Inhibition of Na,K-ATPase-suppressive activity of translationally controlled tumor protein by sorting nexin 6

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Abstract Translationally controlled tumor protein (TCTP) has both extra- and intracellular functions. Our group recently reported that TCTP interacts with Na,K-ATPase and suppresses its activity. Our studies led to the identification of sorting nexin 6 (SNX6) which binds with TCTP as a potential negative regulator of TCTP. SNX6 does not interact directly with any cytoplasmic domains of Na,K-ATPase. However, when overexpressed, it restores the Na,K-ATPase activity suppressed by TCTP. This was confirmed by measurements of purified plasma membrane Na,K-ATPase activity after incubation with recombinant TCTP and SNX6. SNX6 alone has no effect on Na,K-ATPase activity, but activates Na,K-ATPase via inhibition of TCTP. Inhibition of endogenous TCTP by the overexpression of SNX6 or knockdown of TCTP expression by siRNA increased Na,K-ATPase activity above the basal level. The interaction between SNX6 and TCTP thus appears to regulate Na,K-ATPase activity.

Keywords: Na,K-ATPase; Translationally controlled tumor protein; Sorting nexin 6

1. Introduction

Translationally controlled tumor protein (TCTP), also variously known as IgE-dependent histamine-releasing factor (HRF), p23/p21, and fortitin, is distributed in all normal cell types. It exhibits a high degree of homology among various species, suggesting that TCTP may play an essential role in cellular processes [1]. Many studies demonstrated that TCTP is a multifunctional protein [2]. TCTP was reported to be involved, extracellularly in human allergic response as HRF [3], intracellularly in receptor serine/threonine kinase TGF-RI, transforming growth factor-RI, receptor tyrosine kinases of EGFR, PDGFR, IR, and the long form of the leptin receptors [14] such as other members of sorting nexin family believed to function in intracellular trafficking of target proteins [15]. SNX6 is widely expressed in multiple tissues [14]. It is located in the cytoplasm and cell membrane [16], and the nucleus after binding to Pim-1 [12]. However, little is known of the cellular function of SNX6 other than that it inhibits TGF-β signaling.

2. Materials and methods

2.1. Yeast two-hybrid analysis

Yeast two-hybrid screening was performed as described previously [17], using rat TCTP as bait and a mouse thymocyte cDNA library. Protein–protein interaction results in activation of LexAop-Leu2 and LexAop-LacZ reporter genes. Clones encoding TCTP-interacting proteins were identified by DNA sequencing. One of these clones corresponded to SNX6. The cytoplasmic domains (CDs) of rat Na,K-ATPase α1, α2 and β subunits were cloned into the LexA fusion plasmid pEG202 as previously described [7]. Yeast cells were transformed with both resulting in LexA fusion constructs and a SNX6 clone. The binding activity was evaluated by β-galactosidase activity as reported previously [18], using the formula: β-galactosidase unit = A420 x 1000/m (min) x volume of extract (ml) x protein (μg/ml).

2.2. Epitope-tagged constructs

Full-length human SNX6 cDNA was obtained from Dr. Jong-Hoon Park (Sookmyung Women University, Seoul, Korea). Green fluorescence protein (GFP)-tagged SNX6 was generated by PCR
amplification of the coding region of human SNX6 cDNA. The amplified fragments were digested with appropriate restriction enzymes and cloned into corresponding sites of mammalian expression vector pEGFP-N1 (Clontech). His-tagged SNX6 was constructed in bacterial expression vector pRSET (Invitrogen) for recombinant SNX6 protein in same manner. HA-tagged TCTP, GFP-tagged TCTP, and His-tagged TCTP constructs were from our previous study [19].

2.3. Co-immunoprecipitation

HeLa cells were transiently transfected with HA-TCTP construct using LipofectAMINE PLUS™ (Invitrogen) or WelFect-EX™ PLUS (WelGENE). After 24-48 h, the cells were lysed with an ice-cold lysis buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM NaF, 1 mM Na3VO4, and Complete™ protease inhibitor cocktail tablet (Roche Applied Science). After centrifugation, the supernatants were mixed with mouse 12CA5 anti-HA monoclonal antibody and incubated with protein G-agarose. The bound immune complexes were pelleted and washed with ice-cold phosphate-buffered saline (PBS, 136.9 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4). The precipitated proteins were separated on 12% SDS-polyacrylamide gel (SDS-PAGE). The blots obtained were detected with mouse 12CA5 anti-HA monoclonal antibody, mouse anti-Na,K-ATPase α1 monoclonal antibody and rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.). Same experiments were done with untransfected HeLa cells. In this case, after centrifugation of cell lysates, the supernatants were mixed with mouse anti-Na,K-ATPase α1 monoclonal antibody (Upstate Biotechnology) and blots were detected with mouse anti-Na,K-ATPase α1 monoclonal antibody, rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.), and affinity purified rabbit anti-TCTP polyclonal antiserum raised from recombinant His-tagged TCTP in our lab.

