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Master's Thesis

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Master of Science in Molecular Medicine

Characterizing the Role of a GLUT1 Mutation in GLUT1-deficiency Syndrome

Presented by

Jingyuan Cheng

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First Evaluator: Professor Dr. Matthias Selbach

Second Evaluator: Professor Dr. Volker Haucke

Completed at AG Selbach, Max Delbrück Center for Molecular Medicine

Abstract

Glucose transporter-1 (GLUT1) deficiency syndrome is a genetic disorder characterized by impaired glucose transport into the brain. One of the clinically identified pathogenic mutations is a Pro485-to-Leu substitution located in the cytoplasmic carboxyl tail of GLUT1, whose pathogenic mechanisms remain unclear. A previous *in vitro* proteomic screen from our group revealed that this GLUT1 mutation leads to specific interactions with clathrins, which is supported by the further bioinformatic finding that the mutation creates a novel dileucine motif known to mediate clathrin-dependent trafficking.

In this study we used stable inducible HEK293 cells to further investigate the effect of the GLUT1 mutation on the intracellular localization and trafficking of the protein. We showed that the wild type GLUT1 mainly localizes to the plasma membrane, whereas the mutant GLUT1 mislocalizes to intracellular compartments and co-localizes with endocytosed transferrin, as well as early endosomal and late endosomal markers. Moreover, SILAC-based quantitative characterization of proximate proteins also identified increased proximate interaction of the mutant GLUT1 with proteins associated with endocytosis and endosomal compartments. Together, these data suggest that the GLUT1^{P485L} mutation causes internalization of the GLUT1 protein via clathrin-mediated endocytosis, thus leading to GLUT1 deficiency syndrome.

Contents

List of Figures

List of Tables

Introduction

As the primary glucose transporter across the endothelial cells of the blood-brain barrier, the facilitated glucose transporter member 1 (GLUT1) protein plays a central role in the regulation of brain energy metabolism and maintenance of central nervous system homeostasis [1]. Human GLUT1 is encoded by the *SLC2A1* gene on the short arm of chromosome 1 at position 34.2, consist of 492 amino acids and contains 12 transmembrane domains [2, 3].

many of which have been reported to be susceptible to missense mutation that causes GLUT1 deficiency syndrome (G1DS), a genetic disease characterized by hypoglycorrhachia (low glucose concentration in the cerebrospinal fluid), seizures and delayed neurological development [1, 4]. GLUT1 mutations in G1DS patients impair glucose transport into the brain across the blood-brain barrier, resulting in the disease phenotypes.

One of the clinically identified missense mutations in GLUT1 is a Pro485-to-Leu substitution ($GLUT1^{P485L}$) located in the cytoplasmic carboxyl tail [1, 5]. However, the molecular mechanisms by which the mutation causes the disease remains elusive. In a previous proteomic screen study to investigate the impact of disease-causing mutations, the $GLUT1^{P485L}$ mutation was found to lead to an increased binding of clathrins. Sequence analysis revealed that the mutation creates a dileucine motif known to mediate clathrin-dependent endocytosis ([D/E]XXXL[L/I]) in the cytoplasmic tail [6, 7].

Based on these findings, it is hypothesized that the $GLUT1^{P485L}$ mutation causes clathrin-mediated endocytosis and possibly subsequent degradation of GLUT1, leading to the development of GLUT1-deficiency syndrome. The hypothesis will be further investigated in this master thesis.

Materials and methods

Cell culture

HEK293 cells were cultured under standard cell culture conditions. In brief, cells were grown at 37 °C and 5% CO_2 in T-75 flasks (CELLSTAR) at 10% confluency in DMEM (life technologies) complemented with 10% fetal calf serum (Pan-Biotech). Cells used for SILAC based experiments were cultured in SILAC DMEM (life technologies) containing glutamine (Glutamax, life technologies), pyruvate (life technologies), non-essential amino acids (life technologies) and 10% dialyzed fetal calf serum (Pan-Biotech). The SILAC DMEM was supplemented with standard L-arginine (Arg0, Sigma-Aldrich) and L-lysine (Lys0, Sigma-Aldrich)("light") as in 5. Alternatively, Arg6 and Lys4 ("medium") or Arg10 and Lys8 ("heavy") were added in place of their light counterparts.

For the BioID experiments HEK cells were cultured in either light, medium or heavy SILAC medium until fully labeled. The cells were seeded in 15 cm plates with 25% density. Two plates were used for each condition.

For the endocytosis inhibition experiments HEK cells were cultured in a 12-well plate coated with poly-lysine. The cells were

For the degradation inhibition experiments HEK cells were cultured in a 6-well plate. The cells were

Western blot

The Pierce BCA Protein Assay Kit was used for the colorimetric detection and quantification of total protein obtained from extraction. Protein aliquots of 15 µg were heated with sample buffer at 100 °C for 5 minutes, loaded and electrophoresed through pre-cast Mini-PROTEIN TGX gel and subsequently transferred to a PVDF membrane using semi-dry transfer system. After blocking in 5% milk powder in TBST at room temperature for 1 h, the blot was cut into halves, each containing the same samples. The two blots were incubated overnight in 0.1% Anti-MSI2 Antibody (ab50829) solution in TBST and 0.1% Anti-actin antibody in TBST, respectively, at 4 °C. After rinsing the blots 3 times for 8 minutes with TBST, the blots were incubated with 0.02% Anti-mouse IgG VHH Single Domain Antibody (HRP) (ab191866)

solution in TBST at room temperature for 1 h. The Msi2 and actin protein levels were determined by chemiluminescence.

BioID

b

Inhibitor treatment

Fluorescence microscopy

a

Results

3.1 Expression of GLUT1 in stable inducible cell lines

3.2 Proximity labeling of GLUT1 variants

3.3 Subcellular localization study of GLUT1 variants

Discussion

List of Abbreviations

BSA	Bovine serum albumin
CPZ	Chlorpromazine
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
GLUT1	Glucose transporter 1
G1DS	Glucose transporter 1 deficiency syndrome
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SILAC	Stable isotope labeling by amino acids in cell culture
TGN	Tran-Golgi network

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