Human Stem Cell Derived Neural Models for CJD Prion Propagation and Drug Discovery

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Hallmarks of human PrP prion diseases

- \( \text{PrP}^{\text{Sc}} \) accumulation
- Gliosis
- Neuronal loss
- Spongiosis
- Amyloid and plaque formation

We still don’t understand the underlying mechanism of neurodegeneration in human prion diseases due to the lack of relevant model system.
**PRNP codon 129 genotype & CJD**

<table>
<thead>
<tr>
<th></th>
<th>MM</th>
<th>MV</th>
<th>VV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal population</td>
<td>37%</td>
<td>51%</td>
<td>12%</td>
</tr>
<tr>
<td>sporadic CJD</td>
<td>71%</td>
<td>13%</td>
<td>16%</td>
</tr>
<tr>
<td>variant CJD</td>
<td>99%</td>
<td>&lt; 1%</td>
<td>-</td>
</tr>
<tr>
<td>iatrogenic CJD</td>
<td>57%</td>
<td>20%</td>
<td>23%</td>
</tr>
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Tg animal cell culture models for studying CJD

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PrP&lt;sup&gt;Sc&lt;/sup&gt;</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Cerebellar granule cells from Hu tg mice</td>
<td>vCD, sCJD, iCJD</td>
<td>Hannaoui et al., 2013</td>
</tr>
<tr>
<td>Mouse immortalized hypothalamic GT-1</td>
<td>rodent-adapted CJD</td>
<td>Arjona et al., 2014</td>
</tr>
</tbody>
</table>

- Adaptable for high throughput screening

Caveats:

- May not faithfully recapitulate disease conditions of human neurodegenerative diseases

- Small molecules extending survival in mice infected with mouse-adapted prions are ineffective against CJD prions

  (Berry et al., PNAS 2013)
There is no human cell culture model in which human PrP\textsuperscript{Sc} prions replicate

Possible reasons for failure:

- Transformed cells do not represent the phenotypic environment of human prion diseases
- Incompatibility between \textit{PRNP} codon 129 genotype between inocula and cultured cells
- Low PrP\textsuperscript{C} expression
- Protein X
- PrP\textsuperscript{Sc} clearance rate is higher than PrP\textsuperscript{Sc} production
- High rate of cell division
- Tagging of PrP\textsuperscript{C} compromise PrP\textsuperscript{Sc} formation and infectivity \textit{in vivo} & \textit{in vitro}
- Culture conditions
Human neuroglioma H4 line (MM)

3F4 (PrP^C)  
GFAP  
βIII-tubulin

PrP^C expression

40 kDa  
30 kDa  
20 kDa

mAb 3F4
hESC H9 (MV) exposed to prion infection

<table>
<thead>
<tr>
<th>Time points:</th>
<th>BSE</th>
<th>vCJD</th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 hours acute exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>48 hours acute exposure</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>48 hours acute exposure - 24 hours recovery</td>
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</tr>
<tr>
<td>48 hours acute exposure</td>
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<td></td>
</tr>
<tr>
<td>48 hours acute exposure - 48 hours recovery</td>
<td></td>
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</tr>
<tr>
<td>48 hours acute exposure</td>
<td></td>
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<td></td>
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<tr>
<td>72 hours recovery</td>
<td></td>
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</tbody>
</table>

PrP

Krejciova et al., 2011
Human fetal brain-derived neurospheres (MV)
Human fetal brain-derived spheres (MV)

Exposure to sCJD (VV$_2$)

0.4% sCJD (VV2) crude
3 d Exposure & 10 d Recovery
1.2% sCJD (VV2) filtered
3 d Exposure & 10 d Recovery

+ PK

+ EGF/FGF

Loading 1 well/T25 flask
mAb 3F4
Astrocytes contributing to neurodegeneration?

- Astrocytes play an important role in neurovascular and neurometabolic coupling

- In prion diseases neuron-glia interactions are thought to be involved
  (Gomez-Nicola et al., 2013; Asuni et al., 2014; Hennessey et al., 2015; Fang et al., 2016)

- PrPSc replicates in astrocytes in addition to neurons
  (Diedrich et al., 1991; Raebel et al., 1997; Jeffrey et al., 2004; Cronier et al., 2004; Victoria et al., 2016)
Exposure of human astrocytes to CJD brain homogenate

**CJD inoculum**

- Micro-pestle homogenization
- Fast-prep 24 homogenization
- Sonication
- Clarification
- Spin filter centrifugation

