단잠을 깨우시는 사랑하는 외할머니, 그리고 얼마 전 끝내 이북 고향 땅을 다시 밟지 못하시고 타향에서 하느님 곁으로 당신의 마지막 여정을 떠나신 그립고 그리운 친할머니께 마음속 깊이 감사 드리며 글을 맺습니다.

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ABSTRACT

Regulation of the SLC26A3 anion exchanger activity

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The solute linked carrier (SLC) 26A3, also known as the downregulated in adenomas (DRA), functions as a Cl^-/HCO_3^- exchanger and is expressed at the apical membrane of secretory epithelial cells in the intestines, pancreas and salivary glands. In the exploratory yeast two-hybrid assay, the sodium/proton exchanger regulatory factor (NHERF) 4, a PDZ-containing scaffold protein also known as the intestinal and kidney-enriched PDZ protein (IKEPP), was found to interact with the SLC26A3. In this study, the functional role of interaction between NHERF4 and SLC26A3 was investigated using an integrated molecular physiological approach. Immunoprecipitation with the C-terminus-deleted SLC26A3 mutant revealed that the C-terminal PDZ binding motif of SLC26A3 was required for the SLC26A3-NHERF4 interaction. In addition, a direct protein-protein interaction between the C-terminus of SLC26A3 and the third PDZ domain of NHERF4 (NHERF4-PDZ3) was observed in the GST-based pull-down assay. Of note, co-expression of NHERF4 decreased the surface expression of SLC26A3 by accelerating endocytosis and consequently reduced the SLC26A3-mediated Cl^-/HCO_3^- exchange activities. In

contrast, knockdown of the NHERF4 expression by treatment with small interfering RNAs increased the DIDS-insensitive Cl^-/HCO_3^- exchange activities in the HT-29 human colonic epithelial cells. Interestingly, modulation of phosphorylation at the NHERF4-PDZ3 altered the intensity of interaction between NHERF4 and SLC26A3. These results imply that NHERF4 is a physiological regulator of SLC26A3 by affecting its surface expression and that phosphorylation of NHERF4-PDZ3 may be one of the important regulatory factors to control the SLC26A3-NHERF4 interaction.

Key words: PDZ domain, SLC26A3, anion exchanger, NHERF4, endocytosis

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I. INTRODUCTION

The **s**olute **l**inked **c**arrier (SLC) 26A3, which is also known as the **d**own**r**egulated in **a**denomas (DRA), functions as a chloride/bicarbonate (or hydroxyl) anion exchanger at intestinal mucosal epithelium in duodenum, ileum, cecum, and distal colon[1-4]. The anion transport plays important roles in chloride absorption and bicarbonate secretion in the colon[4, 5]. Previous studies reported that the highly conserved C-terminal domain of the SLC26A proteins, which is named a **s**ulfate **t**ransporters and **a**nti**s**igma-factor antagonists (STAS), is an important region for proper ion transport activity. Malfunction of the SLC26A3 caused by various types of mutation on the STAS region is responsible for **c**ongenital ch**l**oride **d**iarrhea (CLD) [2, 6, 7]. Furthermore, many SLC26A proteins including the SLC26A3 have PDZ binding motif sequences at their C-termini, which make connections to various PDZ proteins. The PDZ proteins can coordinate with other regulatory factors[8, 9].

PDZ (**P**DS-95/**d**iscs large/**Z**O-1) domains are the most abundant protein-protein interaction modules in the human genome. PDZ proteins are involved in cellular physiology by assembling multimeric protein complex. PDZ domains compose of two α helixes and six β sheets and bind to short peptide sequences which are usually located at the C-termini of target binding proteins. The short

peptide sequences, PDZ binding motifs, are categorized into three classes: (Class I) -X-S/T-X-Φ, (Class II) -X-Φ-X-Φ, and (Class III) -X-D/E-X-Φ, where Φ represents a hydrophobic residue[10-13]. The PDZ domains have a binding pocket region which is located between the second α helix and the second β sheet. Characteristic amino acid sequences in the binding pocket region are critical to define binding selectivity to their target proteins[14, 15]

The **N**a$^+$/**H**$^+$ **e**xchanger **r**egulatory **f**actor (NHERF) family of epithelial-enriched PDZ domain scaffold proteins is a key modulator for epithelial cell functions with coordination various regulatory factors for ion transport and second messenger cascades. The NHERF family proteins located near the apical regions of epithelial cells and interacted with the class I PDZ binding motifs[16]. The NHERF1 and the NHRF2 compose two PDZ domains and C-terminal **e**zin-**r**adixin-**m**oesin (ERM) binding domains, whereas the NHERF3 and the NHERF4 possess only four PDZ domains. Of the four NHERF family proteins, the NHERF4 was indentified most recently and it has the sequence homology with the other NHERF proteins. The NHERF4 is also well known as the **i**ntestinal and **k**idney-**e**nriched **P**DZ **p**rotein (IKEPP) because of its most restricted tissue expression of NHERF family proteins[16, 17]. For that reason, it is believed that the NHERF4 certainly plays important roles in intestine and kidney. However, its main roles as well as binding proteins have not yet been elucidated.

In this study, yeast two-hybrid screening data showed that the SLC26A3 was a novel NHERF4 binding ion transporter. Thus, regulatory mechanisms of the NHERF4 and physiological significances of their interaction were investigated.

II. MATERIALS AND METHODS

1. Materials, solution, and cells

Standard HEPES-buffered solution A contained (mmol/L) 145 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, and 10 HEPES (pH 7.4 with NaOH). HCO_3^--buffered solution B contained (mmol/L) 120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, 5 HEPES, and 25 $NaHCO_3$ (pH 7.4 with NaOH). Cl^--free solutions were prepared by replacing Cl^- with gluconate. HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. HT-29 cells (KCLB 30038; Korean Cell Line Bank, Seoul, Korea) were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum. The fluorescent pH probe, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), was purchased from Molecular Probes (Eugene, OR). Primary antibodies used for immunostaining and immunoblotting were as follows: anti-Myc monoclonal and polyclonal antibodies, anti-HA monoclonal and polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), anti-His antibody (Calbiochem, Darmstadt, Germany), and anti-NHERF4 (from Dr. Sharon L. Milgram, University of North Carolina). Fluorescein isothiocyanate (FITC)- or rhodamine-conjugated secondary antibodies were from Zymed (Carksbad, CA). Restriction enzymes and peptide-*N*-glycosidase F (PNGase F) were purchased from New England Biolabs (Beverly, MA). All other general chemicals including the siRNA for SLC26A3 and NHERF4 were purchased from Sigma.

2. Plasmid vectors

The coding region of SLC26A3 cDNA was amplified by PCR from the cDNA library of human colon cell line, T84, and subcloned into the pCMV-Myc vector (Clontech, Palo Alto, CA). The SLC26A3 cDNA sequence was verified by nucleotide sequencing, and was identical to a registered sequence (Genbank Accession NM_000111). For deletions of SLC26A3 C-terminal PDZ binding motif, oligonucleotide-directed mutagenesis was performed. pcDNA3.1-HA-NHERF4 was kindly gifted from Dr. Sharon L. Milgram (University of North Carolina, North

Carolina). S329A- or S329D-NHERF4 was generated by oligonucleotide-directed mutagenesis. For GST pulldown assay, SLC26A3 C-terminal 43 amino acids sequence was added to GST using pGEX-KG vector (Amersham Biosciences). 6×His-tagged truncated NHERF4- PDZ1 to PDZ4 pulldown plasmid vectors were gifted from Dr. Sharon L. Milgram.

3. Yeast two-hybrid analysis

The L40 yeast strain harboring the reporter genes HIS3 and LacZ, under control of the upstream LexA DNA-binding domain was used in the assay. To semi-quantify the interaction, HIS3 activity was determined by the percentage of yeast colonies growing on histidine-lacking medium. cDNA sequences containing the carboxyl-terminal ~50 amino acids of CFTR, SLC4A7, SLC26A3, SLC26A6, SLC26A7, and SLC26A9 were amplified by PCR and subcloned into pBHA construct (a bait vector containing the LexA DNA-binding domain). The pGAD10 (a prey vector, Clontech) constructs containing the PDZ domains NHERF1-PDZ1, NHERF1-PDZ2, full-length NHERF2, full-length NHERF3 and full-length NHERF4 were generated.

