Mitochondria Pathology in Amyotrophic Lateral Sclerosis and Huntington’s Disease

Neurodegeneration: Opportunities for Collaboration Across Disease-Specific Research and Development Communities - A Workshop : Session 3

April 30, 2012

Neil W. Kowall, M.D.

VA Boston Healthcare System

Boston University School of Medicine
Mitochondrial Pathology in ALS: Evidence and Potential Mechanisms

- A fatal neurodegenerative disorder most prominently affecting motor neurons leading to progressive muscle wasting and paralysis. About 90-95% of cases are not familial. Mutations in SOD1, TDP-43 and FUS account for 20%-30% of familial cases.

- Two major genes were described last year that cause FTLD +/-ALS: Ubiquilin2 is responsible for X-linked FTLD/ALS. It is thought to target abnormal proteins for lysosomal and proteasomal degradation. A hexanucleotide repeat expansion of C9ORF72 causes between 22 and 50% of familial ALS cases. It encodes a highly conserved protein with no known function. Normal individuals carry up to 23 GGGGCC repeats, whereas affecteds may contain >1,000 repeats leading to toxic RNA inclusions. The relationship of C9ORF72 to mitochondrial function is unknown.

- Mitochondria from mSOD1 motor neurons demonstrate impaired fusion, are smaller, fewer, and have defective membrane potential. These changes are not seen in wtSOD1 motor neurons and do not involve other organelles (Magrane et al J Neurosci 2012). Upon exposure to mSOD1, levels of about 50 mitochondrial proteins, including several ETC components, as well as mitochondrial fusion factor Mfn2 are altered, indicating a possible widespread effect of mSOD1 on mitochondrial function. (Karbowski Acta Neuropath 2012).
Mitochondrial Pathology in ALS: Evidence and Potential Mechanisms II

- Mutant SOD1 compromises mitochondrial membrane integrity leading to release of Cytochrome C only in the presence of Bcl-2. The identification of Bcl-2 as a specific target suggests that inhibiting the formation of the toxic mutant SOD1/Bcl-2 complex may prevent mitochondrial damage in ALS. BH3 and Bcl-2 like peptides are routinely used in cancer therapy; a therapeutic approach with peptides designed to compete or displace Bcl-2 from binding mtSOD should be tested (Pedrini et al HMG 2010)

- Guareschi et al (PNAS 2012), showed that wtSOD1 in patients’ lymphoblasts is modified posttranslationally in sporadic ALS and is over oxidized in a subset of patients with bulbar onset that recapitulates mutant SOD1-like properties and damages mitochondria by forming a toxic complex with mitochondrial Bcl-2. There is a common SOD1-dependent toxicity between mutant SOD1-linked familial ALS and a subset of sporadic ALS. Bcl-2 is a common toxic target of both mutSOD1 and overoxidized SOD1 allows the design of target-based therapies against the SOD1/Bcl-2 complex
Fig. 3. Like mutSOD1, iperOxSOD1 colocalizes and aggregates with Bcl-2 in patient cells. Colocalization of SOD1 and Bcl-2 was evaluated by immunofluorescence with rabbit anti-SOD1 (A–C) and mouse anti-Bcl-2 (D–F) antibodies. The merging signals show colocalization of the two proteins within the aggregates in iperOxSOD1-sALS and mutSOD1-fALS patients after H₂O₂ treatment (G and H, arrows). sALS lymphoblasts with no iperOxSOD1 and no SOD1/Bcl-2 high molecular weight aggregates did not demonstrate colocalization of the two proteins (C, F, and I).
Mitochondrial Pathology in HD: Evidence and Potential Mechanisms I

