The Molecular Pathology of Neurodegeneration

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Objectives

- To understand the concept of neurodegenerative proteinopathies - the basis of neuropathological classification of neurodegenerative diseases

- To understand the basic histological and biochemical characteristics of tau, β-amyloid, α-synuclein, TDP-43 and FUS in neurodegenerative disorders

- To understand some pathogenic implications of abnormal protein aggregates

- To understand the basic molecular and pathological features of the cascade of events in Alzheimer’s disease as an example of neurodegeneration
The ‘proteininopathy cascade’

Mutation, environment

protein → misfolded protein → aggregation

beta-sheet

deposition

Neurodegeneration
Neurodegenerative diseases as ‘proteinopathies’

‘the big four’:

- Tau
- β-amyloid
- α Synuclein
- ?? (ubiquitinated protein(s))

Tolnay & Probst, 1999
Alzheimer’s disease: theories of pathogenesis

- Tau hypothesis
- Amyloid hypothesis
Tau
Normal Tau Protein

- Abundant low molecular weight microtubule (MT) associated protein found mainly in axons
- Promotes MT polymerisation, binds to MTs and stabilises MTs within the cytoskeleton
- Can be phosphorylated at a range of Ser and Thr residues
- Excess phosphorylation of tau inhibits its ability to bind and stabilise microtubules
Normal tau physiology

- Neuron diagram showing normal microtubule network, acetylated-tau, phospho-tau, intact microtubules, tubulin, tau.
- Normal sorting of tau to axons with normal levels of acetylated and phosphorylated tau.

= normal axonal transport and synaptic functioning
Human brain Tau isoforms generated by alternative splicing

There are six isoforms of human CNS tau

PRD = proline-rich domain

M1-M4 = C-terminal repeat microtubule binding domains

Figure from Hanger DP, Anderton BH, Noble W. Trends Mol Med. 2009 Mar;15(3):112-9
PHF-Tau Proteins

- Insoluble
- Form filamentous deposits in neuronal cell bodies/processes and glia
- Aberrantly hyperphosphorylated at Ser/Thr; ubiquitinated
- Shown \textit{in vitro} to be unable to bind to microtubules unless dephosphorylated
Tau pathophysiology

Tau becomes missorted to cell soma and dendrites. Through altered kinase activity, tau is aberrantly phosphorylated. Tau also cleaved by Aβ-induced caspase activity - seeds tau self-assembly into oligomers and then larger insoluble aggregates of NFTs (tangles of PHF).

- Abnormal levels of missorted phosphorylated/acetylated tau = decreased tau-microtubule interactions = microtubule network disruption

= trafficking deficits (e.g. mitochondria), spine & synapse loss
= disrupted hippocampal circuitry and cognitive deficits
PHF-Tau Form NFTs

- Two twisting strands
- alternating width between 8nm and 20nm

Lee et al. Science. 1991; 251:675-8
Analysis of AD brain samples reveals many phosphorylation sites on tau.

Candidate pathological tau kinases in AD include GSK-3, cdk5, CK1 and PKA.
Tau dysfunction and AD pathogenesis

Genetic Factors
- Tau Mutations
- Alteration of 4R/3R Ratio
- Loss of Tau Function
- Gain of Toxic Function
- APP, PS1, PS2 Mutations

Environmental Factors
- Hyperphosphorylation
  - DeP-Tau
  - Kinases
  - Phophatases
  - P-Tau

Tau Dysfunction
- Tau Aggregation/ MT Loss
- Impaired Transport & Neurodegeneration
Neuropathology:
Six “Braak stages” describe the clinical progression of AD, based on the development and spread of neurofibrillary tangles (NFTs).
The spectrum of major tauopathies

(A) Immunochemistry images showing NFTs in AD, Pick bodies in PiD, astrocytic plaques in CBD, and tufted astrocytes in PSP.

(B) Immunoblot analysis of Sarkosyl-insoluble tau before (c) and after (dp) alkaline phosphatase treatment with anti-tau antibody HT7.

