Autophagy, Diet and Cancer

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NO CONFLICTS
Overview

- Autophagy and its regulation
- Autophagy and cancer
- Diet, autophagy + cancer
  - Resveratrol
  - Caloric restriction
- Future research
Definition of Autophagy

Catabolic, lysosomal, self-digestion process responsible for turnover of whole organelles and macromolecules

**Basal**
- Long-lived proteins
- Old/damaged organelles

**Induced**
- Nutrient + growth factor deprivation
- Hypoxia
- Protein misfolding
- Intracellular pathogens
- Cellular damage
Types of Autophagy

- Housekeeping
  - Protein turnover
  - Organelle QC

- Development/Differentiation

- Pathogen defense

- Survival (Stress/Starvation)

- Antigen presentation

Key:
- Protein
- Mitochondrion
- Chaperone
- Lysosomal hydrolase

Macroautophagy

Microautophagy

Chaperone-mediated autophagy

CMA

Trends Cell Biol. 2009

Autophagosome formation

- **Beclin 1**
- **LC3**
- **p62**
- **Amino acids**
- **Protein synthesis**
- **Survival**
- **3-MA**
- **BFA1**
- **ATP**
- **Fatty acids**
- **Autophagic cargo**

References:
Measurement of autophagy
(Klionsky et al., Autophagy 2008)

GFP-LC3 transgenic mice

Starvation response varies

**Brain:** low basal autophagy activity; low during starvation

Regulation of Autophagy

- Growth factors/IGF1
- Insulin
- Amino acids

- PI3KC1
- Akt
- AMPK
- TSC1/2
- Rheb
- PTEN
- mTORC1
- IRS
- PTEN
- LKB1
- Ca++
- CaMKK
- Metabolic stress
- Radiation
- Chemotherapy
- Ras
- Raf
- MEK1/2
- ERK1/2
- FOXO3
- Gαi3
- DAPK
- Beclin 1
- PI3KC3

- Protein Translation

- Ras [AMP]
- Ras [ATP]
- Metabolic stress
- ER stress
- ROS
- Hypoxia

- Oncogenic protein
- Tumor suppressor

- mTORC1
- p27
- Beclin 1
- Atg13/Ulk1
- Protein Translation
Disruption of Autophagy

Contributes to human pathologies:

- Neurodegeneration disorders
  - Alzheimer’s disease
  - Parkinson’s disease
  - Huntington’s disease
- Cardiomyopathy
- Aging
- Infectious diseases
- Alterations in cellular lipid storage/catabolism
- Diabetes/insulin resistance
- Cancer
Autophagy and Cancer Controversy

- Jekyll and Hyde
- Paradox
- Conundrum
- Frustrating
- Trick or Treat
- Two-faced
- Enigma
- Double-edged sword
Autophagy/Cell Death Controversy

- Autophagy: mediator of survival or death?
  - Cell death by autophagy; type II PCD
  - Cell death through autophagy

- Growing consensus: primarily pro-survival pathway (cell death with autophagy)

- Autophagy + cancer: inhibitor or promoter?

  No evidence that physiological cell loss in mammals is executed in vivo by autophagy

Kroemer, Nat. Rev. Mol. Biol. 2008
Scarlatti, Cell Death Different. 2009
Example of variable autophagy/cell death interplay

Mouse Embryonic Fibroblasts
Basal autophagy

Extrinsic apoptosis pathway

*Increased cell death* when autophagy disabled

Wang, J. Biol. Chem. 2008
Increased survival when autophagy disabled

Compensated by increased CMA + other proteolytic pathways

Wang, J. Biol. Chem. 2008
Autophagy-Apoptosis Crosstalk

Beclin 1/ Bcl2 “Rheostat”

Levine, Autophagy 2008
Beclin 1
(Tumor suppressor)

- Mutated in 40-75% human breast, ovarian, prostate cancers (haploinsufficient)

- Beclin 1 mutant mice: increased spontaneous malignancies, cell proliferation, mammary preneoplastic lesions

- Beclin 1 expression + human brain tumor grade inversely related

- Human gastric and colorectal cancers:
  Increased Beclin 1 expression in malignant colorectal and gastric epithelium, not in normal tissue.

