

NNPDF-Funded Research Fellowship # 3

TITLE: Demonstration of NPC1 as a lipid binding protein in Vitro
PROJECT INVESTIGATOR: Nobutaka Ohgami, Ph.D.

PERIOD: 9/15/2004 - 9/14/2006

PROJECT DESCRIPTION

In this application, I propose to test the possibility that NPC1 may directly bind cholesterol and/or other lipid, this study aims at performing photoaffinity labeling of NPC1 with photoactivatable analogues of cholesterol, fatty acids, and sphingolipids. In preliminary studies, I showed that there is direct binding between NPC1 and cholesterol by performing intact cell photoaffinity labeling of cells expressing green fluorescent protein (GFP)-tagged NPC1 with 3H-azocholestanol (AC); the binding does not require NPC2 but requires a functional sterol-sensing domain (SSD) within NPC1. In addition, after partial purification of NPC1-(6xHis)GFP protein with the one round of nickel column chromatography, I showed that partial purified NPC1-GFP protein was specifically cross-linked by 3H-AC in vitro. Furthermore, the result showed that several other proteins with much lower M.W. as well as NPC1 were cross-linked by 3H-AC, suggesting that the protein bands with lower M.W. may consist of NPC2, caveolin-1, and other protein(s) that bind cholesterol specifically. Based on these results, I propose the following 3 specific aims: (1) Does AC bind to the NPC1 within the sterol-sensing domain of NPC1? (2) Does NPC1, NPC2, and/or caveolin-1 form a protein complex in vitro? (3) Does NPC1 protein also bind fatty acids, and/or sphingolipids? Is the in vitro cross-linking between NPC1 and AC affected by the presence of other ligands such as fatty acids or glycosphingolipids? I hope that the outcome of this proposal will help to understand the function of NPC1 protein at the biochemical level.

Final STATUS REPORT

Dated 12/31/2007

Title: Demonstration of NPC1 as a lipid binding protein in Vitro December/30/2007 Nobutaka Ohgami (Current lab: Dept. of Biomedical Sciences, Chubu University, JAPAN : Former lab: TY Chang's laboratory)
Lay summary This present study aims at testing the possibility that NPC1 may directly bind cholesterol and/or other lipid. In vitro (cell free) photo-crosslinking study with the photoactivatable cholesterol analog (azocholestanol: AC) demonstrated that drosophila NPC1 can bind to cholesterol just like mouse NPC1 that has been using for my photo-crosslinking experiments so far. In order to identify a binding site within the SSD of NPC1 protein directly binding to AC, I tried to establish the efficient isolation system to separate the AC-labeled fragments with the biotinylated theta-toxin (BC-theta), which has been known to be the cholesterol-binding toxin. But unfortunately, it did not worked for the isolation purposes, although it did work for the detection of the intracellular cholesterol still as previously reported. Finally, intact cell photo-crosslinking experiment show that the association between 3H-AC

and NPC2 in cells with or without added NPC1 is approximately equal, suggesting that binding between AC and NPC2 does not require NPC1.

PUBLICATIONS:

<http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.cellbio.22.010305.104656>