Protein misfolding and aggregation is a common feature of neurodegeneration

- Alzheimer: Amyloid-β plaques cortex
- Parkinson: α-synuclein substantia nigra
- Amyotrophic lateral sclerosis: Ubiquitin inclusions spinal cord
- Creutzfeldt-Jakob: PrP Sc plaques cerebellum
- Huntington: Poly-Q inclusions striatum

*Modified from Forman et al, Nature Medicine, 2006*
Prion conformational subtypes: Distinct disease phenotypes occurring in patients

Distinct conformations

Subtype 1

Subtype 2
Human prion disease: prion plaques vary in morphology and cells targeted

Sporadic CJD Plaque deposits
Perineuronal deposits
vCJD Plaques
Prion protein aggregates vary by morphology and cells targeted in mice

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PrP
What mechanism underlies the selective cell vulnerability in prion disease?
Heparan sulfate proteoglycans and prion pathogenesis

- Diverse glycoproteins on cell surfaces and in the extracellular matrix
- Promotes the internalization of PrP\textsuperscript{C} and propagation of PrP\textsuperscript{Sc}
- Prolongs prion disease in scrapie-infected rodents
Hypothesis:

PrP\textsuperscript{Sc} interaction with heparan sulfate proteoglycans is a major determinant underlying prion cell tropism
Defining the HS molecules associated with the most common human CJD subtypes

1) Purify PrPSc from 3 brain regions

Prion-infected brain

2) Identify the PrPSc bound heparan sulfate by liquid chromatography-mass spectrometry (LC/MS)

Quantify N-SO₃, 2-O-SO₃, 6-O-SO₃ groups and N-acetylated, N-sulfated and N-unsubstituted glucosamine residues

Amyloid β: cerebral amyloid angiopathy (CAA)

IHC  Congo red  HE
How does the sulfation of HS molecules impact prion replication?

- **Seed PrP<sup>Sc</sup>**
- **Heparan sulfate**
- **PrP<sup>C</sup> produced in RK13 cells**
  - Glycosylated
  - GPI-anchored
  - 3F4-tag
- **Increase in PrP<sup>Sc</sup>**
- **Detection of new PrP<sup>Sc</sup>** using 3F4 tag
- **Amplification of misfolding**
- **Fragmentation of aggregates creates new seeds**
- **Different variably sulfated HS**
Heparin and pentosan polysulfate enhance prion conversion in a dose-dependent manner.
Heparin and PPS lead to a decrease PrP\textsuperscript{Sc} levels in persistently prion-infected cells

Demonstrates the paradoxical effect of HS – in cell lysate amplification versus in live cells and in vivo
The impact of sulfation of HS chains on prion disease progression

Wild type mice

Ext1 +/- : shorter HS chain length

Challenge with prions

Terminal disease

Analysis of brain

Biological properties

Biochemical properties

Histopathology

Ongoing……
Conclusions

• PrP\textsuperscript{Sc} purified for mass spectrometry analysis to identify the heparan sulfate bound to PrP\textsuperscript{Sc}

• Pentosan polysulfate promotes PrP\textsuperscript{Sc} formation in vitro in the PMCA assay

• Heparin and pentosan polysulfate decrease PrP\textsuperscript{Sc} levels in prion-infected cells in culture
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