2.4. Knockdown of TCTP expression by small interfering RNA (siRNA) siTCTP, construct for intracellular expression of TCTP siRNA was made in pSUPER vector (OligoEngine). The functional siRNA corresponding to 5'-AGGTACCGAAAGCACAGTA-3' of human TCTP gene was synthesized inside mammalian cells transfected with siTCTP construct [20]. The level of suppression of TCTP gene was measured from the amount of TCTP protein.

2.5. 86Rb+ uptake assay

HeLa cells were plated in 24-well plate at 5 × 104 cells/well and were transfected with GFP, GFP-TCTP, GFP-SNX6, or siTCTP constructs as mentioned above. After 24-48 h, the cells were incubated for 3 h at 37 °C in serum-free DMEM. The serum-starved cells were washed with 140 mM NaCl, 5 mM KCl, 10 mM Na2HPO4, 1 mM MgSO4, and 2.5 mM glucose, 0.1% BSA, pH 7.4 (KRP). After centrifugation of cell lysates, the supernatants were mixed with mouse anti-HA monoclonal antibody, rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.), and mouse anti-Na,K-ATPase α1 monoclonal antibody and incubated with protein G-agarose. The bound immune complexes were pelleted and washed with ice-cold phosphate-buffered saline (PBS, 136.9 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4). The precipitated proteins were separated on 12% SDS-polyacrylamide gel (SDS-PAGE). The blots obtained were detected with mouse 12CA5 anti-HA monoclonal antibody, mouse anti-Na,K-ATPase α1 monoclonal antibody and rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.). Same experiments were done with untransfected HeLa cells. In this case, after centrifugation of cell lysates, the supernatants were mixed with mouse anti-Na,K-ATPase α1 monoclonal antibody (Upstate Biotechnology) and blots were detected with mouse anti-Na,K-ATPase α1 monoclonal antibody, rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.), and affinity purified rabbit anti-TCTP polyclonal antiserum raised from recombinant His-tagged TCTP in our lab.

2.6. Western blot analysis

Cells were prepared as described for the 86Rb+ uptake assay. The proteins of total cell lysates were separated on 12% SDS-PAGE, blotted, and incubated with rabbit anti-HA monoclonal antibody, rabbit anti-GFP polyclonal antibody (Clontech), and anti-TCTP polyclonal antiserum.

2.7. In vitro Na,K-ATPase assay

The purified plasma membrane Na,K-ATPase fraction was obtained from twenty 100-mm dishes of HeLa cells using the procedure described in a previous report [21]. 0.04 mg/ml of plasma membrane Na,K-ATPase fraction positioned at 30% sucrose was preincubated with various concentrations of ouabain (10^-9–10^-3 M), recombinant TCTP (0.5–2 μg/ml), recombinant SNX6 (0.5–2 μg/ml), or a combination of recombinant TCTP and SNX6 of appropriate concentrations in assay buffer (18 mM histidine, 18 mM imidazole, 80 mM NaCl, 15 mM KCl, 3 mM MgCl2, and 0.1 mM EGTA, pH 7.4). The assay was then started with the addition of ATP to a final concentration of 5 mM. After the incubation for 60 min at 37 °C, the release of inorganic phosphate from ATP was quantified using the colorimetric method described previously [22]. Recombinant TCTP and SNX6 were produced by the bacterial expression pRSET vector system (Invitrogen) which contains the sequence for a His6 tag at the N-terminus of the protein.

2.8. Data analysis

Data are presented as the means ± S.E. Statistical analysis utilized one-way analysis of variance (ANOVA) followed by post hoc Duncan test.

