Alternative Mechanism Behind Low-density Lipoprotein Internalization in Endothelial Cells

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Background

- Cholesterol is an essential compound for all living organisms
- Cholesterol produces steroid hormones, helps form bile, and is incorporated into cell membranes
- Build up within walls of arteries leads to atherosclerosis
- Cholesterol is carried in low-density lipoproteins (LDL) in blood
- LDL internalized into cells by LDL receptor
- Past research ties LDLR uptake exclusively to clathrin mediated endocytosis (CME)
- NPXY signal sequence on LDLR tail is important in CME
- Preliminary findings suggest LDLR can be internalized via another pathway, known as the caveolin pathway and that internalization can be performed independent of NPXY

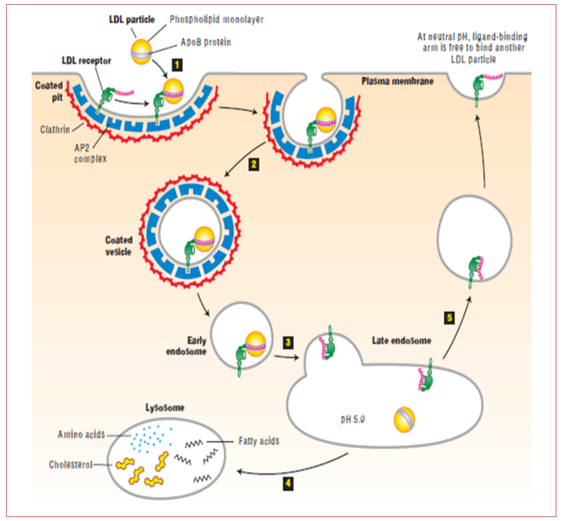

Figure 1. Diagram shows the internalization of low-density lipoprotein via the clathrin mediated endocytosis transport pathway.

Objectives

- Determine what specific signal sequence within LDLR is responsible for its internalization when it is not aided by the clathrin pathway
- Define how the unidentified signal sequence targets the caveolin pathway for LDLR

Methods

Two forms of LDLR sequences → PCR-based truncations w/ synthesized primers → Strata-cloning using pSCA plasmids → Ligate into mammalian plasmids, pAD-CMV → Transfect into mammalian cells (HeLa) → Immunolocalization → Uptake Assay at 10 and 30 min intervals

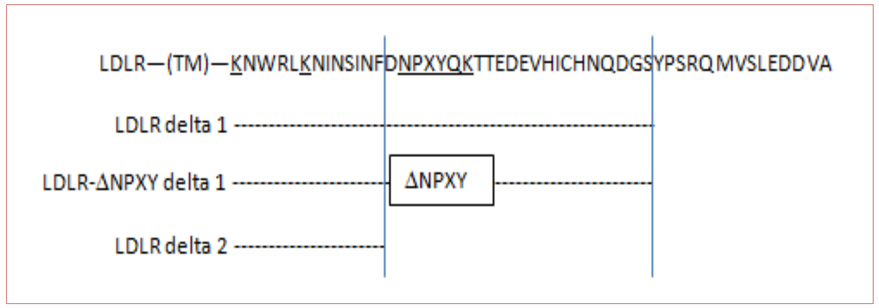

Figure 2. Diagram of PCR generated truncations made from synthesized primers used in experiment. Shows region thought to play a role in internalization via the caveolin pathway.

Results

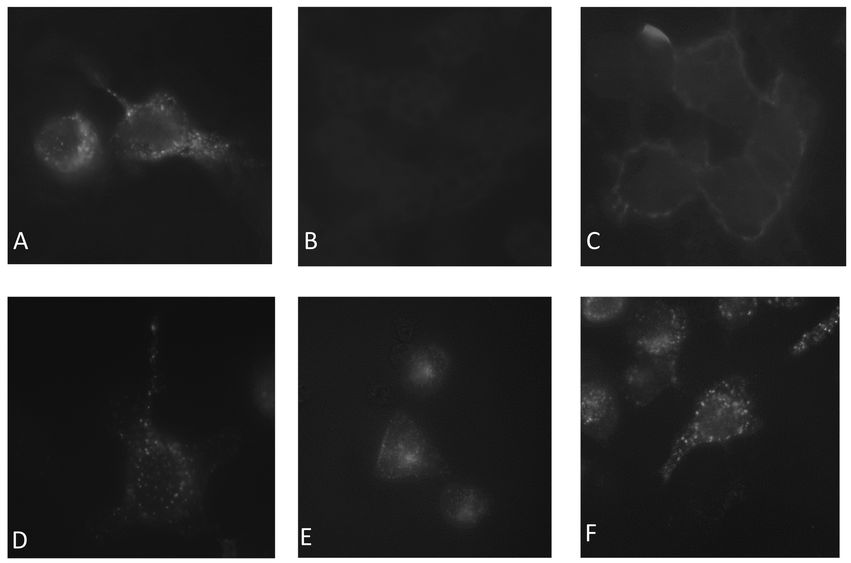

Figure 3. Internalization of LDLR mutants in HeLa cells through an uptake assay. LDLR mutants were tagged with 51.1 antibody and transfected into cells, before being visualized underneath a fluorescent microscope. A: CD8-LDLR (+), B: (-), C: CD8-8A, D: Delta NPXY, E: Delta NPXY SINF, F: Delta NPXY QDGYS

Results Cont.

- Delta NPXY was expressed in mammalian cells
- Strains with SINF and QDGYS truncations were successfully expressed in mammalian cells.
- Donut-shaped endosomes found in cells containing delta NPXY, delta NPXY SINF, and delta NPXY QDGYS

Discussion

- LDLR without NPXY still gets internalized
- SINF and QDGYS truncations internalized into cells (immunolocalization/uptake assay)
- Sequence controlling for internalization, in absence of NPXY, is not where truncations were made
- Deletion of NPXY from LDLR sequence results in LDLR sorting defect within cell (donut-shaped endosomes)

Future Direction

- Perform further truncations closer to the transmembrane domain of the LDL receptor to determine what sequence is important for internalization when not aided by clathrin pathway
- Test LDLR truncation expression using immunoblot
- Conduct further experiments to determine the cause of the sorting defect found when NPXY is deleted from LDLR by targeting the three functions of uptake 1)short term storage 2)long term stage 3)degradation

References

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2. KANG Y.L., YOCHEM J., BELL L., SORENSEN E.B., CHEN L. & CONNER S.D. 2013. Caenorhabditis elegans Reveals a FxNPxY-Independent LDLR Internalization Mechanism Mediated by Epsin1. Mol Biol Cell 24: 308-318.
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