Autophagy

Cure?... or Curse?

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Disclosure Statement:

None

Autophagy—The Basics

Degrades & recycles intracellular components

- -Abnormal proteins
- -Damaged organelles (mitophagy, ribophagy)
- -Infections
  - -For many intracellular bacterial infections, autophagy helps control them
  - -Some use autophagy to their advantage, ie brucella, bartonella, coxiella…Lyme is unknown
  - -Could autophagy induction make these worse?

Can Either Save or Doom the Cell

- -Stress response, enhancing cell survival
- -Also an alternative pathway to cell death
  - —Can cure or cause disease

Autophagy—A Key Player

Impaired autophagy and mitophagy in neurodenerative diseases

Liang P, Le W, 2015

Menzies FM, Fleming A, Rubinsztein DC, 2015

Lionaki E, Markaki M, Palikaras K, et al, 2015

Autophagy reduces carcinogenesis, but can enhance survival of all cells, including cancers

Ryter SW, Choi AM, 2015

Kenific CM, Debnath J, 2015

Autophagy is central to delayed aging in caloric restriction

Madeo F, Zimmermann A, Maiuri MC, et al, 2015

Dong W, Wang R, Ma LN, et al, 2015

Mammalian Target of Rapamycin

Protein kinases: MTOR1 & MTOR2

-Regulates growth, autophagy, & longevity

TOR – Extremely conserved evolutionarily

ALL EUKARYOTES: Yeast, plants, animals

MTOR drives growth, also converts reversible cellular arrest to senescence

Leontieva OV, Demidenko ZN, Blagosklonny MV, 2015

MTOR is central to aging, cancer, and autoimmune dz

Perl A, 2015

Stimulation of MTOR1 in isolation, is sufficient to promote cancer

Menon S, Yecies JL, Zhang HH, et al, 2012.

MTOR—DM2 & Alzheimer's

In animal model, sucrose induces amyloid-β and tauopathy from hyperactive mTOR—sugar consumption & diabetes increased risk of developing Alzheimer's

"…rapamycin, an mTOR inhibitor, prevents the detrimental effects of sucrose in the brain."

Orr ME, Salinas A, Buffenstein R, et al, 2014

"mTOR…involved in cellular senescence, organismal aging, age-dependent diseases."

"mTOR...key players in…the development of Alzheimer disease."

Perluigi M, Di Domenico F, Butterfield DA, 2015

Rapamycin—Sirolimus, & Family

Rapid and Powerful Inhibition of MTOR1

-Does not affect MTOR2 with short term use

-Does inhibit MTOR2 with chronic exposure

Powerfully Induces Autophagy

Immune Suppressant for Organ Transplant

-Related to temsirolimus and everolimus

-NOT related to tacrolimus

Highly Differential Effects are Dose Dependent

Non-cytoxic Anti-cancer Effects

Temsirolimus and everolimus, FDA approved for treating breast and renal cancer, respectively

Other Autophagy Inducers

MTOR Dependent

- -Vitamin D
- -Tetracyclines (mostly)
- -Curcumin
- -Epigallocatechin gallate (EGCG) (mixed)
- -Caloric restriction (CR)
- -Exercise (*in brain, opposite in skeletal muscle*)

MTOR Independent

- -Trehalose
- -Carbamazepine
- -Valproic Acid
- -Lithium (but also stimulates MTOR, so….)

Trehalose—Two Glucose Units

In Many Forms of Life, but Not Mammals

- -In foods, especially mushrooms
- -US approved food additive
- -Metabolized in intestines to glucose, little gets absorbed unchanged

"trehalose is safe for use"

Richards AB, Krakowka S, Dexter LB, et al 2002

"high dose IV trehalose is safe in humans"

Argov Z, Vornovitsky H, Blumen S, et al, 2015

Chronic CNS Infections, Inflammation, Insulin, & Dementia

Chronic CNS Infection/Inflammation, Diet/MTOR, and/or Susceptible Genetics

-Results: Accumulation of abnormal proteins

-Alzheimer's – Amyloid β

-Synucleinopathies – α-Synuclein

Parkinson's, lewy body dementia, & MSA

-TDP-43 Proteinopathies – TDP 43

ALS & FTLD

ALS-FTLD Spectrum-TDP-43

"aggregated proteins are a key feature of…the major neurodegenerative diseases…TDP-43 inclusions in nearly all ALS cases"

"clinical overlap between ALS and frontotemporal lobar degeneration, where ubiquitinated TDP 43 inclusions were first identified."