3. Results

3.1. TCTP interacts with both SNX6 and Na,K-ATPase

To better understand the functional role of TCTP in the regulation of Na,K-ATPase activity, we searched for TCTP-binding proteins using the yeast two-hybrid system. Using this approach, we were able to isolate several cDNA clones. One of these clones corresponded to the gene for SNX6 which encodes 406 amino acids (Fig. 1A). Fig. 1B, which shows the results of β-galactosidase activity assay, suggests that the interaction between TCTP and SNX6 occurred inside the yeast cell, and between TCTP and the third cytoplasmic domain of Na,K-ATPase α1 subunit (α1CD3). We confirmed that the interactions of TCTP with both SNX6 and Na,K-ATPase occur in vivo by co-immunoprecipitation. After checking that HeLa cells transfected with HA-TCTP construct expressed 23 kDa HA-TCTP, the immunoprecipitated complex prepared with anti-HA antibody was blotted with anti-Na,K-ATPase α subunit and anti-SNX6 antibody. HA-TCTP co-precipitated both 100 kDa Na,K-ATPase α subunit and 50 kDa SNX6 (Fig. 1C). We also performed immunoprecipitation with untrasfected HeLa cells to see if endogenous TCTP and SNX6 are complexed with Na,K-ATPase. Immunoprecipitation with anti-Na,K-ATPase antibody detected 50 kDa SNX6 and 23 kDa TCTP (Fig. 1D), suggesting that TCTP associates with SNX6 as well as Na,K-ATPase, and their complex exists in HeLa cells.

3.2. SNX6 does not interact with Na,K-ATPase

To identify whether SNX6 itself interacts with the cytoplasmic domains of Na,K-ATPase like TCTP, individual Ura^-His^- Trp^- transformants were streaked onto Glc Ura His^- Trp^- Leu^- and Gal Ura His^-Trp^- Leu^- plates and assayed for β-galactosidase activity. No transformant of pEG202/cytol plus 5/SNX6 activated the transcription of the respective reporter genes, suggesting that the cytoplasmic domains of Na,K-ATPase α1, α2 and β subunits do not interact with SNX6 (Table 1). Although the cytoplasmic domain of Na,K-ATPase β subunit showed a slight increase in β-gal signal, it did not activate Leu2 reporter. One cannot determine whether the N-terminal portion of Na,K-ATPase α subunit interacts with SNX6 because the yeast cells transformed with pEG202/x1NT or pEG202/x2NT always activated reporter genes.
were grown under galactose induction conditions, respectively.

Fig. 1. TCTP interacts with both SNX6 and Na,K-ATPase. (A) Schematic representation of SNX6. The PX domain is depicted by shaded box and the BAR domain is black box. (B) β-Galactosidase activity in yeast two-hybrid system. Yeast transformants harboring pEG202/the third cytoplasmic domain of Na,K-ATPase α1 subunit (a1CD3) and pJG4-5/TCTP, or pEG202/TCTP and pJG4-5/SNX6 were grown under galactose induction conditions, respectively. β-Galactosidase activity assay was performed to detect activation of LexAop-LacZ reporter gene by protein-protein interaction. The interaction between empty vector pEG202 and pJG4-5 is a negative control and the interaction of a1CD3 with clone 267 (cofilin) is a positive control [17]. Values were converted to percentage of positive control. Data are the average of four independent experiments ± S.E. (C) Immunoprecipitation by HA-TCTP detects Na,K-ATPase and SNX6. HeLa cells were transfected with HA-TCTP construct. Immunoprecipitated complex was prepared from cell extracts employing anti-HA antibody. The blot obtained from total cell extract (20 μg) and immunoprecipitated complex was detected with anti-Na,K-ATPase α subunit, anti-SNX6, and anti-HA. IP, immunoprecipitation. (D) Immunoprecipitation by Na,K-ATPase detects SNX6 and TCTP. HeLa cell extracts were subjected to immunoprecipitation with anti-Na,K-ATPase antibody, followed by immunoblotting with anti-Na,K-ATPase α subunit, anti-SNX6, and anti-TCTP. The whole cell extracts (5 μg) and the precipitates prepared in the absence of anti-Na,K-ATPase antibody were examined as controls.

