Kennedy’s Disease Research at the Lim Lab

- Signaling pathway modulating Androgen Receptor post-translational modifications and its clearance as well as their contribution to KD

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Yale University School of Medicine
Translational Neuroscience

Neurodegenerative Diseases

*successful therapies?*

- pre-clinical trials
  - drug discovery
  - candidate targets for therapeutic intervention

<table>
<thead>
<tr>
<th>SBMA</th>
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<tr>
<td>gene identification</td>
</tr>
<tr>
<td>disease models</td>
</tr>
<tr>
<td>pathogenesis studies</td>
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Basic Neuroscience
Translational Neuroscience

Neurodegenerative Diseases

successful therapies?

pre-clinical trials

drug discovery

candidate targets for therapeutic intervention

SBMA

Basic Neuroscience

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Translational Neuroscience

Neurodegenerative Diseases

successful therapies?

- pre-clinical trials
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SBMA

- gene identification
- disease models
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Basic Neuroscience
Neurodegenerative Diseases

Translational Neuroscience

Pre-clinical trials

Drug discovery

Candidate targets for therapeutic intervention

SBMA

Gene identification

Disease models

Pathogenesis studies

Successful therapies?
Are there any good candidate targets for therapeutic intervention for SBMA?
Are there any good candidate targets for therapeutic intervention for SBMA?

Nemo-Like Kinase (NLK)
Nemo-Like Kinase (NLK)

- Conserved MAPK-like serine/threonine protein kinase
Cargnello et al., 2011

Conventional MAPKs

- ERK1/2
  - Kinase domain

- p38 \(\alpha/\beta\) (\(\gamma/\delta\))
  - Kinase domain
  - TGY

- JNK1/2/3
  - Kinase domain
  - TPY

- ERK5
  - Kinase domain
  - NLS
  - TAD

Atypical MAPKs

- ERK3
  - Kinase domain
  - C34
  - SEG

- ERK4
  - Kinase domain
  - C34
  - SEG

- ERK7/8
  - Kinase domain
  - NLS

- NLK
  - AHQr
  - Kinase domain
  - TQE
Nemo-Like Kinase (NLK)

- Conserved MAPK-like serine/threonine protein kinase

- Linked to several cell signaling pathways and processes (*Wnt*, *TGF-β*, *Notch*, etc.)
Nemo-Like Kinase (NLK)

- Conserved MAPK-like serine/threonine protein kinase
- Linked to several cell signaling pathways and processes (Wnt, TGF-β, Notch, etc.)
- Interacts with many disease-associated proteins
Nemo-Like Kinase as a potential modifier of many neurodegenerative diseases

Lim et al., 2006; unpublished
NLK interacts with both wild-type and polyQ-expanded mutant AR

Todd et al., 2015

NSC-34 motor neuron cells
NLK influences the phosphorylation status of AR in cell culture

Todd et al., 2015
A potential model for the role of NLK in SBMA pathogenesis

SBMA phenotypes
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration
Can NLK modulate SBMA pathogenesis?

Altering *Nlk* expression
or
Modulating NLK activity

? SBMA pathogenesis
NLK promotes polyQ-expanded mutant AR phenotypes in cell culture system

Todd et al., 2015

GFP (AR)
A-FLAG (NLK)
DAPI (nuclei)
NLK modulates polyQ-expanded mutant AR toxicity in a *Drosophila* model of SBMA.
NLK modulates polyQ-expanded mutant AR toxicity in a *Drosophila* model of SBMA

**IB:**
- α-AR (low exposure)
- α-AR (high exposure)
- α-Tubulin

**Control**

**SBMA**

**SBMA; nmo^{adk1/+}**

**SBMA; nmo^{adk2/+}**
Reduced expression of NLK suppresses the toxicity induced by a mutant AR fragment

- trAR112Q: N-terminal 130 amino acids contains polyQ region contains S81 and S94 (purple dots)

Todd et al., in revision

(c) control  (d) trAR^{112Q/+}; nmo^{++/+}  (e) trAR^{112Q/+}; nmo^{adk1/+}  (f) trAR^{112Q/+}; nmo^{adk2/+}
Can loss of \(Nlk\) affect SBMA-related phenotypes \textit{in vivo}? 