β-amyloid
Amyloid hypothesis of AD

- Assumes that neurotoxicity of beta amyloid drives the neurodegenerative process
Beta amyloid

- Beta amyloid is a 39-43 amino acid peptide
- Derived from 700 amino acid amyloid precursor protein (APP)
- APP may be processed to "amyloidogenic" or "non-amyloidogenic" pathways
APP Processing - Aβ production (amyloidogenic)
APP Processing – non-amyloidogenic
Mutations in the APP and presenilin genes that cause familial forms of Alzheimer’s disease alter APP processing and Aβ production

Swedish double APP mutation increases total Aβ production
London APP mutation increases Aβ1-42 production (more amyloidogenic form?)
Dutch APP mutation causes more amyloidogenic Aβ
Presenilin mutations increase ratio of Aβ1-42:Aβ1-40 (i.e. more Aβ1-42 but total Aβ levels unchanged)
‘amyloid cascade’ hypothesis

Cummings JL.
Evaluating the amyloid hypothesis- for and against

- Clinicopathologic correlation
- Genetics of AD
- Cell culture studies
- Animal studies
Pathologic correlates of dementia severity

- Amyloid plaques: poor
- Neurofibrillary tangles: better
- Neuronal loss: as tangles
- Synaptic density: best
Synapse loss is a structural correlate involved early in cognitive decline in mild Alzheimer’s disease.

**Synapse Loss in Frontal Cortex Biopsies in Alzheimer’s Disease: Correlation with Cognitive Severity**

Steven T. DeKosky, MD, and Stephen W. Scheff, PhD

Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment.
Pathologic correlates of dementia severity

- Amyloid plaques: poor
- Neurofibrillary tangles: better
- Neuronal loss: as tangles
- Synaptic density: best

Summary: clinico-pathological studies do not really support the amyloid hypothesis

(Terry, 1991)
Evaluating the amyloid hypothesis - for and against

Genetics of AD----------------------for
  * Autosomal dominant AD associated with mutations in amyloid precursor protein (APP)
  * Trisomy 21 also associated with over-expression of APP and AD
  * “presenilin” initially identified in autosomal dominant AD, has since been shown to be a component of gamma secretase (enzyme which processes APP to beta amyloid)
‘Transgenic’ mice overexpressing a familial mutant form of human APP model some aspects of AD

APP<sub>swe</sub>

Between 6 - 14 months old

- Accumulation of soluble Aβ oligomers
- Impaired synaptic plasticity well before Aβ deposits & plaques
- Learning and memory deficits

The ‘swedish’ mouse model of AD

From 12 -14 months onwards

- Abundant neuritic Aβ plaques
- Astrogliosis, reactive microgliosis
- Some abnormal tau phosphorylation
- But no NFTs!
- No neuronal loss!
Evaluating the amyloid hypothesis- for and against

- Clinicopathologic correlation---against
- Genetics of AD-------------------for
- Cell culture studies--------------for
- Animal studies------------------- mixed
Alzheimer’s disease: environmental risk factors

- Low education
- Head injury
- Depression
- Vascular risk factors (Hypertension, Diabetes mellitus, hypercholesterolemia)
- ....
Multifactorial Diseases

Number of patients

Genetic Environment

Early-onset Late-onset
• There is now good experimental evidence for a causal relationship between Ab aggregates and tau in AD.
• Tau may mediate Ab-induced toxicity in AD.
Evidence suggests that Aβ pathology lies upstream of tau pathology

- Early-onset AD cases caused by mutant PSEN1/2 or APP, which are by definition ‘Aβ-triggered’, are always accompanied by Tauopathy.

- Experimentally, in transgenic mouse models of AD with combined Aβ and tau pathology, Aβ pathology precedes tau pathology.

