Diminished motor neuron activity driven by abnormal astrocytic GLAST glutamate transporter in spinal muscular atrophy is not fully restored after lentiviral SMN delivery

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Contribution of other cell types to SMA motor neuron dysfunction?

**Neuromuscular junction**
- Cholinergic
- ↓ synaptic vesicle release
- ↓ NMJ endplate maturation (AChR clusters)
- Later in disease progression

**Central afferent synapse**
- Glutamatergic
- ↓ in excitatory glutamatergic neurotransmission
- Motor neuron hyperexcitability, but decreased post-synaptic action potentials
- Early in disease progression

**Motor neuron cell autonomous defect?**

**Contribution of other cell types to motor neuron dysfunction?**

**Glial cells?**

Schematic adapted from Hawley et al., 2017
Astrocytes are key regulators of synapse formation and function
- Excitatory
- Glutamatergic

Secreted factors into the synaptic cleft
- Thrombospondin
- Tenascin
- SPARC
- Gliotransmitters
  - Glutamate
  - ATP
  - D-serine
- microRNAs

Cell surface proteins
- Cell adhesion molecules
  - Neuriligin
  - Neurexin
  - Cadherins
  - Ephrins
  - Protocadherins
- Ion channels
  - $K_{i}r4.1$, Na+ K+ ATPases
- Neurotransmitter receptors
  - Metabotropic glutamate receptors (mGluR, P2Y2)
- Neurotransmitter transporters
  - Glutamate (GLT-1 and GLAST)
SMA astrocyte defects correlated to the synapse

- **Mouse studies**
  - Synapse formation and activity is significantly reduced in SMA astrocyte motor neuron direct contact co-cultures (Zhou et al., 2016)
    - Potential candidate: Ephrin B2 reduction (axon guidance and synapse development)
  - Reduced inward rectifier potassium channel Kir4.1 and glutamate transporter EAAT1 in SMA mouse spinal cords. Leads to reduced potassium and glutamate uptake in mouse SMN siRNA astrocytes (Leo et al., 2022)

- **Human induced pluripotent stem cell (iPSC)-derived cultures**
  - Astrocyte-mediated miR-146a may target extracellular matrix proteins within perineuronal nets to decrease motor neuron activity (Welby et al., 2021)
  - Human iPSC-derived astrocytes show increased basal calcium levels and minimal calcium response through ATP stimulation despite presence of purinergic receptors (McGivern et al., 2013)
SMA astrocytes diminish motor neuron activity (evoked)

Induced pluripotent stem cells (iPSCs)

MN and astrocyte co-culture

Microelectrode array (Axion Biosystems)
SMA astrocytes diminish motor neuron activity (evoked)

- SMA patient iPSC-derived astrocytes diminish motor neuron activity in direct contact co-cultures
- Healthy derived astrocytes normalize abnormal burst activity in patient motor neuron cultures
Astrocyte cell surface protein candidates (RNA seq data)

- 2995 significantly differentially expressed genes
  - 1221 upregulated
  - 1734 downregulated

- Downregulated genes in patient-derived astrocytes:
  - Ion buffering
  - Synaptic integrity
  - Glutamate regulation
Disrupted astrocytic glutamate neuromodulation contributes towards central afferent synapse dysfunction?

**Hypothesis**
SMA astrocytes contribute to motor neuron dysfunction due to the lack of important synaptic-related cell surface glycoproteins.

**Strategy**
1. Differences in cell surface transcripts/proteins between healthy and SMA astrocytes (RNA seq/cell surface capture data)
2. Candidate of interest validation
3. Impact on motor neurons (microelectrode array approach)
4. Mechanism: SMN dependent/independent?
Significant reduction of GLAST in SMA patient-derived astrocytes
Astrocyte specific glutamate transporters: GLAST and GLT-1

- Responsible for at least 80% of synaptic glutamate clearance and recycling

- Uptake of glutamate against concentration gradient
  - Na+ dependent glutamate uptake
  - Similar glutamate affinity for both transporters

- Regulated by:
  - Neurons (direct contact and secreted factors)
  - Synaptic activity (e.g. glutamate)
    - Coupled to Na+/K+ ATPases and mGluR
    - Extensive stimuli that affect trafficking of EAAT to and from intracellular pools and lipid rafts (caveolae)
Specific GLAST inhibition fails to decrease intracellular glutamate in SMA patient-derived astrocytes.