SBMA mouse model  

\[\times\]  

Compare the male progeny  

Mice with reduced \(Nlk\) expression
A fifty percent reduction in NLK partially rescues SBMA pathology in mice

Todd et al., 2015
Loss of one copy of Nlk improves the pathogenic change in motor neuronal soma size in SBMA mice

E  
area (µm²)  

F  
perimeter (µm)  

Todd et al., 2015
Loss of one copy of \( Nlk \) significantly extends overall lifespan of AR-BAC mice

Kaplan Meier, log rank test, \( p = 0.00107 \)

Todd et al., 2015
Interim Summary

- NLK interacts with, and phosphorylates, polyQ-expanded mutant AR.

- Increased NLK expression promotes SBMA phenotypes in cell culture and *Drosophila* in a kinase activity-dependent manner.

- Decreased expression of *Nlk* partially, but significantly, suppresses SBMA-related phenotypes in *Drosophila* and mice.
Next questions are …

1. What is the molecular mechanism for the NLK-mediated effects on SBMA?

2. Whether pharmacological inhibition of NLK could suppress SBMA phenotypes?
Next questions are …

1. What is the molecular mechanism for the NLK-mediated effects on SBMA?

2. Whether pharmacological inhibition of NLK could suppress SBMA phenotypes?
A potential model for the role of NLK in SBMA pathogenesis

SBMA phenotypes
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration
NLK influences the phosphorylation status of AR in mice

AR-BAC mouse muscle

S81

polyQ

S94

S256

S308

S424

S515

S630

NTD

DBD

H

LBD

Todd et al., 2015
NLK increases AR-dependent gene transcription in a kinase activity-dependent manner.
Increased NLK elevates AR protein expression

IB: α-AR
IB: α-FLAG
IB: α-GAPDH

IP: α-FLAG

Cell extract (Input)

AR co-IPed

<table>
<thead>
<tr>
<th></th>
<th>HA-AR</th>
<th>25Q</th>
<th>120Q</th>
<th>25Q</th>
<th>120Q</th>
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<tr>
<td>FLAG-NLK-WT</td>
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<tr>
<td>DHT</td>
<td></td>
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</table>

* NSC-34 motor neuron cells

Todd et al., 2015
AR is degraded by the autophagy-lysosomal pathway

**SBMA phenotypes**
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration
Can NLK modulate SBMA via regulating the autophagy-lysosomal pathway?

SBMA phenotypes
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration
Loss of NLK increases lysosome number/size

N2a cells
Loss of *Nlk* leads to up-regulation of a subset of lysosomal genes

- RNA sequencing from WT and Nlk KO N2a cells
- The location or function of lysosomal genes based on Gene Ontology, significantly up-regulated in Nlk KO cells

<table>
<thead>
<tr>
<th>Lysosome</th>
<th>Acp2</th>
<th>Anxa6</th>
<th>Ap5b1</th>
<th>Cd63</th>
<th>Col6a1</th>
<th>Daglb</th>
<th>Dnajc5</th>
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<tbody>
<tr>
<td>Membrane</td>
<td>Dpp4</td>
<td>Dpr137b</td>
<td>Hpse</td>
<td>Litaf</td>
<td>Lnpep</td>
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<td>Sppl2a</td>
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<td>Tmem55a</td>
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<td>Vamp8</td>
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<td>Lysosome</td>
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<td>Bgn</td>
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<tr>
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<td>Sdc3</td>
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<td>Slc30a4</td>
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In preparation
NLK decreases expression of TFEB, a master regulator of autophagy-lysosome biogenesis
Next questions are …

1. What is the molecular mechanism for the NLK-mediated effects on SBMA?

2. Whether pharmacological inhibition of NLK could suppress SBMA phenotypes?
SB23080 can inhibit NLK activity in cell culture

