BIOLOGY OF ALS
LESSONS IN GENETICS

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OVERVIEW

• Introduction

• ALS Clinical Picture

• Pathogenesis of ALS
  • Overview
  • Genetics of ALS – Insights into motor neuron biology and disease
  • Summary

• Targeting the biology of disease
INTRODUCTION

- Understanding of disease mechanisms allows us to understand motor neuron biology
- Overlap with other neurodegenerative diseases
- Provide rationale targets for treatment!
ALS: A MOTOR NEURON DISEASE

**UMN:**
- Primary Lateral Sclerosis (PLS)
- FSP (23 genetic variants-10 AD, 8 Rec, 3 X)
- Bulbar Palsy

**LMN:**
- Spinal Muscular atrophy
- Motor Neuropathy with or without conduction block
- Kennedy’s Disease

**UMN and LMN:**
- ALS
  - Sporadic
  - Hereditary: ALS (32 genetic abnormalities identified)
  - Bulbar Palsy
AMYOTROPHIC LATERAL SCLEROSIS

Symptoms that bring the affected person into the clinic:

- Fatigue
- Weakness
- Stiffness

Underlying condition:

- **Loss of lower motor neurons** (anterior horn of the spinal cord; motor nuclei of the brain stem)
  - Muscle atrophy, weakness, fasciculations
- **Loss of upper motor neurons** (Betz cells of layer V motor cortex)
  - Spasticity, hyperreflexia, jaw jerk, Hoffmann & Babinski signs, loss of coordination, spread of reflexes
- Progressive, spreading
DISEASE CHARACTERISTICS ARE VARIABLE

- **Site of Focal Onset:**
  - Legs (lumbar onset)
  - Hands (cervical onset)
  - Tongue, pharynx (bulbar onset)

- **Age of onset:**
  - Mean age is 54 yrs old
  - Majority of cases are 45-70 yrs (range = 17 - 80 yrs old)

- **Progression:**
  - Bulbar onset tends to progress more rapidly
  - SOD1 mutation: G93A is rapid, A4V is slow
  - Can have “quiet periods”

- **Survival:**
  - 50% die within 3 years from diagnosis
  - 75% die within 5 years
  - 10% last > 10 years
COMPLEXITY OF THE DISEASE (MORE THAN MOTOR NEURON LOSS)

ALS patients can have:

- Sensory symptoms (18%)
- Cognitive involvement
  - Dementia (5%)
- Autonomic involvement:
  - Bladder and bowel dysfunction (30%)
  - Blood pressure, heart rate, GI function
- Substantia nigra/Locus ceruleus cell loss
- Oculomotor deficits/Onuf’s nucleus cell loss
  - Ocular palsy
EPIDEMIOLOGY

- Annual Incidence rate of about 1-2 per 100,000
- Prevalence of about 6-8 per 100,000
- Male-to-female ratio of about 1.3:1
- Familial ALS (FALS): 5-10% of cases can be traced

Occupational clusters:
- Military service during 1991 Gulf War
- Exposure to radiation, electrical shocks, welding or soldering materials
- Employment in the paint, petroleum or dairy industries
- Italian soccer players

Health associations:
- History of trauma to brain or spinal cord
- Strenuous physical activity
- Smoking

Geographic foci:
- Pacific rim islands (Guam, Kii Peninsula of Japan, West New Guinea)
- similar across the globe
GENE DISCOVERY IN ALS

- SOD1
- alsin
- DCTN
- STX1
- ANG
- TDP43
- C9orf72
- UBQLN2
- OPTN
- DAO
- VCP
- ATXN2
- CHGB
- UNC13A
- ELP3
- PON
- FUS
- C9orf72
- UBQLN2

Timeline:
- 1985
- 1990
- 1995
- 2000
- 2005
- 2010
- 2015
## Amyotrophic Lateral Sclerosis

32 genes (26 FALS, 6 SALS)

