“Overview Of TDP-43 Proteinopathies"

John Q. Trojanowski, M.D., Ph.D.
Institute on Aging, Alzheimer’s Disease Center, Morris, K. Udall Parkinson’s Disease Center of Excellence, Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA
Neurodegenerative Diseases Characterized by Filamentous Aggregates of Misfolded Proteins

<table>
<thead>
<tr>
<th>Disease</th>
<th>Lesions</th>
<th>Components</th>
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<tr>
<td>Alzheimer’s Disease</td>
<td>SPs</td>
<td>Amyloid $\beta$</td>
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<td>NFTs</td>
<td>Tau</td>
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<td></td>
<td>LBs</td>
<td>$\alpha$-Synuclein (~50%)</td>
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<td>UBIs</td>
<td>TDP-43 (25-30%)</td>
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<td>Dementia with Lewy Bodies</td>
<td>LBs</td>
<td>$\alpha$-Synuclein</td>
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<td>Multiple System Atrophy</td>
<td>GCIs</td>
<td>$\alpha$-Synuclein</td>
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<td>Parkinson’s Disease</td>
<td>LBs</td>
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<td>Prion diseases</td>
<td>SPs</td>
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<td>Tauopathies</td>
<td>NFTs</td>
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<tr>
<td>Trinucleotide repeat diseases</td>
<td>Inclusions</td>
<td>Expanded polyglutamine</td>
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<td>polyglutamine</td>
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<td>Amyotrophic Lateral Sclerosis</td>
<td>Spheroids</td>
<td>TDP-43</td>
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<td>Frontotemporal Degeneration</td>
<td>Inclusions</td>
<td>TDP-43</td>
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Mechanisms And Consequences Of Protein Misfolding And Brain Amyloidosis In Neurodegenerative Diseases

Background: Frontotemporal Degeneration

FTDs are the 2nd most common cause of dementia < 65 years

Clinical features:
- progressive changes in behavior, personality and language
- FTD may be associated with parkinsonism and motor neuron disease

> 30% of FTD patients have a positive family history
- Chrom. 17: MAPT
- Chrom. 17: PGRN
- Chrom. 9: VCP
- Chrom. 9: unknown
- Chrom. 3: CHMP2B

Early Efforts To Isolate the Disease Protein In FTLD-U Using Proteomics And Other Methods Failed

1) Biochemical fractionation of proteins from brain
   Protein stain after SDS-PAGE
   Ub Immunoblot
2) Aggregate enrichment
3) Sucrose density gradient
4) Protease resistance in limited digestion
5) Affinity purification of ubiquitinated proteins
6) Proteomics Approach
   2-D electrophoresis: DIGE, Ub Immunoblot
   Isotope coded affinity tag-based (ICAT)
   Ion-exchange chromatography
Identification Of TDP-43 As The Disease Protein In FTLD-U & ALS Using The “Brute Force” Monoclonal Antibody Approach

Identification of TDP-43 by LC-MS/MS in FTD-U Cases

TDP-43 Gene (TARDBP)

- TDP-43 is encoded by the TARDP gene located on chromosome 1.
- It is a predominantly nuclear protein that is highly conserved across evolution, and ubiquitously expressed in all tissues including heart, lung, liver, spleen, muscle, and brain.
- Protein structure resembles the domain composition of members of the hnRNP family.
Functions of TDP-43

- Transcription regulation
  - HIV-1 TAR DNA
  - mouse SP-10 gene promoter
- Exon splicing
  - CFTR exon 9
  - Apo AII exon 3
- Neuronal activity-responsive factor
- mRNA stabilization, hNFL
- Others
  - microRNA biogenesis-associated with Microprocessor complex Drosha/DGCR8
  - apoptosis-specific caspase substrate in apoptotic Jurkat T lymphocytes
  - cell division-upregulated in cells infected by RSV
TDP-43 stains all types of inclusions in FTLD-U

Verification of TDP-43 as protein component in FTLD-U inclusions

Type 1

Type 2

Type 3

HDDD2
FTLD-U cases show disease-specific biochemical signature of TDP-43

TDP-43 is hyperphosphorylated in FTLD-U

Neumann M et al, Science 2006
TDP-43 is ubiquitinated in FTLD-U

Neumann M et al, Science 2006
Ubiquitin-inclusions in ALS are TDP-43 positive

Neumann M et al, Science, 2006; Mackenzie et al, 2007
Ubiquitin-inclusions in ALS are TDP-43 positive in all sporadic ALS and most familial ALS except that due to CHMP2B and SOD1 mutations.

