Kennedy’s Disease: Mouse Models and Mechanistic Studies
• Kennedy’s Disease - Spinal Bulbar Muscular Atrophy
• Mouse Models of SBMA
• Transcriptional dysregulation - motor neuron loss
• Role of testosterone in SMBA
• Proteolysis and transcriptional dysregulation
Kennedy’s Disease: What kind of disease is it?
Motor Neuron Disease
Motor Neuron Diseases

- Amyotrophic Lateral Sclerosis or Lou Gehrig’s disease affects both the lower and upper motor neurons—Genetic and environmental factors

- Polio effects the lower motor neurons and is caused by the polio virus

- Spinal Muscular Atrophy (SMA) affects the lower motor neurons—one that minics some of the features of Lou Gehrig’s disease is *Kennedy’s disease or X-linked Spinobulbar Muscular Atrophy (SBMA)*

- There are 1 in 50,000 or 5,500 people diagnosed with motor neurons disease each year in the USA
Movement occurs through the action of motor neurons

Anatomy of motor neuron is special: Very long axon 10,000 times the length of the cell body
How was Kennedy’s Disease Discovered?
William R. Kennedy - neurologist at University of Minnesota

1964 George B. was referred to Kennedy - had a possible diagnosis of ALS

ALS has upper and lower motor neuron loss - George had only lower motor neuron loss symptoms

Progressive weakness and muscle twitches
Kennedy’s Disease Symptoms

EFFECT OF MOTOR NEURON DEGENERATION

• Muscle weakness and wasting
• Swallowing difficulties-bulbar muscles/brain stem
• Speech dysfunction
• Shaky muscles
• Muscle twitches
• Absent reflexes

EFFECT OF MALE HORMONE DYSREGULATION

• Enlarged breast
• Low sperm count
• Shrunken testicles
What is the genetic mutation that causes Kennedy’s Disease?
• SBMA is caused by a polyQ expansion (CAG expansion) in the N-terminus of androgen receptor.
• Expansion > 36-38 polyglutamine repeats causes SBMA
• Longer repeat length earlier age of onset of the disease.

Al La Spada and Kenneth Fischbeck, 1991
At least eight are currently known (HD, DRPLA, SCA-1, 2, 3, 7, 17, and SBMA)

DRPLA is most like HD

Affected brain regions include cortex, basal ganglia, brainstem, cerebellum and spinal cord

The genes share no homology except for the poly-Q repeats

The androgen receptor is a DNA-binding transcription factor

Ross, Neuron 1995
Androgen Receptor Action

1. Steroid binds to receptor
2. Release heat shock proteins
3. Conformational change
4. Phosphorylation
5. Dimerization
6. Binding to DNA
7. Further phosphorylation
8. Activation of transcription
Loss of function mutations in AR in patients causes feminization but not motor neuron loss.
Expansion of polyglutamine repeat has no effect on hormone binding and only slightly reduces its ability to transactivate genes.
Partial loss of function-gynecomastia, predominantly gain of function.
What is the mechanism of motor neuron dysfunction and loss?
Transcriptional Dysregulation - SBMA Neurotoxicity

Ross Neuron 2002

Angelo Poletti
Outline

• Kennedy’s Disease-Spinal Bulbar Muscular Atrophy
• **Mouse Models of SBMA**
• Transcriptional dysregulation-motor neuron loss
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• Proteolysis and transcriptional dysregulation
Generation of SBMA mouse model that recapitulates disease phenotype
SBMA-Mouse Model that Recapitulates Disease
Human AR expressed in Spinal Cord of YACs
SBMA-Mouse Model that Recapitulates Disease

10 month age

- wt, AR20+
- AR100+ (C25)
- AR100+ (C32)
SBMA-Mouse Model AR100 is in the Nucleus

AR100                      Control
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Ross Neuron 2002
Studies of Transcription in PolyQ Disease Models

Steffan…Thompson: CBP and p53-fly models
Paulson: CBP
McCambell…Fischbeck: CBP
Wyttenbach…Rubinsztein: CBP
Nucifora…Ross: CBP/CREB-HD and DRPLA Tg
Shimohata…Tsuji: TAF 130
Li: Sp1
Dunah…Krainc: TAF 130 and Sp1--via soluble interactions?
CBP and Neuronal Signaling

- CBP is activated by cell surface cAMP dependent signaling
- Other signaling pathways also converge on CBP
- CBP activates gene transcription, in part via Histone Acetyl Transferase (HAT) activity
- CBP-mediated transcription is important neuronal survival
- CBP is involved in activating a transcription factor known as hypoxia induced transcription factor (HIF1-α)- motor neurons
**AR-PolyQ dependent binding of CBP**

**Motor neuron cell culture model**

<table>
<thead>
<tr>
<th>DHT</th>
<th>pcDNA3</th>
<th>AR12</th>
<th>AR112</th>
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Input

Anti-AR N-20

IP:CBP

Anti-CBP

IP:CBP

Anti-AR N-20

1  3.0  AR112 / AR12 (-DHT)

1  3.0  AR112 / AR12 (+DHT)

**YAC SMBA model**

<table>
<thead>
<tr>
<th>NTg</th>
<th>AR20</th>
<th>AR100</th>
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IP:CBP

Anti-CBP

IP:CBP

Anti-AR N-441

0.6  1.3  1.9  AR / CBP
VEGF Rescues Mutant Androgen Receptor Neuronal Death

VEGF Rescues Mutant Androgen Receptor Neuronal Death

% ALIVE

% DEAD

pcDNA3 | AR12 | AR112

Control

+ VEGF164

AR112-untreated

2.5 ng/μL VEGF164

5.0 ng/μL VEGF164

10 ng/μL VEGF164

20 ng/μL VEGF164
CBP restores VEGF 164 Levels Suggesting a Critical Role

CBP Rescues Mutant Androgen Receptor Neuronal Death
VEGF and Motor Neuron Survival

- VEGF receptor knockout embryonic lethal...