Baloh RH, 2011

-Autopsy: 64 FTLD patients with or without MND & ALS patients with or without dementia: TDP-43 demonstrated in all

Geser F, Martinez-Lage M, Robinson J, et al, 2009

Alzheimer's—Infection/Inflammation

"neuroinflammation plays a critical role in AD"

Shadfar, Hwang, Lim et al, 2015

"Borrelia…visualized in autopsy brain tissue from a patient with Alzheimer's disease and were cultured"

MacDonald AB, Miranda JM, 1987

"Amyloid metabolism is altered in LNB"

Mattsson N, Bremell D, Anckarsäter R, et al 2010

"spirochetes, contain amyloidogenic proteins"

Miklossy 2008

Parkinson's—Infection/Inflammation

"neuroinflammation is an important contributor to the pathogenesis of the disease [PD]"

Tufekci KU, Meuwissen R, Genc S, et al, 2012

Parkinsonism in brucellosis

Mousa AR, Koshy TS, Araj GF, et al. 1986

Molins A, Montalbán J, Codina A., 1987

Parkinsonism in bartonellosis

Breitschwerdt EB, Maggi RG, Cadenas MB, et al, 2009

Parkinsonism in Lyme

Cassarino DS, Quezado MM, Ghatak NR, et al, 2003

ALS/MND—Infection/Inflammation

Inflammation associated with ALS

Papadimitriou D, Le Verche V, Jacquier A, et al, 2010

ALS patients—Lyme seropositive 5x more frequently, subset responded to antibiotics

Halperin JJ, Kaplan GP, Brazinsky S, et al, 1990

Lyme initially diagnosed as ALS, responded to antibiotics, ALS diagnosis changed

Hänsel Y, Ackerl M, Stanek G, 1995

Motor neuron disease in brucellosis

Bahemuka M, Shemena AR, Panayiotopoulos CP, et al, 1988

Tetracyclines—Randomized Placebo Controlled Trials in Alzheimer's

Large, Multicenter Trial

3 months doxy-rifampin vs placebo

Significantly less decline in treatment arm

Loeb MB, Molloy DW, Smieja M, et al, 2004

Large, Multicenter Trial

12 mos doxy-rifampin, or only doxy, vs placebo

No significant benefits in the treatment arms

Molloy DW, Standish T, Zhou Q, et al, 2013

Tetracyclines—Randomized Placebo Controlled Trials in Synucleinopathy

MSA Parkinson-type –Controlled Trial

Minocycline 48 weeks vs placebo

Treatment decreased microglial activation

-No significant change in rate of functional decline

Dodel R, Spottke A, Gerhard A, et al, 2010

Early Parkinson's Disease—Controlled Trial

Minocycline vs creatine vs placebo x 18 months

-No change in need for starting symptomatic meds

NINDS NET-PD Investigators, 2008

Ceftriaxone—Randomized Placebo Controlled Trial in ALS

ALS – Controlled Trial

Ceftriaxone x 20 weeks vs. placebo

340 participants were randomly allocated to ceftriaxone and 173 to placebo

-No significant difference in rate of decline

Cudkowicz ME, Titus S, Kearney M, et al, 2014

Autophagy—The Rosetta Stone

Autophagy digests aberrant proteins across a range of neurodegenerative disorders

Mitophagy attenuates mitochondrial dysfunction in neurodegenerative disease

Xilouri M, Stefanis L, 2015
Frake RA, Ricketts T, Menzies FM, et al, 2015
Pellegrino MW, Haynes CM, 2015
Lionaki E, Markaki M, Palikaras K, et al, 2015
Wang G, Mao Z, 2014
Metcalf DJ, García-Arencibia M, Hochfeld WE, et al, 2012
Levine B, Kroemer G, 2008