3.3. Overexpression of SNX6 restores the Na,K-ATPase activity suppressed by overexpression of TCTP without change of Na,K-ATPase protein level

To explore whether SNX6 is involved in the regulation of Na,K-ATPase activity through interaction with TCTP, we overexpressed SNX6 in HeLa cells and measured Na,K-ATPase activity using 86Rb+ uptake assay. Because it has been reported that 60% of 86Rb+ uptake in HeLa cells is accounted for by Na,K-ATPase and another 30% by Na+,K+,2Cl− cotransporter, for a total of 90% [23], we determined the individual transporter activity using 0.1 mM ouabain (a specific inhibitor of Na,K-ATPase) and 0.1 mM furosemide (an inhibitor of Na+,K+,2Cl− cotransporter). Fig. 2A shows that Na,K-ATPase activity of GFP-TCTP-transfected cells was 14.5 ± 5.4% lower than that of mock. This confirms the previous report that overexpression of TCTP inhibits Na,K-ATPase activity [7]. However, Na,K-ATPase activity of cells co-transfected with both GFP-TCTP and GFP-SNX6 recovered to the mock level and Na+,K+,2Cl− cotransporter and the residual transporter activities were similar. These results suggest that the overexpression of SNX6 restores the Na,K-ATPase activity decreased by overexpression of TCTP. To clarify whether the increase in Na,K-ATPase activity from overexpression of SNX6 is caused by the change of Na,K-ATPase expression level, Western blot of cells transfected as in the 86Rb+ uptake assay was performed using anti-Na,K-ATPase α subunit antibody. Fig. 2B demonstrates that the expression level of α subunit was the same in all transfected cells. This suggests that recovery of Na,K-ATPase activity in TCTP and SNX6-co-transfected cells is not due to the increase of Na,K-ATPase protein level. Although the expression level of SNX6 as shown in Fig. 2B appeared to be much lower than that of TCTP, SNX6 was able to restore the Na,K-ATPase activity suppressed by overexpression of TCTP. This might be due to different cellular localization of the protein after transfection. Indeed, subcellular fractionation analysis showed that 51–28% of cellular SNX6 is localized in plasma membrane (PM), whereas only 10–7% of cellular TCTP is localized in plasma membrane (unpublished data). Thus, SNX6 localized in PM seems to reverse the inhibition of Na,K-ATPase by TCTP in PM.

3.4. Recombinant SNX6 prevents recombinant TCTP from suppressing purified plasma membrane Na,K-ATPase activity in vitro

Using HeLa cell plasma membrane fraction enriched with ouabain-sensitive Na,K-ATPase by removal of extraneous protein, we investigated the effect of SNX6 and TCTP on purified membrane-bound Na,K-ATPase in vitro. Ouabain effectively decreased the activity of the purified plasma membrane Na,K-ATPase used in this assay (IC50 = 2.85 mM) (Fig. 3A). Addition of recombinant TCTP to the purified Na,K-ATPase resulted in a significant decrease in its activity in a dose-dependent manner (IC50 = 1.84 μM) and finally complete inhibition at 10 μM above (data not shown) as previously reported [7]. However, this inhibition was prevented by recombinant SNX6 (Fig. 3B). Moreover, although the recombinant SNX6 did not affect Na,K-ATPase activity significantly, the purified Na,K-ATPase which existed firstly with recombinant SNX6 was not inhibited by the addition of
activity was calculated by $^{86}$Rb$^+$ uptake assay. As expected, overexpressed in HeLa cells and the individual transporter will be activated. To test this hypothesis, SNX6 alone was SNX6 and TCTP is increased simultaneously, Na,K-ATPase with empty vector pEG202 and pJG4-5 was used as a negative control, and the yeast cell transformed with PEG202/ΔLexAop-Leu2 activation domain. Protein–protein interaction results in activation of as a positive control (25).