- Huntington's disease (HD) is characterized by severe motor, cognitive, and psychiatric symptoms. The etiology of HD is an autosomal dominant CAG-triplet mutation within the huntingtin (htt) gene. Links between mitochondrial dysfunctions and HD pathology are many.
- The physiological role of the polyQ-repeated expansion has been recently explored in mice carrying only seven CAG repeats in the murine Htt gene. Animals have subtle memory and learning deficits & altered energy status caused by changes in mitochondria function.
- mHTT decreases bidirectional mitochondrial motility in both rodent neuronal cultures and transgenic animals. Long mutant HTT protein stretches could interact with cytoskeletal components and transport motors, and specific binding between HTT and milton, a distant homologue of human HAP1 (huntingtin-associated protein 1), which binds to HTT and dynactin.
- mHTT has been shown to affect mitochondrial morphology and the bioenergetic status by altering the balance between mitochondrial fusion and fission under the control of dynamin-related protein or other mitochondria-associated proteins.
- Alterations in mitochondria dynamics are reflected in deficits of the electron transport chain and of cellular respiration. Energy-related supplements, such as creatine, have been tested in HD patients with inconsistent results.
Mitochondrial Pathology in HD: Evidence and Potential Mechanisms II

- Aberrant HTT with the expanded polyQ tract inhibits the expression of the transcriptional co-activator peroxisome proliferator-activated receptor (PPAR) co-activator gamma-1 alpha (PGC-1a), thereby compromising mitochondrial biogenesis and respiration. The combination of altered Ca influx and diminished Ca clearance by mitochondria increases the susceptibility of striatal cells expressing mutant HTT to excitotoxic insults.

- Pandey et al. (2010) report finding mHTT on the outer mitochondrial membrane. Increasing (CAG)n length is associated with increased mitochondrial depolarization in HD lymphoblasts. Reduced electron transport (Complex I), net respiration and ATP levels appear to antedate neuropathology in HD-knock-in mice. Mitochondrial trafficking and integrity (altered fusion and fission) appear altered in HD. There also is reduced calcium loading capacity in HD mitochondria.

- mHTT abnormally interacts with the mitochondrial fission GTPase dynamin-related protein-1 (DRP1) in mice and in humans with HD, which stimulates its enzymatic activity. mHTT–mediated mitochondrial fragmentation, defects in anterograde and retrograde mitochondrial transport and neuronal cell death are all rescued by a dominant-negative DRP1 K38A mutant. Thus, DRP1 might represent a new therapeutic target to combat neurodegeneration in Huntington’s disease. (Song et al, Nature Medicine 2011)

- Pardo et al (PNAS 2012) infused ganglioside GM1 which induce phosphorylation of mutant huntingtin and attenuated toxicity, restoring normal motor function in symptomatic HD mice. Inhibition of PP1/PP2A as a strategy to boost the of mutant HTT phosphorylation may be another approach.

- Meclizine is neuroprotective in an animal model of HD. It’s mechanism of action correlates with its ability to suppress mitochondrial respiration (Vamsi Mootha and colleagues, HMG 2011)
Mitochondrial Pathology in ALS and HD: closing remarks

- Mitochondria are intimately involved in the life and death of the cell and contribute to—or suffer the consequences of—all age-related neurodegenerative diseases including ALS and HD.
- Even if mitochondrial pathology is not a fundamental or primary cause of disease, therapy to preserve, enhance, or correct mitochondrial function will likely be beneficial and delay or prevent cell death and disease progression.
- Many things are yet to be clarified that could be important—individuation of mitochondria in neuronal subsets; the emergent properties of the mitochondrial hive (Braschi and McBride, 2010)
- The challenge is not linking mitochondria to pathogenesis rather the problem may be specificity ("pathoclisis")
A principle the Vogts postulated from their studies of diseased brains was that of ‘pathoclisis’, a genetically determined vulnerability of specific brain areas to toxic or other external agents.
Amyotrophic Lateral Sclerosis (ALS)

