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Rescuing a Mutant Membrane Protein

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Aim of the Project

NBCe1 is a bicarbonate transporter in the kidney that maintains a constant level of bicarbonate in the blood. A mutant NBCe1 protein 'Q913R', identified in a patient with low blood pH, is misfolded and accumulates inside the cell rather than in the plasma membrane. I introduced DNA for wild-type and mutant NBCe1 into a kidney cell-line (HEK) by transfection and cultured the cells either at 37° C or at 30° C; a low-temperature treatment that restores the appropriate trafficking of other mutant membrane protein (e.g., CFTRΔ508 in cystic fibrosis). I studied the distribution of NBCe1 by immunofluorescence microscopy. At 30° C, Q913R showed a pattern of fluorescence around the rim of the cell, similar to the wild type protein. Thus low-temperature is an effective treatment and this system could be used to screen for chemical chaperones that mimic this effect.

Introduction/Background

pH Regulation

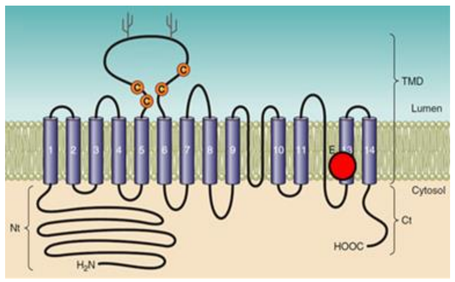
The human body has interesting mechanisms that is able to resist changes in pH. The plasma is supposed to be kept constant at a pH of 7.4. When the body isn't able to produce enough bicarbonate which neutralizes acid in the body that comes from diet and exercise we get metabolic acidosis. Metabolic acidosis can be a complication of chronic kidney disease and can be caused by genetic disease.

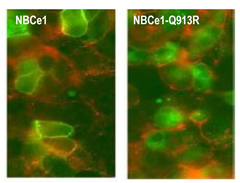
pRTA

Bicarbonate neutralizes acids so mutations in the transporter that stop NBCe1 getting to the cell surface cause a drop in bicarbonate and a drop in blood pH. This is a disease called proximal renal tubular acidosis (pRTA). The lab that I am a part of has identified a new mutation 'Q913R' in the NBCe1 of a patient with pRTA.

NBCe1

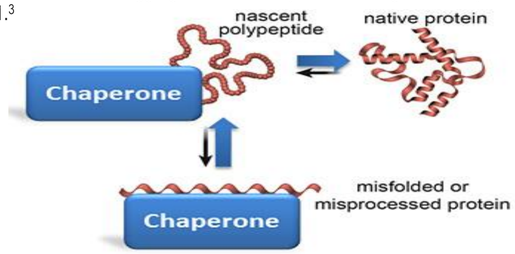
NBCe1 is a bicarbonate transporter in the kidney that maintains a constant level of bicarbonate in the blood.[1] Wild-type NBCe1 must be expressed to the cell surface to perform its function. NBCe1 is processed through the endoplasmic reticulum (ER) where quality-control machinery ensures proper protein folding prior to release and further trafficking to the Golgi apparatus and the plasma membrane. With Q913R they do not traffic to their final destination and are prematurely degraded by the ER- and post-ER quality-control machinery.[2]





Rescue strategies

There have been previous studies done on rescuing mutant membrane proteins over the recent years. A example is the rescue of the most common mutant protein leading to Cystic Fibrosis, the deltaF508 cystic fibrosis transmembrane conductance regulator (CFTR).Chaperones such as DMSO, Glycerol and Temperature reduction have been an effective way of reducing misfolded mutant proteins, reducing the accumulation of aggregates in the body and restoring the activity of transporters related to NBCe1.[3]

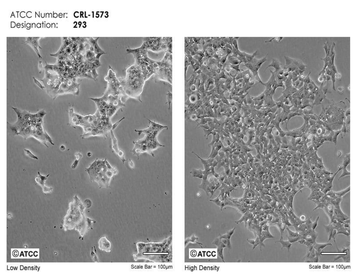


Methods and Results

Methods

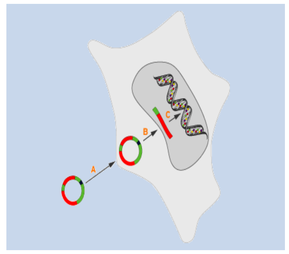
HEK cell type

HEK293 is a cell line derived from human embryonic kidney cells grown in tissue culture. They are also known, more informally, as HEK cells.