- Transgenic mice expressing mutant tau protein develop NFTs. When these mice are crossed with Tg2576 mice expressing mutant APP and high levels of Aβ, the NFT pathology is substantially enhanced.
Clinical Trials
Aβ Immunisation study

Holmes et al., Lancet 2008
The pathological cascade of AD

Clinical symptoms

- Neurofibrillary tangles
- TAU hyperphosphorylation
- Environmental risk factors
- APP
- β-amyloid

Genetic risk factors
- Apo-E
- PS1,2
- Pathogenetic mutations

Cholinergic dysfunction

Neurodegeneration
α-synuclein
Normal α-Synuclein

- An abundant synaptic protein, present to a lesser extent in cell body and axons, but also in oligodendroglia.
- Other members of the synuclein family of synaptic proteins include β and γ-synuclein.
- Function is unknown but may play roles in synaptic transmission.
- Is a phosphoprotein, but role of α-synuclein phosphorylation in its normal function is unknown.
Pathological α-Synuclein

- Forms insoluble filamentous aggregates with the properties of amyloid
- Amino acids 71-82 in the NAC domain are the minimal, essential sequences required for fibrilization
- Filamentous α-synuclein inclusions form in neuronal cell body, processes and in glial cells
- Is abnormally phosphorylated, nitrated and ubiquitinated
Major synucleinopathies

- Parkinson's disease - familial and sporadic
- Dementia with Lewy bodies
- Multiple system atrophy
- Neurodegeneration with brain iron accumulation-1 (formerly Hallevorden-Spatz disease)
- Pure autonomic failure
α-synuclein dysfunction/aggregation & neurodegeneration

Genetic Factors

α-Synuclein Mutations or Duplications

Environmental and/or Genetic risk factors

? Synuclein Dysfunction and/or Aggregation

Neurodegeneration
α-Synuclein aggregation

Fink AL, Acc Chem Res. 2006
Western blot analysis of α-synuclein differentially extracted with Tris HCl, Triton-X, Sarkosyl or urea from cerebral cortices of a patient with dementia with Lewy bodies (DLB) (D) and a normal control individual (C) probed with LB509 (upper panel) or anti-Pser129 (lower panel).
α-Synuclein in pathological inclusions

Lewy bodies

Lewy neurites

Glial cytoplasmic inclusions
TDP-43
Neurodegenerative diseases as 'proteinopathies'

'The big four':
- Tau
- β Amyloid
- α Synuclein
- ? (ubiquitinated protein)
Ubiquitin +ve inclusions in ALS

Lowe et al., 1989
Spectrum of FTLD-U neuropathology detected by anti–TDP-43.

Immunohistochemistry of FTLD-U frontal cortex with anti–TDP-43 reveals robust staining of UBIs in FTLD-U (A) type 1, (B) type 2, (C) type 3, and (D) HDDD2. (E and F) Strong staining of UBIs (arrowheads) in hippocampal dentate granule neurons. Note clearing of nuclear TDP-43 (arrows) in UBI-bearing neurons compared that of with normal neurons (*). TDP-43–positive lentiform (H) and round (G) intranuclear UBIs in HDDD2 and Lewy body–like round inclusions in motor neurons of spinal cord (I). Scale bar in (A) corresponds to 50 μm [(A) to (D) and (G)], 25 μm [(E) and (F)] and 20 μm [(H) and (I)].
ALS

Mackenzie et al., Ann Neurol 2007
TDP-43 proteinopathies

-ve

FTLD-U

Type 1
Type 2
Type 3
Type 4

ALS

SOD-1 fALS
Usually TPD43 -ve

& ALS-PD

AD

DLB

PSP

CBD

Forman 2007
FUS
FUS

Implicated in ALS and FTLD pathogenesis

• Ubiquitin +ve
• TDP-43 –ve (!)
FUS/TLS

- 526 amino acids; 63 kDa
- first identified in human myxoid and round cell liposarcomas as an oncogenic fusion protein
- closely related to Ewing's sarcoma (EWS) protein
- component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complex involved in pre-mRNA splicing and transport of processed mRNA to the cytoplasm
- involved in transcriptional activation and interacts with the RNA polymerase
- C-terminal half of FUS/TLS contains several structural motifs involved in RNA binding (RRM, RGG, Zn-finger)
Mutations in FUS, an RNA Processing Protein, Cause Familial Amyotrophic Lateral Sclerosis Type 6

