Regulation of NHE3 activity by PDZ-based interaction with Shank2

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Regulation of NHE3 activity by PDZ-based interaction with Shank2

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Abstract

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Maintaining intracellular and systemic pH, as well as Na^+ concentration and fluid volume are crucial tasks for a wide variety of cells. One of proton exchangers participating in these tasks is Na^+/H^+ Exchanger 3 (NHE3). Several signaling systems are known to affect NHE3, but the exact mechanism is still to be clarified. However, the most widely accepted and well characterized signaling system is protein kinase A (PKA) mediated inhibition of NHE3 activity. Down regulation of NHE3 activity by PKA requires a multiprotein complex with NHE3 and modular proteins such as EBP50 and Ezrin. Ezrin is a member of the ezrin/radixin/moesin (ERM) family of actin-binding proteins that functions as a cAMP-dependent PKA anchor protein, bringing activated PKA to phosphorylate NHE3. A search for novel NHE3 binding proteins led to the PDZ domain of Shank2 found by the yeast-two hybrid assay. Upon Shank2 expression, cAMP-dependent PKA inhibition of NHE3 activity was attenuated and knock down of Shank2 expression by using RNA interference technique ensued the decrease in NHE3 protein expression as well. From these findings, it can be concluded that Shank2 is a novel NHE3 interacting protein which is involved in the fine regulation of salt and water transport through affecting NHE3 activity.

Key words: NHE3, Shank2, ion transport, PDZ, cAMP, PKA

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I. INTRODUCTION

In all or most prokaryotes to eukaryotes[4-6], maintaining intracellular and systemic pH values close to neutral is a critical task as well as keeping a tight control of other vital parameters such as Na^+ concentration and fluid volume. First demonstrated in 20 years ago[7], members of the mammalian Na^+/H^+ exchanger (NHE) family participate in the regulation of these parameters at both cellular and systemic levels. They are widely expressed and constitute extremely efficient systems for protecting cells against internal acidification by mediating the electroneutral exchange of extracellular Na^+ with intracellular H^+ in a stoichiometry of one to one[8]. NHEs are composed of an N-terminal 10–12 membrane-spanning domain mediating the ion exchange and a large hydrophilic C-terminal domain that serves a regulatory function in ion transport[9, 10].

To date, nine NHE family members have been identified in mammalian cells with unique tissue distribution and functional properties. NHE1 is often referred to as the "housekeeping" isoform, since it is expressed in the plasma membrane of most mammalian cells, responsible for regulation of cytosolic pH and cell volume. Unlike NHE1, NHE2-4 are found in the gastrointestinal tract and the kidney and NHE5 is present in the brain. Recently identified NHE6 is localized in mitochondria whereas NHE7 is found in the trans-Golgi network [11-14]. Most recently, NHE8 and 9 are found to be localized in the mid- to trans-Golgi network and the late recycling endosomes, respectively[14, 15].

As a better characterized isoform among NHE family members, NHE3 is primarily found in the apical membrane of epithelial cells of the renal and gastrointestinal tracts, where it mediates transpeithelial absorption of Na^+ and HCO_3^- [16, 17]. It was reported that NHE3 knockout mice show impaired systemic fluid homeostasis and disturbed acid-base balance. The major changes observed include decreased reabsortion of Na^+ and HCO_3^- by the kidney, reduced blood pressure, mild acidosis, and diarrhea due to absorptive abnormalities in the intestine[18].

NHE3 is known to be regulated by a large variety of hormones such as α-adrenergic receptor agonists, endothelin and growth factors[19, 20] and

several signaling systems, but the exact underlying mechanisms are still only partially understood. Nevertheless, acute regulation of NHE3 activity has been linked to phosphorylation for many years[21-24] and inhibition by cAMP-dependent protein kinase (PKA) has been actively studied by many investigators[25-27].

Recently, it has been reported that adaptor proteins having PDZ domains play an important role in cAMP-regulated ion transport in a number of distinct epithelia[28, 31]. Moreover, it was found that among those adaptor proteins, Ezrin-radixin-moesin (ERM)-binding phosphoprotein 50 (EBP50) and NHE3 Kinase A regulatory protein (E3KARP) are necessary modular proteins in cAMP-dependent PKA phosphorylation of NHE3 by forming a multiprotein signaling complex[28-30].

In recent years, there has been a growing interest in PDZ domains and modular proteins having PDZ domains. PDZ domains are protein-interaction domains that bind to short peptide motifs at the carboxy termini of other proteins and are often found in multi-domain scaffolding proteins[32]. Therefore, PDZ-containing scaffolds can assemble large molecular complexes by binding to specific polypeptides through each domain and those complexes then can be targeted to a specific subcellular site to perform a specialized local function. Previously, we have reported from an exploratory yeast-two hybrid assay that

PDZ domain of Shank2 interacts with NHE3 and that Shank2 is also involved in cAMP signaling pathway[31].

