University of Cyprus
Department of Biological Sciences

Ph.D. Thesis Defense

Student Presentation

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This seminar is open to the public via Zoom at the following link:
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"Functional characterization of the Endoplasmic Reticulum and Nuclear Envelope transmembrane protein TMEM147 in HeLa cells"

The Endoplasmic Reticulum (ER) is the largest membrane-bound cytoplasmic compartment with diverse functionality, including protein synthesis and processing, calcium storage, and lipid and cholesterol biosynthesis. It comprises the nuclear envelope (NE) and the peripheral ER, further divided to the distinct subdomains of flat cisternae sheets and the tubular network. Transmembrane protein TMEM147 has been implicated in muscarinic receptor 3 (M3R) trafficking, in the stabilization of the Nicalin-NOMO protein complex in nodal signaling, and in the activation of NF-κB transcription factors.

In this work, we report TMEM147 as a resident protein of the ER and NE membrane in HeLa cells and describe its physical and functional association with the NE protein lamin B receptor (LBR). We find that downregulation of TMEM147 negatively regulates both gene expression and protein levels of LBR and disrupts its proper inner nuclear membrane (INM) localization, causing its dispersion to the peripheral ER membranes. Silencing of TMEM147 is accompanied by further changes in ER membrane domain organization and changes in nuclear shape and loss of chromatin compaction as well as decreased cell viability. LBR is structurally and functionally a modular protein. Its N-terminus protrudes to the nucleoplasm and is known to be involved in anchoring and organizing heterochromatin, while its C-terminus in the INM extends towards the cytoplasm and hosts a cholesterol reductase activity that we document as essential for the interaction with TMEM147. We determined that DHCR7, another key sterol reductase, catalyzing cholesterol synthesis, also interacts with TMEM147 and, similar to LBR, is downregulated upon TMEM147 depletion at both protein and gene expression levels. Our proteomic analysis of the TMEM147 interactome also revealed an interaction of TMEM147 with sterol reductase TM7SF2, whose gene expression however we found not to be co-ordinated with that of TMEM147. Here we also report that silencing of TMEM147 alters the lipidomic profile of the cells, including total cholesterol levels and cholesteryl ester species and its depletion also modulates the receptor-mediated uptake of cholesterol by cells. Addition of exogenous cholesterol in TMEM147-silenced and lipid starved

cells rescued cell viability and growth while it had a modest ameliorating effect on control cells. Taken together, our findings illuminate TMEM147 as a new likely regulator of cholesterol homeostasis in human cells.

Complementary to this work, we conducted a protein network and pathway analysis of a manually curated list of TMEM147 interactors, co-factors and modulators. Pathway analysis identified four major pathways including G protein-coupled receptor activity, ribosome binding, oxidoreductase activity, and transmembrane transport activity, while protein network analysis identified hub proteins of these corresponding pathways. Association of identified pathways and network hub proteins can be regarded as future research pointers to further TMEM147 functional studies.