- UTX
- 100μM Glutamate

Intracellular glutamate levels (μM)

Healthy astrocytes
SMA astrocytes

↓32.6%  ↓7.2%

Intracellular glutamate levels (μM)

Healthy astrocytes
SMA astrocytes

UTX
100μM Glutamate
100μM Glutamate + 50μM UCPH101

↓32.6%  ***

↓7.2%  ns
Specific GLT-1 inhibition decreases intracellular glutamate in healthy and SMA patient-derived astrocytes

- Healthy astrocytes:
  - UTX: ↓27.5% **
  - 100uM Glutamate: ↓33.1% ****
  - 100uM Glutamate + 50uM DHK: ↓20.2% **

- SMA astrocytes:
  - UTX: ↓33.7% ****
  - 100uM Glutamate: ↓33.7% ****
  - 100uM Glutamate + 100uM DHK
GLAST inhibition mimics diminished neuron activity from SMA astrocyte co-cultures

Healthy MN healthy astrocytes

UCPH101 (selective GLAST inhibitor)
GLAST inhibition mimics diminished neuron activity from SMA astrocyte co-cultures.

**Weighted mean firing rate**

- Pre-Tx: Healthy MN + healthy astrocytes
- Vehicle: Healthy MN + healthy astrocytes
- 50µM UCPH101: Healthy MN + healthy astrocytes
- SMA astrocytes: Healthy MN + healthy astrocytes

**Number of bursts**

- Pre-Tx: Healthy MN + healthy astrocytes
- Vehicle: Healthy MN + healthy astrocytes
- 50µM UCPH101: Healthy MN + healthy astrocytes
- SMA astrocytes: Healthy MN + healthy astrocytes
Lentiviral mediated delivery of SMN into SMA patient astrocytes

GFP signal lost due to acetone/methanol fixation
Partial restoration of GLAST phenotype after SMN re-expression
Partial restoration of GLAST phenotype after SMN re-expression

↓27.4%  ↓33.6%  ↓24.8%  ↓12.4%

UTX
100uM Glutamate
100uM Glutamate + 50uM UCPH101

Intracellular glutamate levels (uM)
Healthy astrocytes
SMN:GFP SMA astrocytes
SMN:FLAG SMA astrocytes
SMA astrocytes
Abnormally upregulated caveolin-1 levels in patient astrocytes

**Caveolin-1 (CAV-1)**

- Scaffold protein required for the formation of caveolae (lipid raft endocytosis)
- Directly interacts with SMN to facilitate local protein translation (Gabanella et al., 2016)
- DJ-1 deficiency impairs caveolin-1 levels, lipid raft endocytosis and glutamate transporter expression and glutamate uptake in Parkinson’s disease astrocytes (Kim et al., 2016)
Partial restoration of CAV-1 phenotype after SMN re-expression
Partial restoration of CAV-1 phenotype after SMN re-expression
Minimal MN activity in SMN:FLAG/GFP patient-derived astrocytes

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Healthy MN healthy astrocytes

Healthy MN:FLAG astrocytes

Healthy MN:GFP astrocytes

SMA MN SMA astrocytes

Healthy MN SMN:FLAG astrocytes

Healthy MN SMN:GFP astrocytes
Conclusions and future directions

- SMA patient-derived astrocytes
  - directly impede motor neuron activity
  - reduced GLAST levels
  - Increased CAV-1 levels

- Disease mechanism involving SMN-CAV-1 regulation of GLAST
  - Impaired local protein translation and turnover of plasma membrane protein -> disrupted glutamate neurotransmission?

- SMN-dependent/associated mechanism?
  - Further SMN modulation needed?
  - Reactivity -> insensitive to SMN restoration?

Other astrocytic defects linked glutamate neurotransmission?
- Related to GLAST regulation (mGluR, Na+/K+ ATPases, calcium signaling)
- Increased glutamate levels

Disrupted glutamate-glutamine cycle
- Impaired synaptogenesis (ephrins)
Conclusions and future directions

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Microscale cell surface capture mass spectrometry: enrichment of synapse-related glycoproteins in healthy-derived astrocytes

Enriched in healthy astrocytes only (59 unique cell surface glycoproteins)

- anchored component of membrane
- synaptic membrane
- cell surface
- cell adhesion
- cell-cell signaling
- membrane depolarization
- metal ion transmembrane transporter activity
- ephrin receptor activity
- transmembrane-ephrin receptor activity

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Microelectrode array system
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Human samples

Human iPSC lines (2 healthy individuals and 3 SMA patients)

Human spinal cord tissue
- University of Maryland Brain and Tissue Bank
- NIH NeuroBioBank

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Questions/Info

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