Shank proteins are a family of newly identified scaffold proteins having five domains that are likely involved in protein-protein interactions: multiple ankyrin repeat domains, an SH3 domain, a PDZ domain, a SAM domain and a long proline-rich region. Currently there are three known members in Shank family: Shank1, Shank2 and Shank3[33, 34]. Therefore, all these multiple sites for protein interaction make Shank2 as a good candidate protein for modulating NHE3 activity.

The present study identifies and characterizes the role of Shank2 on NHE3 activity and show that Shank2 attenuates cAMP-dependent PKA inhibition of NHE3.

II. MATERIALS AND METHODS

1. Cell culture and transfection

NHE-deficient hamster fibroblast cells (PS120) were maintained in DMEM-HG (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and penicillin (50 units/ml)/streptomycin (50 μg/ml). The pCMV-NHE3 constructs were stably transfected into a PS120 cell line using LipofectAMINE Plus Reagent (Life Technologies). NHE3 stable transfectants (PS120/NHE3) were selected in the presence of Geneticin (G418 Life Technologies, Inc.). Shank2 was transiently

transfected into PS120/ NHE3 cells using the same reagent. Caco-2 cells (human colon adenocarcinoma cells) were grown at 37°C in a 5% CO_2-95% air environment. The culture medium consisted of DMEM-HG, 10% FBS, 50 IU/ml penicillin, and 50 μg/ml streptomycin. Cells reached confluence after 5-7 days in culture. They were used for experiments at least 14 days after plating and were fed fresh incubation medium on alternate days.

2. Western blotting (WB) and Immunoprecipitation (IP)

PS120 or Caco-2 cell lysates (~2 mg of protein) were mixed with the appropriate antibodies and incubated overnight at 4°C in lysis buffer. Immune complexes were collected by binding to protein G/A-Sepharose and washed four times with lysis buffer prior to electrophoresis. The immunoprecipitates or lysates (50 μg of protein) were suspended in SDS sample buffer and separated by SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to nitrocellulose membranes, and the membranes were blocked by a 1-h incubation at room temperature in 5% nonfat dry milk in a solution containing 20 mM Tris-HCl, pH 7.5 M NaCl, and 0.05% Tween 20. The membranes were then incubated with the appropriate primary and secondary antibodies, and protein bands were detected by enhanced chemiluminescence solutions.

3. Measurment of Na^+/H^+ exchange activity

Na^+/H^+ exchange activity was measured using a standard protocol with some modifications[2]. Cells grown on glass coverslips were loaded with a fluorescent pH sensitive dye BCECF and measured for the pH changes. The cells were acidified by an NH_4^+ pulse and subsequent perfusion with a Na^+-free solution. The maximal Na^+-dependent pH*i*

recovery was measured in cells acidified to pH 6.4-6.5 with or without 5 μM forskolin/100 μM IBMX treatment. The standard perfusion solution contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose and 10 Hepes (pH 7.4 with NaOH). Na^+-free solutions were prepared by replacing Na^+ with NMG^+. The osmolarity of all solutions was adjusted to 310 mM with the major salt.

4. Cell surface biotinylation Assay

PS120/NHE3 and PS120/NHE3/Shank2 cells were grown to 70-80% confluence in 6-cm petri dishes. Cells were then placed at 4°C, washed twice in phosphate-buffered saline (PBS) and once in borate buffer (in mM: 154 NaCl, 10 boric acid, 7.2 KCl, and 1.8 $CaCl_2$, pH 9). The plasma membrane proteins were then biotinylated by gently shaking the cells in borate buffer containing sulfo-NHS-SS-biotin (Pierce) for 30 min. After cells were being biotinylated, the cells were washed extensively with quenching buffer to remove excess biotin, and then washed twice with PBS. Cells were then lysed and NeutrAvidin solution (UltraLink Immobilized NeutrAvidin Beads 10%, Pierce) was added to the supernatant and mixture was incubated overnight. Avidin-bound complexes were pelleted (13,000 rpm) and washed three times. Biotinylated proteins were eluted in Laemmli buffer, resolved by SDS-PAGE, electrotransferred, and immunoblotted with NHE3 antibody.

5. RNA interference

Cells were grown to 40-50% confluency before transfection. Twenty five base-pair double-stranded RNA oligonucleotides, specific for Shank2, were synthesized by Invitrogen and transfected into cells using Lipofectamin 2000 (Invitrogen). Four days after transfection, cells were harvested in lysis buffer for immunoblotting.

6. *Statistical Analysis* — Results are presented as the means ± S.E. of the indicated number of experiments. The results of multiple experiments were analyzed using the non-paired Student's *t* test or analysis of variance as appropriate.

