Analysis of cholesterol export from purified endosomes in NPC cellular models

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LAY SUMMARY

At the cellular level, the Niemann-Pick type C disease is characterized by the accumulation of cholesterol and other lipids in particular cellular organelles, called late endosomes and lysosomes. Even if the genes (npc1 and npc2) involved in the pathology are known, the molecular mechanisms by which these proteins mediate the export of cholesterol (and possibly other lipids) from late endosomes/lysosomes remain unclear.

The main goal of my research project is to characterize *in vitro* the mechanism of cholesterol export from purified endosomes and to elucidate of the role of the molecular players in the process, including NPC1 and NPC2 proteins with the aim to clarify the direct role of these proteins in cholesterol export from the organelles.

The previous fellowship period was dedicated to the set up and to validate an in vitro assay to measure export of cholesterol from endosomes. I used this assay to compare the kinetics of export in endosomes purified from NPC model cells (NPC1 or NPC2) and I did not observe any difference in the kinetics of cholesterol export in NPC cells compared to control cells. These results indicate that at least on isolated organelles, NPC1 and NPC2 proteins seem not directly implicated in cholesterol export.

I am now performing lipidomics analysis of sub-cellular fractions by mass spectrometry. This type allows the determination of the presence and relative abundance of all lipid species in organelles from NPC model cells.

I am also performing experiments in order to clarify the mechanism of cyclodextrin action at the level of endosomes, specifically focusing of the function of the endosome-specific lipid lysobisphosphatidic acid (LBPA), which is crucial for cholesterol traffic in the endosomes. Present experiments indicate that HPCD treatment strongly modulates LBPA content of endosomes and we are currently investigating the functional significance of these changes in lipid composition and its relevance on HPCD action in NPC cells.

We expect that our project will contribute to a better characterization of the molecular processes at the basis of the disease and the mechanism of action of potentially efficient treatments, like cyclodextrin, and become helpful in the search of new targets for pharmacological intervention.