ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE IN GAUCHER DISEASE

Gali Maor and Mia Horowitz

Gaucher Disease

Due to the defective activity of lysosomal glucocerebrosidase (GCase) in Gaucher disease there is:

- Accumulation of substrate
- ERAD of mutant GCase variants
ERAD of mutant GCase variants

ERAD has been shown for the following genotypes (mutations):


Every ER resident or passenger protein undergoes ERQC and, if needed, ERAD!!!
## Quantitation of ERAD

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>TOTAL AMOUNT (% OF NORMAL) A</th>
<th>AMOUNT IN LYSOSOMES (% OF A) B</th>
<th>AXB</th>
</tr>
</thead>
<tbody>
<tr>
<td>N370S/N370S</td>
<td>47.3</td>
<td>79.8</td>
<td>37.7</td>
</tr>
<tr>
<td>N370S/N370S</td>
<td>70.6</td>
<td>89.8</td>
<td>63.4</td>
</tr>
<tr>
<td>N370S/N370S</td>
<td>49.8</td>
<td>84.9</td>
<td>42.2</td>
</tr>
<tr>
<td>N370S/V394L</td>
<td>45.2</td>
<td>27.2</td>
<td>12.2</td>
</tr>
<tr>
<td>R463C/?</td>
<td>31.9</td>
<td>63.9</td>
<td>20.4</td>
</tr>
<tr>
<td>TYPE 3</td>
<td>14.8</td>
<td>34.5</td>
<td>4.9</td>
</tr>
<tr>
<td>TYPE2</td>
<td>15.5</td>
<td>4.1</td>
<td>0.6</td>
</tr>
<tr>
<td>P415R/L444P</td>
<td>20.3</td>
<td>1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>WT</td>
<td>100</td>
<td>89.8</td>
<td>89.8</td>
</tr>
</tbody>
</table>

There is a correlation between the severity of the disease and the ERAD level.
ERAD of mutant GCase variants may lead to pathogenesis
The Unfolded Protein Response

Test UPR by:
1. Changes in mRNA and protein levels of Bip, CHOP
2. Phosphorylation of eIF2α
3. Splicing of Xbp1 mRNA
Gaucher Disease

- UPR exists in GD derived cells
- UPR also exists in carriers of GD mutations
- UPR upregulates GBA transcription
UPR in GD patients and carriers: Bip and CHOP mRNA levels

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Genotype</th>
<th>Disease type</th>
<th>Relative Chop mRNA</th>
<th>Relative BiP mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WT</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>N370S/N370S</td>
<td>1</td>
<td>5.5±2.6*</td>
<td>6.2±3.1**</td>
</tr>
<tr>
<td>3</td>
<td>L444P/R120W</td>
<td>2</td>
<td>2.8±1.4*</td>
<td>4.2±0.6*</td>
</tr>
<tr>
<td>4</td>
<td>G202R/G202R+M362I</td>
<td>2</td>
<td>3.3±1.6*</td>
<td>3±1.7*</td>
</tr>
<tr>
<td>5</td>
<td>L444P/L415W</td>
<td>2</td>
<td>8.9±2.1**</td>
<td>6±2*</td>
</tr>
<tr>
<td>6</td>
<td>G202R/G202R</td>
<td>2</td>
<td>8.7±3.1**</td>
<td>8.5±4*</td>
</tr>
<tr>
<td>7</td>
<td>K157Q/D140H+E326K</td>
<td>1 (severe)</td>
<td>4.3±2.19*</td>
<td>3.6±1.48 *</td>
</tr>
<tr>
<td>8</td>
<td>K157Q/D140H+E326K</td>
<td>1</td>
<td>9.2±2.94**</td>
<td>7.2±2.87**</td>
</tr>
<tr>
<td>9</td>
<td>N370S/N370S</td>
<td>1 (severe)</td>
<td>4.1</td>
<td>3.4±1.82*</td>
</tr>
</tbody>
</table>
UPR in GD patients: Bip and CHOP proteins

Bip and CHOP mRNA and protein levels are significantly elevated in GD fibroblasts.
Level of Xbp1 splicing is significantly elevated in GD fibroblasts.
UPR in GD patients: Phosphorylation of eIF2α

Levels of phosphorylated eIF2α protein are significantly elevated in GD fibroblasts
Unfolded Protein Response in GD

Substrate accumulation does not lead to UPR
**UPR in GD mice (Grabowski): Bip and CHOP proteins**

Bip and CHOP mRNA levels are significantly elevated only in D409V homozygous mice!!!