III. RESULTS

1. Immunoprecipitation of Shank2 and NHE3

Previously, the protein interaction between the PDZ domain of Shank2 and NHE3 was reported from an attempt to find PDZ-containing scaffolds interacting with epithelial ion transporters by the yeast-two-hybrid assay [31]. To determine whether Shank2 forms a complex with NHE3 in mammalian cells, immumoprecipitation of Shank2 with NHE3 was evaluated in PS120 cells stably expressing NHE3. In total of three experiments, Shank2 formed a complex with NHE3 (Fig. 1). However, there was also a faint band in mock-transfected samples when they were immunoprecipitated with Shank2 and blotted against NHE3.

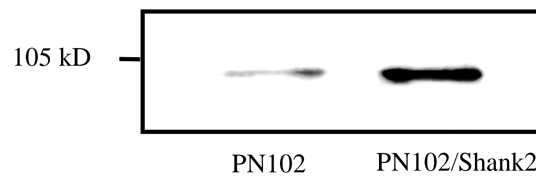
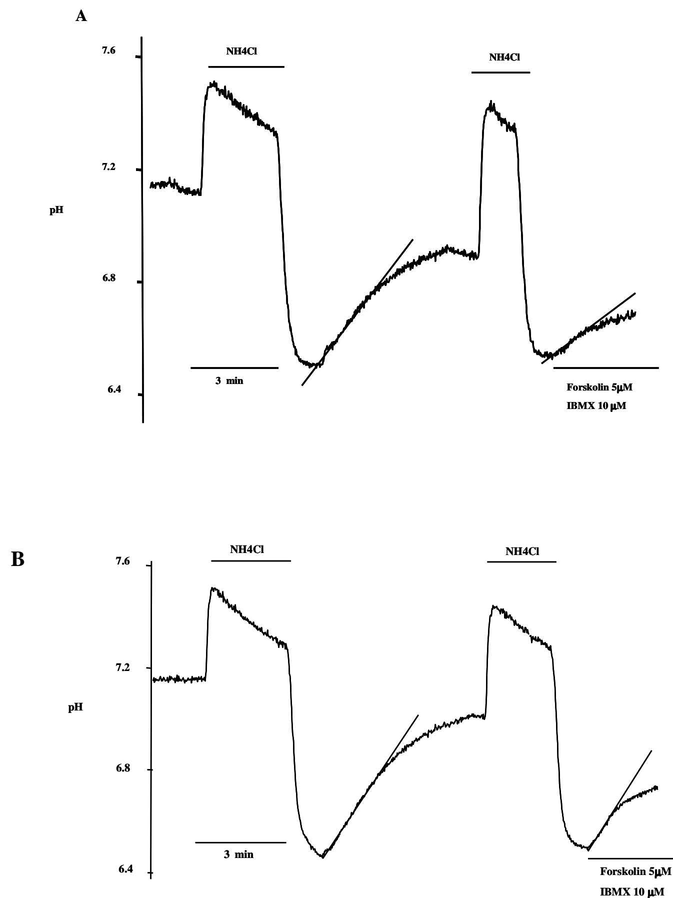


Figure 1. Immunoprecipitation of Shank2 and NHE3 in PS120 cells.
Whole cell lysates of PS120/NHE3 cells and PS120/NHE3 cells transiently transfected with pcDNA3.1 or Shank2 were immunoprecipitated with Shank2 antibody and characterized by immunoblotting with NHE3 antibody.

It can be explained by a small amount of endogenous expression of Shank2. In fact, RT-PCR results revealed the presence of Shank2 mRNA in PS120 cells (data not shown). Nevertheless, we confirmed the direct binding between Shank2 and NHE3 in heterologous expression system.

2. Effects of Shank2 on NHE3 activity

To examine the effects of Shank2 on NHE3 activity, PS120/NHE3 cells are transiently transfected with Shank2, and then Na^{+} dependent pH recovery was measured. To increase NHE3 expression, cells were serum-deprived for 12-16 hr prior to measurements. Since Shank2 is involved in cAMP signaling[31], the effects of cAMP on NHE3 activity were measured when Shank2 was over expressed. Interestingly, when Shank2 was present, cAMP-dependent inhibition of NHE3 activity was remarkably attenuated. Compare to mock transfected cells, cAMP mediated inhibition of NHE3 activity was 22% decreased from Shank2 transfected cells (Fig.2-B, C).



