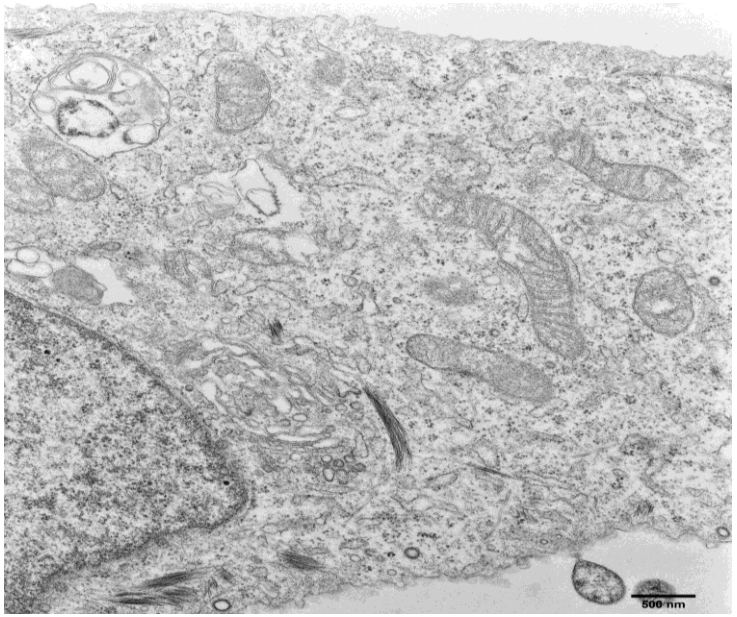
# Autophagy and its Various Roles in Health and Diseases

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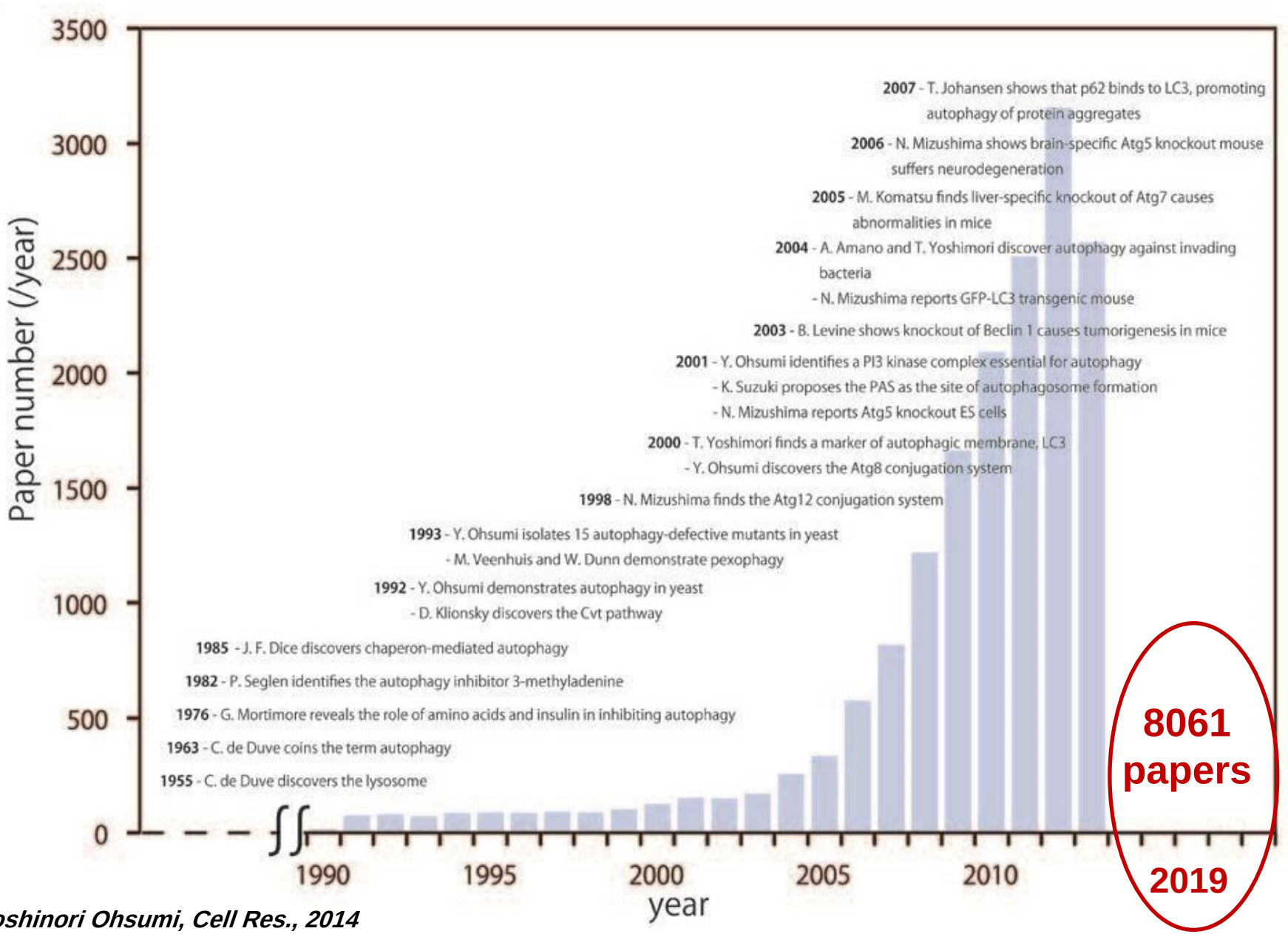
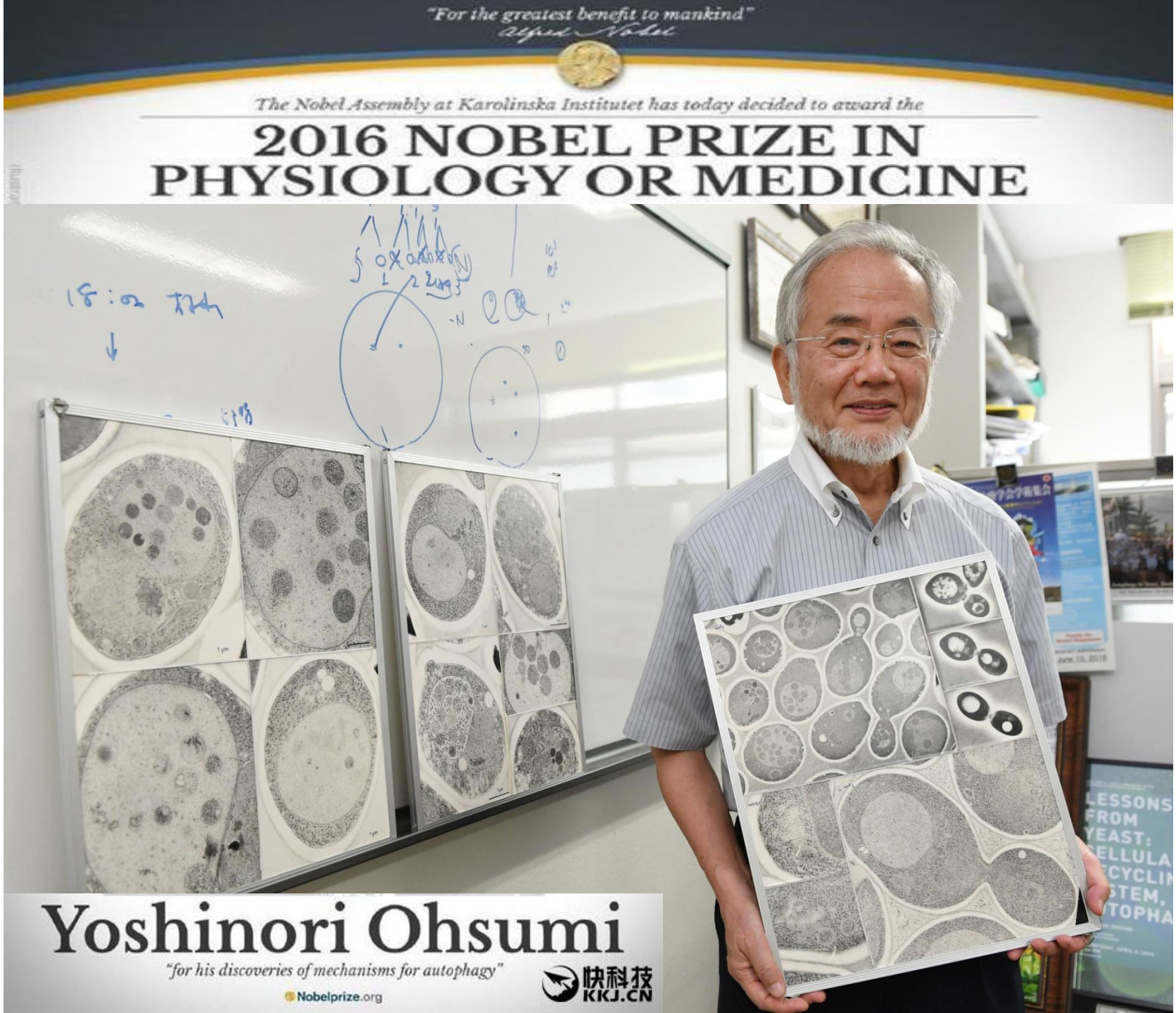
18. Jun. 2020

# Chronology




*Yoshinori Ohsumi, Cell Res., 2014*

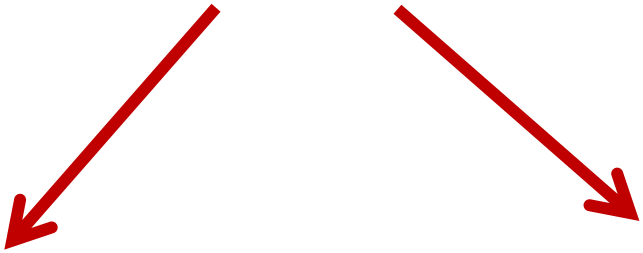

"For the greatest benefit to mankind"
Alfred Nobel
The Nobel Assembly at Karolinska Institutet has today decided to award the
2016 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE
Yoshinori Ohsumi
"for his discoveries of mechanisms for autophagy"
Nobelprize.org
快科技 KKJ.CN

# Cellular Homeostasis

Requirements:

- constant turnover of continuous synthesis of cellular components
- clearance of damaged or superfluous proteins and organelles.

**Degradation pathways**



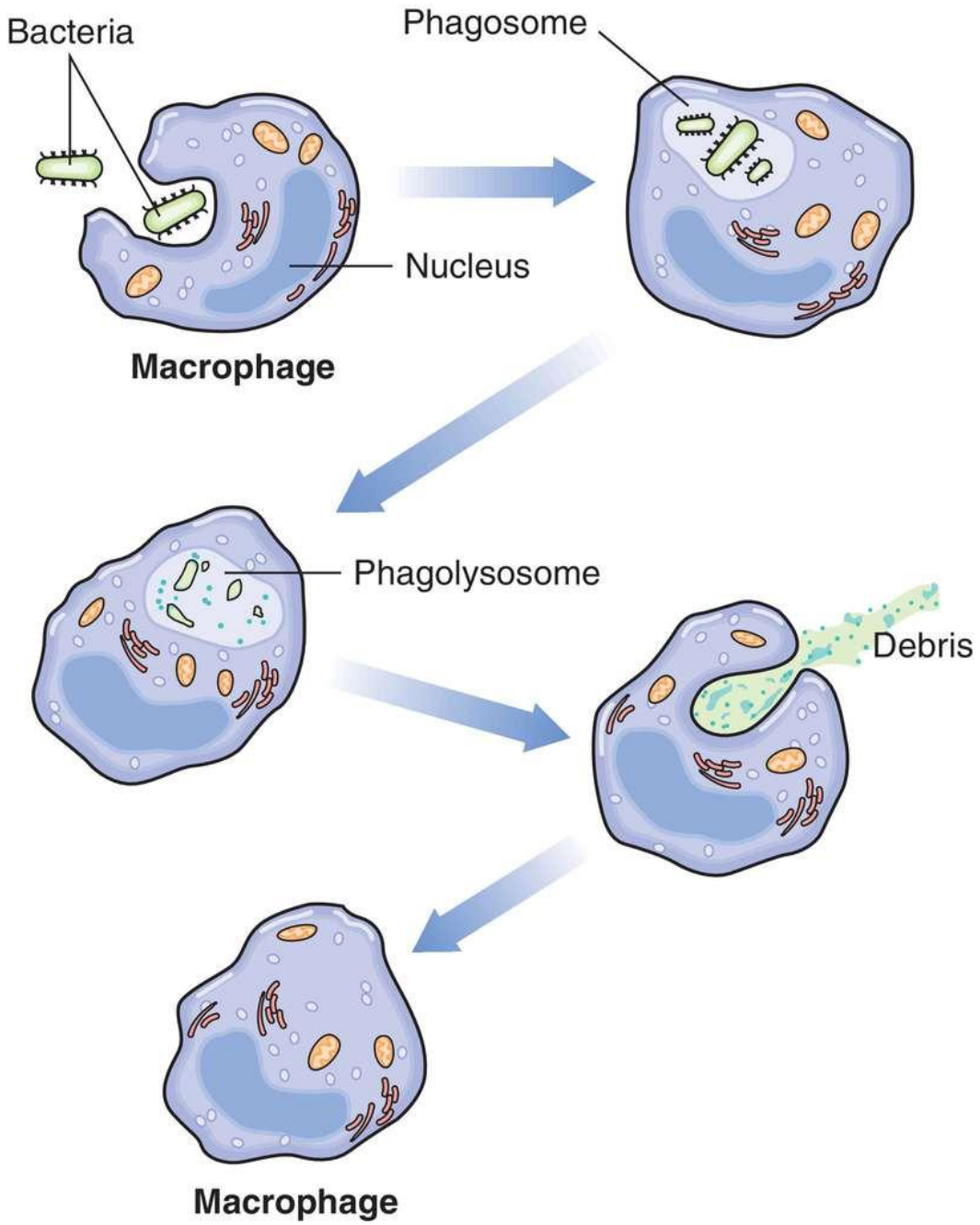
**Ubiquitin-Proteasome System (UPS)**

high selectivity
short-lived proteins

**Lysosomal pathway**

Autophagy

**Phagocytosis**




**Autophagy is totally different**

# What is Autophagy?

“Self-eating”