### Graph

![Graph showing relative mRNA quantities of Bip and CHOP for different genotypes](image)

- **WT**
  - Bip: $0.69 \pm 0.08$
  - CHOP: $0.155 \pm 0.04$

- **D409H/D409H**
  - Bip: $2.02 \pm 0.4$
  - CHOP: $2.7 \pm 0.5$

- **D409V/D409V**
  - Bip: $1.08 \pm 0.3$
  - CHOP: $0.7 \pm 0.06$

- **KO**
  - Bip: $2.91$
  - CHOP: $3.81$

- **WT+Tg**
  - Bip: $2.91$
  - CHOP: $3.81^*$

### Table

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Relative Chop mRNA</th>
<th>Relative Bip mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D409H/D409H</td>
<td>$0.155 \pm 0.04$</td>
<td>$0.69 \pm 0.08$</td>
</tr>
<tr>
<td>D409V/D409V</td>
<td>$2.7 \pm 0.5^*$</td>
<td>$2.02 \pm 0.4^*$</td>
</tr>
<tr>
<td>KO</td>
<td>$0.7 \pm 0.06$</td>
<td>$1.08 \pm 0.3$</td>
</tr>
<tr>
<td>WT+Tg</td>
<td>$3.81^*$</td>
<td>$2.91^*$</td>
</tr>
</tbody>
</table>

* $P < 0.05$
Level of Xbp1 splicing is significantly elevated in D409V homozygous mouse fibroblasts
Gaucher Disease

- UPR exists in GD derived cells
- UPR also exists in carriers of GD mutations
- UPR upregulates GBA transcription
Gaucher Disease

- UPR exists in GD derived cells
- UPR also exists in carriers of GD mutations
- UPR upregulates GBA transcription
Gaucher Disease

- UPR exists in GD derived cells
- UPR also exists in carriers of GD mutations
- UPR upregulates GBA transcription
Unfolded Protein Response in GD carriers

There is UPR in GD carriers even without a detected protein.
Gaucher Disease

- UPR exists in GD derived cells
- UPR also exists in carriers of GD mutations
- UPR upregulates GBA transcription
GBA mRNA levels in GD fibroblasts

There are elevated levels of GBA mRNA in GD derived fibroblasts

Does it result from response to UPR???????
### GBA mRNA levels in GD fibroblasts

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Disease type</th>
<th>Relative mRNA quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>N370S/N370S</td>
<td>1</td>
<td>4.4±1.2*</td>
</tr>
<tr>
<td>L444P/R120W</td>
<td>2</td>
<td>3.3±1.7</td>
</tr>
<tr>
<td>R131C/R131C</td>
<td>2</td>
<td>3±1.1</td>
</tr>
<tr>
<td>L444P/L444P</td>
<td>3</td>
<td>5.1±1.3*</td>
</tr>
<tr>
<td>N370S/WT</td>
<td>-</td>
<td>5.7±0.8*</td>
</tr>
<tr>
<td>WT+CBE</td>
<td>-</td>
<td>0.99±0.43</td>
</tr>
<tr>
<td>WT/84GG</td>
<td>-</td>
<td>1.7±0.1*</td>
</tr>
<tr>
<td>N370S/WT</td>
<td>-</td>
<td>5.3±2.3*</td>
</tr>
</tbody>
</table>

There are elevated levels of GBA mRNA in GD derived and carrier derived fibroblasts.
In UPR there are genes that are upregulated by the transcription factor CHOP.

Does the GBA gene promoter have a UPR Responsive element?
GBA mRNA levels in GD fibroblasts

- GBA
- Vector delivery to living cells

**Luciferase Reporter Vector**

- (pTL-Luc)
- 4.8 kb

**Promoters**

- AP-1
- PEA
- CAAT

**Enhancers**

- CHOP

**Luciferase**

- D-Luciferin
- Oxyluciferin

**Vector delivery to living cells**

**Molecular Mechanism**

- Luciferase + ATP + O₂ → Luciferin
- D-Luciferin + PP_i + AMP → Oxyluciferin + CO₂ + Light
Upon UPR induction (with thapsigargin) normal (but not CAAT mutated) GBA promoter activity elevates. GBA promoter binds CHOP
Gaucher Disease

Since UPR in general may lead to cell death, UPR in GD may lead to death of cells as well.

Dopaminergic cells (Parkinson disease)?!
Conclusions

There is UPR in GD patients

There is UPR in one animal models (out of few we have tested)

There is UPR in GD carriers including the 84GG carriers

There is upregulation of the GBA gene in patients in response to UPR, through CHOP binding

Even without ERAD there is UPR (ER stress) that may lead to death of cells for example in the case of 84GG carriers and PD
ERAD of mutant GCase variants may lead to pathogenesis
Thanks

Thank you for your attention

Gali Maor

Collaborator:
Dr. M. Filocamo

Thank you for your attention