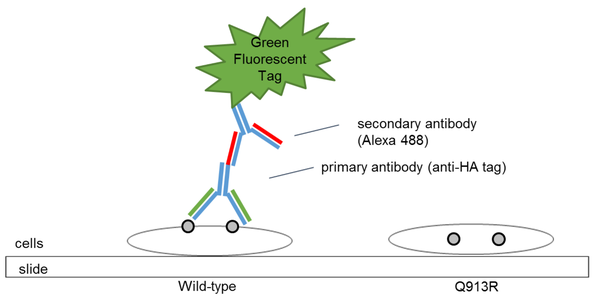
Mammalian Cell Transfection

Transfection is a technique commonly used to express exogenous DNA into a host cell line, in this case being a HEK cell line. Lipofectamine 3000 was used for the transfection process which is a common reagent for transfection.



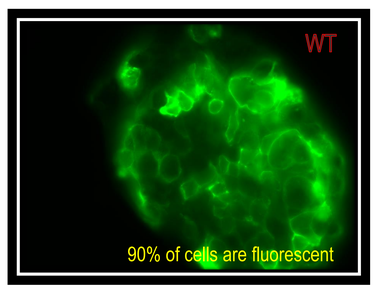
Immunofluorescence + Microscopy

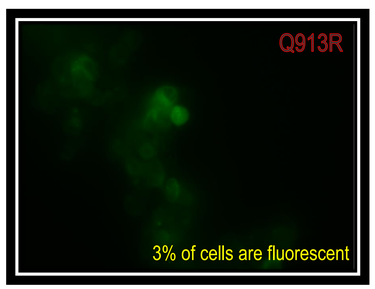
I fixed the cells in paraformaldehyde and applied an antibody against an artificial HA-tag in the extracellular region of NBCe1.I studied the images under a fluorescent microscope(AxioImager Zeiss) to see the fluorescence that denotes extracellular accessibility of the HA-tag (plasma membrane expression of the NBCe1).

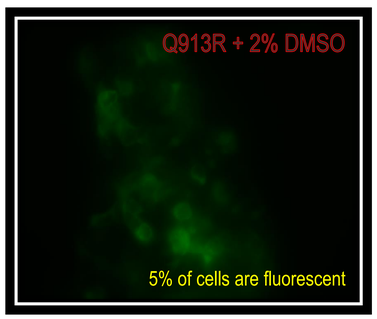


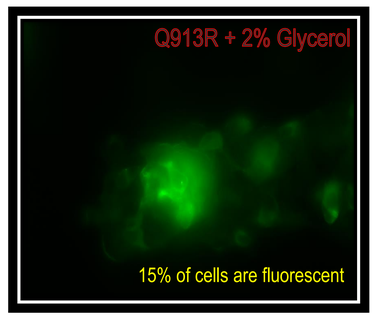
Results

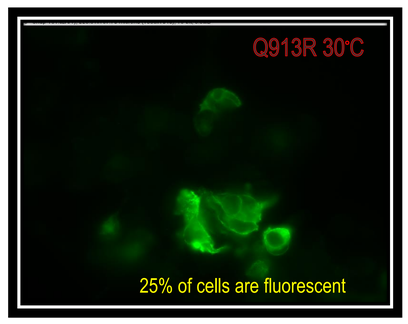
Untreated Q913R-expressing cells and those that had been treated with the addition of DMSO show minimal fluorescence(3-5%). Those treated with glycerol show a greater number of fluorescent cells (15%) that resemble wild-type expression. 25% of cells cultured at 30°C exhibit a wild type distribution.











Discussion

From these preliminary results we can say [1] that low temp and glycerol could be useful to restore trafficking; [2] that this system could be used to screen for drugs that mimic the effect of lower temperature that in the long run could form a new treatment for pRTA. I am in the process of repeating these results to perform statistical analysis of these findings.

Literature cited *[1]Parker and Boron, Physiol Rev 2013, 93: 803-959 [2] Myers, E. J., Yuan, L., Felmlee, M. A., Lin, Y.-Y., Jiang, Y., Pei, Y., Wang, O., Li, M., Xing, X.-P., Marshall, A., Xia, W.-B. and Parker, M. D. (2016), A novel mutant $Na^+/HCO3^-$ cotransporter NBCe1 in a case of compound-heterozygous inheritance of proximal renal tubular acidosis. J Physiol, 594: 6267–6286. [3] Chu CYS, King JC, Berrini M, Alexander RT, Cordat E (2013) Functional Rescue of a Kidney Anion Exchanger 1 Trafficking Mutant in Renal Epithelial Cells. PLOS ONE 8(2): e57062*

Funding sources: *This study was supported by a CURCA award (to AG), start-up funding from the Dean of the School of Medicine and Biomedical Sciences and the Department of Physiology and Biophysics (to MDP).*

Acknowledgements: *Thanks to Dr. Jessica Poulin in the Department of Biology and Wade Sigurdson in the Confocal Microscopy Core Facility.*