Alzheimer's—Rapamycin

"animal model of AD…rapamycin rescues cognitive deficits…by increasing autophagy"

Caccamo A, Majumder S, Richardson A, et al, 2010

"rapamycin…can slow or block AD progression in a transgenic mouse model of the disease"

Spilman P, Podlutskaya N, Hart MJ, et al, 2010

Rapamycin given prophylactically reduces cognitive deficits—In mice with established disease, no effects

Majumder S, Richardson A, Strong R, et al, 2011

Rapamycin started after disease onset improved cognitive function—Treated mice behaved normally

Lin AL, Zheng W, Halloran JJ, et al, 2013

Alzheimer's—Curcumin

Alzheimer's in rural India is rare

Chandra V, Ganguli M, Pandav R, et al, 1998

Alzheimer's mouse model

Curcumin decreased amyloid-β & plaques by 43-50%

Lim GP, Chu T, Yang F, et al, 2001

Human data

34 patients got turmeric vs. placebo x 6 mos

-No change in MMSE scores

Baum L, Lam CW, Cheung SK, et al, 2008

3 patients got turmeric x 1 yr

-1/3 increased MMSE 5 points, 2/3 recognized family

Hishikawa N, Takahashi Y, Amakusa Y, et al, 2012

‘Normal’ Cognition—Curcumin Large Scale Human Data

Large Study: 1,010 Non-demented Participants

“Compared with subjects who had never or rarely consumed curry, subjects with higher levels of curry consumption showed higher crude mean MMSE scores ($p = 0.004$), as well as adjusted mean MMSE scores ($p = 0.023$”

Ng TP, Chiam PC, Lee T, et al, 2006

Alzheimer's—EGCG & Exercise—Mouse Model

Both EGCG and exercise, separately and in combination, attenuated nest building and Barnes maze performance deficits

"Additionally, these interventions lowered soluble Aβ1-42 levels in the cortex and hippocampus."

Walker JM, Klakotskaia D, Ajit D, et al, 2015

Alzheimer's—Carbamazepine

"After three months treatment with CBZ in the APP(swe)/PS1(deltaE9) mice, we demonstrated that the spatial learning and memory deficits in these mice are significantly alleviated."

" We also documented that the cerebral amyloid plaque burden and Aβ42 levels in these mice are significantly reduced. "

Li L, Zhang S, Zhang X, et al, 2013

Alzheimer's—Trehalose

In transgenic mice, trehalose improved dementia & reduced amyloid-β in hippocampus

Du J, Liang Y, Xu F, et al, 2013

In neurons, trehalose induced autophagy, reduced tau aggregation & cytotoxicity

"Thus,trehalose may be a good candidate for developing therapeutic strategies for AD and other tauopathies."

Krüger U, Wang Y, Kumar S, et al, 2013

Alzheimer's Models—Vitamin D

Vitamin D clears amyloid plaques and reduces amyloid toxicity in cortical neurons

Mizwicki MT, Menegaz D, Zhang J, et al, 2012

Vitamin D clears amyloid both on its own and additatively with curcuminoids

Masoumi A, Goldenson B, Ghirmai S, et al, 2009

Vitamin D moves amyloid across blood brain barrier in mouse, reduces amyloid plaques

Ito S, Ohtsuki S, Nezu Y, et al, 2011

Yu J, Gattoni-Celli M, Zhu H, et al, 2011

Alzheimer's—Human Trials
Vitamin D

Vitamin D + Memantine

-Improved MMSE at 6 months

Vitamin D or Memantine Alone

-No change

Annweiler C, Herrmann FR, Fantino B, et al, 2012

RCT of High Dose Vitamin D for 8 weeks

-No benefit

Potential problems: Short term high dose treatment, heterogenous group, ie MMSE 12-24, vitamin D2 was used instead of D3

Stein MS, Scherer SC, Ladd KS, et al, 2011

Cognition NOS—Vitamin D Nonlinear 'U-Shaped' Response

"Participants with both low (<25 nmol/l) and high (≥75 nmol/l) 25(OH)D concentrations at age 45 years performed significantly worse on immediate word recall."

