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Human kidney anion exchanger 1 interacts with adaptor-related protein complex 1 μ1A (AP-1 mu1A)

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ABSTRACT

Kidney anion exchanger 1 (kAE1) mediates chloride (Cl^-) and bicarbonate (HCO_3^-) exchange at the basolateral membrane of kidney α-intercalated cells. Impaired trafficking of kAE1 leads to defect of the Cl^-/HCO_3^- exchange at the basolateral membrane and failure of proton (H^+) secretion at the apical membrane, causing a kidney disease – distal renal tubular acidosis (dRTA). To gain a better insight into kAE1 trafficking, we searched for proteins physically interacting with the C-terminal region of kAE1 (Ct-kAE1), which contains motifs crucial for intracellular trafficking, by a yeast two-hybrid (Y2H) system. An adaptor-related protein complex 1 μ1A (AP-1 mu1A) subunit was found to interact with Ct-kAE1. The interaction between either Ct-kAE1 or full-length kAE1 and AP-1 mu1A were confirmed in human embryonic kidney (HEK) 293T by co-immunoprecipitation, affinity co-purification, co-localization, yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA) and GST pull-down assay. The interacting site for AP-1 mu1A on Ct-kAE1 was found to be Y904DEV907, a subset of YXXØ motif. Interestingly, suppression of endogenous AP-1 mu1A in HEK 293T by small interfering RNA (siRNA) decreased membrane localization of kAE1 and increased its intracellular accumulation, suggesting for the first time that AP-1 mu1A is involved in the kAE1 trafficking of kidney α-intercalated cells.

1. Introduction

Human anion exchanger 1 (AE1 or band 3), encoded by *solute carrier family 4, anion exchanger, member 1 (SLC4A1)* gene, is a plasma membrane transporter functioning in Cl^-/HCO_3^- exchange to regulate intracellular pH and acid–base homeostasis in the human [1]. Two AE1 isoforms, erythroid AE1 (eAE1) and kidney AE1 (kAE1), have been characterized [2]. eAE1 is a major protein on red cell membrane, functioning in both electroneutral anion (Cl^-/HCO_3^-) exchange and cytoskeletal anchorage. It contains 911 amino acids which organize into three structurally and functionally distinct domains: a cytoskeleton-associated amino-terminal domain, a central anion-transporting transmembrane segment, and short cytoplasmic carboxyl-terminal domain [3]. kAE1, which lacks the first 65 amino acids, is expressed at the basolateral membrane of acid-secreting α-intercalated cells of kidney and mediates Cl^-/HCO_3^- transport across the basolateral membrane to balance H^+ secretion across the apical surface into urine [4].

Failure of either acid excretion or bicarbonate reabsorption due to mutations in the gene encoding H^+-ATPase or kAE1, respectively, leads to distal renal tubular acidosis (dRTA), a kidney disorder characterized by an inability to acidify urine resulting in systemic metabolic acidosis and several clinical manifestations such as muscle weakness, failure to thrive, hypokalemia, hypercalciuria, hypocitraturia, and nephrolithiasis or nephrocalcinosis [5]. Genetic studies revealed two modes of inheritance of dRTA attributable to *SLC4A1* mutations: autosomal dominant (AD) and autosomal recessive (AR) dRTA [6], in which molecular mechanisms have been unveiled. The *SLC4A1* mutations causing both forms of dRTA generate mutant kAE1 that almost maintains functional anion-exchange activity but exhibits basolateral trafficking defect and intracellular retention in human embryonic kidney 293 (HEK 293) [7–10] cells and several mutations showed either intracellular retention or apical mis-targeting of kAE1 in polarized Madin–Darby canine kidney (MDCK) cells [11–13].

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The involvement of the C-terminal portion of kAE1 in proper basolateral trafficking was reported [13,14]. A 20-bp deletion in exon 20 of *SLC4A1* leading to a mutation that changes alanine to leucine (A > L) at position 888 and a premature termination codon at position 889 (A888L + 889X), which truncates the protein by 23 amino acids, was identified in two affected brothers with dRTA [15]. R901X (band3 Walton) mutation, a deletion of the last 11 amino acids of AE1, was found to be associated with dominant dRTA in two affected brothers of one family [11,13,16]. Furthermore, tyrosine residue at position 904 (Tyr904) is critical for polarized transport of kAE1 as Y904A or Y904A + V907A mutation caused non-polarized distribution of kAE1 in polarized MDCK cells [11,13]. Removal of the last five amino acids was sufficient to retard kAE1 trafficking in HEK 293 and LLC-PK1 cells [17]. Despite many pieces of evidence suggesting that C-terminal portion of kAE1 is involved in basolateral membrane trafficking, very little information is known for proteins that physically interact with the C-terminal tail of kAE1 [18]. We reported here that kAE1 interacts with AP-1 mu1A, a subunit of AP-1A adaptor complex, in a yeast two-hybrid screening. The interaction was further confirmed by co-immunoprecipitation, affinity co-purification, immunofluorescence staining, protein fragmentation complementation assay (PCA) [19], and GST pull-down assay . The effect of AP-1 mu1A suppression by RNA interference on kAE1 trafficking in HEK 293T cells was also identified.

