Neuronal ERVK protein deposition in ALS: An aspect of TDP-43 misregulation

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Protein Quality Control mechanisms maintain cellular proteostasis

- Mammalian cells contain a large number of undesirable proteins
- Managed by PQC processes to alleviate the risk of proteotoxicity
- Cell-type specific differences in maintaining proteostasis
  - Neurons are the most vulnerable to aberrant protein deposition

Diagram:
- Ubiquitin proteasome system
- Autophagy system
- Stress granule response
When proteostasis goes bad: Protein aggregation in the cell

- Defective PQC pathways lead to protein deposition and aggregation
- Many pathogenic consequences of protein aggregates
- Protein aggregation is a hallmark of major neurodegenerative diseases
Protein aggregation in ALS: Pathological effects of TDP-43 aggregates

- Characterized by aggregation of cellular proteins in affected neurons
- TDP-43 is a major component of cytosolic protein aggregates
- Many pathological effects of cytosolic TDP-43 aggregation in ALS
A retroviral connection to ALS: Pathogenic deposition of neuronal ERVK proteins

- Active ERVK loci in the cortical neurons of patients with ALS produce retroviral reverse transcriptase (RT) enzyme (Douville et al., 2011)

- High levels of RT in the serum and CSF of patients with ALS (McCormick et al., 2008)

- Recapitulated in a murine model of ERVK Env-driven motor neuron damage (Li et al., 2015)
Drivers of neuronal ERVK protein deposition in ALS: Is TDP-43 misregulation involved?

Overexpression of TDP-43 correlates with enhanced ERVK pol RNA levels

TDP-43 and ERVK protein build-up in cortical neurons of patients with ALS

Does TDP-43 activate ERVK transcription?

Does TDP-43 impact ERVK proteostasis?

Douville et al., Ann Neurol, 2011
Drivers of neuronal ERVK protein deposition in ALS:
Are defective PQC pathways involved?

- Viral proteins can also be targeted by autophagy, UPS, and SGs
  - These pathways are disrupted in ALS

- Viruses can also interfere with and usurp cellular PQC strategies

Do PQC mechanisms homeostatically regulate ERVK proteostasis?

Cell-type specific differences in ERVK protein clearance?

Does ERVK interfere with cellular PQC mechanisms?
SPECIFIC AIMS

We sought to evaluate whether wild-type and TDP-43 mutants, as well as select protein clearance pathways, influence neuronal ERVK protein deposition in ALS

- Determine whether TDP-43 binds the ERVK promoter and activates ERVK transcription
- Evaluate cell-type specific differences in clearance of TDP-43 and ERVK protein deposits in human astrocytes and neurons
- Assess whether wild-type and mutated TDP-43 modulate ERVK protein deposition
- Validate findings in autopsy cortical brain tissue from neuro-normal controls and individuals with ALS
TDP-43 binds the ERVK promoter

- ERVK 5’ LTR contains multiple putative TDP-43 binding sites

- TDP-43 has been shown to bind the ERVK promoter and activate ERVK transcription (Li et al., 2015)
TDP-43 binds the ERVK promoter, but is not a transcriptional activator of ERVK

- **Cell lines:**
  - SVGAs: human astrocytic cell line
  - ReNcell CX-derived human neurons

- Overexpression of wild-type or ALS-associated mutant TDP-43 did not increase ERVK gene transcription

- Enhanced binding of TDP-43 to the ERVK promoter with MG132 treatment, and reduced binding with TNFα + MG 132 treatment did not alter ERVK gene transcription
Astrocytes can clear ERVK and TDP-43 protein deposits

- UPS maintains ERVK proteostasis, and inhibition of UPS leads to ERVK protein accumulation
- Astrocytes are able to degrade ERVK and TDP-43 protein deposition with TNFα treatment during MG132-mediated UPS inhibition
Neurons cannot effectively clear ERVK and TDP-43 protein accumulation

Unlike astrocytes, human neurons are unable to effectively degrade ERVK and TDP-43 protein deposition with dual TNFα and MG132 treatment.
Mutant TDP-43 facilitates ERVK protein deposition in astrocytes and neurons

Overexpression of ALS-associated mutant TDP-43 strongly drives ERVK protein aggregation in astrocytes

Manghera et al., Neurobio Dis, 2016
ERVK proteins localize to stress granules

- ERVK RT\(^+\) G3BP1\(^+\) stress granules form in cells treated with TNF\(\alpha\) and MG132
- Stress granule formation may regulate ERVK protein turnover

Manghera et al., Neurobio Dis, 2016
ERVK proteins also localize to autophagic vesicles

- ERVK RT localizes to LC 3B+ autophagic vesicles, but not to the same extent as with G3BP1
- Autophagy also likely regulates ERVK expression

Manghera et al., Neurobio Dis, 2016
Ongoing autophagy fails to clear neuronal ERVK protein accumulation in ALS

- LC 3B levels are markedly enhanced in ERVK RT⁺ cortical neurons from patients with ALS
- Incomplete co-localization of ERVK RT with LC 3B

Manghera et al., Neurobio Dis, 2016
Stress granule response fails to clear neuronal ERVK protein accumulation in ALS

- G3BP1 levels are markedly enhanced in ERVK RT⁺ cortical neurons from patients with ALS
- ERVK RT does not co-localize with G3BP1

Manghera et al., Neurobio Dis, 2016
SUMMARY

- G3BP1 Deregulation of stress granules
- LC3B
- TDP-43 promotes ERVK protein deposition
- RT
- Degradation of ERVK proteins
- Stress granule formation
- Axonal autophagy

Manghera et al., Neurobio Dis, 2016
ERVK protein deposition is a novel aspect of TDP-43 misregulation in ALS

Protein aggregation may serve as a new therapeutic target for ALS

How to prevent or dissolve protein aggregates?

Enhance activity of select cellular proteases?
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Create a world without ALS.