Maddock J, Geoffroy MC, Power C, et al, 2014

"Toddlers in the lowest quintile of cord blood 25(OH)D exhibited a deficit…P = 0.001) …toddlers in the highest quintile of cord blood 25(OH)D also had a significant deficit .P < 0.001)"

Zhu P, Tong SL, Hao JH, et al, 2015

Vitamin D—
Nonlinear 'U-Shaped' Response

U-shaped association between vitamin D and cardiovascular disease

Zittermann A, 2014

Kids ages 6-12, "U-shaped association between vitamin D levels and respiratory health"

Niruban SJ, Alagiakrishnan K, Beach J, et al, 2014

Both low and high vitamin D associated with increased risk of lung and prostate cancer

Kristal AR, Till C, Song X, et al, 2014 Chen GC, Zhang ZL, Wan Z, et al, 2015

Synucleinopathy—Rapamycin

"Since rapamycin, a stimulator of autophagy, increased clearance of alpha-synuclein, it merits consideration as a potential therapeutic for Parkinson's disease"

Webb JL, Ravikumar B, Atkins J, et al., 2003

Rapamycin restores mitochondrial dysfunction and abrogates neurodegenerative features in a rodent model of Parkinson's

Siddiqui A, Bhaumik D, Chinta SJ, et al, 2015

Synucleinopathy—Rapamycin

Parkinson's Mouse Models

"rapamycin is able to prevent the loss of TH positive [tyrosine hydroxylase, enzyme that makes dopamine] neurons and to ameliorate the loss of DOPAC [dopamine metabolite]"

Liu K, Shi N, Sun Y, et al, 2013

Synucleinopathy Mouse Models

Rapamycin improves motor function, reduces abnormal CNS proteins, attenuates synaptic injury in mouse model of synucleinopathy

Bai X, Wey MC, Fernandez E, et al, 2015

Synucleinopathy—Trehalose Parkinson's Mouse Models

Oral trehalose lessens motor asymmetry, dopaminergic neurodegeneration, & α-synuclein accumulation in the nigrostriatal system

He Q, Koprich JB, Wang Y, et al, 2015

Trehalose at low concentrations disaggregates α-synuclein protofibrils and fibrils into small aggregates or even random coil structures.

At high concentrations, slows down transition into β-sheets & completely prevents mature fibrils

Yu WB, Jiang T, Lan DM, et al, 2012

Synucleinopathy—Trehalose LBD Mouse Model

"In Lewy body disease (LBD), which includes Parkinson's disease and dementia with Lewy bodies, insoluble α-synuclein is widely deposited"

Oral trehalose increased the level of the autophagosomal protein LC3, the levels of several chaperon molecules, and decreased the levels of detergent-insoluble α-synuclein

Tanji K, Miki Y, Maruyama A, et al, 2015

Synucleinopathy—Lithium, Valproic Acid, Rapamycin, & Carbmazepine

Rotenone Induced Parkinson's Model

Rapamycin, lithium, valproic acid, and carbamazepine, all autophagy inducers, protected neurons against rotenone toxicity

"Our results suggest that VPA and CBZ, the most commonly used anti-epilepsy and mood-stabilizing medications with low-risk and easy administration might be potential therapeutics for PD."

Xiong N, Jia M, Chen C, et al, 2011

Synucleinopathy/Parkinson's Vitamin D

3173 Men & Women Age 50-79 without PD

-Low vitamin D is a risk for PD development

Knekt P, Kilkkinen A, Rissanen H, et al, 2010

Vitamin D deficiency at Increased Risk for PD

-Vit D supplements & working outside—Less PD

Shen L, Ji HF, 2015

Vitamin D 1200u/day vs. Placebo x 12 mos

-Beneficial in certain VDR genotypes

Suzuki M, Yoshioka M, Hashimoto M, 2013

TDP-43 ALS/FTLD Models

SQSTM1/p62 regulates TDP-43 aggregates

Brady OA, Meng P, Zheng Y, et al, 2011

SQSTM1 zebrafish model of ALS/FTLD

-Rapamycin beneficial

Lattante S, de Calbiac H, Le Ber I, et al, 2015

Drosophila ALS model—TDP-43 ortholog

-Rapamycin beneficial

Cheng CW, Lin MJ, Shen CJ, 2015

Neuronal ALS model—Autophagy induction enhances TDP-43 turnover and survival

Barmada SJ, Serio A, Arjun A, et al, 2014

FTLD-U/FTLD-TDP Mouse Model
Various Autophagy Inducers

Rapamycin & tamoxifen

-MTOR dependent

Spermidine & carbamazepine

-MTOR independent

Benefits to cognitive and motor dysfunction

"Administration of these four chemical drugs to 6 month-old Tg mice for 1 month effectively ameliorates the motor dysfunction."