4. Immunoprecipitation, immunoblotting, and digestion of N-glycosylation

HeLa cells were lysed with lysis buffer (1 % NP-40, 150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.4) containing complete protease inhibitor cocktails (Roche Applied Science, Mannheim, Germany). After lysis, cell debris was removed by centrifugation, and cleared lysates were mixed with the appropriate antibodies and incubated overnight at 4°C. Immune complexes were collected by incubation for 2 hr at 4°C with protein A or G plus agarose and washed four times with lysis buffer to electrophoresis. The immunoprecipitates and cell lysates were suspended in 2× SDS sample buffer and then separated by SDS-PAGE. The separated proteins were transferred to nitrocellulose membranes, and the membranes were blocked by incubation for 1 hr in a solution containing 5% nonfat dry milk in phosphate-buffered saline (PBS) containing 0.1% Tween 20. The membranes were then incubated with the appropriate primary antibodies and secondary antibodies.

Enhanced chemiluminescence solution was used to detect protein bands.

For digestion of glycosylated pendrin by PNGase F, protein samples were first denatured by adding SDS to 0.5% and β-mercaptoethanol to 1%, and incubating for 10 min at 37°C. NP-40 was then added to 1% and N-linked carbohydrates were removed by adding PNGase F (500 U/reaction) and incubating the solution for 2 hr at 37°C.

5. Surface biotinylation and endocytic Assay

For surface biotinylation of the SLC26A3, cells were washed with ice-cold PBS and then incubated with another ice-cold PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ for 5 min. Cells were then biotinylated using EZ-Link Sulfo-NHS-SS-biotin (Pierce, 0.5 mg/ml) for 30 min in dark at 4°C. After washing free biotin with bovine serum albumin-containing (1% w/v) PBS, cells were lysed with lysis buffer. The lysates were centrifuged for 10 min (13,000 × g), and the pellet was discarded. Avidin solution (Streptavidin beads, Pierce, 50 μl) was added to the supernatant (300 μg of protein in 300 μl of lysis buffer), and the mixture was incubated overnight with gentle agitation. Avidin-bound complexes were pelleted (13,000 × g), washed three times with lysis buffer, and immunoblotted.

For endocytic assay of SLC26A3, cells were biotinylated using EZ-Link sulfo-NHS-SS-biotin (Pierce, 0.5 mg/ml) for 30 min in dark at 4°C. Subsequently, in order to induce endosytosis, the cells were warmed to 37°C for 5, 15, 30, and 60 min, and the disulfide bonds on Sulfo-NHS-SS-biotinylated proteins remaining were reduced by 50 mM gluthathione (GSH) added PBS solution six times for total 90 min at 4°C. At this point in the protocol, biotinylated proteins reside within endosomal compartment. Subsequently, the cells were lysed with lysis buffer. The lysates were centrifuged for 10 min (13,000 × g), and the pellet was discarded. Avidin solution (Streptavidin beads, Pierce, 50 μl) was added to the supernatant (300 μg of protein in 300 μl of lysis buffer), and the mixture was incubated overnight with gentle agitation. Avidin-bound complexes were pelleted (13,000 × g), washed three times with lysis buffer, and immunoblotted.

6. Immunocytochemistry

HeLa cells were transfected with plasmids for SLC26A3 or NHERF4 using the Lipofectamine-Plus reagent (Invitrogen, Carlsbad, CA). Immunostaining was performed using anti-Myc primary antibody for the SLC26A3 or anti-HA primary antibody for the NHERF4. Cells were fixed and permeabilized by incubation in cold methanol for 10 min at -20°C, and then stained with the primary antibodies and the fluorescently labeled secondary antibodies. Fluorescent images were obtained with Zeiss LSM 510 confocal microscope. Fluorescence of FITC was excited at 488 nm by argon laser, and emitted fluorescence was detected with 505-530 nm band-pass filter. Fluorescence of rhodamine was excited at 543 nm by He-Ne laser, and emitted fluorescence was detected with 560-615 nm band-pass filter.

7. GST pulldown analysis

GST-fusion SLC26A3 C-terminal 43 amino acid peptides containing protein was expressed in *E. coli* BL21 and purified with glutathione-sepharose (Amersham Biosciences, Sweden). Four 6×His-tagged NHERF4 PDZ domains also were expressed in *E. coli* BL21 and purified with Ni-NTA agarose beads (Qiagen, Germany). Glutathione-sepharose binding to GST-fusion SLC26A3 C-terminal protein was incubated with an equal quantity of each purified NHERF4 PDZ domains overnight with gentle agitation at 4°C. The glutathione-sepharose was pelleted and washed three times with ice-cold lysis buffer prior to resuspension in 2×SDS sample buffer and SDS-PAGE. Immunoblots for 6×His-tagged proteins were executed for detection of 6×His-tagged NHERF4 PDZ domains.

8. Measurement of intracellular pH (pH_i) and Cl^-/HCO_3^- exchange

Measurements of pH_i in HeLa cells transiently transfected with SLC26A3 or NHERF4 plasmids were performed using a pH-sensitive fluorescent probe BCECF with cotransfection of the *trans*-gene marker pEGFP-N1 (Invitrogen, Carlsbad, CA). A cluster of cells showing the green-fluorescent protein (GFP) fluorescence were loaded with BCECF and pH_i was monitored. To minimize the variations from

transfection efficiencies, cells showing GFP fluorescence within 80-120% levels of control cells transfected with pEGFP-N1 were chosen for measuring anion exchange in each set of experiments. After dye loading, the cells were perfused with a HCO_3^--buffered solution B, and BCECF fluorescence was recorded at the excitation wavelengths of 490 and 440 nm at a resolution of 2/s using a recording setup (Delta Ram; PTI Inc. Birmingham, NJ). $Cl^-_i/HCO_3^-{}_o$ exchange activities were estimated from the initial rate of pH_i increase as a result of Cl^-_o removal in the HCO_3^--containing buffer (25 mM HCO_3^- with a 5% CO_2 gassing).

9. Reverse transcription-polymerase chain reaction (RT-PCR)

For confirmation of the expression of SLC26A3 and NHERF4 in HT-29 cells, the messenger RNA (mRNA) transcripts of SLC26A3 or NHERF4 were analyzed by the semi-quantitative RT-PCR. Total RNA was extracted by using a Trizol solution (Invitrogen, Carlsbad, CA) and an equal amount of RNA (2 μg) from each sample was reverse-transcribed by using an oligo (dT) primer and RNase H- reverse transcriptase (Invitrogen). The complementary DNA (cDNA) was amplified with specific primers and a Taq polymerase (Promega, Madison, WI). To semi-quantify the amount of each mRNA transcript, the logarithmic increasing phase of PCR was determined using serially diluted cDNA samples in each set of experiments. The PCR primer sequences were as follows: SLC26A3-sense, CTG GAT CAT CTC AAA GTG TG; antisense, CAT ACT CAG AAT CGG AAA CG ; size of PCR product, 300 base pairs (bp); NHERF4-sense, GCT GGG CAT TGA TAA TCC TG ; antisense, AAG GAC CCT GAT TGC CAT G ; size of PCR product, 470 base pairs (bp); human beta actin-sense, GAC CCA GAT CAT GTT TGA GAC C; antisense, GGC CAT CTC TTG CTC GAA GTC; size of PCR product, 328 base pairs (bp).

10. Protein structure modeling

NHERF4-PDZ3 domain structure modeling (PDB: 2V90) was performed using VMD software package (version 1.8.6) based on the Apple MacOSX 10.4 system.

11. Statistical analysis

The results were presented as the means ± SEM. Statistical analysis was performed with Student *t*-tests using the GraphPad Prism software package (version 4.0). $P < 0.05$ was considered statistically significant.