- A fatal neurodegenerative disorder characterized clinically by a combination of upper and lower motor neuron loss resulting in progressive muscle wasting and paralysis. Incidence is 1-2/100,000/year and may be rising. Death usually occurs within 2-5 years of diagnosis.
- Familial transmission in 6-10% of the cases. Mutations in SOD1, TDP-43 and FUS occur in 20%-30% of familial forms. Two major genes were described last year that cause FTLD +/- ALS:
  - Ubiquilin2 is responsible for X-linked FTLD/ALS. Ubiquilin2 is one of a family of proteins thought to be important in targeting abnormal proteins for degradation via lysosomal and proteasomal routes.
  - A hexanucleotide repeat expansion in C9ORF72 causes chromosome 9p linked FTLD/ALS and is one of the most common causes of familial ALS and is found in between 22 and 50% of cases depending on the geographical origins of the population. It encodes a highly conserved alternatively spliced 481 (full-length) amino acid protein with no known function. Normal individuals usually carry up to 23 repeated GGGGCC repeats, whereas affected individuals carry repeat expansions that may be up to 10 kb in length, containing over 1,000 repeats. C9ORF72 expansion may lead to the development of toxic RNA inclusions. The pathological findings suggest that non-motor features are likely to be extremely common if not universal. The clinical onset is typically bulbar and the prognosis grim. There is evidence for anticipation. ~60 % of ALS patients with the expansion have a family history of dementia. All patients with the C9ORF72 mutation coming to autopsy showed classical ALS pathology with TDP-43 inclusions in spinal motor neurons.
  - Asymmetric atrophy and marked anterior temporal lobe atrophy are more suggestive of a GRN mutation in the context of a positive family history or a low serum progranulin level that can be used as a surrogate marker. In contrast, symmetrical frontal atrophy with additional cerebellar atrophy in the context of prominent psychiatric symptoms should point to a C9ORF72 mutation.

-
Mitochondrial Pathology in ALS: Evidence and Potential Mechanisms

- DNA methyltransferase (Dnmt) Dnmt3a in presynaptic axon terminals and its presence in mitochondria, as well as the presence of 5-methylcytosine in mitochondria, suggesting methylation of the mitochondrial genome by Dnmt3a. They also found upregulation of Dnmts and 5-mC in human ALS, suggesting that aberrant regulation of DNA methylation is part of the pathobiology of ALS (Lee Martin, J Neurosci 2011).

- Miquel at al PLOS April 2012: Mitochondrial dysfunction is one of the pathogenic mechanisms that lead to neurodegeneration in Amyotrophic Lateral Sclerosis (ALS). Dichloroacetate (DCA) improves the functional status of mitochondria through the stimulation of the pyruvate dehydrogenase complex activity (PDH). Their results indicate that improvement of the mitochondrial redox status by DCA leads to a disease-modifying effect, further supporting the therapeutic potential of mitochondria-targeted drugs in ALS.

- A hexanucleotide repeat expansion in C9ORF72 causes chromosome 9p linked FTLD/ALS and is one of the most common causes of familial ALS and is found in between 22 and 50% of cases depending on the geographical origins of the population. It encodes a highly conserved alternatively spliced 481 (full-length) amino acid protein with no known function. Normal individuals usually carry up to 23 repeated GGGGCC repeats, whereas affected individuals carry repeat expansions that may be up to 10 kb in length, containing over 1,000 repeats. C9ORF72 expansion may lead to the development of toxic RNA inclusions.
Session Objectives

• To look at mitochondrial pathobiology across the neurodegenerative diseases and to:
  – Highlight differences and commonalities related to mitochondrial dysfunction and pathology across the diseases.
  – Discuss opportunities for the development of mitochondria-related biomarkers and therapeutic interventions.
  – Identify next steps that research sponsors, investigators, and others should take to facilitate collaborative research and drug development in this area, including frameworks for partnerships and collaboration.
Mitochondrial Function in Neurons

- Oxidative Phosphorylation
- Free radical generation and detoxification
- Cell death pathways
- Inflammation
- Systemic cell death signals (DNA fragments)
Mitochondrial Pathology in ALS: Evidence and Potential Mechanisms

- Dynamics: trafficking, fission, fusion
- Morphology
- Function: ox phos, calcium
- Innate immunity
- Necrotic cell death has also been shown in glutamate-induced excitotoxicity, which is linked to neurological disorders such as Parkinson’s disease, Huntington’s disease and Alzheimer’s dementia. Glutamate-induced neurotoxicity rely on the mitochondrial component cyclophilin D, making it an attractive pharmacological target for clinical practice.