<table>
<thead>
<tr>
<th>Familial (26)</th>
<th>Sporadic (6)</th>
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<tbody>
<tr>
<td>ALS1 21q SOD1</td>
<td>ELP3</td>
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<tr>
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<td>KIFAP3</td>
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<td>ALS 12q HNRNPA1</td>
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<td>ALS 7p HNRNPA2B1</td>
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GENE DISCOVERY IN ALS IS INCREASING

>50% of FALS is attributable to known variants.
DISEASE REFLECTS AN INTERPLAY OF FACTORS

Genotype

Behavior ⇔ Disease ⇔ Chance

Environment
WHAT DO WE KNOW ABOUT MOTOR NEURON DEGENERATION IN ALS?

- Inclusions & aggregates
- Apoptosis
- Gliosis
- Increased glutamate in CSF
- Inflammatory Biomarkers:
  - Cyclo-oxygenase 2 (COX 2)
  - Prostaglandin E2
  - IL-1beta
  - TNF-alpha
- Biomarkers of Oxidative Stress:
  - iNOS
  - nitrosylated proteins

Cytoskeletal pathology and ubiquitinated protein aggregates are seen in ALS motor neurons.
PATHOGENESIS

- Defective glutamate metabolism
- Free radical injury
- Mitochondrial dysfunction
- Programmed cell death (apoptosis)
- Cytoskeletal protein defects
- Immune dysfunction
- Gene defects
- RNA processing
- Misfolded Proteins
EXCITOTOXICITY OF GLUTAMATE

- Glutamate is a chemical used for signaling between neurons.
- It is important for normal nerve cell function but toxic in excess.
- Increased glutamate has been found in ALS patients and in ALS mice.
- Increased glutamate may result from either abnormal “clean up” (transport) of glutamate or increased release of glutamate from nerve cells.
ALS: OXIDATIVE DAMAGE

• Free radicals- molecules with electrical charges
  • unstable and liable to damage cellular structures
  • normal part of cellular life
  • cells are usually neutralize them and keep their numbers in check.

• But in ALS, free radicals build to toxic levels and damage cells, through an attack process called oxidative stress
ALS: OXIDATIVE DAMAGE

- Free radicals are also produced in the powerhouse of the cell called the mitochondria.
- Mitochondria and its genetic material are especially sensitive to the oxidative damage from free radicals.
ALS: OXIDATIVE STRESS

- 20% of FALS patients have mutations in SOD1
- Higher levels of protein carbonyl groups and oxidized nucleic acids in brain homogenates from patients with sporadic ALS
- Fibroblasts from ALS patients with mutant SOD1 appear more sensitive to oxidative stress caused by H2O2
ALS: APOPTOSIS

ALS may be due to an early death of motor neurons or a premature initiation of programmed cell death or suicide (called apoptosis)
ALS: IMMUNE/INFLAMMATORY MECHANISMS

- Trigger increased intracellular calcium and motor neuron degeneration
- Higher incidence of immune disorders in patients
- Blockade of CD-40 receptor and ligand
- Activation of inflammation in the brain of the mouse model of ALS and in people with ALS
  - Cyclo-oxygenase 2 (COX 2) is increased in ALS (Tg mouse and Human)
  - Prostaglandin E2 is increased in ALS
  - Expression of proinflammatory mediators is increased in ALS
    - IL-1beta
    - iNOS
    - TNF-alpha
ALS: NEUROFILAMENTS

- Neurofilaments provide a scaffold structure and for traffic in the nerve cell
- Neurofilaments accumulate in perikarya and proximal axons in amyotrophic lateral sclerosis
- Pathologic features of the disease in transgenic mice overexpressing various filament proteins
- In humans, impaired transport of neurofilaments (resulting from a neurofilament gene mutation or an acquired lesion of the neurofilament subunit proteins) may lead to motor neuron degeneration
PROTEIN MISFOLDING AND ACCUMULATION

• Abnormal protein aggregates in both sporadic and familial ALS
• Includes TARDP43, SOD1, FUS
• In some cases the proteins are mislocalized from the nucleus into the cytoplasm and are important to DNA/RNA processing
TDP-43 INCLUSIONS AND CYTOPLASMIC DISTRIBUTION IN MOTOR NEURONS IN HUMAN ALS

The major neuropathological indicator of ALS?