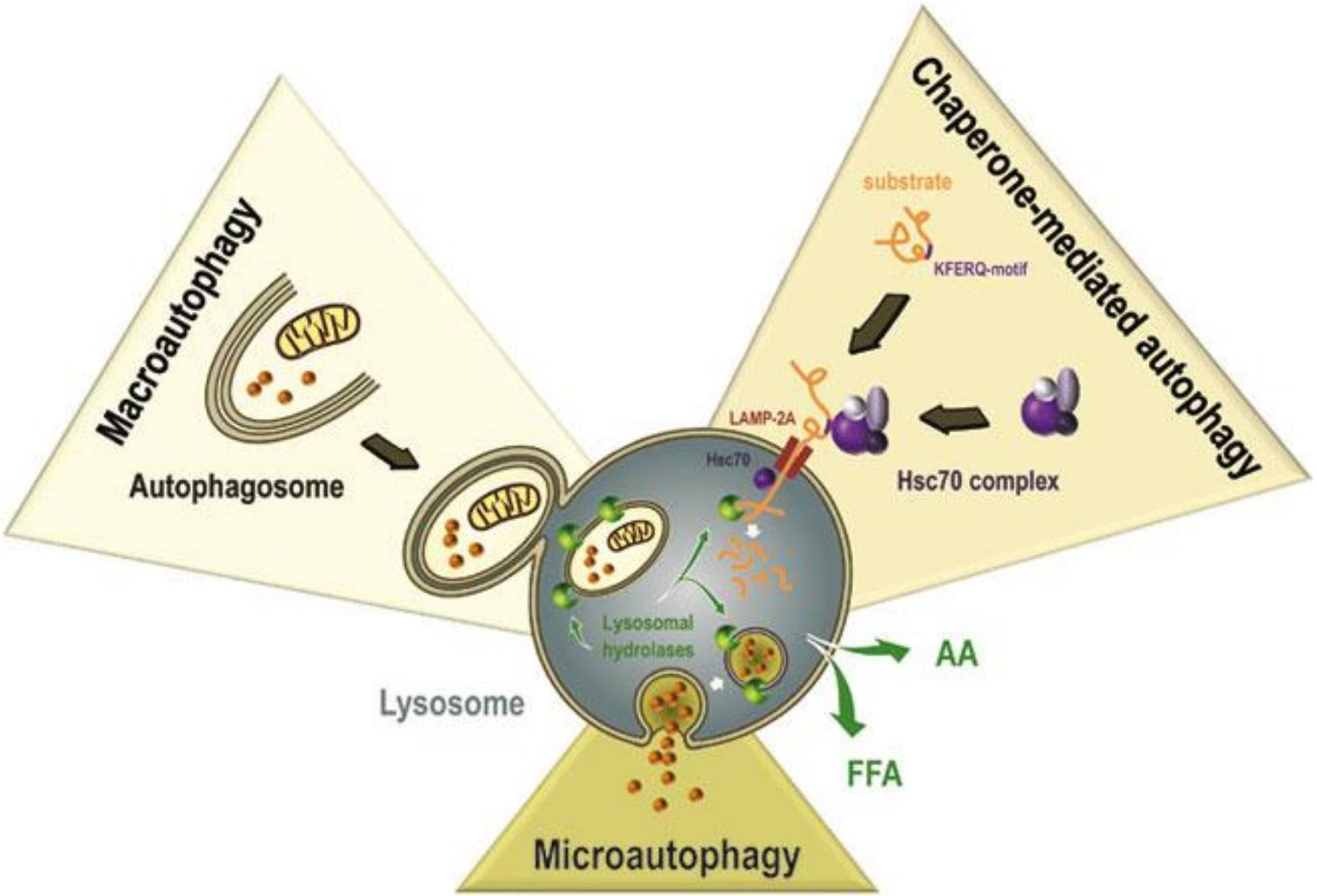
From the Greek words, *auto* "self" and *phagein* "to eat"

“Catabolic process through which the cell recycles its own constituents”

“Pathway that lead to the elimination of cytoplasmic components by delivering them into lysosomes”

“The body’s way of cleaning out damaged cells, in order to regenerate newer, healthier cells”

# Types of Autophagy

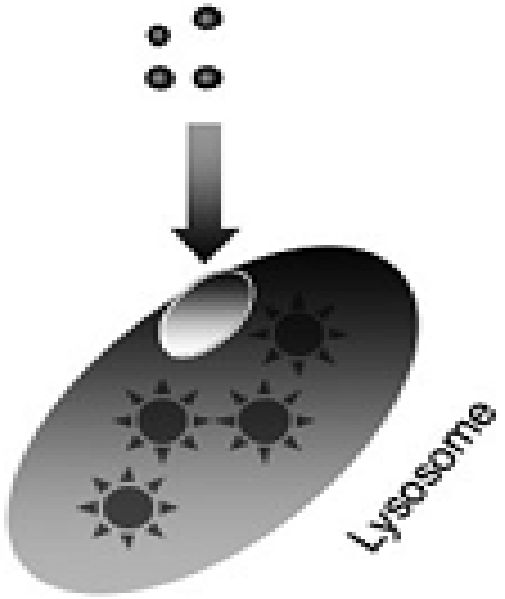



*Wirawan et al., Cell Res., 2012*

# Types of Autophagy

## Micro-autophagy

- By invagination of the lysosome membrane, cytosolic components are directly taken up by the lysosome itself through.
- It could be selective or non-selective.



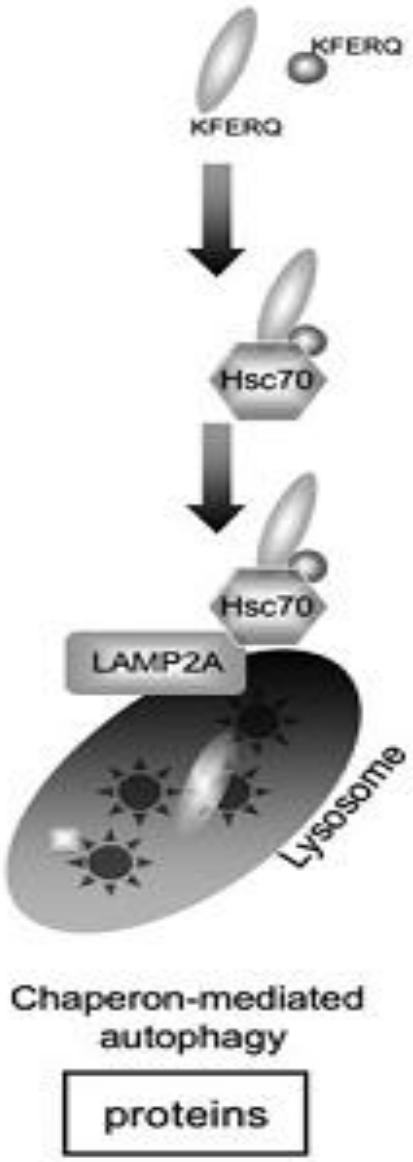

Microautophagy

proteins, lipids, organelles

# Types of Autophagy

## Chaperone-mediated autophagy (CMA)

- Targeted proteins are translocated across the lysosomal membrane in a complex with chaperone proteins (such as Hsc-70) that are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A (LAMP-2A), resulting in their unfolding and degradation.

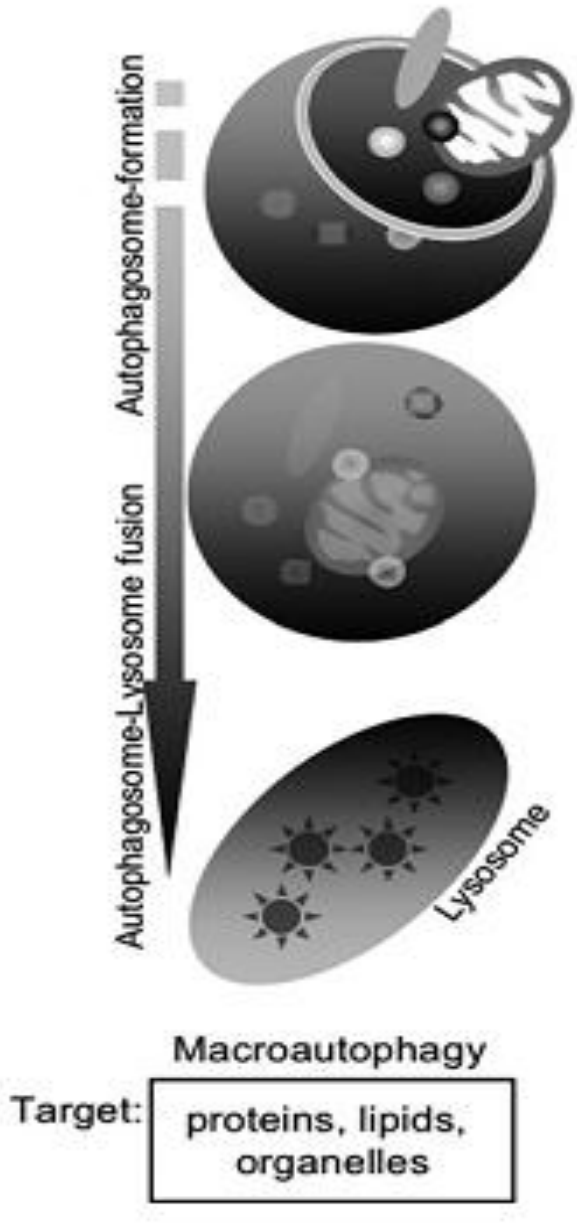


# Types of Autophagy

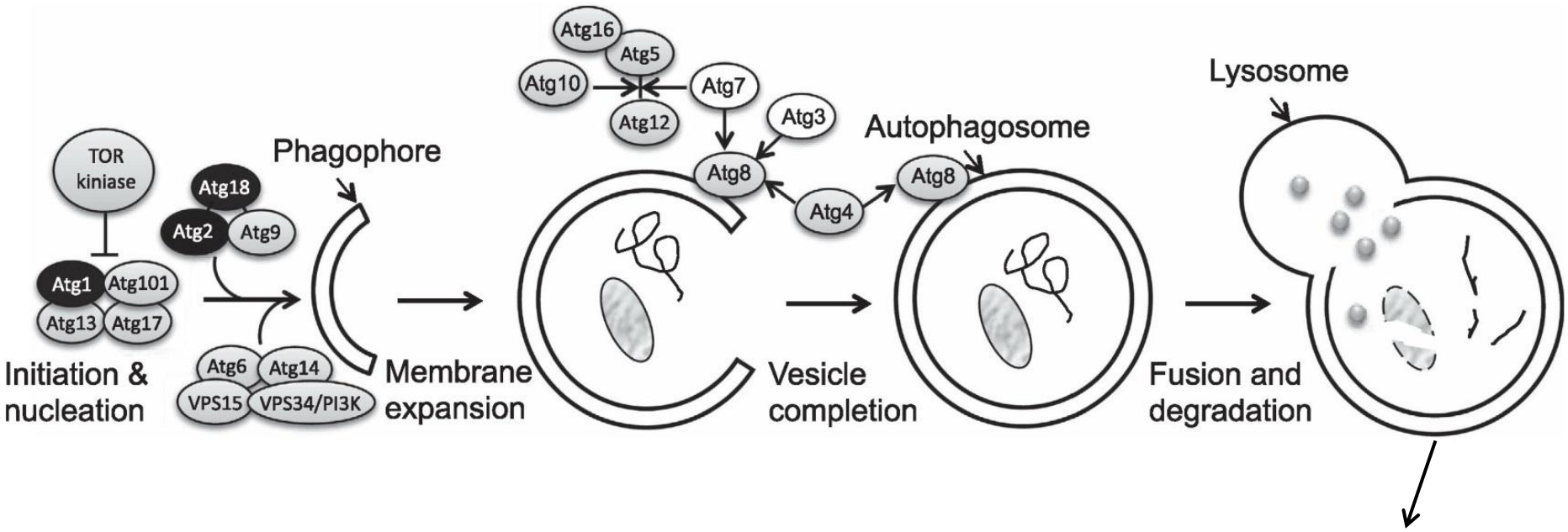
## Macro-autophagy

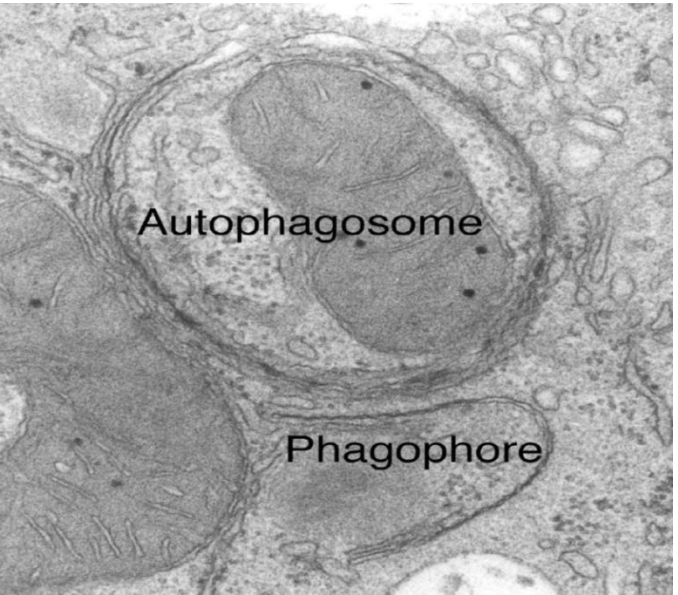
- Delivers cytoplasmic cargo to the lysosome through autophagosome
  (a double membrane-bound vesicle)
- Autophagosome fuses with the lysosome to form an autolysosome.
- It could be selective or non-selective.