2. Materials and methods

2.1. Plasmid construction

pcDNA3-kAE1 (a kind gift from Professor Reinhart Reithmeier, University of Toronto, Canada) containing full-length kAE1 cDNA was used as a template for amplification of a sequence consisting of 108 bp encoding the C-terminal 36 amino acids of AE1 (Ct-kAE1). The *EcoR*I/*Sal*I-digested Ct-kAE1 was subsequently inserted in-frame into pGBKT7 plasmid (Clontech) to generate a bait construct (pGBKT7-Ct-kAE1) expressing a fusion of GAL4-DNA binding domain and Ct-kAE1 in a yeast two-hybrid system. The details of plasmid construction for co-immunoprecipitation, affinity co-purification, double immunofluorescence staining, yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA), GST pull-down assay, and immunofluorescence staining and flow cytometry were described in the Supplementary material.

2.2. Yeast two-hybrid screening

To generate the bait strain, pGBKT7-Ct-kAE1 was transformed into the yeast AH109 strain. The bait construct was tested for correct protein expression prior to library screening. No intrinsic transcriptional activity of the bait construct was observed as measured in an autoactivation test by growing on synthetic dropout (SD)/-His-Ade medium supplemented with X-α-gal. The prey strain, Y187, pre-transformed with the prey plasmids, pACT2, which carried the GAL4-activation domain fused to fragments from a human kidney cDNA library. The yeast two-hybrid screen was performed according to the manufacturer's protocol (Clontech). Mated diploids whose cDNA-encoded products interacted with the bait protein were selected by growth on SD/-Trp-Leu-His-Ade plates and SD/-Trp-Leu-His-Ade plates supplemented with X-α-gal (Clontech) to assay for activation of reporter genes [*HIS3, ADE2, MEL1* (α-galactosidase)]. The positive colonies with strong reporter activities were selected. The prey plasmids rescued from positive colonies were transformed into *E. coli* for PCR amplification. The *Alu*I restriction patterns of PCR products were generated and the representatives from different restriction patterns were chosen for the specificity tests. Specific interactions between the bait protein and the encoded products of isolated preys were tested by re-transforming the prey plasmid into the yeast strain AH109 and then re-mated with the opposite mating strain Y187 harboring either the bait plasmid (Ct-kAE1), empty vector, or two other plasmids containing unrelated genes (p53 and laminC). The cDNA fragments of the positive clones from the specificity tests were sequenced and aligned with the database (http://www.ncbi.nlm.nih.gov) in order to obtain full-length cDNA sequences that were completely matched with the in-put sequences.

2.3. Cell culture and transfection

HEK 293T cells were maintained in complete Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Perbio), 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2. Two days before transfection, the HEK 293T cells were collected and seeded in six-well plates. The cultured cells were transfected with pcDNA3.1 vector or its derivative constructs according to the designed experiments by DEAE-dextran method as previously described [20] or by Lipofectamine transfection method following the manufacture's protocol (Invitrogen). After transfection for 2 days, the cells were collected for further analyses.

2.4. Co-immunoprecipitation and affinity co-purification

Two days post-transfection, the HEK 293T cells were lyzed in lysis buffer containing protease inhibitor cocktail (Roche). Co-immunoprecipitation and affinity co-purification using Co^{2+}-chelated resins (BD Biosciences) were performed as described in our previous study [20].

2.5. Double immunofluorescence staining

HEK 293T cells were grown on coverslips for 1 day, transfected or co-transfected with plasmid constructs using Lipofectamine 2000 (Invitrogen), and cultured for another 2 days. The immunofluorescence staining was performed using rabbit anti-Ct-kAE1, mouse anti-Myc, mouse anti-AP-1 mu1A (Abnova), rabbit anti-calregulin (Santa Cruz) or rabbit anti-TGN46 (Abcam), followed by donkey anti-rabbit IgG conjugated with Cy3 fluoresceine (Jackson Immunoresearch Laboratories) and goat anti-mouse IgG conjugated with Alexa 488 fluoresceine (Molecular Probes) by protocol which was described in our previous study [20].

