Inhibition of Rab prenylation by statins induces cellular glycosphingolipid remodeling

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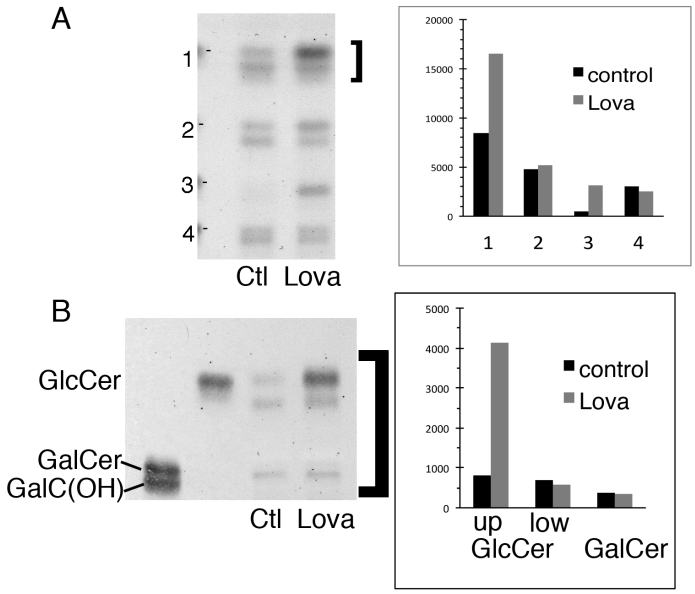
SUPPLEMENTAL METHODS

Qualitative RT-PCR. RNA was extracted from statin-treated and control cells using Trizol. cDNA was reverse-transcribed from 2 μg of DNase I-treated RNA using Superscript III (Invitrogen). PCRs using the primers listed below were performed for the following transcripts: UGCG (GlcCer synthase), BGalT5 (LacCer synthase), BGalT6 (LacCer synthase), BGNT5 (Lc3Cer synthase), PLEKHA8 (Fapp2). Cycle number was below saturation as determined by amplification of serially diluted template.

UGCG_fwd: TATCATCTACACCCGATTACACCTC (529 bp)
UGCG_rev: GAAACCAGTTACATTGGCAGAGATA
BGalT5 fwd: GGCGCCCGGCATAGTGAACA (430bp)
BGalT5 rev: GAGGTGCTCGTGGCGGTTCC
BGalT6 fwd: GCAGCGGCTGGAATTTGCGT (784 bp)
BGalT6 rev: CAGAGCAGGGAGGACGGGTGA
BGNT5_fwd: GGGCAATGAAAATTATGTTCGGTCT (335 bp)
BGNT5_rev: CACGACCAATCCAAAAGTCTTGAAC
GAPDH_fwd: AACTTTGGTATCGTGGAAGGACT (292 bp)
GAPDH_rev: GCTTCACCACCTTCTTGATGTC
PLEKHA8_fwd: CTATTATGATTCTCCTGAAGATGCC (231 bp)
PLEKHA8_rev: TCTTTCTCCTTCTGGGTCCTACT

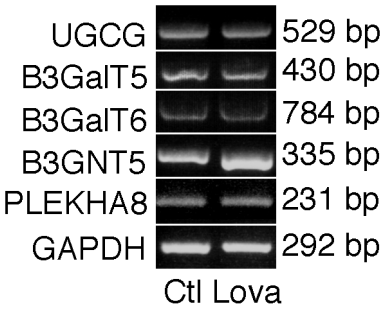
SUPPLEMENTAL FIGURES

Supplemental Figure 1



Analysis of ceramide monohexosides (CMH) of PC-3 Cells. (A) Neutral GSL extracts from vehicle or 10μM lovastatin-treated PC-3 cells were separated by silica TLC in $CHCl_3$:MeOH:H_2O (65:25:4). The bracket on the right indicates the CMH region. Numbers on the left represent migration of GSL with 1 to 4 carbohydrate groups, corresponding to standards GlcCer, LacCer, Gb_3, Gb_4. Intensities of orcinol stained bands were measured using Image J software. (B) Extracts in A were run on sodium tetraborate-impregnated silica TLC plates (same solvent as in A) to separate GlcCer and GalCer. GalCer represented about 20% of the total CMH fraction in untreated cells.

