Outline

1. Introduction on Autophagy
   • Definitions, functions, history and main players involved

1. Signaling pathways regulating Autophagy

2. Implications in Neurodegeneration
   1. Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted by Alzheimer-Related PS1 Mutations
   2. Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits
      • Other neurodegenerative diseases
      • Strategies for potential treatments

3. Implications in Health & Fitness
   3. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis
Autophagy: definitions

- Autophagy is a process of self-degradation of cellular components in which double-membrane autophagosomes sequester organelles or portions of cytosol and fuse with lysosomes or vacuoles for breakdown by resident hydrolases. (Congcong H. et al, 2009 Annual.Rev.Genet)

- Autophagy is a ubiquitous process in eukaryotic cells that results in the breakdown of cytoplasm within the lysosome in response to stress conditions and that allows the cell to adapt to environmental and/or developmental changes. (Klionsky D.J. et al, 2007 Nature Mol. Cell Biology)

- Autophagy is a lysosomal degradation pathway that is essential for survival, differentiation, development, and homeostasis. Autophagy principally serves an adaptive role to protect organisms against diverse pathologies, including infections, cancer, neurodegeneration, aging, and heart disease. (Levine et al, 2008 Cell)
Subtypes of autophagy

- Phagophore
- Autophagosome
- Macroautophagy
- Microautophagy
- Peroxisome
- Macropexophagy
- Mitochondrion
- Mitophagy
- Lysosome (vacuole)
- Micropexophagy
- Ape1 Complex
- Cytoplasm to vacuole targeting (Cvt)
- LAMP-2A
- Chaperone
- Unfolded cargo protein
- Chaperone-mediated autophagy (CMA)
- Piecemeal microautophagy of the nucleus (PMN)
Physiological functions

• Autophagy defends against metabolic stress
  • Autophagy is activated as an adaptive catabolic process in response to different forms of metabolic stress, including nutrient deprivation, growth factor depletion, and hypoxia. This bulk form of degradation generates free amino and fatty acids that can be recycled in a cell-autonomous fashion or delivered systemically to distant sites within the organism.

• Autophagy works as a cellular housekeeper
  • The repertoire of routine housekeeping functions performed by autophagy includes the elimination of defective proteins and organelles, the prevention of abnormal protein aggregate accumulation, and the removal of intracellular pathogens. The autophagy pathway is uniquely capable of degrading entire organelles such as mitochondria, peroxisomes, and ER as well as intact intracellular microorganisms.

• Autophagy in life and death decisions of the cells
  • Autophagy constitutes a stress adaptation pathway that promotes cell survival. An apparent paradox is that autophagy is also considered a form of non-apoptotic programmed cell death called “type II” or “autophagic” cell death. But it is now clear that the mere presence of autophagosomes in dying cells is insufficient to distinguish “cell death with autophagy” from “cell death by autophagy”.

• Autophagy may be a guardian of the genome
  • The autophagic machinery can limit DNA damage and chromosomal instability
Autophagy, the new apoptosis?

- Although initially considered simply a degradative process, recent studies have revealed an integral role for autophagy in human pathophysiology. Accordingly, there has been a tremendous increase in autophagy research in the past 10 years.
History of Autophagy

Molecular machinery

1. **Induction**
   (e.g. low energy, hypoxia, stress, low levels of hormones)

2. **Autophagosome formation**
   (Atg 5,8,9,12,16, Beclin-1)

   Atg8 = LC3 in mammals!! Upon autophagy induction, LC3 exists as the lipid-conjugated form (LC3-II)

3. **Vesicle fusion and autophagosome break-down**
   LAMP2 and the small GTPase Rab7 are needed for autophagosome-lysosome fusion

4. **Degradation**
   acid hydrolase degrades the cargos (e.g. cathepsin B, D, L)

GFP - Atg5
Signaling pathways regulating Autophagy

- In the presence of abundant nutrients and growth factors including insulin, mTORC1 promotes cell growth and metabolic activity while suppressing the ULK1 complex and autophagy.

