GSK-3α is a central regulator of age-related pathologies in mice.

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The glycogen synthase kinase (GSK)-3 family

1. Serine-threonine kinases
2. First identified as a negative regulator of glycogen synthase, the rate-limiting enzyme in glycogen synthesis (Woodgett, 1980)
3. Two isoforms: GSK-3α and GSK-3β with both common and unique functions in various tissues
4. Typically active in unstimulated cells
5. Inhibited by many pathways, including hypertrophic pathways


Targets of GSK-3

22 metabolic and signaling proteins
10 structural proteins
18 transcription factors
GSK-3 as a putative nodal point in hypertrophic signaling

Does GSK-3α regulate aging?

Aging

Aging is a complex biological process controlled by multiple genetic, epigenetic, and environmental factors.
Mechanisms regulating aging

Active processes

Four major theories to explain aging:
1. somatic mutation
2. telomere loss
3. mitochondrial damage
4. waste accumulation

Somatic mutation theory:
Aging proceeds if somatic mutations and DNA damage exceed the capacity for DNA repair
Recruits p53 and p16 which leads to senescence
Protective vis-a-vis cancer
Leads to accelerated aging
Also recruits the senescence-associated secretory phenotype (SASP: Chronic sterile inflammation)
Mechanisms regulating aging

Telomere loss theory:
Telomeres shorten with every round of cell division. Telomere shortening/dysfunction is a cell-intrinsic mechanism leading to activation of DNA damage checkpoints that result in induction of senescence. Although this provides a tumor suppressor function, it can limit regenerative capacity.

Mitochondrial DNA replication errors:
Clonal expansion
Accumulation of these with age impairs energetics and increases ROS production.

Waste accumulation theory:
Aging results from accumulation of damaged proteins or dysfunctional organelles due to age-related impairment of degradative processes (proteasome; lysosome-mediated autophagy).
Virtually nothing is known about the role, if any, of the GSK-3 family in regulating aging.

Why hypothesize?
Signaling pathways involved in aging:

1. Insulin/IGF-1/IRS-1/PI3K/Akt/FOXO
2. mTOR
3. Wnt signaling
4. p53/Sestrins

All are negatively regulated by GSK-3s

Model: Germline GSK-3α knockout mouse.
No developmental abnormalities

Model: GSK-3α pan KO
Cumulative survival curve began with n=57 (KO) and 30 (WT). A survival disadvantage for the KO first became significant at 534 days of age (P=0.0443). The percent survival at termination of the study (24 mos) was 42% for KO and 73% for WT (P=0.0106).

Exaggerated hypertrophy in the GSK-3α KO

Not due to hypertension

Echocardiographic analysis:
LV dysfxn and dilatation
Hemodynamic analysis:
Progressive contractile dysfunction; impaired relaxation

H & E on heart sections:
Myocyte dropout and replacement fibrosis

Trichrome staining on the heart section
Increased superoxide in the KO

P16 is recruited
**H and E staining on heart tissue at 1yr of age**

WT

KO

Vacular degeneration with profound sarcopenia

**Skeletal muscle**

H & E of vastus medialis of quadriceps, showing vacuolar degeneration

**Superoxide production in vastus intermedius**

![Superoxide production graph](image)
EM image of vastus medialis of quadriceps, showing tubular aggregates: Insoluble material from SR and mitos. Protective response to sequester insoluble proteins—ineffective autophagy?

WT                                              KO

Accelerated and advanced osteoarthritis in the KO knee

Inflammatory cytokines in the knee
The liver at 24 mos. of age

Proliferation and senescence in the small intestine
SA-β-gal activity

Proliferation and senescence in the small intestine
SA-β-gal activity is increased in the small intestine of the KO. Crypt number is increased, but the crypts are thinner; the villi are sparse.
The gut at 24 mos. of age

Tyrosine hydroxylase staining

But not in all tissues

Skin appears unaffected. Despite Tyr Hydroxylase findings, brain- no degenerative findings and only moderate behavioral abnormalities.

Consistent with dominance of one isoform over the other in various tissues.
Senescence as a disease of impaired autophagy?
Mitochondrial remnants in the GSK-3α KO

What's the problem?
GSK-3α promotes autophagy in the heart

Alterations in Signaling pathways: mTOR
Autophagosome formation is inhibited by small molecule GSK-3 inhibitor
Autophagy is increased by the mTOR inhibitor, everolimus.

Can the mTOR inhibitor, everolimus, correct the cardiac contractile dysfunction and skeletal muscle abnormalities?
mTORC1 inhibition rescues the aging phenotype in skeletal muscle

Central regulator of hypertrophy/growth: mTORC1
Central regulator of autophagy: mTORC1

Regulation of the aging process

Hypothesis: how GSK-3α regulates aging
Conclusion

GSK-3α is a novel and central regulator of senescence in multiple tissues. This kinase may promote longevity and retard age-related pathologies, at least in part, via inhibition of mTORC1 and, consequently, activation of autophagy.

Knock-in of active GSK-3α.

Summary

Deficiency of GSK-3α accelerates development of age-related pathologies:

- Cardiac hypertrophy;
- Marked cell loss with replacement fibrosis;
- Contractile dysfunction and impaired diastolic relaxation;
- Skeletal muscle degeneration;
- Profound abnormalities of mitochondrial structure;
- Marked SAβ-gal activity in small intestine;
- Early and pronounced osteoarthritis with sterile inflammation;

These phenotypes are associated with a reduced lifespan.

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