

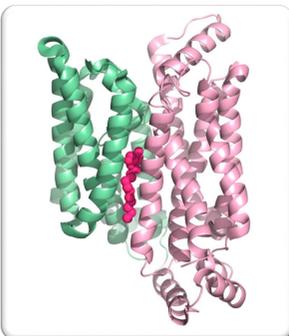
Exploring GLUT-1 Interactions in Human Cell Lines

Thomas Althaus, Eric Arnoys, Brendan Looyenga, Larry Louters, Calvin College

Introduction

GLUT-1 is a membrane-bound protein responsible for the basal uptake of Glucose into most tissues of our body.

"How Sweet! The Structure of the Glucose Transporter 1."
Crystallography365. N.p., 20 June 2014. Web. 29 Apr. 2017.



Abnormalities in this protein can contribute to serious diseases such as diabetes or cancer.

Looking at what factors affect GLUT-1's ability to transport glucose is crucial in understanding its role in cellular physiology and disease.

While there are several hypotheses for how GLUT-1 is regulated, our work this past summer focused on how surrounding proteins may play a role in its activity and presence.

Objectives

- Determine the best detergent for removing GLUT-1 from the membrane without affecting its interactions with other proteins
- Use CRISPR-Cas9 to knock-out potential GLUT-1 interacting protein candidates in HK2 Cell lines
- Use fluorescent imaging to look at GLUT-1 presence in membrane following CRISPR knockdown

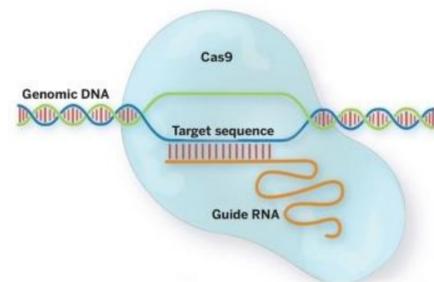
Methods

Western Blot Analysis

- Western Blotting is used to detect the relative amounts of specific proteins within a sample.
- In our study this method allowed us to detect levels of GLUT-1 isolated from detergent-lysed samples as well as confirm the knockout of target proteins from CRISPR.

CRISPR-Cas9 Genome Editing

- CRISPR-Cas9 is a form of gene editing technology which introduces specific mutations in a cell's DNA, knocking out production of a selected protein
- This allow us to edit out the genes of our proteins of interest, which would then possibly have an effect on GLUT-1



<http://cen.acs.org/articles/92/i39/Patent-Picks-CRISPR-Cas9-Gene-Editing.html>

Results

Different detergent types isolated variable amounts of GLUT-1 from the cellular membrane

- To look at the effectiveness of different detergent types we harvested cell lysates from HK2 cells using five types of detergents at varying concentrations. We then performed a Western Blot analysis on all twenty of our samples.
- Detergents TritonX-100 and Beta-d-maltoside appeared to have the greatest GLUT-1 isolation when compared to other detergents at similar concentrations (see Figure 3).

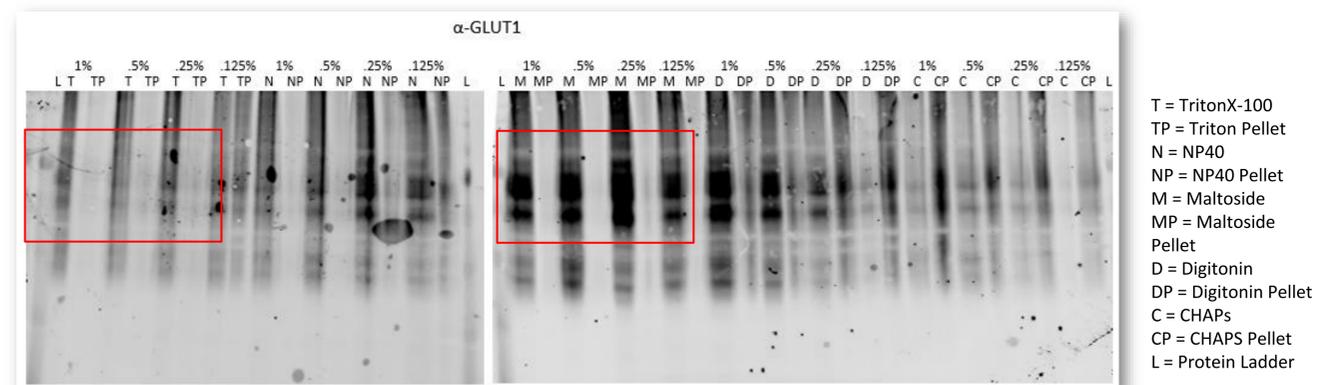


Figure 3 – Western Blot of GLUT-1 for all Five detergents:

The figure above shows GLUT-1 presence in all 20 sample lysates. At each concentration the sample lysate was compared with protein left in the 'pellet' (which is GLUT-1 that remained in the lipid membrane)

T = TritonX-100
TP = Triton Pellet
N = NP40
NP = NP40 Pellet
M = Maltoside
MP = Maltoside Pellet
D = Digitonin
DP = Digitonin Pellet
C = CHAPs
CP = CHAPs Pellet
L = Protein Ladder

CRISPR-Cas9 system resulted in a partial knockout of three of the four target proteins

- Western Blot analysis shows that candidate proteins Scribble, Cask, and TFRC all had variant levels of knockout in HK2 cell lines.
- While there is not evidence of a complete knockout, cell lines showing partial knockout can be selected for in the future for further experimentation.

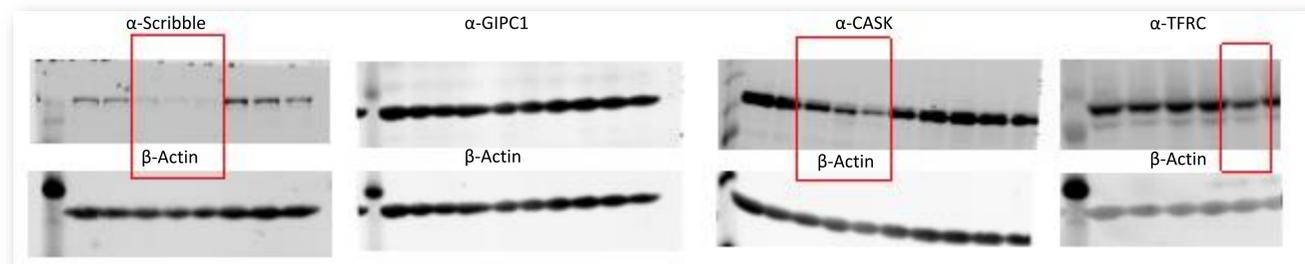


Figure 4 – Western Blot of Target Protein Knockouts

The figure above shows a combination of several experiments attempting to knockout proteins of interest in HK2 cell lines. The lanes highlighted by a red box indicate samples which underwent CRISPR editing for the protein observed. B-Actin serves as a control measure to confirm that empty lanes are not due to lack of sample

Conclusions and Future Directions

- When isolating GLUT-1 from the membrane, detergent type plays a large role. Lower concentrations can now be used for more effective detergents such as TritonX-100.
- We now have cell lines containing successful knockout of our target proteins. Future studies will focus on how the presence and activity of GLUT-1 changes as a result.

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