Wang IF, Guo BS, Liu YC, et al, 2012

Wang IF, Tsai KJ, Shen CK, et al, 2013

ALS—Lithium in Human Trials

44 Patients—Lithium + Riluzole vs. Riluzole

"None of the patients treated with lithium died during the 15 months of the follow-up, and disease progression was markedly attenuated when compared with age-, disease duration-, and sex-matched control patients treated with riluzole"

G93A/SOD1 ALS mouse model demonstrated "marked neuroprotection" associated with a "marked increase in autophagy"

Fornai F, Longone P, Cafaro L, et al, 2008

ALS—Lithium Human Trials

Randomized Controlled Trials

-Lithium showed no benefits & no safety concerns

-Lithium add-on treatment to riluzole in all

Morrison KE, Dhariwal S, Hornabrook R, et al, 2013
Verstraete E, Veldink JH, Huisman MH, et al, 2012
Aggarwal SP, Zinman L, Simpson E, et al, 2010

Riluzole activates heat shock protein 70

Yang J, Bridges K, Chen KY, et al, 2008
Liu AY, Mathur R, Mei N, et al, 2011

"Induction of the 70 kDa heat shock protein stress response inhibits autophagy"

Kanninen TT, Sisti G, Witkin SS et al, 2015

ALS—Riluzole Interference with Neuroprotection

"neuroprotection exerted by memantine, minocycline and lithium…is antagonized by riluzole"

"the inclusion of a group of patients free of riluzole treatment may be mandatory in future clinical trials performed in ALS patients with novel neuroprotective compounds"

Yáñez M, Matías-Guiu J, Arranz-Tagarro JA, et al, 2014

ALS—Lithium + Valproate Human Trials

"lithium and valproate cotreatment significantly increased survival (p=0.016)"

Biochemical markers: Cu/Zn superoxide dismutase, glutathione peroxidase activity, and reduced glutathione were followed

"exerted neuroprotection in our patients because all three markers reached levels that were not significantly different from the matched samples of healthy donors"

Boll MC, Bayliss L, Vargas-Cañas S, et al, 2014

SOD1-ALS Mouse Models

1.5-2% of ALS Patients Have SOD Mutation

Rapamycin worsens disease

-MTOR dependent autophagy induction

Zhang X, Li L, Chen S, et al., 2011

Caloric restriction worsens disease

-MTOR dependent autophagy induction

Patel BP, Safdar A, Raha S, et al, 2010

Trehalose improves disease

-MTOR independent autophagy induction

Zhang X, Chen S, Song L, et al, 2014

‘Normal’ Age Related Cognitive Decline—Rapamycin

Normal Mice

No ‘normal’ cognitive decline if started at 2 mo

-If started at 15 mo, no benefit

Majumder S, Caccamo A, Medina DX, et al, 2012

Normal Mice

Blocked ‘normal’ cognitive decline, even if started at 18 mo

-Treated mice—Less anxiety/depression all ages

Halloran J, Hussong SA, Burbank R, et al, 2012

Rapamycin—Extension of Life and Healthspan in Mice

In Genetically Heterogeneous Mice, Rapamycin Begun Even in Old Age Extends Life

9% in males **14% in females**

(begun at equal to about 65 yrs old in human years)

Harrison, Strong, Sharp, et al., 2009

Improved all age-related conditions in mice

- -Tendon stiffening
- -Cardiac dysfunction
- -Cognitive decline
- -Decreased mobility

Wilkinson, Burmeister, Brooks, et al, 2012

Flynn, O'Leary, Zambataro, et al, 2013

Rapamycin—Extension of Life and Healthspan in Mice

Life extension dose dependent

-At 3x the initial dose studied, genetically heterogeneous mice lived even longer

-23% longer in males & 26% longer in females

-Metabolic changes & hepatic gene expression differ in CR mice vs. rapamycin fed mice suggesting that these two interventions for extending lifespan differ in many respects.