III. RESULTS

1. Screening of NHERF binding proteins

Yeast two-hybrid (Y2H) analysis was used to screen binding target proteins of the NHERF PDZ domains. The C-terminal PDZ binding motifs in various gastric epithelial ion transporters including CFTR, SLC4A7, SLC26A3, SLC26A6, SLC26A7, and SLC26A9 were used as bait, and the full-length PDZ domains of NHERF proteins were used as its prey. The results of Y2H analysis corresponded to previous studies considerably (Table 1)[10, 18, 19]. For examples, many studies have reported that the CFTR interacted with the NHERF -1, -2, and -3. Their interactions played important roles to regulate trafficking to the plasma membrane and functions of CFTR[10, 20]. The results of Y2H analysis showed that the NHERF4 interacted with CFTR, SLC26A3, SLC26A6, and SLC26A9, but not with SLC4A7 and SLC26A7. The interactional intensities with the NHERF4 are as follows: SLC26A3 = SLC26A6 > CFTR = SLC26A9. Among them, I focused on the SLC26A3 as a novel binding ion transporter of the NHERF4 because (1) its interactional intensity was one of the highest among the ion transporters and (2) in previous studies, its expression was well established in intestinal gastric epithelium where the NHERF4 is also expressed[3, 21].

Table 1. Yeast two-hybrid analysis of NHERF binding proteins

Yeast two-hybrid (Y2H) analysis was used to screen binding target proteins of the NHERF PDZ domains. The C-terminal PDZ binding motifs in various gastric epithelial ion transporters including CFTR, SLC4A7, SLC26A3, SLC26A6, SLC26A7, and SLC26A9 were used as bait, and the full-length PDZ domains of NHERF proteins were used as its prey.

Y2H Bait \ Y2H Prey		NHERF1		NHERF2	NHERF3	NHERF4
		PDZ1	PDZ2			
CFTR	His	+++	+++	++	+	++
	β-gal	+++	+++	++	-	++
SLC4A7	His	-	-	-	-	-
	β-gal	-	-	-	-	-
SLC26A3	His	-	-	-	-	+++
	β-gal	-	-	-	-	+++
SLC26A6	His	+++	-	-	++	+++
	β-gal	+++	-	-	++	+++
SLC26A7	His	-	-	-	-	-
	β-gal	-	-	-	-	-
SLC26A9	His	++	+	+	++	++
	β-gal	+	-	-	++	++

HIS3 **+++** **(>60%)** **++** **(30-60%)** **+** **(10-30%)** **-** **(No significant growth)**

β-gal **+++** **(<45min)** **++** **(45-90min)** **+** **(90-240min)** **-** **(>240min)**

2. SLC26A3 interacted with NHERF4

Even though the Y2H analysis is one of the powerful molecular tools for protein-protein interaction study, this technique has an essential limitation that its interaction is based on the yeast system. Therefore, protein-protein interactions in mammalian systems were confirmed by coimmunoprecipitations (co-IPs). The Myc-SLC26A3 and the HA-NHERF4 were overexpressed in HeLa cells and followed by IP with antibodies against the SLC26A3 N-terminal Myc-epitope or the NHERF4 N-terminal HA-epitope (FIG 1A). SLC26A3 proteins were observed in immunoprecipitates with anti-HA antibody. In a converse experiment, NHERF4 proteins were detected in immunoprecipitates with anti-Myc antibody. In case of SLC26A3 proteins, not only an 82 KDa protein band but also higher molecular weight bands (≥105 KDa) were detected. Digestion of SLC26A3 proteins with peptide N-glycosidase (PNGase) F cleared 105 KDa bands and produced a single 82 KDa band (FIG 1B). It showed obviously that the 105 KDa bands were a SLC26A3 glycosylation patterns and the complex glycosylated SLC26A3 also interacted with the NHERF4. In addition, intracellular localizations of SLC26A3 and NHERF4 proteins were examined by the immunocytochemistry in HeLa cells which expressed SLC26A3 and NHERF4 proteins heterologously (FIG 1C). Approximately 36 hr after transfection for NHERF4 and SLC26A3 plasmids in HeLa cells, anti-HA for the NHERF4 and anti-Myc for the SLC26A3 primary antibodies were stained. Rhodamine and fluorescein isothiocyanate (FITC) labeled secondary antibodies were used for fluorescence imaging, respectively. NHERF4 as well as SLC26A3 proteins reached at the plasma membrane regions and both proteins were colocalized considerably. These data indicated that the SLC26A3 was a binding protein of the NHERF4 in the mammalian cellular system and their intracellular localizations were at the plasma membrane regions.

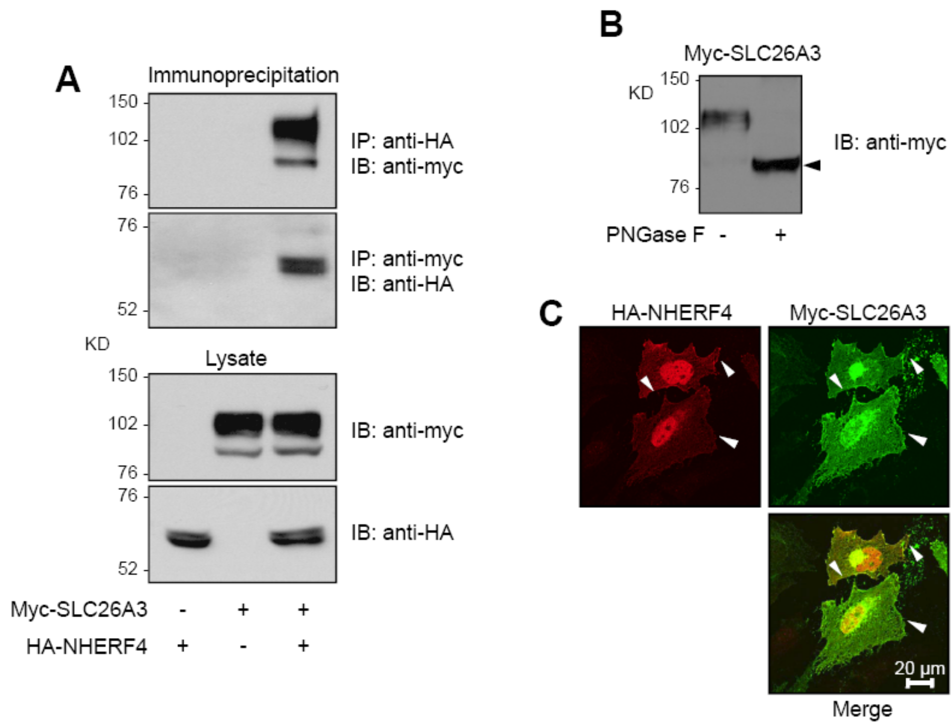

Figure 1. Interaction of SLC26A3 with NHERF4 (A) SLC26A3 and NHERF4 proteins were overexpressed in HeLa cells (lower panels) and subsequently immunoprecipitated from cell lysates using anti-HA for NHERF4 and anti-Myc for SLC26A3 primary antibodies (upper panels). (B) Sample from cells overexpressed of the SLC26A3 was digested with PNGase F. (C) After 36 hr cotransfection with the SLC26A3 and the NHERF4 plasmids in HeLa cells, the cells were incubated with anti-Myc and anti-HA primary antibodies and stained with FITC (green) and Rhodamine (red) conjugated secondary antibodies, respectively.

3. PDZ-based direct interaction between SLC26A3 and NHERF4

To verify that the PDZ based protein-protein interaction between the SLC26A3 and the NHERF4, deletions of the C-terminal PDZ binding motif of SLC26A3 were generated and examined co-IPs with NHERF4 proteins in HeLa cells (FIG 2A and 2C). Deletion of the PDZ binding motif of SLC26A3 (SLC26A3-ΔETKF) did not alter its total amount of expressed proteins as compared to that of the wild-type (WT) SLC26A3 and showed a normal surface expression pattern (FIG 2B). However, the result of co-IPs showed that deletions of the PDZ binding motif of SLC26A3 (SLC26A3- ΔETKF and ΔTKF) limited its interaction with the NHERF4 (FIG 2C).

