

1st PROGRESS REPORT

PROJECT TITLE: Functional Characterization of Cystinosin – LKG

PRINCIPAL INVESTIGATOR: Francesco Emma, M.D.

CO-PRINCIPAL INVESTIGATOR: Anna Taranta, Ph.D.

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The main goal of this project is to increase our knowledge on a new cystinosin isoform, cystinosin-LKG which we have recently described (Taranta et al, Am J Physiol, 2008). We have observed that the cystinosin-LKG intracellular expression is not restricted to the lysosomal compartment. To study the functional role of this isoform we have generated a stable MDCK cell lines, overexpressing cystinosin-LKG or cystinosin conjugated to GFP protein.

Co-localization studies were performed using 24 antibodies, which mark different cell compartments. Cystinosin-LKG co-localizes with the (pre)-lysosomal marker LAMP2. In addition, LAMP2-positive multivesicular bodies were observed in the perinuclear region by immunofluorescence and electron microscopy. These structures resembled autophagosomes, had a “zebra structures”, and were not observed in MDCK cells overexpressing cystinosin conjugated to GFP protein. At the moment, we don't know the cell mechanisms that cause the formation of these structures and further studies are in progress.

Among other markers that have been analyzed, kinesin partially co-localizes with cystinosin-LKG. As a mechanochemical protein, co-localization with kinesin raises the hypothesis that cystinosin-LKG interacts with molecular motors and that intracellular trafficking may be compromised in cystinosis, causing Fanconi syndrome.

Other preliminary data show increased expression of cystinosin-LKG at the cell surface in fast growing non confluent MDCK cells, further indicating functional regulation of the expression of this isoform in the plasma membrane. In polarized MDCK cells, partial localization of cystinosin-LKG was observed in basolateral membrane.

As part of the CRN project, we are generating mutated CTNS-LKG clones to study the potential role of cystinosin-LKG phosphorylation in regulating the expression of the protein.