- The most important type is macro-autophagy, referred to as autophagy.



# Mechanism of Autophagy




*Denton et al., Immunol Cell Biol., 2015*

# Multiple Functions of Autophagy

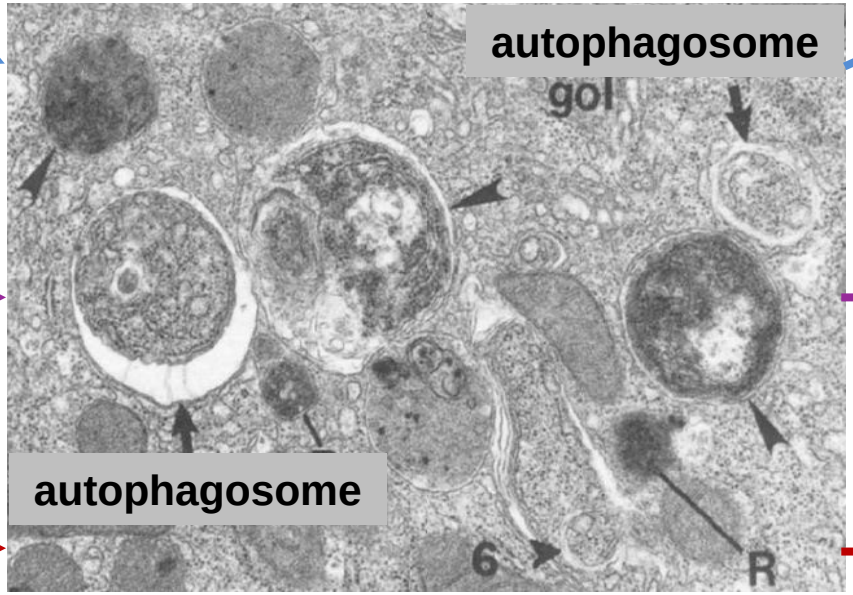
- Occurs in all eukaryotic cells
- Bulk degradative process that ends in lysosomes
- Degradation of intracellular components

Recycling and Cleaning

Basal autophagy
Quality control

Nutrient/
Growth factor
deprivation

Uncontrolled
autophagy

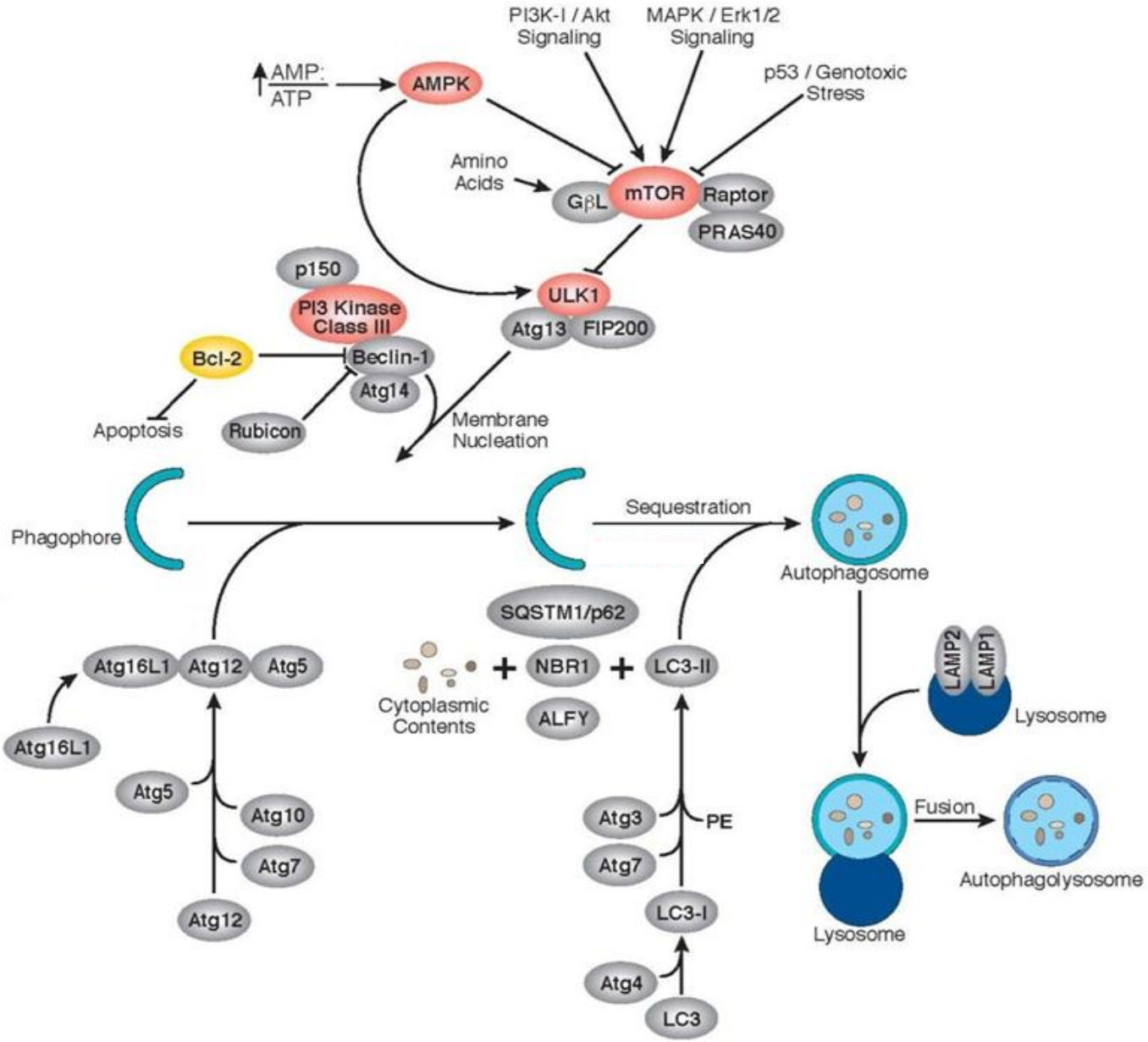



Removal of
obsolete organelles
and protein aggregates

Metabolic substrates
(energy, nutrient)

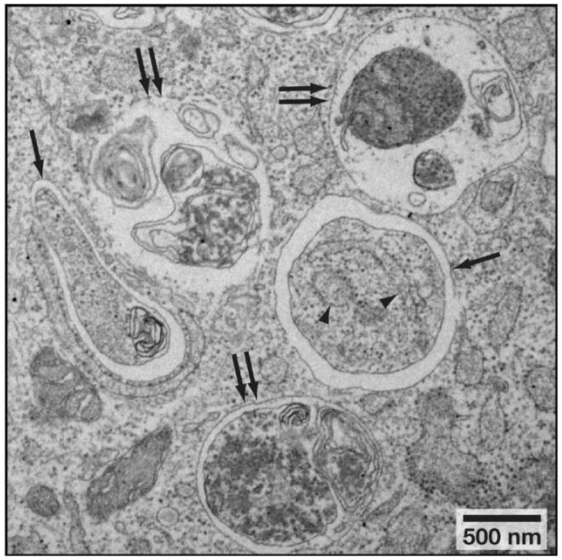
Autophagic
cell death

# Autophagy Signalling Pathway

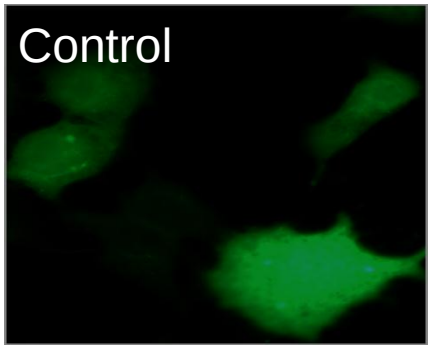


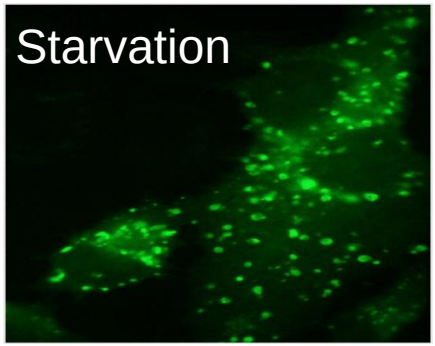
# How can We Monitor Autophagy?