- VEGF required for motor neuron survival-
  Ooshuyse et al 2001 Nature Genet. 28, 131-138
  Deleted hypoxia-response element from the
  Vegf promoter-ALS like phenotype with 30%
  reduction in VEGF levels in spinal cord

- CBP co-activator of hypoxia induced
  transcription factor (HIF-1) which regulates
  growth factor

- Test the hypothesis polyQ AR interferes with
  CBP action on HIF-1 and VEGF levels in the
  spinal cord
VEGF 164 LEVELS ARE ALTERED IN YAC SBMA TG MICE
High VEGF-producing genotypes

High circulating VEGF levels

Direct neurotrophic effect of VEGF

Motor neuron survival

Neuronal perfusion

Reduced direct neurotrophic effect of VEGF

Motor neuron death

Low VEGF-producing genotypes

Low circulating VEGF levels

TRENDS in Molecular Medicine
SBMA-Transcriptional Dysregulation

Normal

SBMA

spinal motor neurons

neurodegeneration
Role of VEGF in Neuroprotection-Motor Neurons

- Deletion of hypoxia-response element from Vegf promoter develop ALS like syndrome.
- Cross-breeding these mice with fALS mice SOD1^{G93A} accelerate phenotype.
- Variations in the human VEGF promoter/leader sequence, which is associated with reduced levels of circulating VEGF also confer increased risk of ALS.
- VEGF reduces glutamate excitotoxicity and production of free radicals.
- VEGF link to motor neuron disease in general provides prospects of new mechanistic insights and treatment of motor neuron disease.
- Mechanism of action not known… trophic support of motor neurons?
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Castration Prevents the Kennedy’s Disease


• Leuprorelin-lutenizing hormone-releasing hormone angonist reduces androgen levels in the testis-medical castration-prevents nuclear translocation of AR
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What is the role of testosterone in SBMA?

Hypothesis: Androgen receptor fragments required for transcriptional dysregulation
Evidence for the Role of Proteolysis in SMBA

- Truncated fragments found in SMBA post-mortem tissue.

- Generation of transgenic model using endogenous human AR promoter and first exon of AR- rapid disease progression. Full-length AR models slow disease progression.

- Caspases cleave androgen receptor.

- Common properties to all polyQ diseases.

- Testosterone results in a polyQ dependent increase in proteolysis.
Evidence for the Role of Proteolysis in YAC100

*Polyglutamine dependent N-terminal fragment
Proteolysis of Androgen Receptor

ANDROGEN RECEPTOR

Poly Q insertion

C4 Zinc finger

hormone binding

NLS

Caspase site DEEDS

~500

910

XXXDX
Androgen Receptor is Cleaved by Caspases

polyQ fragments

polyQ fragments

NH₃   Q  H  CO₂H
Caspase Resistant AR Reduces Cellular Toxicity

% Apoptotic Cells

- pRc/CMV
- AR12
- AR12D146N
- AR50
- AR50D146N
Aggregate Formation in SBMA AR

AR12

AR5O

AR12 D146N +TAM

AR12 +TAM

AR5O +TAM

AR50 D146N +TAM

AR50
Caspase Resistant AR-Transcriptional Dysregulation

% Transactivation  Reporter Assay

pRc/CMV  AR12  AR112  AR112 (1-750)  AR112D146N

0  10  20  30  40  50  60  70  80  90
Generation of SBMA Transgenic Mouse Models

AR12
AR112
AR112 D146N
CBP, p53, Caspase form Complexes-Dysregulation
PolyQ EXPANSION IN AR ALTER LOCALIZATION OF CBP

GFP-AR114  CBP  Merge

FL AR

FL AR + Fragment
FL AR COMPLEX WITH CBP, p53 AND CASPASE-6

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<td><strong>MEK1/2 inhibitor</strong></td>
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**Input**

- Anti-CBP
- Anti-AR N-20
- Anti-p53
- Anti-caspase-6

**IP:CBP**

- Anti-CBP
- Anti-AR N-20
- Anti-p53
- Anti-caspase-6

AR12 fragment
Conclusions:

1) SBMA is a motor neuron disease-testosterone plays a critical role in disease pathology and progression.

2) SBMA treatment:
   - Androgen-blockage drugs used to treat prostate cancer.
   - Growth factors that are required for motor neuron survival-VEGF.
   - Protease inhibitors block production of toxic fragments.

3) VEGF levels play an important role in motor neuron diseases such as fALS and SBMA.
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HDF
MDA
HDSA

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