UNIVERSITÀ
DEGLI STUDI
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MERRY - Exploring mitochondria-endoplasmic reticulum juxtaposition regulation by protein palmitoylation

Endoplasmic reticulum (ER) physically contacts with mitochondria with its specialized subdomain, mitochondria ER contacts (MERCs). MERCs play important roles in lipid and calcium transfer and serve as a reaction hub for insulin signalling. In the previous study, we revealed that the functional MERCs are decreased by 3-hour treatment of free fatty acid (FFA), resulted in impaired insulin response. Therefore, during this fellowship, I will elucidate the mechanism how FFA disrupts MERCs focusing on the posttranslational modification. Since FFA repress the protein palmitoylation, I will explore the novel MERCs regulators from MERCs-localized palmitoylated proteins. First, I will perform comparative proteomic analysis combined with MERCs fractionation and purification of palmitoylated proteins. For the proteins which will be less found in MERCs-localized palmitoylated fraction of FFA-treated cells than that of vehicle-treated cells, I will introduce point mutant to the palmitoylated cysteine of the proteins with CRISPR-Cas9 system and establish their mutant cell lines. The effect of that “nonpalmityolated mutants” on MERCs formation will be evaluated with electron microscopy, calcium flux analysis and FRET-based indicator of ER–mitochondria proximity (FEMP) system, developed by the host laboratory. Moreover, I will assess the impact of the mutants on insulin response and elucidate whether palmitoylation of the proteins affects insulin response. By these analyses, I will reveal how FFA disrupt MERCs formation through the alteration of palmitoylation status. This project will be the first report that reveal the molecular mechanisms how physiological stimulus can affect MERCs formation. This achievement will open the possibility for the analysis of MERCs in not only the congenital hereditary diseases but also the acquired diseases, such as metabolic disease.