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INTERSECTIONS BETWEEN ALPHA-SYNUCLEIN AND CHOLESTEROL: AN UNSOLVED CASE

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A pathological sign of synucleinopathies, including Parkinson's disease, is the aggregation and fibrillation of α-synuclein (α-syn), a small presynaptic protein characterized by a high lipid binding affinity. How α-syn and cholesterol are interconnected in the context of these neurodegenerative disorders remains an open question. In this work, we investigated cholesterol homeostasis in a neuronal model of α-syn overexpression (WT α-syn cells). We found that both free cholesterol and cholesteryl ester levels were increased by α-syn overexpression. The raise in cholesteryl esters was associated with an increased acyl-CoA:cholesterol acyltransferase activity. While cholesteryl esters were part of the lipid droplet core in WT α-syn cells, free cholesterol was located in membrane compartments and lysosomes. Co-staining experiments revealed that cholesterol was accumulated in lysosomes in cells overexpressing α-syn, though the distribution of these organelles and the expression of lysosomal markers, LAMP-1 and Lysotracker, were not altered. In order to determine the mechanism for the increased cholesterol levels, the status of the transcription factor sterol regulatory element-binding protein (SREBP)-2 was evaluated. SREBP-2 nuclear translocation was induced by α-syn overexpression, in agreement with the *in silico* analysis carried out through the MyProteinNet server (Yeger-Lotem lab). The latter showed a relationship between α-syn and sterol regulatory element-binding gen (SREBF-2). Paradoxically, the activation of SREBP-2 was not accompanied by the expected upregulation of involved canonical downstream genes in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and 24-dehydrocholesterol reductase. Moreover, cholesterol content was not altered by the inhibition of HMGCR by mevastatin in WT α-syn cells, thus implying that cholesterolgenesis was not responsible for its increase. Our findings suggest that α-syn overexpression disrupts cholesterol trafficking resulting in the lysosomal sequestration and consequent ER transport impairment. Further studies should be performed to ascertain the mechanisms and functional implications of cholesterol uptake and intracellular trafficking alterations associated with synucleinopathies. *Funding: ANPCyT, CONICET, and UNS.*

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IMPLICATION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2 (S1PR2) IN DIFFERENTIATION AND DEDIFFERENTIATION OF EPITHELIAL RENAL CELLS

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Epithelial cell differentiation is a process that involves the mesenchymal-epithelial transition (MET) and includes cell cycle arrest, cell-cell junction maturation in addition to changes in cell migration capacity. The epithelial-mesenchymal transition (EMT) is a dynamic process by which fully differentiated epithelial cells can acquire a mesenchymal phenotype. During EMT, cell adhesion and apical-basal polarity are lost, and the cytoskeleton is reorganized. Previous results from our laboratory showed that in Madin-Darby canine kidney cells (MDCK) under different culture conditions can achieve different stages of differentiation resembling MET. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid, produced by the phosphorylation of sphingosine by sphingosine kinases (SKs), which is involved in different processes such as proliferation, cell growth, differentiation, and migration. S1P can act both intracellularly as a second messenger or extracellularly as a ligand of 5 different G protein-coupled receptors (S1PR1-5). In the present work, we evaluated the importance of S1P acting on S1PR2 in the modulation of MET and EMT. We found that there are differences in the action of S1PR2 in MDCK cells that depends on the differentiation stage. S1PR2 positively modulates the passage from polarized to differentiated cells through MET. Inhibition of S1PR2 blocks adherens junction establishment, as well as apical and basal polarity. On the other hand, once cells have acquired the differentiated phenotype, S1PR2 induces the dedifferentiation of epithelial cells through EMT. Inhibition of S1PR2 triggers changes in EMT markers, such as rearrangements of the actin cytoskeleton, expression of vimentin, and nuclear translocation of beta-catenin, as well as Slug. The expression levels of S1PR2 in the different stages of differentiation of MDCK cells did not show significant differences. Instead, immunofluorescence studies showed that during cell differentiation, S1PR2 was progressively enriched at the plasma membrane. These results suggest that the location of S1PR2 depends on the stage of cell differentiation, and this determines its role. These findings highlight the great versatility of S1P on the control of physiological and pathophysiological processes.

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ENDOGENOUSLY SYNTHESIZED SPHINGOSINE-1-PHOSPHATE TRIGGERS CELL EXTRUSION IN MDCK CELLS

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One of the mechanisms that ensure epithelial integrity is cell extrusion, a process to remove dying or surplus cells while maintaining the epithelium barrier. This process is triggered by sphingosine-1-phosphate (S1P), which activates S1P receptor