Neumann M et al, Science, 2006; Mackenzie et al, 2007
ALS shows the same biochemical signature of TDP-43 as FTLD-U

Neumann M et al, Science, 2006; Mackenzie et al, 2007
Inclusions in FTLD-U with VCP Mutations (IBMPDBFTD) are TDP-43 Positive

Neumann M et al, J Neuropath Exper Neurol, 2007
TDP-43 reveals additional white matter pathology in FTLD-U

Neumann M et al, J Neuropath Exper Neurol, 2007
TDP-43 Inclusions in FTLD-U do not have Properties of Amyloid
TDP-43 Inclusions Lack The Staining Properties Of Amyloids And Have Scant Fibrils By EM

TDP-43 Pathology Occurs In Synucleinopathies

DLB+AD = 25/80 (31%); PD = 5/69 (7%); PDD = 4/21 (19%); controls = 1/33 (3%); DLB 0/10 (0%); Total = 213

A subset of TDP-43 positive cases show significant CA1/subiculum neuron loss.

*Dementia represents all patients from DLB+AD, DLB, PDD

** significant differences (p<0.05)


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** significant differences (p<0.05)
ALS / ALS-D: FREQUENCY OF TPD-43 PATHOLOGY

ALS Is A Mulytsystem Prtoeinopathy

ALS / ALS-D: THE HEAT MAP

Geser F et al, Arch Neurol, 2008
Guam-ALS-PDC is a multi-system TDP-43 proteinopathy as 100% of cases show TDP-43 pathology albeit less than tau pathology.

Biochemical characterization of novel termini-specific TDP-43 polyclonal antibodies

Characterization of a novel C-terminal specific TDP-43 monoclonal antibody

TDP-43 immunoblotting of sarkosyl-insoluble fractions from different FTLD-U subtype cases before and after alkaline phosphatase treatment

Anti-TDP-43 C-terminal antibodies detect more neuropathology than N-t antibodies in dentate gyrus and frontal cortex of FTLD-U subtypes.

TDP-43 Neuropathology in Spinal Cord Motor Neurons of ALS Cases are Comprised Mostly of Full Length TDP-43

TDP-43 pathology is the same in cortex and spinal cord of FTLD-U with MND and ALS

SUMMARY: TDP-43 pathology was studied in AD = 182; CBD = 39; PSP = 77; PiD = 12, and it was seen in 25.8 % of AD cases (only in dentate gyrus & entorhinal cortex in 75%; more widespread in 25%). PSP and PiD showed no TDP-43 inclusions, but unique glial TDP-43 pathology was seen in 15 % of CBD cases. TDP-43 pathology occurs in tauopathies, albeit to a more limited extent than in FTLD-U and ALS.

TDP-43 pathology in corticobasal degeneration
TDP-43 Proteinopathies

**FTLD-U**
- **Type 1**
  - Pathology: Long, tortuous DN in superficial cortical laminae
  - Relatively few NCI & GCI
  - No/rare NII
  - MND: Variable
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: Unknown

- **Type 2**
  - Pathology: Numerous NCI & GCI in superficial & deep cortex
  - Few DN
  - No/rare NII
  - MND: Frequent
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: 9p13.3-21.3*

- **Type 3**
  - Pathology: Numerous NCI, GCI & short DN in superficial cortex
  - Variable NII
  - MND: Variable
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: PGRN

- **Type 4**
  - Pathology: Numerous NII & DN, superficial > deep cortex
  - Few NCI & GCI
  - MND: Variable
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: VCP