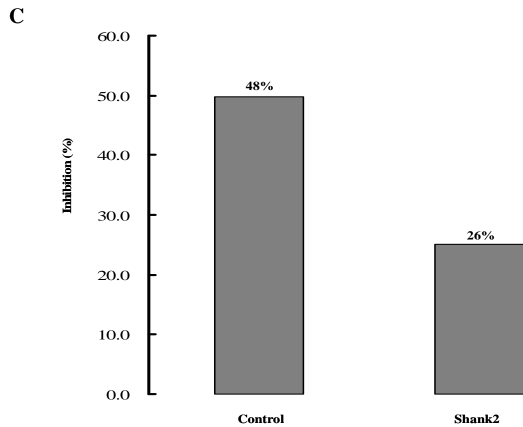
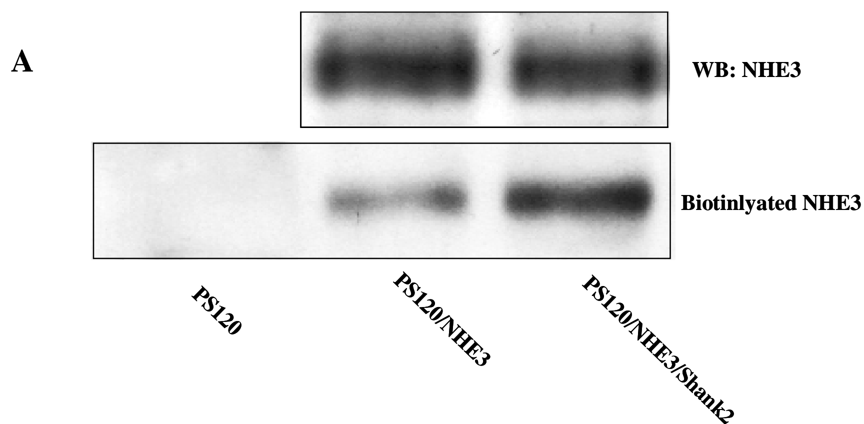


Figure 2. Expression of Shank2 in PS102/NHE3 cells and cAMP-dependent inhibition of NHE3 activity. PS120 cells (A) and PS120/NHE3//Shank2 (B) were acidified twice, before and after stimulation with forskolin and Na^{+}-dependent pH recovery from acidification was measured. Results of three experiments from each set are summarized in (C).

It has been well documented by many[25-27] that NHE3 activity is inhibited by cAMP-dependent PKA. In doing so, multiprotein complex is formed by adaptor protein EBP50 which binds to the C-terminal PDZ binding motif of NHE3 and Ezrin which brings the activated PKA to the vicinity, thus enabling PKA to phosphorylate NHE3 [25, 28, 31, 32]. Based on the above data, it appears that Shank2 competes with EBP50 for its binding. Thus, it will be interesting to look into the relationship between EBP50 and Shank2 on NHE3 activity, since Shank2 is also a multidomain scaffolding protein with PDZ domain and is known to be engaged in sorting, targeting and organizing of target proteins.

3. Surface expression of NHE3

To search for a mechanism responsible for the attenuated c-AMP dependent inhibition of NHE3 upon Shank2 expression, surface level of NHE3 was examined by biotinylating PS120/NHE3 cells transfected with Shank2. Upper panel from Figure 3A shows the whole cell lysates from each sample which were immunoblotted for NHE3 antibody. From this blot, it was determined that Shank2 does not affect the total protein amount of NHE3. However, when biotinylated samples were analyzed, it was clearly seen that Shank2 increased the membrane expression level of NHE3 by almost two fold and this might explain the increased basal activity of NHE3, but not the decreased cAMP dependent inhibition of NHE3.



B

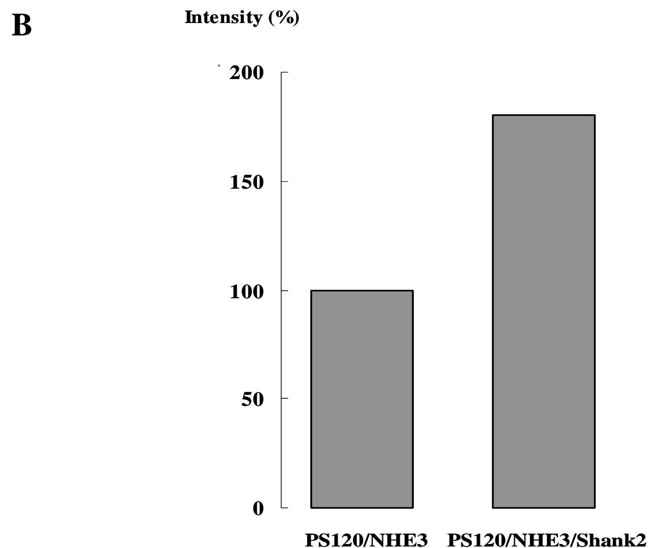


Figure 3. Surface protein level of NHE3 in Shank2 expressed PS120/NHE3 cells. Surface biotinylated NHE3 from PS120, PS120/NHE3 and Shank2 transfected PS120/NHE3 cells was immunoblotted with NHE3 antibody. (A). Each band intensity was determined by densitometry (B).