## EM

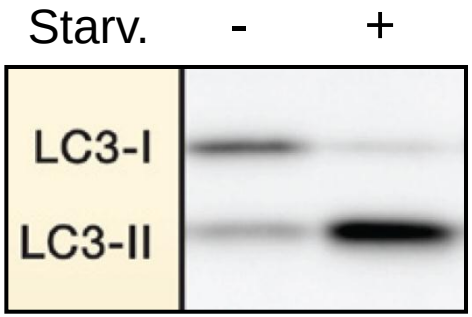



## IF LC3

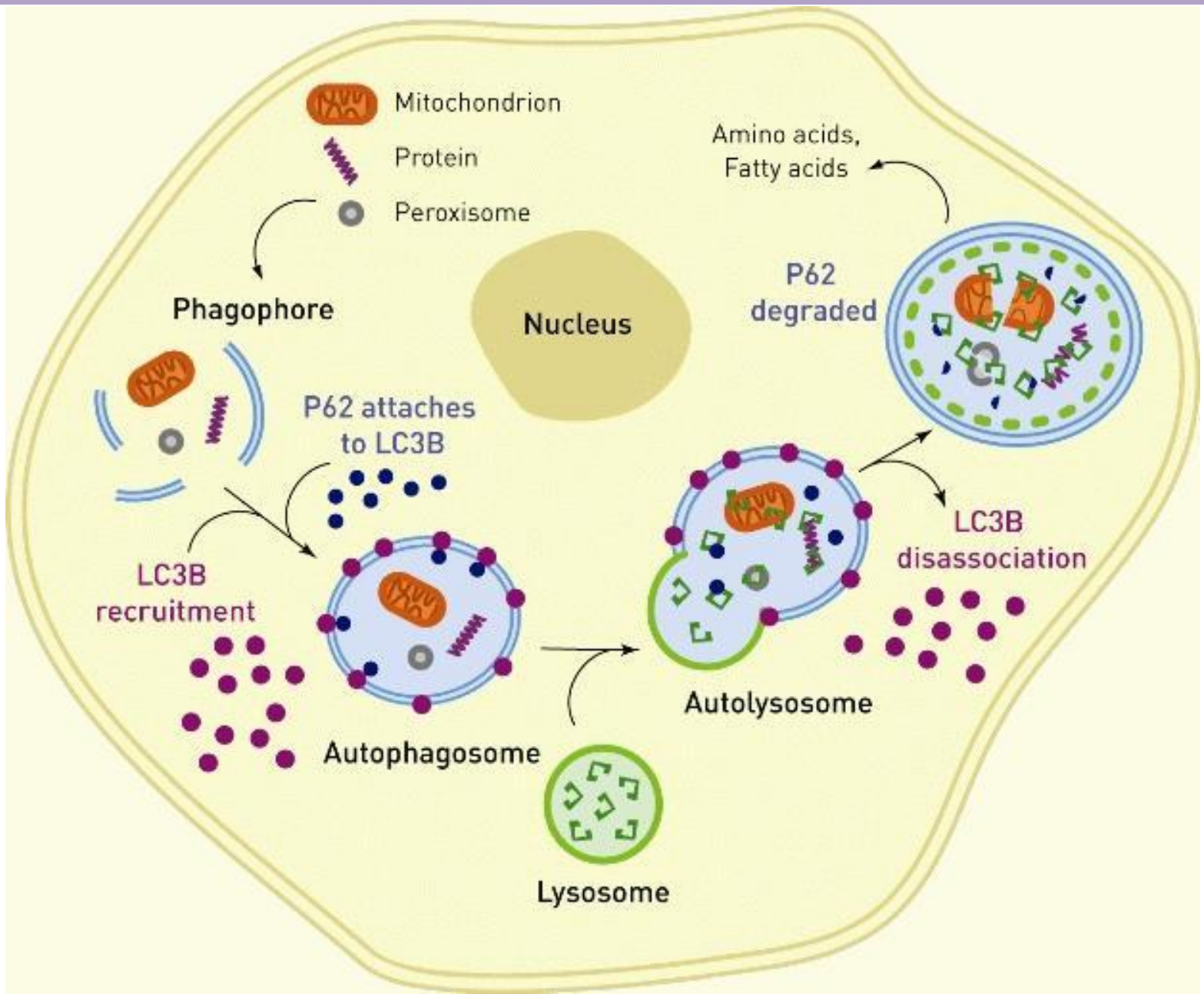


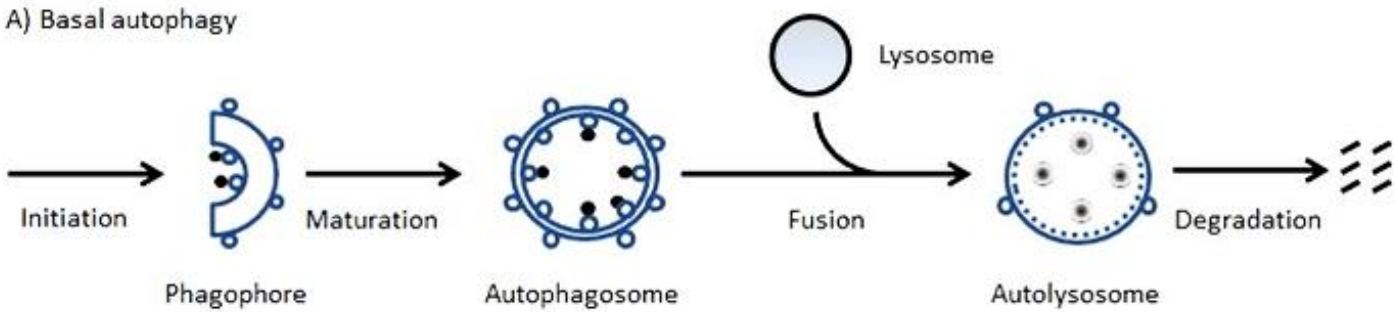

## WB

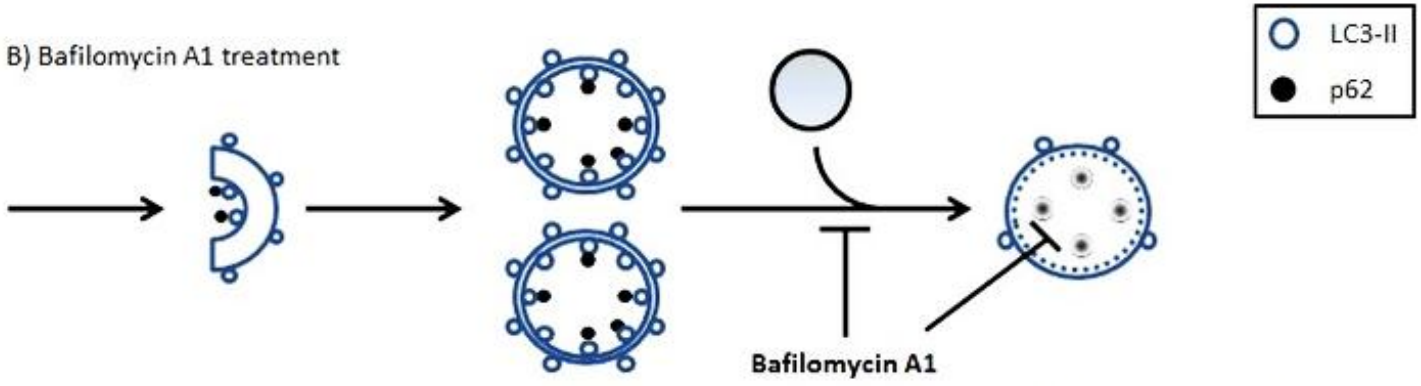


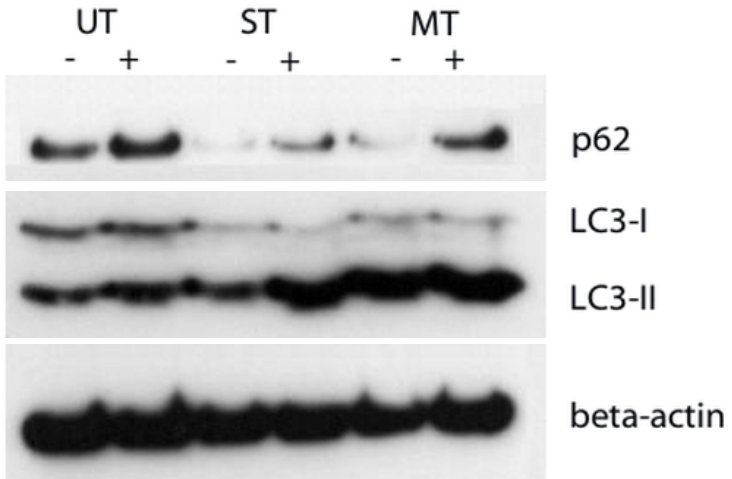
# How can We Monitor Autophagy?



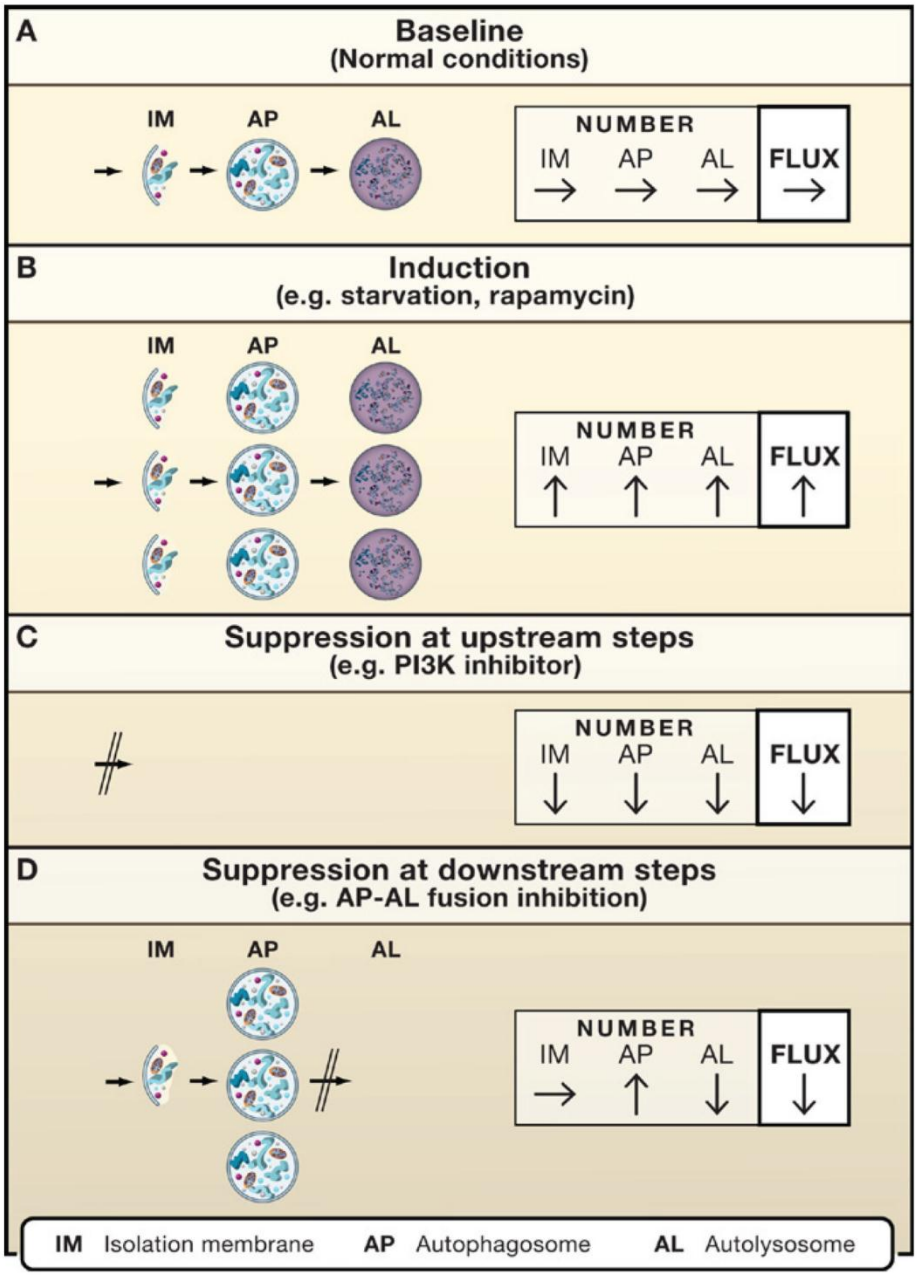
# How can We Monitor Autophagy?



# Dynamic regulation of autophagy

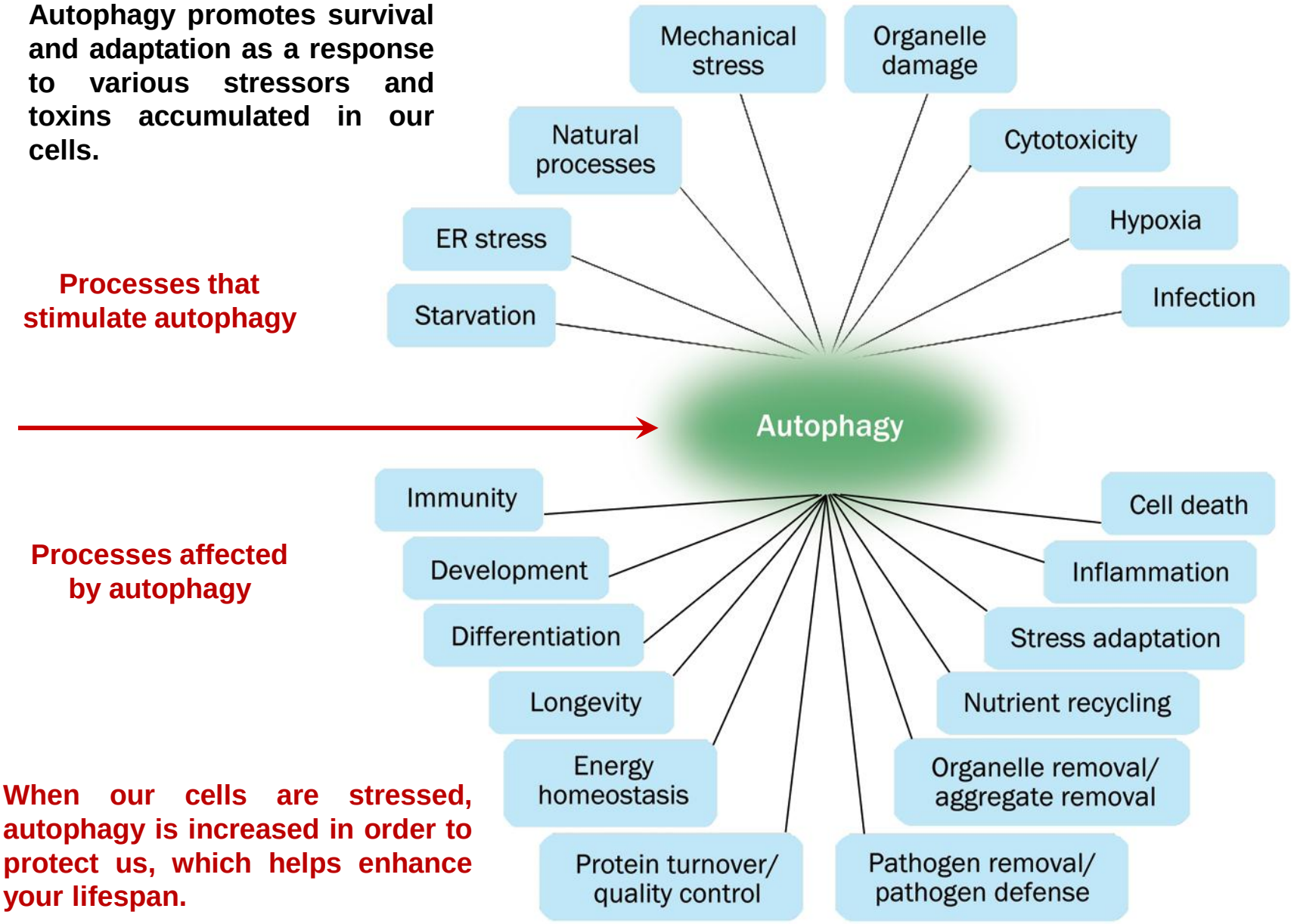



*Mizushima et al, Cell, 2010*

# Induction of Autophagy

**Autophagy promotes survival and adaptation as a response to various stressors and toxins accumulated in our cells.**

**Processes that stimulate autophagy**



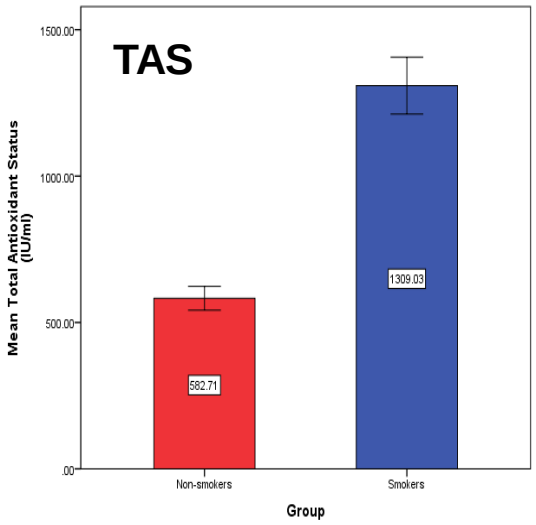

**Processes affected by autophagy**

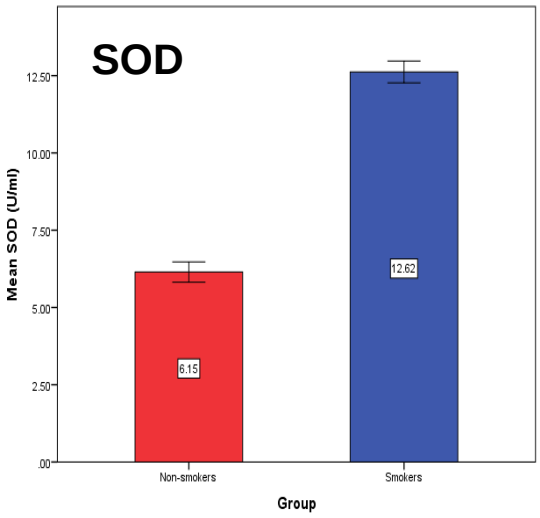
**When our cells are stressed, autophagy is increased in order to protect us, which helps enhance your lifespan.**

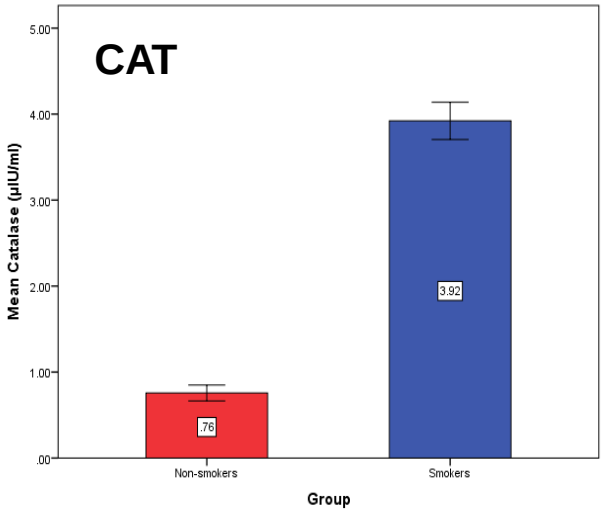
# Induction of Autophagy

## Autophagy and Oxidative Stress in Smokers:

- Our results:

