Investigating the potential of the neuroprotective N1 fragment of the prion protein as a new treatment against prion diseases

„Fighting prions with the prion protein“

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CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease
Fighting prions with the prion protein
The protective role of molecular cleavage events

2016 grant (work ongoing)
The Mary Sue Riffl Memorial Grant
The Neil W. Foster Memorial Grant
The Mary S. Friel and Mary T. Friel Memorial Grant
The CJD Foundation Memorial Grant

2018 grant
The Garry Buttermann IV Memorial Grant
The Davey Kock Memorial Grant
The Jeffrey A. Smith Memorial Grant
The Strides for CJD Grant
“Fighting prions with the prion protein”

The protective role of molecular cleavage events

ADAM10 KO  wild-type  PrP↑ (tga20)

2018 grant

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The α-cleavage of PrP<sub>C</sub> and the N1 fragment

• Responsible protease(s) unknown / controversially discussed:
  - no pharmacological target at the moment
• Protective effects of α-cleavage shown by several groups
• Blocking/neutralizing effects of N1 (mainly towards Aβ)
  convincingly demonstrated by several groups

What about N1-associated protection in prion diseases?

Need for a reliable mouse model
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Generation of N1-overexpressing mice (TgN1)

mRNA level in forebrain:

Biochemical assessment (western blot) of mouse brain homogenates:

TgN1 mice show higher mRNA transcript levels

TgN1 mice show increased N1 amounts at the protein level

Everything so far looked promising, but…
"Fighting prions with the prion protein"

Intracerebral infection of TgN1 mice with prions

Have we been wrong with our strategy?

Primary neurons provide further insight:

<table>
<thead>
<tr>
<th>lysate</th>
<th>precipitated media</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>TgN1</td>
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<tr>
<td>WT</td>
<td>TgN1</td>
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</tbody>
</table>

Similar N1 secretion in TgN1 and WT mice

How can this be explained?

No protection observed in prion diseases
A new finding with general relevance for protein biosynthesis published in parallel to our project.


Inefficient ER-import and secretion of **intrinsically disordered proteins** (such as N1)

- signal peptide remains attached (double band!)
- accumulation in the cytosol / altered signaling
- first *in vivo* proof YET not what we aimed for

Can we overcome this problem?
“Keeping up the fight“
A new mouse model for increased N1 secretion

Work in progress: Generation of transgenic mice expressing the fusion protein N1-Fc

Expression in PrP<sup>C</sup> knockout cells:

<table>
<thead>
<tr>
<th>media (IP)</th>
<th>lysates</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ N1-Fc</td>
<td>+ N1-Fc</td>
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</tbody>
</table>

Molecule part of mouse antibodies (IgG)

An α-cleavage-like event still occurs
Outlook

- Detailed characterization of the N1-Fc mouse model
- Prion inoculation study using N1-Fc mice and controls
- Continue identification of the responsible protease(s)

- Further assessment of the protective shedding by ADAM10 in neurodegenerative diseases
- Substrate-specific stimulation of the shedding
- Influence of physiological/pathological binding partners

Push forward translational aspects and novel therapeutic approaches
Part of future combination therapies?
There is an urgent need for novel therapeutic strategies against prion diseases

Under normal conditions, a fraction of PrP\textsuperscript{C} molecules is cleaved by proteases

Increasing evidence suggests that at least two cleavage events are protective

Mechanistic details of how the resulting prion protein fragments act protective are currently unknown and have to be investigated in detail in order to device novel treatment options (e.g. stimulation of the proteases; administration of PrP fragments)

New transgenic mice overexpressing the N1 fragment of the prion protein have been/will be generated that will show whether N1 also acts protective in prion diseases

Promising advances also have been made with the shedding of PrP\textsuperscript{C} (grant 2016)
Acknowledgements

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