PRION-LIKE PROCESSING OF PATHOLOGICAL PROTEINS AS A UNIFIED BASIS FOR PHARMACEUTICAL DEVELOPMENT IN NEURODEGENERATIVE DISORDERS

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Diseases of protein aggregation

Synthesis and folding

Ribosome
Nascent polypeptide
Folding intermediate
Native protein
Quarternary complex
Environmental stress, mutations or translational errors
Refolding

Misfolding and aggregation

Amyloid fibrils
Prefibrillar aggregates
Partially misfolded
Misfolded
Disordered aggregate
Degradation

X

Different Proteins – Common General Theme

The spread of disorders may depend on the protein:
- α-synuclein for PD,
- tau for AD
- huntingtin inclusions (found in nuclei) for HD.

For FTD, where tau or TDP-43 constitute most cases, the regions affected are frontotemporal, but precise staging not determined yet.
Tau Biology and Pathology as an Example
Normal tau function – axonal cytoskeleton

Tau-Tubulin binding via repeat domain

(A) Tau projection arms microtubules assembled with Tau
(Hirokawa et al 1988 J Cell Biol 107: 1449)

(B) Bundling without Tau
But Tau-KO mice are viable - other MAPs can substitute
(Harada et al 1994 Nature 369:489)

Axonal microtubules essential for transport of synaptic vesicles, mitochondria, tubulin recycling
**MAPT** - the Tau gene

Tau cloned from mouse brain by Gloria Lee (1988). Highly conserved among different species

*MAPT* is over 100 kb on chromosome 17q21-22 having at least 16 exons

Six tau isoforms by alternative splicing of exons 2, 3 and 10

Isoforms are developmentally regulated, the 3R isoforms alone are expressed in fetus.

Exon 10 encodes one of 4 tandem repeat regions of 31-32 aa; 4R tau bind microtubules better than 3R tau
Tau mutations in Frontotemporal dementia with parkinsonism linked with chromosome 17 (FTDP17)

Mutations disrupting tau-microtubule interactions and enhancing tau filament polymerisation (also observed for K280Δ)

- I260V (ATC→GTC)
- G272V (GGC→GTC)
- P301S (CCG→TGC)
- V337M (GTG→ATG)
- L260V (ATC→GTC)
- G272V (GGC→GTC)
- P301L (CCG→CTG)
- E342V (GAG→GTG)
- G389R (GGG→CGG)

Mutations affecting splicing of exon 10 (4R:3R mRNA increased, with the exception of K280Δ, where the ratio is decreased)

- K257T (AAG→ACG)
- K280Δ (ΔAAG)
- N279K (AAT→AAG)
- L284I (CTT→CTC)
- S305N (AGT→AAT)
- S305S (AGT→AGC)
- N296N (AAT→AAC)
- R406W (CGG→TGG)

5 intronic mutations

- +3 [g→a], +12 [c→t], +13 [a→g], +14 [c→t], +16 [c→t]

Mutations surrounding tandem repeat region (tubulin-binding domain)

- Tau protein (441 residues)

- Mutations
  - Human tau exons
  - Attem. spliced exons
  - Untranslated exons
  - Tubulin-binding domains
Tau protein normally stabilises axonal microtubules: but polymerises to form Paired Helical Filaments in AD

- Tau protein normally functions to stabilize axonal microtubules
- These are critical for axonal transport in cortico-cortical association circuits

In AD Tau aggregates to form first oligomers then fibrous aggregates composed of de novo Tau polymers - Paired Helical Filaments (PHFs)
- Alzheimer called these fibrous aggregates “neurofibrillary tangles”
Molecular dissection of Tau pathology of AD
Only 5% of tangle/PHF Tau is phosphorylated ptau

**Tau histopathology of AD**

- Tangle
- PHFs

**Structure of PHF core**

- Phosphorylated tau only in outer coat$^2$, <5% of PHF tau by mass$^2$ and biochemistry$^3$

References:

2. Wischik et al., PNAS, 1988, 85:4884-4888;
PHF core is composed of 3 Tau repeat domain species, all from phase-shifted repeat domain truncated at Glu-391.