2.6. Yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA)

Two days before transfection, the HEK 293T cells were collected and seeded in six-well plates. The cultured cells were individually transfected with 1 μg each of the YFP construct or co-transfected with different pairs of the constructs. Two days after transfection, the cells were fixed in 3% formaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 1% BSA. The coverslips were washed and mounted with Fluorosave. Cell fluorescence images were observed by using a laser scanning confocal Zeiss LSM 510 microscope (Carl Zeiss).

2.7. GST pull-down assay

The pGEX4T-2-GST, pGEX4T-2-GST-Ct-kAE1 and pGEX4T-2-GST-Ct-kAE1 mutants were transformed into *E. coli* BL21 (DE3). The pTrcHisA-AP-1 mu1A were transformed into *E. coli* DH5α. The bacterial cells were grown in Luria broth to express the recombinant proteins. The bacterial cells were lyzed in lysis buffer. The

GST or GST-fusion proteins were conjugated with Glutathione-Sepharose 4B beads (Amersham) then incubated with His-tagged AP-1 mu1A soluble lysate. The unbound proteins were eliminated by serial washing: once with PBS containing 0.1% Triton X-100, 20 mM EDTA, once with PBS containing 0.1% Triton X-100, 10 mM EDTA, and once with PBS. The binding protein complexes were eluted and subjected to analysis by Western blotting using mouse anti-His antibody (Amersham) and rabbit anti-mouse IgG-HRP conjugate (Santa Cruz). Chemiluminescent signals generated by SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) were detected by exposure to an X-ray film.

2.8. RNA interference

Small interfering RNA (siRNA) directed against mu1A subunit of AP-1 (siAP-1 mu1A) has previously been described [21]; its sequence was 5′-TCCGAAGGCATCAAGTATCGGAAGA-3′ (Invitrogen). Transfection of either siAP-1 mu1A or siControl was performed using Lipofectamine 2000 (Invitrogen). The efficiency of AP-1 mu1A knockdown by siRNA was examined by real time RT-PCR and Western blotting (see details in Supplementary material). HEK 293T cells were seeded for 24 h before transfection. The double-stranded siRNA were transfected twice with a 24-h interval. pcDNA3.1-kAE1-HA or pcDNA3-kAE1-Myc was co-transfected with first transfection of siRNA. Cells were harvested 48 h after the second transfection for further study.

2.9. Flow cytometry

Myc epitope inserted at the third extracellular loop of kAE1 would be expressed extracellularly. Thus, expression of kAE1-Myc on the cell surface could be determined by fluorescence staining and flow cytometry. HEK 293T cells were co-transfected with the pcDNA3-kAE1-Myc and siAP-1 mu1A or siControl. Two days after transfections, the cells were collected and determined by flow cytometry as described in our previous study [20].

3. Results and discussion

Trafficking defect of kAE1 has been revealed as a potential molecular basis of dRTA caused by *SLC4A1* mutations [9,10,20,22]. However, to date, the trafficking pathway of kAE1 to cell surface have not been clearly described. In this study, we have firstly reported a novel interaction between kAE1 and AP-1 mu1A, a medium subunit of an adaptor protein complex AP-1A. The interaction of the two proteins was confirmed by *in vitro* and *in situ* studies. The interacting site for AP-1 mu1A on Ct-kAE1 was also identified by YFP-PCA and GST pull-down assay. We therefore examined whether AP-1 mu1A is necessary for kAE1 targeting to the plasma membrane by knocking down AP-1 mu1A using RNA interference.

3.1. Identification of AP-1 mu1A as a Ct-kAE1-binding protein in a yeast two-hybrid screening

To explore the molecular trafficking machineries involved in the basolateral sorting and transport of kAE1, a yeast two-hybrid screening was carried out to search for proteins that bind to the C-terminus of kAE1 (Ct-kAE1, amino-acids 876–911), which contains a putative tyrosine-based sorting signal Y904DEV907. A bait construct, pGBKT7-Ct-kAE1, was used to screen a human kidney cDNA library. One plasmid contained a partial cDNA sequence encoded C-terminal fragment of AP-1 mu1A (amino acids 306–423, Fig. 1A). It is interesting that this region is a part of the AP-1 mu1A segment which reported to directly interact with tyrosine-based sorting signals present in cargo proteins [22]. The prey plasmid containing partial AP-1 mu1A cDNA sequence isolated from the initial screen was re-transformed for re-mating. Mated diploid cells were cultured on SD/-Trp-Leu-His-Ade and SD/-Trp-Leu-His-Ade/X-α-Gal plates as shown in Fig. 1B. Only the diploid cells with both plasmids containing Ct-kAE1 and AP-1 mu1A cDNA sequences activated the expression of reporter genes; hence, they grew and turned blue on plates, respectively.