- In deprivation or stress, numerous signaling pathways inactivate mTORC1 kinase activity. This both suppresses cell growth to reduce energy demand and induces autophagy to enable stress adaptation and survival.

- Upstream of mTORC1 is the cellular energy–sensing pathway controlled by adenosine monophosphate–activated protein kinase (AMPK). High concentrations of AMP signal energy depletion, activate AMPK, and inhibit mTORC1, thus promoting autophagy.

- Hypoxia and activation of hypoxia-inducible factors (HIFs) induce mitophagy.

- Glucagon, a predominant hormone of the fasted state, also triggers autophagy in the liver.
Autophagy and Diseases

**Neurodegeneration**

- **Pro:** Basal autophagy is a homeostatic process that prevents intracellular proteins from accumulating to toxic levels.
- **Con:** Inefficient lysosomal clearance results in intracellular accumulation of autophagosomes, which may process the amyloid precursor protein into toxic forms.

**Cancer**

- **Pro:** Autophagy acts in tumour suppression by removing damaged organelles and possibly growth factors, and reduces chromosome instability.
- **Con:** Autophagy acts as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply.

**Myopathies**

- **Pro:** Autophagy prevents aggregate-prone protein accumulation that leads to physiological dysfunction.
- **Con:** Autophagy may contribute to muscle wasting and defective autophagosome clearance may interfere with cellular function.

**Ageing**

- **Pro:** Autophagy removes damaged organelles and can limit production of reactive oxygen species.

**Liver disease**

- **Pro:** Autophagy can alleviate endoplasmic reticulum stress by degrading portions of the organelle containing misfolded proteins.
- **Con:** Excessive autophagy may cause liver damage.

**Heart disease**

- **Pro:** Autophagy may be protective during ischaemia and pressure overload.
- **Con:** Autophagy is harmful during reperfusion.

**Infection and immunity**

- **Pro:** Intracellular bacteria, viruses and protozoans are removed from host removed from host cells by autophagy, and antigens are processed for MHC class II presentation. Autophagy may prevent auto-immune and inflammatory diseases.
- **Con:** Some microbes have evolved to subvert autophagy to establish a replicative niche.

Mizushima N. et al, 2008 Nature
Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted by Alzheimer-Related PS1 Mutations

Ju-Hyun Lee,1,2 W. Haung Yu,1,2,9 Asok Kumar,1,3 Sooyeon Lee,1,4 Panaiyur S. Mohan,1,2 Corinne M. Peterhoff,1 Devin M. Wolfe,1 Marta Martinez-Vicente,6,10 Ashish C. Massey,6 Guy Sovak,6,11 Yasuo Uchiyama,7 David Westaway,6 Ana Maria Cuervo,6 and Ralph A. Nixon1,2,8,*

- Autophagosome and their contents are cleared upon fusing with lysosomes containing cathepsins, other acid hydrolases, and vacuolar [H]⁺ ATPase.

- Acidification of autolysosomes is crucial for activating cathepsins and effecting proteolysis of substrates.

- Autophagy pathology in Alzheimer’s disease (AD) is exceptionally robust. Autophagic vacuoles (AVs), mostly containing Aβ peptide, collect in massive numbers within grossly distended portions of axons and dendrites of affected neurons >>>> **Defective AV clearance.**

- This lysosome-related pathology is greatly accentuated in early-onset familial AD (FAD) due to mutations of Presenilin-1 (PS1).