90% pure PHFs\(^1\)

Core Tau units all from repeat domain\(^2\)

Glu-391 truncation due to aggregation\(^3\)

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**Tau phase-shift in PHF\(^2\)**

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\(i\) PHF-tau

<table>
<thead>
<tr>
<th>Linker repeats</th>
<th>PHF-core tau linker repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>LKHQPGGGKVQIVYPVDLSKVTSCGSLGN</td>
<td>LKHQPGGGKVQIVYPVDLSKVTSCGSLGN</td>
</tr>
<tr>
<td>IHHKPQGGQVEKVSEKLDFKDRVQSKIGSDLN</td>
<td>IHHKPQGGQVEKVSEKLDFKDRVQSKIGSDLN</td>
</tr>
<tr>
<td>ITHPGGGNNKKIETHKLT</td>
<td>ITHPGGGNNKKIETHKLT</td>
</tr>
</tbody>
</table>

\(\text{Start: 297} \quad \text{End: 301}\)

**Normal tau**

<table>
<thead>
<tr>
<th>Linker segments</th>
<th>Full-length tau tubulin-binding repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Start: 297} \quad \text{End: 301})</td>
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</tr>
</tbody>
</table>

\(\text{Start: 297} \quad \text{End: 301}\)

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\(^1\) Wischik et al., PNAS, 1988, 85:4506-4510

\(^2\) Novak et al., EMBO J, 1991, 88:5837-5841

\(^3\) Wischik et al., PNAS, 1996, 93:11213-11218
Core-oligomer of the Tangle-PHF Drives Template-Directed Proteolysis: Self-Replicating\textsuperscript{1}, ie Prion-like\textsuperscript{2}

\textsuperscript{1} Wischik et al., PNAS, 1996, 93:11213–11218
\textsuperscript{2} Wischik et al., 1997, in Microtubule-Associated Proteins: Modifications in Disease., ed. J. Avila, R. Brandt, K. S. Kosik. pp. 185-241
Template-Directed Proteolysis in Cell Models\(^1\)

Oligomers produced in cells consist of ~100 units

\(^1\) Harrington et al., J Biol Chem, 2015, 290 10862-10875
Formation and transmission of toxic oligomers is common feature of several neurodegenerative diseases
Pattern of spread of Tau pathology in AD = Braak Staging

- Braak stages defined by pattern seen in low-power view of medial temporal lobe

Braak staging\(^1\) measures aggregated Tau load in the brain

Tau aggregation spreads from medial temporal lobe structures to isocortex.

Tau concentrations in neocortex correlated with Braak stage \(^2\).

<table>
<thead>
<tr>
<th>Braak 1</th>
<th>Braak 2</th>
<th>Braak 3</th>
<th>Braak 4</th>
<th>Braak 5</th>
<th>Braak 6 (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild: Transitional Stages</td>
<td>Moderate: Limbic Stages</td>
<td>Severe: Isocortical Stages</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tau load and distribution correlate with clinical severity and neuroimaging defects (PET & SPECT)

Braak stage and scan deficits¹

Braak 2-3

Braak 3-4

Braak 4-6

Braak stage and clinical decline²

Mild AD (MMSE ≥ 24)

Moderate AD (MMSE 15-23)

Severe AD (MMSE ≤ 14)

1 Jobst et al., 1992, J Neurol Neurosurg Psychiat 55, 190-194
Bradley et al., 2002, Br J Radiol 75, 506-513
Nishimura et al. 2007, Ann Nuc Med 21, 15-23

Importance of age-related impairment of mitochondrial turnover by endosomal-lysosomal pathway

- Potential to identify markers of up-stream endosomal-lysosomal dysfunction

Utility of Methylthioninium (MT) as Tau Aggregation Inhibitor
LMTX™ (stable reduced form of MT) overcomes absorption and tolerability limitations of MTC (methylthioninium chloride)\(^1,2\)