As refer to the introduction, the NHERF4 consists of only four PDZ domains. Combination of various amino acids sequence at a binding pocket region of PDZ domain is one of crucial factors to define binding selectivity[13, 14]. Hence each four PDZ domain of the NHERF4 might have different binding selectivity to the PDZ binding motif of SLC26A3. In order to identify a NHERF4 PDZ domain binding to the SLC26A3, a GST-fusion SLC26A3 C-terminal peptide and four NHERF4 truncated 6×His-tagged PDZ domains were used for pull-down assay: PDZ1(58-130), PDZ2(165-235), PDZ3(271-346), and PDZ4(410-482). The GST-fusion SLC26A3 C-terminal peptide consisted of the 43 amino acids sequences of SLC26A3 C-termini (FIG 3A and 3C). The result of pull-down assay revealed that the C-terminal peptide of SLC26A3 interacted with the third PDZ domain of NHERF4 (NHERF4-PDZ3) mainly and there were no interactions with NHERF4-PDZ1 or PDZ2 (FIG 3C). Although the C-terminus of SLC26A3 interacted with the fourth PDZ domain of NHERF4, it was a much weaker interaction compared to the NHERF4-PDZ3. Therefore, the SLC26A3-NHERF4 interaction was PDZ based and the interaction occurred between the SLC26A3 PDZ binding motif and the NHERF4-PDZ3 directly.

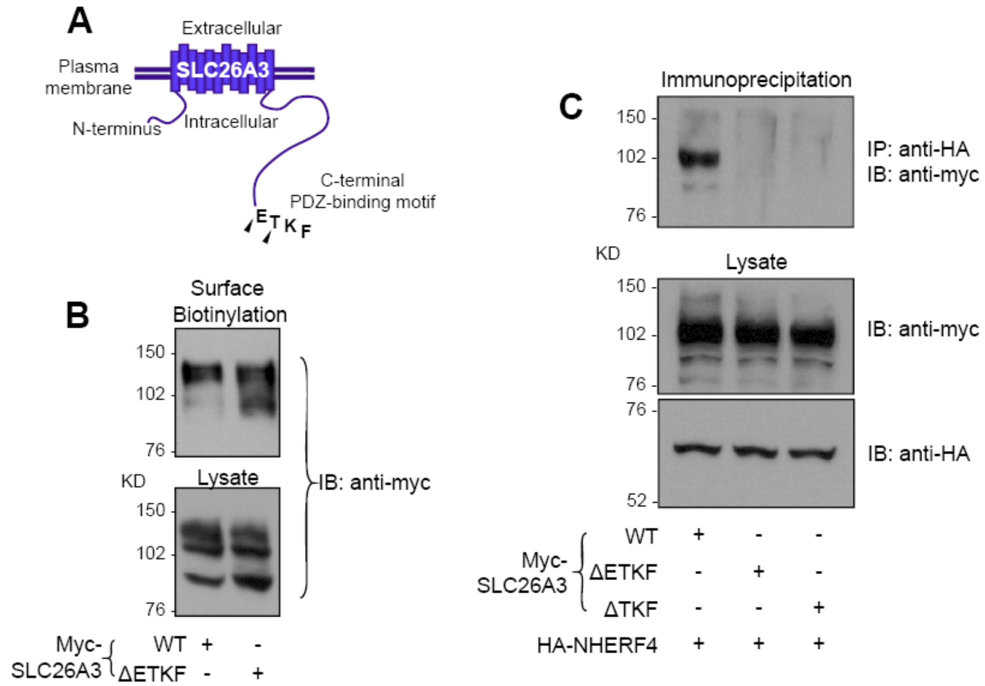

Figure 2. PDZ-based interaction between SLC26A3 and NHERF4 (A) The amino acids sequence of the SLC26A3 C-terminal PDZ biding motif is –ETKF. Arrows on a topology of SLC26A3 indicates the deleted sites of SLC26A3-ΔTKF and SLC26A3-ΔETKF. (B) WT-SLC26A3 or SLC26A3-ΔETKF proteins were overexpressed and the surface biotinylation assay was examined. (C) NHERF4 proteins were cooverexpressed with SLC26A3- WT, ΔETKF, or ΔTKF proteins in HeLa cells (lower panels) and subsequently immunoprecipitated from cell lysates using an anti-HA primary antibody for NHERF4 proteins (upper panels).

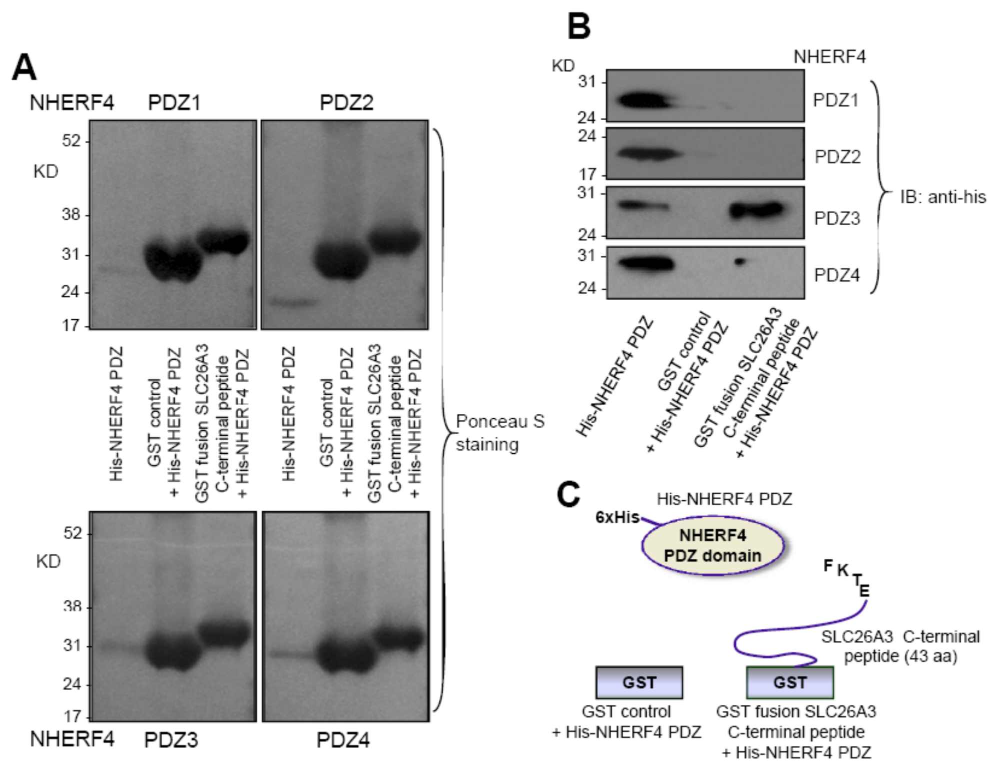

Figure 3. GST pull down assay between SLC26A3 and NHERF4 domains (A) Glutathione-sepharose beads binding to GST-fusion SLC26A3 C-terminal protein were incubated with an equal quantity of purified NHERF4 PDZ domains overnight with gentle agitation at 4 °C. Purified 6×His-tagged NHERF4 PDZ domains, mock GST incubated with 6×His-tagged NHERF4 PDZ domains, and GST fusion SLC26A3 C-terminal peptide protein incubated with 6×His-tagged NHERF4 PDZ domains were separated by SDS-PAGE. After the separated proteins were transferred to nitrocellulose membranes, the membranes were stained with Ponceau S solution. (B-C) Immunoblots for 6×His-tagged proteins were executed for detection of 6×His-tagged NHERF4 PDZ domains.

