Modelling Alzheimer’s disease in vitro

Laura Suter-Dick

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Alzheimer’s disease (AD)

Statistics:

- Worldwide, nearly 44 million people have AD or a related dementia
- Alzheimer’s and other dementias are the top cause for disabilities in later life
- The cost of caring for AD-patients in the U.S. is estimated to be $236 billion in 2016
- The global cost of AD and dementia is estimated to be $605 billion, which is equivalent to 1% of the entire world’s gross domestic product
**Alzheimer’s disease (AD)**

Forms of AD:

- Alzheimer disease (AD): most common form of age-related dementia, progressive memory loss and cognitive impairment

- Familial AD (FAD): Familial, early-onset (<60y.), autosomal dominant forms of AD caused by mutations in \( \beta \)-amyloid precursor protein (APP), presenilin 1 and 2 (PSEN1, PSEN2)

*Castellani & Perry, Biochem Pharm Review, 2014*
Current treatment for AD

Symptomatic treatment

The U.S. Food and Drug Administration (FDA) has approved two types of medications

1) Cholinesterase inhibitors (Aricept, Exelon, Razadyne): They inhibit the acetylcholinesterase from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine

2) Memantine (Namenda): Acts on the glutamatergic system by blocking NMDA receptors. It has been associated with a moderate decrease in clinical deterioration with only a small positive effect on cognition, mood, behavior, etc to treat the cognitive symptoms (memory loss, confusion, and problems with thinking and reasoning) of Alzheimer's disease.

β- and γ- secretase inhibitors

DAPT  N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-S-phenylglycine t-Butyl Ester
A cell-permeable dipeptide that inhibits γ-secretase and suppresses Aβ production (Aβ total IC₅₀ = 115 nM; Aβ₄₂ IC₅₀ = 200 nM)
Reported to reduce extracellular Aβ plaques and intracellular Aβ accumulation in 3xTgAD transgenic mice

Compound E
A cell permeable, γ-secretase inhibitor XXI

β-secretase inhibitor IV
A cell-permeable isophthalamide compound containing hydroxyethylamine motif that binds to BACE-1 active site and potently blocks its proteolytic activity (IC₅₀ = 15 nM for BACE-1, human and 29 nM for sAPP_NF in HEK293-APP⁴⁶₁₇⁹⁸ cells).

SGSM41
γ-secretase modulator, potently inhibiting the generation of Aβ₄₂ and to a lesser extent Aβ₄₀ while concomitantly increasing Aβ₃₈ Aβ₃

Biologicals

Antibodies against A-beta: solanezumab (a humanised monoclonal antibody that promotes β-amyloid clearance in the brain), failed in recent Phase III clinical trials
Key factors in AD

Musiek & Holzman, Nature Neurosciences, 2015
APP encodes for beta-amyloid precursor protein

Swedish double: K670N, M671L described in Swedish families, 1992
increase Aβ levels early onset AD

London mutation: V717I first described in APP, 1991
English and American families most common worldwide
increase Aβ42, early onset AD

http://www.alzforum.org/databases
PSEN1 encodes presenilin-1, a subunit of the γ-secretase.

S290C mutation
T291-S319 del=ΔE9
english family (Perez-Tur et al., 1995)
abrogates the splice acceptor site
so that exon 9 is spliced out of transcripts
early onset AD
In vivo systems: No mouse models fully replicate the human disease

Table 1 - Neuropathological features of the main transgenic mouse models of Alzheimer disease.