- Scientific question: **what is the role of PS1 in the context of FAD?**
About PS1

Mattson M., 2003 Nature
PS1 gene deletion selectively inhibits macroautophagy turnover of proteins

**A**  Radioactivity (dpm x 10^5) for WT and PS1KO.

**B**  Proteolysis (%) for WT and PS1KO.

**C**  Proteolysis (%) over time (hrs) for WT and PS1KO in the presence (+ serum) and absence (- serum) of serum.

**D**  Ratio of proteolysis between serum + and serum - conditions for WT and PS1KO.

**E**  Western blot analysis of total p70S6k and phospho p70S6k (Thr389) for WT and PS1KO with and without serum.

**F**  Fluorescence microscopy images showing control and serum deprivation conditions for WT and PS1KO.

**G**  LC3 punctae per cell for Control, WT, PS1KO, NH₄Cl, and 3MA.

**H**  Western blot analysis of LC3-I, LC3-II, and tubulin for WT and PS1KO with and without serum.
Defective clearance of autophagic vacuoles in PS1 KO blastocysts

Rap = Rapamycin
RC = Rapamycin Removal
Proteolysis deficits in autolysosomes of PS1 KO blastocysts

Is lysosome acidification impaired?
Defective lysosome acidification in PS1 KO blastocysts
Impaired glycosilation and targeting of the v-ATPase V0a1 subunit in PS1 KO cells

Does PS1 play a role in v-ATPase maturation?
Impaired glycosilation and targeting of the v-ATPase V0a1 subunit in PS1 KO cells

Uncleaved PS1 binds to immature v-ATPase. Does PS1 modulate its maturation in the ER and affect its delivery to lysosomes?

Other ER proteins, such as PDI and GRP94, did not interact.

Stable transfection of human PS1 into PS1 KO cells completely restored vesicular compartment acidification, CadD maturation, v-ATPase glycosylation and autophagy response.
Defective vesicle acidification and autophagic pathology in neurons of PS1 hypomorphic and PS cKO mice
PS1 mutations impair macroautophagy and v-ATPase targeting in fibroblast from patients with FAD
Conclusions

• This study defines an essential role for PS1 in the maturation and trafficking of the v-ATPase responsible for lysosomal acidification.

• The normal turnover of protein and organelles by autophagy is impaired if PS1 is ablated or mutated.

• The loss of lysosomal function is accountable for the marked acceleration of autophagy-related dysfunction and neuronal cell death associated with PS1-FAD.

Speculations

• Impaired lysosomal clearance could account for reported PS1-mediated increases in Aβ.

• Similarities between the severe autophagy pathology in PS1-FAD and that developing with a later onset in sporadic AD suggests that lysosomal dysfunction is also a pathogenic mechanism in the common sporadic form of AD.
Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer’s disease ameliorates amyloid pathologies and memory deficits

- TgCRND8 mice, overexpressing a version of APP695 including Swe and Ind mutations and producing more Aβ42 than Aβ40, develop lysosomal system pathology, accumulate intraneuronal Aβ and robustly deposit β-amyloid extracellularly in neuritic plaques.

- TgCRND8 mice were crossed with Cystatin B KO mice (CBKO), in order to relieve inhibition of multiple cathepsins, improving lysosomal proteolytic function in TgCRND8 mice.

- Scientific question: **does improved lysosomal activity ameliorate AD-related pathologies?**
TgCRND8 mice exhibit marked autophagic-lysosomal dysfunction
Deletion of cystatin B enhances lysosomal activities and accelerates protein turnover
Deletion of cystatin B eliminates giant autolysosomes in the brain of CBKO/TgCRND8 mice
Deletion of cystatin B reduces the amyloid load and Aβ level in the brain of CBKO/TgCRND8 mice
Cystatin B deletion restores learning and memory functions in CBKO/TgCRND8 mice

Hippocampus-dependent contextual fear conditioning

CBKO mice can improve contextual memory deficits found in TgCRND8 mice

Odor habituation test

TgCRND8 mice had an increased latency to habituate to novel odours in comparison to age-matched controls. CBKO/TgCRND8 mice did not differ from wild-type mice
Conclusions

- TgCRND8 mice show aggressive amyloidosis and neuritic plaque development, accompanied by extensive autophagic-lysosomal pathology similar to that seen in AD brain, reflecting defective proteolytic clearance of autophagic substrate.

