Exploring the Membrane Lipid Environment for Glucose Transporter 1

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Background

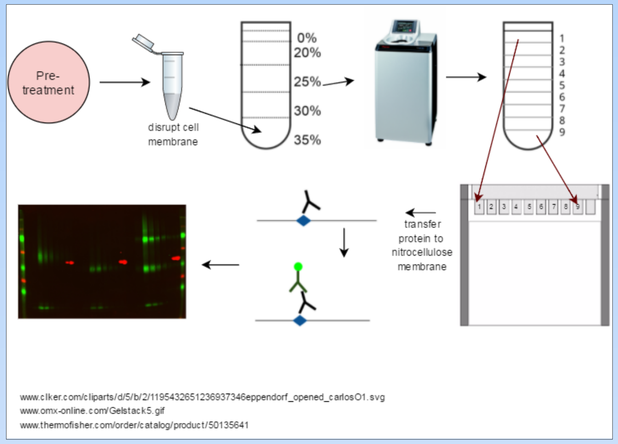
- Glucose is a preferred cellular metabolite and it requires a membrane transport protein, such as GLUT1, to enter cells.
- Abnormal glucose regulation is linked to serious diseases, including cancer, diabetes, and Alzheimer's disease. A deeper understanding of this protein may be important for developing new therapeutic strategies.
- The activity of membrane proteins are influenced by their lipid environment. This study focuses on understanding the association of GLUT1 with compact membrane microdomains called lipid rafts.
- Previous work suggests that lipid rafts play only a subtle role in the regulation of GLUT1 activity. In addition, the GLUT1-containing lipid rafts in L929/EGFP mouse fibroblast cells appeared to be an unusual type of raft.
- The purpose of my research this summer was to investigate the nature of this raft-like structure, and what targets GLUT1 to these domains.

Objectives

1) Determine the composition of the raft-like domains in L929 cells
2) Determine what post-translational modifications might alter GLUT1 membrane location
3) Confirm that my procedures also isolate traditional lipid rafts in other cell lines

Methods

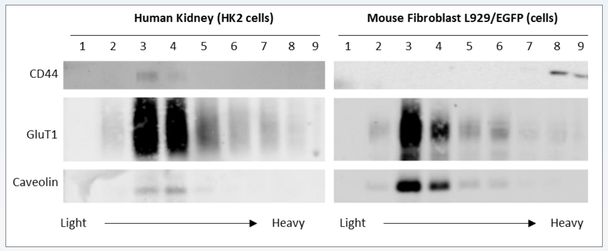
Lipid raft isolation technique



1) Cells are pre-treated
2) Cells are collected and lysed
3) Membrane is broken apart and loaded in an ultracentrifuge tube that has denser liquid towards the bottom
4) Ultracentrifuge is spun; lipid rafts float towards the top due to the lipid that remains associated with the protein
5) Western blot is performed to determine relative GLUT1 amount in each successive density fraction
6) GLUT1 distribution in treatment and control is compared

Results

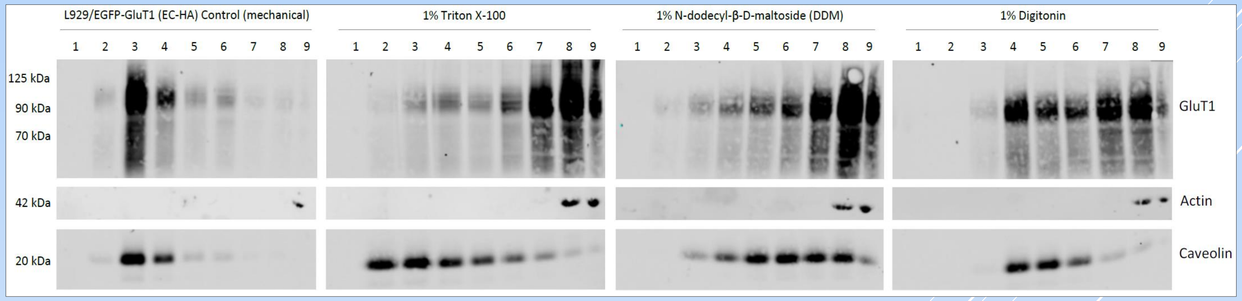
L929/EGFP mouse fibroblast cells do not contain common raft protein markers in the lipid raft fraction



- Both GLUT1 and caveolin (a protein found in a type of low density domain) are in the raft fractions
- CD44, a common raft marker, is still found in raft fractions in HK2 cells (as expected) but pellets down in L929/EGFP cells

Other isolation methods don't capture GLUT1 in low density fractions

Mild detergents are a common way of isolating lipid rafts. However, only mechanically disrupting the cell membrane isolates GLUT1 in low density fractions, suggesting that the low density microdomains in L929/EGFP cells are of unique composition.



Attempts to cause GLUT1 shift in order to investigate composition of domains, or determine what targets GLUT1 to these domains

Drug/treatment	Effect of drug/treatment	Implications	GLUT1 shift?
Triton X-100, DDM, Digitonin	Detergents; permeabilize cell membrane	Is GLUT1 in traditionally isolated lipid rafts?	**yes**
Myriocin	Inhibits sphingolipid synthesis	Is sphingolipid organization an essential component of L929 lipid rafts?	**slight**
Sphingomyelinase	Catalyzes breakdown of a type of sphingolipid	Is sphingolipid organization an essential component of L929 lipid rafts?	**slight**
M-β-CD	Removes cholesterol	Cholesterol helps organize traditional lipid rafts. Are L929 rafts cholesterol enriched?	**no**
Nocodazole	Inhibits microtubule formation	Is microtubule structure essential?	**no**
Latrunculin A	Disrupts actin cytoskeleton formation	Are GLUT1 low density microdomains tethered to the cytoskeleton?	**no**
2-Bromopalmitate	Inhibits palmitoylation	Does palmitoylation target GLUT1 to low density microdomains?	**no**
Kifunensine	Inhibits glycosylation	Does glycosylation target GLUT1 to low density microdomains?	**no**

Conclusions

1) GLUT1 translocation to lipid rafts is not the primary mechanism of activation
2) L929 cells contain a unique type of raft-like structure. Sphingolipids (but not cholesterol or actin) seem to be important in organizing these domains.
3) Neither palmitoylation nor glycosylation targets GLUT1 to L929 "lipid rafts"

Future Directions

- Explore more ways to disrupt these microdomains in order to determine their composition
- Inhibit other post-translational modifications of GLUT1 to try and determine why GLUT1 is in these raft-like structures
- Conduct these experiments on other cell lines

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