Stem Cells and the Study of Neurodegeneration

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Techniques for studying mechanisms of neurological disease

- Animal models
- Human subjects
  - Postmortem analyses, imaging
- Induced pluripotent stem cells
Differential vulnerability to neurodegenerative diseases

- Spinocerebellar ataxia
- Alzheimer’s disease
- Parkinson’s disease
- Huntington’s disease
- Frontotemporal dementia
- ALS (Lou Gehrig’s disease)
- Narcolepsy
Development of stem cells from patients with Alzheimer’s disease

Schematic modified from Nishikawa et al, 2008
Overview of induced pluripotent stem cells (iPSCs)

Muscle cells

Pancreatic cells

Immune cells

Liver cells

Neurons and glia
Human iPSC neural differentiation

- Media
  - iPS/ES (DMEM/F12)

- Neural Induction (N2)

- Media
  - Neural Induction (N2/B27)
  + IGF, +cAMP

- Neural Differentiation (N2/B27)
  + BDNF, +GDNF
  + IGF, +cAMP

<table>
<thead>
<tr>
<th>aggregates</th>
<th>primitive NE</th>
<th>definitive NE</th>
<th>neuronal differentiation</th>
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<tbody>
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<td>iPS Cells</td>
<td>Day 4</td>
<td>Day 10</td>
<td>Day 16</td>
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Differentiation of human iPS cells to neuronal fates

Red = Oct4 = pluripotent cells
Green = MAP2 = neuronal cells
Blue = TOPRO3 = all cells
Living neurons from our patients with neurodegenerative diseases
Vulnerability to degeneration in Alzheimer’s disease differs among brain regions

**Cerebral cortex and hippocampus**
- Memory
- Thought
- Awareness
- Attention
- Consciousness

**Cerebellum**
- Movement
- Balance
- Coordination
- Posture
Aβ and Tau accumulate in the cerebral cortex prior to degeneration.
Mutation of APP causes early-onset Alzheimer’s disease

Genome editing with CRISPR/Cas to generate isogenic lines:
The Amyloid Hypothesis of Alzheimer’s disease pathogenesis

**Early onset/dominantly inherited AD**

- Missense mutations in the APP or Presenilin 1 or 2 genes
- Increased Aβ42 production throughout life
- Accumulation and oligomerization of Aβ42
- Subtle effects of Aβ oligomers on synaptic efficacy
- Microglial and astrocytic activation and attendant inflammatory responses
- Altered kinase/phosphatase activities lead to tau-containing tangles
- Widespread neuronal/synaptic dysfunction and selective neuronal loss, with attendant neurotransmitter deficits
- DEMENTIA

**Late onset AD**

- Failure of Aβ clearance mechanisms (e.g., inheritance of ApoE4; faulty Aβ degradation, environmental increases in Aβ production, etc.)
- Gradually rising Aβ42 levels in brain
- MEMORY LOSS/COGNITIVE DEFECTS
APP mutation does not affect neuronal differentiation

Muratore et al, HMG, 2014
APP mutation leads to increased Aβ42 and Aβ38 production.

Muratore et al, HMG, 2014
APP cleavage products increase over differentiation from immature to mature neuronal fates

[Graphs showing changes in APP cleavage products over differentiation days for control and fAD conditions.]
APP mutation leads to increased $\beta$-secretase cleavage of APP

Muratore et al, HMG, 2014
APP cleavage products increase over differentiation from immature to mature neuronal fates

Muratore et al, HMG, 2014
APP subcellular localization is altered in fAD iPSC-derived neurons

Colocalization coefficient - APP

Control | fAD

***

EEA-1/APP/MAP2/DAPI

Muratore et al, HMG, 2014
Early onset/dominantly inherited AD ("familial")

- Missense mutations in the APP or Presenilin 1 or 2 genes
- Increased A\textsubscript{\beta} production throughout life
- Accumulation and oligomerization of A\textsubscript{\beta}
- Subtle effects of A\textsubscript{\beta} oligomers on synaptic efficacy
- Microglial and astrocytic activation and attendant inflammatory responses
- Altered kinase/phosphatase activities lead to tau-containing tangles
- Widespread neuronal/synaptic dysfunction and selective neuronal loss, with attendant neurotransmitter deficits
- DEMENTIA

Late onset AD ("sporadic")