3.2. kAE1 interacted with AP-1 mu1A in HEK 293T cells as detected by co-immunoprecipitation and affinity co-purification

To establish whether kAE1 interacts with AP-1 mu1A in mammalian cells, full-length kAE1 and AP-1 mu1A cDNA sequences were sub-cloned into mammalian expression vectors and transfected or co-transfected into HEK 293T cells. Fig. 2A shows co-immunoprecipitation of kAE1 with His-AP-1 mu1A by using anti-His antibody; the co-immunoprecipitated kAE1 could be detected by Western blot using anti-Ct-kAE1 antibody (lane 3). Similarly, in the affinity co-purification assay using Co^{2+} column (Fig. 2B), kAE1 was co-purified with His-AP-1 mu1A, which was detected using anti-Ct-kAE1 antibody (lane 3). Lanes 1, 2, and 5 in both figures were lysate inputs.

3.3. Subcellular localization of kAE1 and AP-1 mu1A in HEK 293T cells

Subcellular localization of kAE1 and AP-1 mu1A in HEK 293T cells was investigated by double immunofluorescence staining and confocal microscopy. kAE1-HA and His-AP-1 mu1A showed

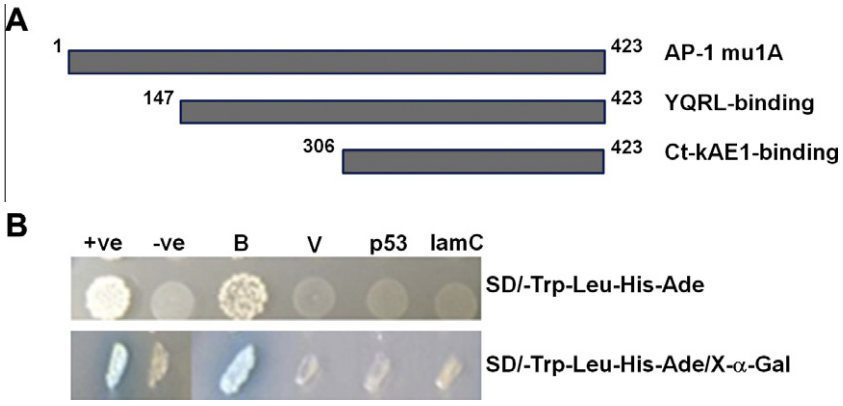

Fig. 1. Identification of AP-1 mu1A as a kAE1 interacting protein by yeast two-hybrid system. (A) Schematic diagram of the identified AP-1 mu1A fragment. (B) Specificity test of Ct-kAE1 and AP-1 mu1A interaction by growth of yeast diploids on synthetic dropout (SD)/-Trp-Leu-His-Ade medium and SD/-Trp–Leu-His-Ade/X-α-Gal agar plates.

