Probing prion disease using case specific-derived neurons

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Background

- **Prion formation**: Conversion of normal prion protein \((\text{PrP}^C)\) into pathogenic protein \((\text{PrP}^\text{Sc})\)

- **Models**: No human-related models so far have been developed

- **iPSCs**: The induced pluripotent stem cells (iPSCs) have been used to probe other familial and sporadic neurodegenerative diseases and to screen potential compounds for therapeutics of the diseases
Insoluble Aggregates and Protease-resistant Conformers of Prion Protein in Uninfected Human Brains*

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PrPSc-like forms (insoluble; aggregates; PK-resistant) ≈ Silent prions
Determination of oligomeric state of PrP by ultracentrifugation in sucrose step gradients

<table>
<thead>
<tr>
<th>Top</th>
<th>Fractions</th>
<th>Bottom</th>
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<tr>
<td>1</td>
<td>2</td>
<td>3</td>
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PrPC (Monomers or small oligomers)  PrPSc-like form (aggregates)

Non-PrD: n=6; sCJD: n=4.

Small amounts of PrP aggregates are present in uninfected human brains

Yuan et al., JBC 2006
PK-resistant PrP is preferentially detected by 1E4 or anti-C but not by 3F4.

Yuan et al., JBC 2006
Oligomeric state of wild-type and mutant PrP from cultured cells

All cells contain PrP aggregates and mutation may facilitate PrP aggregation.
Detection of PK-resistant PrP in cells expressing wild-type and mutant PrP

Yuan et al., *CMLS* 2008

PK-resistant PrP (PrP*²⁰ and PrP*⁷) are present in cells expressing wild-type and mutant PrP
PrP is more resistant to protease in mutant than in wild-type PrP

Yuan et al., CMLS 2008
Inhibition of prion amplification by rHuPrP in PMCA

Yuan et al., 2013

PrPSc (seeds) + -
reProtein - rHuPrP rHuPDI -
PMCA - + - + - + + + +
3F4 34- 29- 20-
Lane 1 2 3 4 5 6 7 8 9
PK + -
MCT - - - +
PMCA - - + - + +
3F4 34- 29- 20-
Lane 1 2 3 4 5 6
PK - + +

Inhibition of PrPSc Amplification (%) vs. rHuPrP23-231 (nM)

- 100
- 60
- 20
0 30 60 120 240 480
rHuPrP23-231 (nM)
rHuPrP inhibits mouse PrP<sub>Sc</sub> in ScN2a cells

Yuan et al., 2013
Aim

To develop case-specific cell models to mimic the conversion event and to screen anti-prion compounds
Generation and application of iPSCs

Cell type
- Blood cell
- Hepatocyte
- Skin fibroblast

Reprogramming factors/delivery method
- Oct4, Sox2, Klf4, c-Myc, Nanog, Lin28
- Plasmids
- Viruses
- Proteins
- Small molecules

iPS cells

Evaluation

Application
- Cell therapy
- Drug screening
- Disease modeling
- Reprogramming mechanism

The Journal of Clinical Investigation
Differentiation of iPSCs into neurons and astrocytes

Diagram of stages and defined markers for isolation of neural stem cells, neurons and glia from neural induction cultures starting with pluripotent stem cells

Yuan SH et al., 2013
Fibroblasts from individuals carrying PrP mutations

- E200K
- D178N
- F198S
- E200K
Genotyping of DNA extracted from fibroblasts of PrP-mutation carriers
iPSC colonies from fibroblasts

Transduction with VSV-g pseudotyped lentivirus
Fibroblasts from KO and Tg mouse expressing human PrP\textsuperscript{N171S}
PrP in fibroblasts from the skin and muscle of KO and Tg mice expressing human PrP<sup>N171S</sup>

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>KO</th>
<th>TgN171S</th>
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<tbody>
<tr>
<td>brain</td>
<td>CTL</td>
<td></td>
</tr>
<tr>
<td>skin msl</td>
<td>27-</td>
<td>17-</td>
</tr>
<tr>
<td>skin msl</td>
<td>17-</td>
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3F4
Conclusions

- Skin samples have been obtained from five asymptomatic mutation-carriers and a sCJD patient.
- iPSC lines have been generated from two mutations (E200K and D178N).
- Fibroblasts have been also generated from KO, Tg N171S and TgWV.
- Profile of fibroblast PrP may be different from that of brain PrP.
Future studies

- To differentiate iPSCs into neurons and astrocytes
- To characterize PrP in fibroblasts, neurons and astrocytes
- To assess susceptibility of iPSC-derived neurons and astrocytes to human prions
- To screen anti-prions compounds
- To conduct gene repair in iPSCs to correct PrP mutation