Propagation of CJD Prions in Cultured Cells Expressing Bank Vole PrP

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The Search for Prion Disease Therapeutics

PrP<sub>C</sub> → PrP<sub>Sc</sub> → Disease

- Therapeutic Strategies:
  - Decrease levels of PrP<sub>C</sub> in the brain
  - Interfere with the conversion of PrP<sub>C</sub> into PrP<sub>Sc</sub>
  - Enhance the clearance of PrP<sub>Sc</sub>
  - Block the toxicity of PrP<sub>Sc</sub>

- The most commonly used cellular model for uncovering anti-prion compounds is N2a neuroblastoma cells infected with RML prions (ScN2a cells)
The Search for Prion Disease Therapeutics

Use automated robotic systems to screen ~100,000 compounds for the ability to reduce PrP^{Sc} levels in ScN2a cells.

Treatment: DMSO 24 81

PK-resistant PrP

IND24  IND81
IND24 and IND81 Greatly Extend the Lifespan of RML Prion-Infected Mice

Inoculate RML prions intracerebrally

Wild-type

Survival (%) vs. Time from inoculation (days)

Vehicle

IND24

IND81
But...IND24 Does Not Extend the Lifespan of Mice Infected with CJD Prions

Inoculate with sCJD MM1 prions

Inoculate with sCJD VV2 prions

Red curves: Vehicle-treated mice
Green curves: IND24-treated mice
**Conclusion:**
Prion disease therapeutics exhibit prion strain-specific efficacy

**Implication:**
To uncover a therapeutic that will be effective at treating CJD patients, you need to perform the primary screen in cells infected with human CJD prions
Cell Lines That Can be Chronically Infected with CJD Prions

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Supports Replication of CJD Prions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human neuroblastoma cells (SH-SY5Y, M17, etc.)</td>
<td>No</td>
</tr>
<tr>
<td>Human glioblastoma cells</td>
<td>No</td>
</tr>
<tr>
<td>N2a cells expressing human PrP</td>
<td>No</td>
</tr>
<tr>
<td>RK13 cells expressing human PrP</td>
<td>No</td>
</tr>
</tbody>
</table>

- Despite an immense research effort, chronic replication of CJD prions in cultured cells has never been achieved.
- Mouse-adapted CJD prion strains do not accurately recapitulate the properties of human CJD prions.
- An alternate strategy seems necessary…
Bank Voles are Uniquely Susceptible to Prion Strains from Various Species

Hamster prions
Human prions
Cervid prions
Sheep prions

Efficient Transmission
No Species Barrier??
Nonno et al., PLoS Pathogens, 2006

Hamster prions
Human prions
Cervid prions
Sheep prions

Poor Transmission
“Species Barrier”
## Generation of Transgenic Mice Expressing Bank Vole PrP (BVPrP)

<table>
<thead>
<tr>
<th>Line</th>
<th>Codon 109</th>
<th>Relative PrP Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg22019</td>
<td>Methionine</td>
<td>~5x</td>
</tr>
</tbody>
</table>

Watts et al., PNAS, 2012
**Tg(BVPrP) Mice are Highly Susceptible to Different Strains of CJD Prions**

<table>
<thead>
<tr>
<th>Prion isolate</th>
<th>Host species</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Passage</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean incubation period ± s.e.m. (d)</td>
<td>Clinical disease</td>
</tr>
<tr>
<td>sCJD MM1</td>
<td>Human</td>
<td>196 ± 4</td>
<td>8/8</td>
</tr>
<tr>
<td>sCJD MM2</td>
<td>Human</td>
<td>244 ± 30</td>
<td>7/7</td>
</tr>
<tr>
<td>sCJD VV2</td>
<td>Human</td>
<td>394 ± 18</td>
<td>7/7</td>
</tr>
<tr>
<td>vCJD</td>
<td>Human</td>
<td>330 ± 15</td>
<td>7/7</td>
</tr>
</tbody>
</table>
PrP Deposition in Tg(BVPrP) Mice Inoculated with Various CJD Strains
CJD Strain Properties are Maintained Upon Passage in Tg(BVPrP) Mice

<table>
<thead>
<tr>
<th>Prion isolate</th>
<th>GdnHCl_{1/2} (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculum</td>
</tr>
<tr>
<td>sCJD MM1</td>
<td>2.1</td>
</tr>
<tr>
<td>sCJD VV2</td>
<td>2.8</td>
</tr>
<tr>
<td>vCJD</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Retrotransmission of Tg(BVPrP)-Adapted CJD Prions

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Recipient animals</th>
<th>Mean incubation period ± s.e.m. (d)</th>
<th>Clinical disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCJD MM1</td>
<td>Tg(HuPrP)2669</td>
<td>149 ± 2</td>
<td>8/8</td>
</tr>
<tr>
<td>sCJD MM1 → Tg22019</td>
<td>Tg(HuPrP)2669</td>
<td>166 ± 1</td>
<td>7/7</td>
</tr>
</tbody>
</table>

Inoculum: MM1 Tg22019

sCJD MM1 → Tg2669

sCJD MM1 → Tg22019 → Tg2669
**Conclusion:**
The properties of CJD prions are maintained upon passage in Tg(BVPrP) mice

**Hypothesis:**
Cultured cells expressing BVPrP will permit the chronic replication of various CJD prion strains
Approach: Generate RK13 Cells Expressing Bank Vole PrP

RK13 cells:
- Rabbit kidney epithelial cells
- Do not express detectable levels of endogenous PrP
- Become susceptible to prions following transfection of the PrP gene from the appropriate species (but not human PrP)

Ex vivo propagation of infectious sheep scrapie agent in heterologous epithelial cells expressing ovine prion protein

D. Vilette*, O. Andreoletti†, F. Archer*, M. F. Madelaine*, J. L. Villette†, S. Lehmann†, and H. Laude†

A cell line infectible by prion strains from different species


Cell-Based Quantification of Chronic Wasting Disease Prions

Jifeng Bian, Dana Napier, Vadim Khaychuck, Rachel Angers, Catherine Graham, and Glenn Telling
**Approach:** Generate RK13 Cells Expressing Bank Vole PrP

**Hypothesis:** RK13-BVPrP #4 cells will be capable of replicating BVPrP-adapted CJD prions
Ongoing and Planned Studies

• Isolation of additional RK13-BVPrP subclones

• Determination of the susceptibility of individual subclones to prion infection using BVPrP-adapted RML prions

• Infection of RK13-BVPrP cells with BVPrP-adapted CJD strains:
  – sCJD MM1
  – sCJD VV2
  – vCJD

• Direct infection of RK13-BVPrP cells with human CJD strains:
  – sCJD MM1
  – sCJD MM2
  – sCJD VV1
  – sCJD VV2
  – vCJD

• Generation of other BVPrP-expressing cell lines
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