Analysis of cholesterol export from purified endosomes in NPC cellular models

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LAY SUMMARY

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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. The genes (npc1 and npc2) and consequently the proteins involved in the pathology have been identified, and yet 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 mostly dedicated to the set up and to validate an in vitro assay to measure export of cholesterol from endosomes. In the last six months period I used this assay to compare the kinetics of export in endosomes purified from NPC model cells (NPC1 or NPC2) or cells treated with drugs that mimic NPC (U18666A). 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.

We are also 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. After preliminary experiments that were performed in the previous semester, we now completed the lipid analysis of sub-cellular fractions in NPC1 and NPC2 model cells.

We expect that our project will contribute to a better characterization of the molecular processes at the basis of the disease and therefore become helpful in the search for targets of pharmacological intervention.