Karen Duff
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Speakers Bureau
None

Clinical Trials
None

Consultant
None currently

I own no stocks or equity in any pharmaceutical company
The pathology of Alzheimer’s disease

- Extracellular deposits (plaques) composed of \( \text{A}_{\beta} \) peptides in brain regions involved in memory.
  - Intracellular neurofibrillary tangles composed of abnormal protein tau in paired helical filaments.

- Inflammatory response (activated astrocytes and microglia).

- Immune response (complement pathway activation).

- Loss of synapses, abnormal cell processes (neurites), degeneration of neurons.
  - Cognitive impairment (memory loss).
Pathology in areas of initial vulnerability spreads to other areas as the disease progresses.

- **Pre-dementia or very early stage**
- **Early-mid stage**
- **Severely affected stage**
Plaque and tangle distribution at different stages of Alzheimer’s disease progression (Braak staging)

Prodromal

transentorhinal
I - II

Early-Moderate

limbic
III - IV

Moderate-Late

isocortical
V - VI

Tangles

Amyloid plaques
Spread of tau pathology follows a distinct neuroanatomical path that suggests network connectivity.
Increased phosphorylation
Accumulation in cell bodies
Redistribution of tau from axonal to somatodendritic compartments
Conformational change
Abnormal tau not removed from cell
dCell dysfunction, death
Age-related degeneration of several systems
Elevated Aβ
Neuropsin-tTA mouse ("activator")
tTA activator is driven by the promoter for the neuropsin gene (Yasuda and Mayford, 2006).

Human tau mouse ("responder")
tau line (rTg4510) consisting of a tetracycline-operon–responsive element (TRE) placed upstream of a cDNA encoding human tau with a mutation that accelerates tangle formation. NO EXPRESSION expected without presence of tTa.
Distribution of tau pathology changes as disease progresses
-correlates with mature tangle formation

MC1
As pathology worsens, tangles spread into extrahippocampal and cortical areas and increase in the CA1.

1. Agranular insular cortex
2. Ectohinal cortex & Secondary somatosensory cortex
3. Granular cell layer of OB
4. Dorsal peduncular cortex
5. Lateral septum
No overt cognitive impairment in the Morris Water Maze at ~22 months
CBV deficit in EC-tau mouse at 24 mo vs control

Deficit in EC and CA1

CBV measured by fMRI (gadolidium contrast T2 weighted imaging) correlates very well with FDG-PET which is the imaging gold-standard measure of metabolic activity.

N=10 EC-tau vs. 10 –tau littermates, ~24 mo, reconstructed global image analysis

Scott Small, CUMC
Hippocampus pathology spreads with progressive disease

As no endogenous human tau is expressed in the granule cells, human tau protein has transferred from EC cells to granule cells across the synapse in the MML.
Tangle-tau can alter the conformation of normal tau by “seeding” (similar to prion - )

And this ability to alter normal tau can spread through the brain-

Mouse injected with normal tau (control)  Mouse injected with abnormal (tangle) tau

stain for tangles
POSSIBLE MODES OF SPREAD

1) Dysfunction in cell A induces dysfunction and *de novo* pathology in neighboring cell B

2) Secretion of tau molecules from cell A induces dysfunction and *de novo* pathology in neighboring cell B

3) Secretion or transfer of tau molecules from cell A to neighboring cell B

Propagation to new cells follows abnormal tau conformation “templating” to normal tau and repeat of cycle

(c) somatodendritic/synapse degeneration releasing tau into the extracellular space?
Is exogenous tau internalized by neurons, and if so, by which compartments?
Microfluidic (Campenot) chambers separate cell populations, and cellular compartments.
Conformers formed from recombinant and endogenously produced tau
Small tau aggregates are taken up by neurons and transported anterogradely.

β-tubulin III (neuron specific)
DAPI

0.4uM hTau40 dimer and trimers for 12 h
...and retrogradely where it coalesces in the cell bodies
Tau aggregates are internalized via endocytosis in neuronal somatodendritic compartments (and axonal terminals – not shown).

Additional controls for endocytosis include temperature block, use of Dynasore to block dynamin mediated endocytosis and Pit2b to test for clatherin mediated endocytosis.
LMW oligomers exist in MultiVesicular Bodies, small vesicles and lysosomes

HeLa cells

Immuno-EM with human tau specific antibody and lysosome marker, LAMP1.
Scale bar=250nm

Sabrina Simoes
Recombinant tau aggregates induce tau pathology in mouse brains- No co-factors involved in uptake/propagation

*rTg4510* mice were injected with recombinant tau aggregates. Tau pathology was examined at 11 weeks post injection.
How does pathology spread for Amyloid differ from tau?
Neuropsin-tTA mouse

tTA activator is driven by the promoter for the neuropsin gene (Yasuda and Mayford, 2006).

Neuropsin-tTa-APP mouse

Generated by crossing the Neuropsin-tTA activator line with a tet inducible APP responder line (line DBo885, Jax labs, strain B6) that encodes a mutant, chimeric mo/huAPP695 (swe/ind KM670, 671NL, and V717F) transgene (Jankowsky et al. 2005).
82E1 – N terminus (Aβ1-5) specific – recognizes β cleaved APP (βCTF, Aβ) but not full length APP, human preferential.
Horikoshi et. al. 2004
20+ month old EC-APP mice make mature and diffuse plaques
Cell-cell propagation to GC layer not apparent in APP mice
AD – A disease of neural networks

How is pathology spread linked to functional spread eg. metabolism, cognitive impairment?
Is AD primarily a disease of pathology, or dysfunction of networks?

Order of events with respect to pathology, synaptic degeneration cell death?

-Why do certain cell populations show initial vulnerability?
  Is there cell type vulnerability to tau propagation?

Why do 4R tauopathies show diversity?

Therapeutic opportunities – can pathology (and dysfunction) spread be attenuated before it gets to regions that cause overt cognitive impairment?

If tau spreads from cell to cell, can spread be attenuated by therapies acting only in the extracellular space – for example antibodies (immunotherapy)?
Duff lab
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Rakez Kayed and Martin Margatti (recombinant tau protein)
GFAP EC-tau 24 mo
Uptake of tau aggregates inhibited by blocker of dynamin mediated endocytosis

Uptake of tau aggregates does not lead to overt caspase (3,7) induction
LMW oligomers bind to cells and are internalized

HeLa

Untreated

hTau40

hTau40 + trypsin 0.25%, 3 min

Graph showing hTau bound cells (% of total cells)
LMW oligomers but not monomer or larger tau aggregates bind to cells

HeLa

MC17

Green=tau
Red=tubulin
Blue= nuclei (DAPI)
LMW tau conformers are internalized with dextran

LMW tau conformers are internalized with dextran

Internalization of Tau and dextran can be blocked by reducing temperature (blocks endocytosis)