Table 1

<table>
<thead>
<tr>
<th>Na,K-ATPase subunit</th>
<th>Construct</th>
<th>Selective media plate</th>
<th>β-Galactosidase activity (Unit)</th>
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<tr>
<td></td>
<td>pEG202 (DNA-binding)</td>
<td>pJG4-5 (transcription activation)</td>
<td>Glc U H T L</td>
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<tr>
<td>Control</td>
<td>Negative</td>
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<td></td>
<td>Positive</td>
<td>–</td>
<td>+++</td>
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<td>α1</td>
<td>NT (Met1-Cys93)</td>
<td>SNX6</td>
<td>+</td>
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<td></td>
<td>CD2 (Glu151-Ile292)</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>CD3 (Lys354-Val759)</td>
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<td></td>
<td>CD4 (Glu825-Arg848)</td>
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<td></td>
<td>CT (Ile935-Tyr1023)</td>
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<td>α2</td>
<td>NT (Met1-Cys91)</td>
<td>SNX6</td>
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<td></td>
<td>CD3 (Lys352-Val756)</td>
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<td></td>
<td>CD4 (Glu822-Arg845)</td>
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<tr>
<td></td>
<td>CT (Ile932-Tyr1020)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β</td>
<td>CD (Ala1-Lys33)</td>
<td>SNX6</td>
<td>–</td>
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The CDs of the Na,K-ATPase α1, α2 and β subunits were fused to the LexA DNA-binding domain. SNX6 was fused to the B42 transcription activation domain. Protein–protein interaction results in activation of LexAop-Leu2 and LexAop-Leu2-Z reporter genes. The yeast cell transformed with empty vector pEG202 and pJG4-5 was used as a negative control, and the yeast cell transformed with pEG202/ΔLexAop-Leu2 and pJG4-5-S267(cofilin) as a positive control (25). β-Galactosidase activity values are the average of four independent determinations converted to percentage of positive control. Gal, galactose; Glc, glucose; U−H−T−L−, without uracil, histidine, tryptophan, leucine; NT, N-terminal portion; CD, cytoplasmic domain; CT, C-terminal portion.

recombinant TCTP (Fig. 3C). These results show that SNX6 specifically inhibits the ability of TCTP to suppress Na,K-ATPase activity, which ultimately results in indirect activation of Na,K-ATPase.

3.5. Overexpression of SNX6 activates Na,K-ATPase above the basal level like knockdown expression of endogenous TCTP

We hypothesized that if the interaction between endogenous SNX6 and TCTP is increased simultaneously, Na,K-ATPase will be activated. To test this hypothesis, SNX6 alone was overexpressed in HeLa cells and the individual transporter activity was calculated by $^{86}$Rb$^+$ uptake assay. As expected, Na,K-ATPase activity of the GFP-SNX6-transfected cells was 18.7 ± 4.2% higher than that of mock (Fig. 4A). Overexpression of SNX6 can promote the interaction between SNX6 and endogenous TCTP, leading to upregulation of Na,K-ATPase above the basal level via inhibition of Na,K-ATPase-suppressive activity of endogenous TCTP. This suggests that inhibition of Na,K-ATPase by endogenous TCTP is a normal physiological event required for the maintenance of cellular Na,K-ATPase activity to basal level under normal environment. We made the siTCTP construct which markedly downregulates the level of endogenous TCTP, and examined whether the activation of Na,K-ATPase occurs in siTCTP-transfected cells. As shown in Fig. 4A, siTCTP caused the activation of Na,K-ATPase as did the overexpression of SNX6, suggesting Na,K-ATPase activity is usually inhibited to basal level by endogenous TCTP. Additionally, Na,K-ATPase activity of cells, co-transfected with both siTCTP and GFP-SNX6, was not different from that of SNX6 or siTCTP alone. Fig. 4B shows that Na,K-ATPase activation by overexpression of SNX6 and decreased expression of TCTP was not caused by increased expression of Na,K-ATPase. In siTCTP-transfected cells, endogenous TCTP expression was reduced by 70–80%. Although siTCTP caused incomplete knockdown of TCTP, co-transfection with both siTCTP and GFP-SNX6 still activates Na,K-ATPase activity. As indicated before, this may be, because, based on the subcellular fractionation analysis of cellular TCTP (unpublished data), only 10–70% of the remaining TCTP is probably localized in plasma membrane. Thus the quantity of the remaining TCTP is probably insignificant.

4. Discussion

Because Na,K-ATPase is closely involved in physiological and pathological phenomena, it is important to understand the mechanisms that regulate its activity. We propose here that Na,K-ATPase activity may be regulated by a complex mechanism involving the interaction of TCTP with Na,K-ATPase and SNX6. Our study suggests the existence of a ternary complex of Na,K-ATPase, TCTP, and SNX6 and that SNX6 acts as the negative regulator of TCTP which was previously identified as a suppressor of Na,K-ATPase [7]. Furthermore, our data demonstrate that the inhibition of Na,K-ATPase by endogenous TCTP is a natural phenomenon existing for the maintenance of cellular Na,K-ATPase activity at normal resting state and that Na,K-ATPase can be activated by inhibition of endogenous TCTP via the interaction with SNX6. Although siTCTP construct caused an incomplete knockdown of the expression of endogenous TCTP (70–80%), this incomplete knockdown had the same effect on the activation of Na,K-ATPase as overexpression of SNX6, implying the breakout of normal resting Na,K-ATPase activity. It was recently reported that knockdown of TCTP promotes apoptosis [24,25]. It appears that the ultimate role of endogenous TCTP is to carry out critical functions for cell survival, although this needs to be confirmed by studying the effect of knocking out the TCTP gene.