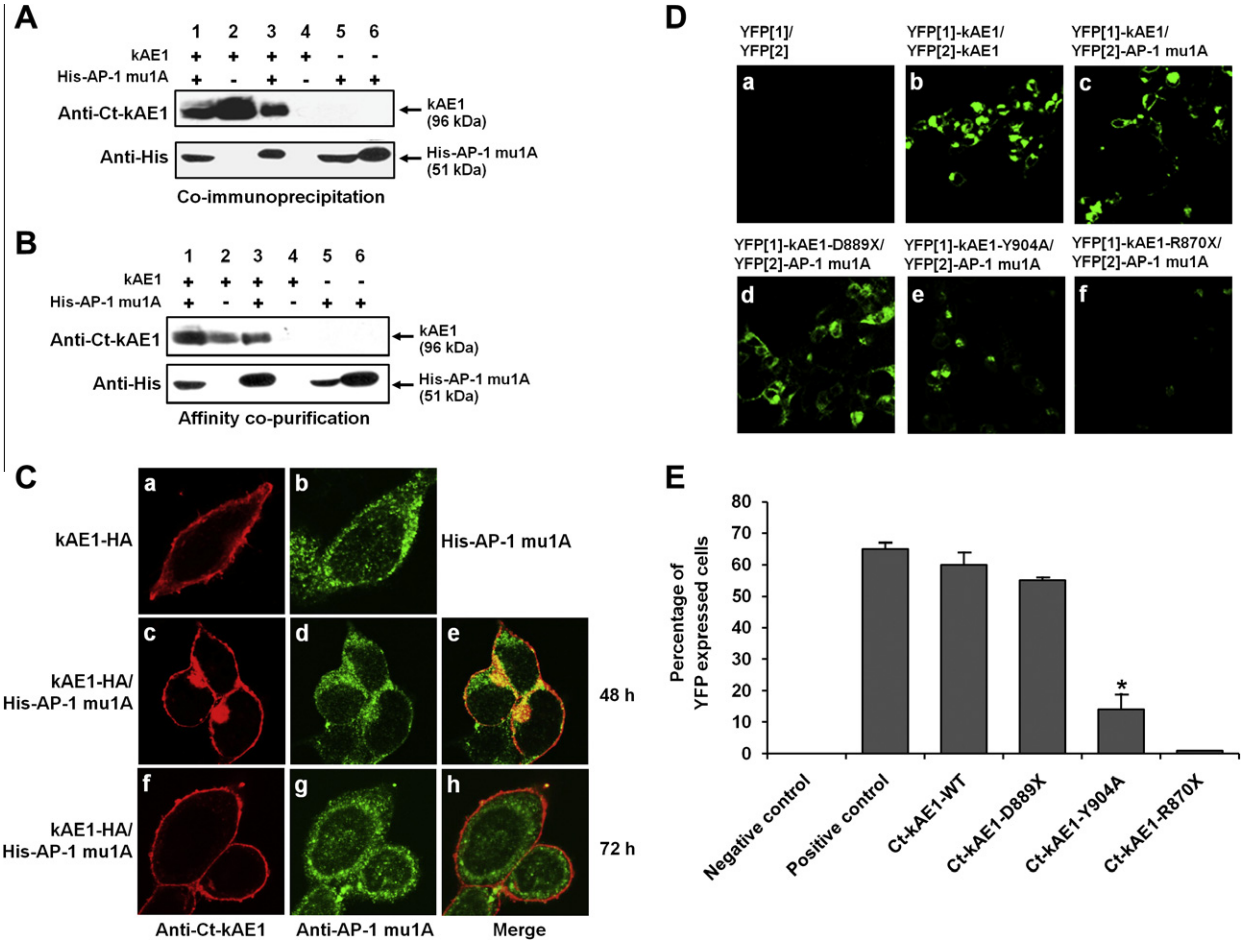

Fig. 2. Verification of kAE1 and AP-1 mu1A interaction by co-immunoprecipiation and co-purification assays and cellular localization of kAE1 and AP1-mu1A. (A) Co-immunoprecipitation of kAE1 and AP-1 mu1A expressed in HEK 293T cells. (B) Affinity co-purification of kAE1 and AP-1 mu1A expressed in HEK 293T cells. (C) Cellular localization of kAE1 and AP1-mu1A. kAE1-HA and His-AP-1 mu1A was individually transfected (a and b, respectively). kAE1-HA and His-AP-1 mu1A were co-transfected HEK 293T cells at 48 h (c–e) and 72 h (f–h). (D) Investigation of *in situ* interaction between kAE1 and AP-1 mu1A expressed in HEK 293T cells by YFP-PCA. (E) Percentage of YFP-expressed cells obtained from (D).

differential localization in the transfected HEK 293T cells in both individually (Fig. 2C(a, b)) or co-transfected to express kAE1-HA and His-AP-1 mu1A (Fig. 2C(c–h)). While His-AP-1 mu1A dispersed throughout the cytoplasm (Fig. 2C(b, d, g)), kAE1-HA showed dynamic distribution. After transfection for 48 h, kAE1-HA might saturate the sorting pathway (Fig. 2C(c)), which was co-stained with

