

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. 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 first six months of the fellowship were mostly devoted to the setting up of a Niemann-Pick C cellular model (based on siRNA-mediated silencing of NPC1 or NPC2 proteins in cultured cells) and setting up of an *in vitro* assay to measure export of cholesterol from endosomes purified from these cells. At present, I did not observe any difference in the kinetics of cholesterol export compared in NPC cells compared to control cells. The physiological relevance of these *in vitro* results is currently under investigation.

In order to combine studies on cholesterol dynamics with a general analysis of the lipid composition of cells and endosomes, 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. Up to date analysis has been performed in control BHK cells and in cells treated with a drug called U18666A (that mimics NPC in cultured cells). We are currently performing experiments on endosomes from cells in which NPC1 and NPC2 proteins have been selectively silenced.

This research project is aimed to characterize the molecular processes at the basis of the disease at the cellular and sub-cellular level convinced that a better knowledge of molecular players in the establishment of the pathology will be helpful in the search for targets of pharmacological intervention.