Progress Report CRF grant: "**Advancing the understanding of the renal Fanconi syndrome in cystinosis**"

Period- Feb 2024-Aug 2024

Abstract

The objective of this project is to unravel the molecular mechanism of the renal Fanconi syndrome in cystinosis. In our previous grant period, we identified in the yeast model a novel interaction of ortholog of cystinosin (Ers1) with the yeast homolog of the sodium/hydrogen (Na/H) exchanger (Nhx1) and showed that this interaction occurred in the endosomes. Ten isoforms (NHE1-NHE10) have been identified within the mammalian NHE family, membrane proteins with identical structural topology but differing tissue and cellular localizations. One isoform of Na/H exchanger, NHE3, is a major absorptive sodium transporter expressed in the endosomal compartment as well as at the apical membrane of the proximal tubules of the kidney and in the gastrointestinal epithelial cells. We thus studied the potential interaction of cystinosin and NHE3 and showed interaction and colocalization of these proteins in proximal tubular cells (PTCs). We also showed that NHE3 was mis-localized in a CTNS-deficient PTC lines and exhibited impaired trafficking to the plasma membrane. These defects could be rescued using lentiviral vectors containing *CTNS* but surprisingly not *CTNS-LKG*. In the *Ctns*$^{-/-}$ mouse kidneys and a cystinosis patient' kidney biopsy, NHE3 appears mis-localized compared to the healthy controls where NHE3 is located at the brush border. Finally, we showed that cystinosin also interacted with NHE2 that is expressed in the kidney and gastrointestinal track too, but not with NHE1 that is expressed ubiquitously. In the CTNS-deficient PTCs and in the *Ctns*$^{-/-}$ mouse kidneys, NHE2 expression increased suggesting a compensatory mechanism for NHE3. In this current application, we first propose to explore the domains of interaction between Ers1 & Nhx1 proteins using the Membrane yeast two-hybrid (MYTH) assay and/or bimolecular fluorescence complementation (BiFC) assays in the yeast model. In human PTCs, we will advance the molecular and functional understanding of the interaction between NHE3 and cystinosin, and the role of cystinosin and cystinosinLKG in its cellular trafficking. Finally, we will further explore the dynamics of NHE3 mis-localization in *Ctns*$^{-/-}$ kidneys at different timepoints. Because NHE3 interacts directly and indirectly with several transporters in PTCs such as megalin, SGLT2 (Sodium Glucose cotransporter 2), and NPT2 (Type IIa sodium dependent phosphate transporter), its mis-localization in absence of cystinosin may lead to a concomitant loss of the major brush border transporters/receptors in the proximal tubules providing a molecular mechanism for the Fanconi syndrome. Thus, to test this hypothesis, we will also investigate the expression and localization of other transporters/endocytic receptors interacting directly or indirectly with NHE3, such as megalin, SGLT2 and NPT2, at different time points to establish the spatio-temporal loss of these proteins at the brush border compared to NHE3. The impact that HSPC transplantation has on these proteins' localization and expression will be tested in the *Ctns*$^{-/-}$ mice.

During this period, we have made progress in **Specific Aim 2.**

The objective of Specific Aim 2 is **domain identification, subcellular localization & function of NHE3 in mammalian system**

Study in mammalian system

We used the Human Kidney 2 (HK-2) cells as a new model of human PTCs, in which *CTNS* has been knocked-out using CRISPR/Cas9 (kindly provided by Dr. Sergio Catz ,The Scripps Research Institute). The knock-out has been confirmed and data presented in our renewal submission. To characterize the defective subcellular localization of NHE3 in PTCs, we transduced the HK-2 cells with LV-NHE3-GFP and studied its localization and subcellular distribution pattern with lysosomes (LAMP1), early endosomes (EEA1), cis-golgi (GM130), and endoplasmic reticulum markers (Figure 1-4). We observed significant difference in the localization pattern of the NHE3 protein, being localized more in the ER and less in the cis-golgi and early endosome under CTNS$^{-/-}$ condition. Thus, suggesting accumulation of the protein in the ER and a defect in its trafficking implemented directly by the absence of cystinosin (Figure 1-4).

Please refer to Figure 1-4.