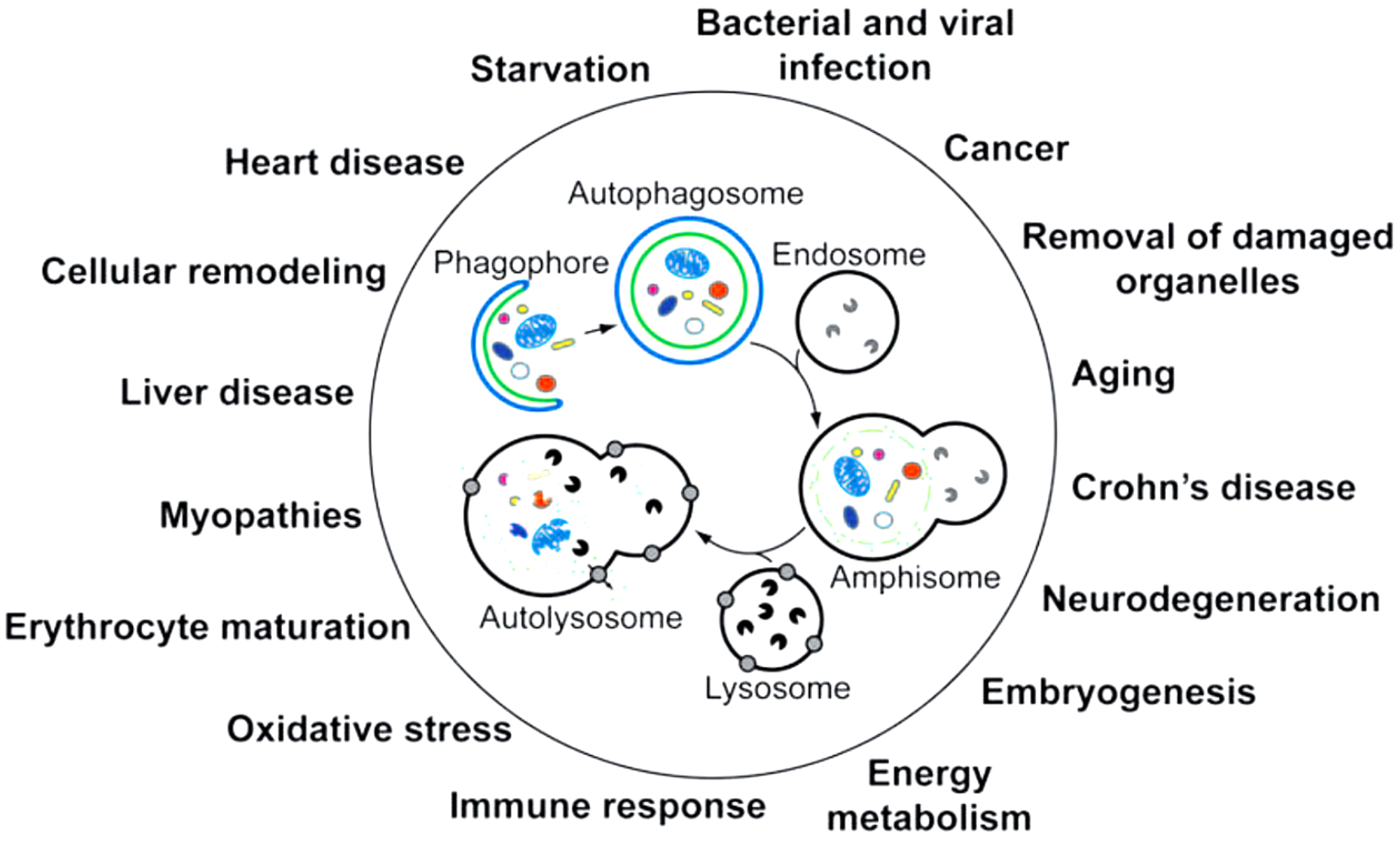


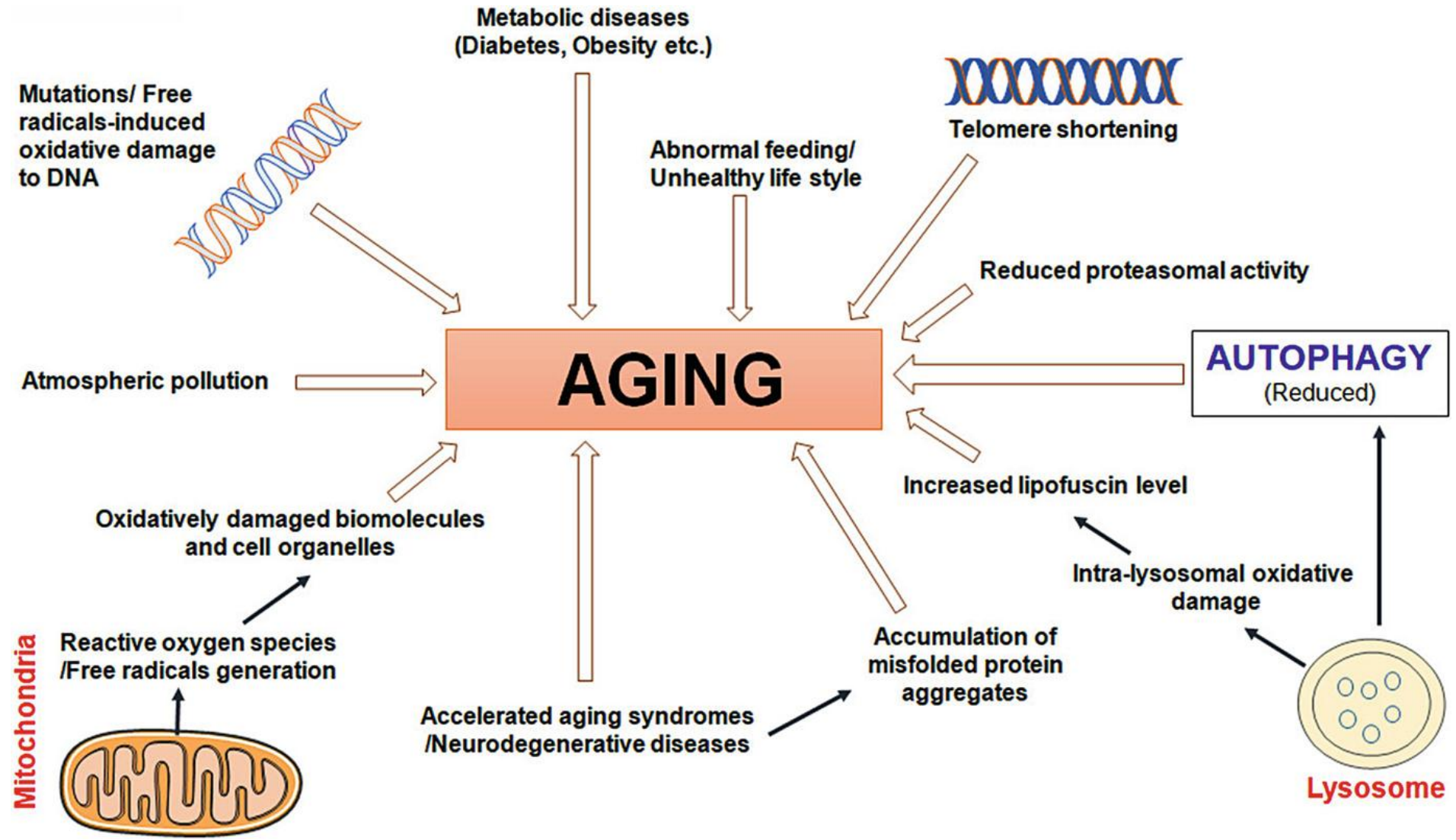

| Groups | Non-Smoker (24) | Smoker (55) | P Value |
|---|---|---|---|
| Beclin 1 | 1.95±0.17 | 12.27±1.57a | <0.0001 |
| Atg5 | 0.69±0.12 | 3.00±0.52 | <0.0001 |
| LC3 I | 0.87±0.17 | 3.43±0.50 | <0.0001 |
| LC3 II | 1.67±0.18 | 4.64±0.38 | <0.0001 |

# Autophagy and Diseases

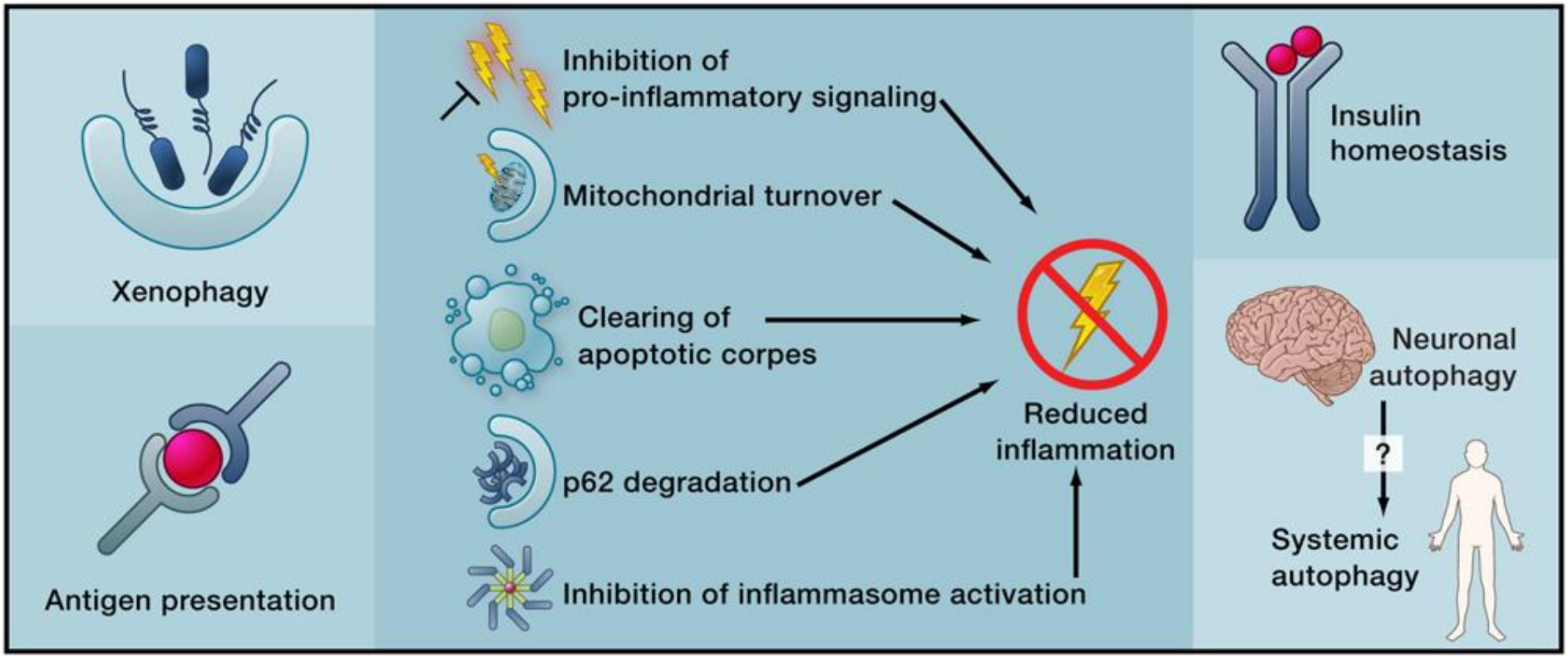



*Klionsky DJ, Dev Cell. 2010*

# Autophagy and Aging



# Autophagy and Aging



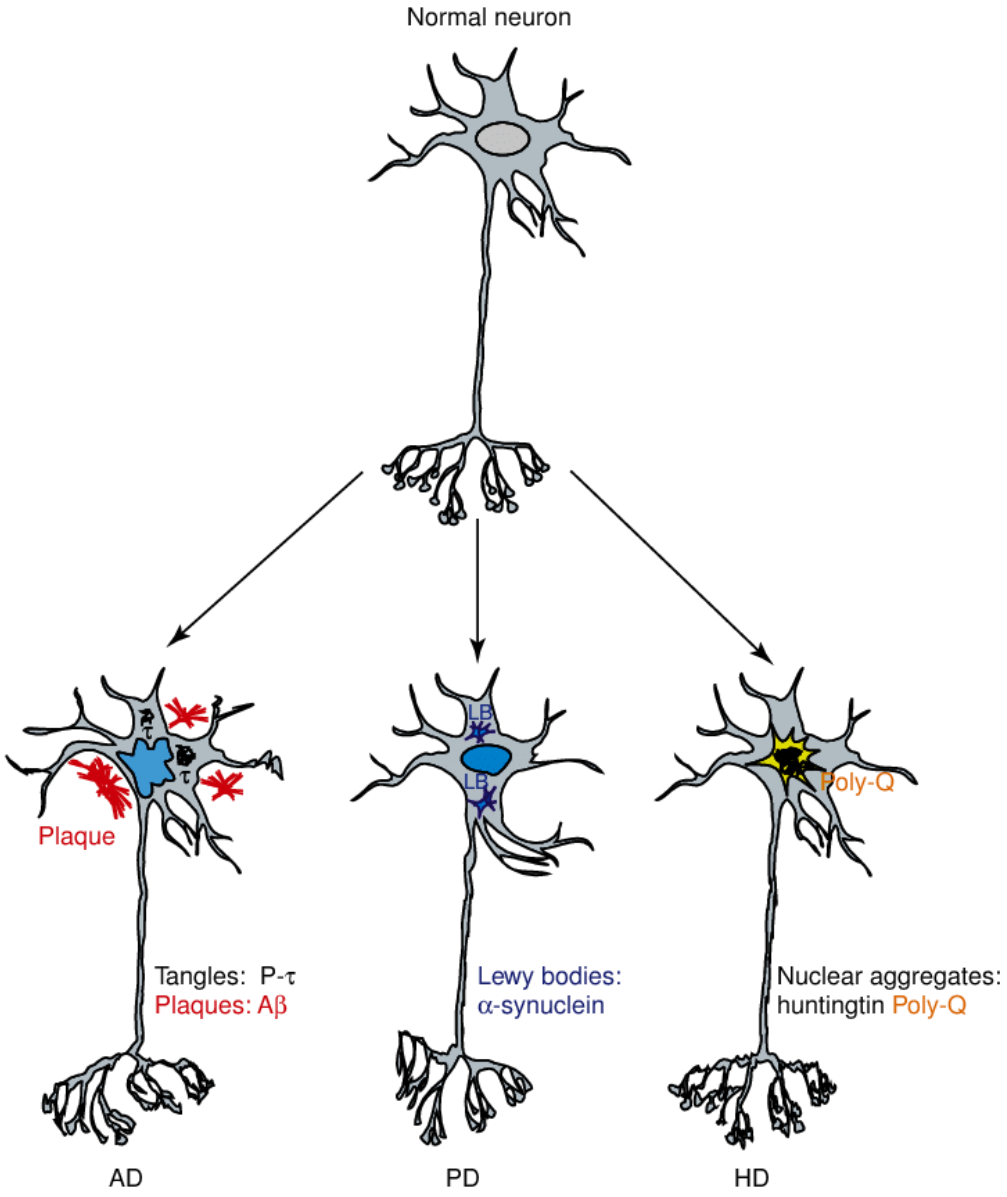

**Systemic Anti-Aging Effects of Autophagy**

Autophagy can reduce age-related dysfunctions through systemic effects. Autophagy may contribute to the clearance of intracellular pathogens and the function of antigen-presenting cells (left), reduce inflammation by several mechanisms (middle), or improve the function of neuroendocrine circuits (right).