4. Shank2 protein expression in Caco-2 cells.

So far, the effects of Shank2 on NHE3 activity were studied in heterologous non-epithelial expression systems. Therefore, it is necessary to examine the effects of Shank2 on NHE3 activity in epithelial cells endogenously expressing NHE3 as well as Shank2. Expression of Shank2 was confirmed by immunoblotting with Shank2 antibody in two clones of Caco-2, one is from ATCC (American Type Culture Collection) and the other one is from KCLB (Korea Cell line Bank) and the latter was used for all the subsequent experiments (Fig. 4).

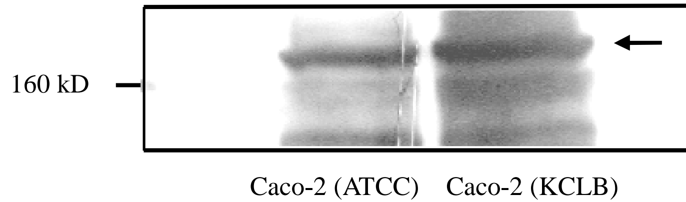


Figure 4. Shank2 expression in Caco-2 cells.

Endogenous expression of Shank2 protein was verified in two different clones of Caco-2 by blotting with Shank2 antibody.

5. Different expression levels of NHE3 in Caco-2 cells.

Differential expression patterns of NHE3 in Caco-2 cells were reported by Janecki et al., and the benefits of using Caco-2 cell clones as an in vitro model for studies on physiology of NHE3 in the intestinal epithelium[35]. To establish the optimal condition for measuring NHE3 activity in Caco-2 cells, the expression patterns of NHE3 was determined by immunoblotting and the results we obtained were quite surprising (Fig. 5).

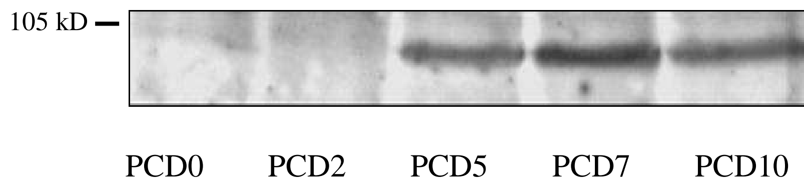


Figure 5. Expression of NHE3 in Caco-2 cells.

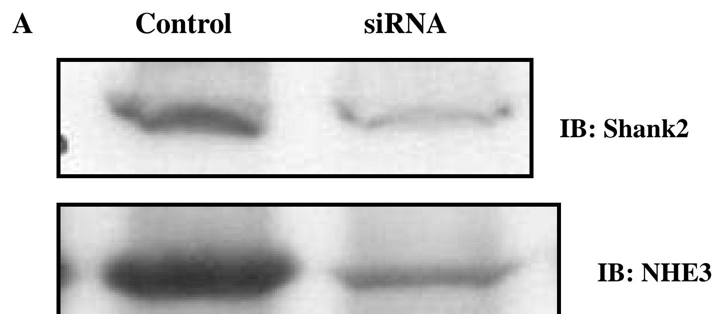
NHE3 expression was verified in Caco-2 cells collected from post-confluent days (PCD) 0, 2, 5, 7 and 10 by immunoblotting with NHE3 antibody.

As it can be seen from Figure 5, NHE3 expression was not detected from Caco-2 cells harvested at PCD 0 and 2. However, NHE3 was expressed from day 5 and at day 7, the protein level was at the highest. This gradual

development of the NHE3 expression is similar to other proteins expressed at the brush border of Caco-2 cells such as sucrase-isomaltase, lactase and alkaline phosphatase[36]. It can be postulated that this gradual increase of NHE3 is due to an increasing number of cells expressing functional NHE3 at the brush border of Caco-2 cells with time. Based on these results, all the subsequent experiments were done in Caco-2 cells at or after PCD 7.

6. Knock down of Shank2 gene by siRNA reduced NHE3 expression in Caco-2 cells.

So far, few aspects of how Shank2 affects NHE3 activity were observed, but it is still not clear whether these findings have any physiological meaning. To determine any physiological relevance Shank2 might have on NHE3 activity, RNA interference technique was used to knock down Shank2 expression in Caco-2 cells and examined how this might affect NHE3.



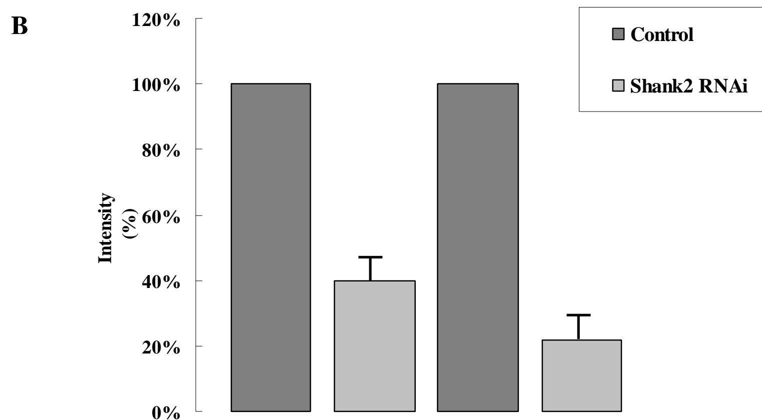


Figure 6. Effect of Shank2 loss on NHE3 expression in Caco-2 cells.