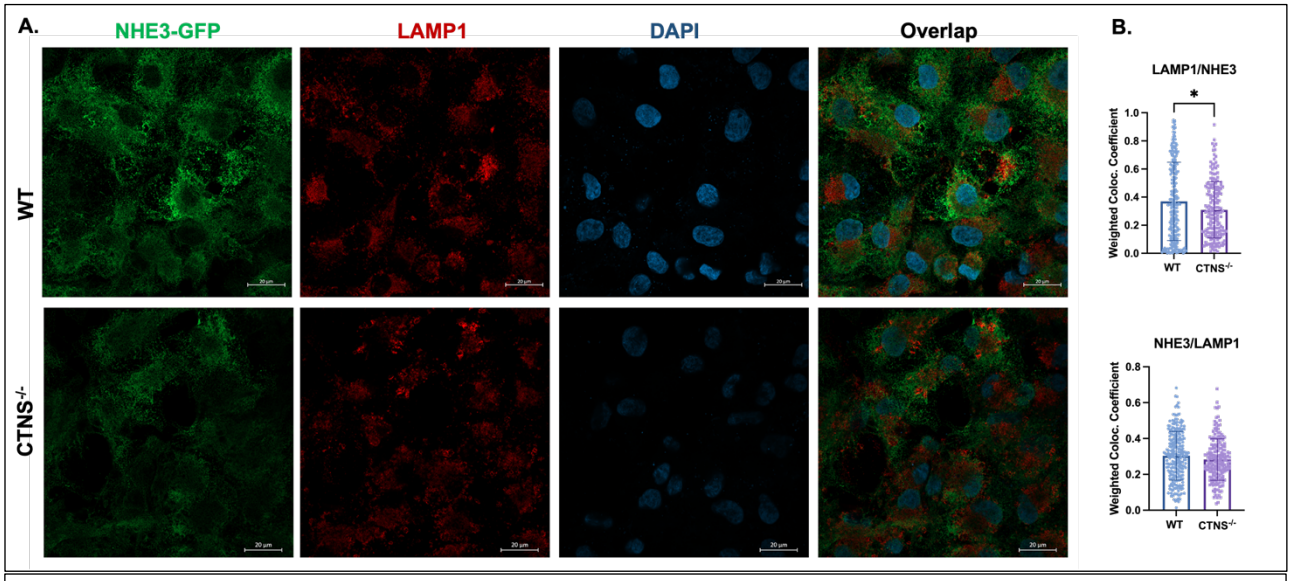

Figure 1: No significant difference was observed in the localization of NHE3 and LAMP1 between HK-2 WT and CTNS $^{-/-}$ cells. A. Immunofluorescent analysis for the expression and subcellular distribution of NHE3-GFP and lysosomal marker Lamp1 in HK-2 WT and CTNS$^{-/-}$ cells. HK-2 WT and CTNS$^{-/-}$ cells stably expressing NHE3-GFP was detected using goat anti-GFP antibody. Endogenous LAMP1 was detected using antibody raised in mouse. **B.** Graph showing the colocalization coefficient between NHE3 and Lamp1 and vice versa.