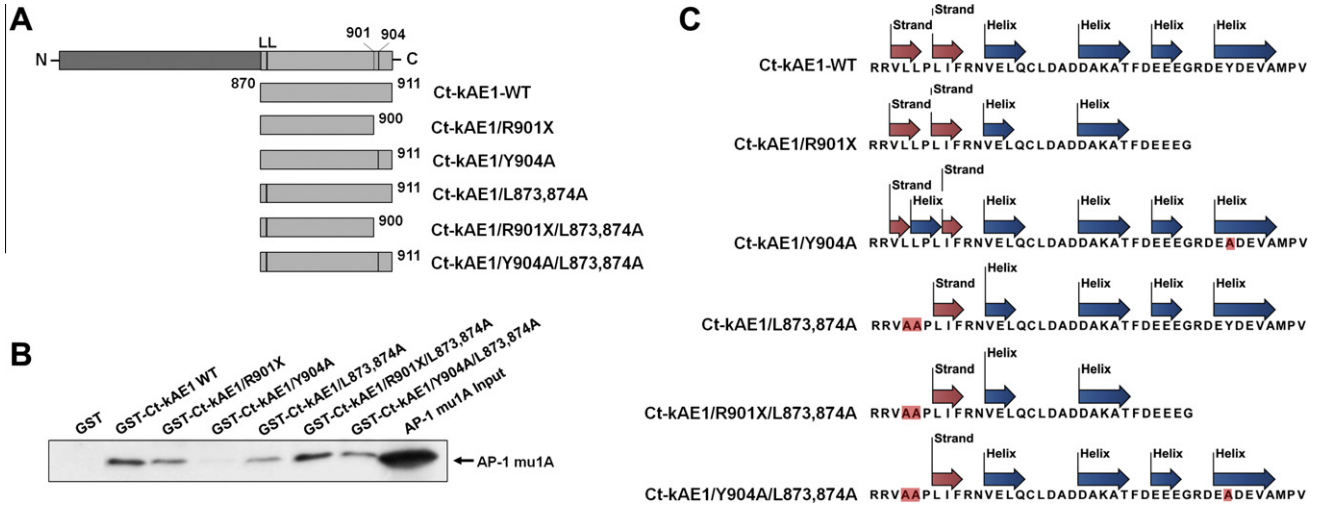

Fig. 3. (A) Illustrations of the carboxyl terminus of kAE1 (Ct-kAE1). Different GST-Ct-kAE1-mutants were generated from GST-Ct-kAE1-WT. (B) GST pull-down binding assay. (C) Prediction of secondary structures of Ct-kAE1-WT and Ct-kAE1-mutants by using CLC Main Workbench software.

His-AP-1 mu1A at Golgi/TGN like and partially stained at the cell membrane (Fig. 2C(e)). However, after transfection for 72 h, kAE1-HA was localized at cell membrane (Fig 2C(f–h)).

3.4. kAE1 interacted with AP-1 mu1A in HEK 293T cells as examined by yellow fluorescent protein (YFP)-based protein fragment complementation assay (PCA)

The interaction between kAE1 and AP-1 mu1A within HEK 293T cells was examined by YFP-PCA. Each of YFP fusion protein was co-expressed in HEK 293T cells and their interactions demonstrated by intracellular green-fluorescent signals. Percentage of YFP-expressed cells was calculated from the number of YFP-expressed cells against the number of nuclear-stained cells by using Hoechst 33258. HEK 293T cells were co-transfected to express YFP[1] and YFP[2] as a negative control (Fig. 2D(a)) and co-transfected to express YFP[1]-kAE1 and YFP[2]-kAE1 as a positive control whose dimerization of kAE1 fusion proteins resulted in YFP[1] and YFP[2] complementation (Fig. 2D(b)) showing intracellular green-fluorescent signals for >60% of the cell population (Fig. 2E). Similarly, when they were co-transfected to express either YFP[1]-kAE1 and YFP[2]-AP-1 mu1A (Fig. 2D(c)) or YFP[1]-kAE1-R889X and YFP[2]-AP-1 mu1A (Fig. 2D(d)), intracellular green-fluorescent signals were captured at >50% of the cell population (Fig. 2E). However, co-expression of YFP[1]-kAE1-Y904A with YFP[2]-AP-1 mu1A showed a reduction in intracellular green-fluorescent signals (Fig. 2D(e)) to be <20% of the cell population (Fig. 2E). Interaction of YFP[1]-kAE1-R870X which lacked C-terminus, and YFP[2]-AP-1 mu1A did not show intracellular green-fluorescent signals (Fig. 2D-f, and E)). It is surprising that deletion of in the YDEV motif did not totally diminish the interaction between kAE1 and AP-1 mu1A but it did for the whole Ct-kAE1 deletion as shown by the result of R870X mutant. These results indicate that although Tyr904 in the YDEV motif is critical for the interaction, the dileucine (Leu873Leu874) motif in Ct-kAE1 might also be used for the interaction.

