Glycogen body metabolism is required for neurons against stresses

Yan Liu, PhD
Research Assistant professor

Center for Cancer and Immunology Research, Children’s National Medical Center and George Washington University, Washington, DC 20010, USA.
Glycogen body (GB) metabolism in mammalian cells

**Hepatic cell**
- Live glycogen synthase (GS2)
- Dephosphorylation
- G6P
- Normal glycogen particles
- Cellular energy store
- Whole body glucose supply

**Most cells/tissues**
- Muscle glycogen synthase (GS2)
- G6P
- Dephosphorylation
- glycogen granules/bodies (GBs)
- Cellular deep energy store
- Provide energy for cells on demands against stresses

Gene deficiency:
- Gbe, Epm2a, Nhlrc1, etc.

Abnormal pathogenic GB:
- Lafora bodies (LBs), deficient in GB degradation
- Polyglucosan bodies (PBs), deficient in glycogen synthesis

Diseases
Difference between normal glycogens and GBs

Soluble glycogen particle

Insoluble glycogen body

Liver glycogen synthase (GS2)

Normal glycogens

Muscle glycogen synthase (GS1)

Glycogen bodies
Glycogen bodies exist in normal tissues of human

Normal brain of human/glycogen

Normal liver of human/glycogen

Normal liver of human/GPBB

The GPBB-binding glycogen bodies may act as a deep glycogen store to supply energy glucose under stress conditions that increase cellular AMP.
What does LB look like

**PAS** (Periodic acid Schiff Stain)

WT (9 Mon)

CA1

Epm2a KO (9 Mon)

pS641/645 GS1
Polyglucosan bodies (PBs) of Gbe-deficient mice

Hippocampus/ CA3  pS641/645 GS1  PBs /pS641/645 GS1  / DAPI
EPM2A and Laforin

*EPM2A*, Epilepsy, Progressive Myoclonus Type 2, encodes the dual specificity phosphatase (DSP) called Laforin.

*EPM2A* is located in chromosome 6q24 in human and 10 in mouse. Loss of heterozygosity (LOH) at 6q22-27 in human occurs at a very high frequency in cancers.

Laforin is comprised of carbohydrate binding domain (CBD) and DSP domain.

Laforin

```
<table>
<thead>
<tr>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>C265S</th>
</tr>
</thead>
</table>
```

CBD and DSP domains are indicated along with the C265S mutation.
Laforin is a phosphatase of GBs

HEK293 cells were transfected with laforin or with its phosphatase-dead mutant C265S for 36h before being stained with antibody of phosphor-S641GS1.
Epm2a knockout causes the accumulation of pS641GS1 bodies.
ER stress induces glycogen bodies in Neuro2a cells.

GS1
DAPI
GS1 glycogen
DAPI
PAS
GS1 is phosphorylated at S641/645 by GSK3

A

p-GS1 (S641/645)

glycogen

DAPI

B

(-) Thapsigargin

<table>
<thead>
<tr>
<th>cytosol</th>
<th>GBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Thap

p-GS1 (S641/645)

GS1

C

p-GS1 S641/645

glycogen

DAPI

GS1-Flag Δ N10 Δ 639 PTG-Flag
GBs are induced in WT neurons by energy deprivation stress

WT/ Glycogen / DAPI

WT+2-DG/ Glycogen / DAPI

No. with seizure score 5

WT (n=10)
P=0.0012

Epm2a KO (n=10)

2-DG (2g/kg)

2-DG (2.5g/kg)

WT

Epm2a-KO

WT-2DG

Epm2a-KO-2DG

GFAP F-Jade-C
GPBB knocked out in neuronal cells causes their death/apoptosis under stresses such as low glucose, 2-DG treatment.

Control, low glucose (5mM) for 48h

GPBB (-/-), low glucose (5mM) for 48h

GPBB was knocked out in Neuro2a cells by sgRNA-guided Cas9-directed genomic editing
The role of Laforin in the formation and accumulation of glycogen bodies (GBs) induced by stress


2. Synthesized GB mingles with GS1 that is phosphorylated by GSK3.

3. Laforin dephosphorylates GB’s GS1 for their degradation against stress persistence.

4. Deficiency of Laforin increases LB phosphorylation and accumulation, accelerates stress-induced LD progression.

5. Stress-induced GB formation and then degradation is a protective process of cells surviving from persistent stress.
Laforin is a phosphatase of GBs and targets phosphorylated GS1.
Proteans’ complex machinery is required for the degradation of pGS1-GBs

GS1

Laforin + Malin

GS1

Poly-Ub-GS1

proteasome

GPBB + AGL1

G1P
Laforin-Malin complex in concert with GPBB and AGL1 degrades GBs completely in vivo
GPBB accumulates in the brain LBs of Epm2a or Nhlrc1 KO mice
Molecular mechanism underlying Lafora disease

Neuron

Glucose

stimulators

Glut3

Glucose

HK1

G6P

Glycolysis

GB synthesis

GS1 (active)

ER stress

Vicious circle

Laforin

Malin

GPBB

AGL1

GB degradation

LB

LD

Cellular energy

ER glycosylation

Glucose

G6P

G1P

Progressive Myoclonus Epilepsy, Neuron degeneration, Demetria.
Acknowledgement:

Yin Wang  
Keni Ma  
Zeng Li  
(General Surgery  
The University of Michigan)

Pan Zheng  
Yang Liu  
(CCIR, Children's National Medical Center)

Helen Zhang  
Jack M. Parent  
(Neurology Department  
The University of Michigan)

Peixiang Wang  
Berge A. Minassian  
(The Hospital for Sick Children and University of Toronto, Canada)