MTC is oxidised MT\(^+\) and suffers from dose-dependent food interference with absorption, but poorly tolerated in the fasted state

LMTX is a distinct chemical entity which delivers the same active moiety

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2 Harrington et al., J Biol Chem, 2015, 290 10862-10875
Cell-free and Cell-based models for identifying Tau Aggregation Inhibitors

Tau-tau binding at central linker

mAb 423

Glu-Ala-390

Inhibition of Tau aggregation in cells

1 Harrington et al., J Biol Chem, 2015, 290 10862-10875
PHFs Isolated from AD Brain are Dissolved by MT and Proteolytic Stability of PHF-core Unit is Reversed¹

¹ Wischik et al., 1996, PNAS 93:11213-11218
PHF-based and Cell-based Activity of MT

Isolated PHF  PHFs dissolved at increasing drug concentration

KI = 0.12 µM

PHT-tau at ~2 µM

Harrington et al., J Biol Chem, 2015, 290 10862-10875
Hypothetical Molecular Dynamic Modelling of MT Action

A. β-sheet stabilised with salt bridges

B. Complexation with MT

C. Disruption of β-sheet and release of MT
Similar Mechanism for Synuclein In Parkinson’s Disease
Lewy bodies


Ubiquitin-immunoreactive Lewy bodies in:
(a) substantia nigra
(b,c) cortex.

▷ LB
▷ melanin
α-Synuclein protein

α-Synuclein: small, thermostable protein, lacking secondary or tertiary structure, but acquires a high level of α-helix on binding to membranes and acidic phospholipids.

Divided into three functional domains

Three pathogenic mutations in the N-terminal domain

- A30P
- A53P
- E46K

Parkinson’s disease mutations:
Similar mechanism in Parkinson’s disease: $\alpha$-Synuclein

- Intracellular lesions
- Aggregation of thermostable protein
- Sporadic PD and rare cases caused by mutations
- Selective neuronal vulnerability

From: Lansbury (2002)
α-Synucleinopathies: neuronal transmission like AD

Lee et al. (2010) Nature Rev Neurol. 6_702
Cell-free assay of synuclein aggregation for aggregation-inhibitor activity

- Lewy bodies in PD are composed of synuclein aggregates
- TauRx’s lead Synuclein Aggregation Inhibitor (SAI) blocks aggregation \textit{in vitro}

<table>
<thead>
<tr>
<th>Compound</th>
<th>( B_{50} ) (( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMTX</td>
<td>130.8 ± 15</td>
</tr>
<tr>
<td>TRx0018</td>
<td>3.8 ± 0.3</td>
</tr>
</tbody>
</table>

\textit{in vitro} inhibition of heparin-induced synuclein aggregation
Cell-based model of synuclein aggregation for aggregation-inhibitor activity

- Lewy bodies in PD are composed of synuclein aggregates
- TauRx’s lead Synuclein Aggregation Inhibitor (SAI) blocks aggregation in cell model

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC$_{50}$ (µM)</th>
<th>LD$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMTX</td>
<td>1.17 ± 0.30</td>
<td>62</td>
</tr>
<tr>
<td>TRx0018</td>
<td>0.08 ± 0.02</td>
<td>38</td>
</tr>
</tbody>
</table>

Cell-based inhibition of synuclein aggregation and truncation
Molecular Dynamic Modelling of Synuclein aggregation: MT disrupts β-sheet structure around aggregation domain

A. High affinity “cage structure” stabilising β-sheet configuration

B. MT H-bonds to β-sheet and disrupts β-sheet backbone

C. Unravelling of β-sheet, and MT released
Effect of LMTX™ on counts of syn-positive cells in motor cortex in synuclein L62 mice

3 months (†)

