The Role of Animal Models in Identifying and Testing Treatments

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Why Use Mice to Study Prion Disease?

• Lack of appropriate cultured cell model
  – Currently, CJD prions cannot be replicated in cultured cells

• Complexity of the brain is not easily mimicked using cultured cells
  – Multiple cell types
  – Non-dividing cells

• Certain features of prion disease cannot be studied using cell or other in vitro models
  – Neuroinvasion
  – Prion neurotoxicity

• Translational considerations when designing therapeutics
  – Blood-brain-barrier
  – Metabolism of compounds
Transmission of Prion Disease to Mice

Intracerebral inoculation of prions

Asymptomatic mice (%) vs. Time from inoculation (days)

Proteinase K: - + - + Uninfected Mice  Prion-Infected Mice  PrP

40 30 20 15
PrPC is Required for Prion Disease

PrPC → PrPSc → Disease

Intracerebral inoculation of prions

PrP knockout mice

No prion disease!!

Büeler et al., Cell, 1993

Mallucci et al., Science, 2003

White et al., PNAS, 2008
Small Molecules Extend the Lifespan of Prion-Infected Mice

- Other effective anti-prion small molecules:
  - anle138b
  - Polythiophenes
  - 2-aminothiazoles (i.e. IND24)

Kawasaki et al., J Virol, 2007
IND24 and IND81 Greatly Extend the Lifespan of Prion-Infected Mice

- IND24-treated mice eventually died of prion disease, but their survival time was almost doubled compared to vehicle-treated mice.
IND24 Greatly Extends the Lifespan of Prion-Infected Mice

<table>
<thead>
<tr>
<th>Day Treatment Started</th>
<th>Mean Incubation Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>118</td>
</tr>
<tr>
<td>1 day post-inoculation</td>
<td>204</td>
</tr>
<tr>
<td>34 days post-inoculation</td>
<td>202</td>
</tr>
<tr>
<td>61 days post-inoculation</td>
<td>221</td>
</tr>
<tr>
<td>90 days post-inoculation</td>
<td>118</td>
</tr>
<tr>
<td>14 days prior to inoculation</td>
<td>452</td>
</tr>
</tbody>
</table>

Giles et al., JPET, 2015

- **Conclusion**: Prion disease therapeutics are most effective when administered early on during the disease course.
But…IND24 Does Not Extend the Lifespan of Mice Infected with Human Prions

• Conclusion: The efficacy of anti-prion compounds is prion-strain specific
Persistent IND24 Treatment Gives Rise to Drug-Resistant Prions

• Prions from mice treated with vehicle (control) are sensitive to IND24

• But…the prions from mice treated with IND24 are now resistant to IND24!

• Conclusion: Like bacterial and viral pathogens, prions can evolve!

Berry et al., PNAS, 2013
Mouse Models: Infectious vs. Spontaneous Prion Disease

**Infectious Prion Disease**
- Prion disease initiated by the injection of pre-formed PrP$^{Sc}$
- ~99% of prion disease studies in mice
- ~1% of human prion disease cases

**Spontaneous Prion Disease**
- Prion disease initiated by the spontaneous formation of PrP$^{Sc}$
- ~1% of prion disease studies in mice
- ~99% of human prion disease cases

Inoculate with prions
Attempts to Generate Tg Mouse Models of Inherited Human Prion Diseases

Criteria for an authentic mouse model of spontaneous prion disease:

1. Develops spontaneous signs of neurological illness
2. The brains of spontaneously sick animals exhibit prion disease-specific neuropathology
3. The brains of spontaneously sick animals contain PK-resistant PrP species
4. The disease is transmissible

Mutations: P102L (GSS), D178N (fCJD/FFI), E200K (fCJD), Octa14 (GSS)
Tg Mice Expressing Mutant BVPrP Develop a Spontaneous, Transmissible Disease

Prion Disease Neuropathology in Spontaneously Sick Mutant BVPrP Tg Mice

- **Conclusion**: Each mutation specifies the formation of distinct PrPSc aggregates

Watts et al., Acta Neuropathologica, 2016
PK-Resistant PrP in Spontaneously Sick Mutant BVPrP Tg Mice

Watts et al., Acta Neuropathologica, 2016
PrP^Sc-Independent Disease Pathogenesis in Tg(FFI) Mice

- Tg(FFI) mice develop spontaneous disease, but highly PK-resistant PrP is not observed in the brain

- The disease is non-transmissible

- However, the mice develop FFI-like sleep abnormalities

- PrP^Sc may not always be necessary for disease pathogenesis!

Bouybayoune et al., PLoS Pathogens, 2015
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Talk Overview

- Mice injected with prions exhibit all the characteristics of human prion diseases
- Mouse studies have revealed that the prion protein (PrP) is an excellent therapeutic target
- Several molecules have been identified that extend the lifespan of mice infected with mouse prions
- As of yet, no molecule has been identified that is capable of extending the lifespan of mice infected with human prions
- Progress has been made towards the development of a mouse model of spontaneous prion disease, but challenges remain