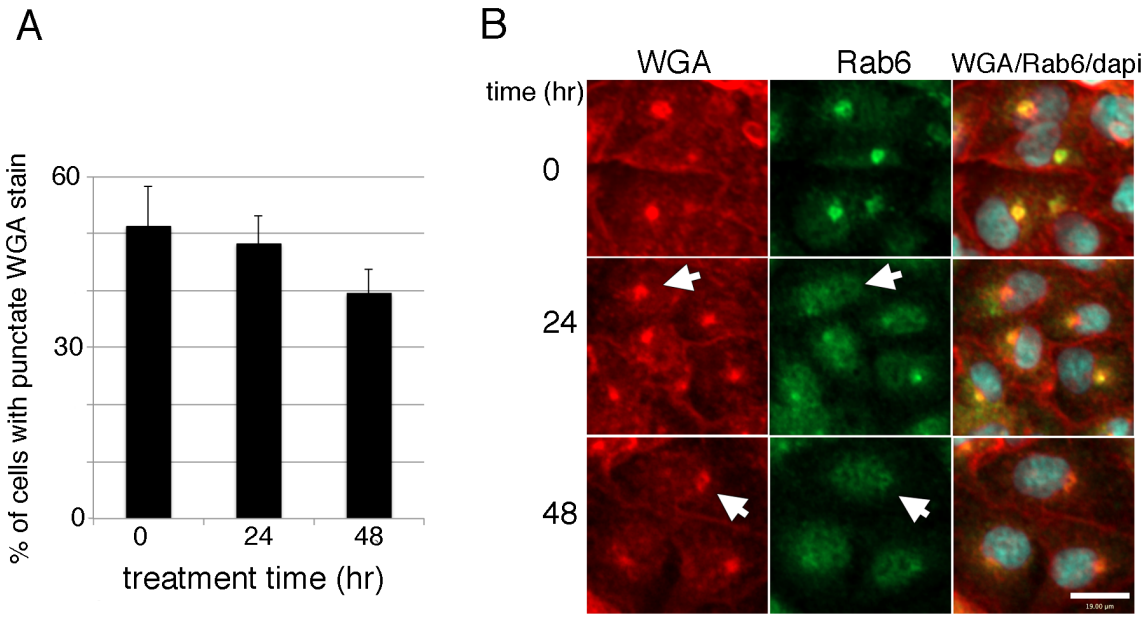
Supplemental Figure 2



Qualitative RT-PCR to detect changes in GSL synthase transcript levels. Changes in transcript levels in PC-3 cells were evaluated by reverse transcription PCR. RNA was collected after 48hr vehicle or 10μM lovastatin treatment (0.1% final DMSO dilution) and processed as described in the Supplemental Methods section. Transcript for B3GNT5 (encoding Lc3Cer synthase) was elevated at this time point however no change was

observed in the transcript levels for GCS, either LacCer synthase, or Fapp2 which is involved in downstream GlcCer metabolism.

Supplemental Figure 3.



Characterization of TGN in statin treated cells by fluorescence microscopy. ACHN cells were treated with 10µM rosuvastatin as indicated then fixed, permeablized with 0.2% Triton X-100 and labeled with Alexa594-WGA or rabbit anti-Rab6. (A) Punctate TGN staining by WGA was quantified and expressed as percentage of total cells, identified by dapi-stained nuclei; Control (n= 10 fields, 325 cells), 24hr lovastatin (n=14 fields, 392 cells), 48 hr lovastatin (n=15 fields, 464 cells). Error bars represent SEM. (B) Cells having intact TGN-WGA staining but having lost TGN association of Rab6 (presumably due to prenylation block) are shown at 24hr and 48hr (arrows). Scale bar=16µm. Double labeling of the TGN by filipin and WGA was not possible as filipin did not permeablize the TGN sufficiently for labeling of luminal glycoproteins by WGA.