T. Arai et al. / Biochemical and Biophysical Research Communications 351 (2006) 602-611
UBIQUITINATED PROTEINS IN ALS MOTOR NEURONS INCLUDE TDP-43

- nuclear protein
- transcription repressor
- part of splicing apparatus
- in early ALS and FTD
  - cleaved
  - hyperphosphorylated
  - ubiquitinated

WHAT ABOUT GENETICS?

- The genetics of ALS mirrors motor neuron biology giving us insight into important pathways
- There are genetic influences in “sporadic” ALS
- The mutations give us an opportunity to develop model systems to study ALS and the disruption of important pathways
- Provide targets for new drugs
- In vivo models for therapeutic testing
GENE DEFECTS AND THEIR EFFECTS ON MOTOR NEURON BIOLOGY

- Some gene defects can implicate many pathways of neuronal death
- Some gene defects can affect axonal transport and vesicle trafficking (also FTD)
- Some gene defects can affect RNA localization and function
- Some gene defects may cause susceptibility to environmental toxins
- Some gene defects clearly demonstrate the convergence of SALS, FALS, and FTD
GENE DEFECTS AND THEIR EFFECTS ON MOTOR NEURON BIOLOGY

• Some gene defects can implicate many pathways of neuronal death
  • SOD1
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OVER-EXPRESSION OF MUTANT SOD1 GENERATES ANIMAL MODELS OF ALS.

SOD1 enzyme activity is normal in these models.

G93A mouse

H46R rat

A4V fly

ALS worm (Rick Morimoto)

ALS zebrafish

SOD1 enzyme activity is normal in these models.
“LYKENS” LADDER CLIMBING BEHAVIOR

Control

Moderately Affected
EARLY IN ALS, MUTANT SOD1 PROVOKES MULTIPLE INTRINSIC TOXICITIES.

- **Abnormal neurite development**

- **Binds mSOD1**
  - ↓ ATP, Ca++
  - → apoptosis

- **Excessive bursting, Excitotoxicity**

- **ER stress, Autophagy**

- **Abnormal axonal transport**

- **Early axonal retraction**
As ALS evolves, SOD$_1^{\text{mut}}$ evokes diverse influences from non-neuronal cells.
GENE DEFECTS AND THEIR EFFECTS ON MOTOR NEURON BIOLOGY

• Some gene defects can implicate many pathways of neuronal death
• Some gene defects can affect axonal transport and vesicle trafficking (also FTD)
  • SOD1, VAPB, dynactin
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• Some gene defects can implicate many pathways of neuronal death
• Some gene defects can affect axonal transport and vesicle trafficking (also FTD)
• Some gene defects can affect RNA localization and function
  • TDP43 - FUS/TLS - C9orf72 (ANG, Ataxin-2, ELP3, ATXN, TAF15, VC P, UBQLN2)
• Some gene defects may cause susceptibility to environmental toxins
• Some gene defects clearly demonstrate the convergence of SALS, FALS, and FTD
BASICS OF DNA TO PROTEIN
FUS/TLS AND TDP43 ARE RNA-BINDING PROTEINS IMPLICATED IN ALS.

