Modelling Alzheimer’s Disease:

A High Throughput-Compatible Assay for Detecting Tau Aggregation Using iPSC-Derived Cortical Neurons

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Outline

- iPSC and related initiatives in Europe: EBiSC
- Alzheimer’s Disease & tau
- Modelling tau aggregation using iPSCs
Induced Pluripotent Stem Cells: *iPSCs*

Nobel Prize in Physiology or Medicine 2012
“for the discovery that mature cells can be reprogrammed to become pluripotent”
A virtually unlimited source of human cells available for drug discovery

Non-embryonic and non-tumoral
European Bank for induced pluripotent Stem Cells

Creating a self financing stem cell repository for Europe

The EBiSC - European Bank for induced pluripotent Stem Cells project has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115582, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. www.imi.europa.eu
What is the EBiSC project?

A €35 million, IMI funded project through which 26 leading European organisations will establish a central facility for the collection, testing and distribution of iPS cells to researchers.
At a Glance
http://www.ebisc.org/

- Pfizer co-ordinates
- Roslin Cells Ltd manages
- International SAB/EAB
- 3 yr. duration
- Total Project Value €32M
- IMI Grant of €22M
- 6 EFPIA members
- 6 SMEs
- 8 Universities
- 5 public agencies
- 1 charity funded institute

Establish a centralized activity in EU for the collection, testing and distribution of iPS lines
Centralized activity which is not for profit & self sustaining
Why create EBiSC?

With EBiSC: better use of research assets

- Research projects creating iPSCs
- EBiSC
  - Creates distribution stocks & ensures quality
  - Provides samples of iPSC lines to EBiSC
- Other researchers
  - Get iPSCs of known quality, faster & at less cost

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What will EBiSC do?

EBiSC : improving the research landscape in Europe

Research projects creating iPSCs

Other researchers

Establish central facilities which use best cell culture technologies to operate at scale

Consent forms & contracts which meet needs of all stakeholders

Common standards for processing and testing cell lines

Data management system which provides extensive data to users but controls access

Create a catalogue of cell lines which meet user needs
• **By end 2016:** validating working pipeline: reception, QC/banking, expansion, distribution and phenotyping

• **Beyond 2016:** expand catalogue to meet user demand leading to a self financing operation by 2019.
More about EBiSC...

Contact us at: ebisc@eurtd.com

Visit our website at: www.ebisc.eu
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Dementia: one of the most significant social and health crisis of 21st century

- Worldwide, **nearly 44 million** people have Alzheimer’s or a related dementia: Denmark, Norway, Sweden, Belgium and The Netherlands (16.8M)

- It cannot be prevented, cured or slowed.

Source: ADI
Alzheimer’s Disease Pathophysiology

Amyloid plaques

“Extracellular deposits of amyloid-β peptide abundant in the cortex of AD patients”

Neurofibrillary tangles (NFTs)

“Intraneuronal aggregates of hyperphosphorylated and misfolded tau”
High correlation between tau lesions and degree of dementia

- Braak stages: Clear **correlation** between the spatiotemporal progression of NFT pathology and cognitive state.

- NFTs, neuron loss, and synaptic loss, parallel the progression of **cognitive decline**

- Specific **genetic variants of tau** are associated with familial forms of frontotemporal dementia (FTD)
Tau – in normal and diseased state

- Tau is expressed in the CNS, primarily in axons of neurons.
- Tau binds to and stabilizes microtubules thus ensuring proper transport of cargo to the nerve endings.
- In Alzheimer’s disease tau is phosphorylated by multiple kinases, detaches from microtubules and aggregates which finally leads to neuronal death.
- It is hypothesized that toxic tau-species can spread from cell to cell thus “infecting” neighbouring neurons and spreading the disease.

Therapeutic approaches

- Blocking aggregation
- Stop spreading
- Promoting clearance

Aggregated tau induces misfolding of normal tau (seeding hypothesis).