Science, 2009

Fig. 2. Patients with FUS mutations develop cytoplasmic FUS immunoreactive inclusions in lower motor neurons. Antibody to FUS immunolabels inclusions within the anterior horn (dotted line) of the spinal cord in patients with FUS mutations (A to F and arrowheads in O). Staining in controls (G, H, and M), mutant SOD1 FALS (I and J), and SALS (K, L, and N) demonstrate diffuse nuclear staining (arrows) with variable intensity without inclusions. Scale bars, 12 μm [(A) to (L)], 50 μm [(M) to (O)].
FUS in aFTLD-U

Neumann M et al., *Brain*, 2009
FUS – a unifying protein in TDP-43 negative FTLD-U cases

aFTLD-U = FTLD-FUS
FUS biochemistry

Figure 3 Biochemical analysis of FUS. Proteins were sequentially extracted from aFTLD-U, FTLD-TDP and control (CO) brains. High salt (lane 1), Triton X-100 (lane 2), RIPA (lane 3), 2% SDS (lane 4) and formic acid (lane 5) fractions were separated by 7.5% SDS-PAGE and immunoblotted with anti-FUS antibody (RP A600-302A). All cases showed a strong ~73-kDa band in the soluble high-salt fraction (lane 1). Although the amount of SDS-soluble FUS (lane 4) was variable within each group, aFTLD-U cases always showed a strong band that was greater than that seen for most of the controls.

Figure 4 Ratio of insoluble to soluble FUS. Band intensities of FUS in insoluble (SDS fraction) and soluble (high salt and Triton-X100 fractions) were analysed and the ratio calculated. Ratios are depicted as a box and whiskers blot that shows the range of values, with the box being subdivided into the 25 and 75% quartiles by the median; circles represent outliers, filled rhombus represent the mean. Although there is some overlap, the aFTLD-U group showed significantly higher ratios compared to both the FTLD-TDP and control groups ($P < 0.05$).

Chromosome 9

De-Jesus Hernandez
p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS

Safa Al-Sarraj · Andrew King · Claire Troakes · Bradley Smith · Satomi Mackawa · Istvan Bodi · Boris Rogelj · Ammar Al-Chalabi · Tibor Hortobágyi · Christopher E. Shaw
Original Article

An MND/ALS phenotype associated with C9orf72 repeat expansion: Abundant p62-positive, TDP-43-negative inclusions in cerebral cortex, hippocampus and cerebellum but without associated cognitive decline

Claire Troakes, Satomi Mackawa, Lokesh Wijesekera, Boris Rogelj, László Siklo,
Christopher Bell, Bradley Smith, Stephen Newhouse, Caroline Vance, Lauren Johnson, Tibor Hortobagyi, Aleksey Shatunov, Ammar Al-Chalabi, Nigel Leigh, Christopher E. Shaw, Andrew King and Safa Al-Sarraj.
Recommended reading

- Fink AL. The aggregation and fibrillation of α-synuclein. Acc Chem Res. 2006; 39:628-34
- Iwatsubo T. Pathological biochemistry of α-synucleinopathy. Neuropathology. 2007; 27:474-8
- Sreedharan J. TDP-43 mutations in familial and sporadic ALS. Science. 2008; 319:1668-1672
An excellent website for the latest AD research can be found at: http://www.alzforum.org/

RESEARCH NEWS

- **Ironing Out Apoptotic Role for New APP-Binding Protein**
  2 November 2012. In a hunt for molecules that bind amyloid-β precursor protein, researchers have discovered a mitochondrial ion transporter that promotes programmed cell death in neurons...

- **Animal Model Redux: New Lessons From Old Transgenics?**
  2 November 2012. At the SfN annual meeting, several presentations raised questions about current animal models and their relationships to disease mechanisms...

- **Growth Factor Stabilizes Cell Skeleton, Rescues Motor Neurons**
  29 October 2012. Ciliary neurotrophic factor (CNTF) differs from other trophins in that it not only boosts cell survival, but also protects axons, and thus neuronal function...

- **ApoE4 Promotes AB Oligomerization**
  26 October 2012. How does apolipoprotein E (ApoE) boost risk for late-onset Alzheimer’s disease (LOAD)?...

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IN THE SPOTLIGHT

- **Student Webinar: Pre-SfN Workshop Offers Leg up on AD Career**
  Before hopping over to the 42nd annual Society for Neuroscience meeting, about 35 international students and