Caco-2 cells are treated with siRNA and immunoblotted against Shank2 (A, upper), then same blot was stripped and re-probed with NHE3 antibody (A, lower). Each band intensity was measured by densitometry and summarized (B).

Upper panel from Figure 6A shows the decreased Shank2 protein expression in siRNA treated Caco-2 cells by Western blotting and the same blot was stripped and reprobed with NHE3 antibody which was seen from the lower panel. The most memorable finding from this study is that knock down of Shank2 also down-regulates NHE3 expression. As it can be seen from Figure 6B, siRNA treatment of reducing Shank2 resulted striking difference in the expression level of NHE3 compared to that of control. Shank2 expression from siRNA treated Caco-2 cells was decreased about 60 % whereas NHE3 expression from same sample was remarkably reduced to about 80%. Shank2 is known to affect sorting, targeting and stability of its target proteins. However, it has not been reported previously that decreased Shank2 gene expression can also diminish the expression of other protein that is very important in maintaining intracellular pH.

7. Effect of Shank2 knock-down on NHE3 activity in Caco-2.

Lastly, the effects of Shank2 loss on NHE3 activity was measured by protocols described earlier in this paper. As it was predicted from the previous results (Fig.6), NHE3 activity was strikingly reduced upon Shank2 loss. As it is seen from Figure 7, even the basal activity of NHE3 was decreased about 40 % compared to control with the loss of Shank2 expression. Therefore, it was observed that Shank2 affects cAMP signaling on NHE3 activity as well as the stability of NHE3 affecting its expression.

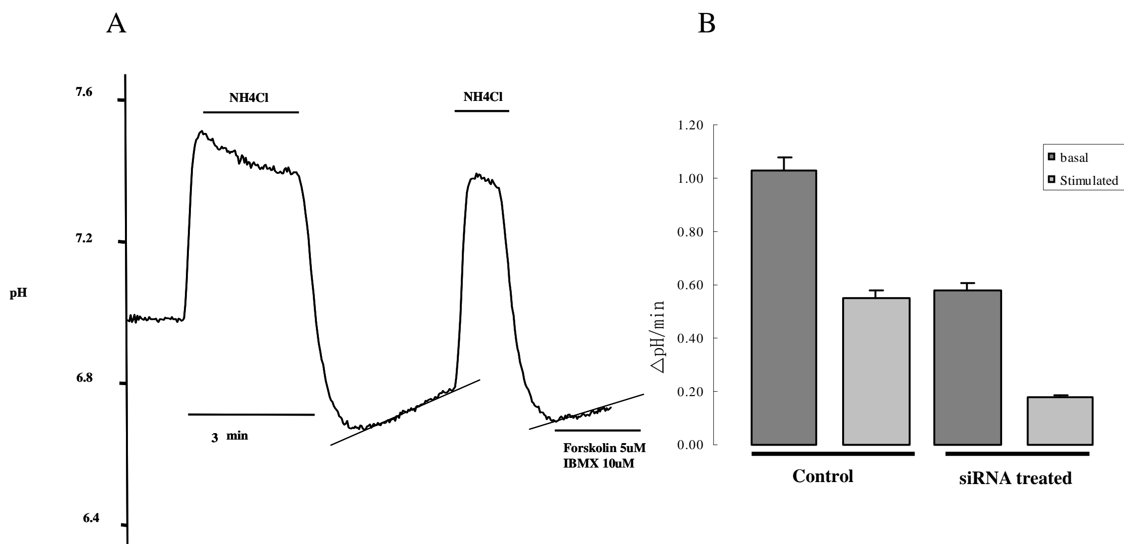


Figure 7. Knock-down of Shank2 and NHE3 activity in Caco-2.

NHE3 activity was measured from Caco-2 cells treated with siRNA (A) and the results are summarized (B).

IV. DISCUSSION

Many hormones and growth factors such as cAMP, cGMP, elevated intracellular calcium, and epidermal and fibroblast growth factors have been identified in various tissues and cell lines as regulators of NHE3. However, NHE3 activity is regulated predominantly via pathways mediated by protein kinases, particularly by PKA[37, 38]. Down-regulation of NHE3 activity by cAMP-dependent PKA involves phosphorylation of c-terminal serine residues of NHE3[23, 39] *in vitro* and *in vivo* and this inhibition only occurs in the presence of PDZ-domain containing modular proteins NHERF1/EBP50 or NHERF2/E3KARP, which link NHE3 to actin cytoskeleton by binding to Ezrin, a PKA anchoring protein. However, mutation studies of those PKA phosphorylation target sites showed only 50% reduction in inhibition of transport activity[40]. Therefore, these findings suggest that direct phosphorylation is an important, but not exclusive mode of regulation of NHE3 activity and that alteration in the number of surface NHE3 by modifying its turn over rate could be another powerful way of regulating NHE3 activity.

