Targeting the unfolded protein response prevents prion neurodegeneration in mice

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Outline

• Background

• Temporal sequence of events

• Prevention of UPR activation
  – genetically
  – Pharmacologically
Neurodegenerative diseases

- Alzheimer’s
- Parkinson’s
- Huntington’s
- ALS
- Prion

WHO: by 2040 second commonest cause of death in developed world
Common themes in neurodegeneration

- aggregation of misfolded proteins
- gliosis
- early synaptic dysfunction and loss
- irreversible neuronal loss

Are there common pathways driving this?
How do we study neurodegenerative disease in mice?

prion disease
Prion neuropathology

Spongiosis

PrP amyloid deposits ‘PrP\textsuperscript{Sc}’

Astrogliosis

Neuronal loss

Hippocampus CA1

GFAP

GSK2606414 Vehicle GSK2606414

ca
e
d
control
b
hf
0
200
400
600

PrP amyloid deposits ‘PrP\textsuperscript{Sc}’
PrP\textsuperscript{Sc} is derived from endogenous PrP\textsuperscript{C}
Removal or PrP\textsuperscript{C} is neuroprotective

- Adult onset removal of PrP is neuroprotective in prion diseased mice (Mallucci et al., 2003 and 2007)

- Injection of shRNA PrP into the hippocampus allowed for neuroprotection and prolonged survival (White et al., 2009)

Critical time-point of intervention?
What are the processes involved?

- Basic descriptive mapping of changes in
  - Synapse number
  - Synaptic function
  - Synaptic proteins
  - Neuronal loss
  - Behavior

[Graph showing prion infection and timeline from 0 to 12 w.p.i.]
Critical point: reduction in synaptic proteins

Moreno et al., Nature (2012)
Is this due to reduction in global protein synthesis?
Temporal sequence of prion disease

prion infection

0
7
8
9
10
11
12 w.p.i.

synapse loss
memory loss
protein synthesis and burrowing
neuronal loss
confirmatory clinical signs death

Synaptic transmission
What is causing this loss protein synthesis?

- Translational control pathways

Unfolded protein response?
Unfolded protein response (UPR)

- Maintains protein folding homeostasis within the ER
- Allowing for proper protein function
- Cellular stress causes activation
  - misfolded proteins
Unfolded/misfolded proteins

ATF6
Vesicular transport to golgi and cleavage
nATF6
Transcription of UPR target genes

Bip

PERK

P

eIF2α
REDUCED TRANSLATION

ATF4

CHOP
Apooptotic pathway

IRE1
mRNA processing

sXBP1
Chaperones, lipid synthesis and ERAD proteins
Unfolded protein response (UPR)
Rising levels of misfolded PrP induce elf2α-P
Unfolded protein response (UPR)

Unfolded/misfolded proteins

- Bip

- PERK

- eIF2α

- GADD34

LV-shPrP

LV-GADD34

- salubrinal

Reduced translation

Synaptic failure

Neurodegeneration
Prion Virus

Control  Prion only  Prion + Salubrinal  Prion + LV-control  Prion + LV-shPrP  Prion + LV-GADD34

Test at 9 wpi

Prion

Virus

5 weeks incubation
PrP knockdown and GADD34 over-expression reduce eIF2α-P levels
Reducing eIF2α-P restores global translation rates.
Reducing eIF2α-P restores synaptic protein levels
Reducing eIF2α-P restores synaptic transmission and behavioural deficits
Reducing eIF2α-P rescues synapse number
Reducing eIF2α-P is neuroprotective
Focal reduction of eIF2α-P levels increases survival
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GADD34

eIF2α

Reduced translation

Loss of critical proteins

Neurodegeneration
GSK2606414 penetrates the blood brain barrier

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Plasma mean (ng/ml ± SD)</th>
<th>Brain mean (ng/g) ± SD</th>
<th>Mean ratio brain: plasma</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>NQ</td>
<td>NQ</td>
<td>-</td>
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<tr>
<td>10</td>
<td>1227 ± 696</td>
<td>557 ± 292</td>
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<tr>
<td>50</td>
<td>13912 ± 5914</td>
<td>7507 ± 2528</td>
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<tr>
<td>150</td>
<td>16002 ± 453</td>
<td>9539 ± 1516</td>
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Moreno et al., 2013 Sci. Trans. Med.
Experimental set-up

Prion infection

0

GSK2606414

7

GSK2606414

8

protein synthesis and burrowing

9

10

neuronal loss

11

12 w.p.i.

confirmatory clinical signs death

synapse loss

memory loss

0.2

0.4

0.6

0.8

1.0

1.2

1.4

1.6

1.8

*
Behavioral deficits rescued by GSK2606414
Clinical signs of disease exacerbated by GSK2606414
Neuronal loss is prevented

<table>
<thead>
<tr>
<th>Hippocampus</th>
<th>Uninfected control</th>
<th>Prion infection: 12 w.p.i.</th>
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<tbody>
<tr>
<td></td>
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Overall brain protection

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<tr>
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<td>Vehicle</td>
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<tr>
<td>Cortex</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Thalamus</td>
<td>e</td>
<td>f</td>
</tr>
<tr>
<td>Brainstem</td>
<td>i</td>
<td>j</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>m</td>
<td>n</td>
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</table>
PERK / eIF2α-P is inhibited
GSK2606414 restores translational failure
GSK2606414 restores synaptic protein levels
Unfolded/misfolded proteins

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Vesicular transport to golgi and cleavage

nATF6

Transcription of UPR target genes

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Apoptotic pathway

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IRE1

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Other branches of the UPR
Levels of PrP$^{C}$ and PrP$^{Sc}$ are unchanged.
• Toxicity of compound?
GSK2606414 treated mice lose >20% body weight
Blood glucose levels rise and pancreas weights decrease with treatment.
Pancreas morphology following 5 weeks of GSK2606414 treatment

- 5 mg/kg
- 10 mg/kg
- 50 mg/kg
Toxic side effects

- Can we reduce toxicity?
  - Dose response studies

- Supplement for loss of pancreas

- Peripheral activation of PERK

- Target other parts of the pathway
Possible new targets for treatment irrespective of specific disease proteins

- Increased eIF2\(\alpha\)-P and PERK-P in AD, PD, ALS and prion patients brains (Hoozemans et al., 2007, 2009; Atkin et al., 2008)

- eIF2\(\alpha\)-P in APOE4 mice (Segev et al., 2013)

- Increasing levels of tau and PERK-P (Abisambra et al., 2013)
Conclusions

• Targeting the UPR genetically and pharmacologically prevents prion neurodegeneration
  – Independent of PrP

• Better compounds need to be identified to target pathway

• Manipulation of this generic cellular pathway may be possible in other mouse models of neurodegeneration
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