- RNA and DNA binding proteins
- Shuttle between nuclear and cytoplasm
- Multifunctional:
  - Cell proliferation, DNA repair, transcription
- Local protein translation at dendritic spines

*Stressor*

* Vaccination, injury, chemical toxicity

C9ORF72: RNA EXPANSION

(1) impair transcriptional regulation
(2) alter mRNA splicing and metabolism
(3) re-distribute, reduce levels of RNA-binding proteins
(4) generate anti-sense transcript
Diverse neuronal gene defects impair function in motor neurons in ALS.
GENE DEFECTS AND THEIR EFFECTS ON MOTOR NEURON BIOLOGY

• Some gene defects can implicate many pathways of neuronal death
• Some gene defects can affect axonal transport and vesicle trafficking (also FTD)
• Some gene defects can affect RNA localization and function
• Some gene defects may cause susceptibility to environmental toxins
  • Paroxonases – Neuropathy Target Esterase
• Some gene defects clearly demonstrate the convergence of SALS, FALS, and FTD
The development of ALS probably reflects an interplay: environment, genetic factors, and chance, aging.

- 10% of ALS: autosomal dominant
- athletic activity
- soccer
- head trauma, smoking
Multiple Complex Pathways. Does “death” differ in different compartments?

Courtesy of Steve Perrin, PhD and ALSTDI
SEVERAL DISABLING, SOMETIMES LETHAL, NEUROLOGICAL DISORDERS ARE CHARACTERIZED BY PROGRESSIVE NEURONAL CELL DEATH.

- Neurodegeneration
  - Amyotrophic Lateral Sclerosis
  - Huntington’s Disease
  - Alzheimer’s Disease
  - Parkinson’s Disease
  - Macular Degeneration
  - Spinocerebellar Ataxias

- CNS Trauma
- Autoimmune Diseases
SHARED FEATURES OF NEURODEGENERATION

1. Age-dependent, progressive
2. Focal onset → spreads
3. Cell death – multifactorial
4. Both sporadic and inherited forms
NEURODEGENERATIVE DISEASES SHARE A SIMILAR CASCADE OF EVENTS CHARACTERIZED BY AXONAL AND SYNAPTIC DYSFUNCTION, FOLLOWED BY NEURONAL DEATH

Changes in the Neurons
• disturbed RNA metabolism
• protein denaturation, ubiquitination, aggregation
• ER stress, unfolded protein response
• autophagy
• excitotoxicity – elevated intracellular calcium
• mitochondrial dysfunction
• activation of death cascades: apoptotic, necrotic

Changes to non neuronal cells
Activation of non-neuronal cellular reactions
• neuroinflammation / microgliosis and astrogliosis
TREATMENT STRATEGIES

- **Anti-Glutamate**
  - Talampanel
  - Ceftriaxone
  - Memantine

- **Anti-apoptotic/neuroprotectant**
  - Minocycline
  - Methylcobalamin
  - Arimoclomol
  - IGF-1
  - Stem cells

- **Anti-oxidant**
  - CoQ
  - R +Pramipexole
  - Hyperbaric oxygen
  - Olesoxime

- **Preserve cAMP and cGMP**
  - Pentoxyfilline

- **Astrocyte Modulator**
  - ONO 2506

- **Anti-inflammatory**
  - Copaxone
  - Lipitor
  - Pioglitazone (Europe)

- **DNA Modulation**
  - Anti-sense SOD1
  - Pyramethamine in SOD1
  - SB 509 (VEGF Upregulation)

- **Other Novel Therapies**
  - NP001 Phase I
  - Cytokinetics Phase 2
  - Anti-Nogo A
  - Ketogenic diet

- **Protein C kinase Inhibitor**
  - Tamoxifen (also anti-apoptotic)
CONCLUSIONS

- There are many mechanisms that contribute to cell death in ALS and neurodegenerative diseases.
- These mechanisms are shared and may reflect the response of the neuron to injury/exposure or genetic mutation or both.
- Genetic mutations in FALS not only define motor neuron biology but also mirror the putative mechanisms.
- Among the most important mechanisms are RNA and protein processing and homeostasis.
- Other mechanisms that play a role are glutamate excitotoxicity and oxidative damage.
- Understanding the biology of ALS will lead to therapeutics that are rational.