Impact of Myosin 5a Mutation In Neurodegenerative Disorders. Rat Model

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EX MORTE VENIT VITA

Externus timor maximum concordiae vinculum. Livy
Berlin-Druckrey (BD-IV) rat model for PD/AD
Potential role of Myo5A in neurodegeneration
Presentation Outline

- Class V Myosins
- Myo5A human/animal diseases
- BD-IV rat genetic analysis
- Myo5A interaction with $\alpha$-syn/tau
- Myo5A dopamine metabolism alteration
- Myo5A miRNA alteration in BD-IV rat
- Conclusions/ Future Directions
Class V Myosins

- Actin-dependent motor proteins
- Involved in intracellular transport of organelles
- Highly Expressed in CNS/PNS
- Three myosin V heavy chain genes (Myo5A,B,C)
Myo5a mutations cause pigmentation and neurological defects in humans and animals

- Mutations in human MYO5A cause Griscelli syndrome, type 1 in humans (Griscelli et al., 1978)
- Mutations in horse MYO5A cause Lavender Foal Syndrome (Brooks et al., 2010)
- Myo5a is mutated in dilute mice, (Mercer et al., 1991)
- Myo5a is mutated in dilute opisthotonus rats (Futaki et al., 2000)
- Myo 5a is mutated in shaker BD-IV rat. Stoica et al.,
Griscelli Syndrome type I

Myo5A

Myo5A
Lavender Foal Syndrome
Myo5a is mutated in mice: *dilute-lethal*
Shaker’s BD-IV hair

Mag:20-x
Myo5A

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<th>BD IV wt</th>
<th>BD IV affected</th>
<th>SD control</th>
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<td>GAPDH</td>
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Mammalian genome contains three myosine V genes-Myo5A, B and C- that display differential expression patterns and tissue-specific alternative splice variants. Myo5A gene encodes the molecular motor protein Myo5A, found on:

- chromosome 1 in horses
- chromosome 15 in humans
- chromosome 9 in mice
- chromosome 8 in rat

Myo5a is a highly conserved protein from plants to human
Myo5A

Myo 5A comprises a homodimer of heavy chains, each of which has six IQ-motifs that bind to calmodulin light chains. The Myo5A heavy chains dimerize via a coiled-coil region (purple) that is interrupted by loops. In neuronal Myo5A, the dimeric light chain dynein light chain 2 (DYNLL2; light blue) binds in the coiled-coil region. Both the coiled-coil region and the globular tail domain (red) are involved in cargo binding.

Rudolf et al., 2011
Myo5A binds actin and produce mechanical force through ATP hydrolysis

- Myo5A regulates organelle transport in both melanocytes and neuronal cells (highly expressed in neurons)
- Is highly expressed in the central and peripheral nervous system
- Myo5A is a motor protein that is involved in local, actin-based organelle transport
- In Purkinje cells Myo5A appears to be involved in transport of smooth endoplasmic reticulum into the spines.

Kneussel and Wagner, Nature Reviews, 2013
Myo5A is associated with mitochondria and secretory vesicles

BD- IV Rat Genetic analysis

- Whole genome sequencing

- Hugo Bellen, Professor&Head, Baylor College of Medicine, Houston
- Chen Rui, Associate Professor, Baylor College of Medicine, Houston
Mutation found in the affected rat (Myo5A) by whole genome sequencing.

Berlin-Druckrey (BD-IV) rats

Control
Control (Carriers) heterozygous
Affected homozygous
Myo5A gene

Results of whole genome sequencing

- located on 8q24 and its size is 118kb (118,043 bp). 182kb (including all the regulatory regions)

- protein size approx. 190 KDa
Myo5A mutation - rats

Ataxic rats were found in a breeding colony of Wistar rats and the abnormal phenotype was shown to be controlled by an autosomal recessive gene. On a pigmented background, mutant homozygotes are distinguishable from their normal littermates at 3–4 days of age by their lighter pigmentation and their subsequent diluted coat color. After 11 days of age, they develop movement disorders such as staggering and difficulty in walking. Around 14–16 days, the symptoms become more severe and mutants manifest chronic convulsion with opisthotonus (state of severe hyperextension and spasticity). Finally, they die at 21–22 days of age, probably due to difficulties with food and water intake. On the basis of this phenotype, we named the mutation dilute-opisthotonus, with the gene symbol dop.

Analysis of the Myo5A gene of the dop genome showed the presence of a complex rearrangement consisting of a 306-bp inversion associated with 217-bp and 17-bp deletions. A 141-bp exon is skipped in the dop transcript, producing a dop cDNA with a 141 in-frame deletion in the sequences encoding the head region.

Takagishi Y, Murata Y, 2006 noted that a Myo5A mutation in rats is an animal model for the human hereditary neurological disease, Griscelli syndrome type 1.
Myosin 5A mutation in BD-IV rat

Scenario 1: internal deletion

Internal deletion generated by the splice donor mutation is likely to be deleterious to the motor domain of Myo5a

If this is the case, RT-PCR of mRNA would allow one to detect a band that is 66bp smaller in the mutant (and even heterozygous) animal compared to homozygous wild-type animals.

http://blackburngen677s13.weebly.com/domains.html
Scenario 2: read-through and early termination

The alternative scenario is that the intronic sequence will be transcribed and translated. This adds 12 random amino acids after E204 and terminate, leading to an early truncation of Myo5a. The protein will most likely be non-functional.

Myo5a is most known for its role in melanosome trafficking. Deletion of 2/3 of the motor domain together with rest of the protein will most likely kill the function of the protein.

