Basic Mechanisms of Disease: Pathology of ALS
Objectives

• To define amyotrophic lateral sclerosis (ALS) as a human disease

• To describe some of the pathological features of ALS

• To introduce possible mechanisms of disease in ALS

• To identify experimental therapeutics for ALS treatment in animal models
ALS Epidemiology

Every 90 minutes someone in the US is diagnosed with ALS and every 90 minutes someone in the US dies from ALS.

- Incidence of 5/100,000
- Lifetime prevalence 1 in 300
- 30,000 Americans have ALS
- 5,600 new cases/year are diagnosed (15 new cases/day)
- Onset at 40-70 years (average 55 years)
- Care costs of $200,000/year
Amyotrophic Lateral Sclerosis (ALS)

ALS is a degenerative disease of lower motor neurons in spinal cord and brainstem and upper motor neurons in cerebral cortex.

Motor Neuron Loss in ALS
Spinal Cord (L4)

Control Spinal Cord

ALS Spinal Cord

Control Anterior Horn

ALS Anterior Horn

Martin et al., 2005

ALS causes paralysis: loss of movement, swallowing, and breathing.
Spinal Cord: Somatotopy Mirrors Affected Muscle in ALS

Human spinal cord Nissl stain

Motor neurons

Nucleus with nucleolus

Nissl Bodies

sensory

motor
ALS is a degenerative disease of upper motor neurons in cerebral cortex.
# Genetics of ALS

Majority (~90%) of adult ALS cases are sporadic with no known genetic component.

About 5-10% of adult ALS is familial.

## Mutant Genes Linked to Familial ALS

<table>
<thead>
<tr>
<th>Locus</th>
<th>Inheritance/Mutations</th>
<th>Gene</th>
<th>Protein Name/ Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS1/21q22.1</td>
<td>autosomal dominant (adult onset) 14-23%, 166</td>
<td>SOD1</td>
<td>Cu, Zn superoxide dismutase/ dismutation of superoxide</td>
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<td>ALS2/2q33.2</td>
<td>autosomal recessive (juvenile onset), 19</td>
<td>Alsin</td>
<td>Alsin/guanine exchange factor for RAB5A and Rac1</td>
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<td>ALS3/18q21</td>
<td>autosomal dominant (adult onset)</td>
<td>?</td>
<td>?</td>
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<tr>
<td>ALS4/9q34</td>
<td>autosomal dominant (adult onset), 9</td>
<td>SETX</td>
<td>Senataxin/helicase, RNA processing</td>
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<td>autosomal recessive (juvenile onset),12</td>
<td>SPG11</td>
<td>Spatacsin, axonal intracellular trafficking</td>
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<td>ALS6/16q11.2</td>
<td>autosomal dominant (adult onset), 42</td>
<td>FUS</td>
<td>Fused in sarcoma, RNA processing, genome stability</td>
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<tr>
<td>ALS7/20p13</td>
<td>autosomal dominant (adult onset)</td>
<td>?</td>
<td>?</td>
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<tr>
<td>ALS8/20q13.3</td>
<td>autosomal dominant, 3</td>
<td>VAPB</td>
<td>VAMP-associated protein B/part of SNARE complex</td>
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<tr>
<td>2q13</td>
<td>autosomal dominant (adult onset, atypical ALS)</td>
<td>DCTN1</td>
<td>Dynactin p150glued/axonal transport, link between dynein</td>
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<td></td>
<td></td>
<td></td>
<td>and microtubule network</td>
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<td>ALS10/1p36.2</td>
<td>Autosomal dominant, 44</td>
<td>TARDBP</td>
<td>TDP-43, DNA/RNA binding protein regulates RNA</td>
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<td></td>
<td></td>
<td></td>
<td>transcription &amp; processing</td>
</tr>
<tr>
<td>9p21.2</td>
<td>ALS/FTD</td>
<td>C9orf72</td>
<td>C9orf72, RNA metabolism</td>
</tr>
</tbody>
</table>
SOD1: FALS Mutations

- 100 mutations in SOD1
- 153 aa protein
- Gain of toxic property
Dynactin Complex

Functions of p150
- increase processivity of dynein
- provide link to cargo
Neuron Design

- Dendrites
- Cell body (soma, perikaryon)
- Initial Segment
- Myelin sheath
- Axon
- Nodes of Ranvier
- Terminals
Inclusions in Cases of ALS

Inclusions / aggregates

- Decreased levels of transporters
- Excitotoxicity
- Dendritic pathology
- Mitochondrial damage
- Inclusions (phosphorylated NF, ubiquitin, other proteins)
- Free radical generation
- Chromatolysis followed by cell atrophy
- Neurofilamentous swellings
- Wallerian degeneration
- Abnormalities of nerve terminals

- Shift in BcL-BAX distributions
- DNA fragmentation, apoptosis
- Impaired axonal transport
- Mitochondrial damage
- ? Loss of trophic support
- Ubiquitin
- NF
- SOD1

- Ubiquitin inclusions
- NF inclusions
- SOD1 inclusions
Mutant SOD1
Potential Toxicities

Bruijn, et al
Synapses on Motor Neurons

Principle of information convergence: 5-10 x more afferent neurons than motor neurons
Glutamate Receptors & Transporters: Roles in Excitotoxicity

