Introduction

Glucose Transporter Protein 1, or GluT1, is a ubiquitous protein in mammalian cells that transports glucose across the cell membrane by facilitated diffusion. GluT1 has cancer and diabetes implications due to the high activity of GluT1 in cancer cells and the need for increased glucose uptake for diabetic patients. Therefore, the mechanism of GluT1 activation could have many medical and biochemical implications.

Models of Activation

1. Oxidation of functional cysteines could activate glucose uptake by forming intramolecular disulfide bonds.

2. Oligomerization could activate glucose uptake by the formation of a multimer.

3. Lipid raft association could activate glucose uptake by GluT1 interactions with the surrounding lipid environment.

Methods

Gibson Synthesis

To explore the role of cysteines in activation of GluT1, Gibson synthesis was performed to mutate either the first three or the second three cysteines to serines. Point mutagenesis also targeted specific cysteine residues for further analysis.

Glucose Uptake Assays

To test GluT1 activation, radioactive glucose assays were conducted using activating and inactivating compounds explored by the Louters’ lab. Glucose uptake assays were conducted with various cell lines with hopes of using the cysteineless mutants generate by the Gibson syntheses mentioned above.

Results

Gibson Synthesis Results

DNA sequencing showed successful synthesis of the following vectors:
- GFP-GluT1(wt) with HA tag
- GFP-GluT1 with the first three cysteines mutated
- GFP-GluT1 cysteineless

TET-Inducible GFP-GluT1

To assist with mechanistic assays, tetracycline inducible cell lines of GFP-GluT1 were also investigated. A stable, TET-inducible cell line would eliminate the need for transfections of GFP-GluT1.

Subsequent western blots show an increase of GluT1 expression with increasing concentrations of TET.

Conclusions

Conclusion

Some Gibson synthesis DNA constructs were successfully synthesized. TET-inducible GFP-GluT1 cell lines have also proved successful as shown by glucose uptakes and western blots.

Future Work

Additional Gibson synthesis mutants will be cloned. Current Gibson synthesis mutants will be tested with glucose uptake assays and FRAP assays to determine whether or not activation has been affected. Mutagenesis DNA constructs may be used for further analysis. TET-inducible cell lines will also be further utilized for increased lab efficiency.

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