In line with the two major regulatory mechanisms for NHE3, present study reports two very remarkable findings by PDZ-containing modular

protein Shank2 on NHE3: 1) Shank2 blunted cAMP-dependent PKA inhibition of NHE3; 2) Shank2 increased membrane expression of NHE3. As it can be seen from Figure 2b-c, Shank2 expression attenuated PKA mediated inhibition of NHE3 activity upon cAMP stimulation. The mechanism of how activated PKA induces changes in the intrinsic activity of NHE3 is still not clear, but it has been shown that acute inhibition by cAMP elevation does not change the number of transporters at the cell surface[41]. Therefore, inhibitory effect of PKA can be ascribed to a decrease in turnover rate of surface NHE3. Based on the data from this study, it can be speculated that binding of a regulatory protein, such as Shank2, can blunt that effect either by changing phosphorylation status of NHE3 or by competing with EBP50 for its binding to NHE3, thus breaching the formation of multiprotein complex that will lead to the decreased PKA dependent inhibition of NHE3 activity. Further studies on NHE3 phosphorylation with Shank2 expression or binding competition between Shank2 and EBP50 will provide new insight in this matter.

Another unique finding that was brought upon by Shank2 was the increased surface expression level of NHE3. As it can be seen from Figure 3a-b, surface membrane level of NHE3 was increased about two-fold upon Shank2 expression. An effective and rapid way of controlling the function of

cell surface proteins is by altering the number of available molecules at the plasma membrane. Recent studies of the subcellular localization of NHE3 by electron microscopy and confocal immuno-fluorecsence microscopy revealed the existence of a substantial intracellular pool of transporters in vesicles as well as at the membrane surface[42, 43], indicating that NHE3 traffics between the plasma membrane and recycling endosomal vesicles under basal and regulated conditions.

There could be many possibilities to explain the increased surface level of NHE3 by Shank2. Since Shank proteins are well characterized for their roles in sorting and trafficking of their target proteins. For instance, it has been reported that PDZ-domain interaction mediates endocytic sorting of internalized β_2-adrenergic receptors between recycling endosomes and lysosomes for degradation[44]. Based on the findings from this study, it can be postulated that cAMP-mediated phosphorylation could regulate membrane trafficking of NHE3 by PDZ-domain interaction between Shank2 and NHE3 and that Shank2 might be involved in endosytic sorting of NHE3. Therefore, reduced cAMP-dependent inhibition of NHE3 activity upon Shank2 expression from Figure 2 can be explained by increased number of functional NHE3 at the plasma membrane. Recently, it was found that Shank2 also

interacts with Dynamin, a GTPase involved in synaptic vesicle recycling, receptor-mediated endocytosis and other membrane trafficking[45]. This observation further supports the possibility that Shank2 might be involved in the trafficking of NHE3, but much work needs to be done to clarify the specific role of Shank2 on NHE3 activity since there is still a possibility that Shank2 might link phosphatase to NHE3, decreasing cAMP-induced phosphorylation of serine residues at C-terminal.

It should be noted that all the above findings were from the heterologous expression system and that it might not have some important factors specific to epithelial cells. Thus, Caco-2 cells were used as a native epithelial cell system to investigate the physiological meaning of Shank2 on NHE3 activity. Caco-2 cells are a colon cancer cell line expressing both Shank2 and NHE3 that has been used to model a number of absorptive functions of intestinal epithelial cell[46].

For a better understanding of its role on NHE3 activity, endogenous Shank2 was knocked-down in Caco-2 cells by siRNA (small interfering RNA) directed against Shank2 which resulted about 60% decrease in protein level detected by immunoblotting. However, the most unforeseen discovery was made when the same blot was reprobed against NHE3. Decreased Shank2

protein level remarkably diminished NHE3 expression in Caco-2 (Fig. 6a-b), implying that Shank2 might play a role in transcriptional/ translational regulation of NHE3.

There have been many reports suggesting the involvement of PDZ containing proteins in transcriptional regulation of target proteins. It has already been reported that EBP50 interacts with beta-catenin through its carboxyl-PDZ domain and promoting Wnt signaling *in vitro* and *in vivo*[47]. Since beta-catenin mediated Wnt signaling plays crucial roles in the development of hepatocellular carcinoma and other cancers such as colorectal cancer, finding a transcriptional coactivator can have significant implications in clinical settings. Another recent finding also reports a new transcriptional coactivator TAZ with PDZ-binding motif that binds to core-binding factor 1 (Cbfa1; also called Runx2), transcription factor belonging to the Runt family of transcription factors that binds to an osteoblast-specific cis-acting element (OSE2) activating the expression of osteocalcin, an osteoblast-specific gene[48]. However, it is still not clear how Shank2 is affecting the transcription or translation of NHE3 at the moment, but it will be further examined.