***David et al., Cell., 2011***

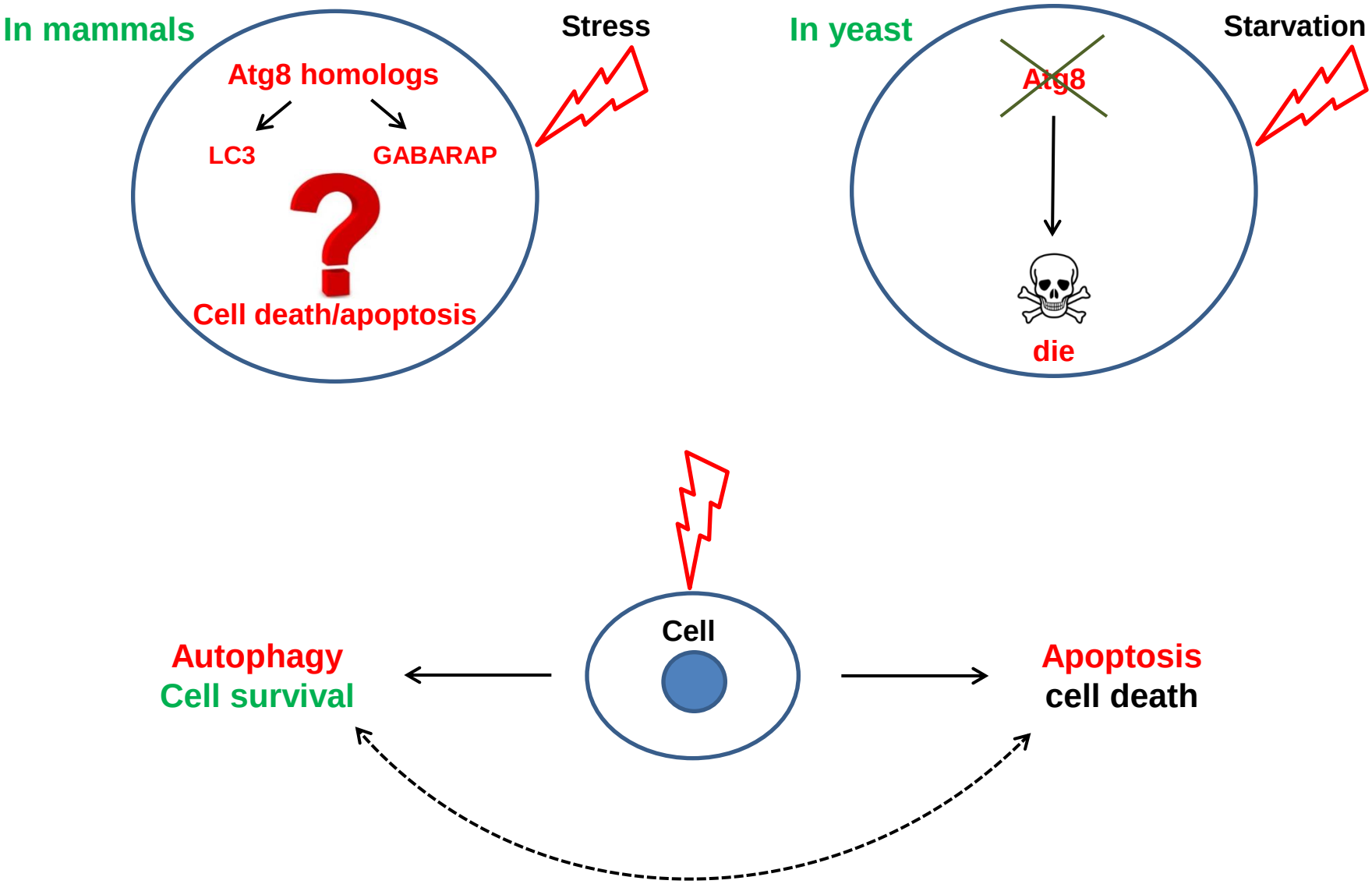
# Autophagy and Neurodegenerative Diseases

- Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) share common features, such as neuronal dysfunction, synapse damage and mechanisms involving death pathways.
- These disorders are characterized by progressive neuronal loss and by deposits of abnormal proteins in the brain, in the form of aggregates or plaques.

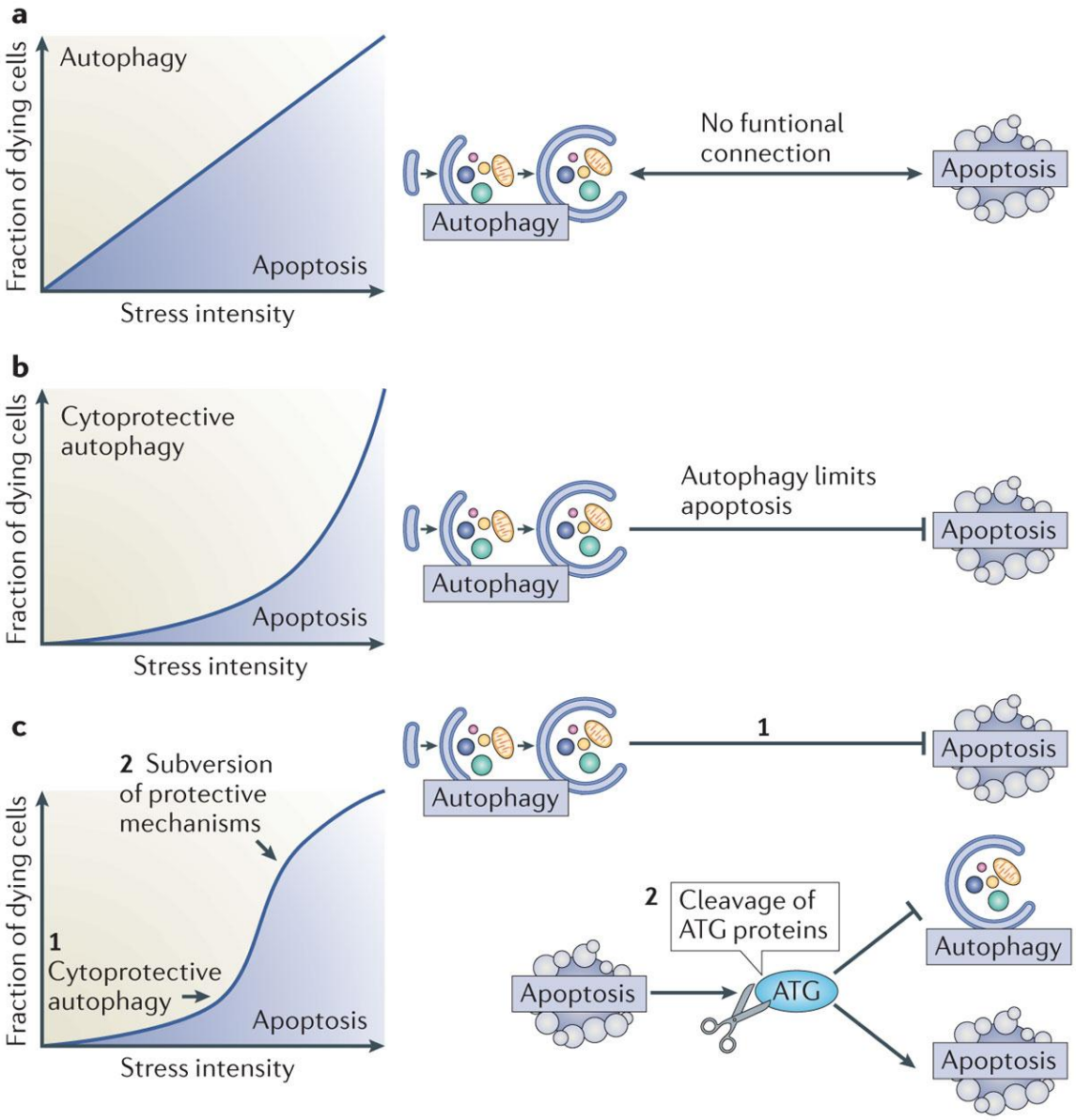



TRENDS in Molecular Medicine

# Autophagy and Apoptosis



# Autophagy and Apoptosis




*Mariño et al., Nat Rev Mol Cell Biol 2014.*

Nature Reviews | Molecular Cell Biology

# Autophagy and Cancer

The connections between autophagy and cancer occur at two aspects:

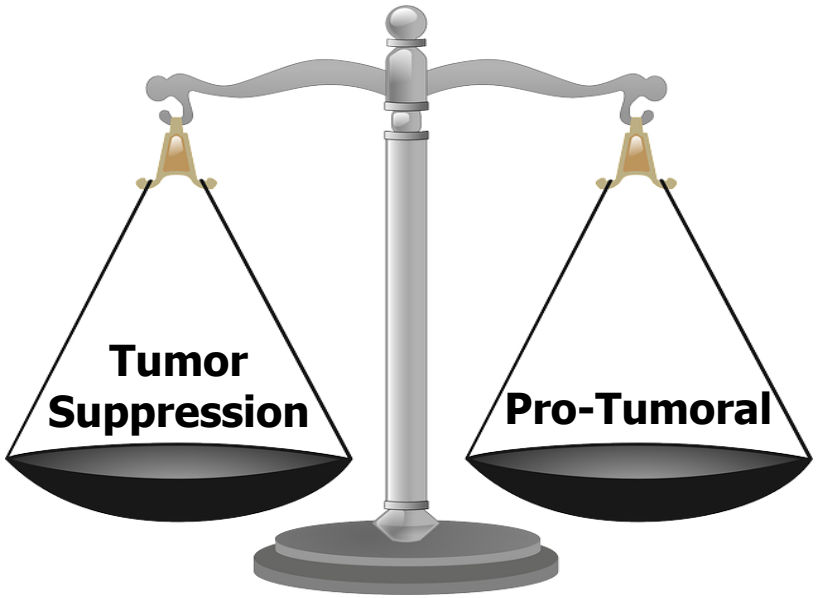
**First** at the level of tumor initiation and progression,

**Second** during cancer treatment.

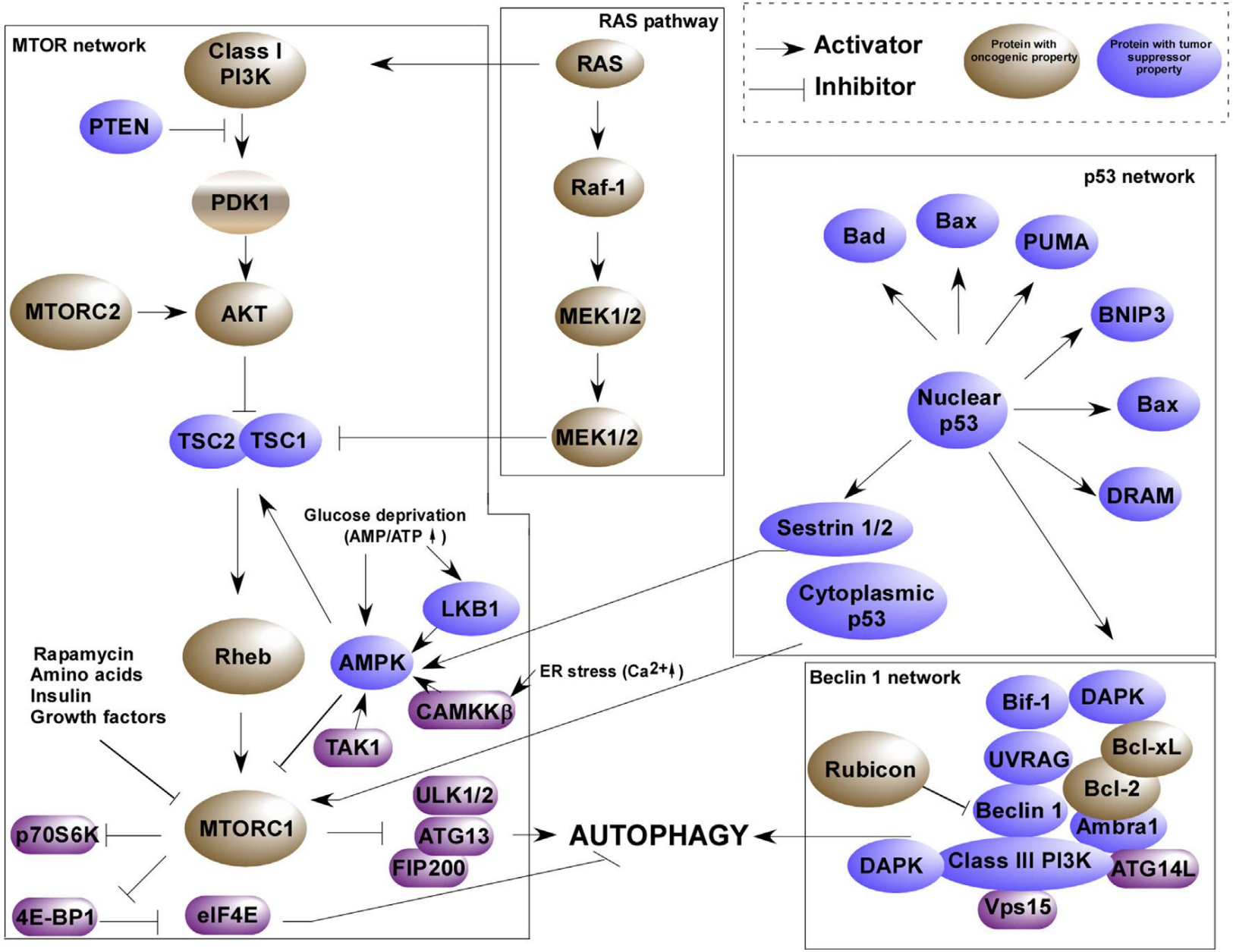
# Autophagy in Tumor Initiation and Progression

The role of autophagy in cancer is complex and likely tissue and genetic context-dependent.