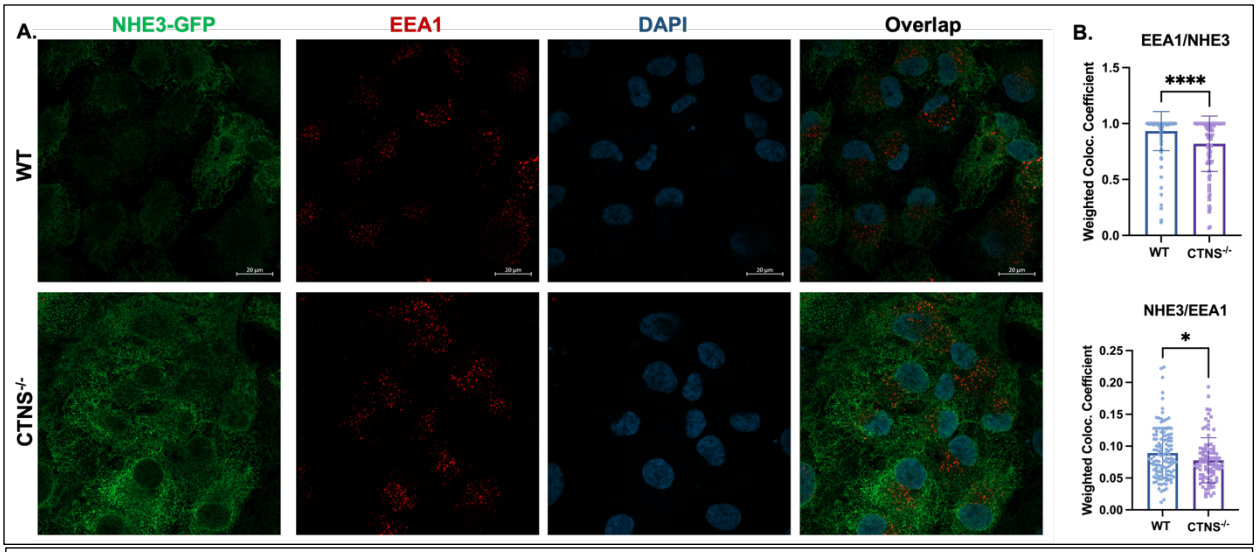

Figure 2: Significant difference was found in the localization of NHE3 and EEA1 between WT and CTNS $^{-/-}$ cells. A. Immunofluorescent analysis for the expression and subcellular distribution of NHE3-GFP and early endosome marker EEA1 in HK2 WT and CTNS$^{-/-}$ cells. HK2 WT and CTNS$^{-/-}$ cells stably expressing NHE3-GFP was detected using goat anti-GFP antibody. Endogenous EEA1 was detected using antibody raised in mouse. **B.** Graph showing the colocalization coefficient between NHE3 and EEA1 and vice versa.

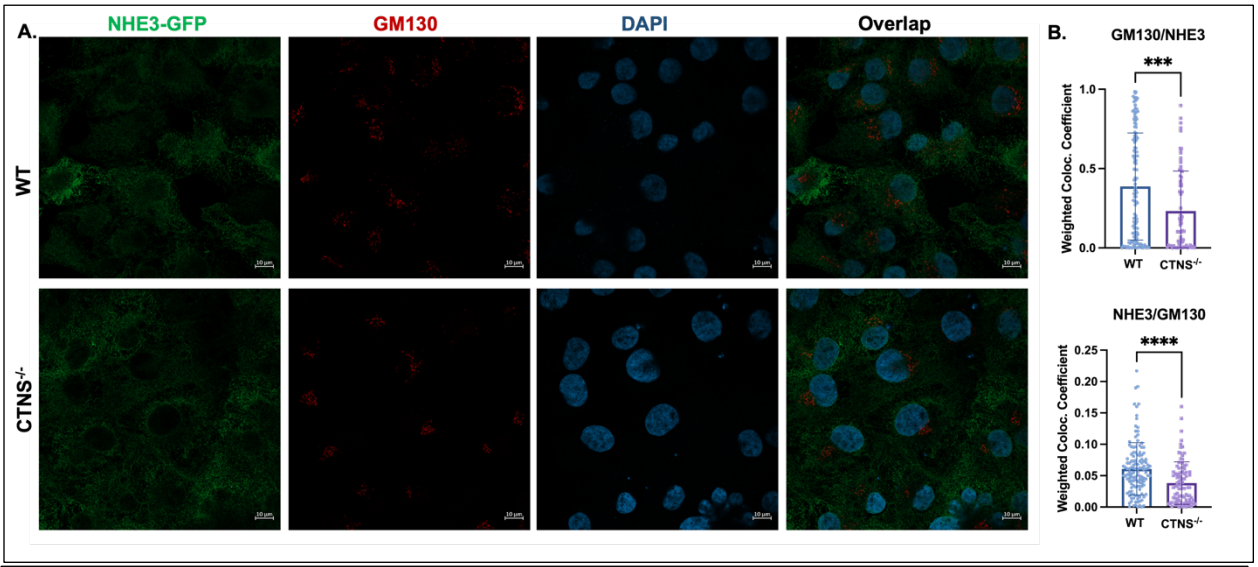

Figure 3: Significant difference was found in the localization of NHE3 and GM130 between WT and CTNS $^{-/-}$ cells. A. Immunofluorescent analysis for the expression and subcellular distribution of NHE3-GFP and cis-golgi marker GM130 in HK-2 WT and CTNS$^{-/-}$ cells. HK-2 WT and CTNS$^{-/-}$ cells stably expressing NHE3-GFP was detected using goat anti-GFP antibody. Endogenous GM130 was detected using antibody raised in mouse. **B.** Graph showing the colocalization coefficient between NHE3 and GM130 and vice versa.