- Similar LC3 accumulation in cancerous pancreatic and colorectal tissues

What are other functions of Beclin 1 in cancerous tissue?
Why the variability?
Autophagy: Different role in stages of carcinogenesis?

Early development vs Solid tumor

![Diagram showing the comparison between apoptosis and autophagy in early development and solid tumors.](image-url)
Autophagy and Carcinogenesis (Early Stages)

♦ Decreased autophagy in premalignant and malignant liver cells vs normal

♦ Cellular autophagic capacity profoundly decreases in azaserine-induced rat pancreatic tumors vs normal

♦ Cancer cell lines often have lower autophagic capacity vs normal and fail to respond to serum/a.a. deprivation or high cell density

♦ Atg4C/- mice have increased susceptibility to develop fibrosarcomas induced by 3-MC

♦ A cell death pathway involving both apoptosis and autophagy is selectively inactivated when normal epithelial cells are immortalized

Autophagy benefits?:
Preserve genomic integrity
Prevent abnormal growth/proliferation
Clearance of dysfunctional proteins/organelles
Autophagy and Solid Tumors: Autophagy is cytoprotective (Pro-Survival/Cancer Preventive?)

Metabolic stress (hypoxia, decreased growth factors, cytotoxic drugs)

Starvation

Autophagy maintain cellular/energy homeostasis: Multiple outcomes?
Metabolically stressed regions of tumor mass (not vascularized ones) have active autophagy

Tumors with **Beclin 1** or **Atg5** deleted have decreased autophagy and increased cell death in metabolically stress regions;

Atg\(^{5+/-}\) cells also show increased **necrosis**, accelerated **DNA damage**, increased **aneuploidy**

Mathew, Meth. Enzymol. 2009
Autophagy in solid tumors

Malignant progression
**Overall Perspective**

- Dormancy?
- Regression?
- Senescence?

Eisenberg-Lerner, Cell Death Different. 2009
Concerns

- Little physiological *in vivo* evidence

- **Measurement shortcomings:**
  - Chemical inhibitors of autophagy are nonspecific
  - Blockage of *Atg* can \(↑\) CMA + alter UPS
  - Non-autophagic functions of *Atg* genes
  - Characterization of cell death is incomplete
Dietary Factors + Drugs

- Vit C
- Vit D
- Vit E
- GEN
- Saponin
- Se
- RES
- SUL
- CUR

Diet/drug
Type cancer
Apoptosis status
Genetic profile

Autophagy ↔ Cancer?
Resveratrol
(phytoalexin in fruits, nuts, wine)
Examples from several cancer cell types

- Glioma cells:
  - + 150μM RES → apoptosis
  - + 150 μM RES → autophagy (MDC, LC3)
  - + 150 μM RES
    + 3-MA → ↑apoptosis
    + bafilomycin A1 → ↓autophagy

Pro-survival function?

Li, BMC Cancer 2009
Resveratrol

Colorectal cancer cells:

- + 100 μM RES  
  - autophagy
  - apoptosis
  - cytosolic cathepsin D
  - Bax to mitochondria

- + 100 μM RES + Beclin 1 siRNA  
  - autophagy
  - apoptosis

- + 100 μM RES + dnPI3KC3  
  - autophagy
  - apoptosis

- + 100 μM RES + Lamp2b siRNA  
  - autophagy
  - apoptosis

**RES: autophagy hyperstimulated**

Autophagy-related proteins mediate apoptosis

Autophagy-dependent cell death?
Resveratrol

- Human breast cancer cells (MCF-7):
  - MCF-7\textsuperscript{casp3+} + 64 \mu M RES
    - \textbf{↓} cell proliferation
    - \textbf{↓} clonal expansion
    - \textbf{↓} apoptosis (caspase 3)
    - necrosis
    - autophagy
  - MCF-7 \textsuperscript{casp3-} + 64 \mu M RES
    - \textbf{↓} cell proliferation
    - \textbf{↓} clonal expansion
    - no apoptosis
    - necrosis
    - autophagy

