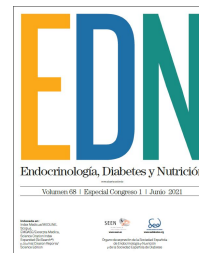




Endocrinología, Diabetes y Nutrición



CO-017 - THE ROLE OF TSC2 ACETYLATION AND ITS SUBCELLULAR LOCALIZATION IN MITOCHONDRIAL TURNOVER OF PANCREATIC β CELLS

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Resumen

Introduction: Eukaryotic cells need to regulate cell growth by the presence of growth factors and a correct sensing of nutrients such as glucose and aminoacids. An alteration in any of these factors generates a dysfunction in cell maintenance and is associated with different diseases such as type 2 diabetes mellitus (T2DM). One of the main controllers of cell size and cell proliferation is the mechanistic target of rapamycin complex 1 (mTORC1). Acetylation is a post-translational modification found in a high variety of proteins, altering its function and stability. Depending on the protein, the introduction of the acetyl group can activate or inactivate its catalytic activity. Our group has previously uncovered that TSC2 acetylation, inhibited its activity and hence, stimulated mTORC1 signaling pathway and downregulated autophagy processes, with important consequences in pancreatic β cells.

Objectives: The main objective is the study of TSC2/mTORC1/p70S6K pathway and the role of TSC2 acetylation in the regulation of pancreatic β cell expansion and viability, focusing on the implication of autophagy and mitochondrial turnover to understand the progression to Type 2 Diabetes Mellitus *in vitro*.

Material and methods: We used different cell lines generously donated by either David Kwiatkowski (MEF TSC2 WT and KO) or Leonard Guarente (MEF SIRT1 WT and KO). Furthermore, we have generated stably transfected MIN6 with either scrambled or TSC2 and SIRT1 shRNA in our laboratory. To perform our studies, cells were treated with different drugs: resveratrol (50 μ M), a sirtuin activator; nicotinamide (5 mM) as a negative control of resveratrol; acetyl CoA in different doses; chloroquine (20 μ M) to block autophagy flux and CCCP (20 μ M) as a mitophagy inducer. Different experiments were performed in fibroblasts (MEF) and pancreatic β cells (MIN6) with a knock out of TSC2 and SIRT1 proteins

Results: We have determined that TSC2, in its unacetylated form due to resveratrol action, can be recruited to the lysosomal membrane, where it inhibits mTORC1 signaling pathway in MEF and in pancreatic β cells. This mechanism of TSC2 doesn't work when TSC2 is hyperacetylated at the basal state using MEF SIRT1^{-/-} cells or β cells with a knock down of SIRT1. In this regard, when we pretreated the cells with acetyl-CoA, we reverted the effect on mTORC1 signaling by resveratrol in a dose-dependent manner. Furthermore, we have observed that resveratrol can activate mitophagy in both TSC2- and SIRT1-dependent manner.

Conclusions: Collectively, our data indicate that resveratrol, by facilitating TSC2 recruitment to the lysosome, is able to inhibit mTORC1 and inducing autophagy. In addition, we have observed a potent activation of mitophagy under these conditions, facilitating mitochondrial turnover, depending on both TSC2 and SIRT1 proteins.