*  
1way ANOVA  
p = 0.0319

6 months (†)

motor cortex  

1way ANOVA  
p < 0.0001

Strong efficacy signal with LMTM at 5 and 15 mg MT/kg/day  
Values are represented as mean ± S.E.
Similar Mechanism for TDP43 in Fronto-Temporal Dementia
rCBF SPECT in FTD

Axial

Sagittal
### Pathology of FTLD syndromes

#### Relationship between Clinical Features, Pathology and Proteinopathies in FTLD sub-syndromes

[modified from Cairns et al, 2007 and Josephs 2008]

<table>
<thead>
<tr>
<th>Sub-syndrome</th>
<th>bvFTD</th>
<th>SD</th>
<th>PNFA</th>
<th>FTD-MND</th>
<th>FTDP-17</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical features</strong></td>
<td>Predominantly behavioural</td>
<td>Predom. language</td>
<td>Predominantly language</td>
<td>Behavioural Language + MND</td>
<td>Behavioural Language +/- EPS</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td>PiD-CBD-PSP-types</td>
<td>FTLD-U types 1-3</td>
<td>FTLD-U types 1-3</td>
<td>PiD-CBD-PSP-types</td>
<td>FTLD-U types 1-3</td>
</tr>
<tr>
<td><strong>Molecular pathology</strong></td>
<td>Tau</td>
<td>TDP-43</td>
<td>TDP-43</td>
<td>Tau</td>
<td>TDP-43</td>
</tr>
</tbody>
</table>
Molecular pathogenesis of TDP-43 proteinopathies: the aggregating species differs from the trigger species

- **GRN mutations**
  - PGRN
  - GRN peptides

- **TARDBP mutations**
  - TDP-43 aggregates

**protease**

- Truncated TDP-43

- TDP-43 aggregates

- Neuronal dysfunction

- Neuronal death
TDP-43 protein aggregation is inhibited by MT

Two SH-SY5Y cell models:  

a) TDP-43(ΔNLS&187-192)  
b) C-terminal TDP-43(162-414)

exhibit phosphorylated/ubiquitinated, TDP-43 aggregate inclusions

TDP-43 aggregates decreased 50% by 50nM MT (in both models)
Similar Mechanism for Huntingtin In Huntington’s Chorea
Autosomal dominant disease – 1:10,000

Personality, cognitive and movement disorder

Caused by an expansion of the polyglutamine-coding CAG trinucleotide repeat in the gene encoding huntingtin (htt)

Mutant htt that is not cleared accumulates as inclusions in nucleus and cytoplasm

MT inhibits aggregation *in vitro* and in several HD models

Huntingtin aggregation inhibited by MT (1)


MT inhibits aggregation of N-term-httEx1Q53 from either monomers or oligomers/fibrils.
Huntingtin aggregation inhibited by MT (2)


MT ameliorates RotaRod phenotype in R6/2 mouse (115 CAG repeats)

MT decreases aggregation in Drosophila expressing Httex1Q93 & increase in number of rhabdomeres per omatidium
Conclusions

• Prion-like processing of aggregating proteins is increasingly recognised as a common underlying mechanism of molecular pathogenesis in a number of progressive neurodegenerative disorders
• This involves a primary conformational change in a critical aggregating domain that becomes self-propagating
• The oligomers produced in this way are able to infect neighbouring neurones, invading previously unaffected brain regions
• The aggregating domain has a distinctive conformation which makes it amenable to pharmaceutical inhibition
• The methylthioninium (MT) moiety has promise for treatment of a range of neurodegenerative disorders of late life
Neurodegenerative disorders potentially treatable with LMTX™

Cognitive disorders:
- AD  Alzheimer’s disease (Tau)
- FTLD  Fronto-temporal dementia (Tau / TDP43)
- CTE  Chronic traumatic encephalopathy (Tau)

Movement disorders:
- PSP  Progressive supranuclear palsy (Tau)
- CBD  Cortico-basal degeneration (Tau)
- PD  Parkinson’s disease (Synuclein)
- HD  Huntington’s disease (Huntingtin / Tau)