- Proof of concept: partially restoring of lysosomal proteolytic function in TgCRND8 mice significantly ameliorates lysosomal system pathology, intraneuronal Aβ accumulation, amyloid plaque formation, and memory and learning deficits in the TgCRND8 model.

- These effects underscore the pathogenic significance of lysosomal system dysfunction in AD and they demonstrate the value of reversing this dysfunction as a potential therapy for AD and other neurodegenerative diseases.
α-Synuclein impairs macroautophagy: implications for Parkinson’s disease

Ashley R. Winslow, Chien-Wen Chen, Silvia Corrochano, Abraham Acevedo-Arozena, David E. Gordon, Andrew A. Peden, Maike Lichtenberg, Fiona M. Menzies, Brinda Ravikumar, Sara Imanisio, Steve Brown, Cahir J. O’Kane, and David C. Rubinsztein

Cargo recognition failure is responsible for inefficient autophagy in Huntington’s disease

Marta Martinez-Vicente, Zsolt Taloczy, Esther Wong, Guomei Tang, Hiroshi Koga, Susmita Kaushik, Rosa de Vries, Esperanza Arias, Spike Harris, David Sulzer & Ana Maria Cuervo

Rapamycin Delays Disease Onset and Prevents PrP Plaque Deposition in a Mouse Model of Gerstmann–Sträussler–Scheinker Disease

Constanza J. Cortes, Kefeng Qin, Julie Cook, Ani Solanki, and James A. Matrianni

Department of Neurology, The University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637
<table>
<thead>
<tr>
<th>Condition</th>
<th>Impairment</th>
<th>Pathway or stage of autophagy</th>
<th>Potential pharmacological treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lafora disease</td>
<td>Loss-of-function mutation in $EPM2A^{37}$</td>
<td>Initiation</td>
<td>Rapamycin</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>Expanded polyglutamine domain of huntingtin protein$^{42}$</td>
<td>Cargo recognition</td>
<td>Rapamycin, rilmenidine, clonidine, carbamazepine, valproate</td>
</tr>
<tr>
<td>Frontotemporal dementia</td>
<td>Mutations in $CHMP2B^{49,50}$</td>
<td>Maturation</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Mutations in $DCTN1$ and $DNCHC1^{43-46}$</td>
<td>Maturation</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Lysosomal storage disorders</td>
<td>Dysfunction of lysosomal hydrolases and other lysosomal proteins$^{54}$</td>
<td>Lysosomal fusion and clearance</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Familial Alzheimer disease</td>
<td>Mutation in $PSEN1$, $PSEN2$ and $APP^{56-61}$</td>
<td>Lysosomal fusion and clearance</td>
<td>Unknown*</td>
</tr>
<tr>
<td>Parkinson disease</td>
<td>Mutations in $PINK$ and $PARK^{70-77}$</td>
<td>Mitophagy</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Upregulation of autophagy might be unwise in patients who have defective autophagosome degradation.
Exercise has beneficial effects on human health, including protection against metabolic disorders such as diabetes.

The cellular mechanisms underlying these effects are incompletely understood.

- During stress, increased levels of autophagy permit cells to adapt to changing nutritional and energy demands through protein catabolism.

- In animal models, autophagy protects against diseases such as cancer, neurodegenerative disorders, infections, inflammatory diseases, ageing and insulin resistance.

- Scientific question: is autophagy implicated in exercise-induced health?
Exercise induces autophagy in skeletal and cardiac muscle.

Similar results were observed in soleus, tibialis anterior, extensor digitorum longus, liver and pancreas.

**Figure captions:**

- **Figure a:** Images of Vastus lateralis muscle at different time points (0 min, 15 min, 30 min, 50 min, 80 min, 110 min).
- **Figure b:** Images of cardiac muscle muscle at different time points (0 min, 15 min, 30 min, 50 min, 80 min, 110 min).
- **Figure c:** Graph showing the number of GFP-LC3 puncta per 2,500 μm² over time for skeletal and cardiac muscle.
### Generation of BCL2 AAA knock-in mice

- Phosphorylation of three sites (Thr69, Ser70, Ser84) in the non-structured loop of human BCL2 is critical for stimulus-induced autophagy.

