miRNA Mediated Regulation of hOrai1 Expression

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Orai1, a calcium channel functions with STIM1, a calcium sensor to mediate calcium influx. Orai1 is at the plasma membrane and STIM1 localizes in a diffuse manner to the ER membrane under resting conditions. Following Ca++ store depletion, STIM1 forms puncta that localize to the cortical ER and binds Orai1 to allow Ca++ influx. This is the predominant pathway for Ca++ influx in non-excitable cells and is referred to as Store-Operated Calcium Entry (SOCE). Mutations in STIM1 and Orai1 cause severe combined immunodeficiencies and are linked to several forms of cancers. Tight regulation of the levels of members of Ca++ signaling pathways is crucial for maintaining the subcellular levels of Ca++ required for its numerous functions.

We are interested in the mechanisms that regulate some of the key players of the SOCE pathway. We have developed a system that allows us to test whether these genes are regulated by miRNAs in different cell types at different stages of the cell cycle. We are using normal human cell lines vs. human cancer cell lines to elucidate different mechanisms of regulation of these genes.

We used a GFP and the 3'UTR of hOrai1 as a transcriptional fusion to transfect HEK 293 cells and GFP without any 3'UTR as a control. As an internal control of transfection efficiency we expressed mCherry from the same vector in both conditions. We found the stoichiometry of GFP and mCherry levels in the experimental conditions for hOrai1 3'UTR to be significantly different from those in the control indicating a miRNA-mediated regulation of hOrai1. We also found the ratio between GFP and mCherry to be constant throughout the cell cycle in the control and variable in the GFP hOrai1 3'UTR transfected cells suggesting a cell-cycle dependent regulation of hOrai1. We are taking a similar approach to determine whether hSTIM1 is regulated by miRNAs.

Intra and Extra Cellular Loops of hENT1 are Dispensable for the Transport of Adenosine and Uridine

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Background: Nucleoside transporters play an important role in regulating the extracellular concentration of Adenosine and salvaging nucleosides. They also play an essential role in the transport of anticancer and antiviral drugs. Nucleoside transporters have been classified into families known as Equilibrative (ENT) and Concentrative (CNT).

Objective: ENTs belong to the SLC 29 transmembrane protein family, with 11 transmembrane domains with intracellular N-terminus and extracellular C-terminus. In order to localize to the plasma membrane they must undergo correct processing, targeting and trafficking. Constructs were designed where the intracellular and extracellular loops were deleted and used to test the efficiency of Adenosine and Uridine transport in Xenopus laevis oocytes using radiolabelled substrates.

Methods: Stage VI Xenopus oocytes were injected with 23ng hENT1 RNA, and incubated for 48 hrs at 18 degrees. 5 uM 14C-labeled Adenosine and Uridine were added to the tube containing 5 oocytes, and incubated for 1-60 min at different time intervals. Concentration dependent study was carried out at different concentrations of Uridine ranging from 100uM - 4mM. The oocytes were washed 6 times in transport buffer and lysed with 1% sodium dodecyl sulfate and counted in a Liquid scintillation counter.

Results and conclusion: Confocal images confirmed the ENT1 protein localization to the plasma membrane. The results of the time-dependent study showed that the deletions were able to transport Adenosine and Uridine, while the concentration dependent study showed no major variation in the Km for the substrates, confirming that the extra and intracellular loops in hENT1 are not essential for the transport of neither Adenosine nor Uridine.