- Failure of A\textsubscript{\beta} clearance mechanisms (e.g., inheritance of ApoE4; faulty A\textsubscript{\beta} degradation, environmental increases in A\textsubscript{\beta} production, etc.)
- Gradually rising A\textsubscript{\beta} levels in brain
- MEMORY LOSS/COGNITIVE DEFECTS
Higher Tau protein levels are observed in fAD-derived neurons

Muratore et al, HMG, 2014
Aβ-specific antibodies (3D6 and AW7) bind secreted Aβ

Aβ bound to antibody:

Little unbound Aβ following antibody treatment:

APPs-α and β do not bind to antibody:

Muratore et al, HMG, 2014
Aβ-specific antibodies (3D6 and AW7) rescue the Tau phenotype

Muratore et al, HMG, 2014
The Amyloid Hypothesis of Alzheimer’s disease pathogenesis

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↓

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**DEMENTIA**

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- Gradually rising Aβ42 levels in brain

↓

**MEMORY LOSS/COGNITIVE DEFECTS**
Examining Glial Activation in Alzheimer’s Disease

[Diagram of the process involving IPSC (DNEMF12), Neural Induction (N2), Neural Induction (N2/B27), Neural Differentiation (N2/B27) with additional factors, and Astrocyte Co-culture, followed by images of Endogenous Astrocytes labeled as DAPI/MAP2/GFAP on Day 42 and Day 100.]

[C and D: Graphs showing relative gene expression A.U. for different genes across different time points (D0, D40, D100) with statistical significance indicated by asterisks (**, ***).]

Muratore et al, PLOS ONE, in press
APPV717I induces GFAP expression in astrocytes-derived from iPSCs

neuronal markers

astrocyte markers

TAU
βIII-tubulin

GFAP
S100B

control
fAD

WB: GFAP
WB: APOE
WB: S100B
WB: MAP2
WB: GAPDH

control 1AD
control 1AD
control 1AD
control 1AD
control 1AD

...
APPV717I induces GFAP expression in human astrocytes

Control neurons

fAD neurons

Add wild type human astrocytes (Sciencell)

![Graph showing GFAP protein levels normalized to glutamine synthetase (% control) for control (ctl) and fAD neurons with and without astrocytes (astros). The graph indicates a significant increase in GFAP levels in fAD neurons with astrocytes compared to control neurons and fAD neurons without astrocytes.](image)
Aβ secreted from fAD neurons induces GFAP expression in human astrocytes.

Control neuron CM

ctl ID Aβ ID

fAD neuron CM

ctl ID Aβ ID

human astrocytes (Sciencell)

![Graph showing GFAP/GS expression comparison between control CM and fAD CM.](chart.png)
The Amyloid Hypothesis of Alzheimer’s disease pathogenesis

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- **DEMENTIA**
Differential vulnerability of neuronal subtypes in Alzheimer’s disease

Susceptible in AD

Cortical/hippocampal forebrain neurons

Cerebellar and lower motor neurons

Relatively spared in AD

iPSCs → NPCs
Directed differentiation of iPSCs to alternate neuronal fates

Susceptible in AD

Relatively spared in AD

iPSCs NPCs

rostral neurons

caudal neurons

+RA/Shh
iPSCs directed to caudal neuronal fates show altered expression profiles
iPSCs directed to a caudal neuronal fate secrete a less toxic ratio of Aβ42/40.
Neurons susceptible to degeneration in Alzheimer’s secrete more toxic Aβ
Measuring $\beta\text{A}$ and sAPP$\alpha$ levels at the single cell level

Technology developed in JC Love lab (MIT) to study cytokine secretion
Current/Future directions: Measuring Aβ and sAPPα levels at the single cell level

Liao et al, unpublished
Current/Future directions: Measuring $A\beta$ and sAPP$\alpha$ levels at the single cell level

Liao et al, unpublished
1. Generation of new stem cell (iPS) lines from patients with early-onset, familial Alzheimer’s disease (fAD)

2. In all fates tested, the fAD APP V717I mutation leads to increased total Aβ, Aβ38 and Aβ42 generation

3. Aβ generation increases as stem cells differentiate to neuronal fates

4. Tau protein levels are increased in fAD neurons directed to a rostral but not caudal neuronal fate and Aβ-specific antibodies are able to rescue this phenotype

5. Aβ from fAD neurons stimulates GFAP expression in astrocytes

6. Directing differentiation to caudal fates versus rostral fates alters APP cleavage

7. Microengraving can be used to determine which cell fates secrete the highest levels of Aβ, and compare drug responsiveness in different cell types
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