- A knock-in mouse was generated; Thr69, Ser70, Ser84 were replaced with three Ala (AAA) -> BCL2 could not free beclin-1.

<table>
<thead>
<tr>
<th>Skeletal muscle</th>
<th>Cardiac muscle</th>
<th>Adipose tissue</th>
<th>Pancreas</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>AAA</td>
<td>WT</td>
<td>AAA</td>
<td>WT</td>
</tr>
<tr>
<td>BCL2</td>
<td><img src="Image" alt="BCL2 WT" /></td>
<td><img src="Image" alt="BCL2 AAA" /></td>
<td><img src="Image" alt="BCL2 WT" /></td>
<td><img src="Image" alt="BCL2 AAA" /></td>
</tr>
<tr>
<td>actin</td>
<td><img src="Image" alt="actin WT" /></td>
<td><img src="Image" alt="actin AAA" /></td>
<td><img src="Image" alt="actin WT" /></td>
<td><img src="Image" alt="actin AAA" /></td>
</tr>
</tbody>
</table>
BCL2 AAA mice do not show exercise- and starvation-induced autophagy

Similar results were observed in liver and pancreas

Non-phosphorylatable BCL2 does not alter basal autophagy \textit{in vivo}, but prevents autophagy activation in response to starvation and exercise.
BCL2 AAA mice show deficient exercise endurance

After 80 min exercise, no differences in weight, fibre cross-sectional area, glycogen content, morphology, and mitochondrial content and functionality.
BCL2 AAA mice show alteration in muscle glucose metabolism

GLUT4 – vastus lateralis muscle

Is defective autophagy directly responsible?
Becn1<sup>+/−</sup> and Atg16l1<sup>HM</sup> mice show a phenotype similar to BCL2 AAA mice.

Data provides strong support for a role of deficient beclin 1 activity, rather than other BCL2-regulated functions, in the impairment of exercise endurance, glucose uptake and AMPK activation in BCL2 AAA mice.

Similar results were provided by Atg16l1<sup>HM</sup> mice (e.g. defects in exercise-induced autophagy associated with decreased AMPK phosphorylation).

Studies in BCL2 AAA, Becn1<sup>+/−</sup> and Atg16l1<sup>HM</sup> suggest that cellular autophagy function is partially required for normal levels of exercise-induced muscle AMPK activation.
The BCL2 AAA mutation did not alter the response of mice to HFD with respect to muscle fiber size, the morphology of liver and pancreas, or the effect of exercise on HFD-induced obesity.
High fat diet (HFD)-induced impaired glucose tolerance in BCL2 AAA mice

Oral Glucose Tolerance Test (OGTT)

BCL2-regulated functions are essential for chronic exercise-mediated protection against HFD-induced glucose intolerance
HFD-fed BCL2 AAA mice are less metabolically active

The HFD study suggests that increased autophagy triggered by exercise may be critical for improving impaired glucose tolerance and metabolism in diet-induced obesity.
Conclusions

• This study demonstrates that exercise is a potent inducer of autophagy, and that acute and chronic exercise enhances glucose metabolism in mice capable of inducing autophagy but not in autophagy-deficient mice.

• BCL2 has now previously undescribed essential roles in the *in vivo* regulation of stimulus-induced autophagy as well as glucose metabolism.

• They propose that manipulation of the autophagy pathway and/or the function of the autophagy inhibitory BCL2 protein may be a logical strategy to mimic the health effects of exercise and to prevent or treat impaired glucose metabolism.

Speculation

• On the basis of this newly discovered link between exercise, autophagy and altered metabolism, autophagy may represent a cellular mechanism by which exercise prolongs life and protects against cancer, cardiovascular disorders and inflammatory diseases.
Train, train, train...... but not too much

THX FOR YOUR ATTENTION!!