Autophagy: GFP-LC3 puncta
- LC-3II protein degradation
- ↓ Akt and mTOR

Scarlatti, J. Biol. Chem. 2004
Scarlatti, Cell Death Different. 2008
Resveratrol

- Human breast cancer cells (MCF-7):
  - MCF-7<sup>casp<sup>3+</sup> / casp<sup>3-</sup> + 64 μM RES + ATG7 siRNA → autophagy
    + BECN1 siRNA → no change
    + PI3KC3 siRNA → no change

- MCF-7<sup>casp<sup>3+</sup> / casp<sup>3-</sup> + 64 μM RES + ATG7 siRNA → n.c. necrosis

- MCF-7<sup>casp<sup>3+</sup> + 64 μM RES + ATG7 siRNA → n.c. apoptosis

Scarlatti, J. Biol. Chem. 2004
Scarlatti, Cell Death Different. 2008
Resveratrol

Human breast cancer cells (MCF-7):

RES triggers different types cell death
RES induces noncanonical Beclin 1-independent autophagy
RES-induced Beclin 1-independent autophagy is unrelated to RES-induced cell death
New type of autophagy insensitive to PI3KC3 inhibitors

Action of RES:
- Cell type?
- Apoptosis status?
- Status of autophagic regulation?
- Other?

Scarlatti, J. Biol. Chem, 2004
Scarlatti, Cell Death Different. 2008
Caloric Restriction (CR)
(potent inducer of autophagy in virtually all species)

- Autophagy, CR and aging:
  - Autophagy and lysosomal activity ↓ in aged animals
    - CR counteracts ↓ activity
  - Humans: autophagy may decline with age and obesity

- Insulin, IGF-1 and autophagy
  - IGF-1 and insulin inhibit autophagy
  - Glucagon/insulin is major regulator of liver autophagy *in vivo*
  - Aging ↓ glucagon action but not insulin activity
Caloric Restriction

- Interplay of CR, autophagy and cancer inhibition?
- CR + mammary tumorigenesis
  - Apoptosis
  - Akt
  - IGF-IR
  - AMPK
  - mTOR

- Differential tumor tissue response to CR:
  - Constitutive activation of PI3KC1-Akt → resistant to CR

Any role for autophagy?

Is autophagy occurring, even if transiently?

Thompson, Cancer Res. 2004
Jiang, Cancer Res. 2008
Kalaany, Nature 2009
Autophagy-cancer outcomes?

- Depend on:
  - Cell/tissue type
  - **Status** of apoptosis/autophagy pathways
  - Oncogene/tumor suppressor profile
  - **Stimulus** of autophagic/apoptotic pathways
  - Autophagy/apoptosis interplay
  - Dietary factor/drug effects
  - Other factors
Unresolved Research Issues

- **In vivo:** measure autophagy— not let it be a “stealth” process
  - Multiple measurement methods (Klionsky et al., Autophagy 2008)
  - Transgenic GFP-LC3 mice
  - Identification of mammalian Atg genes
  - Tissue selective in vivo disabling of Atg

- **In vivo** role of autophagy during temporal development of tumors in established models (esp. compared to normal cells)

- **In vivo** interaction between apoptosis and autophagy
Unresolved Research Issues

- In what cancer cell types and tumor models is there autophagy in response to dietary factors? What is the effect of dose? And is autophagy a mediator of death or survival?

- What role if any in tumor inhibition by CR?
“Natural selection does not have to produce something that makes intuitive sense and a neat story, it just has to produce biological mechanisms that work well enough to be retained as genetically encoded traits.”

Hippert, Cancer Res. 2006
Thank You

“As often happens, the more we learn, the more questions arise.”
Corcelle, Autophagy 2007