**Dual role of Autophagy**



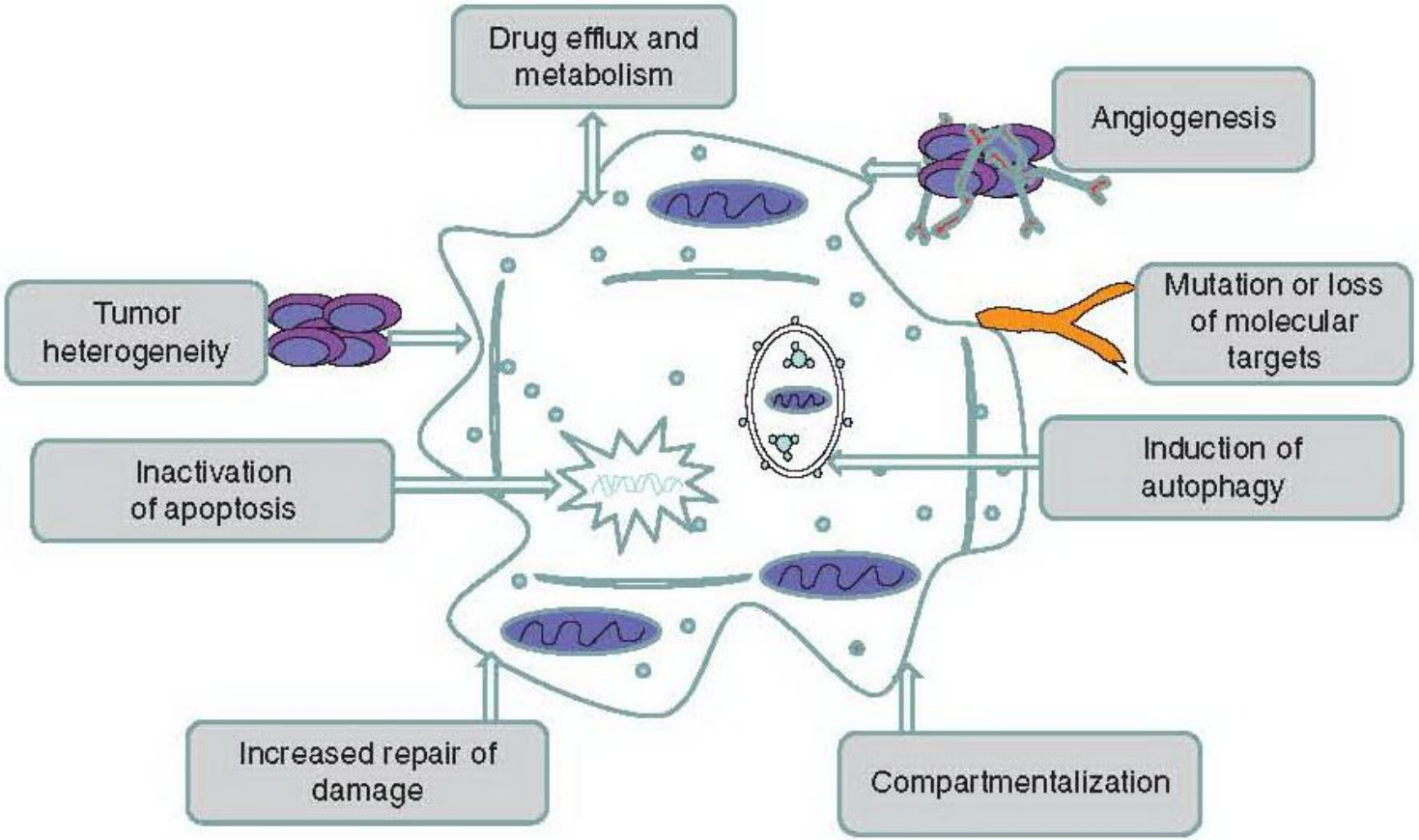
# Principal signalling pathways involved in the autophagy-related cancer interconnections




*Lorin et al., Seminars in Cancer Biology, 2013*

# Autophagy in Cancer Treatment

## Autophagy and chemotherapy resistance




A summary of the approaches by which cancer cells become resistant to chemotherapy and various kinds of genotoxic or metabolic stresses

***X Sui et al., Cell Death Dis., 2013***

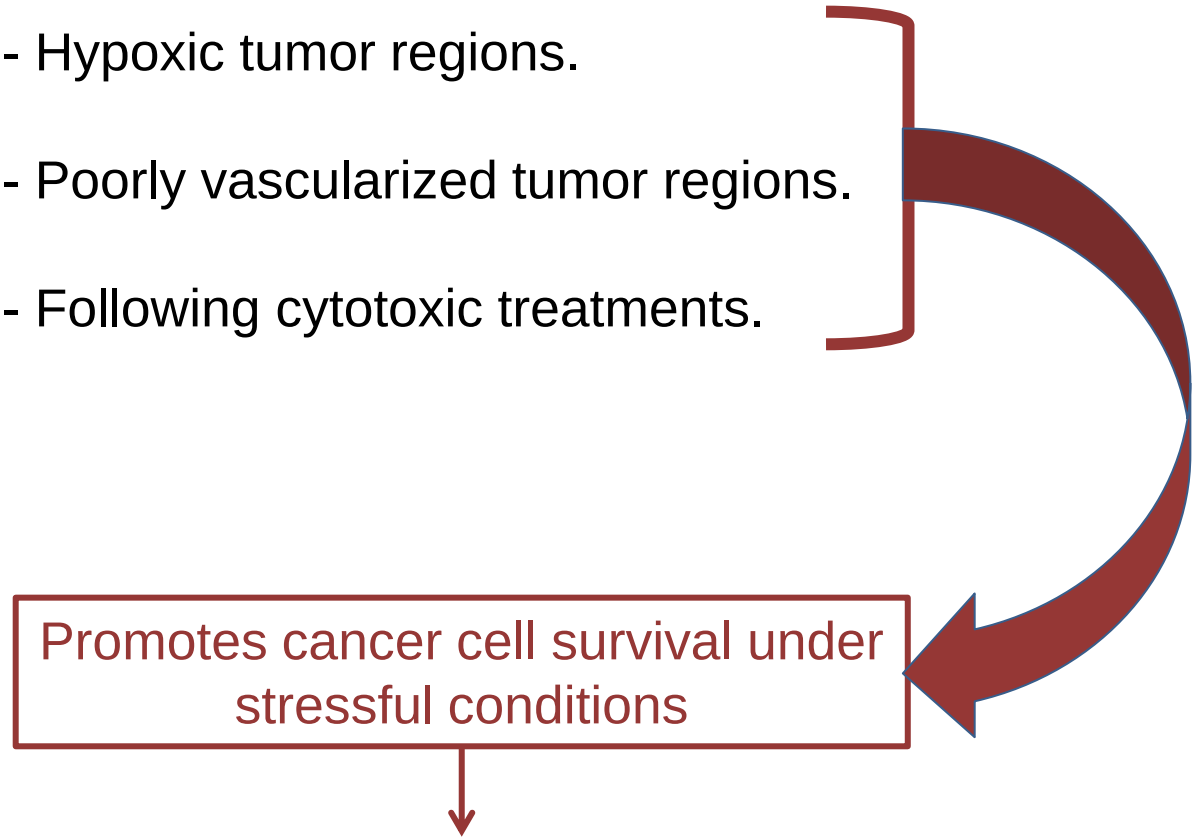
# Autophagy in Cancer Treatment

Autophagy induction have been found to spatially localize to:

1- Hypoxic tumor regions.

2- Poorly vascularized tumor regions.

3- Following cytotoxic treatments.



# Autophagy in Cancer Treatment

The strategies for autophagy inhibition

| Strategies | Target | The effect on autophagy |
|---|---|---|
| *Pharmacological approaches* | | |
| Chloroquine | Lysosomal pH | Inhibit autophagosome fusion with lysosomes and autophagosome degradation |
| Hydroxychloroquine | Lysosomal pH | Inhibit autophagosome fusion with lysosomes and autophagosome degradation |
| Monensin | Change endocytic and lysosomal pH | Inhibit the initiation/expansion stage of autophagy |
| Bafilomycin A 1 | Class III PI3K inhibitor | Inhibit the initiation/expansion stage of autophagy |
| 3-Methyladenine | Class III PI3K inhibitor | Inhibit the initiation/expansion stage of autophagy |
| Wortmannin | Class III PI3K inhibitor | Inhibit the initiation/expansion stage of autophagy |
| LY294002 | Class III PI3K inhibitor | Inhibit the initiation/expansion stage of autophagy |
| Pyrvinium | Class III PI3K inhibitor | Inhibit the initiation/expansion stage of autophagy |
| Genetic silencing of autophagy regulatory genes | | Inhibit the initiation/expansion stage of autophagy |

# Autophagy in Cancer Treatment

| Therapeutic Agent | Model | Autophagy Inhibition | Response |
|---|---|---|---|
| Temozolomide | Human malignant glioma cell lines | 3-Methyladenine<br>Bafilomycin A | Decreased cytotoxicity<br>Increased cytotoxicity |
| Cyclophosphamide | Murine Myc-induced lymphoma cancer | Chloroquine | Increased antitumor response |
| 5-Fluorouracil | Human colon cancer cell lines | 3-Methyladenine | Increased apoptosis |
| 5-Fluorouracil | Human colon cancer cell lines and xenograft | | Increased cytotoxicity |
| 5-Fluorouracil | Human colon cancer cell line (HT29) | Chloroquine | Increased cytotoxicity |
| 5-Fluorouracil | Human hepatic carcinoma cell lines | 3-Methyladenine | Increased apoptosis |
| 5-Fluorouracil | Murine colon cancer cell line and tumor xenograft | Chloroquine | Increased apoptosis |
| 5-Fluorouracil | Human NSCLC cell line (A549) | 3-Methyladenine | Increased apoptosis |
| Cisplatin | Esophageal SSC cell line (EC9706) | 3-Methyladenine | Increased apoptosis |
| Cisplatin | Human cholangiocarcinoma cell lines | 3-Methyladenine<br>Wortmannin | Increased cytotoxicity |
| Cisplatin | Human cervical cancer cell line (HeLa) | 3-Methyladenine<br>Chloroquine | Increased apoptosis |
| Cisplatin | Human hepatic carcinoma cell lines | 3-Methyladenine | Increased apoptosis |
| Cisplatin | Laryngeal cancer cells (Hep-2) | 3-Methyladenine | Increased apoptosis |
| Cisplatin | Human NSLC cell line (A549) | 3-Methyladenine | Increased apoptosis |
| Oxaliplatin | Human colon cancer cell lines and xenograft | Chloroquine | Increased cytotoxicity and tumor control |
| Paclitaxel | Human NSLC cell line (A549) | 3-Methyladenine | Increased apoptosis |
| Etoposide | Human hepatocellular carcinoma cell line (HepG2) | 3-Methyladenine | Increased cytotoxicity |
| Doxorubicin | Human multiple myeloma cell lines, patient-derived multiple myeloma cells, human plasmacytoma xenograft | Hydroxychloroquine<br>3-Methyladenine | Increased apoptosis |
| Epirubicin | Human breast cancer cell line (MCF7) | Bafilomycin A | Increased apoptosis |
| Melphalan | Human multiple myeloma cell lines, patient-derived multiple myeloma cells, human plasmacytoma xenograft | Hydroxychloroquine<br>3-Methyladenine | Increased apoptosis |
| Topotecan | Human NSLC cell line (A549) | Chloroquine | Increased cytotoxicity |
| Camptothecin | Human breast cancer cell lines | Wortmannin<br>3-Methlyadenine<br>Bafilomycin A | Increased apoptosis in selective cell lines |