4. Downregulation of SLC26A3 transport activity by NHERF4

The SLC26A3 functions as a Cl^-/HCO_3^- anion exchanger at the apical membrane of secretory epithelial cells and its anion exchange activities are one of the key factors to determine intestinal water absorption and secretion[4, 8, 22]. Previous studies reported that the NHERF4 regulated its binding membrane proteins by functional inhibition or a disturbance of trafficking to the plasma membrane[16, 17, 23-27]. Therefore, the Cl^-/HCO_3^- anion exchange activity of SLC26A3 depending on the NHERF4 interaction were evaluated. $Cl^-_i/HCO_3^-{}_o$ exchange activities were estimated from the initial rate of pH_i increase (ΔpH unit/min) as a result of Cl^-_o removal in the HCO_3^- containing buffer. Overexpression of SLC26A3 proteins evoked an increase in Cl^-/HCO_3^- exchange activity in HeLa cells (FIG 4A-4B). However, coexpression with the NHERF4 proteins induced a decrease of SLC26A3-mediated Cl^-/HCO_3^- exchange activity significantly. Interestingly, Cl^-/HCO_3^- exchange activities of SLC26A3-ΔETKF were not affected by coexpression with the NHERF4 (FIG 4B). Moreover, the Cl^-/HCO_3^- exchange activity of SLC26A3-ΔETKF was not significantly different from the WT-SLC26A3 activity. These data implied that the SLC26A3-NHERF4 interaction significantly reduced the SLC26A3-mediated Cl^-/HCO_3^- exchange activity and the SLC26A3 C-terminal PDZ binding motif was not a functional regulatory region.

Downregulation of membrane transporter activation could be induced not only by inhibition of transport functions itself but also by disturbance of proper expression at the plasma membrane. Many previous studies reported that the NHERF1 and the NHERF2 were important modulators for trafficking and intracellular localization of their binding proteins using their ERM domain[10, 16, 28, 29]. Furthermore, a few current studies reported that the NHERF3 was also an important regulatory scaffold protein to define intracellular localization of its binding proteins although the NHERF3 did not possess the ERM domain[19, 30]. Thus, I examined whether the NHERF4 affected the localization of the SLC26A3 at the plasma membrane regions using a surface biotinylation assay. Sulfo-NHS-biotins only links surface-expressed proteins using its cellular membrane impermeable properties and the biotinylated proteins can be

separated selectively using avidin immobilized resins. Coexpression of NHERF4 proteins decreased the level of surface-expressed SLC26A3 remarkably (FIG 4C). Interestingly, the level of surface-expressed SLC26A3 was proportioned to the level of NHERF4 expression inversely. On the other hand, the NHERF4 did not affect to the level of surface expressed SLC26A3-ΔETKF.

Most ion transporters at the plasma membrane interact with cytosolic regulatory proteins and the regulatory proteins can modulate functional activities of ion transporters by changing the half-time of surface-expressed ion transporters[10, 18, 31]. Thus, the levels of surface-expressed SLC26A3 depending on the NHERF4 expression were evaluated using the endocytic assay (FIG 4D-4E). In the endocytic assay, cells were surface-biotinylated at 4℃ after SLC26A3 overexpression, and then the cells were incubated at 37℃ for 5, 15, 30, and 60 min to induce endocytosis. In the protocol, biotinylated proteins reside within endosomal compartment at each time. Coexpression of the NHERF4 induced more rapid and quantitative endocytosis of the surface-expressed SLC26A3 at every period of indicated time within 60 min (FIG 4E). It implied that the NHERF4 induced the acceleration of the SLC26A3 endocytosis and reduced the amount of surface-expressed SLC26A3 consequently. These data evidently indicated that a decrease in the SLC26A3 Cl^-/HCO_3^- exchange activity was a result from loss of the functional surface-expressed SLC26A3.

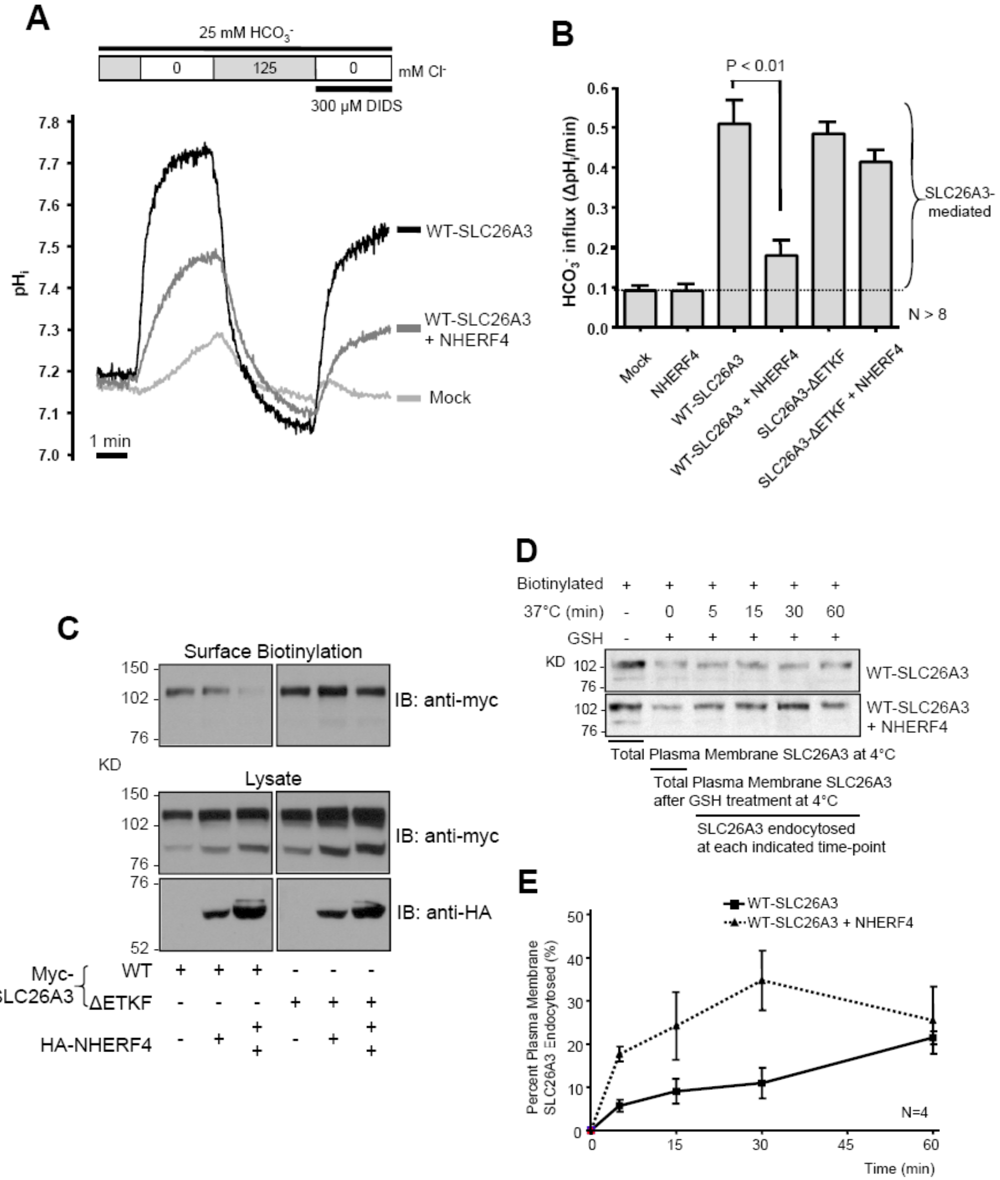
A
25 mM HCO3-
0
125
0
mM Cl-
300 μM DIDS
pHi
WT-SLC26A3
WT-SLC26A3
+ NHERF4
Mock
1 min
B
P < 0.01
HCO3- influx (ΔpHi/min)
SLC26A3-
mediated
N > 8
Mock
NHERF4
WT-SLC26A3
WT-SLC26A3 + NHERF4
SLC26A3-ΔETKF
SLC26A3-ΔETKF + NHERF4
C
Surface Biotinylation
IB: anti-myc
KD
Lysate
IB: anti-myc
IB: anti-HA
Myc-
SLC26A3
WT
ΔETKF
HA-NHERF4
D
Biotinylated
37°C (min)
GSH
KD
WT-SLC26A3
WT-SLC26A3
+ NHERF4
Total Plasma Membrane SLC26A3 at 4°C
Total Plasma Membrane SLC26A3
after GSH treatment at 4°C
SLC26A3 endocytosed
at each indicated time-point
E
WT-SLC26A3
WT-SLC26A3 + NHERF4
Percent Plasma Membrane
SLC26A3 Endocytosed (%)
N=4
Time (min)