**ALS**
- **ALS**
  - Pathology: NCI & GCI in anterior horn
  - Variable NCI, GCI, & DN in extramotor cortex
  - MND: No
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: Unknown

- **ALS-D**
  - Pathology: NCI & GCI in anterior horn
  - Numerous NCI, GCI, & DN in extramotor cortex
  - MND: No
  - Sporadic disease: Yes
  - Familial disease: Yes
  - Gene/locus: Unknown

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*IFT74 is a candidate gene for chromosome 9p-linked ALS-FTLD [12]*

**TDP-43 pathology not identified in fALS with SOD1 mutations**

TARDBP Mutations Are Pathogenic For Familial (G290A, G294A, G298S, A315T, Q331T, M337V, Q343R) And Sporadic (D169G, G287S, G348C, R361S, N390D/S) ALS While An A90V Substitution May Be A Risk Factor For ALS/FTLD

4) Van Deerlin V, et al. Mutations in the TARDBP gene cause familial amyotrophic lateral sclerosis with TDP-43 neuropathology. Lancet Neurol, 2008 (In red)
1) The discovery of TDP-43 by Neumann et al. puts a “molecular face” on the ubiquitin-tagged neuronal inclusions in ALS, ALS-FTLD and FTLD which are part of a spectrum of TDP-43 proteinopathies.

2) The discovery of multiple TARDBP gene mutations in 2008 that are pathogenic for sporadic and familial ALS confirms the initial report that TDP-43 is the disease protein in FTLD/ALS.

3) Thus, TDP-43 now is a target for drug discovery, and these TDP-43 findings open up new opportunities for developing better ways to diagnose and treat ALS, ALS-FTLD and FTLD.

4) These are the lessons learned from similar discoveries that put a “molecular face” on the plaques and tangles of Alzheimer’s disease (AD), i.e. Aβ and tau, respectively, and >60 clinical trials are in progress now to test drugs that target pathways of Aβ and tau mediated AD neurodegeneration.

5) Hence, efforts are needed to ramp up the development of assays to detect TDP-43 in CSF, blood and urine for the early and reliable diagnosis of ALS, ALS-FTLD and FTLD, as well as to map the disease pathways that convert normal TDP-43 into pathological TDP-43, and discover strategies to abrogate TDP-43 mediated neurodegeneration.
The Discovery Of Aβ Opened Up New Avenues For AD Drug Discovery & TDP-43 May Do The Same For FTLD-U/ALS

- Glenner and Wong (1984)- Abeta is the protein entity in cerebrovascular amyloid plaque in DS/AD
- Masters et al. (1985)- Abeta is the protein in cerebral plaque in AD, and forms neurofibrillary tangles (NFTs – but only the plaque part turned out to be correct)
- Robakis et al.; Kang et al. (1987)- cloned human APP gene on chromosome 21
- George-Hyslop et al. (1987)- linkage to same region as APP in 4 FAD families
- Goate et al. (1991)- 1st APP gene mutation identified in FAD (“London mutation”)
- Corder et al.; Strittmatter et al. (1993)- apoE 4 allele association sporadic AD
- Sherrington et al. (1995)- PS 1 mutations discovered in FAD
- Games et al, (1995) – PDAPP Tg mouse model of AD-like amyloid plaques
- Vassar et al, Yan et al. (1999)- identification and characterization of BACE
- Schenk et al, (1999) Abeta vaccine proof of concept study in PDAPP mice
- Etc, etc, etc
- >100 clinical trials on AD now in progress in 2008
Potential Mechanisms of TDP-43 Mediated Neurodegeneration

- Aggregation & sequestration of TDP-43
- Dystrophic neurites
- Cytoplasmic inclusions
- Intranuclear inclusions

Genetics
- PGRN mutations
- VCP mutations
- Locus on chr 9p

Other factors
- Epigenetics
- Environment

- Deregulation of transcription
- Alterations in mRNA splicing or stability
- Alterations in TDP-43-dependent signaling or trafficking
  - ‘Toxic’ gain of function

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