Behavioral Effects of SQSTM1/p62 Overexpression in Mice: Support for a Mitochondrial Role in Depression and Anxiety

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Dementia

Alzheimer's

Age

Confusion

Irritability

Depression

Memory Loss

Dementia

Plagiarism

Delusion

Brai

Delusion
Quick Facts about Alzheimer’s Disease and Dementia:

- Leading cause of death in the United States
- Currently more than 5 million people in the United States are living with Alzheimer’s Disease
- 1 in 3 elderly people will die with Alzheimer’s or another form of dementia

2014 Costs of Dementia:
- $603 BILLION

Projection of Alzheimer’s Cases in the U.S.:
- 6th Change in Deaths (2000-2010)
- 2050 Costs of Dementia:
- $3.9 TRILLION

2015 Worldwide Cases of Dementia:
- 47.5 MILLION

2050 Worldwide Cases of Dementia:
- 150 MILLION

World Health Organization

alz.org | alzheimer's association
A **biomarker**, or **biological marker**, generally refers to a measured characteristic which may be used as an indicator of some biological state or condition.
Pathogenesis of Neurodegeneration

**TRIGGERS**
- Mutations
- UPS Dysfunction
- Prion Transmission
- Ageing
- Oxidative Stress

**PRIMARY RESPONSE**
- Protein recruitment
- Aggresome
- Intranuclear aggregate
- Misfolded protein
- Native state
- Inhibited UPS-mediated proteolysis

**PATHOLOGY**
- PD
- Lewy body
- AD
- Plaque
- Tangles
- Prion
- Plaque
- ALS
- Bunina bodies
- PolyQ
- Inclusions
- Gene Transcription
- Axonal Function
- Synaptic Transmission
- Mitochondrial Survival and Function

Ciechanover and Brundin, 2003 Neuron 40:427

**SQSTM1/p62** is a component of **ALL** aggregates/inclusions
**SQSTM1/p62**


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**Neurodegenerative Pathologies**

*component of specific pathological protein aggregates*

**Ubiquitin – Proteasome Degradation**

*removal of misfolded or damaged proteins*

**Mitophagy/Autophagy**

*removal of defective mitochondria and proteins*
SQSTM1/p62

p62 shows the potential to be used as a biomarker for Neurodegenerative Disease.

Examine the relationship between SQSTM1/p62 and mitochondrial functionality under physiological conditions
p62 Regulation of Mitochondrial Morphology

WT MEF Cells

KO MEF Cells

KO MEF Cells + myc-p62

Mitochondria stained with MitoTracker Red

p62 plays a role in regulating mitochondrial morphology
p62 and Mitochondrial Functionality

p62 not only regulates morphology but also affects mitochondrial metabolism and energy production.
p62 and the Mitochondrial Genome

p62 protects mitochondrial genome integrity by a TFAM import related process.

Dr. Yifeng Du, Wooten Lab
• p62 plays an active role in affecting mitochondrial morphology and functionality.

• Reintroduction of p62 to a null-background restores mitochondrial function.

• **Overexpression** of p62 improves mitochondrial functionality above what is seen in WT.
Overexpress SQSTM1/p62 in a mouse model.

- examine its effects on mitochondrial dynamics
- mitochondrial relationship to mouse behavior
  
  anxiolytic behaviors and
  
  learning and memory
Mitochondrial Function and Behavior Patterns in p62KO Mice

p62KO mice exhibit significant levels of mitochondrial dysfunction.

Loss of p62 results in behavior patterns similar to those seen in Alzheimer’s Disease.
Expression of EGFP-p62 in Mouse Tissue

EGFP-p62 was effectively expressed in mouse brain.

WT

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Mitochondrial Metabolism in OEp62 in Hippocampus

p62 overexpression affects mitochondrial morphology and function.
Mitochondrial Functionality

- SQSTM1/p62 was effectively expressed in the brain, predominantly in the hippocampus, generating an overexpressing mouse model.

- Mitochondrial structure and metabolism improved in the presence of excess SQSTM1/p62.

Does improved mitochondrial function correlate with positive changes in behaviors associated with neurodegenerative disease??
Behaviors Associated with Alzheimer’s Disease

- Excessive worry
- Sleep problems
- Panic attacks
- Compulsive behaviors
- Irrational fears

Anxiety

Depression

Affective Spectrum Disorders
Open Field Maze

Used to measure general locomotor activity and anxiety

Distance traveled in the OFM was the same for overexpressing mice compared to WT.

However, overexpressing mice spent more time in the inner area of the maze reflecting decreased anxiogenic behavior.
Forced Swim Test

Modification of the Porsalt Swim test used to measure despair and depression.

- **Immobile** – floating with no or limited movement
- **Swimming** – active movement around beaker
- **Climbing** – actively trying to escape by climbing walls of beaker

There is no measurable difference in immobility time between genotypes.

However, recorded behaviors during the test did show significant improvement in depression like behaviors.
Affective Spectrum Disorder Behaviors

• Improvement in anxiety related behaviors.

• Distinguishing specific behaviors during the FST show overexpressing mice exhibit decreased depression/despair.

• Overall improvement in affective spectrum disorder behavior patterns in SQSTM1/p62 overexpressing mice.
Learning and Memory
Long Term Potentiation

The long term enhancement of signal transmission between neurons.

Overexpressing mice show improved LTP compared to WT.
Barnes Maze
Noninvasive test for hippocampal dependent spatial learning and memory

ADAPTATION (Day 1) – 4 trials/day; max – 3 min

SPATIAL ACQUISITION (Day 2-5) – 4 trials/day; Max – 3 min

PROBE TRIAL 1 (short term memory) (Day 5) – 2 hours post last acquisition trial; 90 second observation

PROBE TRIAL 2 (long term memory) (Day 12) – 90 second observation

Overexpressing mice showed slight improvement to latency in hidden box search times.

No difference in short term memory was observed, however, long term memory was improved in overexpressing mice.
Learning and Memory

- Overexpression of SQSTM1/p62 shows enhanced LTP compared to WT.
- Spatial learning is slightly improved with overexpression of SQSTM1/p62.
- Spatial long term memory is strengthened with overexpression of SQSTM1/p62.
- Overall, learning and memory are improved with overexpression of SQSTM1/p62 in the brain.
Conclusions

• SQSTM1/p62 levels affect mitochondrial functionality as well as behavior patterns associated with neurodegenerative diseases.

• SQSTM1/p62 appears to be a prime candidate for a protein that changes mitochondrial functionality and also affects behavior making it a potential biomarker for neurodegenerative diseases.

• SQSTM1/p62 could be a novel target for potential drug discovery to treat anxiety and affective spectrum disorders as well as, improve cognitive function associated with neurodegenerative diseases.
Generation of p62OE mice

C57BL/6-Tg(Thy1-SQSTM1)02MCWo/J (Stock# 27258)
**Wish to Thank:**

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- Jin Yan
- Denise Landers

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- Mike Irwin
- Carl Pinkert

- Vishnu Suppiramaniam
- Kodeeswaran Parameshwaran
Indentifying novel targets of intervention

Toxins, aging, Aβ

Mitochondrion → ROS → Neuron → Diseases (e.g., Alzheimer’s)

Qitao Ran, UT Health Science Center