# Autophagy in Cancer Treatment

| Therapeutic Agent | Model | Autophagy Inhibition | Response |
|---|---|---|---|
| Imatinib | Human glioma cell lines | 3-Methyladenine<br>Bafilomycin A | Decreased cytotoxicity<br>Increased cytotoxicity |
| Imatinib | Human Philadelphia chromosome positive CML cells | Chloroquine | Increased cytotoxicity |
| HDACi/vorinostat | Human colon cancer cells and xenografts | Chloroquine | Increased cytotoxicity<br>Decreased growth |
| HDACi/panobinostat | Human triple negative breast cancer cells and xenografts | Chloroquine | Increased cytotoxicity<br>Decreased tumor growth |
| HDACi/SAHA | Human CML cell lines and primary CML cells | Chloroquine | Increased cytotoxicity |
| HDACi/valproic acid | Human t(8;21) acute myeloid leukemia cells | Chloroquine | Increased cytotoxicity |
| HSP90i/DMAG | Human multiple myeloma cell lines | 3-Methyladenine | Increased cytotoxicity |
| Erlotinib | Human glioblastoma cell lines | Chloroquine | Increased cytotoxicity |
| Sorafenib | Human hepatocellular carcinoma cell lines and xenografts | Chloroquine<br>3-Methyladenine | Increased cytotoxicity and decreased tumor growth |
| Sorafenib | Human hepatocellular carcinoma cell lines and xenografts | Chloroquine | Increased cytotoxicity and decreased tumor growth |
| Sunitinib | Rat PC12 cells | Ammonium chloride | Increased cytotoxicity |
| AKTi/AZD5363 | Human prostate cancer cell lines and xenograft | 3-Methyladenine<br>Chloroquine<br>Bafilomycin A | Increased cytotoxicity and decreased tumor growth |
| METi/PHA665752 and EMD1214063 | Human gastric adenocarcinoma cell line | 3-Methyladenine | Increased cytotoxicity |
| Vandetanib | Human glioblastoma cell lines and xenograft | 3-Methyladenine<br>Chloroquine | Increased cytotoxicity and decreased tumor growth |
| Bevacizumab | Human hepatocellular carcinoma xenografts | Chloroquine | Decreased tumor growth |
| Bortezomib | Human multiple myeloma cell line (U266) | 3-Methyladenine<br>Bafilomycin A | Decreased cytotoxicity<br>Increased cytotoxicity |
| Bortezomib | Human hepatocellular carcinoma cell lines and xenografts | Chloroquine | Increased apoptosis |

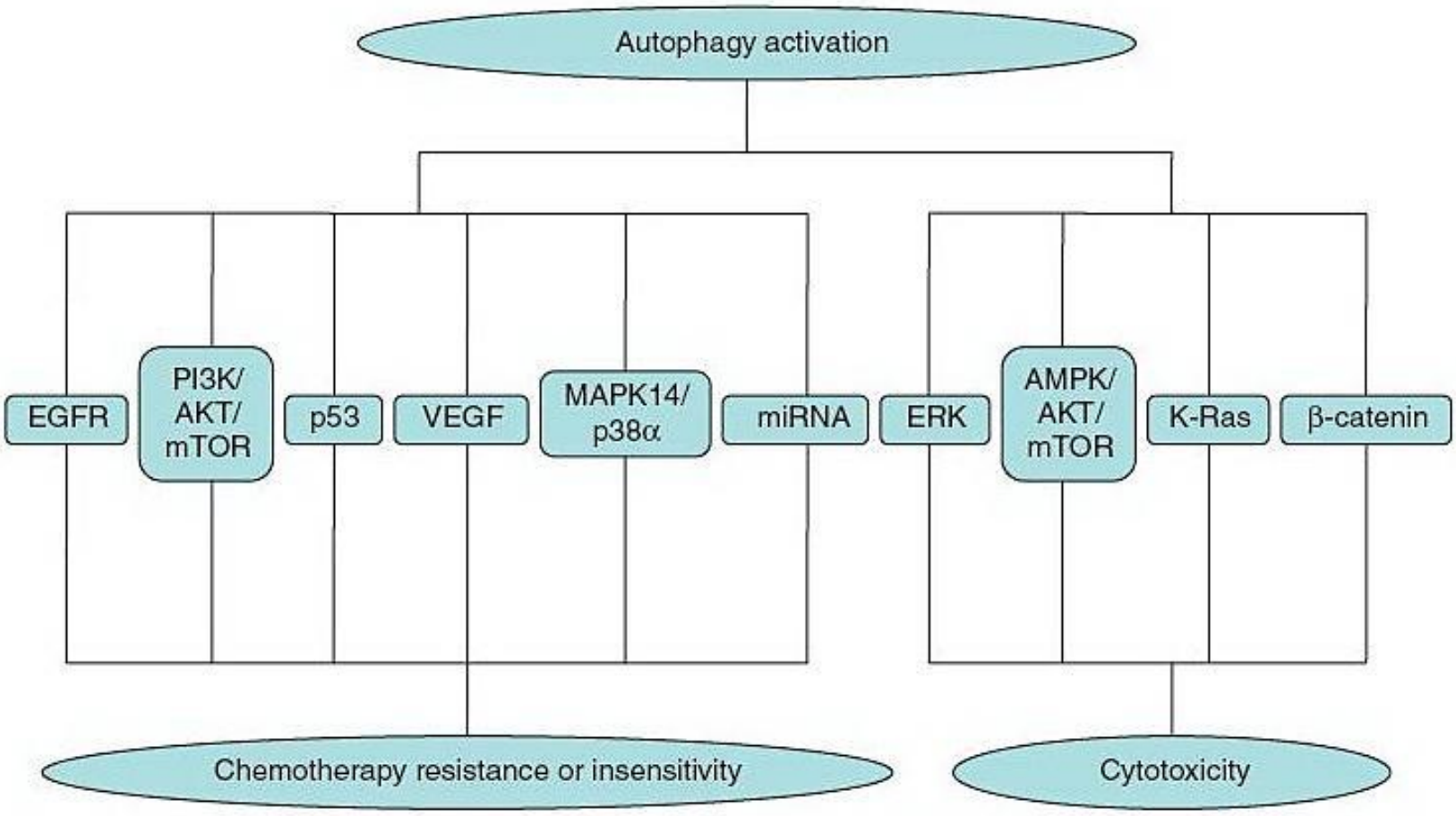
# Autophagy in Cancer Treatment

**Table 2** Active clinical trials combining the autophagy inhibitor HCQ with anticancer therapies

| Identifier | Cancer type | Drugs | Phase | Title |
|---|---|---|---|---|
| NCT00969306 | NSCLC | CQ + cisplatin Etoposide | I/II | Cisplatin, etoposide and escalating CQ in extensive disease SCLC |
| NCT00809237 | NSCLC | HCQ + gefitinib | I/II | Hydroxychloroquine and gefitinib to treat lung cancer |
| NCT01649947 | NSCLC | HCQ + paclitaxel and carboplatin | II | Modulation of autophagy in patients with advanced/recurrent non-small-cell lung cancer – phase II |
| NCT00977470 | Advanced NSCLC and (EGFR) mutations | HCQ + erlotinib | II | Erlotinib with or without hydroxychloroquine in chemonaive advanced NSCLC and (EGFR) mutations |
| NCT00933803 | Advanced or recurrent NSCLC | HCQ + carboplatin, paclitaxel, bevacizuma | | Carboplatin, paclitaxel, bevacizumab and HCQ in advanced or recurrent NSCLC |
| NCT01292408 | Breast cancer | HCQ | II | Autophagy inhibition using hydroxychloroquine in breast cancer patients |
| NCT00765765 | Breast cancer | HCQ + ixabepilone | I/II | Ixabepilone and HCQ in metastatic breast cancer |
| NCT01023477 | DCIS | CQ + tamoxifen | I/II | Neoadjuvant tamoxifen, tamoxifen + CQ, or CQ in DCIS |
| NCT01510119 | Renal cell carcinoma | HCQ and RAD001(p.o. 10 mg/day) | I/II | Autophagy inhibition to augment mTOR inhibition: a phase I/II trial of RAD001 and hydroxychloroquine in patients with previously treated renal cell carcinoma |
| NCT01144169 | Renal cell carcinoma | HCQ + high dose interleukin-2 and other systemic therapies | I | Study of hydroxychloroquine before surgery in patients with primary renal cell carcinoma |
| NCT01550367 | Renal cell carcinoma | HCQ + IL-2 | I/II | Study of hydroxychloroquine and aldesleukin in renal cell carcinoma patients (RCC) |
| NCT00726596 | Prostate cancer | HCQ | II | Hydroxychloroquine in treating patients with rising PSA levels after local therapy for prostate cancer |
| NCT01128296 | Pancreatic cancer | HCQ + gemcitabine | I/II | Study of presurgery gemcitabine + hydroxychloroquine (GcHc) in stage IIb or III adenocarcinoma of the pancreas |
| NCT01273805 | Pancreatic cancer | HCQ | II | Hydroxychloroquine in previously treated patients with metastatic pancreatic cancer |
| NCT01506973 | Pancreatic cancer | HCQ + gemcitabine/abraxane | I/II | A phase I/II/pharmacodynamic study of hydroxychloroquine in combination with gemcitabine/abraxane to inhibit autophagy in pancreatic cancer |
| NCT01128296 | Pancreatic cancer | HCQ + gemcitabine | I/II | Study of Pre-surgery Gemcitabine + hydroxychloroquine (GcHc) in stage IIb or III adenocarcinoma of the pancreas |
| NCT01494155 | Pancreatic cancer | HCQ + capecitabine + photon radiation | II | Short-course radiation therapy with proton beam capecitabine and hydroxychloroquine for resectable pancreatic cancer |
| NCT01206530 | Colorectal cancer | HCQ + FOLFOX/bevacizumab | I/II | FOLFOX/Bevacizumab/Hydroxychloroquine (HCQ) in colorectal cancer |
| NCT01006369 | Metastatic colorectal cancer | HCQ + capecitabine, oxaliplatin, and bevacizumab | II | Hydroxychloroquine, capecitabine, oxaliplatin, and bevacizumab in treating patients with metastatic colorectal cancer |
| NCT00224978 | Glioblastoma | CQ | III | Adjuvant CQ *versus* placebo in glioblastoma |
| NCT00486603 | Glioblastoma | HCQ + temozolomide | I/II | Adjuvant radiation, temozolomide and HCQ in newly resected GBM |
| NCT00962845 | Melanoma | HCQ | No phase specified | Hydroxychloroquine in patients with stage III or Stage IV melanoma that can be removed by surgery |
| NCT00568880 | Multiple myeloma | HCQ + bortezomib | I/II | Hydroxychloroquine and bortezomib in treating patients with relapsed or refractory multiple myeloma |
| NCT01480154 | Advanced solid tumors or prostate or renal cancer | HCQ + MTD of Akt inhibitor MK2206 (MK-2206) | I | Phase I study of Akt inhibitor MK2206 and hydroxychloroquine in patients with advanced solid tumors or prostate or renal cancer |
| NCT00909831 | Metastatic solid tumors | HCQ + temsirolimus | I | Hydroxychloroquine and temsirolimus in treating patients with metastatic solid tumors that have not responded to treatment |
| NCT00813423 | Advanced solid tumors | HCQ + sunitinib | I | Sunitinib and Hydroxychloroquine in treating patients with advanced solid tumors that have not responded to chemotherapy |
| NCT01023737 | Advanced solid tumors | HCQ + vorinostat | I | Vorinostat and HCQ in advanced solid tumors |
| NCT01417403 | Solid tumors undergoing radiation therapy for bone metastases | HCQ | I | Hydroxychloroquine in treating patients with solid tumors undergoing radiation therapy for bone metastases |
| NCT01266057 | Advanced cancer | HCQ + the highest tolerable dose of sirolimus or vorinostat | I | Sirolimus or vorinostat and hydroxychloroquine in advanced cancer |
| NCT00714181 | Metastatic or unresectable solid tumors | HCQ + temozolomide | I | Hydroxychloroquine and temozolomide in treating patients with metastatic or unresectable solid tumors |
| NCT01227135 | CML | HCQ + imatinib | II | Imatinib mesylate with or without hydroxychloroquine in treating patients with chronic myeloid leukemia |
| NCT01634893 | Ovarian cancer | HCQ + sorafenib | I | Oral hydroxychloroquine plus oral sorafenib to treat epithelial ovarian cancer FIGO stage III or stage IV, or extraovarian peritoneal carcinoma, or fallopian tube carcinoma failing or ineligible for first-line therapy |

NSCLC, non-small-cell lung cancer; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; MTD, maximum tolerated dose; HCQ, hydroxychloroquine

# Autophagy in Cancer Treatment




The molecular mechanisms of autophagy activation in response to chemotherapeutic agents. The activation of autophagy either leads to cancer cell chemoresistance via EGFR signaling, PI3K/AKT/ mTOR pathways, p53, VEGF, MAPK14/p38α signaling and microRNA or potentiates autophagic cell death through AMPK/AKT1/mTOR axis, which depends on the tumor types and treatment characteristic

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| 1 | | Not yet recruiting | Autophagy Bladder Cancer | • Bladder Cancer | | |
| 2 | | Unknown † | Autophagy Inhibition Using Hydrochloroquine in Breast Cancer Patients | • Breast Cancer | • Drug: Hydrochloroquine | Phase 2 |
| 3 | | Completed Has Results | Modulation of Autophagy in Patients With Advanced/Recurrent Non-small Cell Lung Cancer - Phase II | • Non-small Cell Lung Cancer<br>• Advanced Non-small Cell Lung Cancer<br>• Recurrent Non-small Cell Lung Cancer | • Drug: Paclitaxel<br>• Drug: Carboplatin<br>• Drug: Hydroxychloroquine<br>• Drug: Bevacizumab | Phase 2 |

# Can you induce autophagy?

- **Fasting and calorie restriction**

Both trigger autophagy by putting cells under stress.

- **Exercise**

Exercise also puts the body's cells under stress. When people exercise, the components of their cells become damaged and inflamed.

There is evidence that exercise increases autophagy in human skeletal muscles.

- **Curcumin**

Scientists have also suggested that curcumin intake triggers autophagy, at least in studies involving mice. For example, one animal study reported that curcumin-induced restoration of autophagy could protect against diabetic cardiomyopathy, a disorder of the heart muscles that affects people with diabetes.


Thank you for attention
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