NHE3 is one of five plasma membrane Na^+/H^+ exchangers, which is encoded by the mouse gene Slc9a3. It is expressed on apical membranes of

renal proximal tubule and intestinal epithelial cells. A major site for NaCl absorption in the gastrointestinal tract is the colon, and much of this activity appears to be mediated by coupled Na^+/H^+ and Cl^-/HCO_3^- exchange. Mice lacking NHE3 function showed slight diarrhea and mild acidosis as well as sharp drop in HCO_3^- and fluid absorption in proximal convoluted tubules[18]. Therefore, lack of the NHE3 impairs acid-base balance and Na^+-fluid volume homeostasis.

With accumulating interests in PDZ domains as protein-protein recognition modules in organizing diverse cell signaling assemblies, another PDZ-domain containing scaffolding protein Shank2 was found to interact with NHE3. Upon cAMP stimulation, Shank2 decreases inhibitory effects of PKA on NHE3 activity by increasing NHE3 to the surface plasma membrane and also affects NHE3 protein expression possibly via modifying transcriptional or translational regulation of NHE3. However, recognizing many regulatory proteins bind directly to NHE3 generates questions regarding whether the binding is dynamic, are there other adaptor proteins modifying their binding and does binding occur all at once or in a competitive manner, such as binding of Shank2 or EBP50 to NHE3. Nevertheless, our findings on Shank2 concerning NHE3 activity will shed new light on its fine regulation of salt and water transport for maintaining epithelial and body homeostasis.

V. CONCLUSIONS

The present study identified a novel interaction between a scaffolding protein Shank2 and NHE3, and characterized the role of Shank2 on NHE3 activity using molecular, biochemical and electrophysiological approaches to conclude:

1. Shank2 interacts with NHE3 in heterologous expression system as well as in native epithelial cell system.
2. Shank2 attenuates cAMP-dependent PKA inhibition of NHE3 activity.
3. Shank2 increases the surface protein expression level of NHE3.
4. Knock down of Shank2 expression remarkably decreases NHE3 expression as well as its basal activity.

From these results, it could be concluded that Shank2 is an important modular protein that is involved in NHE3 activity by affecting cAMP signaling and the stability of NHE3, making Shank2 as an integral part of the regulation of NHE3 activity.

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ABSTRACT (IN KOREAN)

PDZ 에 근거한 Shank2 의 Na^+/H^+ Exchanger3 (NHE3)
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한원선

많은 종류의 세포에서 Na^+ 농도와 세포액의 양뿐만 아니라 세포내 그리고 개체의 pH 를 일정하게 유지하는 것은 매우 중요하다. 이러한 일을 담당하고 있는 proton exchanger 중의 하나가 Na^+/H^+ Exchanger 3 (NHE3) 이다. NHE3 에 영향을 주는 몇 가지 신호전달 기전이 알려져 있기는 하나 명확한 조절 기전은 아직 밝혀지지 않고 있다. 그 중 잘 알려진 신호전달 기전은 protein kinase A (PKA) 를 통한 NHE3 활성 억제이다. NHE3 는 Na^+/H^+ exchanger regulatory factor (NHERF)와 결합하여 복합체를 형성하고 NHERF 는 다시 Ezrin 과 결합하게 된다. Ezrin 은 cAMP 에 의존하는 PKA 연결 단백 역할을 하는 actin 결합 단백인 ezrin/radixin/moesin (ERM) 족의 하나로 활성화된 PKA 를 불러들여 NHE3 를 인산화 시킨다. 이 연구에서는 여러 단백 결합 부위를 가지고 있으면서 cAMP 신호 전달에 관여하는 scaffolding 단백의 하나인 Shank2 가 PDZ domain 을 통하여 NHE3 와 결합하는 것을 yeast-two-hybrid 실험을 통하여 발견하였고 Shank2 과 발현은 PKA 에 의한 NHE3 활성 감소를 억제하는 것을 발견하였다. RNA interference 를 이용한 Shank2 발현 감소는 NHE3 발현 또한 감소 시켰다. 따라서 이상의 결과를 통해 본

연구에서는 Shank2 가 NHE3 의 활성 조절에 관여하는 새로운 단백으로 cAMP 신호와 NHE3 단백의 안정성에 관여하는 중요한 조절 단백임을 밝혔고 NHE3 활성에 대한 Shank2 의 역할은 내피세포와 개체의 항상성을 유지하는데 필수적인 이온과 물 수송체의 미세 조절을 이해하는데 많은 도움을 줄 것으로 생각된다.

핵심되는 말: NHE3, Shank2, PDZ, 이온수송, cAMP, PKA