Figure 4. Downregulation of SLC26A3 transport activity by NHERF4 (A) Representative traces of HeLa cells transfected with mock vector (light gray), WT-SLC26A3 only (black), or WT-SLC26A3 plus NHERF4 (gray) plasmids for measurements of Cl^-/HCO_3^- exchange activity. (B) A summary of Cl^-/HCO_3^- exchange activity. The exchange activities were estimated from the initial rate of pH_i increase. All measurements were taken a minimum of three times on cells overexpressed with each of separate plasmid batches (n>8). (C) Surface biotinylation of SLC26A3 membrane proteins was performed to verify the effect of NHERF4 coexpression. In HeLa cells, an equal amount of WT-SLC26A3 or SLC26A3-ΔETKF proteins and serial levels of NHERF4 proteins were coexpressed. (D) Representative result of the endocytic assay. Overexpressed WT-SLC26A3 proteins were biotinylated using EZ-Link Sulfo-NHS-SS-biotin for 30 min in dark at 4°C. Subsequently, the cells were warmed to 37°C for 5, 15, 30, and 60 min. After incubation as the indicated periods, the cells were washed with GSH added PBS solution six times for total 90 min at 4°C. The cells were lysed, and the biotinylated proteins were pulled down with streptavidin and separated by SDS-PAGE. (E) Percent of the plasma membrane SLC26A3 endocytosed was traced at the indicated time points depending on coexpression of NHERF4. The amount biotinylated SLC26A3 remaining in the plasma membrane after GSH treatment at 4°C was subtracted from the amount of SLC26A3 remaining biotinylated after warming to 37°C. The experiments were performed four times.

5. Effect of NHERF4 knockdown in human colon epithelial cells, HT-29 cells

To verify regulatory effects of the NHERF4 in physiological condition, the changes of Cl^-/HCO_3^- exchange activity by NHERF4 knockdown in a human colon epithelial cell line, HT-29, were monitored. The NHERF4 as well as the SLC26A3 were expressed in the HT-29 cells (FIG 5A). After cDNA of HT-29 cells was synthesized using RT-PCR, the cDNA was amplified with SLC26A3- or NHERF4-specific primers by PCR. SLC26A3- or NHERF4-siRNA administration (100 pmol for 72 hr) reduced the PCR band intensity. Moreover, the result of RT-PCR implied that the NHERF4 knockdown did not affect mRNA transcription of SLC26A3 (FIG 5A). Immnoblot also confirmed that the NHERF4-siRNA administration reduced NHERF4 protein expression effectively (FIG 5B). Cl^-/HCO_3^- exchange activity in HT-29 cells was measured after knockdown of NHERF4 expression (FIG 5C-5D). To analyze the SLC26A3-mediated Cl^-/HCO_3^- exchange activity specifically, the activity measurements were executed at the apical region of monolayered HT-29 cells with 100 μM 4, 4'-diisothiocyanatostilbene-2, 2'-disulfonic acid (DIDS; an anion channel blocker) administration. Prior studies reported that the SLC26A3 expressed at the apical region in epithelial cells and the anion exchange activity of SLC26A3 was insensitive to DIDS at low doses (< 100 μM)[23, 24]. In fact, the recent data in this study also showed that the SLC26A3-mediated Cl^-/HCO_3^- exchange activity was almost insensitive to the concentration of 300 μM DIDS (FIG 4A). Representative trace was shown in FIG 5C and the summarized results were presented in FIG 5D. The knockdown of NHERF4 expression increased DIDS-insensitive Cl^-/HCO_3^- exchange activities significantly (FIG 5D). These results implied that the NHERF4 regulated the level of surface-expressed SLC26A3 in human colon epithelial cells. The reduced NHERF4 expression induced a decrease in the endocytosis of surface-expressed SLC26A3, and thus increased its anion exchange activity.

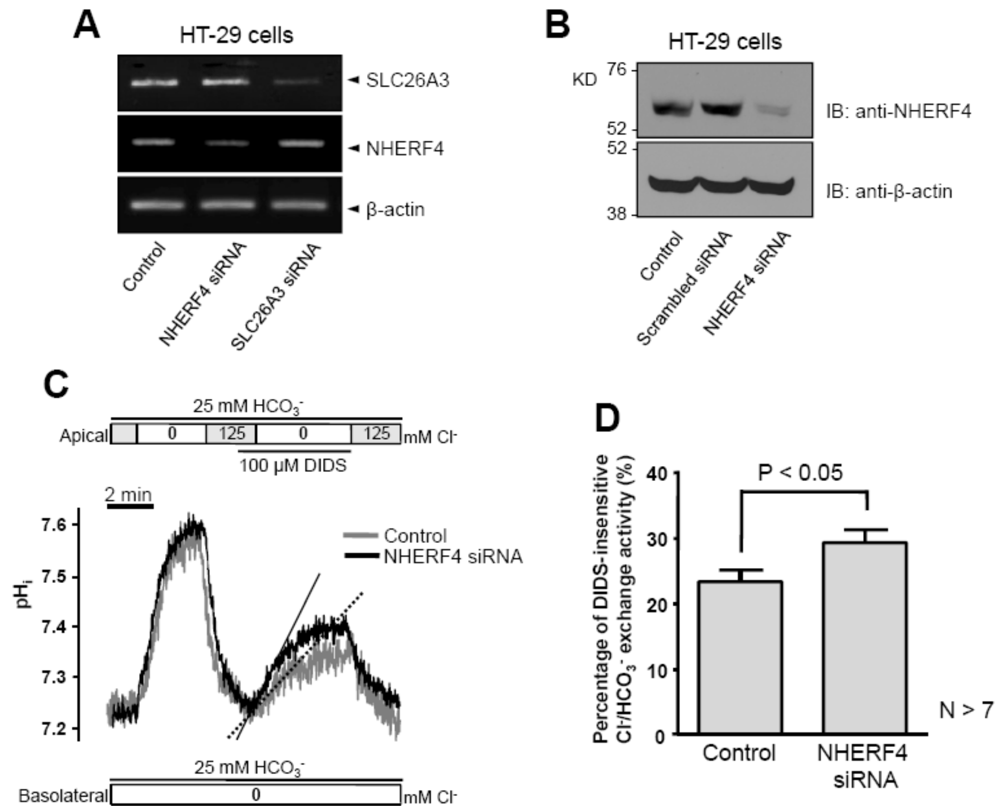

Figure 5. Effect of NHERF4 knockdown in human colon epithelial cells, HT-29 cells (A) After cDNA of HT-29 cells was synthesized using RT-PCR, the cDNA was amplified with SLC26A3- or NHERF4-specific primers by PCR. (B) After administration of scambled- or NHERF4-siRNA in HT-29 cells for 72 hr, the cells were lysed and separated by SDS-PAGE. The proteins were detected with an anti-NHERF4 antibody. (C) Representative traces of Cl^-/HCO_3^- exchange in HT-29 cells. Administration of the scrambled siRNA (gray) was used as a control. The total level of Cl^-/HCO_3^- exchange and the DIDS-insensitive Cl^-/HCO_3^- exchange were measured sequentially. The slope of the lines indicated the Cl^-/HCO_3^- exchange activity. The dashed line represented for control and the solid line represented for NHERF4 knockdown. (D) Summary of the percentage for the DIDS-insensitive Cl^-/HCO_3^- exchange activity of the total Cl^-/HCO_3^- exchange activity.