Miller RA, Harrison DE, Astle CM, et al, 2014

Rapamcyin & Everolimus Improves Immune Function

Rapamcyin in Old Mice

- -Extends lifespan
- -Restores immune function
  - -Better response to influenza vaccination

Chen, Liu, Liu, et al, 2009

Everolimus—Derivative of sirolimus/rapamcyin

Elderly humans treated with low dose everolimus

- -Restored immune function
  - -Better response to influenza vaccination

Mannick, Del Giudice, Lattanzi, et al, 2014

Sirolimus, Everolimus, & Temsirolimus
Differential Immune System Effects

Rapamcyin/Sirolimus—Immune suppressant

Everolimus & temsirolimus both treat cancer, used as immunsuppressants—renal transplant

Skalioti C, Marinaki S, Darema M, et al, 2015
Chueh SJ, Sankari BR, Gonzales-Chambers R, et al, 2014

"…rapamycin and other rapalogs, which for a long time have been viewed (and used in the clinic) as pure immunosuppressants, can mediate robust immunostimulatory functions..."

Bravo-San Pedro JM, Senovilla L, 2013

Rapamycin—Dramatic Reduction of Cancer in Cancer-prone Mice

- Rapamcyin prevents and reduces cancers in multiple studies of genetically cancer-prone mice
  - Popovich IG, Anisimov VN, Zabezhinski MA, et al, 2014
  - Sun ZJ, Zhang L, Zhang W, et al 2013
  - Cen O, Longnecker R, 2011
  - Anisimov VN, Zabezhinski MA, Popovich IG, et al, 2010
  - Mosley JD, Poirier JT, Seachrist DD, et al, 2007
  - Wu Q, Kiguchi K, Kawamoto T, et al 2007
  - Liu M, Howes A, Lesperance J, et al 2005

Rapamycin—Anti-Aging? Or Just Anti-Cancer?

Aging Traits Evaluated—'Young' & Old Mice

-Rapamcyin improved aging traits, but did so in both 'young' and old mice

-Authors' Conclusion: Not an anti-aging effect, lifespan extension was from cancer prevention

'Young' mice translated to 46 human years old

Neff F, Flores-Dominguez D, Ryan DP, et al, 2013

Rapamycin extends life of yeast & drosophila, neither of which get much cancer

Rallis C, Codlin S, Bähler J, 2013

Moskalev AA, Shaposhnikov MV, 2010

Vitamin D & Tetracyclines—Aging

Vitamin D3 increased lifespan of worms by 39% ($p<0.001$)

Messing JA, Heuberger R, Schisa JA, 2013

Mice with too much or too little vitamin D experience premature aging

Tuohimaa P, 2009

Minocycline extends life in drosophila

Oxenkrug G, Navrotskaya V, Vorobyova L, 2012

Doxycycline extends life in C. elegans

-C. elegans & mice—the same mechanism

Houtkooper RH, Mouchiroud L, Ryu D, et al, 2013

Lithium & Trehalose—Aging

"…inverse correlation between drinking water lithium…and all-cause mortality… 1,206,174 individuals (β = -0.661, p = 0.003)"

-Low dose lithium extends life of C. elegans

Zarse K, Terao T, Tian J, et al, 2011

In C. elegans, trehalose starting even in old age extended remaining lifespan by 60%

If started young, lifespan extended by over 30% without side effects

Honda Y, Tanaka M, Honda S, 2010

Summary of Conclusions

MTOR—Central to aging/age related disorders

- -Aging
- -Neuro-degenerative diseases
- -Cancer

-MTOR inhibition may slow or halt these processes—Human research needed

-Induction of autophagy, both MTOR dependent and independent, may be beneficial. If infection is present which subverts autophagy, there may be risk—Human research needed