**Exposure regime**

- Exposure
- 1% spin filtered brain homogenate

**Time points**

- Recovery 8d
- Recovery 3d
- Recovery 0d

**Analysis**

- +PK
- sCJD inocula
- 0d Recovery
- 3d Recovery
- +Gnd
- PrP
- mAb P
hESCs derived astrocytes (MV)

Exposure to sCJD (MM)

Exposure to sCJD (VV₂)

Inocula sCJD MM
24h Exposure
72h Recovery
8d Recovery

Inocula sCJD VV
24h Exposure
72h Recovery
8d Recovery

mAb F20-108a

mAb 3F4
Generation of human cell culture model for studying CJD pathogenesis & drug discovery

Astrocyte progenitor cells (APC) generation from human iPS cells

Krencik & Zhang, Nat Protoc, 2011
Serio et al., PNAS, 2013
Astrocyte Differentiation

Neurospheres → Neurospheres enriched in astroglial progenitors → Mechanical Chopping → EGF+LIF Conversion → EGF+FGF2 Propagation → Astrocyte progenitors → Differentiation in CNTF media 2 wks

**Images**

- **A2B5 GFAP**
- **S100β GFAP**
- **PrPC GFAP**

**Graph**

- Glutamate uptake (nM/hour/cell)
  - HEK293
  - APC EGF+FGF2
  - Astrocytes CNTF

**Western Blot**

- **PrPc**
- **β-actin**
Human astrocytes replicate CJD PrP\textsuperscript{Sc} \textit{in vitro} in a \textit{PRNP} codon 129-dependent manner

Krejciova Z., Alibhai J., et al., (submitted)
Replication of vCJD PrP\textsuperscript{Sc} in human astrocytes is \textit{PRNP} codon 129- and concentration-dependent

Astrocytes

- MM / Inoculum vCJD MM
- MV / Inoculum vCJD MM
- VV / Inoculum vCJD MM

0d post exposure

3d post exposure

mAb 3F4

Krejciova Z., Alibhai J., et al., (submitted)
Guanidine mediates cryptic PrP\textsuperscript{Sc} epitope retrieval

Control

CJD exposed/8d recovery

CJD exposed/8d recovery

-Gnd

-Gnd

-Gnd

inset

+Gnd

+Gnd

+Gnd

inset

20 µm

20 µm

mAb HuM-P

Krejciova Z., Alibhai J., et al., (submitted)
Human astrocytes replicate PrP\textsuperscript{Sc} when the CJD inoculum & cell genotype are matched

**Krejciova, Alibhai, et al., (submitted)**

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**Human astrocytes replicate PrP\textsuperscript{Sc}** when the CJD inoculum & cell genotype are matched.

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**Krejciova, Alibhai, et al., (submitted)**
Human astrocytes (VV) replicate sCJD (VV2) PrP^Sc prions.

Krejcirova Z., Alibhai J., et al., (submitted)

**Signal intensity/cell**

- **sCJD (VV2)**
  - 0 d post exposure
  - 8 d post exposure

- **vCJD (MM)**
  - 0 d post exposure
  - 8 d post exposure

**Astrocytes (VV)**

**mAb HuM-P**
3D reconstruction of PrPSc accumulation in astrocytes (VV) exposed to sCJD (VV2)

control

sCJD (VV2)
8d post exposure

Krejciova Z., Alibhai J., et al., (submitted)
Summary

Proliferating ES, FDC-like and transformed human cells failed to propagate CJD PrP^sc in vitro

Human iPS cell-differentiated astrocytes are susceptible to CJD PrP^sc replication in vitro in a PRNP codon 129 dependent manner

We hypothesize that astrocytes might contribute to prion-induced neurodegeneration in a non-cell autonomous manner, either by impairment of neuroprotective function or gain of neurotoxic function by generating neurotoxic signals in response to prion infection

Our model is providing a new in vitro system for accelerated mechanistic studies and drug discovery
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NIH National Institute on Aging

Daiichi-Sankyo

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Non-Expert Summary

Prions are infectious agents that cause neurodegenerative diseases such as Creutzfeldt-Jakob disease. For four decades, scientists have attempted to produce experimental models that approximate prion diseases – both to develop new therapies and to understand the mechanisms of the disease – but until now these efforts have been unsuccessful. For example, drugs developed using mice infected with prions have been shown to be ineffective against human prions.

Here we show that prions can infect brain cells (so-called astrocytes) derived from human stem cells, and determine which factors influence their susceptibility to prions. These include genetics and the cells’ state of development. Our work thus addresses a long-standing gap, providing a more relevant tool for studying prion diseases and accelerating drug discovery.