6. Serine 329 residue of NHERF4-PDZ3

The PDZ domain consists of two α helixes and six β sheets. The target PDZ binding motif places the binding pocket region between the second α helix and the second β sheet of a PDZ domain[12-14]. Therefore, properties of amino acids sequence at the binding pocket of PDZ domain are crucial to define binding selectivity to its binding proteins. For example, a serine 77 residue located on the surface of the second α helix of the first PDZ domain in the NHERF1, which regulated its binding selectivity to the target PDZ binding motif by phosphorylation[32, 33]. The multiple sequence alignments within all PDZ domains of NHERF family proteins revealed that the NHERF4-PDZ3 had a serine residue on the second α helix same as the NHERF1-PDZ1[17]. However, NHERF4-PDZ1, PDZ2, and PDZ4 had different amino acid residues: arginine, arginine, and glutamine, respectively. The recent results of a pull-down assay revealed that the NHERF4-PDZ3 was a main PDZ domain which bound to the SLC26A3. Moreover, the three-dimensional structure modeling of NHERF4-PDZ3 estimated that the location of the serine residue was very close to the potential amino acids sequences to define its binding selectivity (FIG 6A). To verify whether the serine 329 at the NHERF4-PDZ3 was an important residue to define the SLC26A3-NHERF4 interaction, two substitutions by oligonucleotide directed mutagenesis method were generated: the substitution to an alanine (S329A) and to an aspartate (S329D). The S329A substitution abolished phosphorylation of the serine residue, whereas the S329D mimicked a phosphorylation condition[32, 33]. Co-IPs results showed that the S329A-NHERF4 interacted with SLC26A3 more intensively compared to WT- or S329D- NHERF4 (FIG 6B). The administration of a cell permeable phosphatase inhibitor, calyculin A (10 nM for 30 min), decreased the SLC26A3-NHERF4 interaction, and increased the level of surface-expressed SLC26A3 (FIG 6C-6D). These data highly suggested that phosphorylation of the serine 329 residue at the NHERF4-PDZ3 regulated the binding selectivity to its target protein, SLC26A3.

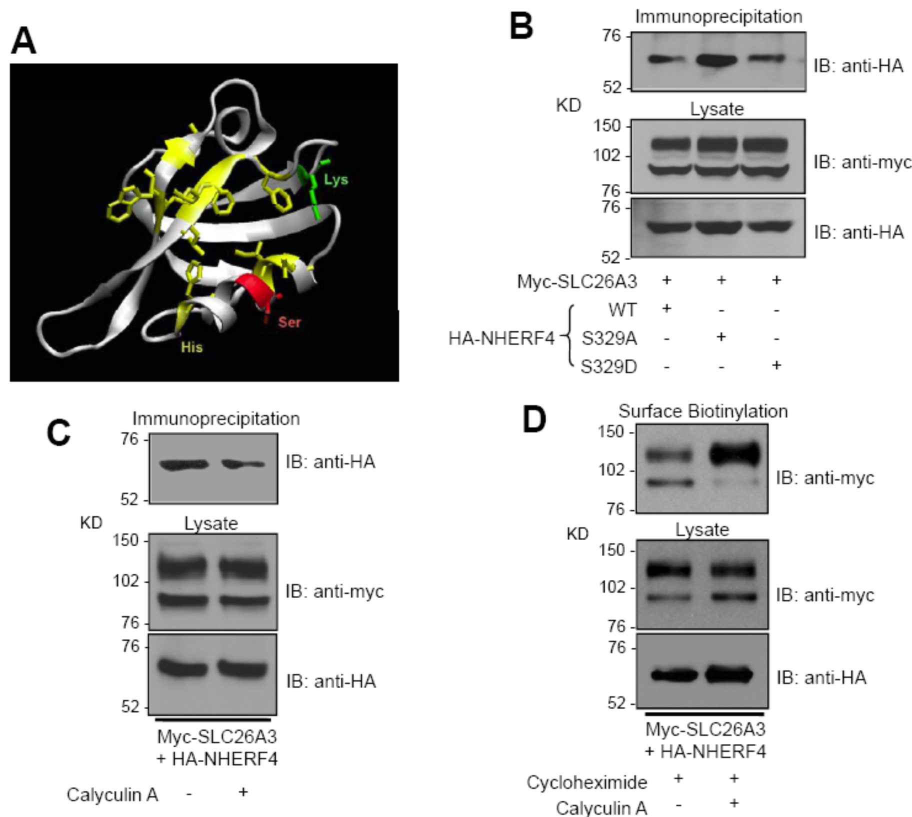

Figure 6. Serine 329 residue of NHERF4-PDZ3 (A) The NHERF4-PDZ3 three-dimensional structure modeling. Yellow-colored residues represented the estimated amino acids which define the binding selectivity. The serine 329 residue (red) was located on the second α helix structure. (B) The WT-, S329A-, or S329D-NHERF4 protein was overexpressed with the SLC26A3 in HeLa cells and immunoprecipitated from cell lysates using anti-HA antibody. (C) After the SLC26A3 and NHERF4 were overexpressed in HeLa cells for 36 hr, a cell permeable phosphatase inhibitor, calyculin A (10 nM for for 30 min), was administrated. Subsequently, the co-IPs was examined from cell lysates using anti-HA antibody. (D) The SLC26A3 and NHERF4 were overexpressed in HeLa cells for 36 hr. After inhibition of the newly synthesized SLC26A3 proteins by treatment of a cycloheximide (50 μg/ml for 60 min), a calyculin A (10 nM for for 30 min) was administrated subsequently. Surface biotinylation was performed using its cell lysates.

IV. DISCUSSION

About ten years ago, the *SLC26* gene family was first identified as a group of sulfate transporters from rat liver. Subsequently, the ten members of *SLC26* gene family were found in mammals, plants, fungi, and bacteria. Special interest in this gene family is focused by its association with inherited human disease: chondrodysplasias (*SLC26A2*), congenital chloride diarrhea (*SLC26A3*), Pendred syndrome and non-syndromic deafness (*SLC26A4*), and non-syndromic hearing impairment (*SLC26A5*) [8, 34, 35]. Several common structural features between SLC26 proteins are found such as the sulfate transport signature and the Saier's motif within hydrophobic transmembranes and the STAS domain in a C-terminal part of the intracellular tail. In addition, many of the SLC26 family proteins including SLC26A3 end with a class I PDZ binding motif. Although these C-terminal PDZ binding motifs are not necessary for transport function, they assemble other transporters or regulatory factors using various PDZ scaffold proteins[8].

In the recent study, comprehensive evidences revealed that the SLC26A3 was regulated by PDZ based protein-protein interaction with the PDZ scaffold protein, NHERF4. The SLC26A3-NHERF4 interaction reduced half-time of the surface-expressed SLC26A3 as a result of accelerating SLC26A3 endocytosis. Therefore, it implied that the NHERF4 derived the downregulation of SLC26A3 activity from loss of the functional SLC26A3 proteins at the plasma membrane rather than from the obstruction of transport function itself. In addition, it suggested that the phosphorylation at the NHERF4-PDZ3 was one of the important factors to define the binding selectivity of the SLC26A3-NHERF4 interaction.

When the *SLC26A3* gene was first discovered in 1993, it was considered one of the tumor suppressor genes in colon cancer because it was significantly down-regulated in colonic adenomas[8, 36]. In addition, the *SLC26A3* gene was found to be defective in CLD patients. The CLD is a recessively inherited defect of intestinal chloride/bicarbonate exchange and a potentially fatal diarrhea. Most mutations in the *SLC26A3* gene of CLD patients are isolated in the STAS domain where proximal part of the intracellular tail of SLC26A3 protein is[34]. The mutations cause

SLC26A3 misfolding and/or mistrafficking[2]. Interestingly, no CLD associated mutations have been found to reside on the PDZ-binding motif and no functional transport obstruction is observed on the artificial truncation of 44 C-terminal amino acids[7, 37]. It agreed with the recent results showing that the deletion of SLC26A3 PDZ binding motif (SLC26A3-ΔETKF) did not significantly affect its trafficking to the plasma membrane (FIG 2B) and Cl^-/HCO_3^- exchange activity as compared to that of WT-SLC26A3 (FIG 4B). Therefore, it appeared that proximal part of the intracellular tail of SLC26A3 protein played important roles to regulate its transport activity by following ways: (1) the STAS domain regulated the functional activity of SLC26A3 directly; and (2) the PDZ bind motif anchored various PDZ proteins which regulate the SLC26A3 activation indirectly; and (3) both regions might offer a place to let other regulatory factors be neighbored. Now, a study to find the regulatory factors binding to the NHERF4 is in progress. The candidates are several endocytic Rab small guanosine triphosphatases and Soluble NSF Attachment protein REceptor (SNARE) proteins[38, 39].