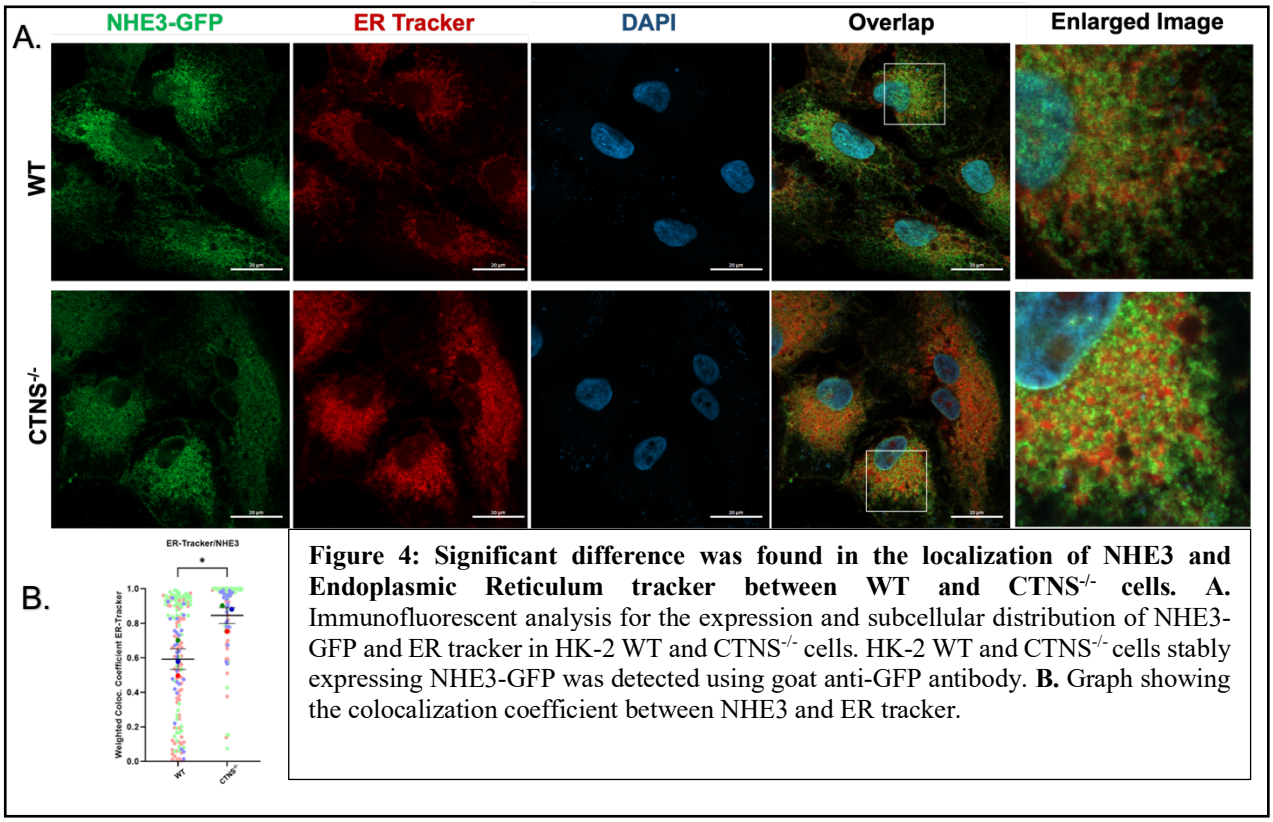

Figure 4: Significant difference was found in the localization of NHE3 and Endoplasmic Reticulum tracker between WT and CTNS$^{-/-}$ cells. A. Immunofluorescent analysis for the expression and subcellular distribution of NHE3-GFP and ER tracker in HK-2 WT and CTNS$^{-/-}$ cells. HK-2 WT and CTNS$^{-/-}$ cells stably expressing NHE3-GFP was detected using goat anti-GFP antibody. **B.** Graph showing the colocalization coefficient between NHE3 and ER tracker.

Conclusion

From our study in human PTCs, it is it is apparent that cystinosin plays a role in NHE3's trafficking, localization and function.

References

1. Gekle M, Drumm K, Mildenberger S, Freudinger R, Gaßner B, Silbernagl S. Inhibition of Na^+-H^+ exchange impairs receptor-mediated albumin endocytosis in renal proximal tubule-derived epithelial cells from opossum. *The Journal of Physiology*. 1999;520(3):709-721. doi:10.1111/j.1469-7793.1999.00709.x