Transporter Subtypes
- EAAT1 (GLAST)
- EAAT2 (GLT-1)
- EAAT3
- EAAT4

Glutamate Receptors
- NMDA
- Non-NMDA
- Metabotropic
Synapses on Dendrites

Electron microscopy: CNS (Martin, 2002)

Forder & Tymianski, 2009
Glutamate Receptor Diversity

Ionotopic GluRs fast, short-lived (1-10msec) EPSC

Metabotropic GluR latencies >100 msec
Excitotoxicity: Mouse Spinal Cord

Quinolinic acid (QA)
NMDA receptor agonist

Kainic acid (KA)
Non-NMDA GluR agonist

Martin, 2011
Mechanisms Leading to Excitotoxic Neuronal Cell Death

- Ionic imbalance
- Kinase and endonuclease activation
- Oxidative/nitrosative stress
- Necrosis, apoptosis, continuum
Mitochondrial Regulation of Cell Death

Mitochondrial permeability transition
- state in which the proton-motive force is disrupted
- formation of the mitochondrial permeability transition pore (PTP)

Martin and Liu, 2004
The mitochondrial permeability transition pore contributes to disease mechanisms in ALS mice

Barry Gertz
The mitochondrial permeability transition pore contributes to disease mechanisms in ALS mice.
Integration (the algebraic summation of inhibitory and excitatory synaptic potentials) occurs in the neuropil. Action potential initiation at the initial segment.
Glycinergic Synapse
Hb9-eGFP Motoneurons in Culture

Chang & Martin, 2011 J Neurosci
Hb9-eGFP Motoneurons for Patch-Clamp Recording

Chang & Martin, 2011 J Neurosci
Single-Cell qRT-PCR and Receptor Clustering

Qing Chang
Dendrites and Axons

Purves et. al.
What are we not seeing about Motoneurons? Adenoviral gene Transfer in Mouse Motoneurons
Mitochondriopathy occurs in the neuropil early in ALS mice

Martin et al 2009
Structural Components in Axon

Long polymers of tubulin dimers: α (53 kDa), β (57 kDa) & 2 GTP; 25-28 nm

NF 68, 145, & 200 kDa; 10 nm

MT & MITOCHONDRIA
Axonal Transport

Anterograde Transport

Fast, forward moving
~400 mm/day

Slow component,
0.5-3 mm/day

Retrograde Transport

Fast, backward moving
~200 mm/day
Impairments in Axonal Transport

Normal transport
Motor or adaptor mutations
Disease-causing mutations

-or-

Stalled vesicles?

Cytosolic or intravesicular fibrils and aggregates?
Motor Neuron Transmitter and Target

1. Synthesis of enzymes in cell body
2. Slow axonal transport of enzymes
3. Synthesis and packaging of neurotransmitter
4. Release and diffusion of neurotransmitter
5. Transport of precursors into terminal

Nucleus
Golgi apparatus
RER
Microtubules
Axon
Terminal
Enzymes
Precursor
Precursor

ChAT: Choline acetyltransferase

EM - neuromuscular junction
Cholinergic Synapse

Purves et. al.
Thy1-YFP (green, motor neuron axon)

Alpha-bungarotoxin (red, NMJ)
Neuromuscular Junction (NMJ)
Fusion of Synaptic Vesicles at Active Zones

Synaptic vesicle diameter: 17-22 nm

Synaptic cleft diameter: 25 nm
Synaptic Transmission

- Docking
- Priming
- Fusion
- Endocytosis
- Receptors
- Vesicle Acidification & Recycling
- Neurotransmitter Uptake

Sudhof, 2004
More than 1000 proteins function in the presynaptic nerve terminal and >100 function in exocytosis

Purves, et. al.
Assembly of SNARE Complex in Synaptic Vesicle Exocytosis

Syntaxin 1 – brown
Synaptobrevin – red
Syntaxin 1 – yellow
SNAP 25 amino – blue
SNAP 25 carboxy - green

Rizo and Sudhoff, Nature Rev 2002
Recycling of Synaptic Vesicles
Motor End Plate

Peters, Palay, & Webster
Nicotinic Acetylcholine Receptor (AchR)
NMJ Degeneration occurs early in ALS mice

Pollari et al., 2014

Martin and Liu 2007
Skeletal muscle role in ALS

Margaret Wong, Wong & Martin, 2010
Astrocyte Role in ALS

Conditional expression of mutant SOD1 using astrocyte promoter does not cause ALS phenotype in mice (Gong et al., 2000)

Rothstein et al., 2005
Microglial Role in ALS

Kevin Chen

Chen et al., 2010

Guillemin, 2010
ALS: Summary

Risk Factors
ALS: age
Gene mutations in FALS: SOD1, ALS2 [Alsin], Dynactin, FUS, TDP43

Clinical Signs
Weakness
Atrophy

Mechanisms
Vulnerable Neurons
Motor Neurons

Cytopathology
Chromatolysis
Soma-dendritic atrophy
Inclusions (aggregates)
Mitochondrial alterations
Excitotoxicity
Cytoskeletal pathology
Axonal transport
Wallerian degeneration

Cell Death
Apoptosis, necrosis, continuum

Models
Axotomy Models
Surrogate Models
Mutant SOD1 Mice
ALS2-/- Mice
Mutant Dynactin Mice
Other Models

Therapeutics
Mitochondrial targeted therapeutics for ALS in mice

GNX = cinnaminic anilide derivative, mPTP inhibitor

Martin et al, in press