The NHERF4 was initially identified by Y2H screening of a human intestinal cDNA library using C-terminus of guanylyl cyclase C[17]. Although the NHERF4 consists of only four PDZ domains, potential roles of the NHERF4 attract attention by reason of its highly isolated tissue expression in the intestine and kidney[16]. Previous studies has shown that the NHERF4 interacted with several apical membrane proteins including the type IIa sodium/phosphate co-transporter (NaPi IIa) and the multi-drug resistance related protein 2 (MRP2), but its regulatory mechanism has not yet cleared[24, 40]. Recently, Zachos et al. showed that the sodium/proton exchanger 3 (NHE3) was a new binding protein of the NHERF4 and its interaction downregulated the NHE3 activity in intracellular calcium-dependent manner[23]. In this study, they implied that (1) the NHE3-NHERF4 complex was localized in the Rab11-positive recycling endosome, (2) increasing intracellular calcium level leaded to disrupt NHE3-NHERF4 interaction, (3) the detached NHE3 from the NHE3-NHERF4 interaction performed exocytosis to the plasma membrane, and (4) it increased the level of surface-expressed NHE3 and stimulated NHE3

activity consequently. It was well correspondent with the recent results that the interaction of NHERF4 reduced the SLC26A3 activation by decreasing functional transporters at the plasma membrane (FIG 4A-4E and FIG 5C-5D). However, the interpretation for the downregulatory role of the NHERF4 was not equal to this recent study. To be elucidating, they are necessary to show that the NHE3 is not anchored to the NHERF4 in recycling endosome on the deletion of NHE3 PDZ binding motif.

The epithelium plays a fundamental role in determining the fluidity of the luminal contents. This function depends on the absorption of nutrients but also on the active secretion and absorption of electrolytes. Physiological controls of the mechanisms that govern luminal fluidity are critical for homeostasis and well-being[41]. For example, the CLD is caused by excessive fluid in the lumen, but on the other hand, the shortage of fluid in the lumen results in the intestinal obstruction. This recent study showed that the NHERF4 would be a physiological modulator of the mechanisms that govern the luminal fluidity by adjusting total amount of the SLC26A3 proteins at the plasma membrane. In a human colon cell line, HT-29, the knockdown of NHERF4 expression induced an increase in SLC26A3 activation significantly (FIG 5C-5D). Moreover, a serine 329 at the NHERF4-PDZ3 was a potential phosphorylation residue to define the SLC26A3-NHERF4 interaction. The substitutions of serine 329 made significant alteration of interactional intensity between the SLC26A3 and the NHERF4 (FIG 6B). The maintenance of phosphorylational condition which was induced by the calyculin A decreased the SLC26A3-NHERF4 interaction and increased total amount of the surface-expressed SLC26A3 proteins (FIG 6C-6D). It was suggested that their interactional properties were changed depending on the phosphorylation at the NHERF4-PDZ3. The potential physiological roles of the NHERF4 were summarized as follows: (1) in normal condition, the SLC26A3-NHERF4 interaction would increase fluid in the intestinal lumen by suppression of total amount of the SLC26A3 proteins at the plasma membrane; and (2) in order to reduce luminal fluidity, phosphorylation of serine 329 in the NHERF3-PDZ3 would be occurred and then increase total amount

of the surface-expressed SLC26A3 proteins which were set free from endocytosis.

Lastly, the results in the recent study can be used as pharmacologic applications. For example, a short chemical peptide, which is identical to the SLC26A3 PDZ binding motif sequence, could be used as a competitor of the SLC26A3-NHERF4 interaction. Specific chemical molecules to induce phosphorylation or dephosphorylation in the NHERF4-PDZ3 could be another candidates. These applications would be used to regulate luminal fluidity and its related diseases in the intestine or kidney.

V. CONCLUSION

The recent study showed that the NHERF4 was an important regulatory scaffold protein of the SLC26A3 activation. The regulation mechanisms and physiological meanings of the SLC26A3-NHERF4 interaction were concluded as follows:

1. The SLC26A3-NHERF4 interaction occurred directly between the SLC26A3 C-terminal PDZ binding motif and the third PDZ domain of NHERF4.

2. The SLC26A3-NHERF4 interaction downregulated the activity of SLC26A3 at heterologously expressing conditions in HeLa cells. In addition, the knockdown of NHERF4 expression significantly increased DIDS-insensitive Cl^-/HCO_3^- exchange activities in human colon epithelial cells, HT-29.

3. The SLC26A3-NHERF4 interaction induced the acceleration of SLC26A3 endocytosis. It reduced total amount of the functional SLC26A3 proteins at the plasma membrane.

4. The substitutions of serine 329 which was one of potential phosphorylation sites at the NHERF4-PDZ3 made significant alteration of interactional intensity between the SLC26A3 and the NHERF4.

These results implied that the NHERF4 was a regulator of the SLC26A3 activity by modulation of the surface-expressed SLC26A3 level by endocytosis. The phosphorylation at the NHERF4-PDZ3 would be one of important regulatory mechanisms to define the SLC26A3-NHERF4 interaction. In addition, it suggested that the physiological role of NHERF4 interaction controlled the luminal fluidity in the intestine by the modulation of the SLC26A3 activation.

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ABSTRACT (IN KOREAN)

NHERF4 PDZ 연결단백에 의한 SLC26A3 이온수송 기능 조절

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소화기 및 비뇨 생식기에서의 이온수송 조절기전은 생명체의 성장에 있어 필수적인 영양분의 흡수 및 체내 부산물의 배출과 관련되어 있기 때문에 많은 연구자들의 관심의 대상이다. Solute linked carrier (SLC) 26A3 는 염소, 중탄산염 등의 음이온을 교환하는 세포막 단백으로 위장, 콩팥 및 췌장과 같은 상피세포에 존재한다. SLC26A3 의 유전적 돌연변이에 인한 기능이상은 선천성 설사질환에 연관되어 있다.

본 연구에서 SLC26A3 는 sodium/proton exchanger regulatory factor (NHERF) 4 라는 PDZ 연결단백과 결합하고 있음을 확인하였고, 이들간의 결합은 SLC26A3 의 기능조절에 중요하게 관여함을 발견하였다. SLC26A3 는 자신의 C-최말단의 PDZ 연결고리을 통해 NHERF4 가 갖고 있는 4 개의 PDZ 도메인 중 세 번째 PDZ 도메인과 직접적으로 결합하고 있다. SLC26A3 와 NHERF4 간의 결합은 세포막에 발현되어 있는 SLC26A3 의 세포이물흡수 반응을 촉진하여 세포막 발현된 SLC26A3 의 총량을 감소시킨다. 이는 결과적으로 SLC26A3 의 활성화 감소를 유도한다. NHERF4 의 세 번째 PDZ 도메인에서 PDZ 결합에 결정적인 역할을 하는 아미노산 서열에는 인산화를 일으킬 수 있는 serine 잔기가 존재함을 3 차원 구조예측을 통해 알아내었다. 이 serine 잔기를 분자적

성질이 서로 다른 아미노산 (alanine 또는 aspartic acid) 잔기들로 치환하거나 탈인산화 억제제를 처리하여 세포 내 인산화 조건을 변화시켰을 때, SLC26A3 와 NHERF4 의 결합력에 변화가 있음을 확인하였다.

이상의 결과를 종합하여 볼 때, SLC26A3 와 NHERF4 간의 PDZ 결합은 SLC26A3 의 세포막 발현 정도를 감소시키고, 결과적으로 SLC26A3 의 활성을 억제한다. 반면, SLC26A3 와 NHERF4 의 결합력은 인산화와 같은 세포 내 반응을 통해 약화 될 수 있으며 이는 SLC26A3 의 세포막 발현 감소의 억제를 유도한다. 그러므로, NHERF4 결합에 의한 SLC26A3 세포막 발현량 조절은 소화기 및 비뇨 생식기에서의 주요 이온수송 및 수분 조절기전 중 하나로 생각한다.

핵심되는 말: PDZ domain, SLC26A3, 음이온교환체, NHERF4, 세포이물흡수

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