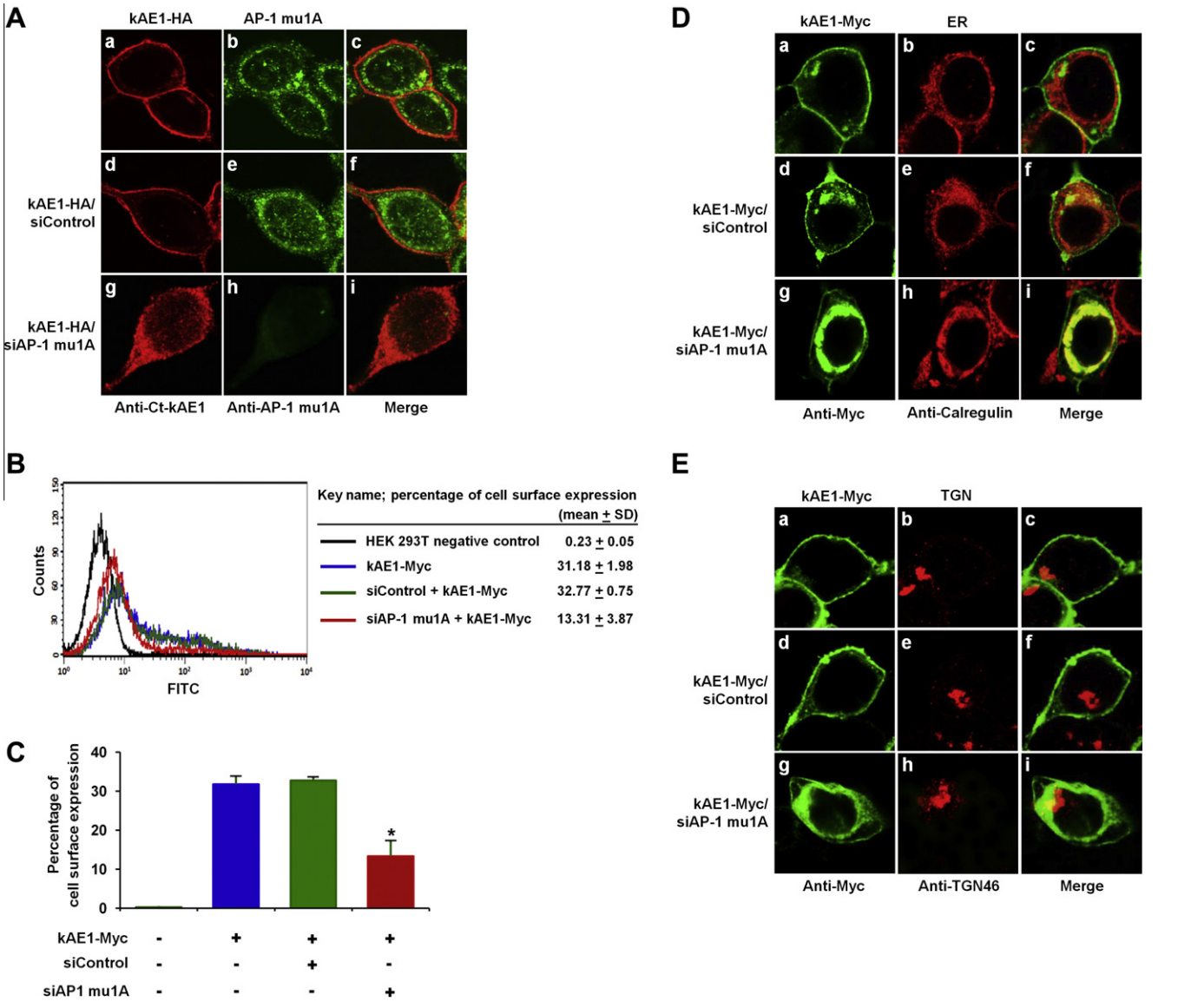

Fig. 4. Subcellular localization of kAE1 and AP-1 mu1A in HEK 293T cells. (A) HEK 293T cells were transfected to express kAE1-HA (a–c) or co-transfected with siControl (d–f) or with siAP-1 mu1A (g–i). (B) Cell-surface expression of kAE1-Myc in transfected HEK 293T cells measured by flow cytometry. Percentages of cell-surface expression of kAE1-Myc (mean ± SD) in different conditions are also indicated. (C) The bar graph showing percentages of cell-surface expression of kAE1-Myc. (D) Localizations of kAE1 and ER marker or (D) TGN marker in HEK 293T cells with suppression of AP-1 mu1A by siRNA.