If this is the case, western blotting or immunohistochemistry using Myo5a will fail to detect a signal, or very detection of a 20-30 kDa protein if the truncated protein is stable.
Myo5a and alpha-synuclein

Myo5A interacts with alpha-synuclein at the presynaptic terminals?

Myo5A influences accumulation and/or aggregation of alpha-synuclein at the presynaptic terminals?

Myo5A plays a role in PD/AD?!

◆ α-syn is the most enriched mRNA associated with Myo5a
◆ RNA localization and local protein synthesis may be involve in neurodegenerative disorders such as PD (Calliari et al., Developmental Neurobiology, 2013)
Morphological changes
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<th>Striatum</th>
<th>SN</th>
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α-synuclein LB
α-synuclein mRNA expression (fold increase in affected vs. control) 

Stoica et al., 2012
TEM: A. Sub.Nigra, B. Striatum, C. Post-synaptic degen., D. OB, autophagy
BD-IV shaker rat: Brain stem and cerebellum nuclei  Ph. Tau
TAU phos. in 30dpn rats

- SD control
- BD IV affected

Bar graph showing TAU phosphorylation levels in different regions (BS, CR, OB, MS, ST, FC) of the brain from 30-day post-natal rats, with controls and BD IV affected groups compared. The graph includes error bars indicating variability.
anti-TAU (ph S396) in the Frontal Cortex

Graph showing the expression levels of anti-TAU (ph S396) in SD control and BD IV affected groups. The y-axis represents the expression levels, and the x-axis represents time points 15 pdn and 30 pdn.

15 pdn:
- SD control: Low expression
- BD IV affected: Moderate expression

30 pdn:
- SD control: Low expression
- BD IV affected: High expression

Additional panels show Western blots for TAUphos and GAPDH at 15 pdn and 30 pdn for SD control and BD IV affected groups.
Alteration of Dopamine metabolism

Catecholaldehyde hypothesis
Ion mobility mass spectrum for blanks (A) as well as normal (B) and affected rat striatal tissues at 11 (C) and 20 dpn (D). The labeled peak was selected for at the reduced mobility of dopamine and at m/z = 154, the mass of the dopamine ion. The striatal level of dopamine in affected rats was reduced by > 90%, relative to control rats.

Serum dopamine levels from control and affected BD-IV rats sacrificed at 24 dpn.

Zhang et al., 2014. Metabolic analysis of striatum tissues from Parkinson’s disease-like rats by electrospray ion mobility mass spectrometry.
Aldehyde dehydrogenase participates in the metabolism of catecholamines including dopamine (DA) and converts 3,4-dihydroxyphenylacetaldehyde (DOPAL), a potentially toxic aldehyde, to 3,4-dihydroxyphenylacetic acid (DOPAC), a non-toxic metabolite.

The decreased levels of aldehyde dehydrogenases were associated with loss of neurons in SN and decline in motor function, supporting the hypothesis that impaired detoxification of biogenic aldehydes are important in the pathophysiology of PD.
DOPAL neurotoxicity and its role in PD was demonstrated both *in vivo* and *in vitro* and supports of “catecholaldehyde hypothesis” as an important link in the pathogenesis of PD.
Goldstein et al., 2012 showed that DOPAL potently oligomerizes α-syn and appears to aggregate mutant form of the protein.

Burke et al., 2008 showed that DOPAL injection into the SN of Sprague-Dawley rats resulted in DA neuron loss and the accumulation of high molecular weight oligomers of α-syn detected by Western blot. These findings support the hypothesis that DA metabolism via DOPAL can cause both DA neuron loss and α-syn aggregation observed in PD.
Conclusions

◆ Aldehyde dehydrogenase decreased
◆ DOPAL levels increased
◆ Catechol aldehyde hypothesis
  proof of concept in this model
The severity of pathology is directly related to the overexpression of \( \alpha \text{-syn/tau} \) and parallel decrease in DA level in striatum and blood.

Stoica G. et al., JNC, 2012
Brain miRNA Expression Profile

(A)

(B)

\( P < 0.05 \)
Brain Nurr-1 Protein Expression

Lungu et al., 2013
Significant reduction in BDNF, major regulator of neuronal survival in the serum and mesencephalon of affected rats

Lungu et al., 2013
Conclusions

- DOPAMINE level decreased
- Perikarya and neurites Lewy bodies
- Neuronal loss
- Gliosis and release of inflammatory cytokines
- Decreased nerve growth factors
- \( \alpha \) -SYN level increased
- \( \alpha \)-syn is the most enriched mRNA associated with Myo5a
- RNA localization and local protein synthesis may be involve in neurodegenerative disorders such as PD (Calliari et al., Developmental Neurobiology, 2013)
Future Directions

- Continue to explore genetic alterations responsible for disease
- Understanding the functions of myosins in neurons is significant for molecular mechanisms at synapses and their plasticity
- Identify proteins/myosins interactions for developing disease-modifying therapies
- Explore the potential involvement of Myo 5A genetic alteration in human neurodegenerative disorders such as: Parkinson’s Disease, Alzheimer and others
- Understanding neuronal functions of myosins help explain how these motors contribute to brain function in health and neurological disorders
A surprise guest! Michael J. Fox addressed the audience to a rousing standing ovation! Fox encouraged the group to interact and do what they need to do to find the answers. He added that the answers don't just fall from the sky—you have to get up on your ladders and get them. (2012 NYAS)

Thank for the generous support from MJ Fox Foundation for Parkinson’s Disease!