It has been reported that TCTP and SNX6 are ubiquitously expressed in multiple tissues and localized throughout the cell
Because the activation of Na,K-ATPase by SNX6 results from the later’s role as an inhibitor of TCTP, any imbalance in the amounts of TCTP and SNX6 affects their interaction which in turn affects Na,K-ATPase activity. SNX9, a member of sorting nexins, was found to be translocated from cytosol to plasma membrane by insulin [26]. Inhibition of TCTP by SNX6 may contribute to upregulation of Na,K-ATPase by stimuli such as insulin. Studies are underway to test this possibility. Moreover, the interaction of TCTP with SNX6 may influence other functions of TCTP involved in growth-related process, apoptosis and allergic response [2].

Currently, little is known of the cellular function of SNX6, other than that SNX6 plays the role of interfering with TGF-β signaling [14]. SNX6 is a member of sorting nexins, a family of cytoplasmic and membrane-associated proteins. A characteristic structural feature of the sorting nexins is the phox homology (PX) domain, a phosphoinositide-binding module [27,28]. Because several sorting nexins with their PX domains localize mainly in PtdIns(3)P-rich endosomal and vacuolar, it has been suggested that they may function in the endocytosis of plasma membrane and/or the trafficking of...
Fig. 4. Overexpression of SNX6 activates Na,K-ATPase above the basal level like knockdown expression of endogenous TCTP. (A) Individual transporter activity was measured by $^{86}$Rb$^+$ uptake assay. HeLa cells were transfected with mock, GFP-SNX6, or siTCTP, or co-transfected with siTCTP and GFP-SNX6 constructs. The experimental procedures for $^{86}$Rb$^+$ uptake assay were described as in the legend of Fig. 2A. The bar graph shows the results of three independent experiments ± S.E. Statistical significance of Na,K-ATPase activity was established by one-way ANOVA and post hoc Duncan test (* P < 0.0005). (B) Effect of SNX6 and knockdown of endogenous TCTP on Na,K-ATPase and TCTP protein synthesis. After HeLa cells were transfected as same condition in (A), the total cell extract (20 μg) was blotted with anti-Na,K-ATPase α subunit, anti-GFP, anti-TCTP.

proteins from one membrane compartment to another [29]. It has been suggested that for some sorting nexins, membrane association also requires their C-terminal coiled-coil regions (BAR domain) as well as PX domain [16]. Several sorting nexins including SNX6 consist of PX domain and a BAR domain [30]. They homo- and hetero-oligomerize and colocalize intracellularly through these domains [31]. Recently, the Bin/Amphiphysin/Rvs (BAR) domain has been described as a dimerization and membrane binding module which can sense membrane curvature [32]. Moreover, this domain has the potential to function as a small G-protein binding module [32,33]. Thus, it is tempting to speculate that the sorting nexins that share PX and BAR domains may share common functions in the cell. However, the fact that individual sorting nexins have different specificities for interacting with the target protein [15] implies that they may serve to select the specific target protein to be conveyed within the particular transport vesicles, or regulate the specific cellular functions unrelated to their normal functions. Our demonstration that SNX6 is involved in cellular ionic homeostasis via inhibition of TCTP may be an example of such new functions.

Further studies are needed to understand the detailed mechanisms underlying the role of SNX6 as a TCTP-inhibitory protein. Elucidation of how Na,K-ATPase activation by SNX6 occurs may lead to more comprehensive approaches to TCTP regulation and the identification of therapeutic targets to diseases involving Na,K-ATPase, such as hypertension.

In conclusion, we identified SNX6 as a TCTP-binding protein and demonstrated that the interaction of TCTP with SNX6 interferes with suppression of Na,K-ATPase by TCTP. SNX6 therefore may be a key molecule serving as the negative regulator of TCTP in the regulation of Na,K-ATPase activity under physiological stimuli. Our demonstration of the indirect regulation of Na,K-ATPase activity by SNX6 suggests a novel molecular mechanism for the functional coupling of SNX6 and TCTP.

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