<table>
<thead>
<tr>
<th>Flag-NLK:</th>
<th></th>
<th>WT</th>
<th>KN</th>
<th></th>
<th>WT</th>
<th>KN</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-hAR:</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Compound X:</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **α-active-NLK**
- **α-Flag (NLK)**
- **α-AR-pS81**
- **α-AR (total)**
- **α-GAPDH**

HeLa cell extract

unpublished
SB23080 can inhibit NLK activity in cell culture
SB23080 can inhibit NLK activity in cell culture

- Flag-NLK: - WT KN - WT KN
- HA-hAR: + + + + + + +
- Compound X: - - - + + + +

α-active-NLK
α-Flag (NLK)
α-AR-pS81
α-AR (total)
α-GAPDH

HeLa cell extract
Working model for the role of NLK in SBMA pathogenesis

- Aggregation
- Cytotoxicity
- Neuromuscular degeneration
Summary

- NLK promotes SBMA phenotypes in a kinase activity-dependent manner.

- Reducing NLK expression is beneficial in SBMA.

- NLK interacts with, and phosphorylates, polyQ-expanded mutant AR.

- NLK can regulate expression levels of AR proteins by controlling the autophagy-lysosomal pathway.
The role of VCP in Kennedy’s Disease

- Supported by 2016 KDA Research Grant
**VCP**

- Valosin-containing protein, also named p97 or cdc48.

- ATPase associated with other activities (AAA+) protein family.

- Implicated in multiple cellular processes
  - cell cycle regulation, DNA replication, organelle biogenesis, and protein degradation, etc.

- Missense mutations in VCP cause IBMPFD (Inclusion Body Myopathy with Paget Disease of Bone and/or Frontotemporal Dementia).
  
  => Multisystem degenerative disorder with three variably penetrant phenotypes.
  - Inclusion Body Myopathy (IBM)
  - Paget’s Disease of the Bone (PDB)
  - Frontotemporal Dementia (FTD)
  - other phenotypes: cardiomyopathy, cataracts and neuropathy
VCP co-localizes with mutant AR in cultured cells

PC12 cells + DHT
VCP co-localizes with mutant AR in SBMA mouse skeletal muscle
VCP expression is increased in SBMA mouse skeletal muscle at symptomatic stages

For 10 weeks, n = 2 vs. 3
For 20 weeks, n = 4 vs. 5
For 30 weeks, n = 7 vs. 8
**p < 0.005 (t-test).
Increased expression of VCP suppresses the toxicity induced by a mutant AR fragment in *Drosophila*
Generation of \textit{Vcp} knockout mice

<table>
<thead>
<tr>
<th></th>
<th>Spinal Cord</th>
<th>Muscle</th>
<th>Cortex</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Vcp}:</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
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</table>

> Backcrossed over 10 generations onto pure C57BL/6J and 129S6/SvEv backgrounds.
Future VCP Questions in SBMA

- Whether, and how, VCP modulates the pathogenesis of SBMA?

1. Can altered expression of VCP modulate mutant AR phenotype and toxicity in SBMA?

2. Is increased VCP expression in SBMA mice beneficial or detrimental?

3. What mechanisms underlie the function of VCP in SBMA?

4. Is AAA+ ATPase enzymatic activity in VCP important for modulating SBMA phenotypes?
Translational Neuroscience

Neurodegenerative Diseases

Kennedy’s Disease

成功疗法？

pre-clinical trials

drug discovery

candidate targets for therapeutic intervention

gene identification
disease models
pathogenesis studies

Basic Neuroscience

NLK, VCP & ……
Acknowledgements

- Lab members:
  - Hiroshi Kokubu
  - Terri Driessen
  - Tingting Dong
  - Ivy Hu
  - Cleo Smeets
  - Xiaowen Yu

- Collaborators:
  - Albert La Spada, UC San Diego
  - Diane E. Merry, Thomas Jefferson University
  - J. Paul Taylor, St. Jude Children’s Research Hospital / HHMI

- Funding: NIH, Kennedy’s Disease Association