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Gene (mutation)</th>
<th>Intraneuronal Aβ</th>
<th>Parenchymal Aβ plaques</th>
<th>Hyperphosphorylated Tau</th>
<th>Neurofibrillary tangles</th>
<th>Neuronal loss</th>
<th>Synaptic loss</th>
<th>CAA</th>
<th>Primary reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDAPP</td>
<td>APP (V717F)</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
<td>Games et al. 1995</td>
</tr>
<tr>
<td>Tg2576</td>
<td>APP (K570N/M671L)</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>Hsiao et al. 1995</td>
</tr>
<tr>
<td>TgCRND8</td>
<td>APP (K570N/M671L, V717F)</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>Chishti et al. 2001</td>
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<tr>
<td>APP/PS1</td>
<td>APP (K570N/M671L), PS1 (M146L)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Holcomb et al. 1998</td>
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<tr>
<td>APP23</td>
<td>APP (K570N/M671L)</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Little</td>
<td>Yes</td>
<td>Yes</td>
<td>Sturchler-Pierrat et al. 1997</td>
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<tr>
<td>Tg-SwDI</td>
<td>APP (E693Q, D694N)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Davis et al. 2004</td>
</tr>
<tr>
<td>APPDutch</td>
<td>APP (E693Q)</td>
<td>-</td>
<td>Little</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Herzig et al. 2004</td>
</tr>
<tr>
<td>APPDutch/PS1</td>
<td>APP (E693Q), PS1 (G384A)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Little</td>
<td>-</td>
<td>-</td>
<td>Herzig et al. 2004</td>
</tr>
<tr>
<td>hAPP-Arc</td>
<td>APP (E693G, K670N/M671L, V717F)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Little</td>
<td>-</td>
<td>Cheng et al. 2004</td>
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<tr>
<td>Tg-ArcSwe</td>
<td>APP (E693G, K670N/M671L)</td>
<td>Yes</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Lord et al. 2006, Knobloch et al. 2007</td>
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<td>APPArc</td>
<td>APP (E693G)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Rönnbäck et al. 2011</td>
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<tr>
<td>TAPP</td>
<td>APP (K570N/M671L), Tau (P301L)</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Lewis et al. 2001</td>
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<tr>
<td>3xTg-AD</td>
<td>APP (K570N/M671L), Tau (P301L), PS1 (M146V)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>Oddo et al. 2003</td>
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<td>APP/PS1</td>
<td>APP (K570N/M671L, V717F), PS1 (M146L)</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Wirths et al. 2002</td>
</tr>
<tr>
<td>APP/PS1KI</td>
<td>APP (K570N/M671L, V717F), PS1 (M233T/L235P)</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Casas et al. 2004</td>
</tr>
<tr>
<td>SxFAD</td>
<td>APP (K570N/M671L, I716V, V717I), PS1 (M146L/L286V)</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Oakley et al. 2006</td>
</tr>
</tbody>
</table>

CAA = cerebral amyloid angiopathy; Dash (-) = not reported.
In vitro systems

2D cultures: secreted amyloid β diffuses into a large volume of media
3D cultures: accelerate amyloid β deposition by limiting its diffusion and allowing for aggregation
Goal of the project

- The goal of the research was to generate an *in vitro* model to recreate key events involved in the pathophysiology of AD
  - Deposition of A-beta plaques
  - Hyperphosphorilation of Tau and intracellular deposition of pTau tangles
  - Neuroinflammation

Walker and Jcker, 2013
Walker and Jcker, 2013
Swartfager et al., 2014
Differentiation of the human neural progenitors ReN VM cells

2 week-differentiated ReN cells
Lentiviral transduction

- Polycistronic lentiviral constructs

APP-SL: APP with both K670N/M671L (Swedish) and V717I (London) mutations
PSEN-DE9: PSEN1 with deleted exon 9

LETTER

A three-dimensional human neural cell culture model of Alzheimer’s disease

Se Hoon Choi*, Young Hye Kim†, Matthias Heblisch‡, Christopher Silwinski‡, Seungheun Lee†, Carla D’Avanzo†, Hechao Chen†, Basavaraj Hooli‡, Caroline Asselin‡, Jullem Muffat‡, Justin B. Klee‡, Can Zhang†, Brian J. Wäniger*, Michael Peitz‡, Dora M. Kovacs‡, Clifford J. Wolff*, Steven L. Wagner‡, Rudolph E. Tanzi§ & Doo Yeon Kim†
**GFP enrichment of the FAD GFP+ cell lines**