3.5. Ct-kAE1 interacted with AP-1 mu1A in GST pull-down assay

To identify specific interaction site of AP-1 mu1A on Ct-kAE1, we conducted a series of GST pull-down assay using Ct-kAE1 containing individual or combinations of truncated and point mutations (Fig. 3A). His-tagged AP-1 mu1A protein was incubated with GST-Ct-kAE1-WT or GST-Ct-kAE1-mutants. All GST-Ct-kAE1-mutants could pull down His-AP-1 mu1A (Fig. 3B, lanes 3–7). However, a very faint band was observed in the binding between GST-Ct-kAE1/Y904A and His-AP-1 mu1A (Fig. 3B, lane 4). This has prompted us to suspect that there might be another potential binding motif on Ct-kAE1 that interacts with AP-1 mu1A. Therefore, we introduced a combination of deletion of the YDEV motif and substitution at the dileucine motif (Fig. 3B, lane 6) or substitutions at both Tyr904 and dileucine motif (Fig. 3B, lane 7) to Ct-kAE1, the binding to AP-1 mu1A still occurred. We then used CLC Main Workbench software (CLC bio) to predict secondary structures of Ct-kAE1-WT and Ct-kAE1-mutants. The prediction showed that Ct-kAE1-WT consists of four helices and two upstream strands (Fig. 3C). It is interesting that in substitution of tyrosine (Y) by alanine (A) at the position 904 in the YDEV motif affected the predicted secondary structure by the presence of a helix between those two upstream strands, where dileucine residues (Leu873-Leu874) and dileucine-like residues (Leu876Ile877) were located (Fig. 3C). However, this dileucine-like motif might not be affected in other Ct-kAE1-mutants. Taken together, the YDEV motif is most likely a primary interaction site of AP-1 mu1A and either dileucine residues (Leu873Leu874) or dileucine-like residues (Leu876Ile877) may also play roles. However, the role of this dileucine-like motif in binding to AP-1 mu1A needed further investigation.

3.6. Suppression of endogenous AP-1 mu1A reduced membranous kAE1 and accumulated kAE1 in the ER of HEK 293T cells

To address the functional significance of AP-1 mu1A in kAE1 trafficking, we employed siRNA-knockdown to suppress endogenous AP-1 mu1A in HEK 293T cells and examined subcellular localization of kAE1. The efficiency of siRNA was determined by real-time PCR and Western blotting (Supplementary Fig. 1).

HEK 293T cells were co-transfected with kAE1-HA and siControl or kAE1-HA and siAP-1 mu1A. Subcellular localization of kAE1 and AP-1 mu1A was examined by immunofluorescence staining using anti-Ct-kAE1 and anti AP-1 mu1A, respectively. The results showed that without AP-1 mu1A suppression, kAE1-HA was mainly located at the cell surface (Fig. 4A(a–f)) but with AP-1 mu1A suppression, kAE1-HA was accumulated in the cytoplasm (Fig. 4A(g–i)).

To quantitatively measure kAE1 expression at the cell surface, we transfected the plasmid construct to express kAE1 containing Myc epitope at the position 557 in the third extracellular loop of kAE1 to allow immunological detection at the surface of intact cells by flow cytometry. The HEK 293T cells individually expressing kAE1-Myc or co-expressing kAE1-Myc with siControl transfection had mean fluorescence intensities of 31.18 ± 1.98% and 32.77 ± 0.75%, respectively (Fig. 4B and C). However, HEK 293T cells with suppression of AP-1 mu1A showed lower levels of kAE1-Myc on the cell surface (13.31 ± 3.87%) than those of the control cells (Fig. 4B and C). The incomplete reduction of cell-surface expression of kAE1 is possibly attributable to the incomplete AP-1 mu1A knockdown. However, this confirms the result of the immunofluorescence staining in the AP-1 mu1A suppressed cells.

To address the location of kAE1-Myc with respect to intracellular organelles when AP-1 mu1A was suppressed, we co-transfected to express kAE1-Myc with siAP-1 and stained the cells with antibodies specific to cellular organelle markers, calregulin for ER and TGN46 for trans-Golgi network (TGN). In the parental and siControl cells, kAE1-Myc was predominantly expressed at the cell membrane (Fig. 4D(a–f) and E(a–f)). In the cells with AP-1 mu1A suppression by siRNA, kAE1-Myc was heavily accumulated in the cytoplasm with calregulin in ER (Fig. 4D(g–i)) but slightly co-localized with TGN46 in TGN (Fig 4E(g–i)). Thus, it is likely that the interaction between kAE1 and AP-1 mu1A normally occurs in Golgi/TGN-like compartment. However, when AP-1 mu1A is suppressed, kAE1 fails to exit from ER resulting in its accumulation in this cellular organelle.

4. Conclusion

We firstly identified and characterized the interaction between human kAE1 and AP-1 mu1A and suggested the amino acid motifs in Ct-kAE1 that are essential for this interaction. A critical role for AP-1 mu1A in kAE1 trafficking to the plasma membrane showed a marked reduction of kAE1 on the membrane and its accumulation in the cytoplasm, particularly in ER. Further studies will be toward the exploration of the role of AP-1 mu1A in kAE1 trafficking to basolateral membrane in polarized cells. The insight into the molecular mechanisms overriding the trafficking of kAE1 to the basolateral membrane will provide a better understanding of the molecular defect of dRTA associated with *SLC4A1* mutations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.09.015.

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