Evidence for seeding in vitro and in vivo
Outline

- iPSC and related initiatives in Europe: EBiSC
- Alzheimer’s Disease & tau
- Modelling tau aggregation using iPSCs
Need for a physiologically relevant/robust model to study TAU aggregation

- Human/Neuronal specific
- High reproducibility (≠primary cultures)
- Sensitive
- Scalable (high throughput)

Verheyen et al., PLoS One, 2015
Medda et al., J Biomol Screen (under review)
Robust differentiation protocol

- Commercially available iPSC lines
- Generation of NPCs (Axol biosciences):
  • Dual SMAD inhibition 12 days
  • Freezing stocks
- Final differentiation: BDNF, GDNF, cAMP
- 384w format (HCl and HTS)
Robust culture conditions to allow standardization:

- Homogeneous cell distribution (HCI)
- Long term stability/adherence (synapse strength)
- Reduced shearing stress due to media change/plate handling
- Scalability/automation (avoid pre-coating)

- Neurospheres/Organoids
  - Intrinsic capacity to form in vitro a “cortical like” structure.

- Scaffolding biomaterials:
  - Matrigel ®
Matrigel scaffold

- Use of matrigel:
  - Homogeneous cell distribution.
  - Synaptic stability/strength
  - Reduces shearing stress
  - Compatible with imaging
Matrigel scaffold

• Low density matrigel:
  - Dilution 1:15 (MG:N2B27)
  - Cells sediment before MG gels
  - Cells are “coated” with matrigel
  - Thickness: 50um (approx.)
  - For TAU aggregation assays

• High density matrigel:
  - Dilution 1:1 (MG:N2B27)
  - No sedimentation, fast gelling (37C)
  - Cells are “embedded” in matrigel
  - Thickness: 200um (approx.)
  - For electrophysiology

7 Days in vitro

7 weeks in vitro
Dynamic culture format to maintain healthy neurons

• 96 well plate, 30K cells/well
• 384 well plate, 10K cells/well
Dynamic culture format to maintain healthy neurons

- IncuCyte®
Differentiated neurons are transducible
Adenoviral-mediated TAU aggregation using iPSC derived neurons

- **Experimental setup:**
  - 10k Cells/well
  - AAV P301L
  - 384WP: 75μL media, 20μL lysate

- **Conditions:**
  - No treated cells (C-)
  - No treated cells + K18 Fibrils (K18)
  - AAV P301L
  - AAV P301L + K18 (P301L + K18)
TAU aggregates in human neurons

*n=3, One-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett’s multiple comparison test versus non seeded control).
How to measure TAU aggregation?

**alphaLISA - TAU**

- alphaLISA is the non-wash ELISA assay alternative
- alpha = amplified luminescent proximity homogeneous assay
- 3-4 hours assay

The higher the emission, the more TAU aggregation
Defining baseline values and sampling time

A

Aggregated TAU (HTAU10/HTAU10)

- No AAVs
- P301L-AAVs + K18
- EGFP-AAVs

RFU ± SEM

B

TOTAL TAU 35 DIV (HT7/HTAU10)

C

Aggregated TAU (HTAU10/HTAU10)

RFU ± SEM

D

Total TAU (HT7/HTAU10)

RFU ± SEM

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).
K18 Dose response
($Z' = 0.52$)
Shortening protocol: 22 days
Assay reproducibility: across cell lines and users

**Aggregated TAU Line #2**

- RFU ± SEM

**Aggregated TAU User #1**
- Relative to control (+/- SEM)

**Aggregated TAU User #2**

**Aggregated TAU User #3**

*n=3, One-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).
New assay to study TAU aggregation

- Human/Neuronal specific
- High reproducibility (≠primary cultures)
- Sensitive
- Scalable (high throughput)
Towards the standardization of iPSC technology for drug development in neuroscience: Perspectives and challenges

1. To tackle different aspects of tau pathology: seeding, aggregation, clearance?

2. Test available mutant cell lines and isogenic controls: less artificial

3. Optimizing maturation process: mature neurons in shorter time, co-culture (astrocytes)

4. Testing chemically defined scaffolding materials: eg. hyaluronic acid
Thanks!

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