- **P3** GFP-high: 45.4%
- **P5** GFP-high: 29.1%
- **P7** GFP-high: 29.6%

- **P0** GFP-APP-PSEN high: 52.6%
- **P2** GFP-APP-PSEN high: 21.3%
- **P4** GFP-APP-PSEN high: 24.5%
- **P7** GFP-APP-PSEN high: 28.3%

- **P0** GFP-APP-PSEN low: 27.9%
- **P2** GFP-APP-PSEN low: 22.7%
- **P4** GFP-APP-PSEN low: 9.1%
- **P6** GFP-APP-PSEN low: 8.8%
Expression of the mutated constructs in enriched FAD GFP+ ReN cells

APP primer binding sites

FACS1 | FACS2

APP

Wt+Mut (380 bp)

London mutation:
V717I

APP London primer binding sites

FACS1 | FACS2

London mutated APP

Mut (357 bp)

APP Swedish primer binding sites

FACS1 | FACS2

Swedish mutations:
K670N; M671L

Swedish mutated APP

Mut (217 bp)

PSEN1 primer binding sites

FACS1 | FACS2

PSEN\AE9:
S290C

Presenilin1

Mut (221 bp)

Wt (308 bp)

Expression of the mutated constructs in enriched FAD GFP+ ReN cells
GFP expression of differentiated FAD 3D-cultures

Cell death increased in GAP High expressing cells after 9 weeks => related to FAD-phenotype?
Viability of the 3D cultures

>8 weeks: reduction of axonal connectivity and cell death in FAD-line
Aβ pathology: production of Aβ42 from 6 weeks onwards
Aβ pathology: Aβ deposits in 6 week-old 3D cultures

GFP-APP-PSEN High (6 weeks)

GFP-APP-PSEN Low
12 weeks
pTau pathology: analysis of 3R/4R Tau isoforms by RT-PCR
pTau pathology: IF on 9 and 10-week old 3D cultures
Neuroinflammation: Inclusion of inflammatory cells (THP-1)

THP-1 cells - conditioned medium

2D THP-1 cultures

3D ReN cultures

THP-1 diff. adapt to ReN medium 24h Activation 1μg/mL LPS 24h

6h: THP-1 diff. adapt to ReN medium 24h Untreated control 24h

control: THP-1 diff. adapt to ReN medium 24h

3D matrigel thin layer ReN culture 10 days in differentiation 48h incubation

THP-1 diff. adapted to ReN medium 24h

Activation 1μg/mL LPS 24h

THP-1 diff. adapted to ReN medium 24h

Untreated control 24h

THP-1 diff. adapted to ReN medium 24h

Activation 1μg/mL LPS 6h

3D ReN cultures

24h: ReN cell culture

6h: THP-1 diff. THP-1 diff.

adapt to ReN medium 24h

Untreated control 24h

24h: THP-1 diff. THP-1 diff.

adapt to ReN medium 24h

Untreated control 24h

THP-1 diff. conditioned medium

THP-1 diff. conditioned medium

TNFα

control

control

6h-THP1 conditioned medium

24h-THP1 conditioned medium

6h-THP1 conditioned medium
Neuroinflammation: Inclusion of inflammatory cells (THP-1)

Differentiated THP-1 were printed on 3D Matrigel-ReN printed cells (3-day differentiated) using a RegenHU Bioprinter and imaged after 5 days in culture (A: opening time: 100μsec; B: opening time: 200μsec).
Neuroinflammation: Inclusion of THP-1 leads to neuronal cell vacuolization

Production of TNF-α

Concentration of TNF-α (pg/mL)

Printed cultures


Conclusions

- Cells expressing FAD mutations are functional in terms of neuronal and glial differentiation
- Phenotype
  - Secretion of Ab42 in 2D and 3D cultures
  - Detectable Abeta deposits in 6week and 12week -old 3D cultures
  - Expression of p-Tau in axons, dendrites and cell bodies in 10week-old 3D cultures
  - Increased neuronal death in FAD-high cell line
- Amenable to co-culture with THP-1 and bioprinting
- Inflammatory stimuli (co-culture) cause neuronal damage

Unparalleled opportunity to study the mechanisms underlying AD and the effects of pharmacological interventions
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